Communauté
UNIVERSITÉ Grenoble Alpes

THÈSE

Pour obtenir le grade de

DOCTEUR DE LA COMMUNAUTE UNIVERSITE GRENOBLE ALPES

Spécialité : **Sciences du médicament**

Arrêté ministériel : 25 mai 2016

Présentée par

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Préparée au sein du **Département de Pharmacochimie Moléculaire (DPM, UMR CNRS 5063, ICMG FR 2607, Université Grenoble Alpes)** dans **l'École Doctorale Chimie et Sciences du Vivant (ED 218), Université Grenoble Alpes**

Développement et élaboration de systèmes innovants pour la voie ophtalmique

Thèse soutenue publiquement le **13 décembre 2019**
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A mon mari Stéphane, à ma fille Emma, à mes parents

REMERCIEMENTS

Une thèse de science n'est pas uniquement faite d'expériences scientifiques, il s'agit également d'une aventure humaine et les rencontres ont été pour moi tout aussi épanouissantes que la recherche proprement dite. J'espère que les personnes qui m'ont encadrée, soutenue et encouragée ou ont contribué de près ou de loin à sa réalisation et à son aboutissement trouveront dans ces remerciements toute ma reconnaissance et l'estime que je peux avoir pour elles.

Je tiens tout d'abord à exprimer toute ma gratitude aux membres du jury pour l'honneur qu'ils me font en acceptant de juger ce travail.

Professeure Valérie SAUTOU et Docteur Stéphane GIBAUD, je vous remercie d'avoir accepté la charge complexe de rapporteur.

Professeure Valérie SAUTOU, je tiens à vous remercier pour l'accueil que vous m'avez réservé dans votre laboratoire en tant qu'interne, pour vos enseignements et pour m'avoir suivie de loin ces dernières années.

Professeure Amélie BOCHOT et Professeur Christophe CHIQUET, je tiens à vous remercier pour votre accompagnement bienveillant lors des comités de suivi de thèse et pour m'avoir fait profiter de votre savoir et de votre expertise.

Professeur Pascal LE CORRE, en siégeant dans mon jury de thèse vous me faites l'honneur d'apporter votre expérience à la critique de ce travail, soyez-en vivement remercié.

Professeur Pierrick BEDOUCH, je ne sais comment te remercier pour la confiance que tu m'as témoignée en me confiant des responsabilités hospitalières et d'enseignement, pour tes conseils précieux et surtout ton soutien constant sur le long parcours hospitalo-universitaire.

Docteur Annabelle GEZE, je vous remercie chaleureusement d'avoir accepté de m'encadrer toutes ces années, du master de recherche à cette thèse. Je voudrais vous

témoigner toute mon estime et ma reconnaissance pour le partage de vos connaissances et votre exigence. Vos conseils pertinents ont guidé ce travail jusqu'au bout.

Professeur Denis WOUESSIDJEWE, je vous remercie d'avoir accepté d'être mon directeur de thèse et pour la confiance que vous m'avez témoigné lors de mes premières années d'enseignement. J'ai pu apprécier votre gentillesse, votre simplicité et votre grande disponibilité. Votre sens de la formule m'a permis de comprendre toute la rigueur et la précision nécessaires à un travail scientifique.

Ce travail n'aurait été possible sans la rigueur scientifique et le soutien de mes collègues et collaborateurs. J'espère sincèrement que cette thèse n'est que le début d'une collaboration longue et fructueuse.

A Delphine et Véronique pour votre bonne humeur et votre aide précieuse au quotidien.

A Luc pour ta grande disponibilité, ta curiosité pour chacun des projets et surtout ton aide en statistique.

A Vincent VERDOOT pour ta grande disponibilité et ton aide efficace lors de la caractérisation rhéologique des formulations.

A Francisco J. Otero-Espinar et toute l'équipe du *Department of Pharmacology* et du *Clinical Pharmacology Group* de Saint Jacques de Compostelle pour notre collaboration sur le radiomarquage des formulations et l'étude de leur biopermanence sur la surface oculaire.

A Arnaud DELORT, inspecteur aux abattoirs de l'Isère et au personnel de l'abattoir de Grenoble pour votre sympathie et votre aide à obtenir des cornées de porc.

A Frédéric LAMARCHE, Emily TUBBS, Inès ZAROURI, Nadia et Jean-Marc MOULIS pour leur conseils précieux lors de l'étude de cytotoxicité sur cellules HCEC.

A Lucile KOWALSKI, Bruna KORESH, Fanny LANTHEAUME, Saoudatouh BAH pour votre participation, en tant qu'étudiant, dans ce projet.

A Marie-Do, vous avez été la première à me faire confiance. Vous m'avez vu grandir professionnellement et personnellement. Je ne saurais comment vous remercier pour votre soutien sans failles, votre écoute active et votre grande disponibilité.

A Marjorie et Audrey, deux amies sincères avec qui j'ai le grand plaisir de travailler. Je vous remercie toutes les deux pour votre soutien, votre bonne humeur et vos conseils avisés.

Je tiens également à remercier l'ensemble de mes collègues du DPM et de l'équipe NOVA, du Pôle Pharmacie, pharmaciens, préparateurs, cadres, internes, externes, agents administratifs pour votre aide et votre soutien tout au long de ce travail de thèse. Vous m'avez permis de me consacrer sereinement à l'élaboration de ma thèse.

Mes remerciements vont enfin à ma famille et à mes amis qui, avec ces deux questions récurrentes « quand vas-tu soutenir cette thèse ? » et « de quoi ça parle déjà ? » bien qu'angoissantes, elles m'ont permis de ne jamais dévier de l'objectif, en tout humilité.

A mes nombreux amis … pour votre présence et votre soutien sans failles lors des soirées de décompression.

A mes parents, mes frères, mes belles-sœurs, mon beau-frère, mon beau-père, toute ma belle-famille, mes cousins, mes oncles et tantes, ma marraine, mon parrain, mes neveux et nièces et mes filleuls pour vos sourires et vos encouragements.

A ma fille Emma, mon petit chat d'amour, pour tes délicates attentions et ta patiente compréhension lors de la rédaction de la thèse.

A mon mari Stéphane pour ton soutien quotidien indéfectible, ta compréhension, tes sourires et ton amour. Notre couple et notre famille ont grandi en même temps que mon projet professionnel, le premier servant de socle solide à l'épanouissement du second.

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Les travaux de cette thèse ont été réalisés au sein de l'équipe Nouveaux Outils de Vectorisation et d'Analyse (NOVA) du Département de Pharmacochimie Moléculaire, UFR de Pharmacie, Université Grenoble Alpes.

Cette thèse a notamment permis de soutenir la recherche clinique sur le médicament et d'augmenter le vivier de diplômés pharmaciens docteurs d'Université susceptibles de se porter candidats sur des postes d'hospitalo-universitaires. Ce projet de recherche a été l'occasion de développer de nouvelles collaborations avec d'autres équipes de recherche universitaires et le CHU de Grenoble Alpes (Pôle Ophtalmologie).

La diffusion des résultats issus de ce travail de thèse s'est faite d'une part à travers la participation à des séminaires et des congrès scientifiques nationaux et internationaux, et d'autre part à travers des publications dans des revues scientifiques.

Articles soumis ou publiés dans des revues internationales à comité de lecture :

- Yaméogo JB, **Mazet R**, Wouessidjewe D, Choisnard L, Godin-Ribuot D, Putaux JL, Semdé R, Gèze A. Pharmacokinetic study of intravenously administered artemisinin-loaded surface-decorated amphiphilic γ-cyclodextrin nanoparticles. Materials Science & Engineering C 106 (2020) 110281 ; doi : 10.1016/j.msec.2019.110281
- **Mazet R**, Choisnard L, Levilly D, Wouessidjewe D, Gèze A. Investigation of combined cyclodextrin and hydrogel formulation for ocular delivery of dexamethasone acetate by means of experimental designs. *Pharmaceutics* (2018);*10* (4), 249; doi : 10.3390/pharmaceutics10040249
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Communications orales (réunions scientifiques nationales avec comité de sélection et ayant donné lieu à la publication d'un résumé)

- **Mazet R,** Gèze A, Choisnard L, Wouessidjewe D. Développement d'outils d'administration topique ophtalmique, 16ème JCD, 2014, Amiens, France.

Communications affichées dans les réunions scientifiques internationales avec comité de sélection et ayant donné lieu à la publication d'un résumé

- **Mazet R**, Gèze A, Choisnard L, Wouessidjewe D. Cyclodextrin based hydrogel formulation of dexamethasone for ocular delivery by means of experimental designs, 5th European Conference on Cyclodextrins, 2017, Lisbonne, Portugal.
- **Mazet R,** Gèze A, Choisnard L, Wouessidjewe D. Design of new cyclodextrin formulations for ocular drug delivery, 4th European Conference on Cyclodextrins, 2015, Lille, France.

Communications affichées dans les réunions scientifiques nationales avec comité de sélection et ayant donné lieu à la publication d'un résumé

- **Mazet R**, Gèze A, Choisnard L, Wouessidjewe D, Cyclodextrin based hydrogel formulation of dexamethasone for ocular delivery by means of experimental designs, JSM, 2018, Grenoble, France.
- **Mazet R**, Gèze A, Choisnard L, Wouessidjewe D. Design of new cyclodextrin formulations for ocular drug delivery, Glyco@alps, 2017, Grenoble, France.
- **Mazet R**, Gèze A, Choisnard L, Putaux JL, Boumendjel A, Wouessidjewe D. Formulation d'un modulateur des récepteurs ABCG2, JRM, 2012, Grenoble, France.
- **Mazet R**, Gèze A, Choisnard L, Putaux JL, Boumendjel A, Wouessidjewe D. Formulation d'un modulateur des récepteurs ABCG2, JSM, 2012, Grenoble, France.

LISTE DES SIGLES ET ABREVIATIONS UTILISES

^{18}F-FDG	^{18}F-Fluordesoxyglucose
AI	Anti-inflammatoire
AINS	Anti-inflammatoire non stéroïdien
AIS	Anti-inflammatoire stéroïdien
BCG	Vaccin bilié de Calmette et Guérin
BCRP	*Breast cancer resistance protein*
BRAF	B-Raf kinase
BSS	*Balanced salt solution*
CAP	Conjonctivite allergique perannuelle
CAS	Conjonctivite allergique saisonnière
CD	Cyclodextrine
CE	Efficacité de complexation
CGP	Conjonctivite gigantopapillaire
CHU	Centre Hospitalier Universitaire
CLHP	Chromatographie liquide haute performance
CMI	Concentration minimale inhibitrice
DMLA	Dégénérescence maculaire liée à l'âge
DXM	Dexaméthasone
DXMa	Dexaméthasone acétate
HAS	Haute autorité de santé
HCEC	*Human Corneal Endothelial Cells*
HPβCD	Hydroxypropyl-β-cyclodextrine
HPγCD	Hydroxypropyl-γ-cyclodextrine
HSV	*Herpes simplex virus*
ICH	*International Council of Harmonisation*
ICPI	*Immune-checkpoint inhibitor*
IFN	Interféron
Ig	Immunoglobuline
IL	Interleukine
ITC	Titrage calorimétrique isotherme
K	Constante d'association
KCA	Kératoconjonctivite atopique
KCV	Kératoconjonctivite vernale
MCP	*Monocyte chemoattractant protein*
MDR	*Multidrug resistance protein*
MEK	*Mitogen activated protein kinase enzyme*
MMP	Matrix metalloproteinase
MRT	Temps de résidence moyen
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetratozolium
NOVA	Nouveaux outils de vectorisation et d'analyse
PEG	Polyéthylène glycol

ROR	Rougeole Oreillons Rubéole
SBEβCD	Sulfobutylether-β-cyclodextrine
TEP	Tomographie à émission de positons
TGF	*Transforming growth factor*
TNF	*Tumor necrosis factor*
VEGF	*Vascular endothelial growth factor*
VHB	Virus de l'hépatite B

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INTRODUCTION GENERALE

L'œil humain est un organe neurosensoriel extrêmement spécialisé dont l'anatomie unique permet de capter et de traiter les informations visuelles. Il présente deux compartiments : le segment antérieur et le segment postérieur [1]. Le film lacrymal, la conjonctive, la cornée, l'iris, le cristallin, et l'humeur aqueuse appartiennent au segment antérieur (Figure 1). Le segment postérieur inclut principalement la rétine, la choroïde, la sclère, la fovéa, la macula et l'humeur vitrée [2]. La cornée est le premier élément réfractif de l'œil et remplit une fonction importante de la vue, en convergeant la lumière en un point focal rétinien. La rétine est une membrane sensorielle composée de photorécepteurs qui transforment les signaux lumineux en signaux électrochimiques. Ces signaux sont transférés au cerveau via le nerf optique puis les voies optiques. Le cristallin est une lentille biconvexe permettant le réflexe d'accommodation et de mise au point par modification de ses courbures.

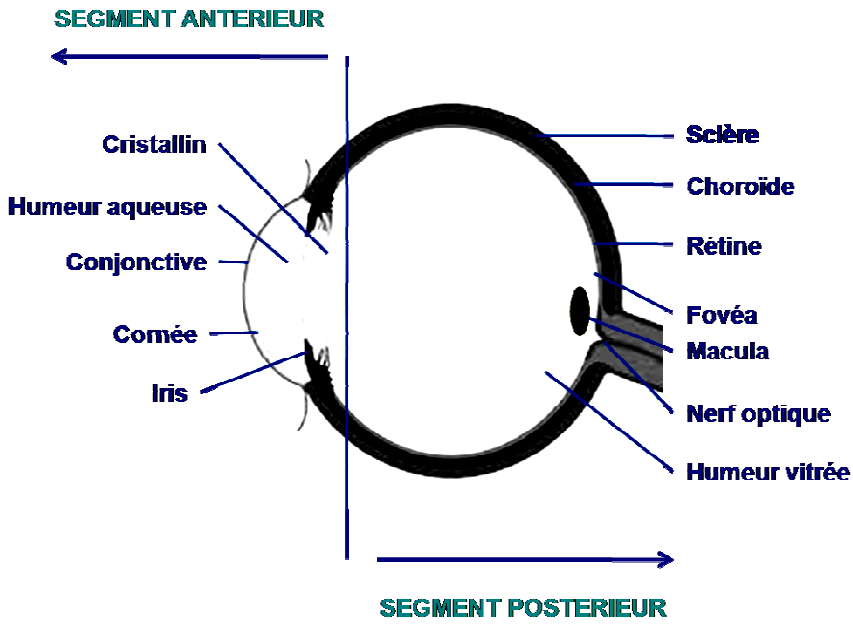

Figure 1. Anatomie de l'œil

La vision est souvent considérée comme le sens le plus précieux et représente une part importante de notre sensorialité [3]. La prévention de la cécité et des déficiences visuelles constitue un enjeu majeur de santé publique. Le *Vision Loss Expert Group* estimait que 36 millions de personnes étaient aveugles en 2015, alors que 216,6 millions de personnes présentaient une déficience modérée ou sévère [4]. L'inflammation ophtalmique est un

symptôme commun à de nombreuses pathologies et est considérée comme l'une des principales causes de cécité [5].

1. Inflammation oculaire

Il est à noter que la réaction inflammatoire oculaire n'est pas spécifique de cet organe. En effet, l'inflammation correspond à un ensemble de mécanismes réactionnels de défense, physiologiques et pathologiques, par lesquels l'organisme reconnaît, détruit et élimine toutes les substances qui lui sont étrangères. Il s'agit d'un processus dynamique comportant plusieurs étapes successives.

a. La réaction inflammatoire

Les principaux instigateurs de l'inflammation aiguë sont l'infection et la lésion tissulaire. Le déclenchement de la réaction inflammatoire peut également faire suite à la présence d'une substance exogène ou d'un agent toxique.

Prenons l'exemple le mieux décrit : la réaction inflammatoire après une infection bactérienne. L'inflammation commence par une réaction dite de « reconnaissance » de l'agent infectieux par les macrophages tissulaires et les mastocytes, entrainant la production de nombreux médiateurs inflammatoires tels que des cytokines, des amines vasodilatatrices, des eicosanoïdes, la cascade du complément et des chimio-attractants. L'effet principal et immédiat de ces médiateurs est de créer un exsudat inflammatoire local contenant des granulocytes neutrophiles et des protéines plasmatiques. Les granulocytes neutrophiles ont accès aux tissus extravasculaires grâce à un endothélium activé entrainant une extravasation sélective. Grâce à la présence de chimio-attractants, ils adhérent à la paroi vasculaire avant de la traverser pour remonter le gradient de chimio-attractants à travers les tissus [6]. Quand ils atteignent le site tissulaire affecté, ils sont activés, soit par contact direct avec les agents pathogènes recouverts d'anticorps, soit par l'action des cytokines. Ils émettent alors des pseudopodes, entourent l'agent infectieux en une vacuole et tentent alors de les tuer en libérant le contenu de leurs granulations dont les effets antimicrobiens sont liés à :

- un pH acide du lysosome qui inhibe la croise ou lyse les bactéries
- des substances bactéricides réactives de l'oxygène ou de l'azote
- un « burst oxydatif » permettant la production d'anion superoxyde ($O_2^{\cdot}$-) [7]

- des enzymes à activité antimicrobienne : protéinases, élastases, métalloprotéinases, ….[8]

Le contenu toxique des granulations est très puissant mais ne fait pas de distinction entre les cibles microbiennes et les tissus hôtes qui subissent inévitablement des dommages [8,9].

Ce phénomène s'accompagne d'une néo-vascularisation, d'une prolifération des fibroblastes et d'une augmentation des protéines de la matrice extracellulaire [10]. Les cellules immunitaires sécrètent également des cytokines et des protéines de régulation avec des fonctions pro ou anti-inflammatoires qui permettent de moduler le processus inflammatoire. Parmi les cytokines pro-inflammatoires, IL-3, IL-6, IL-1b et le *tumor necrosis factor-α* (TNF-α) sont directement impliquées dans le processus inflammatoire en augmentant les facteurs angiogéniques comme le *vascular endothelial growth factor* (VEGF) et le *matrix metalloproteinase*-9 (MMP-9). Dans le groupe des cytokines anti-inflammatoires, IL-10 interagit avec les cellules présentatrices d'antigène en inhibant la production d'IL-1, IL-6, IL-8 et TNF-α [11].

Une réponse efficace à l'inflammation aiguë entraine l'élimination de l'agent infectieux suivie d'une phase de cicatrisation médiée par les macrophages [12].

Parfois l'inflammation aiguë ne permet pas d'éliminer l'agent pathogène, comme par exemple des staphylocoques. La vacuole de digestion se détruit et lyse le neutrophile en libérant le contenu des granules de sécrétions qui peuvent entrainer des lésions tissulaires et causer une inflammation secondaire. Le processus inflammatoire persiste et acquiert de nouvelles caractéristiques : les neutrophiles sont remplacés par des macrophages, des plasmocytes ou des lymphocytes, surtout s'il existe une cause immunitaire. Localement, il se forme un granulome dans lequel les agents pathogènes sont « emmurés » par un tissu lymphoïde tertiaire et le tissu autour est détruit. Il sera remplacé par un tissu fibro-inflammatoire riche en collagène mais n'ayant pas les mêmes propriétés que le tissu initial. Si cette combinaison de cellules est inefficace, le processus inflammatoire se chronicise.

En dehors des agents pathogènes persistants, l'inflammation chronique peut également être liée à des réactions auto-immunes, à la présence de corps étrangers non dégradables, au stress ou à des dysfonctionnements tissulaires [8].

L'inflammation oculaire peut toucher les différents tissus des deux segments et, entre autres, les annexes de l'œil, la surface oculaire, la cornée, la conjonctive, l'iris, l'uvée, le vitrée et la rétine ainsi que les vaisseaux rétiniens, le nerf optique ou l'orbite [13].

L'inflammation oculaire accompagne diverses étiologies : infectieuse, allergique, auto-immune, traumatique, toxique, aigüe ou chronique. De plus, dans des pathologies supposées être non inflammatoires, dont la dégénérescence maculaire liée à l'âge ou l'œdème maculaire diabétique, on retrouve dans l'humeur aqueuse et vitrée des patients des taux élevés d'IL-6, IL-8, VEGF et le *monocyte chemoattractant protein-1* (MCP-1) qui sont des médiateurs inflammatoires [13,14].

b. Inflammation oculaire d'origine Infectieuse

De la paupière au nerf optique, les infections de l'œil sont diverses, tant par leur localisation que par la diversité des microorganismes en cause : bactéries, virus, champignons ou parasites. Les microorganismes induisant une inflammation ne sont pas nécessairement pathogènes. Les bactéries commensales sont une source importante d'inflammation par activation des récepteurs *Toll-like* [8].

L'infection de l'œil peut être isolée ou s'intégrer dans le cadre d'une infection systémique. Elle peut être aigüe ou chronique. Elle se manifeste généralement par un œil rouge, larmoyant, parfois accompagné de douleur, de sécrétions et d'une baisse de l'acuité visuelle (Tableau 1) [15–19].

L'étiologie est différente selon les régions et les facteurs de risques de la population. Ainsi, le trachome, lié à *Chlamydiae,* ou l'onchocercose des rivières, liée à *Onchocerca volvulus,* sont les deux causes majeures de cécité dans les pays à niveau de revenus faible par extension de l'infection au niveau de la cornée [20]. Dans les pays à haut niveau de revenus, les infections ophtalmiques tels que les orgelets, les blépharites, les conjonctivites, les kératites ou les uvéites sont généralement dues à des germes exogènes qui pénètrent au niveau d'un traumatisme oculaire, même minime [17]. Ces infections sont courantes et généralement bénignes. Cependant, certaines peuvent entraîner des séquelles avec baisse de l'acuité visuelle voire la cécité [18]. Dans de très rares cas, dans les pays à haut niveau de

revenus, l'infection peut être endogène. Il s'agit alors d'une infection potentiellement cécitante qui survient lorsque des micro-organismes de la circulation sanguine franchissent la barrière hémato-oculaire et se multiplient au sein des tissus intraoculaires. Cette affection survient habituellement sur un terrain d'immunodépression. Les signes septicémiques généraux précèdent les signes oculaires dans la majorité des cas [21].

Le traitement de l'infection oculaire est adapté à l'origine microbiologique et sa mise en œuvre doit être rapide afin de limiter le risque de complications. Il comprend des lavages ophtalmiques antiseptiques et des anti-infectieux parfois associés à des corticoïdes, utilisés en voie locale ou systémique, selon l'origine endogène ou exogène de l'infection. Les corticoïdes sont prescrits en phase aigüe car l'infection n'est pas seule responsable des séquelles observables. L'inflammation réactionnelle avec le stress oxydatif peuvent entrainer des effets délétères sur l'œil que les corticoïdes peuvent limiter [15].

L'usage d'un antiviral par voie oculaire se restreint aux infections à *Herpes simplex virus* (HSV) avec l'aciclovir, le ganciclovir ou la trifluridine, du fait du risque important de kératite ponctuée d'ulcérations avec baisse de l'acuité visuelle [17].

Le traitement des infections mycosiques est déterminé en fonction de l'identification, des concentrations minimales inhibitrices (CMI) et de l'aspect clinique initial. A l'exception de la natamycine qui existe sous forme de spécialité pharmaceutique, les collyres antimycotiques sont préparés par les pharmacies hospitalières, à partir d'antimycotiques destinés à l'usage systémique [19].

Le traitement des parasitoses oculaires est généralement systémique avec la prise d'ivermectine ou chirurgical [22].

La kératite, l'endophtalmie ou l'uvéite sont des urgences ophtalmiques nécessitant un traitement local adapté, des injections intravitréennes et parfois une vitrectomie et/ou un traitement systémique. Cette prise en charge rapide est indispensable pour sauver l'œil du patient voire sa vie [21]. La perte définitive de la vision peut survenir rapidement si l'infection et l'inflammation réactionnelle détruisent l'architecture fonctionnelle des tissus.

Tableau 1. Origines, tableaux cliniques et traitement des principales infections de l'œil

	Origine		Tableau clinique	Traitement
Infection palpébrale	Bactérienne	Staphylocoques coagulase négative, *Proprionibacterium acnes*	Rougeur +/- œdème palpébrale, nodule +/- Sécrétions +/- prurit	Traitement de l'infection (local) +/- Corticoïdes +/- Exérèse chirurgicale en cas de récidives
	Virale	*Herpes simplex virus*		
	Parasitaire	*Phtirius inguinalis*		
Conjonctivite	Bactérienne	*Cocci* à Gram positif, *Moraxella*, *Chlamydia trachomatis, Haemophilus influenza*	Œil rouge larmoyant avec des sécrétions Impression de sable sous les paupières +/- hyperhémie conjonctivale	Traitement de l'infection (local ou systémique) +/- Corticoïdes
	Virale	*Adénovirus, Entérovirus, Herpes simplex virus, coxsackivirus, poxvirus*		
	Fongique	*Blastomyces dermatitidis, Sporothrix schenckii*		
	Parasitaire	*Leishmania,* microsporidies, *Acanthamoeb*a		
Kératite	Bactérienne	*Cocci* à Gram positif, *Neisseria, Listeria, Shigella, Corynebacterium diphteriae*, *Moraxella, Klebsiella, Enterobater, Proteus, Serratia, Mycobacterium tuberculosis, Treponema pallidum*	Œil rouge larmoyant, douloureux Photophobie Blépharospasme Baisse de la vision	**En urgence** Traitement de l'infection (local ou systémique) +/- Corticoïdes +/- Greffe de cornée
	Virale	*Herpes simplex virus 1, Epstein-Barr virus,* virus de la rougeole, cytomegalovirus		
	Fongique	*Fusarium, Aspergillus, Candida,*		
	Parasitaire	*Acanthamoeba, Onchocerca volvulus, Leishmania,* microsporidies		
Endophtalmie	Bactérienne	*Cocci* à Gram positif, Bacilles à Gram négatif, *Propionibacterium acnes, Corynebacterium, Haemophilus influenza*	Œil rouge, douloureux, Baisse de l'acuité visuelle Œdème conjonctivo-palpébral, inflammation de la chambre antérieure et du vitré	**En urgence** Traitement de l'infection (local ou systémique) +/- Corticoïdes +/- Greffe de cornée
	Fongique	Candida, Aspergillus		
Uvéite	Bactérienne	*Mycobacterium tuberculosis, Borrelia burgdeferei*	Baisse de l'acuité visuelle +- myodésopsies et hyalite +/- opacités vitréennes +/- uvéite antérieure +/- rougeur Parfois asymptomatique	**En urgence** Traitement de l'infection (systémique) +/- traitement local (injection intravitréenne ou implant) +/- vitrectomie
	Virale	Cytomegalovirus, virus varicelle-zona, *Herpes simplex virus*, virus de la rougeole		
	Fongique	*Pneumocystis jirovecii, Cryptococcus neoformans*		
	Parasitaire	Toxoplasma, *Larva migrans, Onchocerca volvulus, Tænia solium*		
Névrite	Virale	Virus varicelle-zona	Eruption cutanée Douleur	Traitement de l'infection (local ou systémique) Antalgique

c. Inflammation oculaire d'origine allergique

L'allergie oculaire touche 15 à 20 % de la population, principalement les enfants et les jeunes adultes ; la conjonctivite allergique en est la manifestation la plus fréquente. L'œil étant ouvert sur l'environnement, il est particulièrement exposé aux aéroallergènes, mais étant également isolé du reste du corps, il réagit de façon indépendante [23].

Le spectre des conjonctivites allergiques est très large et couvre les formes aiguë et chronique (Tableau 2). La conjonctivite allergique saisonnière (CAS) est la plus fréquente. Les allergènes sont le plus souvent les pollens des graminées, d'arbres ou d'herbacés. Elles débutent généralement au printemps [25]. Elles surviennent suite à la liaison directe de l'allergène avec des immunoglobulines-E (IgE) spécifiques ce qui entraine la dégranulation des mastocytes présents en grand nombre dans la conjonctive et la libération de médiateurs, comme l'histamine [23]. L'histamine est responsable des démangeaisons oculaires et provoque une vasodilatation au niveau de la conjonctivite, ce qui déclenche d'autres symptômes d'hypersensibilité immédiate tels que les yeux larmoyants, l'œdème palpébral ou conjonctival et l'afflux vasculaire, provoquant des rougeurs oculaires [26]. En phase tardive, les mastocytes relarguent des leucotriènes, des prostaglandines conduisant à la libération de cytokines pro-inflammatoires [27]. La symptomatologie de la conjonctivite allergique est dominée par les quatre signes cardinaux : prurit, larmoiement, œdèmes palpébral et conjonctival, rougeur conjonctivale [28].

Les conjonctivites saisonnières (CAS) et perannuelles (CAP) sont essentiellement dues à un phénomène d'hypersensibilité de type I, médié par les IgE. Les kératoconjonctivites vernales (KCV) et atopiques (KCA) sont liées à une hypersensibilité retardée de type IV, non IgE dépendante [23,27], principalement due à une infiltration de lymphocytes CD4 positifs et d'éosinophiles [29].

Les traitements courants des allergies oculaires incluent les collyres antiallergiques (cromones ou antihistaminiques) pour les formes modérées. Les formes récurrentes avec atteintes de la cornée nécessitent l'utilisation de corticoïdes, de ciclosporine ou de tacrolimus en collyres afin d'éviter les séquelles visuelles. Des traitements immunomodulateurs en voie systémiques sont utilisés pour les formes chroniques [30,31].

Tableau 2. Classification des conjonctivites allergiques, adaptée de Fauquert [23] et Leonardi [32]

	Présentation	Mécanisme allergique	Symptômes	Allergènes	Traitement
Conjonctivite allergique saisonnière (CAS)	Intermittent	Immédiate IgE médiée, type I	Prurit Larmoiement Œdème palpébral et/ou conjonctival Rougeur	Aéroportés : acariens, moisissures, pollens, phanères d'animaux, poussières Chimiques : Alimentaires Médicamenteux Professionnels	Compresses froides Lavage ophtalmique Larmes artificielles Cromones topiques Antihistaminique topique ± oral Corticoïdes topiques Immunothérapie
Conjonctivite allergique perannuelle (CAP)	Persistant	Immédiate IgE médiée, type I			
Kératoconjonctivite vernale (KCV)	Persistant+/- exacerbations intermittentes	Immédiate IgE médiée ou non	Photophobie majeure Sensation de grain de sable Larmoiement Baisse d'acuité visuelle Amblyopies Papilles géantes	Environnementaux Poussières Pollens	Détersion de la plaque vernale Cromones topiques Antihistaminiques topiques AINS ou AIS topiques Ciclosporine ou tacrolimus topiques
Kératoconjonctivite atopique (KCA)	Chronique		Associée à une dermatite atopique, asthme ou antécédent familial d'atopie Larmoiement Prurit Sécrétions abondantes		Larmes artificielles Décongestionnants topiques Corticothérapie topique Antihistaminiques topiques Cromones topiques AINS topiques
Conjonctivite gigantopapillaires (CGP)	Chronique	Hypersensibilité non spécifique	Présence de papilles géantes Kératite ponctuée superficielle	Port de lentilles de contact	Changement du matériel Application des consignes d'entretien adaptées
Allergies de contact (conjonctivite ou blépharoconjonctivite irritative)	Chronique+/- exacerbations intermittentes		Conjonctivite Eczéma des paupières Symblépharon	Cosmétiques, parfum Vernis à ongles Vapeurs de poisson	Eviction de l'allergène Soin des paupières Larmes artificielles

d. Inflammation oculaire liée à une maladie systémique inflammatoire ou auto-immune

Une inflammation oculaire peut également être non-infectieuse. On la retrouve souvent chez les patients atteints de maladies inflammatoires systémiques ou auto-immunes, telles que la polyarthrite rhumatoïde, le lupus érythémateux disséminé, la sarcoïdose ou la granulomatose avec polyangéite, le syndrome de Sjögren, la périartérite noueuse primitive, le syndrome des antiphospholipides, la maladie de Behçet, le syndrome de Kawasaki, le syndrome de Cogan [14], la spondylarthrite ankylosante, l'arthrite juvénile idiopathique [32,33].

Le plus souvent, l'inflammation survient de manière concomitante aux manifestations extra-ophtalmiques de la maladie. L'inflammation oculaire peut également servir d'indicateur de la gravité de l'inflammation systémique. Elle peut être subaiguë, chronique ou aiguë [14,34].

Le traitement initial des patients présentant une atteinte oculaire auto-immune est basé en première intention sur l'utilisation des corticoïdes [34]. Des immunomodulateurs ou des biothérapies sont une option pour les pathologies réfractaires comme l'uvéite [35].

e. Inflammation oculaire liée à une maladie non inflammatoire

Récemment, il est apparu clairement que certains troubles présumés non inflammatoires, tels que la dégénérescence maculaire liée à l'âge (DMLA) [36] et l'œdème maculaire consécutif à la rétinopathie diabétique [37] ou à l'occlusion de la veine rétinienne, dépendent de certains médiateurs inflammatoires et doivent donc être traités, au moins partiellement, comme des troubles inflammatoires [14]. Ainsi, dans ces pathologies, les produits du stress oxydatif déclenchent un processus inflammatoire chronique de bas grade : une para-inflammation physiopathologique [8]. Au début de la DMLA, les druses contiennent de nombreux médiateurs de l'inflammation chronique de bas grade, tels que la protéine C-réactive, des immunoglobulines et des protéines liées au complément. De nombreux auto-anticorps et la surexpression des récepteurs Fcc jouent un rôle dans l'inflammation à médiation immunitaire chez les patients atteints de DMLA et les modèles animaux. L'infiltration macrophagique à l'interface rétine-choroïde est un facteur protecteur

dans les cas de DMLA précoce et devient un facteur pro-inflammatoire et pro-angiogénique dans la DMLA avancée [36]. Dans les complications oculaires diabétiques, les changements métaboliques, le stress oxydatif et l'accumulation de produits terminaux de glycation sont impliqués [38].

f. Inflammation d'origine traumatique

Les traumatismes oculaires sont un problème majeur de santé publique. Tous les ans dans le monde, 55 millions de traumatismes oculaires entraînent une incapacité temporaire de travail voire une cécité [39]. Les traumatismes oculaires peuvent entrainer un large spectre de blessures où toutes les structures de l'œil peuvent être touchées. Leur gravité est variable. Dans le passé, les traumatismes oculaires survenaient principalement au travail mais ils se produisent maintenant davantage dans le domaine privé, lors d'activités sportives, récréatives ou de traitements cosmétiques [40,41]. Que le globe soit ouvert ou fermé après le traumatisme, il est le siège entre autres de réactions inflammatoires qui peuvent être à l'origine d'un trouble de l'accommodation. L'inflammation associée au traumatisme est souvent de mauvais pronostic.

Le traitement de l'œil traumatisé dépend du traumatisme. Les indications de traitement en urgence sont la suture d'une plaie du globe, l'extraction d'un corps étranger, la plaie du cristallin, l'hypertonie oculaire, une hématocornée, un décollement de rétine ou un hématome sous-rétinien [42,43].

Dans le cas des traumatismes oculaires, seule la prévention, avec le port de lunettes de protection, est efficace mais se heurte à une faible compliance de la population. Les plaintes fréquemment rencontrées sont une vision floue, la présence de buée et la réduction du champ visuel [3,44].

g. Inflammation d'origine iatrogène

La iatrogénie est définie comme toutes « les conséquences indésirables ou négatives sur l'état de santé individuel ou collectif de tout acte ou mesure pratiqués ou prescrits par un professionnel habilité et qui vise à préserver, améliorer ou rétablir la santé » (HAS). Les statistiques médicales actuelles indiquent que l'œil est un organe à haut risque iatrogène avec des atteintes principalement cornéennes et rétiniennes. Parmi les maladies oculaires iatrogènes les plus fréquentes, on retrouve le syndrome de l'œil sec et la kératoconjonctivite per-opératoires ou les kératites chroniques après correction de la vue au laser excimer [14]. Malgré les différences d'étiologie des maladies iatrogènes susmentionnées, le développement d'un stress oxydatif semble être à l'origine d'une réaction inflammatoire locale inhibant de manière significative la régénération tissulaire et entrainant des mécanismes de dégénérescence [45].

Le traitement des atteintes iatrogènes de l'œil passe par l'utilisation de larmes artificielles, d'anti-inflammatoires, d'immunomodulateurs et d'antioxydants [45].

Certains médicaments peuvent également être à l'origine d'effets secondaires oculaires avec un phénomène inflammatoire et des effets délétères pour l'œil. Toutes les tuniques oculaires peuvent être le siège de ces manifestations inflammatoires.
Elles peuvent se développer à bas bruit et se manifester que tardivement. Une surveillance adaptée permet alors de les détecter rapidement et de prendre les mesures nécessaires avant qu'ils ne deviennent sévères et irréversibles. Elles peuvent également induire des pathologies aiguës qui mettent en jeu le pronostic visuel à court terme et nécessitent une prise en charge ophtalmologique urgente [46]. Le Tableau 3 regroupe les principaux médicaments responsables d'atteintes inflammatoires oculaires [46–49].

Ces effets secondaires sont de plus en plus fréquents et l'utilisation croissante des biothérapies tend à augmenter leur prévalence. Un historique médicamenteux permet de les identifier. Généralement, les atteintes oculaires sont rapidement réversibles à l'arrêt du traitement en cause. Cet arrêt peut être associé à la prescription de corticoïdes systémiques ou locaux et à un collyre mydriatique, en fonction de la clinique [49].

Les effets indésirables peuvent être également liés aux conservateurs présents dans les collyres, comme par exemple le chlorobutanol qui est irritant [50] ou secondaire à une phototoxicité, comme c'est le cas avec les fluoroquinolones par voie orale [48].

Tableau 3. Médicaments pouvant être responsables d'atteintes inflammatoires oculaires

	Molécules ou classes thérapeutiques	Atteintes ophtalmiques inflammatoires
Traitements systémiques	Anti-BRAF et anti- MEK (vemurafenib, dabrafenib, trametinib)	Uvéite
	ICPI (pembrolizumab, nivolumab et ipilimumab)	Uvéite
	Cidofovir	Uvéite
	Diéthylcarbamazine	Uvéite antérieure
	Fluoroquinolones orales	Uvéite
	Rifabutine	Uvéite antérieure avec hypopion, panuvéite, vascularite rétinienne
	Sulfamides	Uvéite
	Biphosphonates	Conjonctivite, uvéite antérieure, sclérite antérieure et postérieure, épisclérite, œdème palpébral et orbitopathie inflammatoire
	Topiramate	Uvéite
	Etanercept	Uvéite, sclérite
Vaccins	BCG	Uvéite antérieure granulomateuse ou non
	Grippe	Uvéite, panuvéite bilatérale, panuvéite récurrente
	VHB	Uvéite
	ROR	Uvéite antérieure non granulomateuse
	Varicelle	Uvéite antérieure, kérato-uvéite avec glaucome
	Papilloma virus humain	Uvéite
Autres	Tubertest	Panuvéite
Traitements topiques cutanés	Podophyllum peltatun	Uvéite
	Capsaïcine	Uvéite antérieure non granulomateuse
Traitements topiques ophtalmiques	Métipranolol	Uvéite antérieure granulomateuse
	Corticoïdes	Uvéite, choriorétinite séreuse centrale
	Brimonidine	Uvéite antérieure granulomateuse
	Analogues de la prostaglandine (latanoprost, travoprost, bimatoprost)	Réactivation de kératite à *Herpes simplex*, uvéite antérieure
Traitements intraoculaires	Anti-VEGF (ranibizumab, bevacizumab, aflibercept)	Uvéite
	Triamcinolone	Panuvéite, endophtalmite
	Cidofovir	Uvéite

2. Les traitements de l'inflammation ophtalmique

L'inflammation ophtalmique est donc un symptôme commun à de nombreuses pathologies et est considérée comme un facteur de mauvais pronostic et l'une des principales causes de cécité [5]. Outre le traitement de l'origine de l'inflammation, le syndrome inflammatoire ophtalmique nécessite d'être pris en charge rapidement afin de limiter les effets délétères sur l'œil et le risque de chronicisation. Dans le cas de kératite, d'endophtalmie ou d'uvéite, les traitements sont de véritables challenges car ils doivent être rapides, intenses et associés le traitement de l'atteinte d'origine et celui de l'inflammation [51,52].

La prise en charge thérapeutique de l'inflammation comprend des traitements locaux et systémiques (Tableau 4). La voie d'administration des anti-inflammatoires est souvent déterminée par le tableau clinique. Ainsi, pour une inflammation superficielle, comme on peut le voir dans le syndrome de l'œil sec ou les conjonctivites allergiques, l'administration topique est préférable. Les collyres de corticoïdes ou d'anti-inflammatoires non stéroïdiens (AINS) sont utilisés pour de nombreux types d'inflammation en aigüe ou sur le long terme car leur concentration dans l'œil peut être bien supérieure à celle généralement observée lorsque ces agents sont administrés par voie systémique. Parmi les différents principes actifs anti-inflammatoires, la dexaméthasone est un des plus utilisés et apparait comme une référence. Pour des formes plus sévères ou réfractaires au traitement topique, des implants intravitréens à libération prolongée comme l'OZURDEX® permettent une diffusion lente de dexaméthasone sur plusieurs semaines voire plusieurs mois. Pour les formes les plus sévères ou associées à une maladie inflammatoire ou auto-immune systémique, la voie systémique orale ou injectable est utilisée [53].

Les traitements les plus fréquemment utilisés sont les corticoïdes [54,55], la ciclosporine [56], le tacrolimus [49], le mycophénolate mofétil [56], le méthotrexate [53], le cyclophosphamide [53] ou les biothérapies comme l'infliximab, l'adalimumab ou l'étanercept [35,53,57–59]. Les immunomodulateurs sont utilisés dans les uvéites chroniques, souvent associées à une maladie auto-immune systémique comme la polyarthrite rhumatoïde ou le lupus. L'utilisation des biothérapies est reconnue par l'*American Uveitis Society* pour différentes uvéites comme dans la maladie de Behçet ou l'uvéite antérieure associée à la spondylarthrite ankylosante [53].

Tableau 4. Principaux traitements utilisés pour traiter les inflammations ophtalmiques [36,50]

Classes thérapeutiques	**Mécanismes d'action** **Effet** **Voie(s) d'administration**	**Type d'inflammation**	**Effets indésirables**
Corticoïdes	Mécanisme d'action : inhibition de la phospholipase A2, de la production de prostaglandines et de leucotriènes Effet : vasoconstriction, inhibition du chimiotactisme, stabilisateur de membrane, antifibrotiques, anti-angiogénique Voies d'administration : locale ou systémique	Traumatique Allergique Infectieuse (herpès Immunologique Rejet de greffe de cornée Sécheresse oculaire	Réactivation virale Cataracte sous-capsulaire postérieure Hypertonie oculaire Retard de cicatrisation Ulcération Hypersensibilité locale ou systémique
Anti-inflammatoires non stéroïdiens	Mécanisme d'action : inhibition de la cyclooxygenase Effet : anti-inflammatoire et antalgique Voies d'administration : locale ou systémique	Allergique Sécheresse oculaire Sclérite Laser de surface cornéen	Retard de cicatrisation Ulcération Iatrogénie cornéenne
Immunosuppresseurs : **Inhibiteurs de la calcineurine** **Ciclosporine, tacrolimus**	Mécanisme d'action : inhibition la prolifération et la différenciation des lymphocytes T et diminue la production d'IL-2, IL-4 et IL-5 et IFN-γ Effet : anti-inflammatoires et antalgiques Voies d'administration : locale ou systémique	Sécheresse oculaire Kératoconjonctivite phlycténulaire Kératoconjonctivite limbique, virale, atopique et vernale Syndrome de Gougerot-Sjögren Rejet de greffe	Sensation de brûlures Hypersensibilité Aggravation d'infection virale Risque infectieux Risque tumoral
Immunosuppresseurs **Mycophénolate mofétil**	Mécanisme d'action : Inhibiteur de la biosynthèse des purines Effets : diminue la prolifération des lymphocytes T et B et la production d'anticorps Voie d'administration : systémique	Immunologique Choriorétinopathie de type Birdshot	Leucopénie, Thrombopénie Risque infectieux Risque tumoral Tératogène
Anti-TNF-α	Mécanisme d'action : Anticorps monoclonaux dirigés contre les cytokines Effets : Bloque la réponse immunitaire Voie d'administration : systémique		Risque infectieux Risque tumoral Hypersensibilité
Inhibiteurs d'Il-6 **Tocilizumab**			

3. Limites de la biodisponibilité pour la voie topique ophtalmique

L'œil étant un organe faiblement vascularisé et en contact avec l'environnement extérieur ; il présente de nombreuses barrières anatomiques et physiologiques qui limitent la biodisponibilité des principes actifs [51,60] (Figure 2). En effet, après instillation, environ 90% de la dose appliquée est évacuée soit par le drainage nasolacrymal, la dilution par les larmes ou le renouvellement des larmes [61]. La biodisponibilité des actifs ainsi administrés est généralement inférieure à 5 %, obligeant les patients à de fréquentes instillations de manière à maintenir des concentrations thérapeutiques [62].

Parmi les barrières dynamiques, on retrouve le drainage, le film lacrymal et le renouvellement des larmes, le clignement des paupières, le flux sanguin conjonctival et le flux lymphatique [2,49]. Ce sont des mécanismes protecteurs. Le drainage lacrymal est une barrière très efficace car il limite le temps de présence des médicaments sur la surface oculaire à 5 minutes [63]. Des mucines libres, présentes dans le film lacrymal, forment une couche hydrophile qui se déplace sur le glycocalyx de la surface oculaire et éliminent les substances exogènes [49].

Les barrières statiques limitent la diffusion des actifs dans l'œil. Il s'agit de la conjonctive, la cornée et des barrières hémato-oculaires. La barrière cornéenne représente la barrière principale car elle est composée de six couches successives dont trois de polarité différente :

- un épithélium lipophile caractérisé par des jonctions serrées. C'est un obstacle important pour les molécules hydrophiles,
- un stroma hydrophile composé de kératocytes, de protéoglycanes et de fibrilles de collagène dont la régularité et la répartition spatiale permet la transparence de la cornée,
- un endothélium ayant les mêmes caractéristiques que l'épithélium [64].

Les barrières hémato-oculaires ne permettent que le transport passif. La diffusion des médicaments est plus facile dans l'humeur aqueuse, expliquant une plus grande concentration des actifs dans le vitré antérieur que dans le vitré postérieur. À l'état physiologique, peu de médicaments traversent la barrière hémato-rétinienne, des capillaires vers le vitré, sauf dans les cas de diabète, d'hypertension artérielle maligne ou d'œdème maculaire [65]. Dans le cas du diabète, l'hyperglycémie non contrôlée va favoriser la production de cytokines pro-

inflammatoires, comme le VEGF, qui altèrent l'intégrité de la barrière hémato-rétinienne interne et affaiblissent les jonctions serrées des cellules endothéliales entrainant ainsi un œdème maculaire et une baisse de l'acuité visuelle [49].

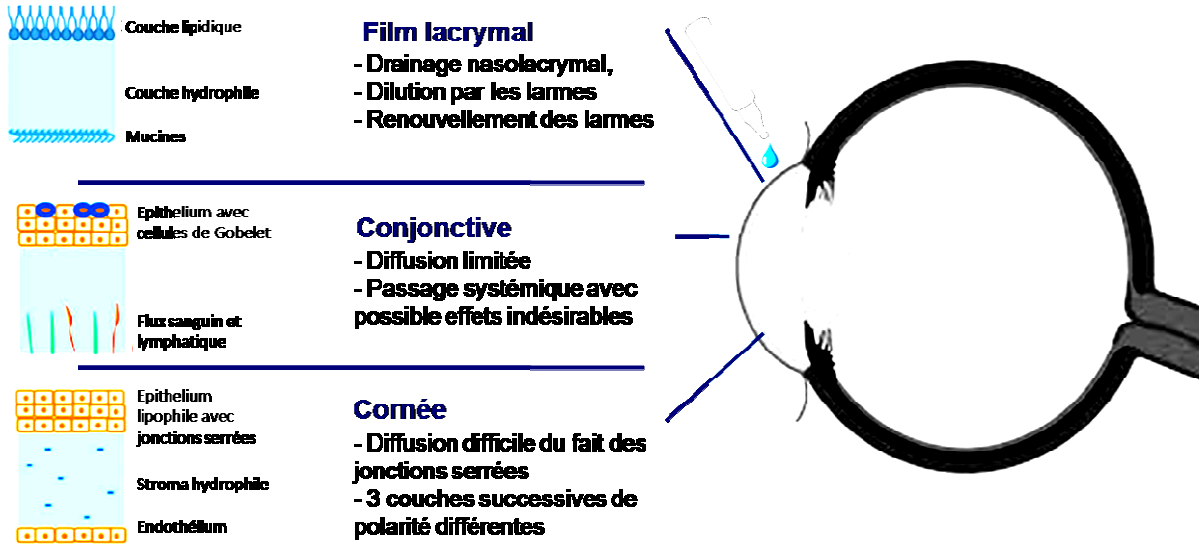

Figure 2. Barrières oculaires limitant la biodisponibilité des actifs après instillation oculaire, d'après Di Tommaso *et al.* [60]

Les barrières métaboliques limitent enfin de manière significative la diffusion des actifs par les pompes d'efflux comme les glycoprotéines *multidrug resistance protein*-1 (MDR-1) ou *breast cancer resistance protein* (BCRP).

Tous ces phénomènes sont des obstacles à la thérapie locale.

4. Notre projet de recherche : objectifs, stratégies, justifications

Dans ce contexte de thérapie locale de l'inflammation oculaire, les anti-inflammatoires stéroïdiens ont démontré leur efficacité tant dans le traitement de la surface que du segment antérieur de l'œil. Cependant, la voie topique ophtalmique est largement sous-dotée en spécialités pharmaceutiques anti-inflammatoires par rapport à d'autres voies d'administration, comme décrit dans la partie Revue bibliographique. En effet, la voie topique ophtalmique requiert une formule adaptée et complexe du fait d'une part de spécifications réglementaires exigeantes, au regard de la fragilité de l'œil, et d'autre part de la faible biodisponibilité oculaire des médicaments. En outre, les anti-inflammatoires stéroïdiens

présentent une faible solubilité en milieu aqueux, une élimination pré-cornéenne importante entraînant une biodisponibilité inférieure à 5 %. De plus, les anti-inflammatoires ont une faible pénétration oculaire après administration topique [66,67]. Cette voie d'administration reste malgré tout très intéressante et incontournable car elle est non invasive, bien tolérée et facile d'accès. L'enjeu majeur de la prise en charge thérapeutique de l'inflammation oculaire est le traitement rapide de l'affection oculaire afin de réduire le risque d'handicap visuel tout en limitant les effets indésirables.

Parmi les corticoïdes, la dexaméthasone (DXM) est l'une des plus puissantes et des plus efficaces dans le traitement de l'inflammation. Elle est utilisée pour le traitement de l'inflammation oculaire aiguë et chronique, y compris l'inflammation postopératoire ou l'uvéite [68,69]. Elle agit sur les cellules trabéculaires en inhibant la phospholipase-A2 et ainsi la synthèse des prostaglandines [70,71]. Cependant, la DXM présente un défi de formulation car il s'agit d'un composé très faiblement soluble dans l'eau [72,73].

La DXM est commercialisée sous forme de suspensions, MAXIDEX® à 0,1% (m/v) (Novartis Pharma, Rueil-Malmaison, France), ou sous forme de solution, en utilisant une prodrogue hydrosoluble, le phosphate de DXM à 1% (m/v), DEXAFREE® (Laboratoires Théa, Clermont-Ferrand, France) [69]. L'acétate de DXM (DXMa) (Figure 3) est un dérivé lipophile, potentiellement intéressant pour une utilisation topique ophtalmique, mais non disponible à ce jour en spécialité pharmaceutique.

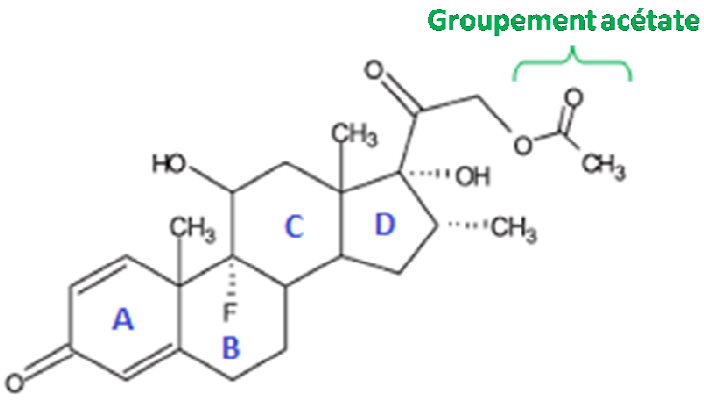

Figure 3. Structure chimique de la dexaméthasone-21-acétate, A = cycle A, B = cycle B, C = cycle C et D = cycle D

Cet ester de dexamethasone présente une bonne pénétration transcornéenne et s'hydrolyse en DXM lors de son absorption [74]. Leibowitz *et al*. ont démontré également que la forme acétate est plus efficace que la forme phosphate dans le traitement des kératites. En outre, cet effet thérapeutique n'est pas associé à une plus grande propension à augmenter la pression intraoculaire, l'un des effets secondaires les plus fréquents des glucocorticoïdes [75]. Cependant, le DXMa présente l'inconvénient d'être moins soluble dans l'eau que la forme phosphate.

Les objectifs de ce projet sont donc de développer, d'élaborer et d'évaluer un système galénique innovant pour la voie topique ophtalmique afin d'améliorer la prise en charge thérapeutique de l'inflammation ophtalmique et ainsi conduire à une réduction du risque potentiel de cécité afférente. Le défi des formulations proposé est d'améliorer la solubilité apparente en milieu aqueux de l'acétate de dexaméthasone (DXMa) et d'augmenter son temps de résidence sur la surface oculaire, tout en lui conférant des propriétés de réservoir, afin d'accroître son efficacité et de diminuer la fréquence d'instillation.

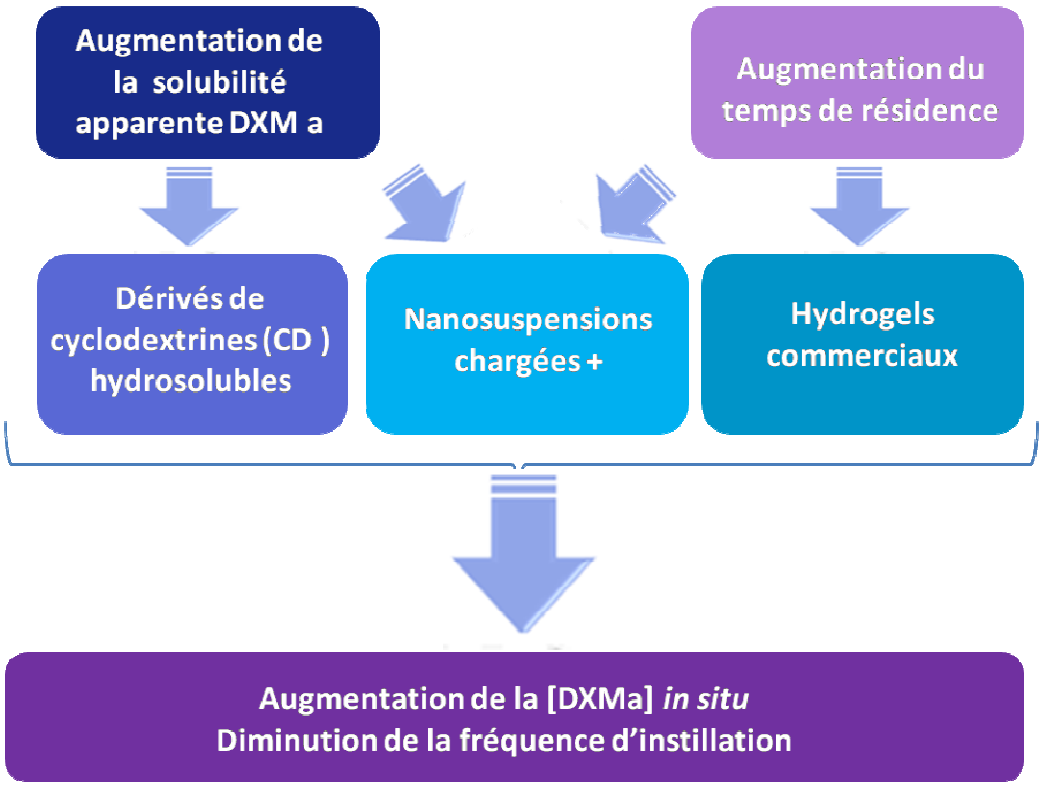

Figure 4. Stratégies de formulation

Différentes méthodes peuvent être mises en œuvre pour améliorer la solubilité des substances actives peu ou pas solubles dans l'eau, telles que la co-solvatation, la micellisation ou l'utilisation de cyclodextrines (CD) [76,77]. Notre choix s'est orienté vers la mise en œuvre des cyclodextrines hydrosolubles commerciales. Ces excipients présentent le grand avantage d'améliorer la solubilité apparente des molécules hydrophobes, leur biodisponibilité, tout en étant biocompatibles [78,79]. Cette stratégie semble plus adaptée et a déjà été validée pour la voie topique oculaire, notamment avec la disponibilité sur le marché de la spécialité INDOCOLLYRE®, collyre anti-inflammatoire d'indométacine contenant l'hydroxypropyl-β-cyclodextrine.

Afin d'augmenter le temps de résidence topique des formulations, nous avons incorporé la DXMa dans des gels bioadhésifs. Ces gels adhérent à la paroi ophtalmique et limitent la dispersion rapide de l'actif sur la surface cornéenne [80]. Nous avons opté pour la mise en œuvre de gels commercialisés, car ils présentent des profils physicochimiques et toxicologiques favorables déjà établis.

Enfin, de manière à favoriser le temps de contact précornéen tout en prenant en charge une quantité supplémentaire de substance active, nous avons élaboré et incorporé dans les formulations des nanostructures cationiques associant le DXMa. La présence de charges positives à la surface des nanoparticules favoriserait l'interaction de type électrostatique avec la surface anionique de la cornée et augmenterait le temps de résidence de l'actif en agissant comme un dépôt [80] pouvant améliorer la biodisponibilité [81]. En outre, la littérature montre que les systèmes nanoparticulaires, même non chargés, constituent d'excellents outils de formulation pour relever partiellement ou totalement certains défis galéniques et présentent les avantages :

- d'augmenter la solubilité des molécules faiblement hydrosolubles en offrant une surface spécifique élevée,
- de favoriser le passage transcornéen des actifs,
- d'agir comme un réservoir,
- d'être transparente ou légèrement trouble, les suspensions colloïdales ne troublent pas la vision des patients [82].

Ainsi, les systèmes colloïdaux permettent–dans la voie topique ophtalmique de réduire le nombre d'instillations et le risque d'effets indésirables [83] et d'augmenter la compliance des patients [60].

De nombreux travaux relevés dans la littérature tentent d'augmenter le temps de résidence précornéen de la dexaméthasone. Parmi ces travaux, il est possible de citer notamment les formulations à base de micelles [84], des nanostructures [85,86], des nanostructures associées à un gel [87–89] ou des suspensions de nanogels [90]. Dans la plus part des cas ces formulations procurent quelques avancées comparativement aux formes ophtalmiques conventionnelles, cependant leur efficacité dans le traitement des inflammations oculaires demeure parcellaire [91]. Cette situation justifie l'intensification de la recherche en formulation afin de répondre au grand défi thérapeutique des inflammations ophtalmiques.

C'est dans ce cadre que se situe ce travail de thèse. Nous avons retenu la dexaméthasone acétate (DXMa) comme substance active. Elle est décrite dans la littérature comme présentant une absorption oculaire supérieure à la dexaméthasone. De plus, elle aurait une moindre propension à induire une augmentation de la pression intraoculaire. Il faut rappeler que la DXMa est pratiquement insoluble dans l'eau : 0,021 mg/mL à 25 °C.

Notre approche de formulation est graduelle et combinatoire. Nous avons d'abord cherché à résoudre le problème de la très faible solubilité de la DXMa en la complexant avec deux dérivés hydrosolubles des cyclodextrines commerciales, l'hydroxypropyl-βCD (HPβCD) et l'hydroxypropyl-γCD (HPγCD). Ensuite nous avons associé les composés d'inclusion obtenus à des gels oculaires commerciaux, réalisant ainsi un système mixte solubilisant la DXMa et potentiellement rémanent au niveau cornéen. Enfin, une formulation de nanostructures cationiques à base de cyclodextrine bioestérifiée, d'un amphiphile cationique et de DXMa a été réalisée. Un système final innovant mixte, combinant complexe d'inclusion/gel commercial/nanostructure cationique a été envisagé.

De manière un peu plus détaillée et complète, l'ensemble de la thèse s'est agencée selon les phases suivantes : Dans un premier temps, à travers des études préliminaires dites de pré-

formulation, nous avons développé puis validé les méthodes analytiques de dosage de la DXMa. Les diagrammes de solubilisation de la DXMa avec les deux cyclodextrines ont été réalisés, ce qui nous a permis de déterminer les constantes d'association et les paramètres thermodynamiques des complexes DXMa/HPβCD et DXMa/HPγCD.

Dans un second temps, nous avons optimisé deux complexes d'inclusion à base d'HPβCD et HPγCD associés à deux gels commerciaux à partir de plans d'expériences. L'objectif des deux plans de mélange réalisés étaient d'augmenter la fraction de DXMa solubilisée, tout en conservant une osmolalité compatible avec l'œil, soient des valeurs d'osmolalité comprises dans la fourchette 200 < X < 450 mOsm/Kg [92]. Nous avons ensuite caractérisé les deux formulations retenues d'un point de vue physicochimique et rhéologique. Les systèmes mixtes ont également été étudiés pour leur aptitude à relarguer *in vitro* la DXMa. Concernant les évaluations biologiques *ex-vivo* et *in vivo*, nous avons évalué la biopermanence des systèmes sur l'œil de rat, leur cytotoxicité sur les cellules HCEC (*Human Corneal Endothelial Cells*). Enfin, le passage transcornéen de la DXMa à partir des systèmes a été mesuré sur la cornée de porc isolée. En outre, nous avons réalisé une étude de stabilité physicochimique et microbiologique des systèmes à 25 °C, selon les recommandations ICH Q1A (R2) [93].

Dans un troisième temps, nous avons mis au point une formulation de nanoparticules cationiques de DXMa dans le double objectif, d'une part d'augmenter le temps de permanence sur la surface oculaire par des interactions électrostatiques et d'autre part de favoriser le passage transcornéen de la DXMa. Cette formulation nanoparticulaire a été caractérisée de la même manière que les précédentes formulations.

Ce document de thèse est subdivisé en trois parties.

La première partie est une revue bibliographique, présentée sous la forme de publication dans laquelle nous avons répertorié les différentes formulations commerciales d'anti-inflammatoires, stéroïdiens et non stéroïdiens, en France, en Europe et aux Etats Unis. Les formulations développées dans le but d'augmenter la biodisponibilité de ces substances actives sont examinées ainsi que certaines innovations galéniques. Enfin, nous faisons le point sur les modalités et les méthodes d'évaluation des formulations topiques ophtalmiques.

La deuxième partie est consacrée au travail expérimental et les résultats correspondants, rédigée sous la forme de trois articles.

La publication n°1 porte sur la pré-formulation et la formulation de deux systèmes galéniques mixtes optimisés à base de complexes DXMa /HPβCD et DXMa/ HPγCD associés à des gels commerciaux ainsi que leur lyodisponibilité.

La publication n°2 est consacrée aux évaluations *in vitro*, *in vivo* et *ex vivo* des deux formulations mixtes optimisées ainsi qu'à l'étude de leur stabilité physicochimique et microbiologique à 25°C.

La publication n°3 est consacrée aux évaluations *in vitro*, *in vivo* et *ex vivo* d'une formule combinant complexe d'inclusion/gel commercial/nanostructure cationique.

Dans la troisième partie, une conclusion générale reprend le contexte, les objectifs et les principaux résultats de ce travail. Cette partie permet de dresser un bilan des objectifs atteints et ouvre également sur les perspectives de ce travail de thèse.

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REVUE BIBLIOGRAPHIQUE

Titre : Recent advances in the design of anti-inflammatory delivery system for topical ophthalmic administration and their evaluation

Résumé : Les anti-inflammatoires stéroïdiens et non stéroïdiens ont largement démontré leur efficacité tant dans le traitement de la surface que du segment antérieur de l'œil.

Cependant, la voie topique ophtalmique est largement sous-dotée en spécialités pharmaceutiques anti-inflammatoires, par rapport à d'autres voies d'administration comme les voies orale ou injectable.

Dans cet article qui constitue une introduction justifiée du sujet sur lequel portera notre travail expérimental, les différentes spécialités d'anti-inflammatoires commercialisées en France, en Europe et aux Etats Unis ont été répertoriées. Les stratégies de formulation visant à augmenter la biodisponibilité et l'efficacité des anti-inflammatoires ont été également recensées. Enfin, les modalités d'évaluations des formulations topiques ophtalmiques ont été présentées.

Article de revue bibliographique en préparation pour soumission

Review

Recent advances in the design of anti-inflammatory delivery system for topical ophthalmic administration and their biopharmaceutical evaluation

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Abstract: Ocular inflammation is one of the most common symptom of eye disorders and diseases. The therapeutic management of this inflammation must be rapid and effective in order to avoid deleterious effects for the eye and the vision. Steroidal (SAID) and non-steroidal (NSAID) anti-inflammatory drugs have been shown to be effective in treating inflammation of ocular surface and anterior segment of the eye by topical administration. However, it is well established that the anatomical and physiological ocular barriers are limiting factors for drug penetration. In addition, the SAIDs and NSAIDs, are generally characterized by a very low aqueous solubility, resulting in low bioavailability as only 1 to 5% of the applied drug permeates the cornea. The present review gives an updated insight on the SAIDs and NSAIDs conventional formulations, i.e. ointments, eye drops, solutions, suspensions, gels and emulsions, based on the 88 commercial products available on the US, European and French markets. As well, sophisticated formulations and innovative ocular drug delivery systems will be discussed. Promising results are presented with micro- and nanoparticulated systems, or combined strategies with polymers and colloidal systems, which offer a synergy in bioavailability and sustained release. Finally, different tools allowing the physical characterizations of all these delivery systems as well as *in vitro*, *ex vivo* and *in vivo* evaluations will be considered with regards to the safety, the tolerance and the efficiency of the drug products.

Keywords: anti-inflammatory; SAID; NSAID; topical ophthalmic formulation; recent advances; biopharmaceutical evaluation

1. Introduction

Ocular inflammation is considered as a major eye disorder and many reports demonstrates that topical administration of anti-inflammatory drugs, steroidal (SAIDs) [2] and non-steroidal (NSAIDs) [1] are effective in treating ocular surface and anterior segment inflammation, including pain and post-operative inflammation, seasonal allergic conjunctivitis [3,4] or age related macular degeneration [5]. The major challenge in the therapeutic management of ocular inflammation is the rapid treatment in order to reduce the risk of visual impairment while limiting side effects. Topical administration is the most preferred route for management of ocular inflammations as it is (i) easy to handle, (ii) non-invasive, (iii) rather well-tolerated [1] and (iv) it provides sufficient ocular drug concentrations, when avoiding the systemic side effects associated with the oral administration.

Nevertheless, the ocular drug bioavailability in conventional topical formulations is notoriously poor; only 1–5 % of drug applied to the surface penetrates the cornea. This is the consequence of various protective mechanisms and multiple barriers to drug entry, such as the fast nasolacrymal drainage due to high tear fluid turnover and lid blinking, the corneal structure with a hydrophilic stroma sandwiched between the lipophilic epithelium and endothelium, the epithelial drug transport barriers, the efflux pump and the clearance from the vasculature in the conjunctiva [6,7]. Besides these ocular anatomical and physiological constraints, another limiting factor encountered with anti-inflammatory drugs is their poor water solubility [8,9]. Thus, topical anti-inflammatory drugs for ophthalmic route require complex formula adapted to regulatory specifications, due to the eye fragility, their low ocular bioavailability and their poor water solubility. As a consequence, a limited number of drugs are marketed as well as a few drug associations such as anti-inflammatory and ant-infective molecules [10,11].

Despite these drawbacks, many strategies have been investigated in order to improve ocular topical bioavailability of SAID and NSAID drugs, such as the physicochemical modifications of active principle ingredient (API) in order to favor their absorption or the development of formulations ensuring a prolonged corneal residence time of drug product.

Concerning the physicochemical modifications of drug molecules, one approach is based on the synthesis of new API from the chemical structures of well-known available SAIDs and NSAIDs. For instance, new drug molecules were synthesized from propionic acid derivatives NSAID such as pranoprofen [12], pyranoprofen [13], suprofen [1]. Other molecules were derived from SAIDs such as clobetasone butyrate [14], difluprednate [15,16], loteprednol [17]. Unfortunately, these new synthesized molecules did not lead to expected enhanced ocular penetration [1], are more irritating in nature, [18] or have an increased higher risk of side effects [19]. The prodrug approach is another chemical way to enhance the drug permeability. Indeed the synthesized inactive prodrug exhibits a better corneal penetration and once *in situ*, is either chemically and/or enzymatically metabolized to become active [20]. As an example, nepafenac, an amide prodrug of amfenac, belongs to pharmacological NSAID class of arylacetic derivatives and is commercially available. *In vitro* nepafenac demonstrates a nearly six-fold greater permeation coefficient than diclofenac [21]. *In vivo,* nepafenac easily crosses corneal and retinal tissues following topical ocular administration. Thereafter nepanefac is hydrolyzed to amfenac which shows high anti-inflammatory property when used to treat pain and inflammation associated with cataract surgery [22]. Several lipophilic esters of dexamethasone were developed and evaluated for permeability and bioreversion across the rabbit cornea and bovine conjunctival epithelial cells (BCEC). The permeability of phosphate and metasulfobenzoate esters of dexamethasone were restricted across BCEC due to their hydrophilic and ionic character. On the contrary, the prodrugs including acetate, propionate and butyrate esters demonstrated a better permeability increasing with the ester lipophilicity. The valerate ester conjugate being highly lipophilic easily crosses the corneal epithelium while the hydrophilic stroma acts as a barrier and allows a depot of lipophilic prodrug until hydrolysis to parent dexamethasone. The hydrolysis of valerate ester is very slow in the cornea suggesting for this prodrug a possible use as a sustained drug release system [23]. The prodrug approach is tailor-made to improve solubility, stability, or permeability characteristics to lead molecules without causing any damage to the

biological barriers involved. Despite increased research work, there are only a few prodrug products due to their poor stability in aqueous environment [24].

Ocular retention of drug product combined or not with corneal penetration enhancers can also improve drug bioavailability. These approaches are carried out through conventional formulations *i.e.* eye drop solution or suspension, ointments and hydrogels for example by using mucoadhesive agents into these formulations. Furthermore, other sophisticated drug delivery systems have been achieved such as liposomes, micro-polymer systems, or solids inserts. Iontophoresis is a non-invasive technique, applied with ionized active ingredient for anterior and posterior ocular disorders. It can achieve higher bioavailability and reduce clearance as compared to topical eye drops [25]. In parallel with these novel drug delivery systems, the researchers focused on the development of new functional materials as well as innovative formulations based on the use of combined strategies. Finally, the ideal drug delivery system should administer accurate and therapeutic concentrations of drug over a specified time, correlated with the ophthalmic affection disorder. It should also be easy to handle and manufacture, and should remain stable over the whole ocular surface, be biocompatible, preferably be biodegradable, and free of toxic side effects [26]. The present review gives an updated insight on the SAIDs and NSAIDs conventional formulations for topical ophthalmic administration. As well, sophisticated formulations and innovative ocular drug delivery systems will be discussed. Finally, the different tools will be described in order to characterize, to evaluate *in vitro, ex vivo* and *in vivo* and to assess if the drug product is safe, sure, well tolerate and efficient.

2. NSAID and SAID

2.1. Chemical family

Corticosteroids have a C21 structure, presenting a steroid nucleus derived from cholesterol [27]. From this backbone, numerous drugs vary from differing functional groups and oxidation state [28]. Topical corticosteroids used in ophthalmology can be classified as ketone or ester steroids. Loteprednol is the only ester steroid drug presenting an ester instead of a ketone group at C20-position, responsible for the cataractogenic side effect [29,30]. Unlike corticoids, NSAIDs do not include a steroid nucleus and are a heterogeneous group of compounds of different chemical classes. Table 1 summarizes the anti-inflammatory drug molecules commercially available for oral, parenteral and topical ophthalmic administrations in France, EU and USA as well as their chemical class [1,31–35].

On February, 26, 2019, a total of 40 NSAID or SAID were marketed for oral, parenteral or topical ocular administrations, only 14 (35 %) of which concerned the topical ocular route. Among these 14 drugs, 5 of them (12.5 %) are actually available only for the topical ophthalmic route. Those are bromfenac, difluprednate, fluorometholone, loteprednol etabonate and nepafenac. Table 2 includes all the brand name products of NSAID or SAID marketed for the ophthalmic topical route and used in USA, EU and France as of February 26, 2019, except the generic specialties. The combinations of anti-inflammatory drugs with other pharmacological classes of molecules are also listed.

Table 1. NSAIDs and SAIDS marketed for oral, parenteral and topical ophthalmic administration as of 26th February, 2019, in France, EU and USA and their chemical class

DCI	NSAID/SAID	Chemical class	Topical ocular route
Aceclofenac	NSAID	Aryl-acetic acid derivatives	-
Alminoprofen	NSAID	Propionic acid derivatives	-
Betamethasone	SAID		-
Bromfenac	NSAID	Aryl-acetic acid derivatives	-
Celecoxib	NSAID	Selective cylooxygenase -2 inhibitors	Only
Deflazacort	SAID		-
Dexamethasone (base and phosphate sodium)	SAID		-
Dexketoprofen	NSAID	Propionic acid derivatives	Yes
Diclofenac	NSAID	Aryl-acetic acid derivatives	-
Difluprednate	SAID		Yes
Etodolac	NSAID	Indole and indene derivatives	Only
Etoricoxib	NSAID	Selective cylooxygenase -2 inhibitors	-
Fluocortolone	SAID		-
Fluorometholone (base and acetate)	SAID		-
Flurbiprofen	NSAID	Propionic acid derivatives	Only
Hydrocortisone	SAID		Yes
Ibuprofen	NSAID	Propionic acid derivatives	Yes
Indomethacin	NSAID	Indole and indene derivatives	-
Ketoprofen	NSAID	Propionic acid derivatives	Yes
Ketorolac tromethamine	NSAID	Aryl-acetic acid derivatives	-
Loteprednol etobonate	SAID		Yes
Meclofenamate sodium	NSAID	Fenamic acid derivatives	Only
Mefenamique acide	NSAID	Fenamic acid derivatives	-
Meloxicam	NSAID	Enolic acid derivatives	-
Methylprednisolone	SAID		-
Morniflumate	NSAID	Fenamic acid derivatives	-
Nabumetone	NSAID	Non acidic derivatives	-
Naproxen	NSAID	Propionic acid derivatives	-
Nepafenac	NSAID	Aryl-acetic acid derivatives	-
Niflumic acid	NSAID	Fenamic acid derivatives	Only
Oxaprozin	NSAID	Propionic acid derivatives	-
Parecoxib	NSAID	Selective cylooxygenase -2 inhibitors	-
Piroxicam	NSAID	Enolic acid derivatives	-
Prednisolone (acetate and sodium phosphate)	SAID		Yes
Prednisone	SAID		-
Salicylic acid	NSAID	Salicylic acid derivatives	Yes
Sulindac	NSAID	Indole and indene derivatives	-
Tenoxicam	NSAID	Enolic acid derivatives	-
Tiaprofen	NSAID	Propionic acid derivatives	-
Tolmetin	NSAID	Aryl-acetic acid derivatives	-
Triamcinolone	SAID		Yes

Only: NSAID or SAID actually available only for the topical ophthalmic route, Yes: NSAID or SAID actually available for oral, parenteral or topical ocular administrations

Table 2. US, European and French marketed NSAID and SAID medicines listed as of 26th February, 2019 for topical use in ophthalmology

DCI	NSAID / SAID	Product name in USA, EU and France
Bromfenac	NSAID	BROMSITE EQ®, PROLENSA EQ®, YELLOX®
Dexamethasone (base or sodium phosphate)	SAID	CHIBRO CADRON®, DEXAFREE®, DEXASPORIN®, DEXTENZA®, FRAKIDEX®, MAXIDEX®, MAXIDROL®, MAXITROL®, STERDEX®, TOBRADEX®
Diclofenac	NSAID	VOLTAREN®, VOLTAREN®OPHTA, VOLTAREN®OPHTABAK
Difluprednate	SAID	DUREZOL®
Fluorometholone (acetate or base)	SAID	FLUCON®, FML®, FML FORTE® FLAREX®
Flurbiprofen	NSAID	OCUFEN®
Hydrocortisone		SOFTACORT®
Indomethacin	NSAID	INDOCOLLYRE®, INDOBIOTIC®
Ketorolac tromethamine	NSAID	ACULAR®, ACULAR LS®, ACUVAIL®,
Loteprednol etabonate	SAID	ALREX®, INVELTYS®, LOTEMAX®, LOTEMAX SM®, ZYLET®
Nepafenac	NSAID	ILEVRO®, NEVANAC®
Prednisolone (acetate or sodium phosphate)	SAID	BLEPHAMIDE®, BLEPHAMIDE S.O.P.®, OMNIPRED®, PRED FORTE®, PRED MILD®
Salicylic acid	NSAID	ANTALYRE®, CIELLA®
Triamcinolone		CIDERMEX®

2.2. *Mechanism of action*

Inflammation corresponds to a set of mechanisms of defense, physiological and pathological, by which the organism recognizes, destroys and eliminates all the substances foreign to it. It is a dynamic process with several successive steps in which the membrane phospholipids will be degraded in arachidonic acid by phospholipase A2, resulting in the release of pro-inflammatory mediators including prostaglandins, thromboxanes, leukotrienes and eicosanoids. The corticosteroids and NSAIDs both inhibit prostaglandin formation but their pharmacological properties differ by their place of action in the inflammatory cascade (Figure 1).

The corticosteroid agents inhibit the arachidonic acid pathway indirectly through the induction of lipocortin synthesis which inhibit the phospholipase A2 enzyme, therefore preventing production of all the pro-inflammatory mediators including arachidonic acid cited above [1,30,36]. Despite their chemical heterogeneity, NSAIDs share similar therapeutic properties. They act solely on the action of cyclooxygenase (COX), inhibiting among others the formation of prostaglandins [1,37,38]. Conventional NSAID agents inhibit both COX-1 and COX-2 in a nonselective way.

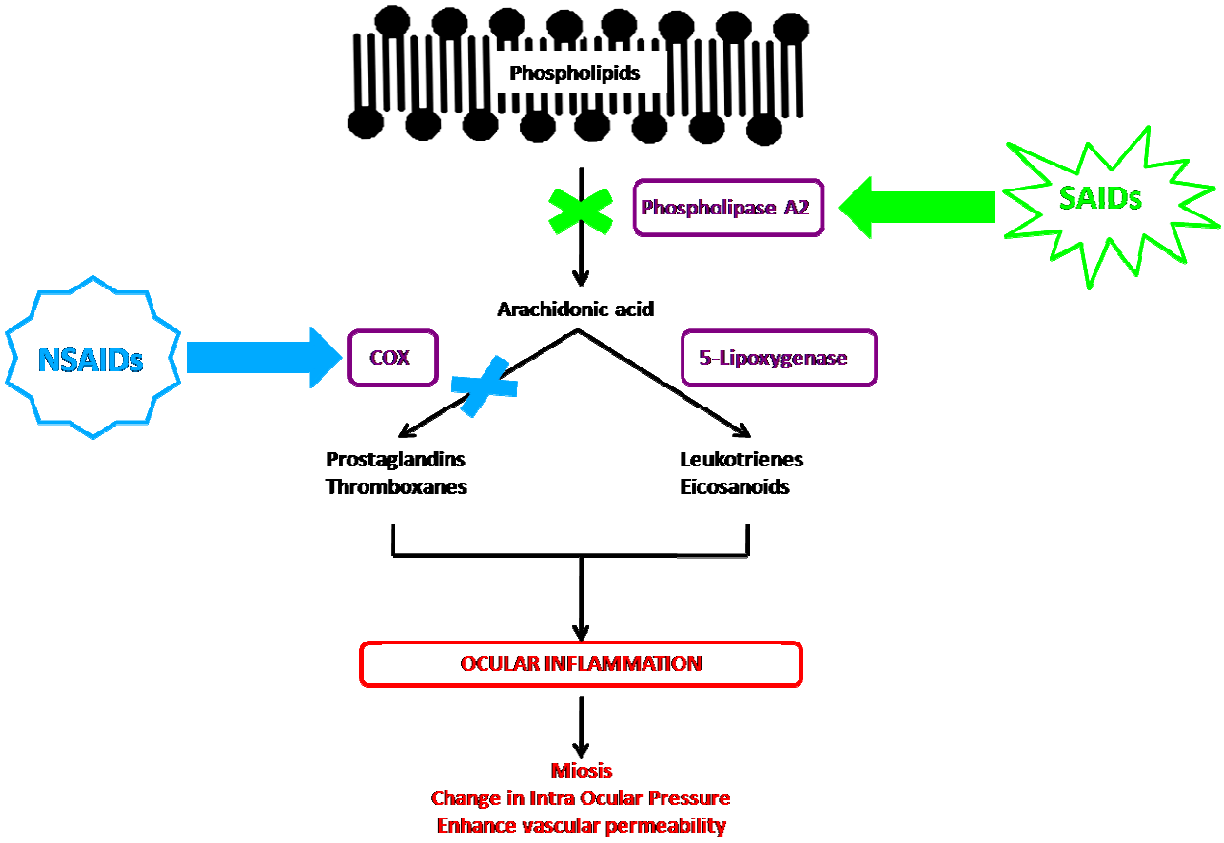

Figure 1. NSAIDs and SAIDs mechanisms of action in the inflammatory cascade

2.3. Sites of action / Therapeutic uses

Topical SAIDs are widely prescribed as anti-allergic or anti-inflammatory drugs for the anterior segment of the eye (Figure 2), combined or not with anti-infectious drugs (Table 3). In order to treat conjunctival diseases, SAIDs can be used to treat allergic conjunctivitis, blepharoconjunctivitis and corneo-conjunctival burns. Regarding corneal diseases, the indications are the treatment of immune and bacterial keratitis, in any case herpetic or mycotic. The anti-inflammatory effect is highly used in post-operative inflammation such as cataract or glaucoma surgery or in prevention of corneal graft rejection, as immunosuppressive agent [39,40].

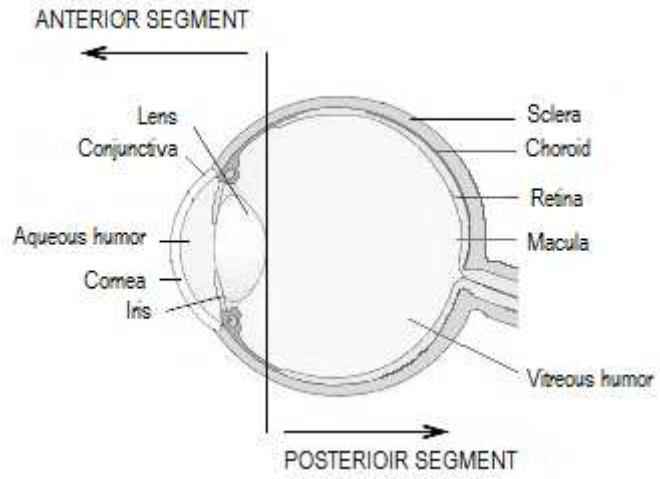

Figure 2. Anatomy of the eye

Topical ophthalmic NSAIDs are sometimes indicated, but are less prescribed to treat post-operative inflammation e.g. following cataract surgery. They also have shown benefits by preventing intraoperative miosis, improving treatment of seasonal allergic conjunctivitis and reducing post-operative pain [1,3].

Table 3. Sites of action and therapeutic use of the widely prescribed anti-inflammatory drugs

Indications			Commonly used anti-inflammatory drugs
Management of post-operative inflammation			Diclofenac, ketorolac, SAIDs [1,40]
Prevention of intra-operative miosis			Flurbiprofen, ketorolac [41]
Anterior segment	**Conjunctiva**	Treatment of allergic conjunctivitis	Ketorolac, SAIDs [1,40]
		Treatment of blepharoconjunctivitis	SAIDs [40]
		Treatment of corneo-conjunctival burn	SAIDs [40]
	Cornea	Prevention of corneal graft rejection	Dexamethasone [39,40]
		Control of pain after refractive surgery	Diclofenac, ketorolac [1]
		Treatment of immune keratitis	SAIDs [40]
		Treatment of bacterial keratitis	SAIDs [40]
Posterior segment	**Macula**	Prevention and treatment of cystoid macular edema	Diclofenac, ketorolac, indomethacin [1,41]

3. Formulation for topical ophthalmic drug delivery systems

3.1. Conventional formulation

Most conventional ophthalmic dosage forms include ointment, solutions, emulsions and suspensions, which together account for nearly 90 % of currently available formulations in the United States and Europe. It is usual that water-soluble drugs are delivered through topical instillation in an aqueous solution and water insoluble drugs are administered topically as ointments or aqueous suspensions [42]. Among the topical dosage forms for ophthalmic drug delivery, eye drops solutions are quite popular since they are relatively well tolerated by patients, simple to prepare, to filter and to sterilize. On February 26, 2019, we have identified 88 commercial drugs on the US, European and French markets. Among these specialties, 35 contain an NSAID as API, 23 contain SAID and 30 correspond to anti-inflammatory API associated with anti-infective drugs (1 association with NSAID and 29 associations with SAID). They are reported in Tables 4, 5 and 6. In these Tables, it should be noted that the first line of inactive ingredients corresponds to the preservatives present in the formulation. The composition of some marketed formulations is unfortunately not currently available.

3.1.1. Ointments

The ophthalmic ointment base is made generally of mineral oil and petrolatum. Due to their composition, they present the great advantage to increase contact time of drug (two to four times longer) [43]. The ointment bases are generally either monophasic bases in which the vehicle forms one continuous phase, or biphasic systems, in which an emulsion of oil and water is created. The ointments may cause discomfort to patients. They blur the vision due to refractive index difference

between the tears and the non-aqueous nature of the ointment and inaccurate dosing [44,45]. Consequently, they are less marketed, only 15 of anti-inflammatory specialties are counted among the 88 products listed in the Tables 4, 5 and 6.

3.1.2. Eye drops

3.1.2.1. Solutions

Most of topical ophthalmic preparations available today are in the form of aqueous solutions. A homogeneous solution dosage form offers many advantages including the simplicity of large-scale manufacture, easy handle and the good tolerance. The factors that must be taken into account while formulating aqueous solution include selection of appropriate salt of the drug substance to achieve the therapeutic concentration required. The compatibility of other formulation components such as preservative or buffer salts is to be considered as well as the inertia of the primary packaging. Some typical physical parameters including pH, osmolality, viscosity, color and appearance of the product must be suitable with ocular administration. Usually, aqueous solutions are easily manufactured by dissolution of active and inactive compounds before sterilization by filtration or autoclaving. Nevertheless most of the recently developed drugs are hydrophobic and have limited solubility in water [45]. 40 anti-inflammatory specialties among the 88 listed in Tables 4, 5 and 6 are formulated as ophthalmic solutions.

3.1.2.2. Suspensions:

Ophthalmic suspensions may be defined as a fine dispersion of insoluble API in water which is considered as the most suitable solvent for ocular administration. Eye drop suspensions appear to be an unavoidable alternative to formulate some interesting API which are hydrophobic and then have limited solution in water. What is expected with administered suspensions is that solid drug particles will be retained in the conjunctival *cul-de-sac* and then improving dug contact time compared to eye drop solution. Solid drug particles dissolve progressively leading to improve bioavailability [23,46]. However, it must be emphasized that the formulation of eye drop suspensions is a real challenge. One of the main parameters to take into account is the size of solid API suspended. For reasons of patient comfort, the average particle size in most eye drop suspensions is below 10 μm [46]. Likewise, the morphology of solid particles i.e. irregular shape and crystallinity must be considered with regards to irritation of ocular mucosa. In respect of the tolerate particle size, due to a larger surface area deployed, smaller size drug particles dissolve more or less quickly in the precorneal pocket liquid and the drug is absorbed into ocular tissues while the larger particles dissolve more slowly prolonging the contact time and the availability of the drug.

Another concern of the formulations is the addition of adequate inactive ingredients for many beneficial reasons *i.e.* preservative to prevent microbiological contamination, suspending agents to limit rapid particle settling or caking and surfactants used as wetting or stabilizing agents. In some formulations, hydrophilic cyclodextrins (CD) have been added as complexing agents for solubilizing hydrophobic drug molecule. The CD may also act as absorption promoters [46]. Finally, the redispersibility of drug particles by shaking the container must be effective to ensure the mean dose and the uniformity of amounts administered under therapeutic conditions. In addition of the complexity of the formulations, the technological aspects of the manufacture of suspensions are also to be considered. Indeed, the fabrication of these dosage forms is unconventional and requires specific equipment such as suspension aseptic ball milling. The sterile product is subsequently filled into sterile containers which are hermetically sealed under aseptic environment i.e. class A grade.

Despite all the difficulties encountered in the formulation and manufacture of eye drop suspensions, some very interesting pharmaceutical products have already reached the market. To our knowledge almost 27 suspensions are marketed in Europe and in the USA. Most of these specialties are from 20th century, but some of them are relatively recent, showing the interest of these ophthalmic dosage forms (Tables 4, 5 and 6). One representative example is NEVANAC® which was launched in the USA market in 2007 for the treatment of post-operative inflammation after cataract surgery. NEVANAC® is a 0.1 % suspension of a nepafenac which is described chemically as 2-amino-3-benzoylbenzeneacetamide. This API is an amid liophilic prodrug which is expected to be deaminated by hydrolytic enzymes in aqueous humor to amfenac (2-amino-3-benzoylbenzeneacetic acid) known as NSAID which has unique time-dependent inhibitory properties for COX-1 and COX-2. The prodrug nepafenac is less polar or unionized and offer better corneal penetration [47]. Note that new suspension formulation of nepafenac is a 0.3 % has already been developed by Novartis in USA (ILEVRO®) but not commercialized in European Union (Table 4).

Another example is the eye drop suspension TOBRADEX® which is a combination product of two API, an antibiotic, tobramycin (0.3 %) and a steroid, dexamethasone (0.1 %). This commercial product represents one of the widely used steroid, indicated when superficial bacterial ocular infection or a risk of bacterial ocular infection exist. It is interesting to note that TOBRADEX® which came to the market in 1997 continued to be improved recently. Indeed, a new formulation was developed and launched as TOBRADEX®ST by Alco Laboratories, Inc., with the scope to increase pharmacokinetic characteristics as well as the patient compliance compared to TOBRADEX®. The combination of API in the TOBRADEX®ST was tobramycin 0.3 % and dexamethasone 0.05 % which is half of TOBRADEX® content. Concerning the formulation, the main change was the replacement of the suspending agent Hytellose (hydroxyethylcellulose) present in TOBRADEX® by xanthan gum in TOBRADEX® ST. The consequences of these modifications were that anti-inflammatory and anti-infective activities were improved by the new suspension formulation. The explanations could be that xanthan gum which is an anionic polysaccharide with repeating unit of two D-glucose, two D-mannose and one D-glucuronic acid residues forms an ionic interaction with tobramycin to decrease the viscosity of the suspension. This interaction reduces sedimentation of dexamethasone and improves suspension characteristics. After eye drop was instilled, the pH 7 and ionic content of tears disrupt interactions between xanthan gum and tobramycin leading to enhanced viscosity of eye drop that increase it ocular retention and then improves bioavailability of the drugs [23,48]. Despite these substantial differences, TOBRADEX®ST appears to be clinically equivalent to the older formulation [49]. Through these two examples, it is possible to say that eye drop suspension may be of particular interest for the ocular formulation of some API.

Table 4. Topical ocular pharmaceutical forms and compositions of SAID containing medicines in the US, European or French markets listed as of 26th February, 2019

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
ALREX 0.2%	Loteprednol etabonate	Benzalkonium chloride,	suspension/drops	USA	1998
Multidose bottle 2.5, 5 and 10 mL		Edetate disodium, glycerin, povidone, purified water, tyloxapol, hydrochloric acid and/or sodium hydroxide to adjust the pH			
DEXAFREE 0.1%	Dexamethasone phosphate		solution/drops	Fr	2006
Single use vial 0.4 mL		Edetate disodium, sodium phosphate dibasic, sodium chloride, water for injection			
DEXAMETHASONE SODIUM PHOSPHATE EQ 0.1% PHOSPHATE	Dexamethasone phosphate	Sodium bisulfite, phenylethyl alcohol, benzalkonium chloride,	solution/drops	USA	1996
Multidose bottle 5 mL		Sodium citrate, sodium borate, creatinine, polysorbate 80, edetate disodium dihydrate, purified water, hydrochloric acid			
DEXTENZA 0.4MG	Dexamethasone		intracanalicular insert	USA	2018
Single dose		4-arm polyethylene glycol (PEG) N-hydroxysuccinimidyl glutarate (20K), trilysine acetate, N-hydroxysuccinimide-fluorescein, sodium phosphate dibasic, sodium phosphate monobasic, water for injection			
DUREZOL 0.05%	Difluprednate	Sorbic acid,	emulsion	USA	2008
2.5 mL in 5 mL multidose bottle 5 mL in 5 mL multidose bottle		boric acid, castor oil, glycerin, polysorbate 80, purified water, sodium acetate, sodium EDTA, sodium hydroxide to adjust the pH			
FLAREX 0.1%	Fluorometholone acetate	Benzalkonium chloride,	suspension/drops	USA	1986
5 mL in 8 mL multidose bottle 10 mL in 10 mL multidose bottle 15 mL in 15 mL multidose bottle		sodium chloride, monobasic sodium phosphate, edetate disodium, hydroxyethyl cellulose, tyloxapol, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
FLUCON 0.1%	Fluorometholone	Benzalkonium chloride,	suspension/drops	Fr	1980
Multidose bottle 3 mL		monobasic sodium phosphate, dibasic sodium phosphate, polysorbate 80, sodium chloride, edetate disodium, polyvinyl alcohol, hydroxypropylmethylcellulose, hydrochloric acid and/or sodium hydroxide to adjust the pH			
FML 0.1%	Fluorometholone	Phenylmercuric acetate,	ointment	USA	1985
3.5 g tube		mineral oil, petrolatum alcohol, lanolin alcohol, white petrolatum			
FML 0.1%	Fluorometholone	Benzalkonium chloride,	suspension/drops	USA	1972
5 mL in 10 mL multidose bottle 10 mL in 15 mL multidose bottle 15 mL in 15 mL multidose bottle		edetate disodium, polysorbate 80, polyvinyl alcohol, purified water, sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, sodium hydroxide			
FML FORTE 0.25%	Fluorometholone	Benzalkonium chloride,	suspension/drops	USA	1986
5 mL in 10 mL multidose bottle 10 mL in 15 mL multidose bottle 15 mL in 15 mL multidose bottle		edetate disodium, polysorbate 80, polyvinyl alcohol, purified water, sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, sodium hydroxide			
INVELTYS 1%	Loteprednol etabonate		suspension/drops	USA	2018
2.8 mL in 5 mL multidose bottle		Glycerin, sodium citrate dihydrate, poloxamer 407, sodium chloride, edetate disodium dihydrate, citric acid			

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
LOTEMAX 0.5% Multidose bottle 2.5, 5, 10 and 15 mL	Loteprednol etabonate	Benzalkonium chloride, edetate disodium, glycerin, povidone, purified water, tyloxapol, hydrochloric acid and/or sodium hydroxide to adjust the pH	suspension/drops	USA	1998
LOTEMAX 0.5% 3.5 g tube	Loteprednol etabonate	 Mineral oil, white petrolatum,	ointment	USA	2011
LOTEMAX 0.5% 5 g in 10 mL multidose bottle	Loteprednol etabonate	 Boric acid, edetate disodium, glycerin, polycarbophil, propylene glycol, sodium chloride, tyloxapol, water for injection, sodium hydroxide to adjust to the pH	gel	USA	2012
LOTEMAX SM 0.38% 5 g in 10 mL multidose bottle	Loteprednol etabonate	Benzalkonium chloride, boric acid, edetate disodium dihydrate, glycerin, hypromellose, poloxamer, polycarbophil, propylene glycol, sodium chloride, water for injection,	gel	USA	2019
LOTEPREDNOL ETABONATE 0.5% Multidose bottle 5, 10 and 15 mL	Loteprednol etabonate	Benzalkonium chloride, edetate disodium, glycerin, povidone, purified water, hydrochloric acid and/or sodium hydroxide to adjust the pH, tyloxapol	suspension/drops	USA	2019
MAXIDEX 0.1% Multidose bottle 3 mL	Dexamethasone	Benzalkonium chloride, sodium phosphate monobasic, polysorbate 80, edetate disodium, sodium chloride, methylhydroxypropylcellulose, citric acid, purified water	suspension/drops	Fr	1992
MAXIDEX 0.1% Multidose bottle 5 mL	Dexamethasone	Benzalkonium chloride, hypromellose, sodium chloride, dibasic sodium phosphate, polysorbate 80, edetate disodium, citric acid and/or sodium hydroxide to adjust the pH, purified water	suspension/drops	USA	1962
OMNIPRED 1% Multidose bottle 5 and 10 mL	Prednisolone acetate	Benzalkonium chloride, hypromellose, dibasic sodium phosphate, polysorbate 80, edetate disodium, glycerin, citric acid and/or sodium hydroxide to adjust the pH, purified water	suspension/drops	USA	1973
PRED FORTE 1% 5 mL in 10 mL multidose bottle 10 mL in 15 mL multidose bottle 15 mL in 15 mL multidose bottle	Prednisolone acetate	Benzalkonium chloride, boric acid, edetate disodium, hypromellose, polysorbate 80, purified water, sodium bisulfite, sodium chloride, sodium citrate	suspension/drops	USA	1973
PRED MILD 0.12% 5 mL in 10 mL multidose bottle 10 mL in 15 mL multidose bottle	Prednisolone acetate	Benzalkonium chloride, boric acid, edetate disodium, hypromellose, polysorbate 80, purified water, sodium bisulfite, sodium chloride, sodium citrate	suspension/drops	USA	1972
PREDNISOLONE SODIUM PHOSPHATE EQ 0.9% 5 mL in 10 mL multidose bottle 10 mL in 15 mL multidose bottle 15 mL in 15 mL multidose bottle	Prednisolone sodium phosphate	Benzalkonium chloride, hypromellose, monobasic sodium phosphate, dibasic sodium phosphate, sodium chloride, edetate disodium dihydrate, purified water, hydrochloric acid and/or sodium hydroxide to adjust the pH	solution/drops	USA	1994
SOFTACORT 0.335 % Single use vial 0.4 mL	Hydrocortisone	 Sodium phosphate dibasic, monobasic sodium phosphate, edetate disodium, hydrochloric acid to adjust the pH, water for injection,	solution/drops	Fr	2017

Table 5. Topical ocular pharmaceutical forms and compositions containing NSAID medicines in the US, European or French markets listed as of 26thFebruary

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
ACULAR 0.5% Multidose bottle 5 mL	Ketorolac trometamol	Benzalkonium chloride, sodium chloride, edetate disodium, octoxynol 40, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water	solution/drops	Fr	1991
ACULAR 0.5% Multidose bottle 5 and 10 mL	Ketorolac tromethamine	Benzalkonium chloride, edetate disodium, octoxynol 40, purified water, sodium chloride, hydrochloric acid and/or sodium hydroxide to adjust the pH	solution/drops	USA	1992
ACULAR LS 0.4% Multidose bottle 5 and 10 mL	Ketorolac tromethamine	Benzalkonium chloride, edetate disodium, octoxynol 40, purified water, sodium chloride, hydrochloric acid and/or sodium hydroxide to adjust the pH	solution/drops	USA	2003
ACUVAIL 0.45% Single use vial 0.4 mL	Ketorolac tromethamine	Carboxymethylcellulose, sodium chloride, sodium citrate, purified water, hydrochloric acid and/or sodium hydroxide to adjust the pH	solution/drops	USA	2009
ANTALYRE 0.1% Single use vial 0.4 mL	Salicylic acid	Borax, boric acid, sodium chloride, purified water	solution/drops	Fr	2004
BROMFENAC SODIUM EQ 0.09% ACID 1.7 mL in 6 mL multidose bottle	Bromfenac sodium	Benzalkonium chloride, boric acid, edetate disodium, polysorbate 80, povidone (K30), purified water, sodium borate, sodium sulfite anhydrous, sodium hydroxide to adjust the pH	solution/drops	USA	2014
BROMSITE EQ 0.075% ACID 5 mL in 7.5 mL multidose bottle	Bromfenac sodium	Benzalkonium chloride, boric acid, sodium borate, citric acid anhydrous, sodium citrate dihydrate, poloxamer 407, polycarbophil, sodium chloride, edetate disodium, sodium hydroxide, water for injection	solution/drops	USA	2016
CIELLA 0.1 % Multidose bottle 5 mL	Salicylic acid	Borax, sodium chloride, boric acid, rose-flavored water, purified water	solution	Fr	2004
DICLOFENAC SODIUM 0.1% Multidose bottle 2.5 and 5 mL	Diclofenac sodium	Polyoxyl 35 castor oil, boric acid, tromethamine, sorbic acid, edetate disodium, purified water	solution/drops	USA	2008
DICLOFENAC SODIUM 0.1% Multidose bottle 5 mL	Diclofenac sodium	Polyoxyl 35 castor oil, boric acid, tromethamine, sorbic acid, edetate disodium, purified water	solution/drops	USA	2015
DICLOFENAC SODIUM 0.1% Multidose bottle 5 mL	Diclofenac sodium	Polyoxyl 35 castor oil, boric acid, tromethamine, sorbic acid, edetate disodium, purified water	solution/drops	USA	2007
DICLOFENAC SODIUM 0.1% Multidose bottle 5 mL	Diclofenac sodium	Polyoxyl 35 castor oil, boric acid, tromethamine, sorbic acid, edetate disodium, purified water	solution/drops	USA	2008

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
DICLOFENAC SODIUM 0.1%	Diclofenac sodium		solution/drops	USA	2008
Multidose bottle 5 mL		Polyoxyl 35 castor oil, boric acid, tromethamine, sorbic acid, edetate disodium, purified water			
FLURBIPROFEN SODIUM 0.03%	Flurbiprofen sodium	Thimerosal,	solution/drops	USA	1995
Multidose bottle 2.5 mL		citric acid, edetate disodium, polyvinyl alcohol, potassium chloride, purified water, sodium chloride, sodium citrate, hydrochloric acid and/or sodium hydroxide to adjust the pH			
ILEVRO 0.3%	Nepafenac	Benzalkonium chloride,	suspension/drops	USA	2012
1.7 mL in 4 mL multidose bottle		boric acid, propylene glycol, carbomer 974P, sodium chloride, guar gum, carboxymethylcellulose sodium, edetate disodium, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
INDOCOLLYRE 0.1 %	Indomethacin	Thimerosal,	solution/drops	Fr	1996
Multidose bottle 5 mL		arginine, hydroxypropylbetadex, hydrochloric acid, purified water			
INDOCOLLYRE 0.1%	Indomethacin		solution/drops	Fr	1997
Single use vial 0.35 mL		arginine, hydroxypropylbetadex, hydrochloric acid, purified water			
KETOROLAC TROMETHAMINE 0.4%	Ketorolac tromethamine		solution/drops	USA	2009
NA					
KETOROLAC TROMETHAMINE 0.4%	Ketorolac tromethamine		solution/drops	USA	2009
NA					
KETOROLAC TROMETHAMINE 0.4%	Ketorolac tromethamine		solution/drops	USA	2009
NA					
KETOROLAC TROMETHAMINE 0.4%	Ketorolac tromethamine		solution/drops	USA	2018
NA					
KETOROLAC TROMETHAMINE 0.5%	Ketorolac tromethamine	Benzalkonium chloride,	solution/drops	USA	2009
5 mL in 11 mL multidose bottle 10 mL in 11 mL multidose bottle		edetate disodium, octoxynol 40, sodium chloride, hydrochloric acid and/or sodium hydroxide to adjust the pH, water for injection			
KETOROLAC TROMETHAMINE 0.5%	Ketorolac tromethamine	Benzalkonium chloride,	solution/drops	USA	2009
Multidose bottle 5 and 10 mL		edetate disodium, octoxynol 40, purified water, sodium chloride, hydrochloric acid and/or sodium hydroxide to adjust the pH			
KETOROLAC TROMETHAMINE 0.5%	Ketorolac tromethamine	Benzalkonium chloride,	solution/drops	USA	2009
Multidose bottle 3, 5 and 10 mL		edetate disodium, octoxynol 40, sodium chloride, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
KETOROLAC TROMETHAMINE 0.5%	Ketorolac tromethamine	Benzalkonium chloride,	solution/drops	USA	2009
3 mL in 5 mL multidose bottle 5 mL in 5 mL multidose bottle 10 mL in 10 mL multidose bottle		edetate disodium, octoxynol 40, water for injection, sodium chloride, hydrochloric acid and/or sodium hydroxide to adjust the pH			

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
NEVANAC 0.1%	Nepafenac	Benzalkonium chloride,	suspension/drops	EU	2007
Multidose bottle 3 mL		boric acid, propylene glycol, carbomer 974P, sodium chloride, guar gum, carboxymethylcellulose sodium, edetate disodium, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
NEVANAC 0.1%	Nepafenac	Benzalkonium chloride,	suspension/drops	USA	2005
3 mL in 4 mL multidose bottle		boric acid, propylene glycol, carbomer 974P, sodium chloride, tyloxapol, edetate disodium, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
OCUFEN 0.03%	Flurbiprofen sodium		solution/drops	Fr	1991
Single use vial 0.4 mL		Polyvinyl alcohol, sodium chloride, sodium citrate, potassium chloride, citric acid, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
OCUFEN 0.03%	Flurbiprofen sodium	Thimerosal,	solution/drops	USA	1986
2.5 mL in 5 mL multidose bottle		citric acid, edetate disodium, polyvinyl alcohol, potassium chloride, purified water, sodium chloride, sodium citrate, hydrochloric acid and/or sodium hydroxide to adjust the pH			
PROLENSA EQ 0.07% ACID	Bromfenac sodium	Benzalkonium chloride,	solution/drops	USA	2013
1.6 mL in 7.5 mL multidose bottle 13 mL in 7.5 mL multidose bottle		boric acid, edetate disodium, povidone, sodium borate, sodium sulfite, tyloxapol, sodium hydroxide, water for injection			
VOLTAREN 0.1%	Diclofenac sodium		solution/drops	USA	1991
Multidose bottle 5 mL		Polyoxyl 35 castor oil, boric acid, tromethamine, sorbic acid, edetate disodium, purified water			
VOLTARENOPHTA 0.1%	Diclofenac sodium		solution/drops	Fr	1995
Single use vial 0.3 mL		Cremophor EL, tromethamine, boric acid, water for injection			
VOLTARENOPHTABAK 0.1%	Diclofenac sodium		solution/drops	Fr	2005
Multidose bottle 10 mL		Cremophor EL, tromethamine, boric acid, water for injection			
YELLOX 0.09%	Bromfenac	Benzalkonium chloride,	solution/drops	EU	2011
Multidose bottle 5 mL		boric acid, borax, sodium sulphite anhydrous (E221), tyloxapol, povidone, edetate disodium, water for injections, sodium hydroxide to adjust the pH			
YELLOX 0.09%	Bromfenac sodium	Benzalkonium chloride,	solution/drops	Fr	2011
Multidose bottle 5 mL		boric acid, borax, sodium sulphite anhydrous (E221), tyloxapol, povidone, edetate disodium, water for injections, sodium hydroxide to adjust the pH			

Table 6. Topical ocular pharmaceutical forms and compositions of NSAID or SAID associated with anti-infective drugs in the US, European or French markets listed as of 26th February

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	SAID/ NSAID	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
BACITRACIN-NEOMYCIN-POLYMYXIN W/ HYDROCORTISONE ACETATE 400 UNITS/GM;1%; EQ 3.5MG BASE/GM;10,000 UNITS/GM	Hydrocortisone acetate, Bacitracin zinc, Neomycin sulfate, Polymyxin B sulfate	SAID		ointment	USA	1981
-			Ointment			
BLEPHAMIDE 0.2 %; 10%	Prednisolone acetate, Sulfacetamide sodium	SAID	Benzalkonium chloride,	suspension/ drops	USA	1961
5 mL in 10 mL multidose bottle 10 mL in 15 mL multidose bottle			edetate disodium, polysorbate 80, polyvinyl alcohol, potassium phosphate monobasic, purified water, sodium phosphate dibasic, sodium thiosulfate, hydrochloric acid and/or sodium hydroxide to adjust the pH			
BLEPHAMIDE S.O.P. 0.2%; 10%	Prednisolone acetate, Sulfacetamide sodium	SAID	Phenylmercuric acetate,	ointment	USA	1986
3.5 g multidose tube			mineral oil, petrolatum alcohol, lanolin alcohol, white petrolatum			
CHIBRO CADRON 0.1%; 3 500 UNITS/ML	Dexamethasone sodium phosphate, Neomycin sulfate	SAID	Benzododecinium bromide,	solution/drops	Fr	1992
Multidose bottle 5 mL			sodium citrate, polysorbate 80, hydroxyethylcellulose, sodium hydroxide, sodium chloride, purified water, sodium citrate dihydrate			
CIDERMEX 0.1%; 3 500 UNITS/GM	Triamcinolone, Neomycin sulfate	SAID		ointment	Fr	1991
3 g multidose tube			Mineral oil, white petrolatum			
DEXASPORIN 0.1%; EQ 3.5MG BASE/ML; 10 000 UNITS/ML	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID	-	suspension/ drops	USA	1995
-						
FRAKIDEX 0.1%; 6 300 UNITS/ML	Dexamethasone sodium phosphate, Framycetine sulfate	SAID	Benzalkonium chloride,	solution/drops	Fr	1997
Multidose bottle 5 mL			sodium citrate, polysorbate 80, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
FRAKIDEX 0.1%; 3 150 UNITS/GM	Dexamethasone sodium phosphate, Framycetine sulfate	SAID		ointment	Fr	1998
5 g multidose tube			Mineral oil, white petrolatum			
INDOBIOTIC 0.1%; 3 000 UNITS/ML	Indomethacin, Gentamicin sulfate	NSAID		solution/drops	Fr	2000
Single use vial 0.35 mL			Hydroxypropylbetadex, arginine, hydrochloric acid, purified water			
MAXIDROL 0.1 %; 3500 UNITS/ML; 6 000 UNITS/ML	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID	Benzalkonium chloride,	suspension/drops	Fr	1991
Multidose bottle 3 mL			methylhydroxypropylcellulose, sodium chloride, polysorbate 20, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	SAID/ NSAID	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
MAXIDROL 0.1 %; 3500 UNITS/GM; 6 000 UNITS/GM	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID	Methylparaben, propylparaben,	ointment	Fr	1997
3.5 g multidose tube			lanolin, white petrolatum			
MAXITROL 0.1%; EQ 3.5MG BASE/ML; 10 000 UNITS/ML	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID	Benzalkonium chloride,	suspension/ drops	USA	1963
5 mL in 8 mL multidose bottle			hypromellose, sodium chloride, polysorbate 80, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
MAXITROL 0.1%; EQ 3.5MG BASE/GM; 10 000 UNITS/GM	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID	Methylparaben, propylparaben,	ointment	USA	1963
3.5 g multidose tube			white petrolatum, anhydrous liquid lanolin			
MAXITROL 0.1%; EQ 3.5MG BASE/ML; 10 000 UNITS/ML	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID		suspension/ drops	USA	1984
-						
NEOMYCIN AND POLYMYXIN B SULFATES AND DEXAMETHASONE 0.1%; EQ 3.5MG BASE/GM; 10 000 UNITS/GM	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID	Methylparaben, propylparaben,	ointment	USA	1994
3.5 g multidose tube			white petrolatum, lanolin, mineral oil			
NEOMYCIN AND POLYMYXIN B SULFATES AND DEXAMETHASONE 0.1%; EQ 3.5MG BASE/GM; 10 000 UNITS/GM	Dexamethasone, Neomycin sulfate, Polymyxin B sulfate	SAID		ointment	USA	1989
-						
NEOMYCIN AND POLYMYXIN B SULFATES AND HYDROCORTISONE 1%; EQ 3.5MG BASE/ML; 10 000 UNITS/ML	Hydrocortisone, Neomycin sulfate, Polymyxin B sulfate	SAID	Potassium metabisulfite,	suspension/ drops	USA	1988
Multidose bottle 10 mL			glycerin, propylene glycol, hydrochloric acid, water for injection			
NEOMYCIN AND POLYMYXIN B SULFATES, BACITRACIN ZINC AND HYDROCORTISONE 400 UNITS/GM; 1%; EQ 3.5MG BASE/GM; 10 000 UNITS/GM	Hydrocortisone acetate, Bacitracin zinc, Neomycin sulfate, Polymyxin B sulfate	SAID		ointment	USA	1995
3.5 g multidose tube			Mineral oil, white petrolatum			
NEOMYCIN AND POLYMYXIN B SULFATES, BACITRACIN ZINC AND HYDROCORTISONE 400 UNITS/GM; 1%; EQ 3.5MG BASE/GM; 10 000 UNITS/GM	Hydrocortisone acetate, Bacitracin zinc, Polymyxin B sulfate Neomycin sulfate,	SAID		ointment	USA	2012
3.5 g multidose tube						
PRED-G EQ 0.3%; 0.6%	Prednisolone acetate, Gentamicin sulfate	SAID	Chlorobutanol,	ointment	USA	1989
3.5 g multidose tube			mineral oil, petrolatum, lanolin alcohol, white petrolatum			
PRED-G EQ 0.3%; 1%	Prednisolone acetate, Gentamicin sulfate	SAID	Benzalkonium chloride,	suspension/ drops	USA	1988
5 ml in 10 mL multidose bottle 10 ml in 15 mL multidose bottle			edetate disodium, hypromellose, polyvinyl alcohol, polysorbate 80, purified water, sodium chloride, sodium citrate dihydrate, hydrochloric acid and/or sodium hydroxide to adjust the pH			

TRADE NAME AND PRESENTATION	ACTIVE SUBSTANCE	SAID/ NSAID	EXCIPIENTS	PHARMACEUTICAL FORM	MARKETED IN	YEAR OF AUTHORIZATION
STERDEX	Dexamethasone, Axytetracycline	SAID		ointment	Fr	1997
Single dose vial			Mineral oil, white petrolatum			
TOBRADEX 0.1%; 0.3%	Dexamethasone, Tobramycin	SAID	Benzalkonium chloride,	suspension/ drops	USA	1988
10 ml in 15 mL multidose bottle			tyloxapol, edetate disodium, sodium chloride, hydroxyethyl cellulose, sodium sulfate, sulfuric acid and/or sodium hydroxide to adjust the pH, purified water			
TOBRADEX 0.1%; 0.3%	Dexamethasone, Tobramycin	SAID	Chlorobutanol,	ointment	USA	1988
3.5 g multidose tube			mineral oil, white petrolatum			
TOBRADEX ST 0.05%; 0.3%	Dexamethasone, Tobramycin	SAID	Benzalkonium chloride,	suspension/ drops	USA	2009
Multidose bottle 2.5, 5 and 10 mL			xanthan gum, tyloxapol, edetate disodium, sodium chloride, propylene glycol, sodium sulfate, hydrochloric acid and/or sodium hydroxide to adjust the pH, purified water			
TOBRADEX 0.1%; 0.3%	Dexamethasone, Tobramycin	SAID	Benzalkonium chloride,	suspension/ drops	Fr	1997
Multidose bottle 5 mL			edetate disodium, sodium chloride, sodium sulfate, tyloxapol, hydroxyethylcellulose, sulfuric acid and/or sodium hydroxide to adjust the pH, purified water			
TOBRAMYCIN AND DEXAMETHASONE 0.1%; 0.3%	Dexamethasone, Tobramycin	SAID	Benzalkonium chloride,	suspension/ drops	USA	1991
Multidose bottle 2.5 and 5 mL			sodium sulfate, sodium chloride, hydroxyethylcellulose, tyloxapol, edetate disodium, purified water, sulfuric acid and/or sodium hydroxide to adjust the pH			
ZYLET 0.5%; 0.3%	Loteprednol etabonate, Tobramycin	SAID	Benzalkonium chloride,	suspension/ drops	USA	2004
5 mL in 7.5 mL multidose bottle 10 mL in 10 mL bottle			edetate disodium, glycerin, povidone, purified water, tyloxapol, sulfuric acid and/or sodium hydroxide to adjust the pH			
PREDNISOLONE SODIUM PHOSPHATE EQ 0.23%; SULFACETAMIDE SODIUM 10%	Prednisolone sodium phosphate, Sulfacetamide sodium	SAID		solution/drops	USA	1993
-						
PREDNISOLONE SODIUM PHOSPHATE EQ 0.23%; SULFACETAMIDE SODIUM 10%	Prednisolone sodium phosphate, Sulfacetamide sodium	SAID	Thimerosal,	solution/drops	USA	1995
Multidose bottle 5 and 10 mL			poloxamer 407, boric acid, edetate disodium, purified water, hydrochloric acid and/or sodium hydroxide to adjust the pH			

3.1.3. Gels

Gels are intended to be introduced into the conjunctival *cul-de-sac* or to be applied to the conjunctiva. These semi-solid pharmaceutical presentations are made of polymers presenting the ability to swell in aqueous solvents which make it possible to increase the contact time of the preparation, reduce the elimination rate and obtain a prolonged release of the active ingredient [50]. They reduce the frequency of administration and side effects and consequently improve compliance. They have formed a popular strategy in the early research stages of ocular drug delivery. Mainly hydrophilic gels (hydrogels) have the advantage of being transparent and therefore less disturbing to vision than ointments. However, the drying of the preparation over time and especially at night leads to the formation of deposits that are often not well accepted by the patient. For this reason, it is preferable to use gels during the day rather than at night.

The main inactive ingredients (excipients) used are viscosity modifiers, which slightly increase the viscosity of the product. As previously described, these latter can also be used to stabilize suspensions or as a substitute for tears (artificial tears). These polymers form transparent gels, are sterilizable, water-miscible and have rheological properties adapted to be easily spread on the surface of the eye [50]. A distinction is made between preformed gels (already in the form of gels at the time of application) and in situ gels, applied as a solution, whose gelling mechanism takes place after instillation, due to physicochemical change inherent to the ocular environment (variation in pH, temperature or ions). Among the most common polymers used to obtain preformed gels are cellulosic derivatives (hydroxypropylmethylcellulose (hypromellose), methylcellulose, hydroxyethylcellulose, carboxymethycellulose), polyvinylalcohol (PVA), carbomers and hyaluronic acid. Sometimes a combination of polymers is possible. *In situ* gels are instilled in liquid form, like a simple eye drops allowing an accurate and precise administration. They provide good sustained release properties. Once- or twice-a-day dosing is the typical expectation from these gel systems. For example, polymers such as gellan gum and sodium alginate are able to form gels in the presence of mono or divalent cations while poloxamer rare temperature-responsive polymers [51].

The anti-inflammatory eye gels on the market fall into the category of preformed gels with the example of LOTEMAX® (loteprednol etabonate) containing polycarbophil (cross-linked polyacrylic acid) as viscosifying agent (Table 4). One can note that some pharmaceutical compositions contain hydrophilic polymer agents (polyvinyl alcohol, carboxymethylcellulose, hydroxypropylmethylcellulose) so that they are less fluid, without being classified as gels in the summary of product characteristics. OCUFEN® 0.03 % flurbiprofen sodium), ACUVAIL® 0.45 % (ketorolac trometamine) and PREDNISOLONE SODIUM PHOSPHATE EQ 0.9% are some examples listed in Tables 4 and 5 and are classified as eye drops, aqueous solutions. The effect of viscosity enhancers on drug bioavailability is minimal in humans and their clinical significance is modest [44]. Today, they continue to be used in formulations of ophthalmic product, but their function is more for patient comfort and/or reasons of bioadhesion rather than viscosity enhancement [42]. Marketed SAID/NSAID-based ocular gels are either presented in preservative-containing multi-dose or single dose packaging. Effort is being made to develop multi-dose packaging free of preservatives.

3.1.4. Emulsions:

Emulsions are systems composed of liquid droplets of a liquid A dispersed in another liquid B along with surfactants. Two types of emulsion are described: water-in-oil and oil-in-water emulsion. These systems are useful, particularly oil-in-water emulsion, in the delivery of poor water soluble drugs. By keeping the drug in solution, the issue of potential absorption because of slow dissolution of solid drug particles is avoided. In addition, the blurred vision caused by oils is minimized by the water in the external phase. Furthermore, the concentration of the drug in the oil phase can be adjusted to maximize thermodynamic activity, thus enhancing drug penetration and bioavailability [52].

DUREZOL® is a topical corticosteroid that is indicated for the treatment of inflammation and pain associated with ocular surgery. The product approved by the US FDA in June 2008 is a sterile preserved ophthalmic oil-in-water emulsion. It contains a nonionic emulsifying surfactant polysorbate 80 (4 %, w/v), sorbic acid as preservative and castor oil (5 %, w/v) as oily vehicle. Emulsions eye drops offer advantages over suspensions of solubilizing hydrophobic drug in the oily emulsion vehicle, providing uniform doses without need of shaking before use. William Stringer and Roy Bryant studied dose uniformity of DUREZOL® emulsion 0.05 % versus branded prednisolone acetate ophthalmic suspension 1 % (PRED FORTE®) and it generic under different simulated patient usage conditions. All the results of their study showed that dose uniformity of DUREZOL® emulsion was predictable, within 15 % of declared concentration whereas drop concentration of PRED FORTE® and generic prednisolone acetate ophthalmic suspensions were highly variable throughout the study depending if the bottle of eye suspension was stored upright or inverted as well as the shaking or not of the bottle before use [53]. Furthermore, regarding the *in vivo* corneal penetration of difluprednate, Yamaguchi *et al.* found that within 30 min of instillation the emulsion achieves a concentration 7.4 times higher compared to the suspension. Also after 1 hour instillation, the emulsion led to a higher difluprednate concentration (5.7-fold) in aqueous humor compared to the suspension [54].

As previously discussed by Ding *et al*., oil-in-water emulsions are particularly useful in the delivery of water insoluble drugs which solubilized in the internal oil phase [42]. By choosing appropriate inactive ingredients, *i.e.* new type of emulsifiers or polymeric emulsifiers which are safe and non-irritating, novel ophthalmic emulsion formulations could be achieved having good stability and improved drug bioavailability.

However, castor oil is the inactive ingredient commonly used as the lipophilic phase of the emulsions, although some cytotoxicity towards conjunctival cells has been observed. Indeed Said *et al.* showed in an *in vitro* study that incubating human conjunctival cells with castor oil vehicle during 15 min period of time induced significant cell death. The authors think that this *in vitro* cytotoxicity could explain the side effects observed in some patients and suggest to choose other lipophilic vector to replace castor oil in emulsion based ophthalmic formulations [55].

3.1.5. Use of penetration enhancers :

The use of absorption enhancer transiently increases drug permeability across ocular membranes by decreasing barrier resistance. The surfactants alter physical properties of cell membranes, by disrupting tear film and mucin layer as well as the epithelia by loosening tight junctions or by modifying the cell membrane. The benzalkonium chloride (BAC) which is commonly used in formulations for ocular drugs as a preservative is a cationic surfactant. BAC is known as irritant even used at low concentration (< 0.01 %). It destabilizes the tear film removing its protection properties. As well, it acts on phospholipid bilayer of cell membranes, inducing morphological changes in the epithelium. Concerning EDTA, also found in ocular formulation as chelating agent, itis able to disrupt the tight junctions via extraction of Ca^{2+} [56,57]. Therefore, formulations have changed over the past decade by removing preservatives such as BAC and adapting multi-dose primary packaging, for example: COMOD® or ABAK® or by developing single-dose forms. As well, the cyclodextrins (α, β and γ), which are cyclic oligosaccharides, are able to extract cholesterol and lipids from ocular membranes [57,58]. Finally, crown ethers, bile acids, bile salts (deoxycholate, glycocholate, taurodeoxycholate) and cell penetrating peptides (TAT, penetration, poly(arginine), poly(serine) have been the subject of research that shows their role as penetration enhancers. Nevertheless, none of these are currently used in ocular medicines [57]. However, the safety of these enhancers has to be proven before clinical trials and particularly considering a long-term exposure of the ocular tissues to enhancers.

3.2. *Original formulations*

Original formulations are still in development in order to increase residence time, decrease instillation frequency and finally increase bioavailability of ophthalmic dosage forms [59].

3.2.1. Contact lens

Contact lenses are curved shaped discs prepared from polymeric materials originally designed for vision correction. They can be subdivided in several groups according to their consistency (rigid, semi-rigid, soft) and the polymers used (e.g., poly methyl methacrylic acid, copolymer of hydroxyl ethyl methacrylic acid: poly(vinyl pyrrolidone). The drugs can be added to contact lens allowing an innovative and relevant approach for the treatment of ocular pathologies. Generally the drug molecules are bound to contact lenses by presoaking them in drug solutions [60]. The drug-loaded contact lenses offer advantages of increasing the drug residence time on the ocular surface and sustain drug release. Due to the close proximity of contact lens with the cornea the drug molecules are available for absorption. Finally contact lens soaked with drug could offer highest bioavailability compared to the other noninvasive ophthalmic medication such as eye drop solutions [25]. Addo *et al.* reported that postlens tear film allows drug release from the lens and enhance their precorneal residence time of at least 30 min**.** The bioavailability increase to about 50 % with contact lens [61].

3.2.2. Ophthalmic insert

Ophthalmic inserts are solid or semi-solid sterile devices whose size and shape are specially designed to be placed into the cul-de-sac or conjunctival sac of the eye to deliver active ingredients. They offer many advantages among which the increase of ocular residence and the extension of the drug release into the eye are the most relevant. They also improve patient compliance due to the reduction of dosing frequency. The inserts can be classified according to their solubility behavior in two main categories soluble and insoluble inserts. Insoluble inserts can be a matrix or reservoir form. After release of the active ingredient in predetermined rate the empty insert must be removed from the eye. Bioerodible inserts do not need removal as these devices are made of polymers that undergo gradual hydrolysis of chemical bonds and dissolution

While releasing the drug, inserts can be considered as technical advances for the ocular delivery of drugs. To our knowledge and particularly concerning the ocular anterior segment, only one anti-inflammatory product has reached the market. This is DEXTANZA® a preservative-free, resorbable hydrogel insert containing 0.4 mg of dexamethasone (Table 4). DEXTANZA® was the first FDA-approved intracanalicular insert, a novel route of administration that delivers drug to the surface of the eye. The product originally received FDA approval in November 2018 for the treatment of ocular pain following ophthalmic surgery. Recently DEXTANZA® received a FDA supplemental new drug application for the therapeutic management of ocular inflammation [25,60,62].

3.2.3. Micro and nanocarriers for ocular drug delivery

Despite many efforts made by galenic scientists and pharmaceutical companies, effective commercially drug available to manage affections of the anterior segment of the eye remain a challenge. In this context nano-and microparticle based-ocular formulations could offer several improvements such as a substantial increase in residence time and bioavailability, which are the main limitations of the conventional ocular dosage forms. Therefore the literature now report extensive research on several types of micro- and nanoparticles carriers developed for topical ophthalmic administration in order to enhance drug release and improve the bioavailability through the biological membranes of the anterior segment of the eye [63]. Particularly concerning nanosystem, the use of

different biocompatible materials (phospholipids, polymers, dendrimers, cyclodextrins, lipids, proteins) made it possible to propose liposomes, nanoparticles, nanosuspensions, nanowafers, nanosponges, nanoemulsions and nanomicelles as tools with auspicious outcome for topical ocular delivery of drugs [64]. Tables 7 and 8 group several micro- and nano-formulations of SAIDs and NSAIDs described in the literature. Interesting research and review articles have been published highlighting the benefits of nanosystems in optimizing ocular administration of active ingredients [25,65–67]. In some of these publications, the nanocarriers are studied in several points of view: composition, physicochemical characteristics, association and release of active ingredients, potential interests in ocular use. Many potential benefits are therefore expected from ophthalmic topical nanocarriers.

First of all, they may enhance the solubility of hydrophobic drugs. As an example, Jansook et *al.*, formulated dexamethasone with γCD and HPγCD-poloxamer under the form of nanoaggregates which further exhibit a 15-fold higher concentration than marketed formulation [68].

Their second advantage is their ability to improve precorneal retention through adhesive properties and active uptake by the corneal and conjunctival epithelia leading to enhance ocular permeation [66] in order to produce a rapid anti-inflammatory effect. Gonzalez-Pizzaro *et al.* investigated the benefits of a nanoparticulate formulation of fluorometholone based on PLGA and Pluronic 188 in pigs. The nanoformulation was administered 30 min after the induction of ocular inflammation and was found to produce a greater anti-inflammatory effect up to 120 min compared to ISOPTOFLUCON®, an eye drop suspension of fluorometholone 1mg/mL (Alcon, Barcelona, Spain) as measured by ocular inflammation score according to Draize modified scoring system. This could be attributed to a greater and faster transcorneal permeation [69]. Furthermore, Baba *et al.* suggest a 50-fold greater ocular penetration of fluorescein diacetate for nanoparticles of hydrolysable dye compared to microparticles [70].

The third attribute of nanoparticles is their capacity to enhance drug bioavailability by increasing their residence time at the desired sites [71] in order to prolong the effect. Therefore, N-trimethyl chitosan nanoparticles encapsulating diclofenac sodium showed a 2.5-fold increase in AUC and a sustained residence time with therapeutic concentration was detected up to 12 h in the aqueous humor of rabbit as compared with marketed formulation [72].

Moreover, coating nanoparticles with positively charged bioadhesive polymers is a strategy designed to enhance the interaction between nanoparticles and the negative charges on the corneal surface and to increase precorneal residence time and absorption of drug. Chitosan is the most widely used cationic polymer because of its unique properties, such as acceptable biocompatibility, biodegradability and ability to enhance the paracellular transport of drugs [73]. Badawi *et al.* demonstrate *in vivo* that indomethacin chitosan coated nanoparticles were able to contact intimately with the cornea providing slow gradual indomethacin release with long-term drug level thereby increasing delivery to both external and internal ocular tissues [74].

In Tables 7 and 8, selected studies are described quickly in terms of biocompatibility, entrapment efficiency, transcorneal permeation of drug, aqueous humor drug's concentration and anti-inflammatory effect *in vitro* and / or *in vivo*.

Nanocarriers for ocular drug delivery appear very promising for the treatment of the anterior segment of the eye and particularly for the inflammatory diseases. However, to our knowledge and despite fruitful research leading to a wide range of proposed nanosystems, there are still no clinical trials in progress in 2017 [75]. Apart from the technological effort to be overcome for the scale-up fabrication of these nanosystems, the complexity of the dossiers to be submitted to the authorities for placing on the market is a limiting factor, in particular concerning the toxicological aspects

Table 7. NSAID formulated in micro or nanocarriers for topical ophthalmic administration and their main components from the literature

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
ACECLOFENAC	Nanoparticles	EUDRAGIT®RS 100, Polysorbate 80, mannitol, water	High entrapment efficiency (>90 %) Sustained drug release *in vitro* 2-fold higher transcorneal permeation *ex vivo* as compared with aceclofenac solution Higher anti-inflammatory activity *in vivo* than marketed formulation	[76]
		EUDRAGIT®RL 100, Polysorbate 80, mannitol, water	High entrapment efficiency (>95 %) 2-fold higher transcorneal permeation *ex vivo* as compared with aceclofenac solution Higher anti-inflammatory activity *in vivo* than marketed formulation	[77]
AMFENAC	Nanoparticles	Catechin, $HAuCl_4$, tris acetate buffer, water	No irritation effect *in vivo* and no cytotoxic effect *in vitro* Higher efficiency in DED treatment *in vivo* than marketed formulation of ciclosporin A	[78]
BROMFENAC SODIUM	Liposomes	L-α-distearoylphosphatidylcholine, dicetylphosphate, cholesterol, acetate salt solution, Hank's balanced salt solution, 2-morpholinoethanesulfonic acid monohydrate, chitosan, water	Good entrapment efficiency (>75 %) Sustained drug release without burst effect *in vitro*	[79]
CELECOXIB	Nanoparticles	Poly-ε-caprolactone, poloxamer 188, Sorenson's phosphate buffer, water	High entrapment efficiency (> 89 %) Sustained drug release without burst effect *in vitro* ≈ 2-fold higher corneal permeation *ex vivo* Higher anti-inflammatory activity *in vivo* than marketed formulation	[80]
	Solid lipid nanoparticles	Lipid glyceryl monostearate, PVA, polysorbate 80, poloxamer 188, Sorenson's phosphate buffer, water	Entrapment efficiency (65<X<94 %) Sustained drug release with burst effect *in vitro* ≈ 2-fold higher corneal permeation *ex vivo* Higher anti-inflammatory activity *in vivo* than marketed formulation	[80]
DEXIBUPROFEN	Nanoparticles	PLGA-PEG 5%, PVA, water	No irritant effect *in vitro* and in vivo High entrapment efficiency (>85 %) Sustained drug release up to 12 h *in vitro* and *ex vivo* Sustained anti-inflammatory activity *in vivo*	[81]
DICLOFENAC	Nanoparticles	Methoxy poly(ethylene glycol)-poly(ε-caprolactone)-chitosan copolymer, sodium chloride, water	No cytotoxic effect *in vitro* no irritation effect *in vivo* High entrapment efficiency (>95 %) Sustained drug release up to 8 h *in vitro* ≈1.4-fold higher corneal penetration *ex vivo* than marketed formulation 2.3-fold higher concentration in aqueous humor *in vivo* than marketed formulation	[82]
		NaOH, $Zn(NO_3)_2\,6H_2O$, $Al(NO_3)_3\,9H_2O$, PVP K30, trichlorobutanol, water	No irritation effect *in vivo* High corneal penetration *ex vivo* High apparent permeability coefficient and prolonged precorneal retention time *in vivo*	[83]
DICLOFENAC SODIUM	Liposomes	Phosphatidylcholine, cholesterol, phosphatidylserine low molecular weight chitosan and sodium chloride, water	No irritation effect *in vivo* High entrapment efficiency (>95 %) ≈2-fold higher corneal penetration at 6 h *ex vivo* than diclofenac solution	[84]
	Micelles	Methoxypoly(ethylene glycol)-poly(ε-caprolactone), water	No irritation effect *in vivo* Good entrapment efficiency (>70 %) Sustained drug release *in vitro* up to 24 h 17-fold higher corneal penetration *ex vivo* 3-fold higher concentration in aqueous humor *in vivo* 2-fold higher bioavailability *in vivo*	[85]

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
DICLOFENAC SODIUM	Nanoparticles	N-trimethyl chitosan, phosphate buffer, polysorbate 80, sodium tripolyphosphate, water	No irritating effect *in vitro* and *in vivo* Entrapment efficiency >70 % Sustained drug release *in vitro* ≈2-fold higher concentration in aqueous humor *in vivo* at 1 h	[72]
	Nanoparticles	PLGA, poly[Lac(Glc-Leu)], polysorbate 80, benzalkonium chloride, mannitol, water	No irritants effect *in vivo* Sustained drug release *in vitro* up to 14 h	[86]
	Solid lipid nanoparticles	PHOSPHOLIPON 90G®, goat fat, polysorbate 80, sorbitol, thimerosal, water	High entrapment efficiency (≈90 %) Sustained drug release *in vitro* Higher corneal permeation flux	[87]
FLURBIPROFEN	Cubosomes	Glyceryl monooleate, poloxamer 407, glycerol, water	No irritation effect *in vivo* High entrapment efficiency (>98 %) Sustained drug release without burst effect *in vitro* 2.5- and 2-fold higher apparent permeability *ex vivo* 2-fold higher aqueous humor concentration *in vivo* at 3 h	[88]
	Liposomes	Chitosan, egg phosphatidylcholine, cholesterol, SOLUTOL®HS-15, HCl, water	No irritation effect *in vivo* High encapsulation efficiency (>90 %) 4.59-, 3.56- and 2.36-fold higher apparent permeability *ex vivo* 4.11- and 2.19-fold higher prolonged retention time *in vivo*	[89]
	Nanoemulsion	PLGA, poloxamer 188, water	High entrapment efficiency (>85 %) Sustained drug release *in vitro* ≈1.7-fold increase corneal permeation *ex vivo* than marketed formulation	[90]
	Nanoparticles	EUDRAGIT®RS 100 and RL 100, polysorbate 80, phosphate buffer, benzalkonium chloride, water	No irritation effect *in vivo* High entrapment efficiency (>85 %) Sustained drug release without burst effect Higher concentration in aqueous humor than with marketed formulation	[91]
		PLGA, poloxamer 188, PVA, water	No irritation effect *in vivo* Good entrapment efficiency (>75 %) Sustained drug release *in vitro* Higher anti-inflammatory activity *in vivo* than marketed formulation	[92]
		Poly-ε-caprolactone, poloxamer 188, water	Entrapment capacity (>75 %) Sustained drug release *in vitro*	[93,94]
		PLGA or poly-ε-caprolactone, water	Good entrapment efficiency (>85 %) ≈ 3.9- and 7.6-fold increase corneal permeation *ex vivo*	[95]
		PLGA, poloxamer 188, water	No irritation effect *in vitro* High entrapment efficiency (>90 %) Sustained drug release *in vitro*	[96]
		Poly-ε-caprolactone, poloxamer 188 , trehalose or PEG3350, water	No irritating effect *in vitro* and *in vivo* Good entrapment efficiency (>85 %) Sustained drug release *in vitro* Enhance corneal permeation *ex vivo* Higher anti-inflammatory activity *in vivo*	[97]
	Solid lipid nanoparticles	Stearic acid, MIGLYOL® 812, castor oil, polysorbate 80, water	No irritation effect *in vivo* Good entrapment efficiency (>75 %) Sustained drug release without burst effect *in vitro*	[98]

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
FLURBIPROFEN AXETIL	Nanoemulsion	Castor oil, polysorbate 80, glycerin, carbomer 974P, sodium acetate, boric acid, sorbic acid, water	High entrapment efficiency (>98 %) Better ocular biocompatibility than marketed formulation Higher anti-inflammatory activity *in vivo* than marketed formulation	[99]
IBUPROFEN	Liposomes	Soybean phospholipids, cholesterol, octadecylamine, water	72.9 % entrapment efficiency 1.64-fold higher corneal permeation *ex vivo* at 6 h 1.53-fold higher aqueous humor concentration *in vivo*	[100]
	Liposomes	Cotton-like silk fibroin, phosphate buffer, purified soybean lecithin, cholesterol, stearylamine, water	No cytotoxic effect *in vitro* Entrapment efficacy (59<X<86 %) Sustained release *in vitro* and sustained corneal permeation *ex vivo*	[101]
	Solid lipid nanoparticles	Polyoxyl-35 castor oil, COMPRITOL® 888 ATO, Gelucire 44/14 or TRANSCUTOL® P or sterarylamine, MIGLYOL® 812, water	High entrapment efficiency (>90 %) 4.19-fold higher corneal apparent permeability *ex vivo* 3.99-fold increase of aqueous humor drug concentration *in vivo*	[102]
IBUPROFEN SODIUM SALT	Nanoparticles	EUDRAGIT®RS 100, polysorbate 80, water	Higher anti-inflammatory activity *in vivo* than marketed formulation ≈ 1,5-fold higher aqueous humor concentration *in vivo* than with ibuprofen solution	[103]
	Nanoparticles	EUDRAGIT® RS 100, polysorbate 80, benzalkonium chloride, water	Good ocular tolerability High entrapment efficiency (>90 %) Sustained drug release Higher aqueous humor concentration *in vivo*	[104]
INDOMETHACIN	Microparticles/ Nanoparticles	Zirconia beads and Bead Smash 12, benzalkonium chloride, mannitol or methylcellulose, HPβCD, sodium chloride, water	Better ocular tolerance than marketed formulation *in vitro* ≈ 6-fold higher corneal penetration *in vitro* ≈ 10-fold higher corneal penetration *in vivo*	[105]
	Nanoemulsion/ Nanoparticles	NC : Poly-ε-caprolactone, lecithin, MIGLYOL® 840, poloxamer 188, water NE : Lecithin, MIGLYOL® 840, poloxamer 188, water NP : Poloxamer 188, water	Good tolerance *in vivo* High entrapment efficiency (>89 %) Sustained drug release 4-5-fold higher corneal penetration *ex vivo* than marketed formulation	[106]
	Nanoemulsion/ Nanoparticles	NP : Chitosan with tripolyphosphate, acid acetic, water NE : Chitosan, lecithin soya, MIGLYOL® 840 and Poloxamer 188 or PVA or polysorbate 80, sorbitol, benzalkonium chloride, water	Good entrapment efficiency (>75 %) Sustained release *in vitro* 30-fold higher corneal concentration *in vivo* at 1 h with NE than with solution 13-fold higher aqueous humor *in vivo* at 6 h post instillation with NE than with solution	[74]
	Nanoparticles	Poly-ε-caprolactone, lecithin, MIGLYOL® 840, poloxamer 188, poly-L-lysin or chitosan, water	Good tolerance *in vivo* High entrapment efficiency (>90 %) Rapid release *in vitro* 4-6-and 4-7-fold higher corneal and aqueous humor concentrations *in vivo* after 30 and 60 min post-instillation than marketed formulation	[107]
	Solid lipid nanoparticles	COMPRITOL® 888 ATO, poloxamer 188 and/or polysorbate 80, glycerin, NaOH or HCl, water	Entrapment efficiency (>70 %) 3- 4.5-fold higher corneal permeability *ex vivo* than marketed formulation	[108]

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
KETOROLAC TROMETHAMINE	Micelles	*N*-isopropylacrylamide, vinyl pyrrolidone, acrylic acid, water	30 % entrapment efficiency Sustained release *in vitro* 2-fold higher corneal permeation *ex vivo* Higher anti-inflammatory activity up to 3 h and PMN migration *in vivo*	[71]
	Nanoparticles	Chitosan, acetic acid, NaOH, tripolyphosphate, water	Entrapment efficiency (34<X<41 %) Sustained drug release	[109]
		Chitosan, acetic acid, tripolyphosphate, NaOH, water	Entrapment efficiency (5<X<75 %) Sustained release *in vitro* up to 6 h 3.77-fold lower permeation parameters lower than solution *ex vivo*	[110]
NAPROXEN	Microparticles	Sodium alginate, carbomer 974P, hydroxypropyl methylcellulose, paraffin, calcium chloride, water	Good entrapment efficiency (63<X<76 %) Sustained release *in vitro* without burst effect	[111]
	Nanoparticles	PLGA, PVA, water	High entrapment efficiency (>80 %) Sustained drug release *in vivo* without burst effect *in vitro*	[112]
NEPANEFAC	Nanoaggregates	PVP, PVA, carboxymethylcellulose, hydroxypropylmethylcellulose, methyl cellulose, tyloxapol, γCD, HPβCD, EDTA, benzalkonium chloride, sodium chloride, water	Good entrapment efficiency (>60 %)	[113]
PHOSPHO-SULINDAC	Nanoparticles	Methoxy poly(ethylene glycol)-poly(lactide), sodium cholate, water, phosphate buffer	Entrapment efficacy 46.4 % Sustained drug release *in vitro* up to 24h	[114]
PIROXICAM	Microparticles	Albumin, sodium chloride or sorbitol, water	High entrapment efficiency (>99 %) Sustained release *in vitro* 1.8-fold higher bioavailability *in vivo* than marketed formulation	[115]
	Nanoparticles	EUDRAGIT®RS 100, hydroxypropyl methylcellulose, PVA, sodium chloride, water	Sustained release *in vitro* Great anti-inflammatory activity *in vivo* up to 12 h but no difference compared with microsuspension	[116]

α-CD: α-cyclodextrin, βCD: β-cyclodextrin, γCD: γ-cyclodextrin, HPβCD: hydroxypropyl-β-cyclodextrin, HPγCD: hydroxypropyl- γ-cyclodextrin, RMβCD: randomly methylated-β-cyclodextrin, PEG: polyethylene glycol, PLGA: poly(lactic-co-glycolic acid), Poly[Lac(Glc-Leu)]: poly(lactide-co-glycolide-leucine), PVA: polyvinyl acetate , PVP: polyvinylpyrrolidone, EDTA: ethylenediaminetetraacetic acid, HCl: hydrochloric acid, NaOH: sodium hydroxide

Table 8. SAID and SAID associated with anti-infective formulated in micro or nanocarriers for topical ophthalmic administration

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
DEXAMETHASONE	Cubosomes	Monoolein, poloxamer 407, glycerol, water	Good tolerance *in vitro* High entrapment efficiency (>95 %) 4.5 - 3.5-fold higher apparent permeability *in vitro* 1.8 fold increase the concentration in aqueous humor *in vivo*	[117]
	Microemulsion	Isopropyl myristate, polysorbate 80, propylene glycol, chitosan, acetate buffer, water	No irritation effect *in vivo* High entrapment efficiency (>95 %) Sustained drug release with burst effect *in vitro* Higher anti-inflammatory activity *in vivo* than marketed formulation	[118]
	Microparticles/ Nanoparticles	Zirconia beads and Bead Smash 12,methylcellulose, propyl p-hydroxybenzoate, methyl p-hydroxybenzoate, water	No cytotoxic effect *in vitro* ≈ 5.1-fold higher corneal penetration of nanoparticles than marketed formulation *in vivo*	[119]
	Nanogels suspension	HPγCD, γCD nanogels, EDTA, benzalkonium chloride, hydroxypropylmethylcellulose, sodium chloride, pH adjuster, water	No irritation effect *in vitro* and *in vivo* Sustained drug release without burst effect ≈ 80-fold increase concentration in tear fluid at 6h *in vivo* 3-fold increase concentration in aqueous humor *in vivo*, 2 h after instillation	[120]
		N-tert-butylacrylamide, methylcellulose, nitric acid, cerium ammonium nitrate, water	No cytotoxic effect *in vitro* High entrapment encapsulation efficiency (>95 %) Sustained drug release without burst effect	[121]
	Nanomicelles	Polyoxyl-40-stearate, polysorbate 80, water	No irritation effect *in vivo* Sustained drug release *in vitro*	[122]
	Nanoparticles	Ethyl cellulose or EUDRAGIT® RS or ethyl cellulose/EUDRAGIT® RS, PVA, water	No toxicity, except for ethylcellulose particles Entrapment efficiency (12<X<87 %) Sustained drug release without burst release	[123]
		Propylene glycol, phosphate buffer, EDTA, poloxamer 188, hydroxyethylcellulose, benzalkonium chloride, water	Higher intensity of drug action Higher extent of drug absorption	[124]
		γCD, HPγCD, poloxamer 407, benzalkonium chloride, EDTA, sodium chloride, water	15-fold higher concentration than marketed formulation	[68]
	Nanosponges	βCD nanosponge, water	No irritation or toxic effect *ex vivo* Entrapment efficiency (3<X<10 %) Sustained drug release without burst effect ≈2-fold higher corneal permeability *ex vivo*	[125]
	Solid lipid nanoparticles	Soy lecithin, soybean oil, glycerol, poloxamer 188+/- chitosan, water	No irritation effect *in vivo* Entrapment efficiency (30<X<70 %) Sustained drug release *in vitro* 4.69-fold higher concentration in aqueous humor from L/NPs with chitosan than aqueous solution *in vivo*	[126]

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
DEXAMETHASONE SODIUM PHOSPHATE	Microparticles	RMβCD or γCD, benzalkonium chloride, EDTA, sodium chloride, hydroxypropylmethylcellulose, water	No irritation effect *in vivo* 3-8-fold higher concentration in aqueous humor 2 h after instillation *in vivo* than marketed formulation	[127]
	Nanoparticles	Chitosan, sodium tripolyphosphate, acid acetic, phosphate buffer, hyaluronic acid, water	No irritation effect *in vivo* Entrapment efficiency (58<X<73 %) Sustained drug release *in vitro* Prolonged precorneal retention *in vivo* ≈ 8-fold increase the aqueous concentration at 6 h *in vivo*	[128,129]
		Quaternary ammonium-chitosan conjugate or its thiolated derivative, acid hyaluronic, phosphate buffer, water	No irritation effect *in vivo* Entrapment efficiency (18<X<35 %) Sustained drug release *in vitro* Sustained residence time in tear fluid *in vivo*	[130]
FLUOCINOLONE ACETONIDE	Liposomes	α-, β and HPβCD, water, dextrose, glucose, phosphatdyl choline, triolein, cholesterol, L-lysine, phosphate buffer, water	Entrapment efficiency (7<X<52 %) Sustained release *in vitro* up to 180 h for FA-HPβCD complex	[131]
	Nanoparticles	PLGA P 5002 or 7502, poloxamer 407, phosphate buffer, chitosan HCl, water	No irritation effect *in vivo* Entrapment efficiency (> 50 %) Sustained drug release *in vitro* ≈ 2.5-fold higher concentration in tear sample *in vivo* at 1 h	[132]
FLUORO-METHOLONE	Nanoparticles	PLGA, poloxamer188, water	No irritation effect *in vitro* and *in vivo* High entrapment efficiency (>99 %) Sustained drug release *in vitro* ≈2.2-fold higher increase corneal permeation *ex vivo* than marketed formulation Higher anti-inflammatory activity *in vivo* at 30 min than marketed formulation	[69]
HYDROCORTISONE	Micelles/ Nanoparticles	Albumin, glutaraldehyde, sodium metabisulfite, glucose, polysorbate 80, phosphate buffer, water	Entrapment efficiency (16<X<70 %) Sustained corneal permeation *ex vivo* Neither higher AUC values nor prolonged release *in vivo*	[133]
	Nanoparticles	Propylene glycol, isotonic phosphate buffer, EDTA, hydroxyethylcellulose, benzalkonium chloride, poloxamer 188, water	Higher intensity of drug action Higher extent of drug absorption	[124]
		Gelatin A or B, water, HCl or NaOH, sodium metabisulfite, HPβCD, glutaraldehyde, water	Entrapment efficiency (35<X<45 %) Sustained drug release *in vitro* closed to zero order, 30 % in 200 min	[134]
LOTEPREDNOL ETABONATE	Nanogels suspension	N-boc ethylenediamine, polysorbate 60, chitosan, succinic anhydride, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, phosphate buffer, water	No cytotoxic effect *in vitro* Good entrapment efficiency (67<X<70 %) Sustained release *in vitro*	[135]
	Nanoparticles	PLGA, PVA, water	Good entrapment efficiency (>70 %) Improve *ex vivo* transcorneal penetration	[136]
METHYL-PREDNISOLONE ACETATE	Nanoparticles	EUDRAGIT®RS 100, PVA, sodium chloride, hydroxypropylmethylcellulose, water	No irritation effect *in vivo* Sustained release *in vitro* Higher anti-inflammatory activity up to 36 h *in vivo*	[137]

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
PIRFENIDONE	Nanoparticles	Monoolein, poloxamer P 407, oleic acid, NaOH, glycerin, water	No irritation effect *in vitro* Entrapment efficiency (6<X<36 %) Sustained release *in vitro* Reduction in ocular lesions associated with a reduction of inflammatory cells *in vivo*	[138]
PREDNISOLONE	Micelles	Quaternary ammonium palmitoyl gycol chitosan, poloxamer 407, water	45 % entrapment efficiency 10-fold aqueous humor concentration *in vivo*	[139]
	Nanoparticles	Poly-ε-caprolactone or EUDRAGIT® RS100, castor oil and mineral oil, sorbitan monostrearate, polysorbate 80, water	No irritation effect *in vitro*, no cytotoxic effect *in vitro* Entrapment efficiency (45<X<52 %) Sustained release *in vitro*	[140]
		Propylene glycol, phosphate buffer, EDTA, hydroxyethylcellulose, benzalkonium chloride, poloxamer 188, water	Higher intensity of drug action Higher extent of drug absorption	[124]
PREDNISOLONE ACETATE	Liposomes	1,2-dipalmitoyl-sn-glycerol-3-phosphocholine, cholesterol, stearylamine, water	High entrapment efficiency (78<X<90 %) Sustained release *in vitro* 1.2-2.8-fold lower apparent corneal permeability *ex vivo* than solution ≈ 3-5-fold higher aqueous humor concentration at 3 h *in vivo* than solution Higher anti-inflammatory activity *in vivo* with positively charged unilamelar liposome	[141]
PREDNISOLONE ACETATE OR PHOSPHATE	Ethoniosomes	SPAN® 60, cholesterol, phosphate buffer, water	No irritation effect *in vivo* Entrapment efficiency >85% for prednisolone acetate and 25<X<46% for Prednisolone phosphate Sustained release *in vitro* Higher corneal permeation than marketed formulation Lower bioavailability than marketed formulation Quicker anti-inflammatory activity than marketed formulation	[142]
PREDNISOLONE GATIFLOXACINE	Nanoparticles	EUDRAGIT®RS 100, RL 100, hyaluronic acid, benzalkonium chloride, EDTA, water	Good entrapment efficiency (>60 %) Sustained release *in vitro* 5.23-fold higher and sustained concentration in aqueous humor *in vivo* than marketed formulation	[143]
TRIAMCINOLONE ACETONIDE	Nanoparticles	Methoxypoly(ethylene glycol)-poly(dl-lactic-co-glycolic acid),PVA, water	Non cytotoxic effect *in vitro* 77 % entrapment efficiency Sustained release maintained for 45 days *in vitro* anti-inflammatory activity *in vivo*	[144]
		Poly-ε-caprolactone, poloxamer 188, water	No cytotoxic effect *in vitro* 60 % encapsulation efficiency Sustained release *in vitro* anti-inflammatory activity *in vivo*	[145]
		PLGA, PVA, water	Poor entrapment efficiency (12<X<32 %) Sustained release *in vitro* Similar anti-inflammatory activity *in vivo* than intravitreal injection	[146]

3.2.4. Combined strategies

The last decades were extremely fructiferous regarding therapeutic developments for ocular disease treatments. Particularly, the incorporation of drug's nanocarrier into a polymer matrix creates a system that combines the advantages of micro or nanocarrier and gel:

- convenient administration with good tolerance
- protection of the drug from the enzymatic metabolism present in the tear film [69,123,147]
- longer retention time on the ocular surface [148]
- sustained release [149]
- bioavailability improvement [150]
- increase in drug's penetration in anterior and posterior segments of the eye.

The most used polymers are alginates, chitosan, cellulose derivatives, poloxamer, hyaluronic acid and carbomer. They are mainly used in order to prolong the retention time on the ocular surface as observed with the gamma scintigraphy study of Gupta *et al.* The authors demonstrated that PLGA nanoparticles of levofloxacin incorporated in chitosan have a good spreading, a better retention on the eye, stay for a longer time on the eye and finally present a better bioavailability than marketed formulation [151].

As seen previously, positively charged bioadhesive polymers can be used to enhance the interaction with negative charges on the corneal surface and to increase precorneal residence time and drug absorption. Overall, these systems constitute a suitable strategy for the delivery of drugs in order to enhance drug's bioavailability. As an example, Ibrahim *et al.* demonstrated that their nanoparticles included in gels, made of chitosan or poly-ε-caprolactone, showed 4.8-29.7-folds increase celecoxib bioavailability compared to celecoxib suspension in rats. The improved bioavailability was indicated in extent and in duration compared with marketed formulation. On one hand, this can be due to the high viscosity and the bioadhesive properties of chitosan, which prevent the rapid drainage of the formulations and so increased their contact time with the ocular surface. On the other hand, celecoxib-loaded nanoparticles act as drug reservoirs for sustained drug release. Furthermore, these combined formulations increased celecoxib concentration in both anterior and posterior segments of the eye. This penetration-enhancing property might be due to chitosan and this ability to open the tight junctions and to increase the permeability of cell membrane[150].

As previously described, we identified published reports on the micro or nano delivery systems of NSAID and SAID combined with polymers for topical ophthalmic administration through a systemic search of PubMed from inception until September 2019. We examined the retrieved reports and included in this review those which presented preclinical research on micro or nanocarriers combined with polymer. In Tables 9 and 10 selected formulations are described quickly in terms of biocompatibility, entrapment efficiency, transcorneal permeation of drug, aqueous humor drug's concentration and anti-inflammatory effects.

Table 9. NSAID formulated in combined strategies for topical ophthalmic administration

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
Celecoxib	Nanoparticles in gel	Lecithin, poloxamer 188, PVA, poly-ε-caprolactone or PLA or PLGA, trehalose, hydroxypropylmethylcellulose or methylcellulose, phosphate buffer, benzalkonium chloride, water	No cytotoxic effect *in vitro* Good entrapment efficiency (>79 %) Sustained drug release without burst effect *in vitro*	[152]
Celecoxib	Nanoparticles in gel	Chitosan or sodium alginate, poly-ε-caprolactone or PLA or PLGA, lecithin, PVA, poloxamer 188, trehalose, hydroxypropylmethylcellulose or methylcellulose, phosphate buffer, benzalkonium chloride, water	≈ 5-fold higher concentration in aqueous humor *in vivo* 4.8 -29.7-fold higher bioavailability *in vivo* than marketed formulation	[150]
Celecoxib	Nanoparticles in gel	Chitosan or poly-ε-caprolactone, sodium alginate, lecithin, PVA or poloxamer 188, acetic solution, trehalose, hydroxypropylmethylcellulose or methylcellulose, benzalkonium chloride, water	No cytotoxic effects *in vitro* Entrapment efficiency (>75 %) Sustained drug release without burst effect *in vitro*	[153]
Diclofenac	Micelles in gel	Methoxypoly(ethylene glycol)- poly-ε-caprolactone copolymer,αCD, water	Low cytotoxic effects *in vitro* No irritant effects *in vivo* Sustained drug release *in vitro* up to 216 h 2.37-fold higher concentration in aqueous humor *in vivo* 1h after instillation compared to micelles	[154]
Flurbiprofen	Solid lipid nanoparticles in gel	COMPRITOL® 888 ATO, saturated fatty acid of C18, Gelificante PFC carbomer, MIGLYOL® 812, castor oil, Polysorbate 80, glycerol, NaOH, water	No irritation effects *in vivo* Good entrapment efficiency (>70 %) Sustained release without burst effect *in vitro* Higher corneal permeation *ex vivo*	[155]
Ibuprofen	Solid lipid nanoparticles in-situ forming gel	COMPRITOL® 888 ATO, MIGLYOL® 812, cetyltrimethylammonium bromide, Polysorbate 80, poloxamer 407, water	No cytotoxic *in vitro* High entrapment efficiency (>90 %) Sustained release *in vitro*	[156]
Ketorolac tromethamine	Nanoparticles in-situ forming gel	EUDRAGIT®RL 100, poloxamer 407, hydroxypropylmethylcellulose, citrate-phosphate buffer, PVA, water	No irritation effect *in vivo* Entrapment efficiency (51<X<92 %) ≈ 3-fold higher corneal permeation *ex vivo* ≈ 4-fold higher concentration in aqueous humor *in vivo* at 4 h	[157]
Meloxicam	Nanoaggregates in contact lens	Bovine serum albumin, polysorbate 80, NaOH, HCl, 2-HEMA monomer, tetraethylene glycol dimethacrylate, ethylene glycol, sodium metabisulfite, ammonium persulfate, water	No irritation effect *in vivo* Sustained drug release without burst effect *in vitro* Reduce corneal penetration *ex vivo*	[158]
Nepanefac	Nanoparticles in-situ forming gel	Tetraethyl orthosilicate, cetyltrimethyl ammonium bromide, ammonia, polysorbate 80, poloxamer 407, Pluronic F67 or chitosan, water	No cytotoxic effect *in vitro* High entrapment capacity (>98 %) Sustained drug release *in vitro* 3.68-fold higher corneal permeation *ex vivo*	[159]
Piroxicam	Microparticles /Microparticles in gel	Pectine, polyacrylate gel, water	Entrapment efficiency (41<X<46 %) ≈ 5-6-fold higher residence time *in vivo* ≈ fold increase bioavailability in aqueous humor *in vivo* than marketed formulation	[160]
Pranoprofen	Nanoparticles in gel	PLGA, PVA, carbomer 934P, glycerol, glycerin or azone, water	No irritation effects *in vitro* and *in vivo* High entrapment efficiency (>80 %) Sustained release *in vitro* Greater anti-inflammatory effect in the cornea *in vivo* than marketed formulation	[161]

Table 10. SAID formulated in combined strategies for topical ophthalmic administration

DRUG	SYSTEM	MAIN COMPONENTS	KEY RESULTS	REF.
Dexamethasone	Nanoparticles in-situ forming gel	Poloxamer 188, poloxamer 407, water	No irritation effects *in vivo* Sustained drug release *in vitro* 2.56-fold higher corneal permeation *ex vivo* ≈3-fold higher concentration in aqueous humor *in vivo*	[162]
Dexamethasone	Solid lipid nanoparticles in gel	Soybean oil, glycerol, poloxamer 188, poloxamer 407, water	No irritation effects *in vivo* Entrapment efficiency >50 % Sustained drug release 2.56-fold increase corneal permeability ex vitro ≈3-fold higher concentration in aqueous humor *in vivo* at 6 h after instillation than marketed formulation	[163]
Dexamethasone acetate	Nanoparticles in film hydrogel	Kaolin, hydroxypropyl methylcellulose 5 and 15000cps, triethanolamine, water	Poor entrapment efficiency (8.89 - 9.8 %) Controlled drug releases *in vitro* up to 6 h without burst effect Kaolin extends the corneal permeation up to 6 h *ex vivo* Sustained anti-inflammatory activity *in vivo*	[164]
Fluorometholone	Nanoparticles in-situ forming gel	PLGA, poloxamer 407, sodium alginate, sodium carboxymethylcellulose, benzalkonium chloride, water	No irritation effect *in vitro* and *in vivo* Sustained drug release *in vitro* Higher corneal residence time than marketed formulation *in vivo* 2-3-fold higher concentration in aqueous humor than marketed formulation *in vivo* Greater capacity in decreasing OII than marketed formulation *in vivo*	[165]
Loteprednol etabonate	Nanoemulsion in-situ forming gel	Propylene glycol monocaprylate, poloxamer 407, poloxamer 188, benzalkonium chloride, artificial tear fluid, acetate buffer, cetalkonium chloride, glycerin, water	Zero-order drug release kinetics No irritation *in vitro* High entrapment efficiency (>95 %) 2.54-fold higher bioavailability compared to marketed formulation *in vivo*	[166]
Prednisolone acetate	Nanoparticles in gel	Acetic acid, PVA, sodium deoxycholate, methylparaben, propylparaben, hydroxypropylmethylcellulose, water	Entrapment efficiency (35<X>60 %) Sustained drug release *in vitro* Greater anti-inflammatory effects *in vivo* than marketed formulation	[167]

4. Current techniques for evaluating the topical ophthalmic formulations of NSAID and SAID

Regulatory specifications of topical ophthalmic preparation are very restrictive concerning tolerance, stability and sterility, with regard to the fragility of the eye. Ophthalmic formulations are also complex, adapted to the specific requirements and must be well characterized. Examinations which have to be performed in order to determine the properties of each formulation, may be divided into four parts: physico-chemical characterization, biocompatibility evaluation, pharmacokinetics studies and efficacy regarding to the anti-inflammatory effect.

4.1. Physico-chemical characterizations

4.1.1. Sterility assay

Sterility is one of the essential requirements for drug dosage forms applied on the eye ball. The sterility assay is well described in the European Pharmacopoeia [168]. It involves inoculation in aseptic conditions of the sample examined on two microbiological media:

-Fluid thioglycollate medium with resazurin, used for growth of aerobic and anaerobic bacteria incubated at 30 - 35 °C

-Soy-bean casein digest medium, used for growth of aerobic bacteria and fungi incubated at 20 - 25 °C.

The samples are incubated for a time not shorter than 14 days. Two methods are described, the direct inoculation or the membrane filtration. The number of containers to be tested is fixed by the Pharmacopoeia: 5 % of the batch, minimum 2 and maximum 10 containers per media. The minimum quantity of each container to be tested is also fixed, as an example for liquids: half of the contents of each container but not less than 1 mL per media.

Finally, the sterility assay is conform if no growth of microorganism occurs at 14 days. The procedure for the sterility assay must be performed previously by a suitability test method. The aim of this test is to prove that drug do not exhibit microorganism growth. As described below, the product must be examined using exactly the same methods. After transferring the content to be tested, an inoculum of a small number of viable micro-organisms is added to the media. The inoculums must be < 100 CFU of *Pseudomonas aeruginosa, Clostridium sporogenes, Staphylococcus aerus, Baccilus subtilis, Candida albicans, Aspergillus brasiliensis* per media. The incubation for this test is not more than 5 days.

4.1.2. Clarity examinations

Clarity examination involves the visual assessment of formulation in suitable lighting on white and black background. It is well described in Pharmacopoeia and performed for liquid forms, with the exception of suspensions. This examination applies to eye drops and in situ gels before and after gelling [169].

4.1.3. Osmolality and pH:

Osmolality can be measured by the freezing-point depression method. The pH is most often determined using a potentiometric method. pH and osmolality acceptance are 3 - 8 and 250 - 450 mOsm/kg for the topical ophthalmic administration [170].

4.1.4. Rheological characterization:

Rheological characteristics of ophthalmic formulations are examined at high shear rates using continuous shear techniques and in the viscoelastic region using oscillation techniques. These experiments are currently performed with a controlled stress using cone and plate geometry and the temperature is controlled by a Peltier plate.

The steady-state flow experiments are performed in the range of 0.11 to 100s^{-1}. The frequency sweep method is usually performed between 0.1 Hz and 10 Hz, with a shear strain, while the table of shear rate method is performed by increasing the shear rate from 0.1 s^{-1} to 100 s^{-1}or more. The shear stress is measured by this method and the apparent viscosity is calculated by dividing the shear stress by the shear rate. If the relationship between the shear stress and the strain rate is linear, the fluid is Newtonian. If it is non-linear, the fluid is non-Newtonian.

Oscillation frequency tests can be realized over a frequency range of 0.1 – 10 Hz at constant stress amplitude under linear viscoelastic region (5 Pa), which was previously determined by the oscillation stress sweep tests. From the results of oscillation frequency, we obtain G′ and G″ modulus. If G′ modulus is even greater than G″, the gel exhibits a viscous-like mechanism spectra *a contrario* if G″ modulus is even greater than G′, the gel exhibits a fluid-like mechanism spectra.

Rheological parameter can influence the bioavailability of drugs and the comfort after instillation. The fluids or solutes are eliminated from tears in a few minutes, which results in a short contact time with the eye and high drainage rates and bioavailability for the drugs. To increase the residence time, the viscosity can be increased from 10 to 100 mPa.s but it may cause discomfort due to blurred vision, foreign body sensation and damage to ocular epithelia due to an increase in shear stress during blinking, resulting in a faster elimination due to reflex tears and blinks [171].

4.1.5. Mucoadhesion tests

There are many methods that have been developed for mucoadhesion measurement. Some are similar to the *in vivo* situation and are useful when comparing different materials and formulations to find out which may give the longest residence time. Others have been employed to study the mechanisms of mucoadhesion. The usefulness of the different methods depends on the characteristics of the dosage form and what kind of information is being sought.

Some *in vivo* methods assess the residence time at the application site using gamma scintigraphy, positron emission tomography or fluorescence, while others involve measurement of the transit time using radioisotopes or fluorescence. The successful use of tracers added to the formulation relies upon the properties of the vehicle remaining unchanged and, therefore, behaving in a manner that is identical to that in the absence of the tracer. So, the results obtained are a genuine reflection of the residence time of the dosage form. The low use of *in vivo* methods may be explained by the fact that they do not distinguish between mucosal adhesion and other factors affecting residence time, they are expensive and they are often accompanied by large standard deviations [59].

The mucoadhesion can be evaluated *in vitro* by viscosity, rheology and ZP measurements [172]. When using these *in vitro* methods, not only the method must be chosen, but also the mucus substrate. It could be either an excised tissue or a mucus preparation.

Mucoadhesiveness can be determined *ex vivo* using corneal buttons cutout from freshly isolated porcine eye and fluorescence. A fluorophore is added to formulations, dropped on the corneal surface and then exposed to a continuous stream of normal saline solution at a rate of 10 mL/min for 5 min to 4 h. This continuous irrigation was followed in order to mimic the blink-induced shear stress on the ocular surface. At the end of the pre-determined exposure time, cryostat sections of the cornea were prepared by embedding the corneal button in optimum cutting temperature compound and frozen at −20 °C for at least 24 h. The corneal buttons are then sectioned at 5 μm using a cryostat, placed on slides, and imaged to be visualized by fluorescence microscopy [173].

4.1.6. Characterization of particle size and morphology:

Multiple methods are used for particle size measurements: optical microscopy (microscopic particle count test), light obscuration particle count test, dynamic imaging analysis, laser diffraction particle analyzers, electron microscopy (scanning electron microscopy, transmission electron microscopy and atomic force microscopy), DLS (dynamic light scattering), Coulter Counter test, and nanoparticle tracking analysis [169].

Suspensions or colloidal suspensions require a homogenous and monodisperse population of particles of a certain size, in order to ensure their suitability for *in vitro* and *in vivo* applications and their physical stability. With respect to particle size distribution characterization, a parameter used to define the size range is called the "polydispersity index" (PDI).

The morphology of particles can be examined by transmission electron microscopy (TEM), Cryo-transmission electron microscopy or scanning electron microscopy with negative staining. Briefly, the samples are prepared by wetting a carbon-coated copper grid with a small drop of diluted formulation (5 – 10 µL). Upon drying, they are stained with 1 % uranyl acetate and 2 % phosphotungstic acid, air-dried at room temperature and viewed by TEM. Imaging viewer software is used to perform the image capture and analysis [174].

4.1.7. Zeta potential measurement:

The electrophorectic mobility of nanoparticles is determined by using a Zetasizer and transformed into Zeta potential by using the Smoluchowski equation [175].

4.1.8. Drug and preservative contents

The drug and preservatives contents must be determined by an analytical drug quantification's methodology and validated according to ICH Q2 (R1) guidelines in order to evaluate specificity, linearity, repeatability, intermediate fidelity, limit of detection (LOD) and limit of quantification (LOQ) [176]. The most frequent method used is HPLC [169].

If it is a nanoparticulate formulation, the entrapment efficiency (EE %) must be determined. The EE % is found by subtracting free drug from the total concentration found in the nanosuspension [169].

4.1.9. Stability study

ICH Q1A (R2) defines the stability data package for a new drug substance or drug product that is sufficient for a registration application within the three regions of the EC, Japan, and the United States.

The purpose of stability testing is to provide evidence on how the quality of a drug substance ordrugproductvarieswithtimeundertheinfluenceofavarietyofenvironmentalfactorssuchastemperature, humidity, light and to establish are test period for the drug substance or a shelf life for the drug product and recommended storage conditions. Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy.

The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative content (*e.g.* antioxidant, antimicrobial preservative). A stability study consists of following these parameters at different pre-determined times (e.g. T0 and 3, 6, 9 and 12 months) and stored at one or more controlled temperature and with a controlled humidity. An approach for analyzing the data on a quantitative attribute that is expected to change with time is

to determine the time at which the 95 % one-sided confidence limit for the mean curve intersects the acceptance criterion [177].

4.1.10. In vitro drug release study

In vitro release characteristic can be investigated using dialysis membrane, whose molecular weight cut-off is between 1,000 and 14,000, in Franz cell [100] or modified rotating paddle apparatus [169]. The release medium is generally made of phosphate buffer solution (PBS, pH 7.4) and sometimes PBS is containing polysorbate 80 in order to facilitate drug's solubilization by increasing its wettability in PBS and to keep sink condition. The dialysis membrane and cell are maintained at 35 – 37 °C. At predetermined times a volume of release medium is withdrawn and samples are measured [178].

Finally, other specific tests must be useful to be performed, according to the pharmaceutical form. For example the gelification ability to form the gel in contact with the eye must be performed for *in situ* gelling system or the swelling index for inserts [169].

4.2. Toxicity and biocompatibility tests

Corneal damages vary from irritation and inflammation causing mild discomfort to tissue corrosion resulting in irreversible blindness. During drug evaluation, eye irritation potential and eye toxicity of eye drops must be tested to ensure the safety of drug product before clinical trials in humans.

4.2.1. *In vitro* tests

In vitro testing models using cultured cells area present numerous advantages compared to in *vivo* or *ex vivo* testing as they are relatively inexpensive, simple and quick to implement.
Most *in vitro* ocular toxicity assays consist of a monolayer of cultured cells and a cytotoxicity assessment in response to a test material. Among the methods of assessing cytotoxicity are MTT assay, LDH assay, fluorescein leakage tryptan blue exclusion, fluorescent staining with propidium iodide and neutral red uptake/release tests or ALAMAR® BLUE assay [179]. Each of these methods has their advantages and limitations. In general, a combination of two or more of these methods is normally used to assess cytotoxicity.

For example, MTT assay in short time exposure (STE) according to the Organization for Economic Co-operation and Development (OECD) guideline is performed after a 24 h stabilization of the cells, then fresh medium containing either different concentrations (5 and 0.05 %) of the formulation, blank or formulation without drug are added. Cells are incubated 5 min at 37 °C in order to compare the cytotoxicity of different concentrations and incubation times on cells. After incubation, media is removed and fresh medium and MTT solution are added to each well. Incubation is allowed for another 4 h in darkness at 37 °C. Since living cells metabolize the MTT and form blue formazan crystals, DMSO is added to dissolve the formazan crystals. Absorbance can be read with any filter in the wavelength range of 550 - 600 nm, and percentage of viability can be calculated. The viability of the treated cell cultures is expressed as a percentage of control untreated cell cultures assumed to be 100 %. According to OECD, Table 11 summarizes the prediction model of STE [180]. Note that the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS) is a system proposing the classification of chemicals (substances and mixtures) according to standardized types and levels of physical, health and environmental hazards. This system is addressing corresponding communication elements, such as pictograms, signal words, hazard

statements, precautionary statements and safety data sheets. UN GHS Category 1 corresponds to "Serious eye damage", UN GHS Category 2 corresponds to "Eye irritation" and finally, UN GHS No Category corresponds to chemicals that are not classified as UN GHS Category 1 or 2 (2A or 2B).

Table 11. Prediction model of the STE method inspired from OECD guideline [180]

Cell viability		UN GHS Classification	Applicability
At 5%	At 0.05%		
> 70 %	>70%	No category	No serious damage nor eye irritation effect
≤ 70 %	>70%	No prediction can be made	No prediction can be made, eventual eye irritation
≤ 70 %	≤ 70 %	Category I	Serious eye damage

It is an ethical alternative to *in vivo* studies but do not represent the variability observed in animal and human trials. Generally *in vitro* cell culture models can be classified into three different groups namely primary cell cultures, immortalized cell lines and reconstructed tissue cultures. According to Rökkö *et al.* Table 12 summarizes advantages and disadvantages of each type of cells. The most frequent used cells are Y79 [156], HEK 293 [153] , SIRC [180] or HCEC [181].

In vitro assays and models provide useful data that complement *in vivo* studies allowing for significant reductions in the numbers of animals used. Numerous *in vitro* methods are used to predict biocompatibility or irritation effects of formulations for topical administration, according or not to OECD guidelines: reconstructed human cornea-like epithelium eye irritation test, fluorescein leakage test method, VITRIGEL® EIT method, EPIOCULAR® time to toxicity, OCUL® IRRITECTION, neutral red release or red blood cell test [182].

Table 12. Advantages and disadvantages of each type of cells

	Primary cell cultures	Immortalized cell line	Reconstructed tissue culture
Obtention	From rabbit's corneal tissue or human corneal epithelial cells by excising the tissue and allowing it to adhere	By maintaining the harvested cells in suitable growth medium and transfecting them with a viral vector to induce cell division	From bovine or human corneal tissue construct
Advantages	Relatively cheap and easy	Good correlation with excised rabbit cornea	Morphology similar to excised cornea
Disadvantages	Are not a true representation of the whole cornea	Exhibit abnormal gene expression and/or biological function	More accurate way to mimic the cornea

4.2.1. *Ex vivo* tests

Several *ex vivo* models have been developed as excised rabbit, porcine or bovine corneas, since human corneas are generally reserved for transplant purpose only. They exhibit interspecies variations due to difference in their anatomy and morphology, however with some caution, they can be used to establish good qualitative comparisons of different drug transport pathways.

Rabbit's eyes, although smaller than human eyes, are most preferred for *ex vivo* models as they can also conveniently be used for *in vivo* studies, facilitating *ex vivo - in vivo* correlations. As rabbit eyes lack Bowman's layer; thus, penetration is generally much higher and cannot be correlated well to humans.

The pig eyes are structurally the most similar to human eyes in terms of globe size, corneal thickness, globe diameter to corneal length ratio and the presence of Bowman's layer.

Bovine eyes, on the other hand, are significantly larger than human eyes, and the corneal epithelium is almost twice as thick. Furthermore, it is important to keep in mind that human and animal corneas may significantly differ in metabolic enzymes and transporters present on their surface affecting bioavailability [183].

To date neither *in vitro* nor *ex vivo* test is capable of classifying chemicals as the Draize test. Currently only a limited number of ocular toxicity assays have resulted in validation and regulatory acceptance: bovine corneal opacity and permeability (BCOP), isolated chicken eye (ICE), fluorescein leakage (FL) and STE have been accepted by ICCVAM and OECD.

4.2.2. *In vivo* tests

Live animals have been used to assess and evaluate potentially harmful products to eyes since the 18th century. The international standard assay for acute toxicity is the rabbit *in vivo* Draize eye test which was developed in the 1940s by the Food and drug administration (FDA). New Zealand white (NZW) rabbits are most commonly used. The procedure involves the application of 0,1 mL (or 0,1 g solid) test substance onto cornea and *cul-de-sac* conjunctival of one eye of a conscious rabbit for up to 72 h while the other eye serves as untreated control [184]. The original Draize protocol used at least six rabbits per test, but this was reduced to three animals or a single when serious ocular damage is expected, those with severe lesions being "humanely" euthanized. The latest Draize test guidelines including the application and delivery of analgesics and anesthetics was introduced in 2012 [185] to reduce animal pain and suffering. The rabbits are observed at selected intervals for up to 21 days for signs of irritation, including redness, swelling, cloudiness, edema, hemorrhage, discharge and blindness [179]. In fact, the Draize testing is the only test formally accepted and validated to assess the full range of irritation severity. Both reversible and irreversible ocular effects can be identified using this test [186].

The observed degree of irritancy allows to classify the substances, based on the subjective scoring of the effect on the cornea, the conjunctiva and iris, ranging from non-irritating to severely irritating.

Despite its "gold standard" status, it is often criticized due to its subjective and time consuming nature, lack of repeatability, variable estimates, insufficient relevance of test chemical application, high dosages and over-prediction of human responses primarily due to interspecies differences [187,188]. Since many years, the legislation of many countries as the European directive 2010/63/EU tries to reduce, refine and replace animal testing in biological experiments and promote alternatives. However, the reduction of animal use is primarily concentrated on toxicology studies since no government agency to date has eliminated animal use in basic pharmaceutical development.

One of the alternative *in vivo* tests is low volume eye-irritation test (LVET). It was developed in response to a recommendation from the National research Council [189]. It is a refinement of the Draize test with lower volume: 0.01 mL/0.01 g applied on corneal surface of the right eye of the animal without forced eyelid closure employed and not on the conjunctival sac. It is less stressful for the animal. However the LVET is still criticized for the use of animal and the risk of false negative results

and it is not considered to be a valid replacement nor recommended for prospective ocular safety testing [187].

4.2.3. *In silico* tests

In silico models are computer generated models that can play a useful role in predicting the ocular toxicity of a substance, using quantitative structure-activity relationships (QSAR) [187].

4.3. Pharmacokinetic studies:

For ocular drug products, there is no requirement for pharmacokinetics studies in human subjects. This is because the relevant target or surrogate tissues cannot be sampled serially. For the same reasons, during development, pharmacokinetics data are relied on the use of animal's models, such as rabbit, monkey, dog and pig.

4.3.1. *Ex vivo* transcorneal permeation studies

Transcorneal permeation studies are carried out by putting the eye drops (0.4 to 1 mL) on a freshly excised cornea. The cornea is freshly excised and fixed between the clamped donor and receptor compartments of an all-glass modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The receptor compartment is filled with freshly prepared simulated tear fluid (pH 7.4). The permeation study is carried out for 4 h, and samples are withdrawn from the receptor and analyzed. At the end of the experiment, the corneal hydration of each cornea must be evaluated [190]. Different excised cornea can be used as bovine, porcine, rabbit's, goat, sheep or buffalo [191].

4.3.2. In vivo tests

The most used *in vivo* pharmacokinetics tests are tear fluid or aqueous humor sampling [117]. Some protocol exhibit to evaluate the pharmacokinetics of drug in eye tissues but animals need to be euthanized [150]. The pharmacokinetics study is also conducted using a single-dose response design. The rats are used to evaluate uveitis while rabbits are used to evaluate conjunctivitis. The animals are divided in two groups: verum and control. The animals are lightly sedated. Each formulation is instilled into the inferior conjunctival sac of right eyes of the animals, whereas left eyes served as control by application of the plain dosage form. The eyes are held opened for at least 20 s to allow for adequate ocular surface contact of the formulations and to prevent excessive blinking during application of dosage form, and then the 2 eyelids were held together for additional 10 s to avoid rapid loss of the formulations. A part of the animals is euthanized at predetermined time and then scarified by thoracic opening. Blood samples are collected.

Both eyes are enucleated and dissected while fresh to separate different eye tissues cornea, conjunctiva, anterior sclera, aqueous humor, lens, iris, vitreous body, and posterior eye cup. The amount of drug retained from the different parts of the eye must be further quantified [150].

Some *in vivo* methods assess the transcorneal permeation by radiolabelling and imaging by gamma scintigraphy [100] or positron emission tomography (113). The successful use of tracers added to the formulation relies upon the properties of the vehicle remaining unchanged and, therefore, behaving in a manner that is identical to that in the absence of the tracer so that the results obtained are a genuine reflection of the residence time of the dosage form. The low use of *in vivo* methods may be explained by the cost and the large standard deviations of the method [59].

Another alternative approach includes microdialysis. The microdialysis probe is generally placed in liquid compartments of the eye, such as aqueous humor and vitreous humor and so allows continuous sampling, making it possible to access pharmacokinetic parameters [100,192–194].

4.4. Efficacy testing

The anti-inflammatory efficacy test for topical ophthalmic formulations consists in administering a proinflammatory substance to animals, i.e. carrageenan [164] or arachidonic acid and more specific induced inflammation model exist as autoimmune uveitis [195] or ethanol burn [138].

Usually, rabbits are used for conjunctivitis and rats for uveitis [76,81]. Inflammation is induced to marked extent one hour after carrageenan injection and 30 min after sodium arachidonate instillation.

For example, a usually protocol consists in comparing the formulation to commercial drug and control group (NaCl 0.9% or BSS). The assay is carried out using New Zealand albino male rabbits (n = 6 per group). The study is conducted with the application of 50 µL of 0.5 % sodium arachidonate dissolved in PBS in the right eye, using the left eye as control. After 30 minutes of exposure, 50 µL of each formulation are instilled. In order to evaluate the prevention of inflammation, the evaluation of inflammation is performed from the application of formulation up to 150 min according to Draize modified scoring system. It includes histopathological examination, such as inhibition of polymorphonuclear leukocytes migration and lid closure scores and the alterations of interleukin (IL)-17 and IL-10 at mRNA and protein levels in either aqueous humor or serum [144].

5. Conclusion

Still today the ocular administration of drugs remains a huge challenge for ophthalmologists and galenic scientists. This review mainly devoted to the management of inflammation of the anterior segment of eye offers a complete view on the conventional anti-inflammatory products marketed in France, Europe and USA. Furthermore, the review highlights the progress of therapeutic efficacy expected with implementation of new delivery systems. In addition, the main *in vitro*, *ex vivo* and *in vivo* study methods for the development of ophthalmic anti-inflammatory products are considered. Finally, through the literature cited in this review, scientists have an up-to-date background of informations to improve the efficacy and tolerability of future topical anti-inflammatory products for the anterior segment of the eye.

Author Contributions: R.M., Y.J., L.C., D.W. and A.G., contributed to draft the manuscript and intellectually to the development of the project.

Fundings: This research received no external private funding.

Acknowledgments: This work was supported by Labex ARCANE (ANR-11-LABX-0003-01) and Institut de Chimie Moléculaire de Grenoble (FR 2607) and the Glyco@Alps program (ANR-15-IDEX-02).

Conflicts of Interest: The authors declare no conflict of interest.

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PUBLICATION DES RESULTATS

Publication des résultats N°1

Titre : Investigation of combined cyclodextrin and hydrogel formulation for ocular delivery of dexamethasone acetate by means of experimental designs

Cette partie a consisté à développer deux formulations combinant des cyclodextrines (CD) avec des hydrogels.

Résumé : Les cyclodextrines hydroxypropyl-β-CD (HPβCD) et hydroxypropyl-γ-CD (HPγCD) ont permis d'augmenter la solubilité de l'acétate de dexaméthasone (DXMa) en solution aqueuse de 500 et 1500 fois. Nous avons souhaité conserver les deux complexes DXMa/HPβCD et DXMa/HPγCD pour la suite des travaux. Il faut noter que seule l'HPβCD présente une monographie dans les Pharmacopées européenne et américaine. A ce jour, l'HPγCD n'a encore pas été enregistrée comme excipient par aucune des principales pharmacopées. Néanmoins, ce dérivé est déjà utilisé dans la composition du collyre VOLTAREN® OPHTHA, commercialisé dans certains pays, comme la Nouvelle Zélande.

A partir des résultats satisfaisants de l'augmentation de solubilité de la DXMa, nous avons associé les complexes d'inclusion à des hydrogels bioadhésifs afin d'augmenter le temps de résidence précornéen des systèmes mixtes. Pour ce faire, nous avons choisi trois hydrogels commerciaux (CELLUVISC®, VISMED® et GEL-LARMES®) pour leur compatibilité avec la DXMa. Nous avons ensuite optimisé les formulations en termes de prise en charge de la DXMa et d'osmolalité des mélanges (maximum 400 mOsm/kg) par la réalisation de deux plans de mélange.

Nous avons pu ainsi sélectionner deux gels contenant 0,7 % de DXMa (HPβCD) et 2 % de DXMa (HPγCD) avec des profils de libération différents de la substance active.

Article publié dans *Pharmaceutics* (2018)

pharmaceutics

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Investigation of Combined Cyclodextrin and Hydrogel Formulation for Ocular Delivery of Dexamethasone Acetate by Means of Experimental Designs

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Received: 22 October 2018; Accepted: 20 November 2018; Published: date

Abstract: Dexamethasone acetate (DXMa) has proven its efficiency to treat corneal inflammation, without a great propensity to increase intraocular pressure. Unfortunately, its poor aqueous solubility, associated with a rapid precorneal elimination, results in a low drug bioavailability and a low penetration after topical ocular administration. The main objective of this study was to improve the apparent aqueous solubility of DXMa using cyclodextrins. First, hydroxypropyl-β-CD (HPβCD) and hydroxypropyl-γ-CD (HPγCD) were used to enhance DXMa concentration in aqueous solution. The β and γ HPCD derivatives allowed the increase of the DXMa amount in solution at 25 °C by a factor of 500 and 1500, respectively. Second, with the aim of improving the persistence of the complex solution after instillation in the eye, the formulations of DXMa-based CD solutions with marketed ophthalmic gels (CELLUVISC®, GEL-LARMES®, and VISMED®) were investigated and optimized by means of special cubic mixture designs, allowing the defining of mixed gels loaded with 0.7% (HPβCD) and 2% (HPγCD) DXMa with osmolality within acceptable physiological range. Finally, *in vitro* drug release assays from the mixed gels were performed and compared with reference eye drops. Similarly to MAXIDEX® and DEXAFREE®, in the case of mixed gel containing HPβCD, more than 90 % of the drug was released within 2 h, while in mixed gel containing HPγCD, the release of DXMa was partial, reaching ≈60% in 2 h. This difference will have to be further addressed with *ex vivo* and *in vivo* ocular delivery experiments.

Keywords: dexamethasone acetate; cyclodextrins; eye drops; hydrogels; experimental design; phase solubility; dissolution assay

1. Introduction

Ocular inflammation is the consequence of many potential eye disorders among which uveitis is believed to be the cause of about 10% of the cases of severe visual handicap in the United States [1].

Topical administration of anti-inflammatory drugs, steroidal (SAID) and non steroidal (NSAID), is the most frequently used method to treat ocular surface and anterior segment inflammation as it presents an easy accessibility, a simplicity of use, a non invasive way, and generally a good tolerance. Nevertheless, the ocular drug bioavailability in conventional eye drops is notoriously poor; only 1 – 5 % of drug applied to the surface penetrates the cornea. This is the consequence of various effective protective mechanisms and multiple barriers to drug entry, including a fast naso-lachrymal drainage due to high tear fluid turnover and lid blinking, the corneal structure with a hydrophilic stroma sandwiched between the lipophilic epithelium and endothelium, epithelial drug transport barriers, and clearance from the vasculature in the conjunctiva [2,3].

Numerous strategies have been developed to increase the bioavailability of ophthalmic drugs. One of them is to prolong the contact time between the drug and the corneal/conjunctival epithelium by the use of mucoadhesive hydrogels [4]. An enhanced residence time will increase the time over which absorption can occur and the total amount of drug absorbed and has been shown to result in prolonged effect and increased bioavailability in several studies [5]. As an example, this strategy is used in marketed eye drops such as TIMOPTOL LP® and GELTIM LP®, which are instilled once daily *vs.* twice daily with TIMOCOMOD®.

Among corticoids, dexamethasone (DXM) has one of the highest potencies and effectiveness on inflammation. DXM is used for the treatment of acute and chronic eye inflammation, including postoperative inflammation or uveitis [6,7]. DXM acts in the human trabecular meshwork cells by inhibiting phospholipase-A2, i.e., prostaglandins synthesis, which causes inflammation [8,9]. Unfortunately, DXM presents a formulation challenge, since it is a water-insoluble compound [10,11]. DXM is soluble to a limited extent in aqueous eye drops. Thus the drug is frequently used as suspensions, such as 0.1 % *w*/*v* MAXIDEX® (Novartis Pharma, Rueil-Malmaison, France) or as solutions using a hydrophilic water-soluble prodrug, such as 1 % *w*/*v* DXM sodium phosphate DEXAFREE® (Laboratoires Théa, Clermont-Ferrand, France) [7]. The lipophilic derivative DXM acetate (DXMa), currently unavailable for topical use, has been shown to readily permeate the cornea and hydrolyze to DXM during absorption [12]. As well, Leibowitz *et al.* demonstrated that the acetate form was more effective compared to phosphate derivative in suppressing inflammation in the cornea. This therapeutic effect was not associated with a greater propensity to increase intraocular pressure, one of the most frequent side effects of glucocorticoids [13]. Therefore, DXMa (Figure 1) was selected in this study to be formulated for topical ocular administration. Methods such as pH adjustment, cosolvency, micellization, complexation, or use of cyclodextrins (CD) are among the most commonly used approaches for drug solubilization allowing the formulation of eye drops solution [14]. Cyclodextrins present the great advantage of enhancing both bioavailability and the apparent solubility of poorly water-soluble drugs while being biocompatible [15,16]. In this context, the two hydrophilic cyclodextrin derivatives, hydroxypropyl-β-cyclodextrin (HPβCD) and hydroxypropyl-γ-cyclodextrin (HPγCD), were used to enhance the molecular DXMa fraction in aqueous solution. Mixtures of CD/DXMa solutions with marketed mucoadhesive gels were investigated as topical drug vehicles to the eye, with the objectives to achieve therapeutically effective DXMa dosage form with a reduced frequency of instillation.

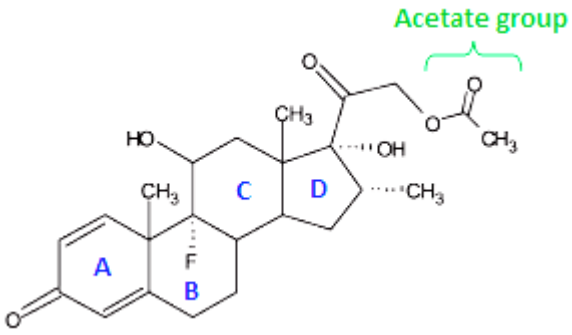


Figure 1. Chemical structure of dexamethasone-21-acetate with perhydro-cyclopentano-phenanthrene ring system, A = A-ring, B= B-ring, C= C-ring, and D = D-ring.

In this study, we first evaluated the association constants DXMa/HPβCD or HPγCD. Then, the mixtures of these HPβCD or HPγCD/DXMa solutions with marketed gels (CELLUVISC®, GEL-LARMES®, and VISMED®) were further investigated by means of two mixture experimental designs in order to define optimized DXMa formulations in terms of water-soluble drug fraction content and osmolality. Finally, the *in vitro* release profiles from selected mixed hydrogels were evaluated.

2. Materials and Methods

2.1. Materials

DXMa was purchased from LA COOPER (Melun, France). Hydroxypropyl-γ-cyclodextrin (HPγCD, W8HP, DS = 0.6 and Mw = 1576 Da) was a kind gift from ASHLAND (Schaffhausen, Switzerland) and Hydroxypropyl-β-cyclodextrin (HPβCD, KLEPTOSE DS = 0.63 and Mw = 1391 Da) was obtained from ROQUETTE (Lestrem, France). CELLUVISC® (sodium carboxymethylcellulose), GEL LARMES® (Carbopol 974P) and VISMED® (sodium hyaluronate) are marketed gels used for the treatment of dry eye syndrome. DEXAFREE® (DXM sodium phosphate 1% solution eye drops), MAXIDEX® (DXM 0.1% suspension eye drops) are human authorized ocular medicines. Methanol (HiPerSolvCHROMANORM for HPLC grade) was purchased from BDH, PROLABO (Leuven, Belgium. Purified water was prepared by DIRECT-Q® 3UV water purifier (MILLIPORE, Molsheim, France). All other solvents and chemicals were of HPLC and analytical grade, respectively.

2.2. Methods

2.2.1. Quantitative Determinations

Quantitative determinations were performed on a reversed-phase, high-performance liquid chromatographic (HPLC) component system LC 2010 AHT (SHIMADZU, Kyoto, Japan) consisting of a pump with degasser, an autosampler, a UV-VIS detector, and a column XTERRA©MS C8 5 µm particles 150 × 4.6 mm with C8 cartridge. This method was adapted from that previously reported by Urban *et al.* and validated in DXMa, DXM sodium phosphate (DXMp) and dexamethasone (DXM) concentrations ranging from 0.001 to 1 mg/mL [17]. The mobile phase made of methanol:water (70:30 *v*/*v*) was set at the rate of 0.8 mL, the temperature at 25 °C and the detection wavelength at 240 nm. The calibration curves are presented in Table 1.

Table 1. Calibrations curve, retention time, correlation coefficient and variability of DXM, DXM sodium phosphate and DXMa quantitative determinations by HPLC.

Drugs	Retention Time	Calibration Curve	Correlation Coefficient	Intra-Day Variability (CV%)	Inter-Day Variability (CV%)
DXM	4.8	$y = (3 \times 10^7)x + 27.867$	0.999	< 1 %	< 3 %
DXM sodium phosphate	3.8	$y = (5 \times 10^6)x - 312.7$	0.999	< 1 %	< 2 %
DXMa	6.3	$y = (3 \times 10^7)x + 39.464$	0.999	< 1 %	< 3 %

2.2.2. Phase Solubility Diagrams

The phase solubility studies were carried out according to Higuchi and Connors [18]. Briefly, an excess amount of the drug was added to aqueous solutions containing increasing amounts, 0 to 60 % (*w*/*v*) of HPβCD or HPγCD. After 24 h under magnetic stirring at 25 °C, the drug suspensions were ultracentrifugated for 1 h at 35,000 rpm (Optima L-80 XP Ultracentrifuge BECKMAN COULTER, Brea, California, USA). Note that our operating conditions are limited to 24 h according to preliminary studies, showing that 24 h and 72 h did not change the equilibrium. The supernatant was then diluted at 1:50 in the mobile phase and analyzed by HPLC. The experiments were repeated three times for each cyclodextrin derivative.

The apparent stability constant of the drug/cyclodextrin complex (D/CD), assuming that one molecule of drug forms a complex with one molecule of cyclodextrin ($K_{1:1}$), can be calculated from the slope of the linear phase-solubility profiles and the intrinsic drug solubility in the complexation media [18,19] in the absence of the cyclodextrins as presented in Equation (1):

$$K_{1:1} = \frac{\text{Slope}}{S_0 \times (1 - \text{Slope})} \quad (1)$$

The complexation efficiency (CE) can be calculated by applying the following Equation (2), which also refers to the slope of the linear phase-solubility profiles [20] and intrinsic solubility:

$$\text{CE} = K_{1:1} \times S_0 = \frac{\text{Slope}}{(1 - \text{Slope})} \quad (2)$$

2.2.3. Chromatographic Determination of the Association Constants

The mobile phase consisted of a mixture of methanol:water (70:30 *v*/*v*) with various HPβCD and HPγCD concentrations (0, 0.5, 2.5, 5, 7.5 and 10 mM). Standard solutions of DXMa (0.85 $mg.mL^{-1}$) were freshly prepared in a mixture of methanol:water (70:30 *v*/*v*). The chromatographic system was allowed to equilibrate for at least 1 h prior to each experiment. 10 µL of this standard solution was injected and the retention time collected. All the experiments were carried out in triplicate for each temperature (25, 30, 35 and 40 °C) and each cyclodextrin concentration.

The chromatographic determination of the association constants with high-performance liquid chromatography is based on the partitioning of the solute between the mobile and the stationary phase. When cyclodextrin is added to the mobile phase, solute retention is split into two main physicochemical processes, namely, solute complexation by cyclodextrin and transfer of free (uncomplexed) solute from the mobile to the stationary phase. The association constant *K* (M^{-1}) between compound and cyclodextrin can be determined by using the established Equation (3) [21].

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K[CD]^x}{k_0}, \quad (3)$$

where *k* (min) is the solute retention factor, k_0 (min) the solute retention factor without cyclodextrin in the mobile phase, [CD] (M) the concentration of cyclodextrin in the mobile phase, and *x* the stoichiometry of the complex. For an inclusion complex with a 1:1 stoichiometry (*x* = 1), a linear plot of 1/*k* versus [CD] must be obtained and the *K* value calculated.

2.2.4. Thermodynamic Parameters for the DXMa/Cyclodextrin Complexes

According to the previous chromatographic conditions, the retention factor was determined in triplicate at the following temperatures: 25, 30, 35 and 40 °C. $\Delta H°$ and $\Delta S°$ are, respectively, the standard enthalpy and entropy of transfer of DXMa from the mobile phase to the cyclodextrin cavity. These energies can be calculated using the following thermodynamic relationships as described in Equation (4) [21,22]:

$$\ln K = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R}, \quad (4)$$

where T is the temperature and R the gas constant. For a linear plot of ln$K$ versus 1/T, the slope and the intercept are, respectively $-\Delta H°/R$ and $\Delta S°/R$.

2.2.5. Experimental Designs and Data Analysis

Experimental designs were used in order to determine the optimized formulations based on HPβCD and HPγCD. In this study, the goal of optimization was respectively focused on maximization of the DXMa solubility and adjustment of osmolality within acceptable physiological range from 250 to 450 mOsm/Kg [23]. Experimental domain was obtained by fixing the minimum and maximum proportions of each component (% *w*/*w*) as presented in Table 2.

Design-Expert software version 10.0.8 (State Ease, Inc., Minneapolis, MN, USA) package was used to establish a special cubic mathematical model which exhibits the relationships between response and formulation components, allowing the optimum operational conditions to be obtained via a statistical analysis.

Two experimental designs (1 and 2) were performed, one per CD derivative (Table 2). Each experimental design included 29 experiments. Each experiment was performed according to the procedure described in Figure 2. Briefly, a mixture (2 g) containing DXMa/CD solution and hydrogel(s) was stirred for 2 h under magnetic stirring at room temperature. Then, a large excess of DXMa was added to the mixture and agitated during 12 h. The drug suspension was ultracentrifugated at 15 °C during 1h at 35,000 rpm (Optima L-80 XP Ultracentrifuge BECKMAN COULTER, Brea, CA, USA). The supernatant was collected and the osmolality measured (Model 2020, ADVANCED INSTRUMENT, Norwood, MA, USA) before a dilution at 1:50 with the mobile phase in order to assay the rate of DXMa by HPLC.

Table 2. Low and high levels of formulation components for special cubic mixture designs.

	Component	Low Level (%)	High Level (%)
Experimental Design 1	CELLUVISC®-Gel$_1$	0	70
	GEL-LARMES®-Gel$_2$	0	70
	VISMED®-Gel$_3$	0	70
	HPβCD 600 mg/mL with DXMa 10 mg/mL	30	100
Experimental Design 2	CELLUVISC®-Gel$_1$	0	70
	GEL-LARMES®-Gel$_2$	0	70
	VISMED®-Gel$_3$	0	70
	HPγCD 600 mg/mL with DXMa 30 mg/mL	30	100

The special cubic model coefficients were estimated in accordance with the established Multi Linear Regression (MLR), which allows fitting of the observed response with the analytical model [24].

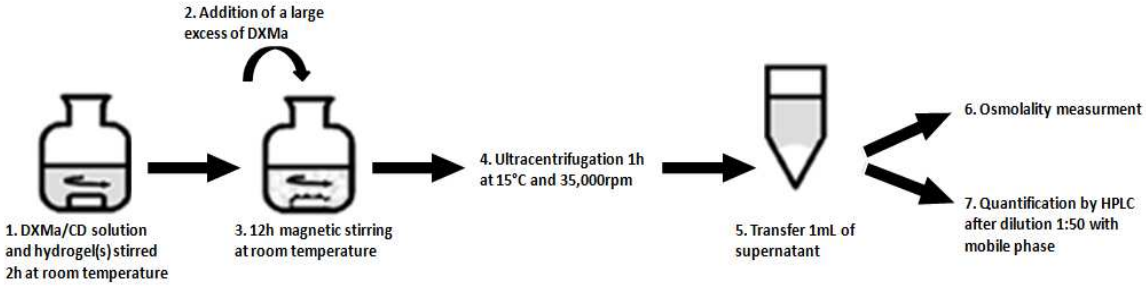


Figure 2. Steps implemented for the experimental design with 29 experiments.

The full mixture cubic model including all coefficients was refined using stepwise technique [25]. This procedure involves removing step by step each eligible coefficient to find the model that best fits the data according to some criteria. The corrected Akaike Information Criterion (AICc) and the Bayesian Information Criterion (BIC) minimization are the likelihood statistics criteria used to compare the different models.

The fitness of the models can be validated using statistical parameters as R-square (R^2), adjusted R-square (R^2_{adj}), predicted R-square (R^2_{pred}), and adequation precision (*AdeqPrec*) values. The R^2 value, as shown in Equation (5), refers to the ratio of the sum of squares regression (SS_R) to the total sum of squares (SS_T) from the ANOVA table. The R^2 value explains the total variation of the data around the average, and its value is in the range of 0–1.0. A value of R^2 close to 1.0 indicates that the models have good fit. Nonetheless, the value of R^2 is directly related to the number of terms in the model. Therefore, the additional checking criteria (R^2_{adj}) and (R^2_{pred}) are also needed (Equations (6) and (7)). In Equation (6), p denotes the number of factors plus one and SS_E is the error or residual sum of squares while PRESS in Equation (7) is the predicted residual error sum of squares. Generally, R^2_{adj} decreases as insignificant terms are added to the model and R^2_{pred} decreases when the model considers too many insignificant terms. Therefore, these two criteria are the primary concerns in response modeling, where both values should be close to 1.0 and within 0.2 of one another [26]. The adequation precision (*AdeqPrec*) measures the signal-to-noise ratio (Equation 8). $\hat{\sigma}^2$ denotes the residual mean square from ANOVA table, $\max(\hat{Y})$ and $\min(\hat{Y})$, respectively, are the maximal and minimal response predicted for the experimental design run conditions. A ratio greater than 4 is desirable.

$$R^2 = \frac{SS_R}{SS_T}, \tag{5}$$

$$R^2_{Adj} = 1 - \frac{\frac{SS_E}{n-p}}{SS_T/(n-1)} = 1 - \frac{n-1}{n-p}(1-R^2), \tag{6}$$

$$R^2_{pred} = 1 - \frac{PRESS}{SS_T}, \tag{7}$$

$$AdeqPrec = \frac{\max(\hat{Y}) - \min(\hat{Y})}{\sqrt{\frac{p\hat{\sigma}^2}{n}}}, \tag{8}$$

In complement, scatter plots of Actual *vs.* Predicted are used to evaluate how the model predicts over the range of data. Ideally, the predicted values should be close to the actual values

and then all points should be close to a regressed diagonal line. Furthermore, the points should be symmetrically scattered about the line, as expected if the errors are normally distributed.

2.2.6. Rheological Characterization

The viscosity of optimized mixed gels A and B (n = 3) were determined by using a rotational viscometer (RM 100, LAMY, Champagne au Mont d'Or, France). The viscosity measurements were performed at controlled temperature (22 °C) at increasing shear rates (from 12.9 to 1936 s^{-1}).

2.2.7. *In Vitro* DXMa Release Profiles

The drug release experiments were carried out using a Sotax Dissolutest AT7 (SOTAX, Aesch, Switzerland). A sample of optimized mixed gels A or B or MAXIDEX® or DEXAFREE® was dropped in the extraction cell, which was placed at the bottom of the vessel filled with the dissolution medium. The experiments were conducted for 24 h at 35 °C, in 250 or 500 mL of phosphate buffer saline (PBS 1X pH 7.4). The speed of the rotating paddle was set at 100 rpm. The DXM, DXMa, and DXMp solubilities were previously determined in triplicate after 2 h agitation of aqueous drug suspensions in PBS at 35 °C. After filtration (0.2 µm), the solubilized drug content was quantified by HPLC at 240 nm. The amounts of sample used in the cell were 1.5 g for gel A, MAXIDEX®, and DEXAFREE®, and 0.5 g for gel B. At the set time points (30 min, 1 h, 2 h, 4 h, 8 h, 24 h), aliquots of 1 mL filtered medium were withdrawn and DXMa content measured by HPLC.

3. Results and discussion

DXMa, a poorly water-insoluble steroid, was selected in this study to be formulated for topical ocular administration. In this context, the two hydrophilic cyclodextrin derivatives, HPβCD and HPγCD, were used to enhance the molecular DXMa fraction in aqueous solution.

3.1.Solubility Determinations of Dexamethasone Acetate

The phase-solubility study is one of the most common methods applied in order to evaluate the solubilization ability of CDs. Figure 3 was obtained by plotting the total concentration of dissolved cyclodextrin (mM) HPβCD or HPγCD versus apparent DXMa concentrations at equilibrium (mM). The obtained profiles were then classified according to Higuchi and Connors [18]. For both cyclodextrin derivatives, the phase-solubility profiles are linear ($R^2 \geq 0.995$), indicating that the apparent solubility of DXMa increases with an increase of the cyclodextrin concentration. Thus these linear curves refer to AL-type phase-solubility profiles according to Higuchi and Connors [18]. The solubility studies indicated that the DXMa probably forms water-soluble complexes with the two CDs. Indeed, our results showed a dramatic increase of DMXa solubility induced by the complexes. Typically, 600 mg/mL (380 mM) HPβCD and HPγCD (430 mM) aqueous solutions at 25 °C solubilize 10.91 ± 0.16 mg/mL and 30.48 ± 0.12 mg/mL of DXMa, respectively, which correspond to increasing in solubility of about 520- and 1450-fold compared to aqueous solubility of uncomplexed DXMa at 25 °C (i.e. S_0 = 0.021 mg/mL). Usayapant *et al.* and Vianna *et al.* also studied interaction between DXMa and cyclodextrins [12,27]. Especially, Usayapant *et al.* found that at 260 mM HPβCD, the solubility enhancement for DXMa was 1016-fold. For their part, Vianna *et al.* indicated a 88-fold increase of DXMa with a maximum of 53 mM HPβCD. The differences between these solubility values are probably related to the experimental conditions of phase-solubility studies such as pH, ionic strength, temperature, the time necessary to reach equilibrium, the range of CD concentration, the graphical determination of S_0 and analytical method used. The degree of substitution of the HPβCD used is also to be considered. Despite these differences, HPβCD complexation allowed the DXMa water solubility to be enhanced significantly. So far there are few studies concerning complexation of HPγCD with DXMa, hence our study, which showed a remarkable increase of apparent solubility of DXMa up to 30.48 mg/mL in the presence of 600 mg/mL HPγCD, is of promising interest. Concerning the type of phase-solubility diagram, according to our solubility profiles, we assumed that both cyclodextrin derivatives lead to A_L-type.

It is to highlight that Vianna *et al.* also described an A_L-type phase-solubility profile for DXMa/HPβCD complex while Usapayant *et al.* claimed an A_P-type phase-solubility profile.

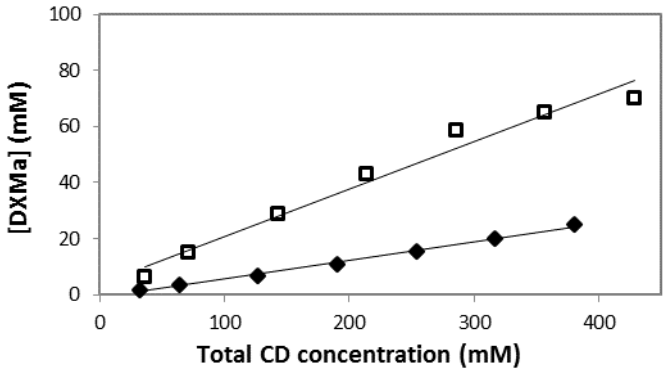


Figure 3. Phase-solubility diagrams of DXMa in water under various concentrations of HPβCD (◆) or HPγCD (□). Each data point represents a mean (n = 3), with SD smaller than the symbol size.

The stoichiometric and binding or association constant K, as well as the complexation efficiency (CE), are important characteristics of the complex. Based on the phase A_L-type phase-solubility diagram, a stoichiometry of 1:1 was assumed for DXMa/HPβCD and DXMa/HPγCD. Usayapant *et al.* claimed that 1:1 and 1:2 complexes where present when HPβCD interacted with DXMa in solution [12]. However, Usayapant *et al.* also indicated in their work that the formation of 1:2 complex was less favored due to the very low value of the constant binding $K_{1:2}$ inferior to 20 M^{-1}. When referring to the literature, some authors have described the geometry of inclusion complexes of steroids with cyclodextrins. Usually it is reported that inclusion occurred primarily at the A–B ring, especially when A ring bears a ketone (Figure 1) [27–29]. Concerning specially the DXMa, it is not totally excluded that the acetyl group can interact with the cyclodextrin cavity [12,27].

In this study, assuming the formation of 1:1 complex, the apparent stability constant of the DXMa/cyclodextrin complex was first calculated from the slope of the linear phase-solubility profiles and the intrinsic drug solubility (S_0 = 0.021 mg/mL = 0.048 mM) in the complexation media [18] in the absence of the cyclodextrins, as presented in Equation (1).

The complexation efficiency (CE) was calculated by applying Equation (2), which also refers to the slope of the linear phase-solubility profiles [20] and intrinsic solubility of DXMa.

The results of calculated $K_{1:1}$ and CE, as well as the slope of phase-solubility diagrams, are shown in Table 3. The $K_{1:1}$ values were 1462 and 5368 M^{-1} for DXMa/HPβCD and DXMa/HPγCD, respectively. The $K_{1:1}$ value reported by Usayapant *et al.* for DXMa/HPβCD was 2240 M^{-1} and slightly higher. This difference can be explained by the fact that phase-solubility studies are influenced by various factors such as the operating conditions, that is, pH, ionic strength, temperature, and analytical method, that do not allow a strict comparison of binding constants K obtained from different studies. The K value of DXMa/HPγCD complex is about four times higher than that of DXMa/HPβCD complex. This result could be explained by the larger size of the HPγCD 8.3 Å, which better accommodates with the A–B ring, while the size of HPβCD cavity is smaller, 6.5 Å, to allow a strong interaction with this cyclodextrin [29]. So far, K value of DXMa/HPγCD complex has not been reported in the literature, however, a comparison could be done at least with the binding constant of the complex DXM/HPγCD equal to 5190 M^{-1}, determined by Jansook *et al*.

Table 3. Apparent stability constant *K* and the complexation efficiency (CE) of DXMa/cyclodextrin complexes at 25 °C.

CD Type	Slope	Correlation Coefficient	$K_{1:1}$ (M^{-1})	CE
HPβCD	0.066	0.995	1462	0.071
HPγCD	0.206	0.999	5368	0.259

Regarding the pharmaceutical applications of cyclodextrins, it is important to choose the derivative exhibiting the higher solubilizing efficiency. The complexation efficiency was calculated as 0.071 and 0.259 for DXMa/HPβCD and DXMa/HPγCD complexes, respectively. CE of 0.071, approximately 0.1, suggests that 1 out of 11 HPβCD molecules forms a complex with DXMa, and CE of 0.259, approximately 0.3, suggests that 3 out of 4 HPγCD molecules are involved in forming a complex with DXMa [30]. From a strict point of view of the drug formulation, it would therefore be advantageous to choose HPγCD as host agent instead of HPβCD.

3.2.Chromatographic Determination of the Association Constants Between Dexamethasone Acetate and HPβCD or HPγCD

Using the solute retention time and the void time, the *K* values were determined for all the cyclodextrin concentrations at temperatures of 25, 30, 35 and 40 °C. The coefficients of variation of the *k* values were <0.5%, indicating a high reproducibility and a good stability for the chromatic system. The 1/*k vs.* [HPβCD] or [HPγCD] plots were determined and the values of the linear regression coefficients R^2 were calculated. The R^2 values were higher than 0.934 in all cases. For example, Figure 4 shows the two plots corresponding to the two cyclodextrin derivatives at 40°C. The results of the association constants *K* are presented in Table 4 together with those found in the literature. From these results, it appears clearly that the interaction of DXMa with the two cyclodextrin derivatives is well described by the 1:1 stoichiometry model as claimed by other authors [12,27]. As expected, our results showed that *K* values of DXMA/HPγCD complex are higher than those of DXMa/HPβCD complex. We find that the *K* value of DXMa/HPγCD obtained from phase-solubility diagram at 25 °C is about two fold higher than that calculated by chromatographic study. Concerning the complex DXMa/HPβCD, our *K* value obtained from phase-solubility studies is not very far from that reported by Usayapant *et al.* [12].

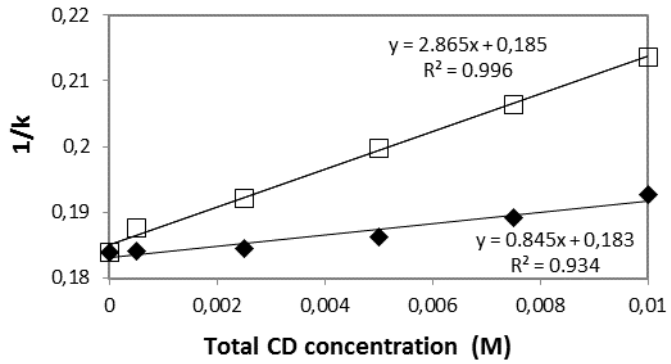


Figure 4. Plots of 1/*k vs.* [HPβCD] (◆) or [HPγCD] (□) (assuming 1:1 stoichiometry) for dexamethasone acetate at a column temperature equal to 40 °C. Stationary phase: phenyl silica gel; mobile phase: mixture methanol: water (70:30 *v*/*v*).

Table 4. Apparent association constants *K* of the complexes DXMa/HPβCD and DXMa/HPγCD determined by chromatographic procedure at various temperatures compared to literature data.

Method	Chromatographic Experiments				Phase Solubility Studies		UV Spectroscopy
Reference	Present study				Present study	[12]	[12]
Solution	methanol:water (70:30)				water	water 0.1 M citrate buffer (pH 6.0)	water 0.1 M citrate buffer (pH 6.0)
Temperature (°C)	25	30	35	40	25	25	25
HPβCD	1807	1421	1234	1020	1462	2240	2445
HPγCD	2541	2195	1883	1787	5368	-	-

Finally and regardless of the characterization methods implemented, it is clear that the affinity of DXMa for HPγCD is greater than that for HPβCD.

3.3.Thermodynamic Parameters for the DXMa/Cyclodextrin Complexes

In order to gain information about the mechanistic aspect of the difference in the solute affinity for HPβCD and HPγCD, the thermodynamic parameters were obtained from Van't Hoff plots. The ln*K vs.* 1/*T* plots were obtained for the two cyclodextrins. Figure 5 shows linear Van't Hoff plots with correlation coefficient higher than 0.988. Table 5 presents Δ*H*° and Δ*S*° for the two complexes with the corresponding Gibbs free energy Δ*G*° at 25 °C.

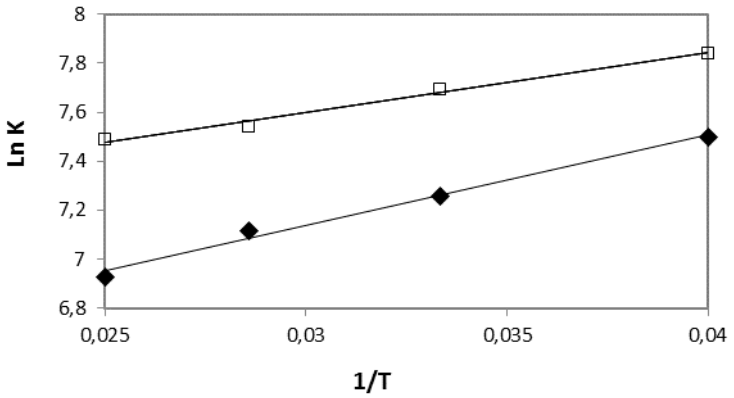


Figure 5. Van't Hoff plots (ln*K vs.* 1/*T*) for DXMa/HPβCD (◆) or DXMa/HPγCD (□) associations.

Table 5. Thermodynamic parameters Δ*H*°, Δ*S*° and Δ*G*° at 25 °C for DXMa/HPβCD and DXMa/HPγCD complexes.

DXMa/CD Complexes	Δ*H*°		Δ*S*°		Δ*G*° (kJ/mol)
	kJ/mol	Contribution to Δ*G*°	J/mol.K	Contribution to Δ*G*°	
DXMa/HPβCD	−20.3	54%	+57.1	46%	−3.3
DXMa/HPγCD	−30.7	67%	+50.1	33%	−15.7

For both DXMa/HPβCD and DXMa/HPγCD associations, Δ*H*° exhibits weak negative values while Δ*S*° ones are positive. These values demonstrate that the association phenomenon is both enthalpically and entropically driven (Table 5). At 25 °C, in the case of DXMa/HPβCD, the

contributions of enthalpic and entropic terms to the Gibbs free energy are almost identical, suggesting that the DXMa/HPβCD association is dependent on the hydrophobic effect between non polar groups of solute and the hydroxypropyl groups of the cyclodextrin derivative. Similar observations have been reported with NSAID association and HPβCD [21]. In the case of DXMa/HPγCD, the contribution of the enthalpic term to the Gibbs free energy is higher, close to 70 %.

All the results described above clearly indicated that complexation between HPβCD or HPγCD with DXMa significantly increases the water solubility of the guest molecules. Although the complexation efficiency of HPβCD was lower than that of HPγCD, we decided to continue the eye drop formulation studies keeping the two pairs of complexes DXMa/HPβCD and DXMa/HPγCD. It should be noted that the European Pharmacopoeia and USP/NF have published monographs for HPβCD. The natural γCD has a monograph in the Japanese Pharmaceutical Codex and the USP/NF. So far, HPγCD has not been registered as excipient by any of the major Pharmacopoiea [15]. Nevertheless, one can note that this derivative is already used in the composition of the marketed eye drop VOLTAREN OPHTHA®.

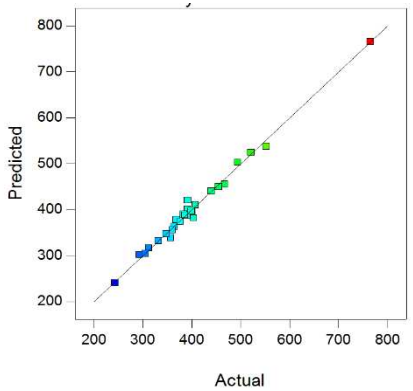
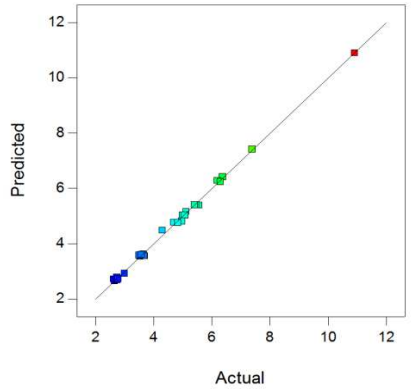
3.4.Special Cubic Mixture Designs

Using the DXMa solubility and osmolality of each experiment (Table S1 and S2), the experimental designs for both cyclodextrin derivatives were analyzed using Design-expert software and the results are reported in Table 6.

Based on these results , the R^2, R^2_{adj}, and R^2_{pred} values for the osmolality and [DXMa] for HPβCD are higher than 0.98, indicating that the refined model has high regression accuracy. For HPγCD, the R^2, R^2_{adj}, and R^2_{pred} statistics values are higher than 0.85, indicating that the refined model has good regression accuracy. For all considered responses, the *AdeqPrec* values are up to 31.45, which indicates an adequate signal to noise. The analysis of variance (ANOVA) results are described in supplementary materials (Table S3) and evidenced that the reduced models were highly significant (p-value < 0.05) [31]. The scatter plots of Actual *vs*. Predicted responses are useful to detect misspecifications in the structural model. Here, this figure does not reveal any significant bias. We can observe that the points are lying around the line along the total length of the line, that the amount of variation around the line does not change along the length of the line, and that there are no outliers.

In addition to the experimental design points, a set of supplementary trials at a single combination of factors settings are added to ensure the accuracy of the reduced mixtures models. The desirability function proposed by Derringer and Suich [32] is used to realize the simultaneous optimization of both osmolality and DXM solubility responses. In this study, the goal of optimization was respectively focused on maximization of the DXM solubility and adjustment of osmolality within acceptable physiological range. A Nelder–Mead simplex-algorithm-based numerical optimization is used to identify the best subset of variable setting combination that maximizes the desirability function [33,34]. Finally, the selected levels of variables used as the model confirmation samples are reported in Table 7.

Table 6. Best models containing the best subset of the predictors after backward stepwise selection, overall quality of model fit and the corresponding predicted against actual plot, Gel_1: CELLUVISC®, Gel_2: GEL-LARMES® and Gel_3: VISMED®.

		Final Equation in Terms of Actual Components	Model Evaluation	Predicted *vs.* Actual Plot
HPβCD	**Osmolality**	Osmolality(mOsm/Kg) = +342.68 × Gel_1 +369.84 × Gel_2 +242.96 × Gel_3 +765.94 × [HPβCD] −504.45 × Gel_1 × [HPβCD] −452.83 × Gel_2 × [HPβCD] −757.14 × Gel_3 × [HPβCD]	R^2 = 0.9900 R^2_{adj} = 0.9872 R^2_{pred} = 0.9849 *Adeq Prec* = 98.87 BIC = 232.56 AICc = 228.17	
	[DXMa]	[DXMa] (mg/mL) = (+1.47×10^{-3}) × Gel_1 +0.50 × Gel_2 +1.16 × Gel_3 +10.90 × [HPβCD] −2.93 × Gel_1 × [HPβCD] −6.01 × Gel_2 × Gel_3 −4.29 × Gel_2 × [HPβCD] −5.49 × Gel_3 × [HPβCD] +17.45 × Gel_2 × Gel_3 × [HPβCD]	R^2 = 0.9980 R^2_{adj} = 0.9972 R^2_{pred} = 0.9932 *Adeq Prec* = 155.01 BIC = −37.76 AICc = −41.50	

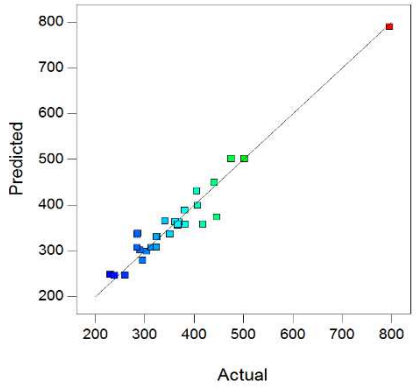
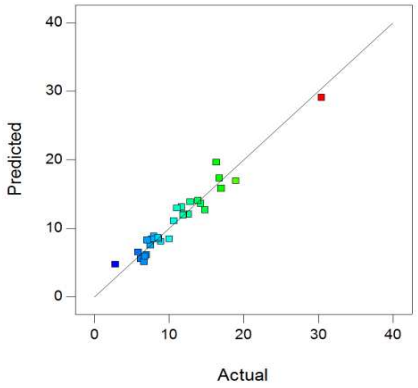
HPγCD	**Osmolality**	Osmolality (mOsm/Kg) = +768.79 × Gel_1 +57.80 × Gel_2 +329.10 × Gel_3 +789.73 × [HPγCD] −1367.82 × Gel_1 × Gel_2 −1986.36 × Gel_1 × [HPγCD] −140.20 × Gel_2 × [HPγCD] −1053.47 × Gel_3 × [HPγCD] +5990.04 × Gel_1 × Gel_2 × [HPγCD]	R^2 = 0.9403 R^2_{adj} = 0.9165 R^2_{pred} = 0.8517 *AdeqPrec* = 31.45 BIC = 297.68 AICc = 293.94	
	[DXMa]	[DXMa] (mg/mL) = +4.36 × Gel_1 −5.70 × Gel_2 −4.60 × Gel_3 +29.10 × [HPγCD] −20.34 × Gel_1 × [HPγCD]	R^2 = 0.9450 R^2_{adj} = 0.9357 R^2_{pred} = 0.9055 *AdeqPrec* = 42.94 BIC = 108.39 AICc = 104.58	

Table 7. Variables setting combination used for models confirmation samples (n = 2).

CD Type	Gel_1 CELLUVISC® (%)	Gel_2 GEL-LARMES® (%)	Gel_3 VISMED® (%)	CD (%)	Actual Osmolality	Predicted Osmolality	Actual[DXMa] (mg/mL)	Predicted[DXMa] (mg/mL)
HPβCD	0.000	0.000	0.300	0.700	429	450.045	6.973	6.826
HPβCD	0.000	0.145	0.215	0.640	450	449.858	6.319	6.305
HPβCD	0.454	0.000	0.000	0.546	435	448.735	4.651	5.226
HPγCD	0.089	0.089	0.098	0.724	519	489.326	17.153	19.188
HPγCD	0.000	0.425	0.000	0.575	447	444.396	12.813	14.310
HPγCD	0.244	0.201	0.000	0.555	436	448.831	13.492	13.314

The average of response (n = 2) of the confirmation sample is compared to the 95 % prediction interval. For both cyclodextrin derivatives, the reduced cubic models for osmolality and [DXMa] are experimentally validated because the average observation of the supplementary experiments proposed in the Table 7 are within the confirmation node's prediction interval.

Table 8 reports the quantitative composition of two mixed gels containing either HPβCD or HPγCD, resulting from the experimental designs, achieving high DXMa content and acceptable osmolality. These formulations are further denoted optimized mixed gels A (HPβCD) and B (HPγCD).

Table 8. Composition of optimized mixed gels A and B.

	Components	Quantity (g)
Optimized mixed gel A	VISMED®-Gel_3	0.300
	HPβCD 600 mg/mL with DXMa	0.700
	Optimized mixed Gel A contains 7 mg/g of DXMa and an osmolality of 449 mOsm/kg	
Optimized mixed gel B	CELLUVISC®-Gel_1	0.151
	VISMED®-Gel_3	0.085
	HPγCD 600 mg/mL with DXMa	0.764
	Optimized mixed gel B contains 20 mg/g of DXMa and an osmolality of 425 mOsm/kg	

3.4.Rheological Characterization

The administration of an ophthalmic formulation should not influence the pseudoplastic nature of precorneal film, or the influence should be negligible. Figure 6 shows the apparent viscosity of the mixed gels A and B as a function of shear rate. They both showed pseudo plastic behavior. The apparent viscosity value was lower as the speed gradient increased. At 22 °C and 12.9 s^{-1} (the lowest shear rate allowing stable value to be obtained), the mean ± SD apparent viscosity (n = 3) of optimized formulation gels A and B was 98 ± 2 mPa.s and 78 ± 1 mPa.s, respectively.

This viscosity range and the non-Newtonian behavior is fruitful for ophthalmic use due to the fact that the ocular shear rate is highly variable, ranging from 0.03 s^{-1} during interblinking periods to 4250–28,500 s^{-1}during blinking. If the viscosity at a high shear rate is too high, this will result in irritation. On the other hand, if the viscosity is too low, it will give rise to increased drainage. The rheological property

of these formulations should be in favor of sustaining drainage of drugs from the conjunctival sac of the eye without blinking difficulty in undergoing shear thinning [6,35].

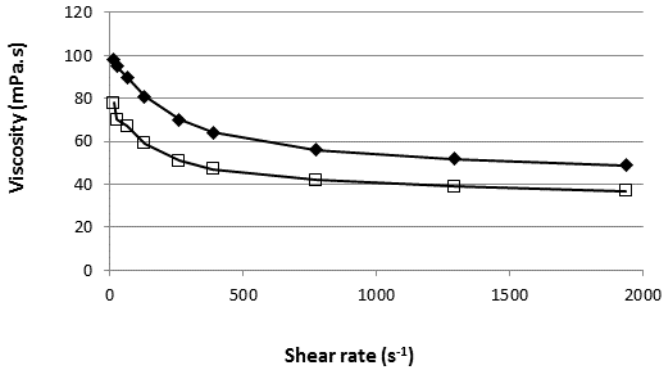


Figure 6. Rheological profiles of the two optimized mixed gels based on HPβCD (◆) or HPγCD (□).

3.5.In Vitro DXM Release Studies

In vitro release assessment was performed on several formulations, namely, both optimized mixed gels A and B, as well as two reference marketed eye drops, MAXIDEX® and DEXAFREE®. The drug solubilities were, respectively, 0.1 mg/mL, 0.015 mg/mL, and higher than 10 mg/mL for DXM, DXMa, and DXMp in PBS at 35 °C. On this basis, and in order to ensure sink condition dissolution testing, all the experiments were performed in sufficient volume media related to either DXM, DXMa, or DXMp to be dissolved. Therefore, the highest concentration corresponding to 100% drug release could not exceed 0.05 mg/mL, 0.01 mg/mL, and 5 mg/mL for DXM, DXMa, and DXMp, respectively, so that the dissolution media solution would not reach saturation. As shown in Figure 7, a complete drug release was observed at 24 h (1440 min) for DEXAFREE® with the major part of drug released within 30 min (92 %). As well, MAXIDEX® exhibited similar DXM release profile with 90 % of the drug recovered in the release medium at 30 min and 10 % remaining released over 24 h. Optimized mixed gel A exhibited DXMa release comparable to the reference eye drops, the mixed gel A being superimposable to the MAXIDEX® one. The release experiments observed for mixed gel A showed that upon high dilution conditions, the DXMa molecules could freely diffuse from the delivery system, which is a prerequisite for local biological activity. When looking at the mixed gel B behavior, 56 % of the drug diffused in the external medium after 2 h. The missing DXMa fraction was recovered in the cell extraction, meaning that a part of the mixed gel B remained stuck to the cell surface during the experiment, limiting further DXMa release. Indeed, the amount of DXMa very slightly increased with time, achieving 60 % and 62 % after 24 h and 48 h, respectively (unshown result). This retention phenomenon was only observed in the case of HPγCD containing mixed gel. This characteristic will have to be addressed in future *in vivo* bioremanence studies.

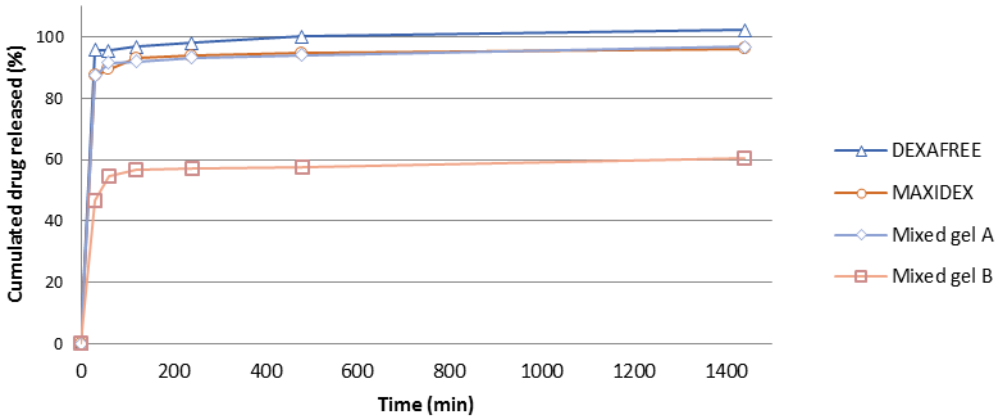


Figure 7.*In vitro* drug release from MAXIDEX®, DEXAFREE® and optimized mixed Gels A and B in PBS, at 35 °C.

4. Conclusions and Future Prospects

The formulation of dexamethasone acetate, a highly lipophilic corticosteroid prodrug, was investigated for topical ocular delivery. High drug contents in aqueous solution were achieved by using HPβCD or HPγCD at a concentration of 600 mg/mL, allowing the increase by a factor of around 500 and 1500, respectively, of the DXMa amount in water at 25 °C. The mixtures of these HPβCD or HPγCD/DXMa solutions with marketed gels were further investigated by means of two mixture experimental designs. New mixed gels loaded with 0.7% and 2% DXMa were developed made of sodium hyaluronate and/or carbopol with HPβCD or HPγCD, respectively. Both mixed gels released the drug *in vitro* after dilution in PBS at 35 °C, more or less completely depending on the composition of the vehicle. Next steps of the study will focus on mucoadhesion properties of DXMa mixed gel formulations as well as cytotoxicity studies. The statistical approach by experimental designs and the good prediction power of the models will be helpful to further adjust the compositions of the mixed gels as a function of future *in vitro* and *in vivo* results.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Optimization of mixed gel based on HPβCD, Table S2: Optimization of mixed gel based on HPγCD, Table S3: Analysis of variance for Reduced Special Cubic Mixture models (Partial sum of squares Type III).

Author Contributions: R.M., L.C., D.L., D.W. and A.G. carried out all the experiments, designed all the studies, oversaw all the experiments and contributed to draft the manuscript and intellectually to the development of the project.

Fundings: This research received no external private funding.

Acknowledgments: This work was supported by Labex ARCANE (ANR-11-LABX-0003-01) and Institut de Chimie Moléculaire de Grenoble (FR 2607) and the Glyco@Alps program (ANR-15-IDEX-02).

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary Materials: Investigation of Combined Cyclodextrin and Hydrogel Formulation for Ocular Delivery of Dexamethasone Acetate by Means of Experimental Designs

Roseline Mazet, Luc Choisnard, Delphine Levilly, Denis Wouessidjewe and Annabelle Gèze

Table S1. Optimization of mixed gel based on HPβCD.

	Gel$_1$: CELLUVISC®		Gel$_2$: GEL-LARMES®		Gel$_3$: VISMED®		HPβCD 600mg/mL with DXMa 10 mg/mL		Adjunction of DXMa (mg)	Osmolality (mOsm/Kg)	DXMa solubility (mg/mL)
Experiments	Theoretical proportion (%)	Real proportion (%)	Theoretical proportion (%)	Real proportion (%)	Theoretical proportion (%)	Real proportion (%)	Theoretical proportion (%)	Real proportion (%)			
1	70.0	69.9	0.0	0.0	0.0	0.0	30.0	30.1	10.676	364	2.657
2	0.0	0.0	70.0	69.8	0.0	0.0	30.0	30.2	10.811	399	2.625
3	0.0	0.0	0.0	0.0	70.0	69.4	30.0	30.6	10.228	243	2.992
4	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	10.154	765	10.912
5	35.0	35.0	35.0	35.3	0.0	0.0	30.0	29.8	11.148	368	2.743
14-1	23.3	23.5	23.3	22.8	23.3	23.7	30.0	30.0	11.598	324	2.861
6	35.0	35.2	0.0	0.0	35.0	34.8	30.0	30.0	10.274	293	2.735
7	35.0	35.0	0.0	0.0	0.0	0.0	65.0	65.0	10.122	494	6.375
8	0.0	0.0	35.0	35.9	35.0	34.7	30.0	29.5	10.078	312	2.725
9	0.0	0.0	35.0	35.1	0.0	0.0	65.0	64.9	10.014	521	6.195
10	132.9	0.0	0.0	0.0	35.0	35.1	65.0	64.9	10.356	407	6.292
14-2	23.3	23.5	23.3	23.7	23.3	23.3	30.0	29.4	10.018	321	2.677
11	0.0	0.0	23.3	22.7	23.3	24.2	53.3	53.1	10.964	391	5.118
12	23.3	24.2	0.0	0.0	23.3	23.1	53.3	52.7	10.172	387	4.988
13	23.3	23.8	23.3	23.4	0.0	0.0	53.3	52.8	10.104	467	5.067
14	23.3	23.5	23.3	23.4	23.3	23.6	30.0	29.5	10.332	332	2.766
15	43.8	43.3	8.8	9.8	8.8	8.3	38.8	38.5	10.007	376	3.531
14-3	23.3	23.0	23.3	22.7	23.3	22.3	30.0	31.9	10.008	337	2.939
16	8.8	9.0	43.8	43.9	8.8	8.4	38.8	38.6	10.832	383	3.678
17	8.8	9.3	8.8	9.6	43.8	42.8	38.8	38.2	10.195	305	3.640
18	8,8	8,9	8,8	8,8	8,8	9,0	73,8	73,3	10.075	552	7.387
19	8,8	8,4	20,4	20,0	20,4	20,2	50,4	51,4	10.255	400	4.963
20	20,4	47,1	8,8	5,7	20,4	13,7	50,4	33,5	10.746	390	4.685
14-4	23,3	24,3	23,3	22,8	23,3	22,7	30,0	30,1	10.244	330	2.760

21	20,4	19,9	20,4	8,7	8,8	21,2	50,4	50,2	10.517	392	4.828
22	20,4	20,9	20,4	21,5	20,4	20,4	38,8	37,2	10.732	360	3.496
23	26,3	25,5	26,3	26,7	8,8	9,1	38,8	38,6	10.041	404	3.667
24	26,3	25,9	8,8	9,1	26,3	26,2	38,8	38,9	10.152	357	3.500
25	26,3	25,6	8,8	8,2	8,8	8,5	56,3	57,7	10.391	440	5.553
14-5	23,3	22,4	23,3	23,5	23,3	23,7	30,0	30,5	10.179	327	2.746
26	8,8	8,6	26,3	27,2	26,3	25,9	38,8	38,3	10.043	348	3.569
27	8,8	8,5	26,3	25,9	8,8	9,0	56,3	56,5	10.610	455	5.458
28	8,8	8,7	8,8	8,9	26,3	26,0	56,3	56,4	10.163	399	5.398
29	17,5	17,9	17,5	18,0	17,5	17,4	47,5	46,7	10.196	386	4.295
14-6	23,3	23,4	23,3	24,0	23,3	22,3	30,0	30,3	10.176	329	2.791
14-7	23,3	24,0	23,3	22,6	23,3	23,1	30,0	30,3	10.029	321	2.717

Table S2. Optimization of mixed gel based on HPγCD.

Experiments	Gel 1 : CELLUVISC®		Gel 2 : GEL-LARMES®		Gel 3 : VISMED®		HPγCD 600mg/mL with DXMa 30 mg/mL		Adjunction of DXMa (mg)	Osmolality (mOsm/kg)	DXMa solubility (mg/mL)
	Theoretical proportion (%)	Real proportion (%)	Theoretical proportion (%)	Real proportion (%)	Theoretical proportion (%)	Real proportion (%)	Theoretical proportion (%)	Real proportion (%)			
1	70.0	70.2	0.0	0.0	0.0	0.0	30.0	29.8	5.454	382	7.510
2	0.0	0.0	70.0	70.3	0.0	0.0	30.0	29.7	5.113	230	2.804
3	0.0	0.0	0.0	0.0	70.0	69.8	30.0	30.2	5.032	239	6.223
4	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	5.001	796	30.448
5	35.0	34.8	35.0	35.4	0.0	0.0	30.0	29.9	5.625	367	6.972
6	35.0	35.9	0.0	0.0	35.0	34.6	30.0	29.5	5.295	290	5.866
7	35.0	35.1	0.0	0.0	0.0	0.0	65.0	64.9	5.355	326	16.978
8	0.0	0.0	35.0	35.2	35.0	34.6	30.0	30.2	5.318	260	6.640
9	0.0	0.0	35.0	36.1	0.0	0.0	65.0	63.9	2.101	502	18.965
10	0.0	0.0	0.0	0.0	35.0	37.0	65.0	63.0	5.350	381	16.727
11	0.0	0.0	23.3	23.7	23.3	23.4	53.3	52.9	5.063	370	11.688
12	23.3	24.3	0.0	0.0	23.3	23.5	53.3	52.2	5.212	304	11.079
13	23.3	23.2	23.3	23.4	0.0	0.0	53.3	53.4	5.161	441	14.819
14	23.3	23.7	23.3	23.9	23.3	22.7	30.0	29.7	5.049	313	6.771
15	43.8	43.6	8.8	9.3	8.8	9.0	38.8	38.1	5.326	284	7.963
16	8.8	8.7	43.8	43.8	8.8	9.0	38.8	38.5	5.611	351	8.886
17	8.8	9.7	8.8	8.7	43.8	44.0	38.8	37.6	5.456	296	10.039
18	8.8	8.6	8.8	10.0	8.8	9.5	73.8	71.9	5.064	475	16.334
19	8.8	8.5	20.4	20.7	20.4	19.9	50.4	50.8	5.209	362	12.604
20	20.4	20.3	8.8	9.5	20.4	20.0	50.4	50.1	5.124	286	11.972
21	20.4	21.6	20.4	19.4	8.8	9.4	50.4	49.6	5.144	407	11.885
22	20.4	21.0	20.4	20.1	20.4	19.8	38.8	39.2	5.268	324	8.571
23	26.3	26.2	26.3	26.0	8.8	9.6	38.8	38.2	5.008	341	7.706
24	26.3	26.0	8.8	9.4	26.3	26.0	38.8	38.6	5.429	284	8.550
25	26.3	26.6	8.8	9.3	8.8	8.5	56.3	55.7	5.269	446	14.230
26	8.8	9.1	26.3	26.9	26.3	26.2	38.8	37.8	5.228	324	7.088
27	8.8	8.9	26.3	26.2	8.8	9.7	56.3	55.2	2.281	405	12.869
28	8.8	9.2	8.8	9.2	26250.0	27.0	56.3	54.5	5.188	367	13.874
29	17.5	18.3	17.5	17.6	17.5	17.5	47.5	46.6	5.147	418	10.633

For both cyclodextrin derivatives, the analysis of variance (ANOVA) was carried out to determine the statistical significance of the fitted special cubic model and the coefficient terms. Basically, the values of "p-value Prob> F" less than 0.05 indicated that the selected model term were statistically significant and the values larger than 0.05 reflected that the model terms were not significant towards the output of responses [31]. All reduced models were highly significant with p-value < 0.0001 and then can be used to predict responses within the given range of factors (Table 7).

Table S3. Analysis of variance for Reduced Special Cubic Mixture models (Partial sum of squares—Type III).

		Source	Sum of square	Df	Mean square	F value	p-value Prob> F	Results
HPβCD	**Osmolality**	Model	$2.536.10^5$	6	42262.67	361.69	< 0.0001	**significant**
		Linear Mixture	$2.379.10^5$	3	79289.97	678.57	< 0.0001	
		X_1X_4	3896.27	1	3896.27	33.34	< 0.0001	
		X_2X_4	3139.69	1	3139.69	26.87	< 0.0001	
		X_3X_4	8777.33	1	8777.33	75.12	< 0.0001	
		Residual	2570.67	22	116.85			
		Cor Total	$2.561.10^5$	28				
	DXMa]	Model	90.45	8	11.31	1239.87	< 0.0001	**significant**
		Linear Mixture	89.78	3	29.93	3281.65	< 0.0001	
		X_1X_4	0.13	1	0.13	14.41	0.0011	
		X_2X_3	$7.392.10^{-3}$	1	7.392E-003	0.81	0.3787	
		X_2X_4	0.22	1	0.22	24.58	< 0.0001	
		X_3X_4	0.37	1	0.37	40.28	< 0.0001	
		$X_2X_3X_4$	0.043	1	0.043	4.74	0.0416	
		Residual	0.18	20	9.119E-003			
		Cor Total	90.63	28				

HPγCD	**Osmolality**	Model	$3.034.10^5$	8	37927.80	39.40	< 0.0001	**significant**
		Linear Mixture	$2.307.10^5$	3	76889.67	79.87	< 0.0001	
		X_1X_2	2243.58	1	2243.58	2.33	0.1425	
		X_1X_4	48040.28	1	48040.28	49.90	< 0.0001	
		X_2X_4	239.33	1	239.33	0.25	0.6235	
		X_3X_4	16942.75	1	16942.75	17.60	0.0004	
		$X_1X_2X_4$	5092.80	1	5092.80	5.29	0.0323	
		Residual	19252.87	20	962.64			
		Cor Total	$3.227.10^5$	28				
	[DXMa]	Model	767.80	4	191.95	102.81	< 0.0001	**significant**
		Linear Mixture	761.46	3	253.82	135.94	< 0.0001	
		X_1X_4	6.34	1	6.34	3.39	0.0778	
		Residual	44.81	24	1.87			
		Cor Total	812.61	28				

Publication des résultats N°2

Titre : Biopharmaceutical Assessment of Dexamethasone acetate-based Hydrogels Combining Hydroxypropyl Cyclodextrins and Polysaccharides for Ocular Delivery

Résumé : Dans la précédente étude, nous avons développé deux formulations contenant 0,7 % et 2 % de DXMa, constituées respectivement d'hyaluronate de sodium et/ou de carboxymethylocellulose et d'HPβCD ou d'HPγCD afin d'augmenter le temps de résidence précornéen et la biodisponibilité de la DXMa après administration topique ophtalmique.

L'objectif de cette deuxième étude est de réaliser l'évaluation biopharmaceutique de ces deux formulations par rapport aux formes commerciales MAXIDEX® et DEXAFREE®. Dans un premier temps *in vitro*, nous avons mesuré la mucoadhésion des deux formulations et leur cytotoxicité sur cellules épithéliales de la cornée humaine. Nous avons également évalué leur stabilité physico-chimique et microbiologique sur 12 mois à 25 °C. Ensuite, nous avons évalué le passage transcornéen sur cornée de porc isolée. Enfin, nous avons étudié *in vivo* la biodistribution oculaire des deux formulations, à l'aide de l'imagerie moléculaire par tomographie à émission de positons (TEP). Cela nous a permis d'établir le profil pharmacocinétique des deux formulations, marquées au ^{18}F-Fluorodésoxyglucose (FDG).

Les données fournies dans cette étude démontrent que les gels A et B sont stables 12 mois à 25 °C, que le gel B semble biocompatible et mucoadhésif. Il présente, en outre, une bonne perméabilité transcornéenne sur la cornée de porc isolée, 3,22 fois plus élevée que le DEXAFREE® et 4,04 fois plus élevée que le MAXIDEX®. L'évaluation *in vivo* de la biopermanence sur la surface cornéenne montre une augmentation du temps de demi-vie par rapport aux larmes artificielles ce qui pourrait permettre d'augmenter la biodisponibilité *in vivo* de l'acétate de dexaméthasone. Ces bons résultats doivent être confirmés, *in vivo* chez le rat, par des études pharmacocinétiques, d'efficacité et de tolérance.

Article scientifique en préparation pour soumission

Article

Biopharmaceutical Assessment of Dexamethasone acetate-based Hydrogels Combining Hydroxypropyl Cyclodextrins and Polysaccharides for Ocular Delivery

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Abstract: We developed previously two optimized formulations of dexamethasone acetate (DXMa) hydrogels by means of special cubic mixture designs for topical ocular administration. These gels were elaborated with hydroxypropyl-β-CD (HPβCD) and hydroxypropyl-γ-CD (HPγCD) and commercial hydrogels in order to enhance DXMa water solubility and finally DXMa's ocular bioavailability and transcorneal penetration. The main objective of this study was to characterize them and to evaluate *in vitro, ex vivo* and *in vivo* their safety, biopermanence and transcorneal permeation. Gels A and B are Newtonian fluids and display a viscosity of 13.2 mPa.s and 18.6 mPa.s, respectively, which increase their ocular retention, according to the *in vivo* biopermanence study by PET/CT These hydrogels could act as corneal absorption promoters, as they allow a higher transcorneal permeation of DXMa through porcine excised cornea, compared to DEXAFREE® and MAXIDEX®. Cytotoxicity assays showed no cytotoxic effects on HCE cells. Furthermore, Gel B is clearly safe for the eye but the effect of Gel A on human eye cannot be predicted. Both gels were also stable 12 months at 25 °C after sterilization by filtration on 0.22 μm PVDF filters. These results demonstrate that the developed formulations present a high potential for the topical ocular administration of dexamethasone acetate.

Keywords: dexamethasone acetate; cyclodextrins; eye drops; hydrogels; rheology, cytotoxicity studies, transcorneal permeation, radiolabeled ocular biopermanence

1. Introduction

Dexamethasone (DXM) is one of the most prescribed anti-inflammatory drug in the treatment of acute and chronic eye inflammation due to its high potency and effectiveness [1]. DXM acts by binding with the corticosteroid receptors present in the human trabecular meshwork cells and inhibits phospholipase-A2 and so prostaglandins synthesis. DXM eye drops, as MAXIDEX® 1mg.mL^{-1} DXM (Novartis Pharma, Rueil-Malmaison, France) and DEXAFREE® 1mg.mL^{-1} DXM phosphate (Laboratoires Théa, Clermont-Ferrand, France) are effective to treat postoperative inflammation, keratitis, uveitis [2] and prevention of corneal graft rejection [3]. Despite the many advantages offered by this route of administration, these marketed formulations present a major disadvantage by requiring frequent administrations (up to 6 times/day) [2]. This is due to the presence of various anatomical and physiological barriers, which lead to a poor bioavailability of the ophthalmic drugs; only 1 – 5 % of drug instilled reaches in aqueous humor [4].

In order to enhance DXM bioavailability, the lipophilic derivative DXM acetate (DXMa), currently unavailable for ophthalmic topical use, could be very interesting. Indeed, DXMa has shown to readily permeate the cornea and be hydrolyzed into DXM during absorption [5]. As well, Leibowitz *et al*. demonstrated that the acetate form was more effective compared to phosphate derivative in suppressing inflammation in the cornea. This therapeutic effect was not associated with a greater propensity to increase intraocular pressure, one of the most frequent side effects of glucocorticoids [6].

Furthermore, for the topical administration of DXMa into the eyes, we developed previously, by means of experimental designs, two optimized formulations based on HPβCD or HPγCD/DXMa solutions and marketed gels, with the aim of increasing DXMa bioavailability and reducing instillation frequency. HPβCD or HPγCD have considerably enhanced DXMa solubility in water, 500 and 1550-fold [7]. CELLUVISC® (sodium carboxymethylcellulose) and VISMED® (sodium hyaluronate) have both been used as artificial tear in order to stabilize the tear film on the ocular surface [8]. Carboxymethylcellulose (CMC) and sodium hyaluronic (NaHA) present the great advantages to be mucoadhesive, biodegradable and biocompatible [9]. These properties exhibit an enhance of the precorneal residence time and a reduce in the nasolacrymal drainage due to increased viscosity [10]. In addition, NaHA has been shown to modulate the inflammation response of the ocular surface in dry eye syndrome [11].

In the present study, the optimized formulations were characterized. The ocular *in vitro* cytotoxicity and mucoadhesion properties were evaluated as well as *ex vivo* transcorneal permeation of DXMa. As well, *in vivo* precorneal drug kinetics were investigated by radiolabelling with ^{18}F-FDG in order to evidence the benefits of the newly designed formulations.

2. Materials and Methods

2.1. Materials

DXMa was purchased from LA COOPER (Melun, France). Hydroxypropyl-γ-cyclodextrin (HPγCD, W8HP, DS = 0.6 and Mw = 1576 Da) was a kind gift from ASHLAND (Schaffhausen, Switzerland) and Hydroxypropyl-β-cyclodextrin (HPβCD, KLEPTOSE DS = 0.63 and Mw = 1391 Da) was obtained from ROQUETTE (Lestrem, France). CELLUVISC® (sodium carboxymethylcellulose) and VISMED® (sodium hyaluronate) are marketed gels used for the treatment of dry eye syndrome. DEXAFREE® (DXM sodium phosphate 1 % solution eye drops), MAXIDEX® (DXM 0.1 % suspension eye drops) and BSS® (Alcon Laboratories, Rueil-Malmaison, France) are human authorized ocular medicines. Normal Human Primary Corneal Epithelial Cells (ATCC PCS 700-010), medium (ATCC PCS-700-030), growth kit (ATCC PCS-700-040), PBS (ATCC 30-2200), trypsin EDTA (ATCC PCS-999-003 and 005) and antibiotics (gentamicin, streptomycin and amphotericin BATC PCS-999-002) were obtained from ATCC®. Thioglycollate with résazurine medium and Tryptic soy broth were obtained from BIOMERIEUX (Craponne, France). ALAMAR BLUE® was purchased from BIO-RAD (Marnes-la-Coquette, France) and DMSO from SIGMA-ALDRICH (Lyon, France). Purified water was prepared by DIRECT-Q®3 UV water purifier (MILLIPORE, Molsheim, France). All other solvents and chemicals were of HPLC and analytical grade, respectively.

2.2. Methods

2.2.1. Gels composition

The composition of optimized mixed Gels A and B were obtained by means of experimental design as previously described [7] (Table 1). Briefly, the mixed gels are obtained as follows: 600mg/mL HPβ- or γCD solutions are prepared at room temperature. Then DXMa was added to the solutions before introducing CELLUVISC® and/or VISMED®.

Table 1. Composition of optimized mixed Gels A and B.

	Components	Quantity (g)
Optimized mixed Gel A	VISMED®	0.300
	HPβCD 600 mg/mL with DXMa	0.700
	Optimized mixed Gel A contains 7 mg/g of DXMa and an osmolality of 449 mOsm/kg	
Optimized mixed Gel B	CELLUVISC®	0.151
	VISMED®	0.085
	HPγCD 600 mg/mL with DXMa	0.764
	Optimized mixed Gel B contains 20 mg/g of DXMa and an osmolality of 425 mOsm/kg	

2.2.2. Sterilization step

Two different methods were investigated with Gels A and B, i.e. : autoclaving (SANO CLAV from ADOLF WOLF, Überkingen, Germany) at 121 °C during 20 min or double sterilizing filtration (CME or PVDF 0.22 µm filter, ROTH, Karlsruhe, Germany) and conditioned in sterile vials under laminar air flow of an ISO 4.8 microbiological safety cabinet.

2.2.3. Physicochemical characterizations

2.2.3.1. *Drug quantification*

The drug quantification's methodology was adapted from that previously reported [7,12] and validated in DXMa concentrations according to ICH Q2 (R1) guidelines in order to evaluate specificity, linearity, repeatability, intermediate fidelity and limit of detection (LOD) and limit of quantification (LOQ) [13]. Quantitative determination were performed on a reversed-phase, high-performance liquid chromatographic (HPLC) component system LC 2010 AHT (SHIMADZU, Kyoto, Japan) consisting of a pump with degasser, an autosampler, a UV-VIS detector, and a column XTERRA®MS C8, 5 µm particles, 150 × 4.6 mm with C8 cartridge. The mobile phase made of methanol:water (70:30 v/v) was set at the rate of 0.8 mL/min. The column was thermo-regulated at 25 °C. The detection wavelength was set up at 240 nm.

Method Validation

The method was validated according to International Conference on Harmonization (ICH) guideline Q2(R1) "Validation of Analytical Procedures" [13].

Linearity and accuracy studies

Five standard samples at different concentration values were prepared using 0.1 mg/mL DXMa as a solution stock. Table 2 contains the different sample concentration levels for Gels A and B.

Table 2. Sample concentration levels for Gels A and B

	Level 80%	**Level 90%**	**Level 100%**	**Level 110%**	**Level 120%**
Gel A (µg/mL)	56	63	70	77	84
Gel B (µg/mL)	160	180	200	220	240

These calibration levels were analyzed twice a day during three days [14]. The peak area was plotted against the concentration at each level and a calibration curve was generated by a linear least square regression analysis.

Specificity

The specificity of the developed method was first established by verifying that all the components of gels are separated from the DXMa chromatographic peak. In complement, to exclude potential interference of degradation products with DXMa quantification, DXMa 1mg/mL solutions, Gel A and Gel B were subjected to forced degradation conditions, according to SFSTP

guidelines [15]: 0.5 N hydrochloric acid or sodium hydroxide, at 80 °C for 60 min, in 3 % hydrogen peroxide at 80 °C for 4 h and under visible and ultraviolet light for 6 h.

Precision

Intra-day (repeatability) and inter-day (intermediate) precision assays were determined by preparing a model solution at 100 % concentration level (70 µg/mL for Gel A and 200 µg/mL for Gel B). Each solution was analyzed 6 times a day for 3 days.

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from the standard deviation of the response as well as the slope, according to ICH guidelines. The estimated results were not empirically verified.

2.2.3.2. Rheological Measurements

Rheological characteristics of both gels were examined at high shear rates using a high sensitivity pressure cell ARES-G2 rheometer from TA Instruments (New Castle, USA) equipped with a coaxial cylinder geometry (SN402525.001, TA Instruments, New Castle, USA) with APS kit and Couette system from TA Instruments (New Castle, USA). The measuring cup diameter (33.985 mm)/measuring bob diameter (32 mm) corresponds to 1.0620 according to ISO 3219. The gap length is 2mm and the sample volume > 5.2 mL. The temperature is controlled at 35 °C by a Peltier plate.

The steady-state flow experiments were performed in the range of 0.11 to 100 s^{-1}. The frequency sweep method was performed between 0.1 Hz and 10 Hz, with a shear strain of 10 % for both formulations, while the table of shear rate method was performed by increasing the shear rate from 0.1 to 100 s^{-1}, at 35 °C. The shear stress was measured by this method and the apparent viscosity was calculated by dividing the shear stress by the shear rate.
An oscillatory amplitude sweep and frequency testing was performed using this equipment.

The amplitude sweep conditions used were shear strain between 0.1 % and 100 % with the frequency of 0.1 Hz. It was concluded that the linear-viscoelastic region (LVER) was at shear strain of 10 %. In the frequency testing, the frequency range used was between 0.1 – 10 Hz with a shear strain of 10 %.

2.2.4. Mucoadhesion

In this study, mucin was rehydrated with water by gentle stirring until complete dissolution to yield a dispersion of 10 % (w/w) at 20 – 25 °C. The mucoadhesion was evaluated by the effect of mucin on zeta potential (ZP) values of Gel A ± mucin (1:1), Gel B ± mucin (1:1), DXMa/HPβCD ± mucin (1:1), DXMa/HPγCD ± mucin (1:1). A volume of 40 µL of Gel A, Gel B, DXMa/HPβCD or DXMa/HPγCD were diluted in either 2 mL of sterile purified water [16–18]. The ZP values of the different mixtures were measured using a Zetasizer Nanoseries Nano ZS (Malvern Instruments, Malvern, UK) at 35 °C. All the experiments were done in triplicate.

2.2.5. Cytotoxicity studies

Two different cellular toxicity assays were used, based on cell viability in relation to mitochondrial enzymes [19] i.e. the methylthiazolyldiphenyl-tetrazolium bromide conversion (MTT) and ALAMAR BLUE® assays. The experiments were performed using Normal Human Primary Corneal Epithelial Cells (HCEC) obtained from ATCC® and maintained in an incubator (37 °C and 5 % CO_2 saturation). HCEC were kept in corneal epithelial cell growth culture medium with gentamicin and amphotericin B, without fetal bovine serum. All the experiments were performed in between steps 4 and 8. Three thousand cells per well (96 wells per plates) were incubated for 24

hours at 37 °C and 5 % CO_2 in order to have between 80 to 90% of cell confluence, according to ATCC® protocol.

Subsequently, during the MTT assay, the original culture medium was aspirated and different concentrations (25 µL/200 µL, and 0.25 µL/200 µL) of different formulations: Gels A and B with or without DXMa, HPβCD (600 mg/mL) and HPγCD (600 mg/mL) aqueous solutions, DEXAFREE® eye drops solution and MAXIDEX® eye drops suspension were added to different wells and incubated during 30 min and 2 h. Each concentration was tested in 3 individual wells. After 30 min, 2 h and 24 h, the supernatant was removed and 200 µL of MTT solution (5 mg/mL in PBS and then diluted to 1/10 in complete medium) was added to each well and then incubated for 3 h at 37 °C to allow the formation of formazan crystals. The medium was then removed, and blue formazan was eluted from cells by 200 µL of DMSO. The plates were shaken in order to solubilize the crystals of formazan. The liquid was aspirated to another new 96-wells plate and measured directly at 590 nm with Clariostar (BMG Labtech, Champigny sur Marne, France). Each plate was duplicated.

Additionally, the ALAMAR BLUE® was performed after 2 h of incubation at 37 °C, 5 % CO_2, with the IC50 concentrations as determined by the MTT assay. 20 µL of ALAMAR BLUE® reagent were added in each well before 2 h of incubation at 37 °C, 5 % CO_2. Fluorescence was measured with excitation wavelength at 530-560 nm and emission wavelength at 590 nm with Clariostar (BMG Labtech, Champigny sur Marne, France). Each plate was duplicated.

The % of reduction of ALAMAR BLUE® was calculated by the following Equation 1:

$$\%\,\text{Reduction} = \frac{(\text{Experimental RFU value}) - (\text{Negative control RFU value})}{(100\%\ \text{reduced positive control RFU value}) - (\text{Negative control RFU value})} \times 100, \quad (1)$$

2.2.6. *Ex vivo* evaluation of the corneal permeation

The transcorneal permeation experiment was performed for Gels A and B, DEXAFREE® and MAXIDEX®, using Franz diffusion cells with an available diffusion area of 1.131 cm^2. The porcine corneas were recovered from the slaughterhouse in accordance to ethical regulations. The corneas were removed and then mounted onto diffusion cells, with the epithelial layer exposed to the donor chamber. The latter was filled with 0.4 g of each ophthalmic formulation; whereas the receptor chamber was filled with 13 mL artificial tear fluid BSS. According to Wen *et al.* [20], the experiment was performed at 35 ± 1 °C in a thermostatic water bath with a moderate speed of rotation maintained for 24 h. Three corneas per formulation (n=3) were used. A 1 mL sample was removed at predetermined time intervals (15 min, 30 min, 1 h, 2 h and 4 h) and replaced with an equal volume of fresh medium to maintain the sink conditions. The withdrawn samples from receptor chamber were analyzed by HPLC. The cumulative amount of drug appearing in the receptor compartment (*Qn*) was plotted as a function of time (t_n) and calculated using the following Equation 2:

$$Qn = V_0 \left(C_n + \frac{V}{V_0} \sum_{i=1}^{n-1} C_i \right) = V_0 C_n + V \sum_{i=1}^{n-1} C_i, \quad (2)$$

C_n: Drug concentration at t time points (µg.mL^{-1}),
C_i: Drug concentration at sampling points,
V_0: Volume of the medium in the receiving chamber,
V: sampling volume

The corneal hydration level (% HL) was measured with a relative Humidity Analyzer MB45 OHAUS® (Parsippany, USA).

2.2.7. *In vivo* evaluation of the residence time on the ocular surface

In vivo studies were carried out on male Sprague-Dawley rats with an average weight of 250 g supplied by the animal facility at University of Santiago de Compostela (Spain). The animals were treated according to the guidelines for laboratory [21,22]. The experiments were approved by the Galician Network Committee for Ethics Research following the Spanish and European Union (EU) rules (86/609/CEE, 2003/65/CE, 2010/63/EU, RD 1201/2005 and RD53/2013). The animals were kept in individual cages at controlled conditions of temperature and humidity (22 °C and 60 %) with free access to water and food, with day-night cycles regulated by artificial light.

Each component of the optimized formulations, i.e. CELLUVISC®, VISMED®, DXMa (10 mg/g)/HPβCD(600 mg/mL) and DXMa (30 mg/g)/HPγCD (600 mg/mL) aqueous solutions, Gel A and Gel B were radiolabeled by incorporating 100 µL of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in a volume of 1 mL of either hydrogel or cyclodextrin based aqueous solution until homogenization. Randomly taken samples from each labeled component were measured using a high-precision dose calibrator (Atomlab 500, Biodex Medical System, Inc., New-York, NY, USA) in order to control radiotracer uniformity.

Positron emission tomography and computerized tomography (PET/CT) images were acquired using the Albira PET/CT. Preclinical Imaging System (Bruker Biospin, Woodbridge, Connecticut, USA). The anesthetized animals were positioned into the imaging bed and 7.5 µL of each formulation labeled with ^{18}F-FDG was instilled into the conjunctival sac eye using a micropipette. The administered radioactivity was 0.35 ± 0.08 MBq. Therefore, the ^{18}F-FDG labeled component (CELLUVISC®, VISMED®, DXMa (10 mg/g)/HPβCD (600 mg/mL) and DXMa (20 mg/g)/HPγCD(600 mg/mL) aqueous solutions as well as the ^{18}F-FDG labeled optimized gels (A or B) were tested. Static PET frames at different times were acquired during 5 hours following instillation. Three animals (6 eyes) were tested for each formulation.

The results were corrected to radioactive decay. Graphical representations of radioactivity versus time were obtained. The fitting of the remaining formulation versus time to a monoexponential decay equation using a single compartmental model was performed using pK Solver [23]. A non-compartmental analysis was also performed calculating the mean residence time (MRT) and the total area under the curve (AUC) of the remaining formulations (%) versus time. All data are expressed as mean value ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA test, and the level of significance was set at 5 %.

2.2.8. Stability studies

Both formulations were prepared using sterile water, HPβCD, HPγCD, DXMa, VISMED® and CELLUVISC® under laminar air flow of an ISO 4.8 microbiological safety cabinet. 2 mL of each gel were conditioned into 5 mL glass vial, previously autoclaved, closed with a polypropylene cap and sealed with an aluminum ring. Two batches of each gel were prepared and submitted to either a double filtration with a PBS 0.22 µM filter.

The stability of each gel was studied in unopened multidose eyedroppers for 12 months at 25 °C in a climate chamber (BINDER GmbH, Tuttlingen, Germany). 4 units per formulation were subjected to visual inspection, DXMa quantification, sterility, osmolality and pH measurements at times 0, 14 and 30 days, 2, 6, 9 and 12 months.

More precisely, for each unit, color and aspect were checked. DXMa was quantified by HPLC and degradation product sought using a stability indicating method [13]. Gels A and B were previously diluted by 1/100 with mobile phase. Osmolality was measured using a 2020 freezing point osmometer (Advanced Instruments, Norwood, United States). PH measurements were made with a SevenMulti® pH-meter with InLab electrode (Mettler-Toledo, Viroflay, France).The sterility test was carried out according to the European Pharmacopeia sterility assay (2.6.1) [24]. Briefly, the multidose eyedroppers were opened under the laminar air flow of an ISO 4.8 microbiological safety

cabinet and the content was divided into two equal parts, each transferred in a fluid Thioglycollate with résazurine medium and Tryptic soy broth and incubated respectively at 30 - 35 °C and 20 - 25 °C for 14 days. The culture medium was examinated every day.

3. Results and discussion

3.1. Drug quantification before and after sterilization

DXMa presents a retention time of 3.2 ± 0.2 minutes and their chromatograms are presented in Figure 1.

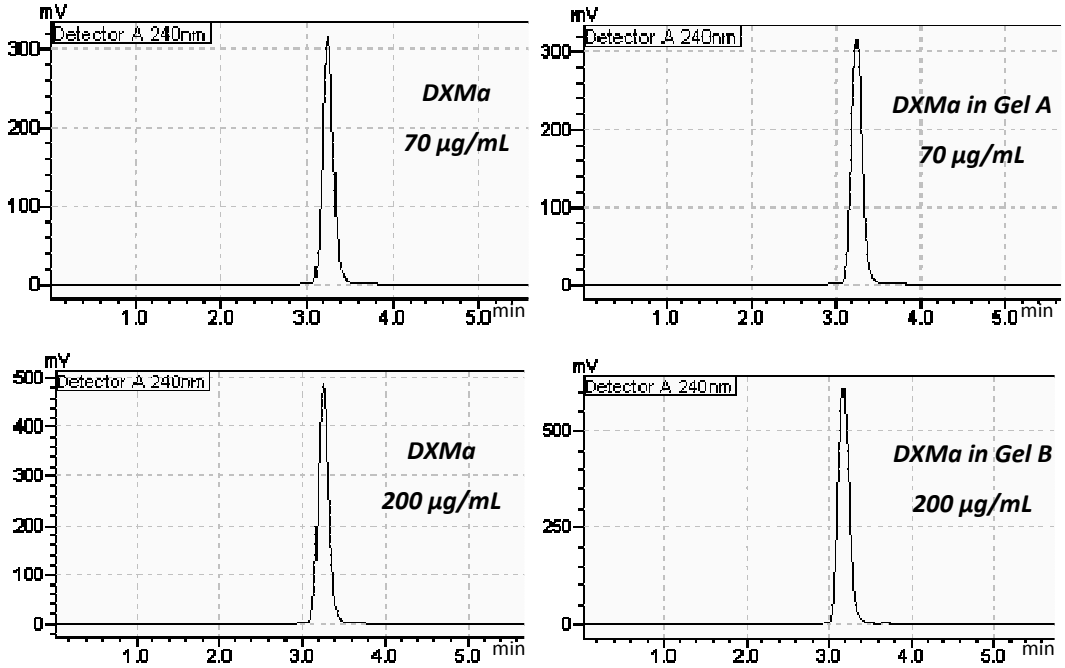


Figure 1. A, B, C and D show the chromatogram of DXMa for Gel A diluted to 70 µg/mL , DXMa in Gel A diluted to 70 µg/mL and DXMa for Gel B diluted to 200µg/mL and DXMa in Gel B diluted to 200 µg/mL obtained with the chromatographic methods used

Method validation studies

The RP-HPLC method used to analyze the DXMa in Gels A and B was validated according to current ICH Q2 (R1) [13].The performed validation tests proved the suitability of the method for its intended purposes. Validation tests including specificity, linearity and range parameter, accuracy, precision, LOQ, LOD. Original validation data are reported in supplementary material.

Linearity

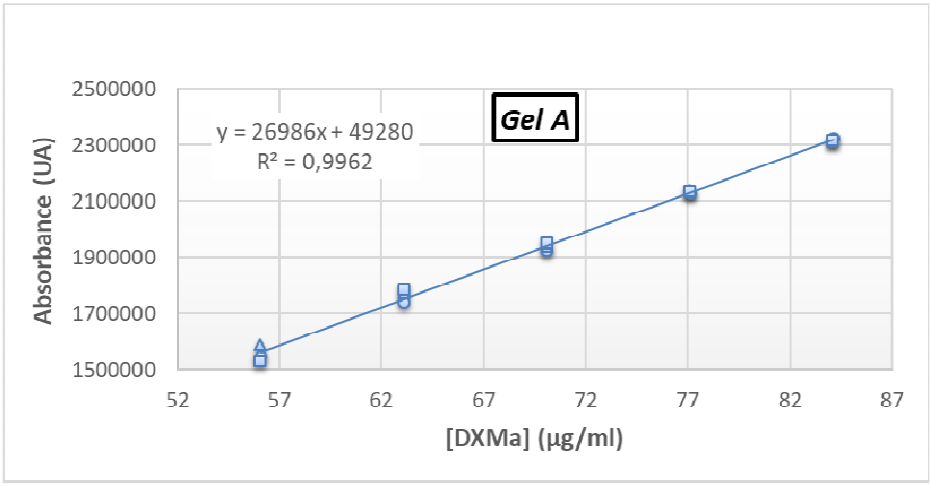
The linearity was required to demonstrate that the detector response is directly proportional to the analyte concentration over a specific range. The evaluation of calibration curves was made with five different known concentrations of DXMa (80, 90, 100, 110 and 120 % of the specification level), daily injected in duplicates, three days during. The standard calibration curves plotted the obtained mean peak area as a function of the concentration of DXMa are reported in Figure 2 for both Gel A and B.

The regression parameters of the lines are reported in Table 3.

Table 3. Calibration curves of DXMa in Gel A and Gel B

Gel	Range of linearity (µg/mL)	Slope	intercept	Correlation coefficient R^2
Gel A	56 - 84	26986	49280	0.996
Gel B	160 - 240	30783	-545504	0.999

Slopes were significantly different from zero (p-value < 5 %) and interceptions were not significantly different from zero (p-value > 5 %). The determination coefficient (R^2) value was found to be > 0.99. Hence, the method has linear response over the performed concentration range.



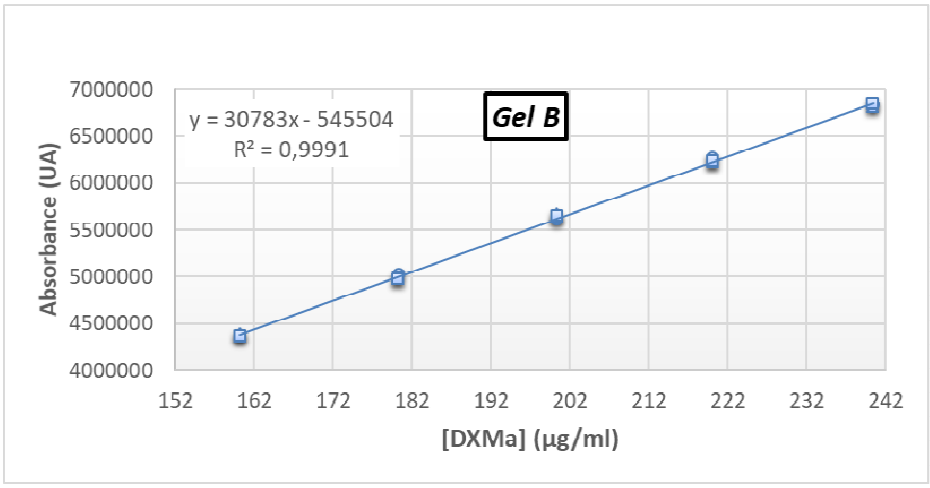


Figure 2. Calibration curves for DXMa in Gel A and Gel B (3 days/5 levels a day).

Accuracy (Bias %)

The accuracy studies were performed to verify the closeness of the agreement between the expected and the determined values. The DXMa concentration spiked in Gels A or B were determined using a linear regression(y = ax + b). The accuracy was evaluated by calculating first the percentage recovery and then the percentage of relative standard deviation (RSD) of recovery. The recovery results obtained from the five standards of calibration levels were between 98.48 and 101.07 % for DXMa in Gel A and between 98.40 and 101.01 % for DXMa in Gel B. The values are

within the limit of acceptance (95 – 105 %). The RSD (%) of all five levels were 1.31 % for DXMa in Gel A and 0.97 % for DXMa in Gel B. The results were lower than the limit of acceptance (2 %), indicating that the method is accurate.

Specificity

Specificity was examined by analyzing only the excipients of each gel (Gel A or B without DXMa). The absence of interference with DXMa was demonstrated (chromatogram not shown). In complement, to prove the specificity of the method, the degradation studies under relevant stress conditions were also performed and degradation products were observed after stress treatment (Figure 3).

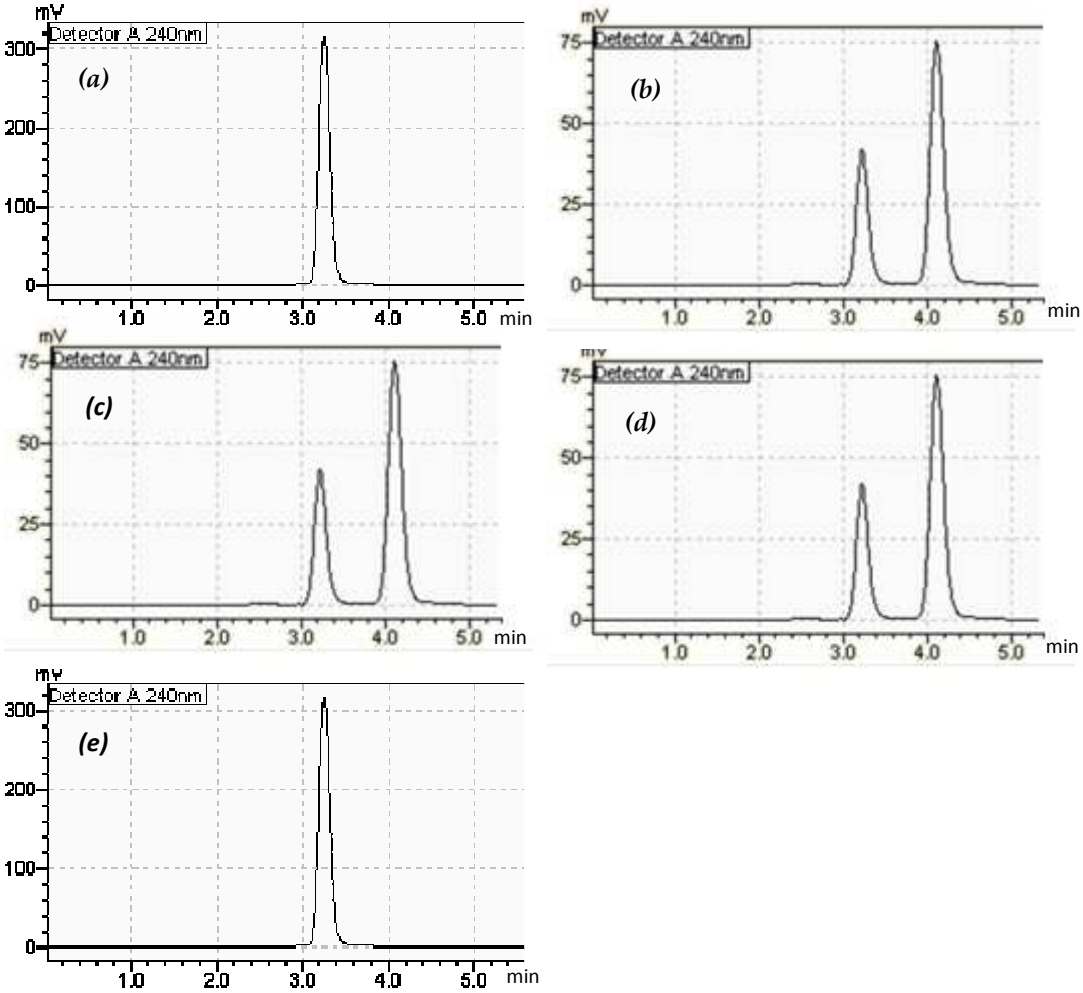


Figure 3. Chromatograms obtained for DXMa in Gel A and B after applying different stress conditions. (a) No stress, (b) HCl 0.5 N at 80 °C during 1 h, (c) NaOH 0.5 N at 80 °C during 1 h, (d) H_2O_2 3 % at 80 °C during 4 h and (e) UV light for 6 h

None of the observed peaks interfered with the DXMa peak in terms of retention time (resolution greater than 1.5). The used methods are therefore capable of identifying degradation products separately from DXMa. It should be noted that at this stage, we did quantify these degradation products.

Precision

Synthetic blend solutions representing 100 % of the target concentration of the method were used. The precision parameter was evaluated by performing both repeatability (intra-day variability) and intermediate precision (inter-day variability).

The repeatability characterizes the reproducibility of a given analytical procedure for the same sample preparation, as performed by the same analyst using the same instrument during a relatively short period time (intra-day). The repeatability was demonstrated by preparing six sample solutions (100 %) measured by HPLC and calculating the relative percentage of standard deviation (RSD). For both formulations, the repeatability RSD values were 0.29 % (Gel A) and 0.36 % (Gel B). The RSD (%) values for intra-day are found to be < 2 %, which were considered acceptable.

The intermediate precision characterizes the reproducibility of results obtained in the same laboratory during a prolonged period. It was established by preparing six assay sample solutions similar to repeatability (level 100 %) injected into a HPLC system as per proposed method on 3 different days. The RSD (%) of assay results was calculated. The intermediate precision results are 0.44 % for Gel A and 0.55 % for Gel B. The RSD (%) values for inter-day precision were found to be lower than 2 %, which indicates that method is also reproducible. The method was considered to be precise.

Limit of detection and limit of quantification

Detection and quantification limits are the lowest detectable and quantifiable concentration that a method can achieve (Table 4). As per ICH guideline, the LOD and LOQ were determined based on the standard deviation of the response (σ) and the slope (s) in accordance with the equations: LOD = 3.3 × σ/s and LOQ = 10 × σ/S.

Table 4. Limit of detection and quantification for Gels A and B

Gel	LOD (µg/mL)	LOQ (µg/mL)
Gel A	2.16	6.55
Gel B	3.06	9.26

In conclusion, the chromatographic method described was validated for quantitative assay determination of DXMa in Gels A and B as per ICH Q1A (R2) guideline.

The developed method is specific, accurate, precise, and reproducible. All the degradation products formed during stress conditions were well separated from the DXMa peak demonstrating that the developed method was specific. The method, according to international guidelines, can be used to determine DXMa content over time since no interference with degradation products was observed.

3.2. Sterilization step

Two different methods were investigated with Gels A and B i.e. : autoclaving (SANO CLAV from ADOLF WOLF, Überkingen, Germany) at 121 °C during 20 minutes or double sterilizing filtration (CME or PVDF 0.22 µm filter, ROTH, Karlsruhe, Germany). The sterile filtered product was packaged in sterile vials under laminar air flow of an ISO 4.8 microbiological safety cabinet. The choice of the sterilization steps is primordial and was evaluated in terms of change in chromatographic profile and in % of drug loss.

As seen in Figure 4, a peak of degradation product appears and DXMa peak was reduced. Therefore, it excludes autoclaving as a sterilization method of DMXa. DXMa seems to be heat labile, a similar result is reported in the literature for dexamethasone sodium phosphate [25].

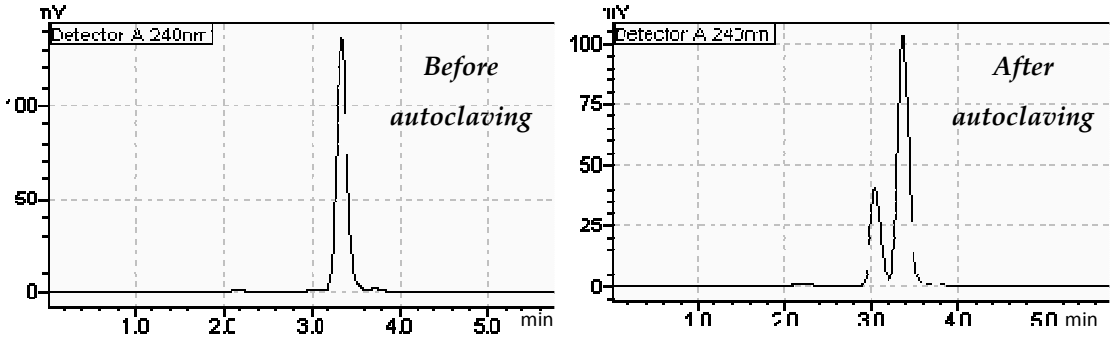


Figure 4. Chromatograms before and after autoclaving

The CME filters were discarded because leading after filtration to a loss of 12.9 ± 0.5 % DXMa with Gel A and 5.3 ± 0.3 % with Gel B, while the filter PVDF resulted in only a loss of 0.6 ± 0.02 % DXMa with Gel A and 0.4 ± 0.02 % with Gel B.

The PVDF filters are therefore retained and were confirmed by demonstrating the repeatability of the sterilization step without a great loss of DXMa. Indeed, six samples of each gel were prepared and DXMa was quantified by HPLC before and after double filtration steps with PVDF 0.22 µm filters. The relative percentage of standard deviation (RSD)of drug quantification was calculated from these quantifications. For both formulations, the drug loss was < 0.3 % and the repeatability RSD values were 0.96 % (Gel A) and 0.95 % (Gel B). The RSD (%) values are found to be < 1 %, which were considered acceptable.

3.3. Rheological measurements

The administration of an ophthalmic formulation should not influence the pseudoplastic nature of precorneal film, or the influence should be negligible. Figure 5 a and b present the dynamic viscosity of each formulations as a function of shear rate (0.11–100 s^{-1}) at 35 °C, measuring 5 points per decade and with 20 s equilibration's time. The both formulations exhibit newtonian behavior in contrast to our previous published results at 25 °C [7]. At shear gradients greater than 70-80 s^{-1}, centrifugal forces come into play, which results in a fall in axial force. The apparent rheofluidifying behavior past 100 s^{-1} is therefore an artifact caused by these centrifugal forces. For shear rates of less than 1 s^{-1}, the crust formed by the eye drops when drying opposes a resistance to the rotational movement of the geometry, which is no longer negligible compared to the measured torque, which explains the slight rise in the curve between 0.1 and 1 s^{-1}.

Below 0.3 s^{-1}, this crust makes measurements imprecise and so between 0.3 and 100 s^{-1}, Gels A and B are presenting a Newtonian behavior, Gel A displays a viscosity of 13.2 mPa.s ± 1 0% and Gel B a viscosity of 18.6 mPa.s ± 10 % (Figure 5). These viscosities are well appreciated by patients because it does not lead to a blurred vision. As demonstrated by Zaki *et al.,* the retention on eye surface began to increase only after a viscosity exceeding a critical value of about 10 mPa.s [26].

Although increasing fluid viscosity improves the residence time, it may also cause discomfort and damage to ocular epithelia due to an increase in the shear stresses during blinking. Carboxymethylcellulose and sodium hyaluronate are well known for their viscosifying properties. Furthermore, sodium hyaluronate, present in Gel A, Gel B and VISMED®, is a shear thinning fluid. Sodium hyaluronate should contribute to enhance viscosity while avoiding excessive stresses

during blinking [27]. Additionally, these viscosities, lower than < 30 mPa.s, are well tolerated by patients because it does not lead to blurred vision and foreign body sensation, resulting in a faster elimination due to reflex tears and blinks [28].

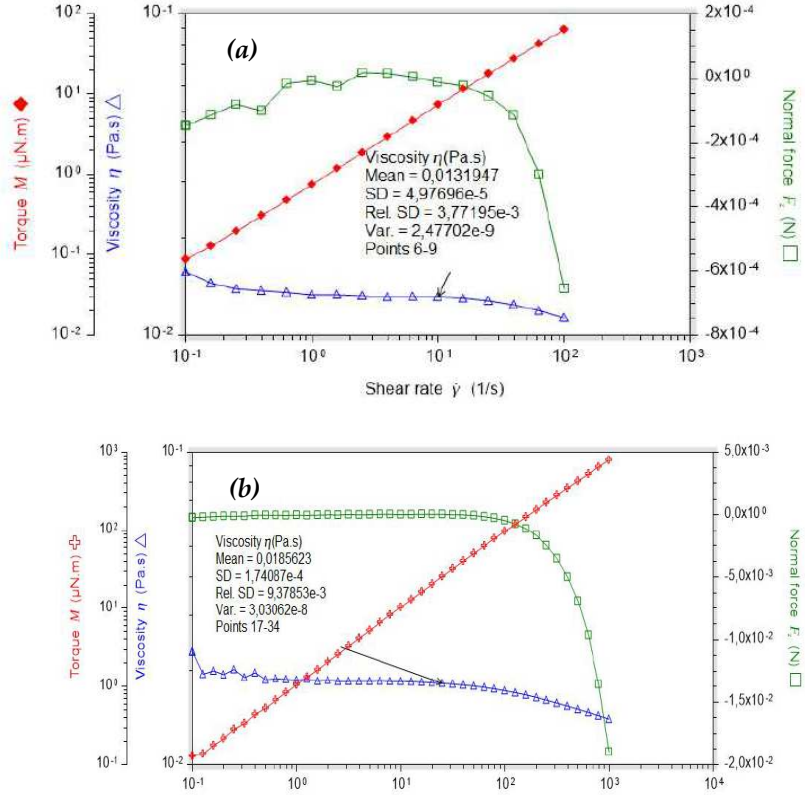


Figure 5. Dynamic viscosity of (a) Gels A and (b) B performed in the range of 0.11 to 100 s^{-1} at 35 °C

Before oscillation frequency sweep, an amplitude sweep test was performed to define the fluid's LVER, and the results showed that this region was at 10 % shear strain for both formulations. Indeed, for the Gel A the amplitude sweep test performed at 1 Hz between 0.1 and 100 % does not indicate any output of the linear domain. For the Gel B, the oscillation measured between 0.1 and 100 % of deformation do not show any upper limit and so, caution should be used to avoid not being below 1.5 % deformation with this rheometer (Figure 6). At 0.1 Hz, the storage module is negligible, which explains why some points are missing on the graphs (negative values cannot be displayed on a logarithmic scale). For both at low amplitudes, the signal becomes lower than the sensitivity of the material (0.01 µNm).

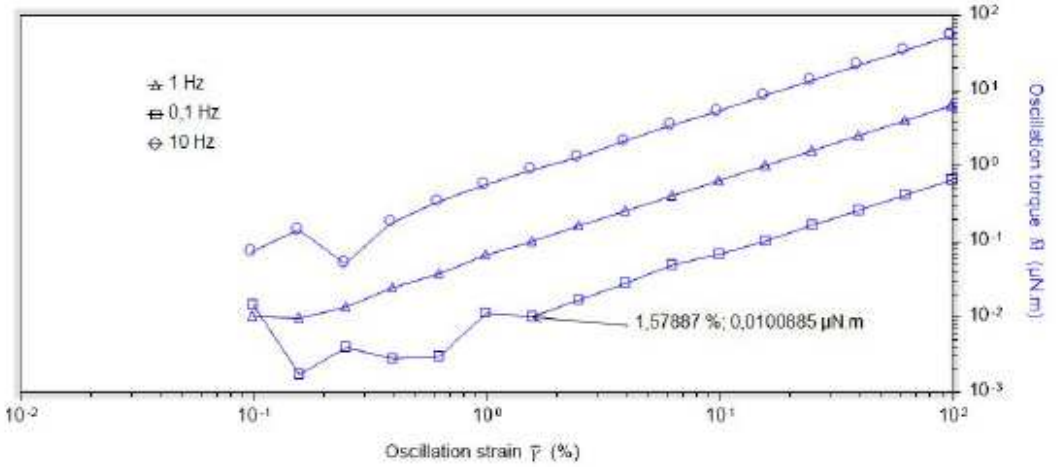


Figure 6. Amplitude sweep test performed with Gel B at 0.1, 1 and 10 Hz at 35 °C

With these results, the Gels A and B can be further characterized using a frequency sweep proving more information about the effect of colloidal forces[29]. Figure 7 presents oscillation frequency performed between 0.1 – 10 Hz with a shear strain of 10 % at 35 °C. Both formulations exhibited fluid-like mechanism spectra with G″ modulus even greater than G′, being both frequency dependent.

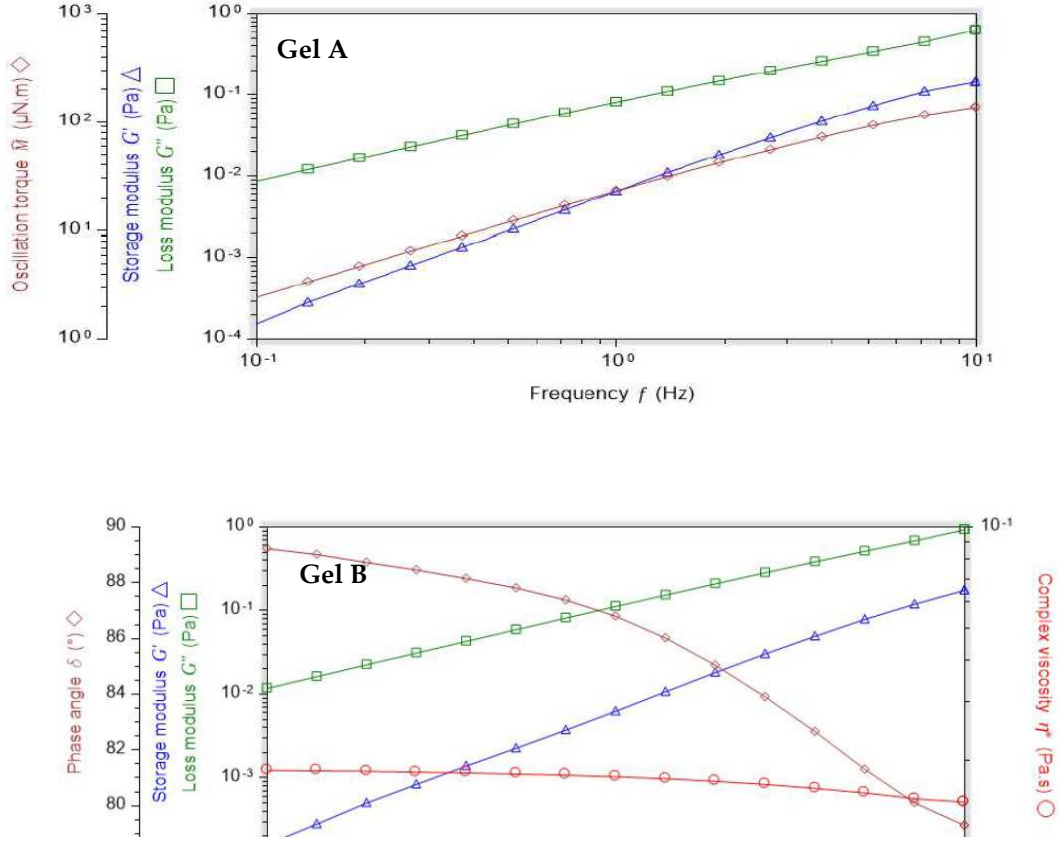


Figure 7. Oscillation frequency performed with Gel A and B between 0.1 – 10 Hz with a shear strain of 10 % at 35 °C

3.4. Mucoadhesion

Zeta potential (ZP) value is related to the measurement of the surface charge that a specific material possesses or acquires when suspended in a fluid. This study demonstrated that the ZP values of Gel A and Gel B are quite different. Indeed, Gel B ZP value (- 41.1 ± 2.3 mV) is much more negative than Gel A (-23.9 ± 0.7 mV) (Figure 8). These negative values are in accordance with the anionic nature of the hyaluronic acid due to the presence of carboxylic groups. HA is present in VISMED®, Gel A and Gel B. The mucins also present a negative ZP value due to their carboxyl and sulfate groups. The obtained value is quite different from the one described in literature, which is approximately -10 mV [16]. This difference could be explained by a different degree of hydration [30]. When the mucin 5 % (w/v) suspension is added to Gel B an increase of the negative charge is observed, showing the reduction of electrostatic repulsion and indirectly an interaction of the vehicle with the mucins [31].

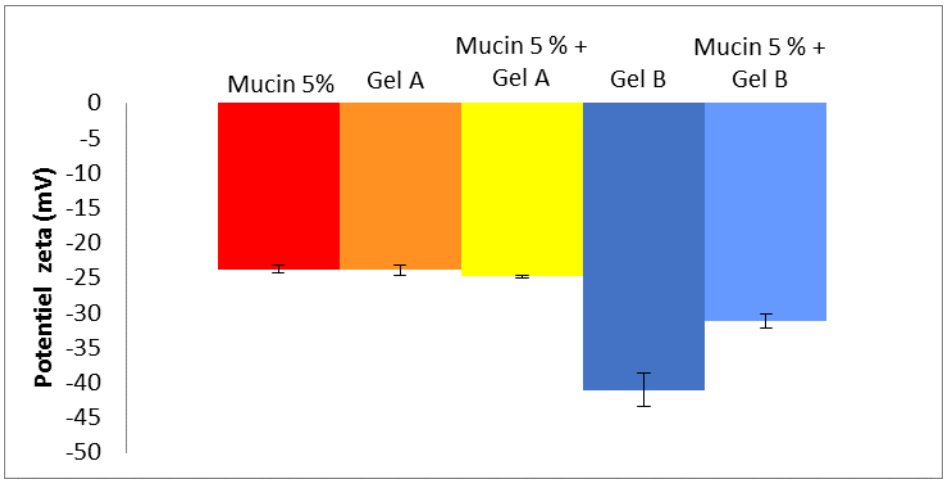


Figure 8. ZP values of mucin 5 %, Gel A, Gel B and mucin 5 % + Gel A or Gel B

3.5. Cytoxicity studies

3.5.1. MTT

To evaluate *in vitro* cell toxicity of Gels A and B with or without DXMa, HPβCD (600 mg/mL) and HPγCD (600 mg/mL) aqueous solutions, DEXAFREE® and MAXIDEX®, HCE cells grown in the presence of each formulation were evaluated by quantitative determination of living cells, after 30 min, 2 h and 24 h at 5 and 0.05 % concentration (Figure 9). The results are analyzed according to the Organization for Economic Co-operation and Development (OECD) guidelines for short time exposure *in vitro* test method [32] (Table 5).

Table 5. Prediction model inspired by the short time exposure according to OECD guideline [32]

Cell Viability		UN GHS Classification	Applicability
At 5 %	**At 0.05 %**		
> 70 %	> 70 %	No category	No serious damage nor eye irritation effect
≤ 70 %	> 70 %	No prediction can be made	No prediction can be made, eventual eye irritation
≤ 70 %	≤ 70 %	Category 1	Serious eye damage

As shown in Figure 9, Gel B showed an acceptable level of cytotoxicity to HCE cells and is considered rather well tolerated by HCEC. As well, DEXAFREE® presents a cell viability higher than 70 % at 5 and 0.05 % after 30 min, 2 h and 4 h. At the opposite, the Gel A is classified in the non predictable category since the cell viability was lower than 70 % at 5 % and higher than 70 % at 0.05 %. A similar cytotoxicity profile was observed in the case of the reference suspension MAXIDEX®. The cytotoxic effect of Gel A is time and concentration dependent and seems to be caused mainly by HPβCD (600 mg/mL) aqueous solution. Indeed, the cell viability of Gel A with or without DXMa and HPβCD (600 mg/mL) aqueous solution are relatively similar at each time and each concentration with values decreasing from around 30 %, 15 % and 10 % at 30 min, 2 h and 24 h respectively. Furthermore, each CD derivative at the concentration of 600 mg/mL presents a cytotoxic effect more or less pronounced. These observations could be attributed to the known capacity of CDs to extract and solubilize cholesterol from membranes, potentially causing destruction of phospholipid bilayers [33]. One can note that in the present study, HPγCD has a much less pronounced effect than HPβCD, showing a cell viability higher than 65 % against lower than 30 % for HPβCD. Moreover, the cytotoxicity of HPβCD is enhanced with increasing exposure time [34]. These differences may be attributed to the higher propensity of the βCD derivative to solubilize cholesterol from membranes compared to γCD [33]. Moreover, the clear decreased cytotoxicity observed in the case of Gel B may be related to the less extend of free cavities available for complexation in the case of HPγCD for which a higher complexation efficiency value was previously described [7], allowing Gel B to be relatively safe for HCEC. Therefore, in the future, it will be possible to consider a lower concentration of HPβCD in Gel A in order to improve ocular tolerance [34], even if this means reducing the solubilized DXMa fraction.

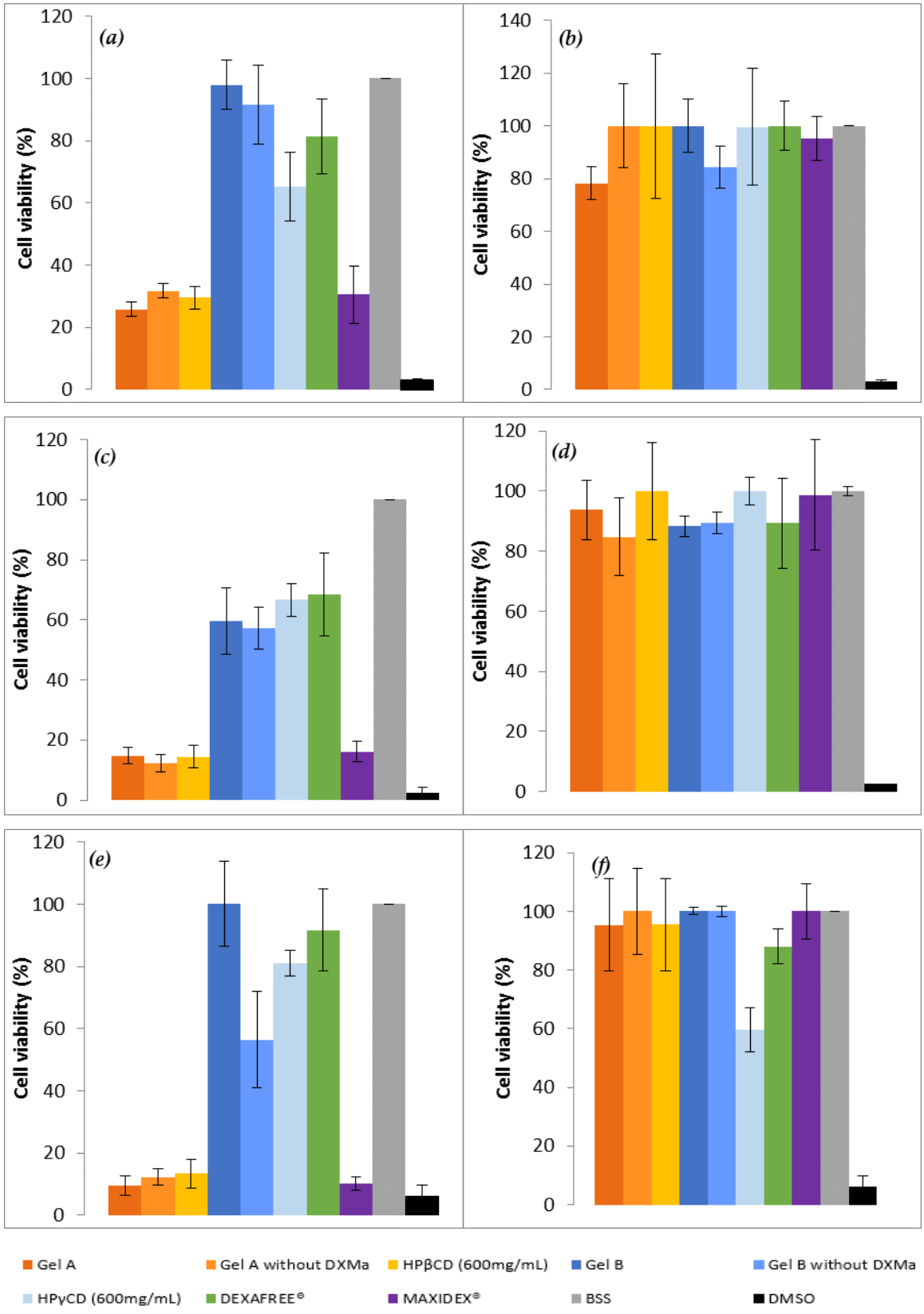


Figure 9. Cell viability of Gels A and B with or without DXMa, HPβCD (600 mg/mL) and HPγCD (600 mg/mL) aqueous solutions, DEXAFREE® and MAXIDEX®. (a) 5 % concentration during 30 min, (b) 0.05 % during 30 min, (c) 5 % during 2 h, (d) 0.05 % during 2 h, (e) 5 % during 24 h, (f) 0.05 % during 24 h

3.5.2. ALAMAR BLUE® assay

To complete *in vitro* cell biocompatibility study, ALAMAR BLUE® assay was performed by using fluorescence, which is proportional to the number of cells with metabolic activity (Figure 10). Gel B, Gel B without DXMa and HPγCD showed acceptable levels of metabolic activity as DEXAFREE®, with a cell viability even > 70 % after 2 h of exposure. Unfortunately Gel A, Gel A without DXMa and HPβCD (600 mg/mL) showed a low metabolic activity, < 30 %, and could cause serious eye damage. According to these results, we can demonstrate different biocompatibility profiles between Gel A and Gel B, probably related to the difference in biocompatibility profile between HPβCD and HPγCD. Hence Gel B is considered as biocompatible and the formulation of Gel A might be optimized regarding the effect of HPβCD on HCEC.

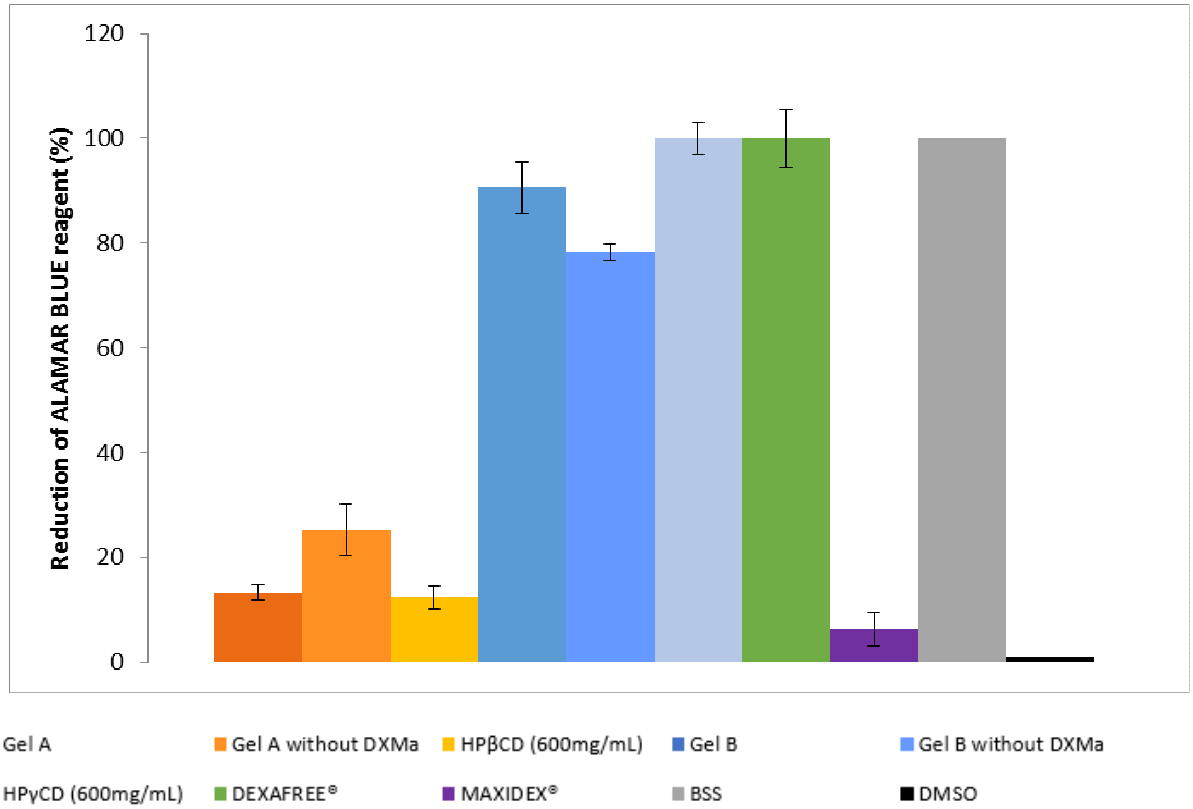


Figure 10. Reduction of ALAMAR BLUE® reagent (%) of Gels A and B with or without DXMa, HPβCD (600 mg/mL) and HPγCD (600 mg/mL) aqueous solutions, DEXAFREE® and MAXIDEX®

3.6. Ex-vivo evaluation of the corneal permeation

Ex vivo permeation of Gel A, Gel B, DEXAFREE® and MAXIDEX® were evaluated using the excised porcine cornea. The amount of DXMa permeated through the excised cornea from Gel B was higher than that of the other formulations (Figure 11). With Gel B, a maximum of 71.71 µg of DXMa permeates (i.e. 0.89 % amount of drug applied) and it is nearly 3.2-fold higher than DEXAFREE® and 4-fold higher than MAXIDEX®. Gel A presents also a good corneal permeation with a maximum of 40.48 µg (i.e. 1.44 % amount of drug applied) which is 1.8-fold higher than DEXAFREE® and 2.5-fold higher than MAXIDEX®. This suggests that both Gels A and B might be more effective than reference marketed formulations to treat corneal inflammations. Moreover, these results are associated to a good corneal hydration level, between 76 - 80 %.

Dexamethasone is a highly potent long acting drug requiring a far lower dosage compared to other intermediate and short acting glucocorticoids, i.e. nearly 5 times lower than prednisolone, methylprednisolone and 25 times lower than hydrocortisone, to elicit a biological response [35,36]. As demonstrated by Djalilian *et al*., dexamethasone inhibits inflammatory cytokines in human corneal epithelial cell and fibroblast cell lines with a concentration range of 0.1 to10 µM [37]. The marketed formulation DEXAFREE® contain 1 mg/mL drug, i.e. 1.9 mM. As previously described, Gel B released DXMa allowing a maximum drug amount of 71.71 µg to be permeated across excised cornea. As well, Gel A allows a permeated drug amount of 40.48 µg.

Therefore, considering the normal tear volume to be about 6 to 10 µL, assuming no tear drainage and similar release behavior as observed in 13 mL of PBS, 71.71 µg and 40.48 µg of DXMa (Mw = 434.5 g/mol) in 10 µL of tears, would theoretically be almost 16.6 mM and 9.3 mM, which is about 8- and 5-fold higher than the concentration provided by DEXAFREE®. These latter results warranted to be clinically relevant and within the therapeutic index [37].

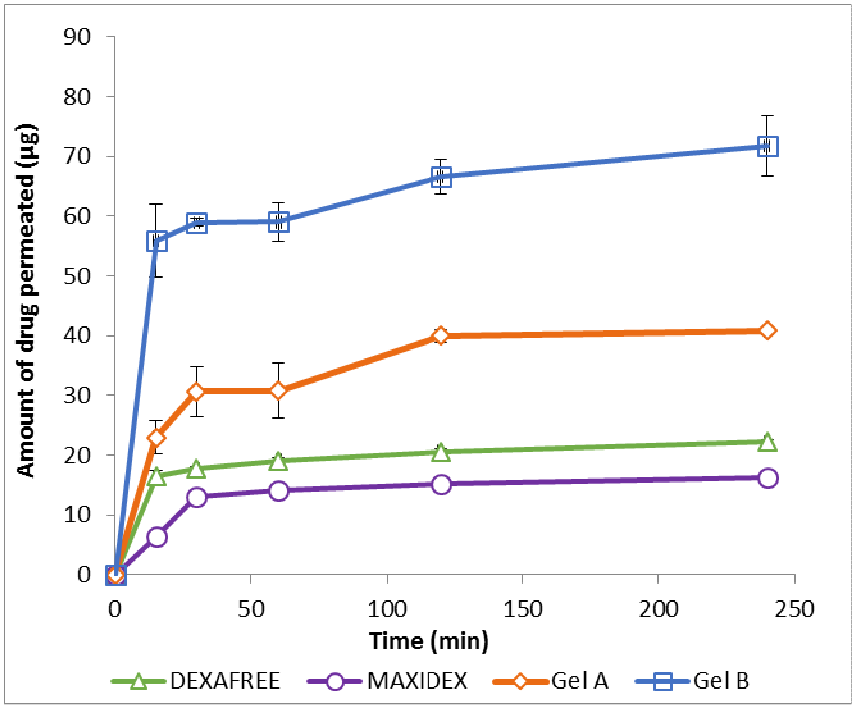


Figure 11. Amount of drug permeated through excised cornea of Gels A and B, DEXAFREE® and MAXIDEX®

3.7. *In vivo evaluation of the residence time on the ocular surface*

The biopermanence of Gels A and B, DXMa (10 mg/mL)/HPβCD (600 mg/mL), DXMa (30 mg/mL)/HPγCD (600 mg/mL), VISMED® and CELLUVISC® was characterized on the ocular surface of rats by ^{18}F-FDG radiolabeling followed by radioactivity in PET over 5 h (300 min) . It is an non invasive tool for pharmacokinetics studies of biopermanence of topical ocular drug delivery systems [38,39]. In the present study, all the formulations tested present a higher biopermanence than the control solution Balanced Salt Solution, (BSS), whose composition is close to tears Indeed, in Figure 12, it can be observed that after 30 min of contact, 23 % of the BSS remains in the ocular surface against 60 to 100 % remaining doses for the other formulations.

These observations are in accordance with the PET data described by Luaces-Rodriguez *et al*. in the case of tacrolimus eye drops [40]. According to the literature, increasing fluid viscosity increases

the residence time to some extend, by delaying the tear action [26]. This is in agreement with our observations since the reference marketed gels sodium carboxymethylcellulose and sodium hyaluronate more viscous than the other components, present a higher ocular residence time with a MRT of 197 and 134 min respectively. As well, the CD solutions present a slight viscosity of around 6 mPa.s, which result in a significant increase in $T_{1/2}$ and MRT values as compared to BSS. These results The MRT value for Gel B (112 min) was in between the values obtained for CELLUVISC® (197 min), VISMED® (134 min) and DXMa/HPγCD solution (101 min). Also, the presence of both CMC and HA associated to higher gel B viscosity, seems to promote ocular remanence. The low MRT value of 67 min obtained in the case of gel A is rather surprising with respect to the observed HPγCD solution MRT value (118 min). This would merit further investigation since a high variability in the results. Furthermore, sodium hyaluronate, present in Gel A, Gel B and VISMED®, is a shear thinning fluid. Sodium hyaluronate contributes to enhance viscosity while avoiding excessive stress during blinking [27].

The data summarized in Table 6 show that pharmacokinetic parameters such as $T_{1/2}$, MRT, and k, are significantly different between each Gel and BSS, at $p < 0.05$. The data collected from 3 to 240 min are significantly different between Gels A and B, DXMa (10 mg/mL)/HPβCD (600 mg/mL), DXMa (30 mg/mL)/HPγCD (600 mg/mL), VISMED® and CELLUVISC®, at $p < 0.05$ (Figure 12).

Table 6. Ocular biopermanence parameters measured *in vivo* for Gels A and B, DXMa (10 mg/mL)/HPβCD (600 mg/mL), DXMa (30 mg/mL)/HPγCD (600 mg/mL), VISMED® and CELLUVISC® versus BSS

Components	Viscosity at 35 °C (mPas)	k (min^{-1})	$T_{1/2}$ (min)	MRT	R^2
CELLUVISC®	167 – 260	0.007 ± 0.003	136.5 ± 95.5	196.9 ± 137.8	0.9738
VISMED®	16.8	0.008 ± 0.003	92.7 ± 26.7	133.7 ± 38.5	0.9404
Gel B	18.6	0.0096 ± 0.036	77.4 ± 28.8	111.6 ± 41.5	0.9837
Gel A	13.2	0.015 ± 0.002	46.6 ± 4.8	67.2 ± 6.9	0.9365
DXMa/HPβCD (10 mg/mL/600 mg/mL)	6.4	0.015 ± 0.014	81.7± 59.0	117.9± 85.2	0.9866
DXMa/HPγCD (30 mg/mL/600 mg/mL)	6.5	0.11 ± 0.003	70.2 ± 21.9	101.3 ± 31.6	0.9697
BSS	1.5	0.046 ± 0.015	16.0 ± 5.2	23.1 ± 7.6	0.9965

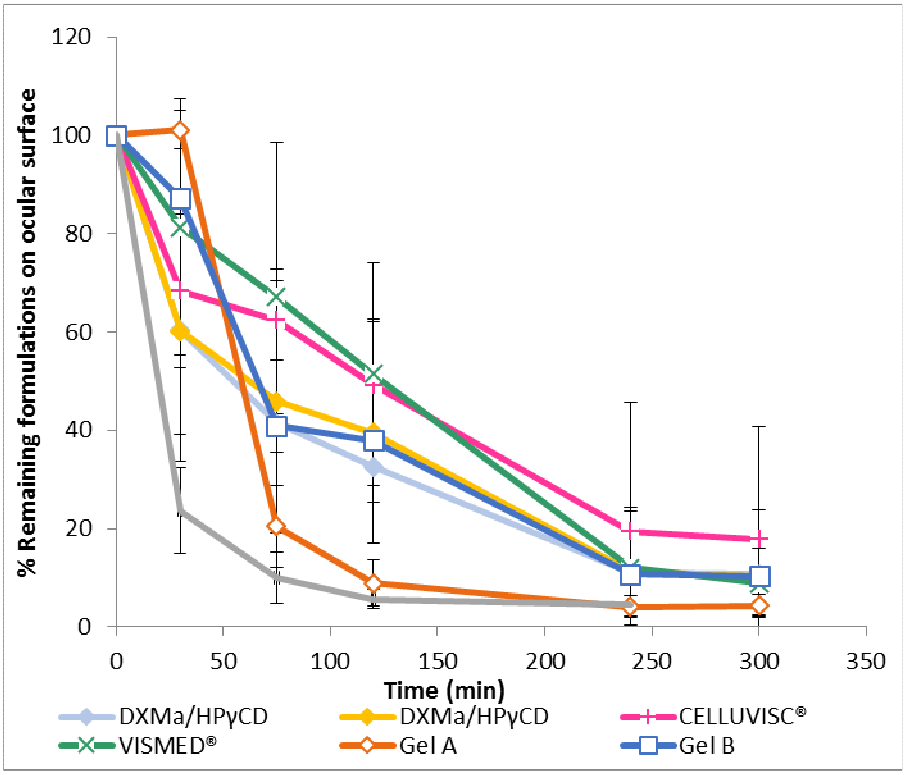


Figure 12. Ocular biopermanence of Gels A and B, DXMa (10 mg/mL)/HPβCD (600 mg/mL), DXMa (30 mg/mL)/HPγCD (600 mg/mL), VISMED® and CELLUVISC® versus BSS

3.8. Stability

The stability of Gels A and B was assessed using the following parameters: visual inspection, presence or absence of visible particles, DXMa concentration, presence or absence of breakdown products, pH and osmolality. The study was conducted according to ICH Q1A (R2) methodological guidelines for stability studies [15,41]. A variation of DXMa concentration outside 90 - 110 % interval of initial concentration was considered as being a sign of a significant DXMa concentration variation. The observed gels must be limpid, of unchanged color, and clear with no visible signs of haziness or precipitation. pH values were considered to be acceptable if they did not vary by more than one pH unit from initial value.

Gels A and B stayed limpid and there was no appearance of any visible particulate matter, haziness or gas development. Every Gel A presented a slightly yellowish tinge throughout the study.

The DXMa concentrations during 12 months are presented in Figure 13. Throughout the dosage times, Gel A and B did not vary by more than 10 % of initial concentrations at 25 °C; with low variability as 95 % confidence intervals.

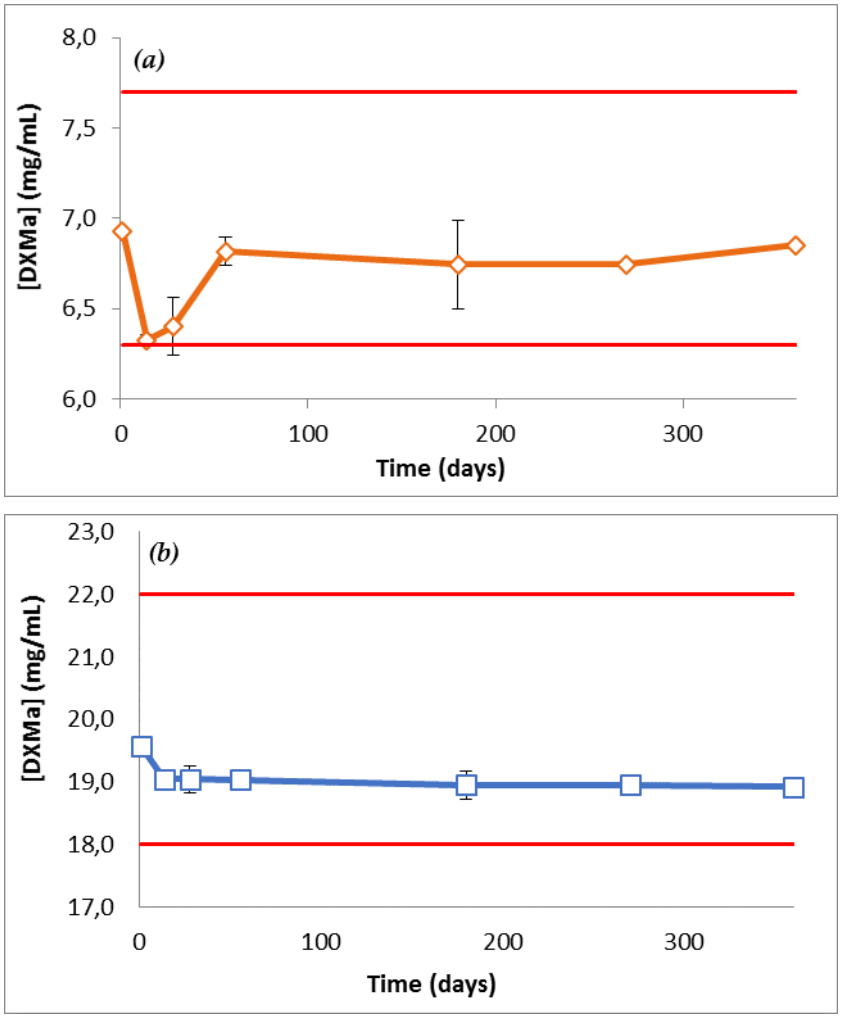


Figure 13. DXMa concentrations (mg/mL) during 12 months for (a) Gel A and (b) Gel B at 25 °C

For each gel, pH did not vary by more than 0.3 pH units from D0 to M12. pH Gel A is 7.5 and pH Gel B is 7.0. At 12 months, osmolality's of Gel A and B had not varied by more than 2.5% of initial osmolality. Both pH and osmolality did not vary during 12 month and stay in acceptable physiological range.

None of the 4 analyzed gels conserved at 25 °C in unopened bottles at day 0, 14 days, 30 days and 2, 6, 9, 12 months showed any signs of microbiological growth.

4. Conclusions and Future Prospects

In conclusion, the data provided in this study demonstrate that the use of hydrogels combined with hydrosoluble cyclodextrins is relatively safe, increase ocular retention and could act as penetration promoters for DXMa. Indeed, both gels present a good corneal permeation which is 3.22-fold higher than DEXAFREE® and 4.04-fold higher than MAXIDEX® for Gel B and 1.8-fold higher than DEXAFREE® and 2.5-fold higher than MAXIDEX® for Gel A. Furthermore, they are stable at 25 °C during 12 month after filtration sterilization. These good results have to be confirmed *in vivo* with pharmacokinetic, efficacy and tolerance studies.

Author Contributions: R.M., P.AF., L.C., V.V., F.B., F.J.O.E., A.F.F., D.L., V.B.M. carried out all the experiments, R.M.,L.C., D.W. and A.G., designed all the studies, oversaw all the experiments and contributed to draft the manuscript and intellectually to the development of the project.

Fundings: This research received no external private funding.

Acknowledgments: This work was supported by Labex ARCANE (ANR-11-LABX-0003-01) and Institut de Chimie Moléculaire de Grenoble (FR 2607) and the Glyco@Alps program (ANR-15-IDEX-02).

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary material 1: Original data for validation of analytical dosage methods

Data concerning linearity of DXMa					
Group i (Level)	**Assay j (day)**	**Concentration x_{ij}(µg/mL)**	**AUC $y_{ij\,1}$**	**AUC $y_{ij\,2}$**	**Average AUC y_{ij}**
1	**1**	56,090	1588077	1588124	1588101
1	**2**	56,090	1547002	1546820	1546911
1	**3**	56,09	1520954	1539070	1530012
2	**1**	63,101	1774759	1770105	1772432
2	**2**	63,101	1735297	1741892	1738595
2	**3**	63,101	1787189	1781234	1784212
3	**1**	70,112	1937731	1938898	1938315
3	**2**	70,112	1924412	1924942	1924677
3	**3**	70,112	1948512	1946367	1947440
4	**A**	77,120	2130570	2141625	2136098
4	**2**	77,120	2128707	2128697	2128702
4	**3**	77,120	2125759	2138035	2131897
5	**1**	84,130	2310641	2313094	2311868
5	**2**	84,130	2324057	2325693	2324875
5	**3**	84,130	2313642	2316523	2315083

Data concerning linearity of DXMa in Gel A					
Group i (Level)	**Assay j (day)**	**Concentration x_{ij}(µg/mL)**	**AUC $y_{ij\,1}$**	**AUC $y_{ij\,2}$**	**Average AUC y_{ij}**
1	**1**	56,000	1553272	1552935	1553104
1	**2**	56,000	1551302	1543942	1547622
1	**3**	56,000	1566062	1555866	1560964
2	**1**	63,100	1772311	1774311	1773311
2	**2**	63,100	1777449	1783165	1780307
2	**3**	63,100	1743901	1743134	1743518
3	**1**	70,112	1979040	1980674	1979857
3	**2**	70,112	1959011	1959320	1959166
3	**3**	70,112	1942446	1947436	1944941
4	**A**	77,120	2102508	2128252	2115380
4	**2**	77,120	2094166	2074850	2084508
4	**3**	77,120	2089469	2102097	2095783
5	**1**	84,130	2325019	2323786	2324403
5	**2**	84,130	2324102	2325355	2324729
5	**3**	84,130	2324567	2335678	2330123

Data concerning intermediate fidelity of DXMa in Gel A			
Group i (Level)	**Assay j (day)**	**Concentration x_{ij}(µg/mL)**	**AUC $y_{ij\,1}$**
1	**1**	70,112	1937731
1	**2**	70,112	1938898
1	**3**	70,112	1944643
1	**4**	70,112	1944588
1	**5**	70,112	1945897
1	**6**	70,112	1934567
2	**1**	70,112	1924412
2	**2**	70,112	1924942
2	**3**	70,112	1930883
2	**4**	70,112	1933463
2	**5**	70,112	1934576
2	**6**	70,112	1945632
3	**1**	70,112	1948512
3	**2**	70,112	1946367
3	**3**	70,112	1948860
3	**4**	70,112	1939592
3	**5**	70,112	1945631
3	**6**	70,112	1949087

Data concerning linearity of DXMa					
Group i (Level)	**Assay j (day)**	**Concentration x_{ij}(µg/mL)**	**AUC $y_{ij\,1}$**	**AUC $y_{ij\,2}$**	**Average AUC y_{ij}**
1	**1**	160,260	4390650	4388550	4389600
1	**2**	160,260	4376427	4374207	4375317
1	**3**	160,260	4331524	4407674	4369599
2	**1**	180,290	4976139	4976872	4976506
2	**2**	180,290	5030549	5033908	5032229
2	**3**	180,290	4978558	4981948	4980253
3	**1**	200,320	5654170	5690387	5672279
3	**2**	200,320	5613164	5614173	5613669
3	**3**	200,320	5604309	5686397	5645353
4	**A**	220,040	6227256	6227115	6227186
4	**2**	220,040	6269243	6277131	6273187
4	**3**	220,040	6234668	6235969	6235319
5	**1**	240,380	6804130	6807503	6805817
5	**2**	240,380	6839372	6839752	6839562
5	**3**	240,380	6853296	6848154	6850725

Data concerning linearity of DXMa in Gel B					
Group i (Level)	**Assay j (day)**	**Concentration x_{ij}(µg/mL)**	**AUC $y_{ij\,1}$**	**AUC $y_{ij\,2}$**	**Average AUC y_{ij}**
1	**1**	159,530	4437552	4383902	4410727
1	**2**	159,530	4440132	4374644	4407388
1	**3**	159,530	4390134	4381900	4386017
2	**1**	179,990	4935648	4917118	4926383
2	**2**	179,990	4921226	4885186	4903206
2	**3**	179,990	4900604	4878454	4889529
3	**1**	199,860	5664654	5658696	5661675
3	**2**	199,860	5665840	5665940	5665890
3	**3**	199,860	5670640	5688068	5679354
4	**A**	220,030	6239162	6228308	6233735
4	**2**	220,030	6246426	6234646	6240536
4	**3**	220,030	6222518	6225324	6223921
5	**1**	240,020	6803372	6806470	6804921
5	**2**	240,020	6813104	6808300	6810702
5	**3**	240,020	6826300	6826300	6826300

Data concerning intermediate fidelity of DXMa in Gel B			
Group i (Level)	**Assay j (day)**	**Concentration x_{ij}(µg/mL)**	**AUC $y_{ij\,1}$**
1	**1**	200,320	5654170
1	**2**	200,320	5690387
1	**3**	200,320	5679719
1	**4**	200,320	5674248
1	**5**	200,320	5687689
1	**6**	200,320	5663442
2	**1**	200,320	5613164
2	**2**	200,320	5614173
2	**3**	200,320	5636475
2	**4**	200,320	5631799
2	**5**	200,320	5658997
2	**6**	200,320	5648976
3	**1**	200,320	5604309
3	**2**	200,320	5686397
3	**3**	200,320	5684617
3	**4**	200,320	5654929
3	**5**	200,320	5642356
3	**6**	200,320	5678493

Publication des résultats N°3

Titre : Topical Formulation of Nanoparticles in Gel vehicle for Dexamethasone acetate Ocular Delivery

Résumé : Dans la précédente étude, nous avons caractérisé et évalué, d'un point de vue biopharmaceutique, les deux formulations contenant 0,7 % et 2 % de DXMa, constituées respectivement d'hyaluronate de sodium et/ou de carboxymethylocellulose et d'HPβCD ou d'HPγCD afin d'augmenter le temps de résidence précornéen et la biodisponibilité de la DXMa après administration topique ophtalmique.

L'objectif de cette troisième étude est de réaliser l'évaluation biopharmaceutique du système d'administration combinant HPβCD, hyaluronate de sodium, carboxymethylcellulose et nanoparticules chargées positivement, obtenues par co-nanoprécipitation de cyclodextrines bioestérifiées et d'un composé cationique amphiphiles. Cette troisième formulation, le Nano-Gel B, sera comparée à la formules optimisée Gel B ainsi qu'aux formules commerciales MAXIDEX® et DEXAFREE®. L'étude *in vitro*, nous permettra d'apprécier sa mucoadhésion et sa cytotoxicité sur cellules épithéliales de la cornée humaine. Ensuite, nous avons évalué le passage transcornéen sur cornée de porc isolée. Enfin, nous avons étudié *in vivo* la biodistribution oculaire de cette formulation, à l'aide de l'imagerie moléculaire par tomographie à émission de positons (TEP). Cela nous a permis d'établir le profil pharmacocinétique des deux formulations, marquées au ^{18}F-Fluorodésoxyglucose (^{18}F-FDG).

Les données fournies dans cette étude démontrent que les nanoparticules cationiques sont mucoadhésives et permettent d'améliorer le temps de résidence cornéenne. La formulation Nano-Gel B présente une bonne perméabilité transcornéenne, 3,8 fois et 5,2 fois plus élevée que le DEXAFREE® et le MAXIDEX®. Ces bons résultats doivent être confirmés, *in vivo* chez le rat, par des études pharmacocinétiques, d'efficacité et de tolérance.

Article scientifique en préparation pour soumission

Article

Topical Formulation of Nanoparticles in Gel vehicle for Dexamethasone acetate Ocular Delivery

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Abstract: Conventional topical formulations of anti-inflammatory drugs are often associated with a poor bioavailability due to their high elimination rate in tear fluid and a low drug permeation. The aim of our work was to develop an innovative formulation of dexamethasone acetate (DXMa) which is incorporated in γCD-C10-nanoparticles into hydrogel (Nano-Gel B) in order to enhance DXMa bioavailability and transcorneal penetration. A novel formulation of self-assembled γCD-C10-nanoparticles with a new synthetized lipid cationic, MAP-103a, was developed and characterized by a mean size range of 90 - 110 nm, a zeta potential value of about +40 mV and an enhanced drug retention time on ocular surface. The Nano-Gel B presents a sustained drug release and no cytotoxic effects. Furthermore, Nano-Gel B displays a viscosity of 9.6 mPa.s, which cannot explain to itself the great extent of transcorneal penetration, 3.8-fold higher than DEXAFREE® and 5.2-fold higher than MAXIDEX®. These good results have to be confirmed *in vivo* with pharmacokinetic, efficacy and tolerance studies.

Keywords: dexamethasone acetate; nanoparticles, cyclodextrins-polysaccharide-based hydrogel; eye drops; rheological/mucoadhesion properties, cytotoxicity studies, transcorneal permeation

1. Introduction

Ocular inflammation results from various clinical causes, such as infection, physical or chemical involvement, or may be the primary reason for the lesions. One of the particularities of their clinical management is the need to treat this inflammatory component very early to avoid its harmful consequences on the vision [1]. In this context, the administration of anti-inflammatory drugs is most often done by topical ophthalmic route because of its many advantages : easy to handle, non-invasive, rather well-tolerated and providing sufficient ocular drug concentrations [2]. Nevertheless, the conventional topical formulations, i.e. ointments, eye drops, gels and emulsions, present a very poor drug bioavailability; about 1 – 5 % [3] and therefore require frequent instillations. This phenomenon is

due to various protective mechanisms, anatomical and physiological barriers encountered in the eye [5].

Nanotechnology is one of the best tool used to enhance drug ocular bioavailability through their various properties to minimize drug degradation in tear film layer [6], to decrease ocular elimination rate and to increase drug permeation [7]. Furthermore, nanotechnology is known to be suitable for poorly water-soluble drug and to target drugs and control their release [8].

Several investigations attempted to improve corneal drug penetration through the development of various colloidal drug delivery system, such as liposomes [9], nanoaggregates [10], nanoemulsion [11], nanogels [12], nanomicelles [13], nanoparticles [14], nanocapsules [15], solid lipid nanoparticles [16] or nanosponges [17]. Unfortunately, these colloidal drug delivery were not able to solve the problem of rapid loss of drug with tear drainage and still have a short residence time [6].

In the context of nanomedicine, amphiphilic γ-cyclodextrin-based nanoparticles were previously investigated by our group in order to develop a parenteral formulation of artemisinin [18]. Cyclodextrin (CD) derivatives were grafted with decanoic alkyl chains (CD-C_{10}) by a one-step bioesterification method [19–22]. Using the solvent displacement methodology, the derivatives yielded a variety of nanostructures with a size ranging from 70 to 220 nm [18–23], that can be easily surface modified by co-nanoprecipitation of CD-C_{10} with amphiphilic molecules. [23]. In this context of ocular delivery, a cationic amphiphile, allowing to yield cationic nanoparticles with higher mucoadhesive properties. The use of mucoadhesive nanocarriers represents a promising strategy toward the treatment of various ophthalmic disorders. They present the ability to avoid major drawbacks of conventional topical ophthalmic drug delivery systems [6] by a high mucus-permeating properties [24]. Recently, a new combination strategy involving nanocarriers into a polymer matrix was investigated [25] allowing to prolong the retention time on the ocular surface [26].

The aim of our study was to develop an innovative formulation of dexamethasone acetate (DXMa) by using a mixed vehicle of cationic γCDC_{10} nanoparticles and a hydrogel containing either HPβCD or HPγCD and polysaccharides, recently developed by our group for ocular delivery DXMa which is not available for topical ophthalmic route despite its powerful anti-inflammatory action. It is poorly-water soluble (0.021 mg/mL at 25 °C) and lipophile (log P = 2.92). This study reports the formulation and physicochemical characterization of the nanoparticles and their combination with gel vehicles. As well, the *in vitro* drug release studies, cytotoxicity tests, *in vivo* residence time on ocular surface and *ex vivo* transcorneal permeation assays were investigated.

2. Materials and Methods

2.1. Materials

DXMa was purchased from LA COOPER (Melun, France). Hydroxypropyl-γ-cyclodextrin (HPγCD, W8HP, DS = 0.6 and Mw = 1576 Da) was a kind gift from ASHLAND (Schaffhausen, Switzerland). γCD-C_{10} fatty ester (Mw = 2714 Da, total degree of substitution = 9.2) was synthesized in our laboratory by an enzymatically assisted pathway using thermolysin as catalyser and decanoic vinyl esters (C_{10}) as acyl donors, according to the procedure described by Choisnard *et al*. [19] (Figure 1). Thermolysin (EC 3.4.24.27), a protease type X isolated from *Bacillus thermoproteolyticus rokko*, anhydrous DMSO (99 %), vinyl decanoate (95 %) were obtained from Sigma-Aldrich (L'Isle d'Abeau Chesnes, France). (*S*)-2,6-diamino-*N*-((*S*)-3-hydroxy-1-(octadecylamino)-1-oxopropan-2-yl)hexanamide dihydrochloride) is a cationic amphiphile called MAP-103a (Figure 2) and synthetized by chemists from our group.

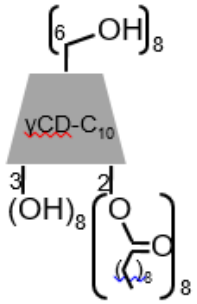

Figure 1. γCD-C10

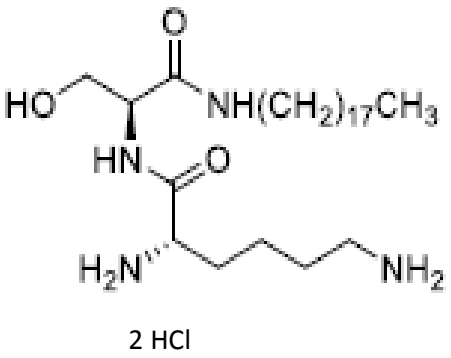

Figure 2. MAP-103a

CELLUVISC® (sodium carboxymethylcellulose) and VISMED® (sodium hyaluronate) are marketed gels used for the treatment of dry eye syndrome. DEXAFREE® (DXM sodium phosphate 0.1 % solution eye drops), MAXIDEX® (DXM 0.1 % suspension eye drops) and BSS® (Alcon Laboratories, Rueil-Malmaison, France) are human authorized ocular medicines. Normal Human Primary Corneal Epithelial Cells (ATCC PCS 700-010), medium (ATCC PCS-700-030), growth kit (ATCC PCS-700-040), PBS (ATCC 30-2200), trypsin EDTA (ATCC PCS-999-003 and 005) and antibiotics (gentamicin, streptomycin and amphotericin BATC PCS-999-002) were obtained from ATCC®. Thioglycollate with résazurine medium and Tryptic soy broth were obtained from BIOMERIEUX (Craponne, France). ALAMAR BLUE® was purchased from BIO-RAD (Marnes-la-Coquette, France) and DMSO from SIGMA-ALDRICH (Lyon, France). Purified water was prepared by DIRECT-Q®3 UV water purifier (MILLIPORE, Molsheim, France). All other solvents and chemicals were of HPLC and analytical grade, respectively.

2.2. Methods

2.2.1. Formulation

2.2.1.1. DXMa-loaded surface-decorated γCD-C10 nanoparticles

γCD-C10-based nanosphere suspensions were prepared using the solvent displacement technique adapted from Yaméogo *et al.* [18,28]. Briefly, for the preparation of nanospheres, the method consists in injecting, under magnetic stirring (500 rpm) at 25 °C, an organic solution of 4 mL acetone containing γCD-C10 (10 mg) and DXMa (2 mg) into 4 mL of distilled water containing MAP-103 (1 mg), a cationic amphiphile. The nanoparticles spontaneously formed, and the organic solvent was then removed under reduced pressure. The suspension was concentrated at 40 °C under vacuum until a final aqueous volume equal to 60-70 % of initial distilled water volume. The aqueous suspension was then filtered through 0.8 µm PVDF filters.

2.2.1.2. Gel vehicle formulation

The composition of optimized mixed Gel B was obtained by means of experimental design as previously described [27] (Table 1). Briefly, the mixed gel is obtained as follows: 600 mg/mL HPγCD solution is prepared at room temperature. Then DXMa was added to the solution before introducing CELLUVISC® and VISMED®.

Table 1. Composition of optimized mixed Gel B.

Components	Quantity (g)
CELLUVISC®	0.151
VISMED®	0.085
HPγCD 600 mg/mL with DXMa	0.764
Optimized mixed Gel B contains 20 mg/g of DXMa and an osmolality of 425 mOsm/kg	

2.2.1.3. γCD-C_{10} nanoparticles in gel vehicle formulation

The Nano-Gel B was prepared from the aqueous nanosuspension. Briefly, γCD-C_{10}/MAP-103a loaded with DXMa were prepared according to the procedure described above. Then, HPγCD (1.5 g) was added to the aqueous colloidal suspension (2.5 mL) under magnetic stirring (500 rpm) at 25 °C until complete HPγCD dissolution. DXMa (99 mg) was introduced in the mixture under constant magnetic stirring by successive additions of 5 mg drug. Once the DXMa powder completely disappeared, VISMED® (421 mg) and CELLUVISC® (823 mg) were added successively and the preparation was left under stirring for another 10 minutes at 25 °C. The Nano-Gel B was filtered on 0.8 µm PVDF filter.

2.2.2. Physicochemical characterizations

2.2.2.1. *Drug quantification*

The total concentration of DXMa recovered in the colloidal suspensions (Ct) and the DXMa concentration after ultracentrifugation at 140,000 g, 15 °C for 1 h (OPTIMA™ L-80 XP Beckman Coulter, rotor SW60 Ti. DXMa content in supernatant (Cs) were determined, after a sample dilution with methanol:water (70:30 v/v) by HPLC as previously described by Mazet *et al*. [27] The drug encapsulation efficiency EE (%) and the drug association DA (%) were estimated as follows (Equations 1 and 2):

$$EE\ (\%) = \frac{C_t}{C_i} \times 100, \quad (1)$$

$$DA\ (\%) = \frac{(C_t - C_s)}{C_t} \times 100, \quad (2)$$

where C_t corresponds to the DXMa's concentration in colloidal suspension. The physical stability of the nanosuspensions was achieved over 2 months.

The DXMa content in Nano-Gel B was assayed by HPLC according to the method previously described at T0 and 1 month. During this period, the visual appearance of the Nano-Gel B was checked to identify any signs of instability.

2.2.2.2. Size, zeta potential and morphology

The mean size, polydispersity index (PI) and zeta potential were determined after appropriate dilution using a Zetasizer (Nano ZS, Malvern Instruments) [28]. The potential zeta was measured after dilution of the nanosuspensions in PBS (pH 7.4) and NaCl 0.009 %. The unloaded nanosystems were observed by transmission electron microscopy (TEM) after negative staining of the preparations with uranyl acetate, using a Philips CM200 'Cryo' microscope operating at 80 kV. The images were recorded on Kodak SO163 films [21].

2.2.2.3. Rheological measurements

Rheological characteristics of both gels were examined at high shear rates using a high sensitivity pressure cell ARES-G2 rheometer from TA Instruments (New Castle, USA) equipped with a coaxial cylinder geometry (SN402525.001, TA Instruments, New Castle, USA) with APS kit and Couette system from TA Instruments (New Castle, USA). The measuring cup diameter (33.985 mm)/measuring bob diameter (32 mm) corresponds to 1.0620 according to ISO 3219. The gap length is 2mm and the sample volume > 5.2 mL. The temperature is controlled at 35 °C by a Peltier plate.

The steady-state flow experiments were performed in the range of 0.11 to 100 s^{-1}. The frequency sweep method was performed between 0.1 Hz and 10 Hz, with a shear strain of 10 % for both formulations, while the table of shear rate method was performed by increasing the shear rate from 0.1 to 100 s^{-1}, at 35 °C. The shear stress was measured by this method and the apparent viscosity was calculated by dividing the shear stress by the shear rate.

2.2.2.4. Mucoadhesion

In this study, mucin was rehydrated with water by gentle stirring until complete dissolution to yield a dispersion of 10 % (w/w) at 20 – 25 °C. The mucoadhesion was evaluated by the effect of mucin on zeta potential (ZP) values of Gel B ± mucin (1:1), Nano-Gel B ± mucin (1:1) and DXMa/HPγCD ± mucin (1:1). A volume of 40 µL of Gel B, Nano-Gel B and DXMa/HPγCD were diluted in either 2 mL of sterile purified water [29–31]. The ZP values of the different mixtures were measured using a Zetasizer Nanoseries Nano ZS (Malvern Instruments, Malvern, UK) at 35 °C. All the experiments were done in triplicate.

2.2.3. *In vitro* DXM release studies

The drug release experiments were carried out using a Sotax Dissolutest AT7 (SOTAX, Aesch, Switzerland). A sample of Gel B, Nano-Gel B, MAXIDEX® and DEXAFREE® was dropped in the extraction cell, which was placed at the bottom of the vessel filled with the dissolution medium. The experiments were conducted for 24 h at 35 °C, in 250 or 500 mL of phosphate buffer saline (PBS 1X pH 7.4). The speed of the rotating paddle was set at 100 rpm. The DXM, DXMa, and DXMp solubilities were previously determined in triplicate after 2 h agitation of aqueous drug suspensions in PBS at 35 °C. After filtration (0.2 µm), the solubilized drug content was quantified by HPLC at 240 nm [27]. The amounts of samples used in the cell were 1.5 g for MAXIDEX®, and DEXAFREE®, and 0.5 g for Gel B and Nano-Gel B. At the set time points (30 min, 1 h, 2 h, 4 h, 8 h, 24 h), aliquots of 1mL filtered medium were withdrawn and DXM content assayed by HPLC.

2.2.4. Cytotoxicity studies

Two different cellular toxicity assays were used, based on cell viability in relation to mitochondrial enzymes [32] i.e. the methylthiazolyldiphenyl-tetrazolium bromide conversion (MTT) and ALAMAR BLUE® assays. The experiments were performed using Normal Human Primary Corneal Epithelial Cells (HCEC) obtained from ATCC® and maintained in an incubator (37 °C and 5 % CO_2 saturation). HCEC were kept in corneal epithelial cell growth culture medium with gentamicin and amphotericin B, without fetal bovine serum. All the experiments were performed in between steps 4 and 8. Three thousand cells per well (96 wells per plates) were incubated for 24 hours at 37 °C and 5 % CO_2 in order to have between 80 to 90 % of cell confluence, according to ATCC® protocol. Subsequently, during the MTT assay, the original culture medium was aspirated and different concentrations (25 µL/200 µL, and 0.25 µL/200 µL) of different formulations: Nano/DXMa, Nano-Gel B, Gel B with or without DXMa, HPγCD (600 mg/mL) aqueous solutions, DEXAFREE® eye drops solution and MAXIDEX® eye drops suspension were added to different wells and incubated during 30 min and 2 h. Each concentration was tested in 3 individual wells. After 30 min, 2 h and 24 h, the supernatant was removed and 200 µL of MTT solution (5 mg/mL in PBS and then diluted to 1/10 in complete medium) was added to each well and then incubated for 3 h at 37 °C to allow the formation of formazan crystals. The medium was then removed, and blue formazan was eluted from cells by 200 µL of DMSO. The plates were shaken in order to solubilize the crystals of formazan. The liquid was aspirated to another new 96-wells plate and measured directly at 590 nm with Clariostar (BMG Labtech, Champigny sur Marne, France). Each plate was duplicated.

Additionally, the ALAMAR BLUE® was performed after 2 h of incubation at 37 °C, 5 % CO_2, with the IC50 concentrations as determined by the MTT assay. 20 µL of ALAMAR BLUE® reagent were added in each well before 2 h of incubation at 37 °C, 5 % CO_2. Fluorescence was measured with excitation wavelength at 530 - 560 nm and emission wavelength at 590 nm with Clariostar (BMG Labtech, Champigny sur Marne, France). Each plate was duplicated.

The % of reduction of ALAMAR BLUE® was calculated by the following Equation 3:

$$\%\ \text{Reduction} = \frac{(\text{Experimental RFU value}) - (\text{Negative control RFU value})}{(100\%\ \text{reduced positive control RFU value}) - (\text{Negative control RFU value})} \times 100, \quad (3)$$

2.2.5. *Ex vivo* evaluation of the corneal permeation

The transcorneal permeation experiment was performed for Gel B, Nano-Gel B, DEXAFREE® and MAXIDEX®, using Franz diffusion cells with an available diffusion area of 1.131 cm^2. The porcine corneas were recovered from the slaughterhouse in accordance to ethical regulations. The corneas were removed and then mounted onto diffusion cells, with the epithelial layer exposed to the donor chamber. The latter was filled with 0.4 g of each ophthalmic formulation; whereas the receptor chamber was filled with 13 mL artificial tear fluid BSS. According to Wen *et al.* [33], the experiment was performed at 35 ± 1 °C in a thermostatic water bath with a moderate speed of rotation maintained for 24 h. Three corneas per formulation (n=3) were used. A 1 mL sample was removed at predetermined time intervals (15 min, 30 min, 1 h, 2 h and 4 h) and replaced with an equal volume of fresh medium to maintain the sink conditions. The withdrawn samples from receptor chamber were analyzed by HPLC. The cumulative amount of drug appearing in the receptor compartment (Qn) was plotted as a function of time (t_n) and calculated using the following equation (Equation 4):

$$Qn = V_0 \left(C_n + \frac{V}{V_n} \sum_{i=1}^{n-1} C_i \right) = V_0 C_n + V \sum_{i=1}^{n-1} C_i, \quad (4)$$

C_n: Drug concentration at t time points (µg/mL),
C_i: Drug concentration at sampling points,
V_0: Volume of the medium in the receiving chamber,
V: sampling volume

The corneal hydration level (% HL) was measured with a relative Humidity Analyzer MB45 OHAUS® (Parsippany, USA).

2.2.6. *In vivo* evaluation of the residence time on the ocular surface

In vivo studies were carried out on male Sprague-Dawley rats with an average weight of 250 g supplied by the animal facility at University of Santiago de Compostela (Spain). The animals were treated according to the guidelines for laboratory [34,35]. The experiments were approved by the Galician Network Committee for Ethics Research following the Spanish and European Union (EU) rules (86/609/CEE, 2003/65/CE, 2010/63/EU, RD 1201/2005 and RD53/2013). The animals were kept in individual cages at controlled conditions of temperature and humidity (22 °C and 60 %) with free access to water and food, with day-night cycles regulated by artificial light.

Each component of the optimized formulations, i.e. CELLUVISC®, VISMED®, DXMa (20 mg/g)/HPγCD (600 mg/mL) aqueous solutions, Nano/DXMa and Gel B were radiolabeled by incorporating 100 µL ^{18}F -fluorodeoxyglucose (^{18}F-FDG) in a volume of 1 mL of either hydrogel or cyclodextrin based aqueous solution until homogenization. Randomly taken samples from each labeled component were measured using a high-precision dose calibrator (Atomlab 500, Biodex Medical System, Inc., New-York, NY, USA) in order to control radiotracer uniformity. Positron emission tomography and computerized tomography (PET/CT) images were acquired using the Albira PET/CT Preclinical Imaging System (Bruker Biospin, Woodbridge, Connecticut, USA).

The anesthetized animals were positioned into the imaging bed and 7.5 µL of each formulation labeled with ^{18}F-FDG was instilled into the conjunctival sac eye using a micropipette. The administered radioactivity was 0.35 ± 0.08 MBq. Therefore, the ^{18}F-FDG labeled component (CELLUVISC®, VISMED®, DXMa (20 mg/g)/HPγCD (600 mg/mL) aqueous solutions as well as the ^{18}F-FDG labeled Nano/DXMa and Gel B were tested. Static PET frames at different times were acquired during 5 hours following instillation. Three animals (6 eyes) were tested for each formulation.

The results were corrected to radioactive decay. Graphical representations of radioactivity versus time were obtained. The fitting of the remaining formulation versus time to a monoexponential decay equation using a single compartmental model was performed using pK Solver [36]. A non-compartmental analysis was also performed calculating the mean residence time (MRT) and the total area under the curve (AUC) of the remaining formulations (%) versus time. All data are expressed as mean value ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA test, and the level of significance was set at 5 %.

3. Results and discussion

3.1. Formulation and physico-chemical characteristics

3.1.1. DXMa-loaded surface-decorated γCD-C_{10} nanoparticles

DXMa was loaded in positively charged γCD-C_{10} nanoparticles through co-nano-precipitation with a cationic amphiphile derivative (namely MAP-103a), taking advantage of the interaction

between the aliphatic segments of the amphiphiles [28]. The size distribution (mean size and polydispersity index PI), zeta potential (ζ) and drug content are reported in Table 2. The colloidal system was characterized by a mean size range of 90 - 110 nm, which is compatible with a topical ocular administration. Indeed, this value is well below the threshold required for this route of administration. Moreover, a positive value of zeta potential close to + 40 mV was obtained for the nanoparticles. This surface charge is expected to improve adhesive properties of the nanosystem, which could prolong the residence time of the drug in the *cul-de-sac*, prevent tear washout (due to tear dynamics), and increase ocular bioavailability [8]. Also, the nanosuspension had substantial and stable charge loading in DXMa corresponding to drug levels reaching 0.8 mg/mL, a value 38 times the solubility of DXMa in water and close to the DXM amount present in conventional eye drops (DEXAFREE® and MAXIDEX®). The DXMa amount in the colloidal suspensions correspond to an EE % close to 98.75 % and a drug association (DA) of 100 % since no DXMa was detected in the supernatant. The high EE % and DA values confirm that the formulation design and production process are suitable for dexamethasone acetate encapsulation.

The low DXMa water solubility is in favor of an DXMa loading in γCD-C_{10} based nanoparticles. Nevertheless, it is well known that the solvent displacement technique allows to associate significant amounts of drug with a large part that is adsorbed on the surface. No DXMa desorption was observed with time at 25 °C, suggesting that DXMa did not form stacking layers at the nanoparticle surface. The formulation conditions were optimized in order to avoid this phenomenon. Indeed, a higher initial DXMa amount in the conditions of nanoprecipitation did not yield stable γCD-C_{10} nanostructures (unshown results). γCD-C10 nanoparticles were observed by TEM in order to observe their morphology. (Figure 3). As expected, the nanoparticles were spherical, no aggregation phenomena were visible.

Table 2. Physicochemical characteristics of DXMa-loaded surface-decorated γCD-C10 nanoparticles (mean ± SD, n = 3)

Formulation	Mean size (nm)	PI	ζ (mV ± SD)	EE (%)	DA (%)
DXMa-loaded in *γCD-C_{10}*/MAP 103a nanoparticles	102 ± 10	0.017 ± 0.02	+ 38 ± 5	98.75	100

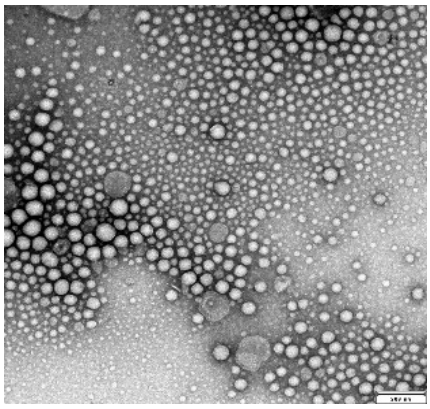

Figure 3. TEM image of negatively stained unloaded loaded cationic γCD-C_{10} nanoparticles

3.1.2. Optimized formulations of Nano-Gel B and Gel B

In order to obtain a topical mixed vehicle for DXMa, combining Gel B and the nanoparticles, two operating procedures were investigated. The main objective was to avoid the dilution of Gel B by directly mixing the colloidal suspension with preformed Gel B. The first methodology consisted in using the HPγCD/DXMa solution as the aqueous dispersing phase in the co-nano-precipitation process. The second operating approach was the successive addition of the different components of Gel B into a preformed concentrated DXMa loaded γCD-C_{10} nanoparticles. Only the second strategy made it possible to achieve a satisfying result, without floculation nor precipitation phenomena, ensuring a high drug percentage. As a result, the newly designed Nano-Gel B was slightly opalescent and presented a DXMa concentration of 21 mg/g as well as a pH and osmolality values (Table 3) that meet the requirements of the ophthalmic topical route [37].

Table 3. Physicochemical characteristics of Nano-Gel B and Gel B formulations

Formulation	Gel B	Nano Gel B
DXMa content (mg/g)	20	21
pH	7.0	7.0
Osmolality (mOsm/Kg)	425	403

3.1.3. Rheological measurements

Figure 4 a and b present the dynamic viscosity of Nano-Gel B as a function of shear rate (0.11 – 100 s^{-1}) at 35 °C, measuring 5 points per decade and with 20 s equilibration's time. Gel B and Nano-Gel B exhibit a Newtonian behavior. Nano-Gel B displays a viscosity of 9.6 mPa.s ± 10 % and Gel B a viscosity of 18.6 mPa.s ± 10 %. So, the combination with the cationic nanoparticles decreased the viscosity of the Gel B. Nevertheless, this range of viscosities is well tolerated by patients because it does not lead to a blurred vision nor foreign body sensation, often leading to a faster elimination due to tears reflex and blinks [38].

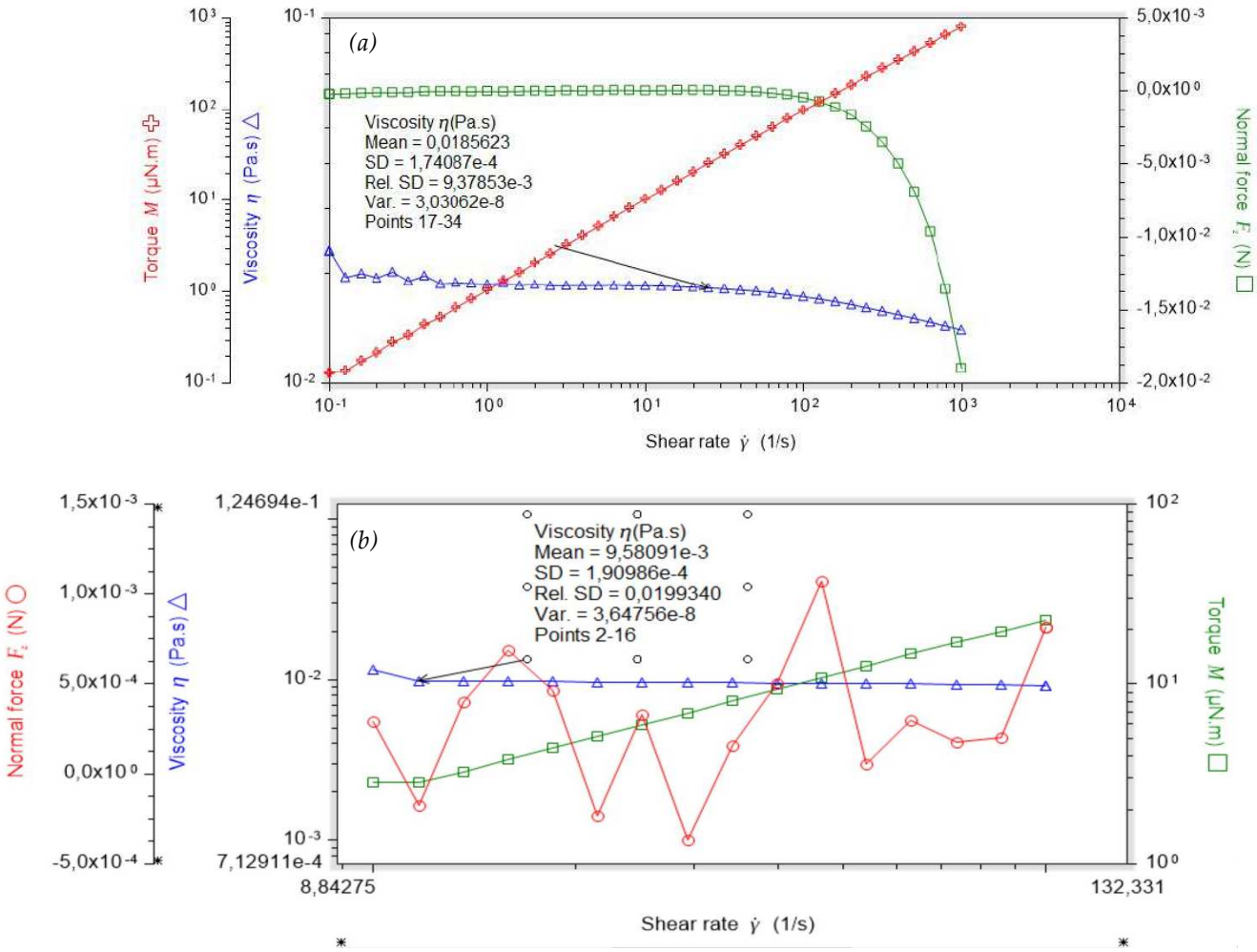

Figure 4. Dynamic viscosity of (a) Gel B and (b) Nano-Gel B performed in the range of 0.11 to 100 s^{-1} at 35 °C

The tears present a viscosity of 1.5 mPa.s, but a non-Newtonian flow due to the presence of mucins and other macromolecules. Increasing the viscosity of a preparation may influence drug bioavailability by improving ocular retention time [39]. However, some studies demonstrated that a viscosity below 10 mPa.s, as the one of Nano-Gel B, leads to undetectable changes in drainage rate and does not affect ocular retention time [40,41]. Anyway, the viscosity of the solution is not the only factor that influences solution drainage. The rheological, spreading and film properties of the solution are also important as the mucoadhesive properties of the main components of the formulation [42].

3.1.4. Mucoadhesion

As known, the conjunctiva and the cornea are protected by the tear film, a multi-layered structure and the layer closed to the cornea is an aqueous-mucin gel layer. Mucins are negatively charged and change the hydrophilic corneal surface to an hydrophilic surface by adhering the glycocalyx to the cornea. Mucins can also play an important role in drug ocular bioavailability depending of its behavior as barrier or retention site [43].

Therefore charged nanoparticles increase retention time in mucosal surface due to interaction between nanoparticles and mucosa, as demonstrated by Shen *et al.* [44].

A simple and indirect method used to evaluate this interaction is ZP measurement with and without mucin suspension. Zeta potential (ZP) value is related to the measurement of the surface charge that a specific material possesses or acquires when suspended in a fluid. This study demonstrated that the ZP values of Nano-Gel B and Gel B are quite similar. Indeed, Gel B ZP value (- 41.1 ± 2.3 mV) is close to Nano-Gel B ZP value (- 41.6 ± 1.1 mV) (Figure 5). Although present in the mixed nano-gel, the low concentration of positive charges of the nanoparticles are certainly masked and did not impact the overall negative zeta potential value. These negative values are in accordance with the anionic nature of the polysaccharides hyaluronic acid (HA) and carboxymethylcellulose due to the presence of carboxylic groups. HA is present in VISMED®, Nano-Gel B and Gel B. The mucins also present a negative ZP value due to their carboxyl and sulfate groups. The obtained value is quite different from the one described in literature, which is approximately -10 mV [29]. This difference could be explained by a different degree of hydration [45]. An important reduction, superior to 15 mV, in ZP absolute value after adding mucin 5 % (w/v) suspension to Nano-Gel B highlighted the interaction between mucin and nanoparticles [30,46]. Both Nano-Gel B and Gel B presented a reduction of the negative charge with the addition of mucin, showing the reduction of electrostatic repulsion [29].

This method is a characterization tool for mucoadhesive formulation screening. Given the complex composition of the tear fluid and its evolution during inflammatory phenomena, a definite *in vivo* correlation is difficult to establish. Further *in vivo* studies are necessary to confirm real mucoadhesion properties [32].

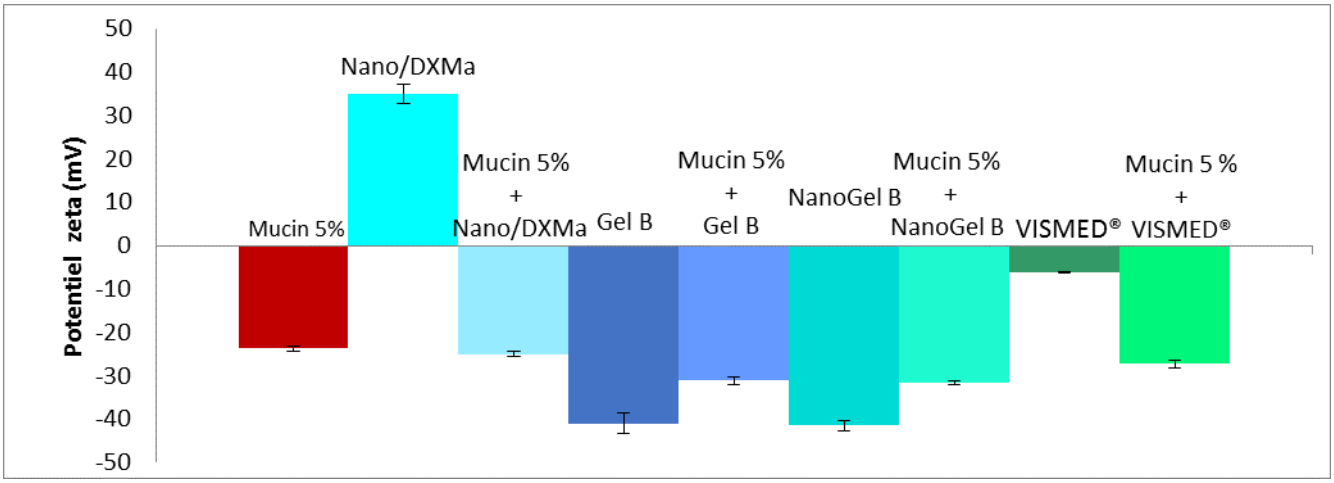

Figure 5. ZP values of mucin 5% and Nano/DXMa, Nano-Gel B, Gel B and VISMED® with or without mucin 5 %

3.2. *In vitro* DXMa release profile

In vitro release assessment was performed on several formulations, namely, Gel B, Nano-Gel B as well as two reference marketed eye drops, MAXIDEX® and DEXAFREE®. The sink conditions for dissolution testing were respected. As previously described, a complete drug release was observed at 24 h (1440 min) for DEXAFREE® and MAXIDEX® with the major part of drug released within 30 min with 92 % and 90 %, respectively (Figure 6). For Gel B, 56 % of the drug diffused in the external medium after 2 h. The missing DXMa fraction was recovered in the cell extraction, meaning that a part of Gel B remained stuck to the cell surface during the experiment, limiting further DXMa release. This characteristic was not present with Nano-Gel B, so the DXMa loaded nanoparticles impacted the release profile of mixed Nano-Gel. In this case, a sequential release of

DXMa was observed, showing a first rapid release of 40 % DXMa, followed by a slowed down drug release of 30 % over 8 hours and final 15 % drug release during 16 hours. This type of multiphasic release profile should make it possible to meet the therapeutic requirements of ocular inflammations requiring a large loading dose followed by a later release that makes it possible to maintain an effective concentration of the active ingredient over a longer period. In addition, it should reduce the frequency of instillations and thus improve the comfort of patients.

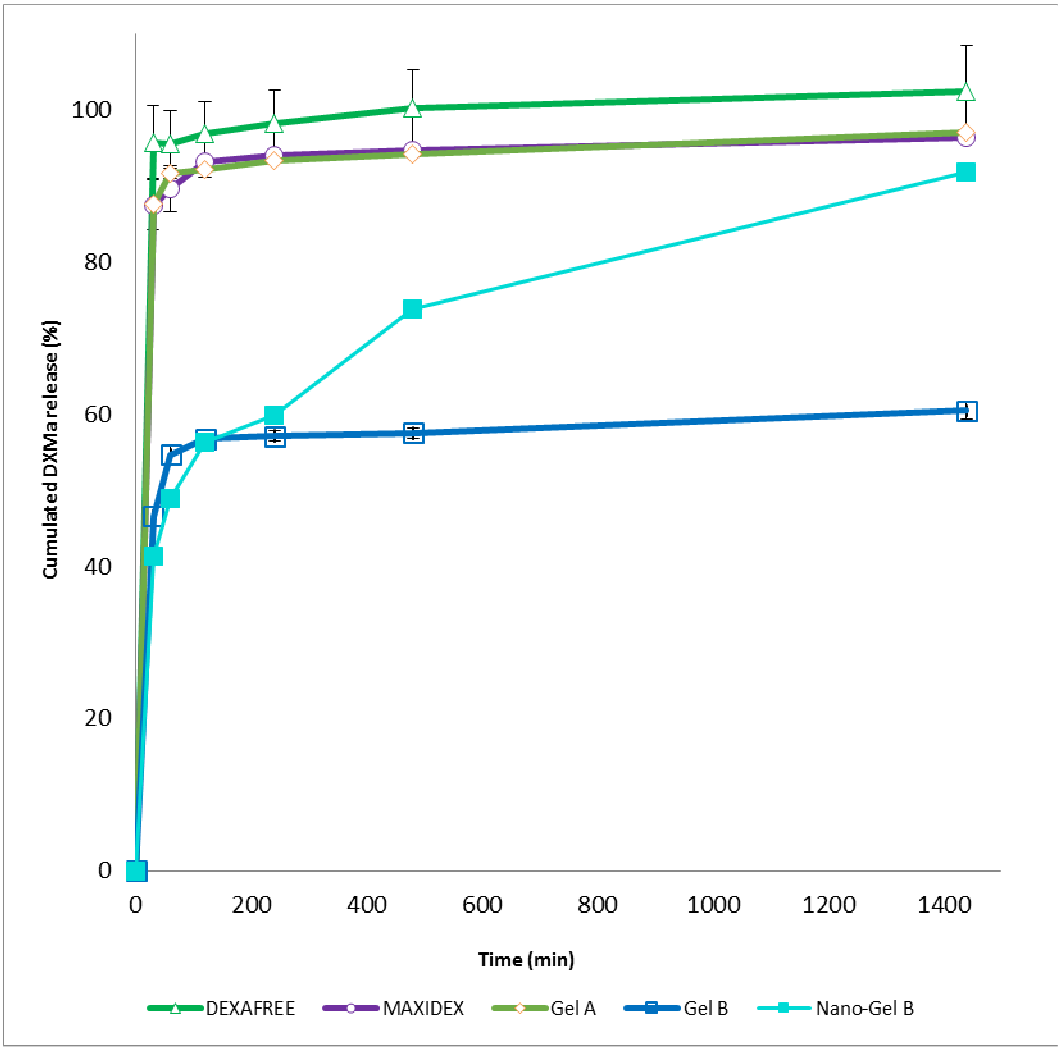

Figure 6. *In vitro* drug release from MAXIDEX®, DEXAFREE®, Nano-Gel B and Gel B in PBS, at 35 °C.

3.3. Cell toxicity

3.3.1. MTT assay

To evaluate *in vitro* cell toxicity Nano/DXMa, Nano-Gel B, Gel B with or without DXMa, HPγCD (600 mg/mL), DEXAFREE® and MAXIDEX®, HCE cells grown in the presence of each formulation were evaluated by quantitative determination of living cells, after 30 min, 2 h and 24 h at 5 and 0.05 % concentration (Figure 7). The results are analyzed according to the Organization for Economic Co-

operation and Development (OECD) guidelines for short time exposure *in vitro* test method [47] (Table 4).

Table 4. Prediction model inspired by the in short time exposure according to OECD guideline [47]

Cell Viability		UN GHS Classification	Applicability
At 5 %	At 0.05 %		
> 70 %	> 70 %	No category	No serious damage nor eye irritation effect
≤ 70 %	> 70 %	No prediction can be made	No prediction can be made, eventual eye irritation
≤ 70 %	≤ 70 %	Category 1	Serious eye damage

As shown previously, Gel B present an acceptable level of cytotoxicity to HCE cells and is considered rather well tolerated by HCEC. As well, DEXAFREE® presents a cell viability higher than 70 % at 5 and 0.05 % after 30 min, 2 h and 4 h. At the opposite, the Nano/DXMa and Nan-Gel B are classified in the non predictable category, according to UN GHS [47] . Indeed, their cell viability were lower than 70 % at 5 % and higher than 70 % at 0.05 %. Their effect on HCEC appeared time and concentration dependent. A similar cytotoxicity profile was observed in the case of the reference suspension MAXIDEX®. Furthermore, Nano-Gel B seemed to present a better cell viability which is still superior to that of DXMa-loaded nanoparticles. Gel B seems to be relatively safe for HCEC.

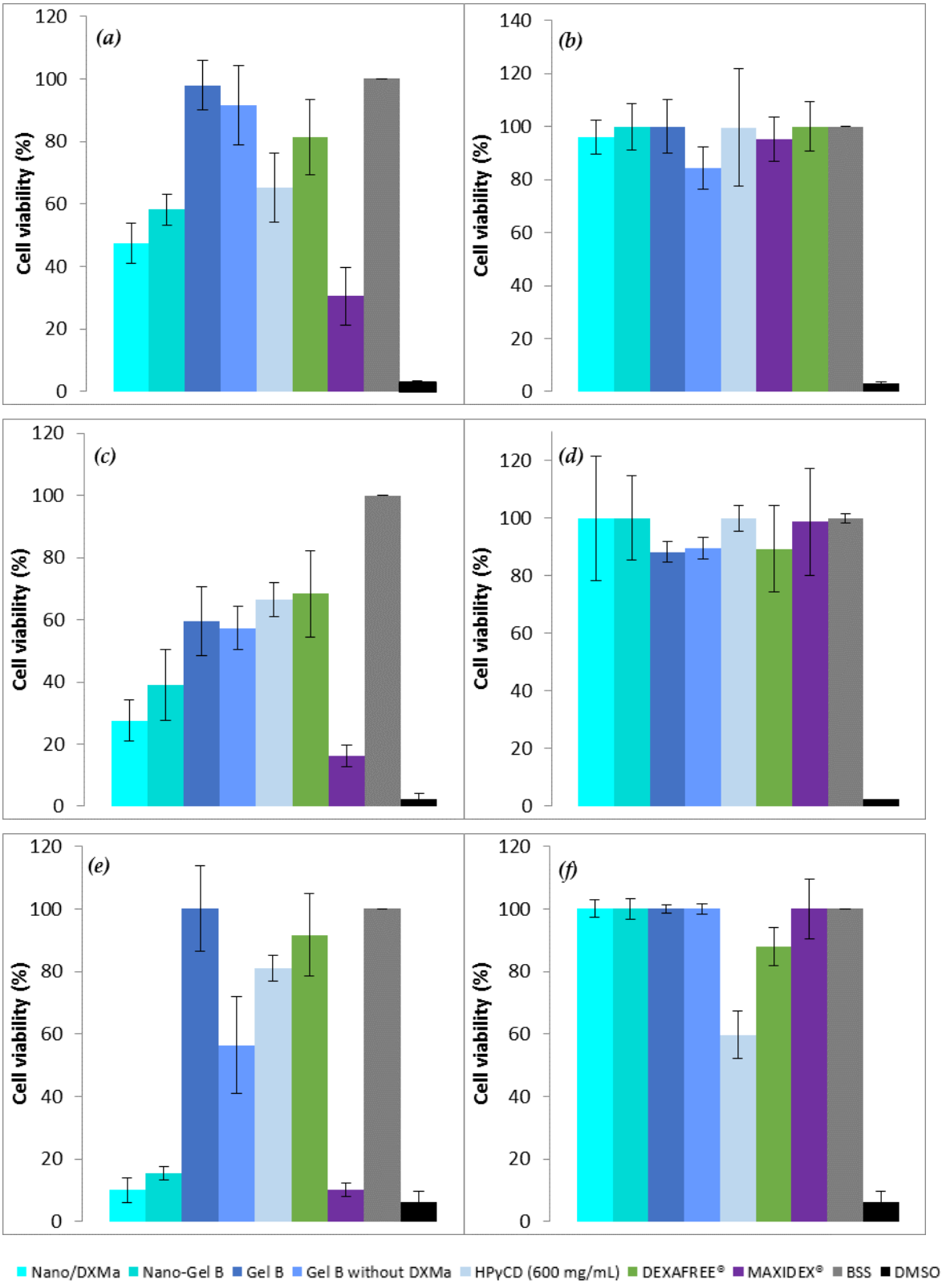

Figure 7. Cell viability of Nano/DXMa, Nano-Gel B, Gel B with or without DXMa, HPγCD (600 mg/mL), DEXAFREE® and MAXIDEX® versus BSS and DMSO. (a) 5 % concentration during 30 min, (b) 0.05 % during 30 min, (c) 5 % during 2 h, (d) 0.05 % during 2 h, (e) 5 % during 24 h, (f) 0.05 % during 24 h

3.3.2. ALAMAR BLUE® assay

To complete *in vitro* cell biocompatibility study, ALAMAR BLUE® assay was performed by using fluorescence. This assay is based on the ability of metabolically active cells to convert the ALAMAR BLUE® reagent into a fluorescent and colourimetric indicator. Damaged and non-viable cells have lower innate metabolic activity, and generate a proportionally lower signal (Figure 8). Nano-Gel B, Gel B with or without DXMa, HPγCD (600 mg/mL), DEXAFREE® and MAXIDEX® showed acceptable levels of metabolic activity as DEXAFREE®, with a cell viability even > 70 % after 2 h of exposure. The Nano/DXMa, showed a lower metabolic activity, close to 60 %, and its effect on eye cannot be predicted. According to these results, we can demonstrate that Gel B seems to be considered as biocompatible. Both formulations have to be evaluated *in vivo* by a Draize test or low volume eye-irritation test (LVET) in order to observe degree of irritation and identify reversible and irreversible ocular damage [48].

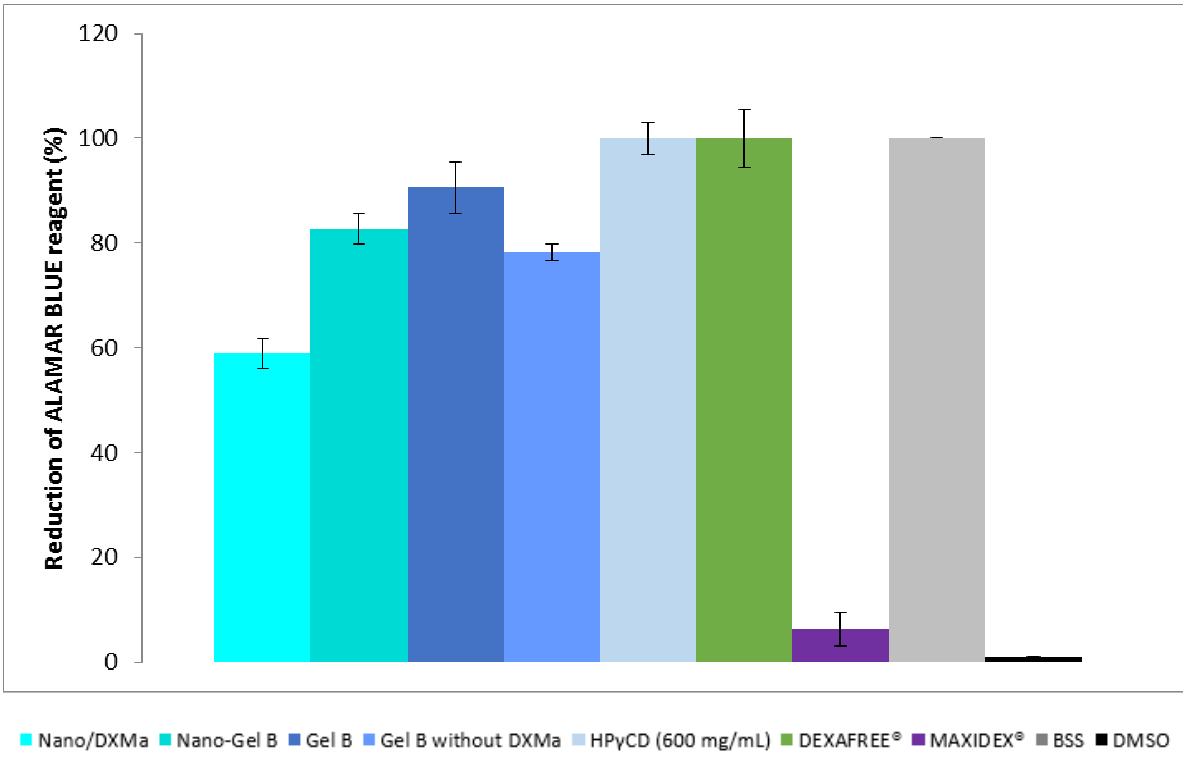

Figure 8. Reduction of ALAMAR BLUE® reagent (%) of Nano/DXMa, Nano-Gel B, Gel B with or without DXMa, HPγCD (600 mg/mL), DEXAFREE® and MAXIDEX® versus BSS and DMSO

3.4. *Ex-vivo* evaluation of the corneal permeation

Ex vivo permeation of Nano-Gel B, Gel B, DEXAFREE® and MAXIDEX® were evaluated using the excised porcine cornea. The amount of DXMa permeated through the excised cornea from Nano-Gel B was higher than other formulations (Figure 9). With Nano-Gel B, a maximum of 85.04 µg (i.e. 1.01 % amount of drug applied) which is about 3.8-fold higher than DEXAFREE® and 5.2-fold higher than MAXIDEX®. As previously described, Gel B present a good corneal permeation of 71.71 µg of DXMa permeates (i.e. 0.89 % amount of drug applied) and it is nearly 3.2-fold higher than DEXAFREE® and 4-fold higher than MAXIDEX®. This suggests that both Nano-Gel B and Gel B might be more effective than marketed formulations to treat corneal inflammations. Moreover, these results are associated to a good corneal hydration level, between 76-80 %.

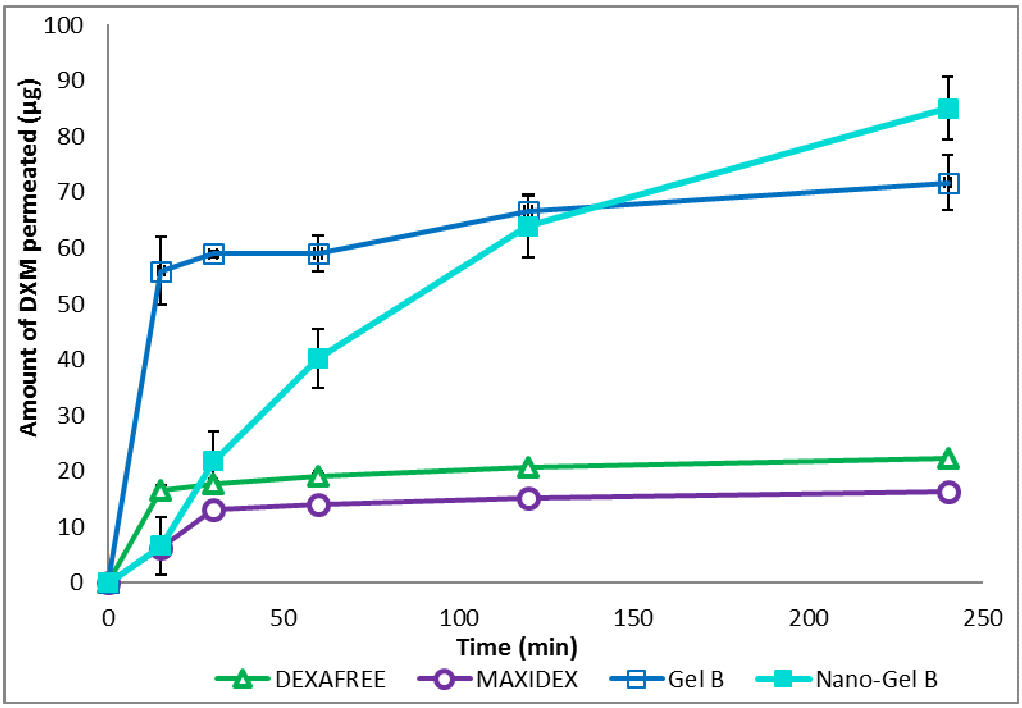

Figure 9. Amounts of DXM permeated through excised cornea of Nano-Gel B, Gel B, DEXAFREE® and MAXIDEX®

The correlations between *in vitro* release and amount of DXMa permeated of Nano-Gel B have been depicted in Figure 10. The correlation coefficient was evaluated to 0.984 and regression equation of *in vitro* release vs *ex vivo* permeation graph was calculated to y = 3.2984 x – 117.36 and indicated a good correlation between *in vitro* release and *ex vivo* permeation.

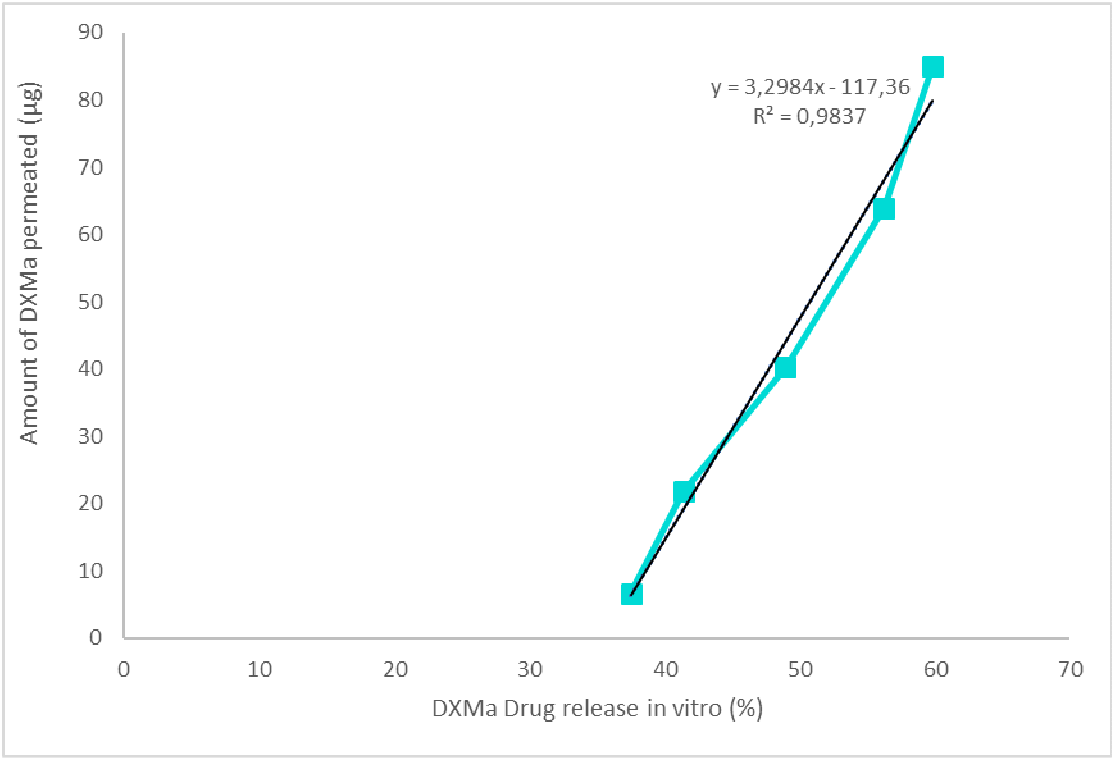

Figure 10. Correlation between *in vitro* drug release and *ex vivo* permeation of Nano-Gel B

Dexamethasone is a highly potent long acting drug requiring a far lower dosage compared to other intermediate and short acting glucocorticoids, i.e. nearly 5 times lower than prednisolone, methylprednisolone and 25 times lower than hydrocortisone, to elicit a biological response [49,50]. As demonstrated by Djalilian *et al.*, dexamethasone inhibits inflammatory cytokines in human corneal epithelial cell and fibroblast cell lines with a concentration range of 0.1 to 10 µM. The marketed formulation DEXAFREE® contain 1 mg/mL drug, i.e. 1.9 mM. As previously described, Nano-Gel B released DXMa allowing a maximum drug amount of 85.04 µg to be permeated across excised cornea. As well, Gel B allows a permeated drug amount of 71.71 µg. Therefore, considering the normal tear volume to be about 6 to 10 µL, assuming no tear drainage and similar release behavior as observed in 13 mL of PBS, 85.04 and 71.71 µg of DXMa (Mw = 434.5 g/mol) in 10 µL of tears, would theoretically be almost 19.5 and 16.6 mM, which is about 10- and 8-fold higher than the concentration provided by DEXAFREE®. These latter results warranted to be clinically relevant and within the therapeutic index [51].

3.5. *In vivo* evaluation of the residence time on the ocular surface

The biopermanence of Nano/DXMa and Gel B, HPγCD (600 mg/mL), VISMED® and CELLUVISC® was characterized on the ocular surface of rats by 18F-FDG radiolabeling followed by radioactivity in PET over 5 h (300 min). All the formulations tested present a higher biopermanence than a control solution, Balanced Salt Solution, (BSS), whose composition is close to tears (Figure 11).

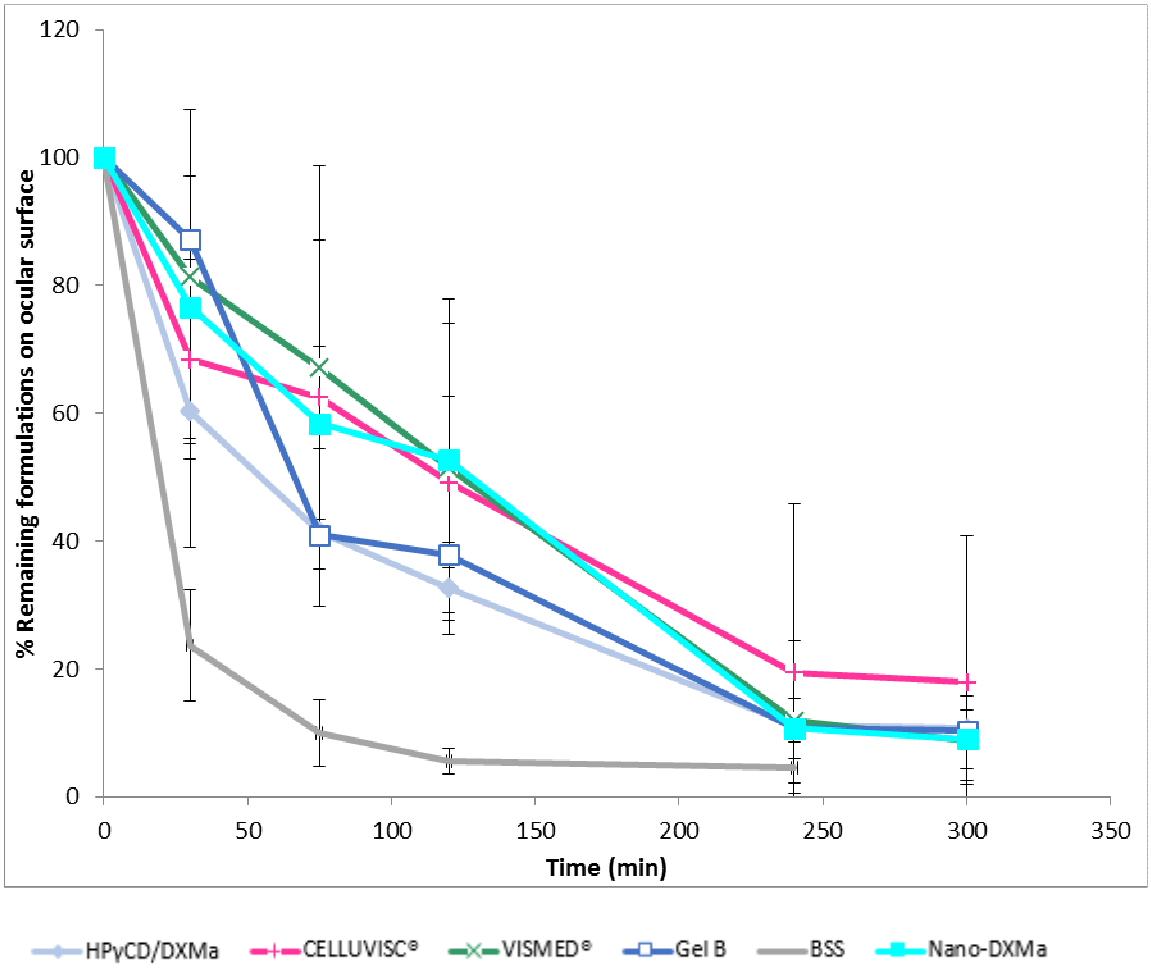

Figure 11. Ocular biopermanence of Nano/DXMa, Gel B, HP-γ-CD (600 mg/mL), VISMED® and CELLUVISC® versus BSS

According to the literature, increasing fluid viscosity increases the residence time to some extent, by delaying the tear action [41]. This is in agreement with our observations since the reference marketed gels carboxymethylcellulose and sodium hyaluronate more viscous than the other components, present a higher ocular residence time with a MRT of 196.9 and 133.7 min respectively. The Nano/DXMa present a slight viscosity of around 9.5 mPa.s but the impact on $T_{1/2}$ and MRT values is really significant and higher than Gel B values. Also, the presence of nanoparticles suspension really prolongs the ocular surface residence. We will test rapidly the Nano-Gel B, but we expect that the effects of nanoparticles and viscosities enhancers will act synergistically. Nano-Gel B will present a higher $T_{1/2}$ and MRT values, which will explain a part of the great extend in transcorneal permeation.

The data summarized in Table 5 show that pharmacokinetic parameters such as $T_{1/2}$, MRT, and k, are significantly different between each Gel B, Nano/DXMa and BSS, at $p < 0.05$. The data collected from 3 to 240 min were significantly different between Nano/DXMa, Gel B, DXMa (30 mg/mL)/HPγCD (600 mg/mL), VISMED®, CELLUVISC® and BSS, at $p < 0.05$.

Table 5. Ocular biopermanence parameters measured *in vivo* for Gels A and B, HPβCD (600 mg/mL), HPγCD (600 mg/mL), VISMED® and CELLUVISC® versus BSS

Components	Viscosity at 35 °C (mPas)	k (min^{-1})	$T_{1/2}$ (min)	MRT	R^2
CELLUVISC®	167 – 260	0.007 ± 0.003	136.5 ± 95.5	196.9 ± 137.8	0.9738
VISMED®	16.8	0.008 ± 0.003	92.7 ± 26.7	133.7 ± 38.5	0.9404
Gel B	18.6	0.0096 ± 0.036	77.4 ± 28.8	111.6 ± 41.5	0.9837
Nano/DXMa	9.6	0.093 ± 0.006	93.9 ± 44.3	135.4 ± 63.8	0.9738
DXMa/HPγCD (30 mg/mL/600 mg/mL)	6.5	0.11 ± 0.003	70.2 ± 21.9	101.3 ± 31.6	0.9697
BSS	1.5	0.046 ± 0.015	16.0 ± 5.2	23.1 ± 7.6	0.9965

4. Conclusions and Future Prospects

In conclusion, the data provided in this study demonstrate that Nano-Gel B cause no adverse effects to the eye and is mucoadhesive. Nano-Gel B and Gel B present a good corneal permeation which is 3.2-3.8-fold higher than DEXAFREE® and 4-5.2-fold higher than MAXIDEX®. These good results have to be confirmed *in vivo* with pharmacokinetic, efficacy and tolerance studies.

Author Contributions: R.M., P.AF., L.C., V.V., F.B., F.J.O.E., A.F.F., D.L., V.B.M. carried out all the experiments, R.M.,L.C., D.W. and A.G., designed all the studies, oversaw all the experiments and contributed to draft the manuscript and intellectually to the development of the project.

Fundings: This research received no external private funding.

Acknowledgments: This work was supported by Labex ARCANE (ANR-11-LABX-0003-01) and Institut de Chimie Moléculaire de Grenoble (FR 2607) and the Glyco@Alps program (ANR-15-IDEX-02).

Conflicts of Interest: The authors declare no conflict of interest.

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DISCUSSION GENERALE

Dans ce contexte de thérapie locale de l'inflammation oculaire, les anti-inflammatoires stéroïdiens ont démontré leur efficacité tant dans le traitement de la surface que du segment antérieur de l'œil. Cependant, en dépit des nombreux efforts déployés par les chercheurs et les industries pharmaceutiques, les ressources thérapeutiques disponibles sur le marché demeurent restreintes pour la voie topique ophtalmique. Bien que cette voie d'administration soit non invasive, bien tolérée et facile d'accès, elle relève de nombreux défis galéniques. En effet, les anti-inflammatoires, dont l'acétate de dexaméthasone, présentent généralement une faible solubilité en milieu aqueux (0,021 mg/mL à 25 °C) et une faible pénétration oculaire [1,2]. En outre l'élimination pré-cornéenne importante des médicaments déposés sur la cornée, entraîne une biodisponibilité, généralement inférieure à 5 % [3]. D'un point de vue clinique, des instillations fréquentes deviennent alors nécessaires pour atteindre des concentrations efficaces. Ainsi dans le traitement de rejet aigu de greffe ou des états inflammatoires sévères, le collyre DEXAFREE® (phosphate de dexaméthasone) peut être administré à raison d'une goutte toutes les heures pendant 24 à 48 h, puis une goutte toutes les 4 heures lorsqu'une amélioration est observée [4].

1. Relever les défis galéniques

a. Améliorer la solubilité apparente de la DXMa

Différentes approches galéniques sont envisageables pour améliorer la solubilité des substances actives peu ou pas solubles dans l'eau, telles que la co-solvatation, la micellisation ou l'utilisation de cyclodextrines (CD) [5,6]. Après différents essais préliminaires, notre choix s'est orienté vers la mise en œuvre des cyclodextrines hydrosolubles commerciales. Ces excipients présentent le grand avantage d'améliorer la solubilité apparente des molécules hydrophobes et ainsi leur biodisponibilité, tout en étant biocompatibles [7,8]. Cette stratégie est déjà utilisée pour la voie topique ophtalmique, notamment dans la spécialité INDOCOLLYRE® où l'indométhacine est solubilisé en présence d'hydroxypropylβCD. Au cours de nos essais, plusieurs cyclodextrines ont été testées, notamment la βCD, la γCD, la sulfobutyletherβCD (SBEβCD), l'HPβCD ou HPγCD (Figure 5). Nos meilleurs résultats de solubilisation ont été obtenus en présence d'HPβCD et d'HPγCD. Les associations DXMA/HPβCD et HPγCD/DXMa contenant 600 mg/mL de chaque

cyclodextrine ont permis de multiplier par un facteur 500 et 1550 la solubilité apparente à 25 °C de la DXMa, soit 10 mg/mL de DXMa solubilisée par l'HPβCD et 30 mg/mL par l'HPγCD. Cette augmentation substantielle de la solubilité apparente de la DXMa est très probablement attribuable à son inclusion dans la cavité de la cyclodextrine correspondante.

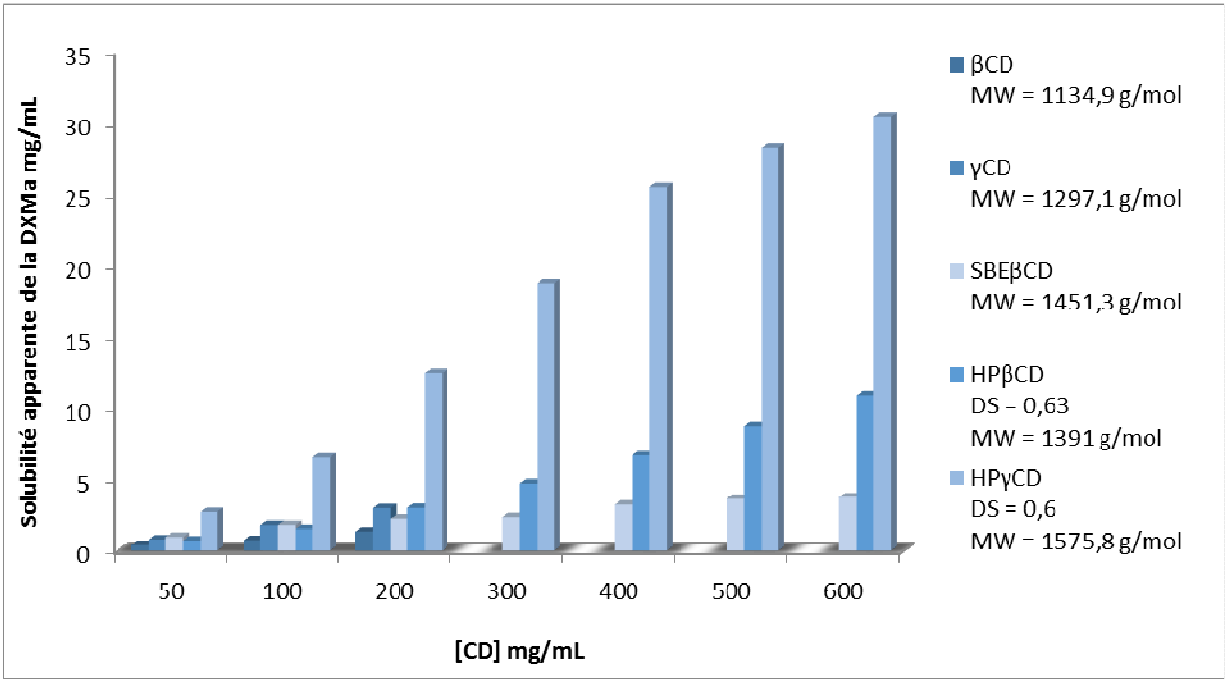

Figure 5. Impact des cyclodextrines : βCD, γCD, SBEβCD, HPβCD et HPγCD sur la solubilité apparente de l'acétate de dexaméthasone (DXMa) dans l'eau à 25 °C

Les constantes d'associations des deux complexes les plus intéressants du point de vue de l'augmentation de la solubilité, DXMa/ HPβCD et DXMa/ HPγCD, ont été calculées par la méthode de diagramme de solubilité et par CLHP en assumant une stœchiométrie 1:1 [9]. Les efficacités de complexation (*Complexation Efficiency*, CE) ont également été calculées. Les résultats sont regroupés dans le Tableau 5. Il apparait clairement que l'affinité de la DXMa pour la cavité de la cyclodextrine est plus forte pour l'HPγCD que pour l'HPβCD. En effet, le CE de 0,071 suggère qu'une molécule d'HPβCD sur 11 forme un complexe avec la DXMa alors que le CE de 0,259 suggère que 3 molécules d'HPγCD sur 4 sont complexées à la DXMa [10].

Nous assumons de ne pas avoir étudié de manière très approfondie les composés d'inclusion entre la DXMa et les cyclodextrines. Une étude du complexe solide n'a pas été envisagée sachant que les composés seraient incorporés sous forme liquide dans les formulations. Concernant les complexes en milieu liquide, la mise en évidence de la formation de composés d'inclusion par RMN du proton et du carbone 13 aurait été difficile et complexe compte tenu de la grande statistique des protons dans les dérivés cyclodextrines HPβCD et HPγCD pour lesquels les degrés de substitution (DS) sont eux-mêmes des valeurs moyennes. Des études d'interaction en titrage calorimétrique isotherme *(Isothermal Titration Calorimetry, ITC)* seront réalisées ultérieurement.

Tableau 5. Pentes, constantes d'association K et CE des complexes DXMa/cyclodextrine hydrosolubles à 25 °C

Type de CD	Pente	Coefficient de corrélation R^2	$K_{1:1}$ (M^{-1})	CE
HPβCD	0.066	0.995	1462	0.071
HPγCD	0.206	0.999	5368	0.259

A ce stade de notre recherche, nous disposons de deux complexes DXMa/ HPβCD et DXMa/ HPγCD, qui permettent d'augmenter significativement la solubilité apparente de la DXMa. Dans l'hypothèse de l'élaboration d'un médicament, il serait judicieux de choisir le complexe DXMa/HPβCD pour des raisons réglementaires. En effet, l'HPβCD possède une monographie dans les Pharmacopées Européenne et Américaine. Le complexe DXMa/ HPγCD est intéressant dans la mesure où sa capacité à solubiliser la DXMa est supérieure à celle de l'HPβCD. Le dérivé HPγCD, lui, ne possède pas de monographie à la Pharmacopée Européenne. Cependant, il est déjà utilisé dans la spécialité VOLTAREN®OPHTA, commercialisée notamment en Nouvelle Zélande. Cette utilisation pour la voie topique ophtalmique permet de croire que le profil toxicologique et la tolérance ophtalmique de ce dérivé sont satisfaisants. Les osmolalités mesurées de ces deux solutions de cyclodextrines à 600 mg/mL sont de 756 mOsm /kg pour l'HPβCD et 796 mOsm/kg pour l'HPγCD ce qui est incompatible pour l'œil [11]. Ces résultats sont donc à prendre en considération pour la suite

de la formulation. Finalement, nous avons choisi de conserver les deux complexes HPβCD/DXMa et HPγCD/DXMa.

b. Augmenter le temps de résidence

Dans un second temps, nous avons décidé d'incorporer les solutions d'HPβCD/DXMa (600 mg/mL /10 mg/mL) et HPγCD/DXMa (600 mg/mL /30 mg/mL) dans des gels bioadhésifs. Ces gels adhèrent à la paroi ophtalmique et limitent ainsi l'élimination rapide de l'actif de la surface cornéenne [12]. Nous avons opté pour la mise en œuvre de gels commercialisés (CELLUVISC® - Carmellose sodique, GEL-LARMES® - Carbomère 974P et VISMED®- Hyaluronate de sodium), car ils présentent des profils physicochimiques et toxicologiques favorables déjà établis. Le mélange des solutions à base d'HPβCD ou d'HPγCD/DXMa avec les gels commerciaux a été étudié plus en détail à l'aide de deux plans de mélange afin de définir deux formulations optimisées de DXMa. L'objectif de ces deux plans d'expériences était d'augmenter la fraction de DXMa solubilisée tout en ayant une osmolalité compatible avec l'œil, comprise entre 200 et 450 mOsm/Kg [11].

Les deux plans d'expériences ont présenté une excellente précision de la régression avec des valeurs R^2, R^2 ajusté et R^2 prédictive supérieures à 0,85, confirmant ainsi la prédictivité satisfaisante des deux modèles [13]. Les compositions des deux formules sont présentées dans le Tableau 6.

Tableau 6. Composition des formules optimisées des Gels A et B

	Composition	Quantité (g)
Formulation optimisée Gel A	VISMED®	0,300
	HPβCD 600 mg/mL avec DXMa 10 mg/mL	0,700
	Formulation contenant 7 mg/g de DXMa avec une osmolalité de 449 mOsm/kg	
Formulation optimisée Gel B	CELLUVISC®	0,151
	VISMED®	0,085
	HPγCD 600 mg/mL avec DXMa 30 mg/mL	0,764
	Formulation contenant 20 mg/g de DXMa avec une osmolalité de 425 mOsm/kg	

c. Améliorer la charge de la formulation en substance active ainsi que son temps de résidence cornéenne

De manière à favoriser le temps de contact précornéen tout en prenant en charge une quantité supplémentaire de substance active, nous avons élaboré des nanostructures cationiques associant la DXMa qui ont ensuite été combinées aux formulations initiales associant gels commerciaux bioadhésifs /cyclodextrines hydrosolubles/DMXa. La présence de charges positives à la surface des nanoparticules favoriserait l'interaction de type électrostatique avec la surface anionique de la cornée et augmenterait le temps de résidence de l'actif tout en agissant comme un réservoir-dépôt [12] et par conséquent, elle pourrait améliorer la biodisponibilité [14]. En outre, la littérature rapporte que les systèmes nanoparticulaires, même non chargés, constituent d'excellents outils de formulation pour relever partiellement ou totalement certains défis galéniques de la voie oculaire en augmentant la solubilité des substances actives hydrophobes, en leur servant de réservoir, en favorisant le passage transcornéen des principes actifs. Par ailleurs, ces suspensions colloïdales peuvent être plus ou moins transparentes ou très légèrement opalescentes et donc troublent moins la vision des patients après leur instillation [15].

Ainsi, l'utilisation des systèmes colloïdaux dans la voie topique ophtalmique permettrait de réduire le nombre d'instillations et le risque d'effets indésirables [16] et d'augmenter la compliance des patients [17].

Dans le cadre de notre travail, nous avons utilisé le dérivé γCD-C_{10} comme molécule de base pour la préparation des nanoparticules. Ce dérivé est obtenu par greffage de chaînes alkyle en C_{10} sur la face secondaire de la γCD selon une voie de synthèse enzymatique développée au laboratoire. La γCD-C_{10} est capable d'auto-organisation sous la forme de structures nanométriques dans les conditions standard de nanoprécipitation [18,19]. Afin d'incorporer des charges positives dans les nanoparticules de γCD-C_{10} nous avons mis en œuvre la méthode de co-nanoprécipitation développée au laboratoire et qui consiste à injecter dans la phase aqueuse une phase organique contenant la γCD-C_{10} et un amphiphile chargé. Les premiers amphiphiles chargés mis en œuvre ont été des lipides cationiques PEGylés ou non (DMPE-PEG_{2000} amine, DSPE-PEG_{5000} amine et la stéarylamine). Dans les

assemblages obtenus, le segment lipophile de l'amphiphile cationique est ancré dans le réseau matriciel tout en orientant la partie chargée vers l'extérieur. On obtient des nanoparticules chargées positivement [20–22]. Les résultats obtenus avec cette première série d'amphiphiles n'ont pas été concluants. En effet, les systèmes colloïdaux se sont avérés instables après 7 à 14 jours de stockage à température ambiante.

Par la suite, nous avons testé un nouveau composé amphiphile cationique synthétisé au sein de notre groupe de recherche (Département de Pharmacochimie Moléculaire, UMR CNRS 5063). Il s'agit du ((S)-2,6-diamino-N-((S)-3-hydroxy-1-(octadecylamino)-1-oxopropan-2-yl)hexanamide dihydrochloride) dont la structure chimique est la suivante :

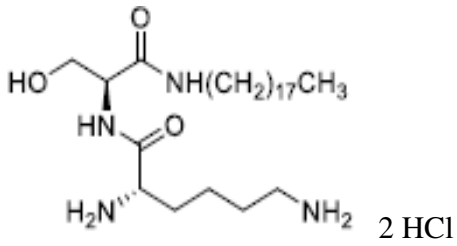

Figure 6. MAP-103a

Après optimisation des conditions de co-nanoprécipitation, des nanoparticules chargées positivement (+ 40 mV), présentant une taille de l'ordre de 100 nm et capables d'associer une quantité substantielle de DXMa ont été obtenues. Ces nanoparticules cationiques se sont révélées stables pendant au moins 2 mois à température ambiante.

Un nouveau travail de formulation a été réalisé afin d'associer les nanoparticules cationiques aux gels commerciaux et aux cyclodextrines hydrosolubles. Une formule optimisée a permis de combiner nanoparticules chargées et Gel B et de générer la formulation Nano-Gel B dosée à 21 mg/g soit une augmentation de 5 % comparé au Gel B initial (20 mg/g). La formule Nano-Gel B s'est avérée stable sur une période d'un mois. L'association nanoparticules cationiques avec le Gel A n'a pas été concluante, les préparations présentant systématiquement une instabilité physique dans les premières 24 heures.

A l'issue de ce travail de développement galénique, trois formulations, Gel A, Gel B et Nano-Gel B, ont été retenues en vue de leur évaluation biopharmaceutique.

2. Evaluation biopharmaceutique des trois formulations

Les différents tests comparatifs mis en œuvre lors de l'évaluation biopharmaceutique sont les suivants :

- Etude de la lyodisponibilité,

- Etude rhéologique, menée en partenariat avec le Laboratoire Rhéologie et Procédés de l'Université de Grenoble Alpes, France,

- Etude indirecte *in vitro* de la mucoadhesion par mesure du potentiel zêta

- Etude *in vitro* de la cytotoxicité sur cellule HCEC par le test MTT et l'ALAMAR BLUE®,

- Etude *ex vivo* du passage transcornéen sur cornée de porc isolée,

- Etude *in vivo* de la biopermanence sur la surface oculaire de rat par radiomarquage au ^{18}F-FDG et suivi de la radioactivité par tomographie à émission de positons (TEP) couplée à un scanner. Cette étude a été menée en collaboration avec le Département de pharmacologie, pharmacie et technologie pharmaceutique de la Faculté de Pharmacie et le Centre Hospitalier de l'Université de Saint Jacques de Compostelle, Espagne,

- Etude de la stabilité physicochimique et microbiologique des Gels A et B à 25 °C.

Les principales caractéristiques physicochimiques des trois formulations retenues sont compatibles avec l'administration topique ophtalmique (Tableau 7).

Les trois préparations sont des fluides Newtoniens avec des viscosités comprises entre 9 et 19 mPa.s. Ces niveaux de viscosité sont suffisants pour augmenter le temps de résidence cornéenne. Par ailleurs, ces niveaux de viscosité ne devraient pas entrainer de vision floue, ni de sensation de corps étranger. Cela limiterait donc le renouvellement des larmes et le clignement réflexe de paupières qui sont des phénomènes favorisant l'élimination des xénobiotiques [23,24].

L'objectif d'accroitre le temps de résidence oculaire est atteint. En effet, le temps de résidence augmente bien pour le Gel A et le Gel B, parallèlement à leur viscosité. Cette augmentation est confirmée par les résultats de l'étude *in vivo* de la biopermanence sur la surface oculaire. En effet, les temps de résidence moyen (MRT) du Gel A (67 min) et du Gel B (112 min) sont 2,9 et 4,8 fois supérieurs au *Balanced Salt Solution* (BSS) équivalente de larmes artificielles, dont le MRT est de 23 min.

Comme attendu, l'utilisation des nanoparticules cationiques seules ont amélioré le temps de résidence cornéenne par le biais des interactions de type électrostatique avec la couche de mucines chargées négativement. Leur MRT étant de 135 min, la contribution des nanoparticules à l'augmentation du temps de résidence oculaire apparait supérieure à celle des polysaccharides. L'étude de la biopermanence de la combinaison nanoparticules cationiques/ Gel B sera réalisée ultérieurement et permettra d'évaluer la synergie d'action des nanoparticules mucoadhésives et des polymères viscosants.

Tableau 7. Principales caractéristiques biopharmaceutiques des trois formulations retenues

Caractéristiques		Gel A	Gel B	Nanoparticules associées au Gel B
Composition (pour 1 g)		0,3 g de VISMED® 0,7 g d'HPβCD (600mg/mL)/DXMa (10mg/mL)	0,151 g de CELLUVISC® 0,085 g de VISMED® 0,764 g d'HPγCD (600mg/mL)/DXMa (30mg/mL)	0,151 g de CELLUVISC® 0,085 g de VISMED® 0,764 g d'HPγCD (600mg/mL)/DXMa (30mg/mL) 2,5 mL de suspension colloïdale DXMa/γCD-C10/MAP
Quantité de DXMa (par g)		7	20	21
Caractéristiques physico-chimiques	pH	7,5	7,0	7,0
	Osmolalité (mOsm/kg)	449	425	403
	Taille des particules (nm)	NA	NA	102 ± 10 nm, IP = 0,017 ± 0,02
Rhéologie	Viscosité (mPas)	13,2	18,6	9,6
	Type de fluide	Newtonien	Newtonien	Newtonien
Mucoadhésion	Potentiel Zeta sans mucine 5% (mV)	-24	-41	-41,6
	Potentiel Zeta avec mucine 5% (mV)	-24,8	-31,2	-31,6
Etude de cytotoxicité *in vitro*		Pas de prédiction	Biocompatible	Pas de prédiction
Etude *ex vivo* du passage transcornéen	Qté maximale de DXMa passée au travers de la cornée (µg)	40,48 ± 0,7	71,71 ± 5,0	85,04 ± 5,5
	Par rapport au DEXAFREE®	1,8	3,2	3,8
	Par rapport au MAXIDEX®	2,5	4,4	5,2
Etude de biopermanence *in vivo* par TEP/CT	K	0.015 ± 0.014	0.0096 ± 0.036	Non réalisé
	$T_{1/2}$ (min)	46.6 ± 4.8	77.4 ± 28.8	Non réalisé
	MRT (min)	67.2 ± 6.9	111.6 ± 41.5	Non réalisé
Stabilité physico-chimique et microbiologique		Stable 12 mois à 25 °C	Stable 12 mois à 25 °C	Non réalisé

NA : Non applicable

L'étude du passage transcornéen *ex vivo* réalisée sur la cornée excisée de porc avait pour objectif d'une part de vérifier le franchissement par la DXMa de cette barrière et d'autre part de mesurer l'intensité et le profil de ce passage. Les trois formulations, Gel A, Gel B, Nano-Gel B ont été retenues pour l'étude et comparées aux formulations commerciales MAXIDEX® (dexaméthasone) et DEXAFREE® (phosphate de dexaméthasone). Il est important de signaler que les comparaisons des passages transcornéens rapportés dans notre travail doivent être considérées comme relatives. En effet, les formes de dexaméthasone sont différentes et il ne nous était pas possible de reformuler les produits commerciaux. Par ailleurs, pour des raisons pratiques d'administration, nous avons déposé des quantités équivalentes de produit sur les cornées de manière à recouvrir approximativement la même surface et par conséquent, les taux de principe actif sont différents. En dépit de ces différences, les profils illustrés dans la Figure 7 montrent des passages transcornéens supérieurs correspondant à des quantités 2,5 à 5,2 fois et 1,8 à 3,8 fois celles observées dans le cas de MAXIDEX® et DEXAFREE®, respectivement. Ces résultats semblent garantir la pertinence potentielle des trois formulations, compte tenu de l'index thérapeutique de la DXMa [28]. Il est à noter l'important passage de la DXMa à partir du Nano-Gel B qui pourrait s'expliquer par la synergie entre les nanoparticules et les polymères viscosants qui promeut le passage transcornéen.

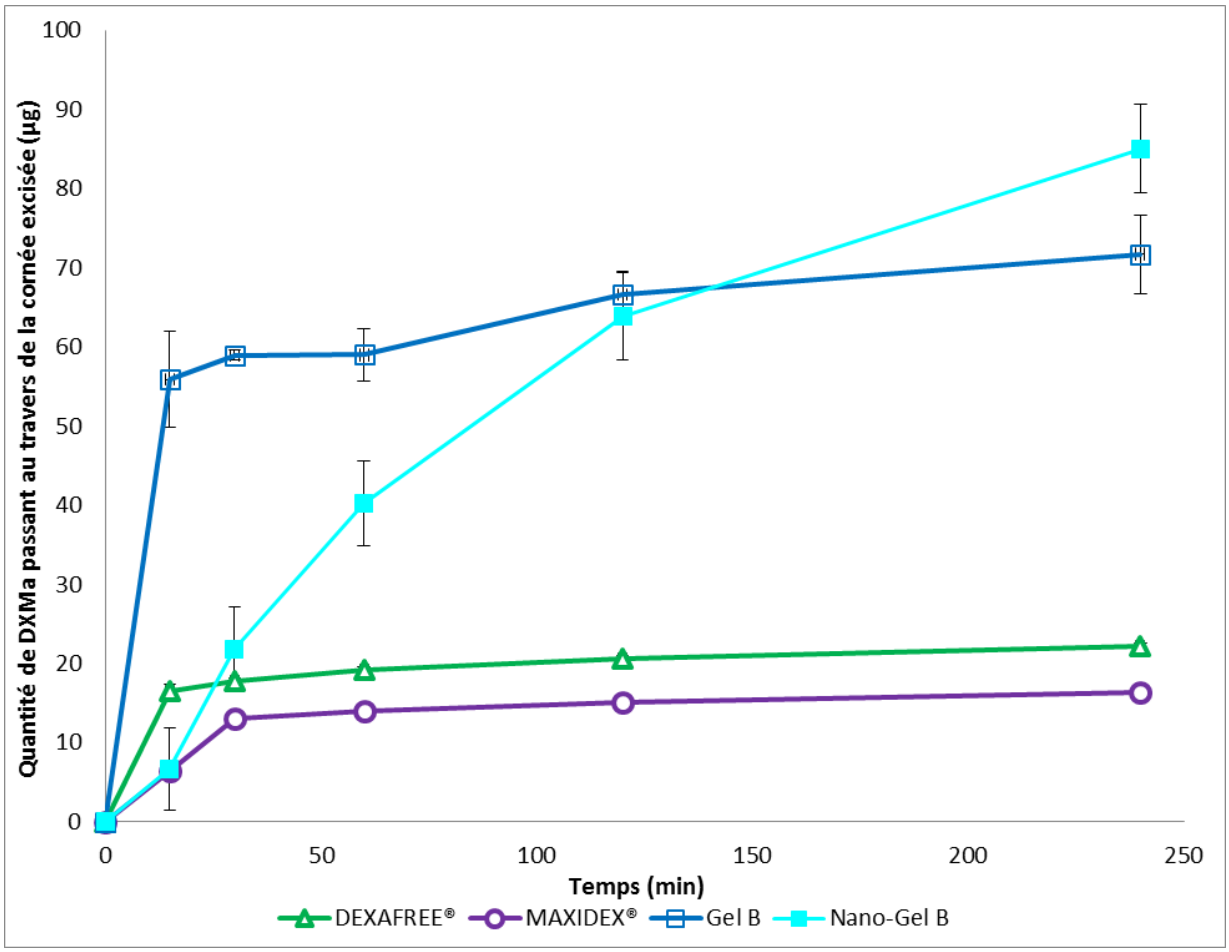

Figure 7. Profils de passage transcornéen *ex vivo* des Gels A et B, des nanoparticules combinées au Gel B en comparaison des formules commerciales MAXIDEX® et DEXAFREE®

Il est intéressant de noter également que les passages transcornéens ont des profils assez proches de ceux obtenus lors des études de libération *in vitro*. En effet, les Gels A et B ainsi que les spécialités commerciales ont une dissolution *in vitro* et un passage *ex vivo* rapides et massifs, supérieurs à 90 % après 30 minutes. Tandis que le Nano-Gel B présente des profils plus ralentis, avec une libération ou un passage inférieur à 40 % en 30 min, mais soutenue puisque 92 % sont libérés après 24 h.

Ces deux profils de libération permettent de répondre aux exigences thérapeutiques des inflammations oculaires nécessitant une dose de charge importante. Cependant, seule la

formulation Nano-Gel B offre un effet réservoir permettant de maintenir une concentration efficace en principe actif sur une période plus longue.

Les résultats de ces études *in vitro* et *ex vivo* constituent incontestablement une avancée importante dans l'amélioration du temps de résidence sur la surface oculaire et le passage transcornéen. Cette approche novatrice combinant nanoparticules chargées, polysaccharides et cyclodextrines amphiphiles permet potentiellement d'augmenter la biodisponibilité de la DXMa, de prolonger son action par un effet réservoir et ainsi pourrait réduire la fréquence des instillations tout en améliorant son efficacité. Ces résultats prometteurs devront être confirmés *in vivo* par une étude pharmacocinétique et des tests d'efficacité.

Les tests MTT et ALAMAR BLUE® réalisés ont un intérêt prédictif et ont permis de mettre en évidence des profils toxicologiques et donc de biocompatibilité différents pour les trois formulations. Il est important de noter qu'aucune des trois formulations n'entraine d'effets avérés pour l'œil et seul le Gel B est apparu biocompatible. Concernant le Gel A et le Nano-Gel B, les tests ne peuvent permettre à ce stade de prédire leurs effets sur l'œil *in vivo*. Il sera donc essentiel d'évaluer nos trois formulations par un test de Draize *in vivo*, seul test formellement accepté et validé pour apprécier l'irritation oculaire et identifier les effets réversibles et irréversibles sur l'œil [27].

La tolérance des formulations pour la voie topique ophtalmique étant un point clé du développement pharmaceutique, nous avons veillé à ne pas utiliser de conservateurs antimicrobiens, de type chlorure de benzalkonium, connaissant ses effets délétères sur l'épithélium cornéen [26].

Enfin, l'étude de stabilité physicochimique et microbiologique a permis de s'assurer d'une stabilité sur 12 mois à 25 °C des Gels A et B après stérilisation par double filtration stérilisante et conditionnement en flacon de verre de type I. Ayant proscrit l'utilisation de conservateurs antimicrobiens dans nos formulations et les Gel A et B étant filtrables sur PVDF 0,22µm, nous envisageons un conditionnement de nos formulations soit dans des emballages unidoses, soit dans des dispositifs spéciaux multidoses de type : système ABAK®

ou système COMOD® ou NOVELIA®. Ces conditionnements multidoses contiennent un filtre ou un système anti-retour ou un système à pression variable pour éviter une contamination microbiologique de la formulation au cours de l'utilisation. En outre ces conditionnements offrent un avantage économique et écologique indéniable, malgré leur petite taille, leur capacité est équivalente à celle de 100 unidoses [29].

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CONCLUSION ET PERSPECTIVES

Les objectifs de ce projet étaient donc d'élaborer, de développer et d'évaluer un système galénique innovant pour l'administration topique ophtalmique de l'acétate de dexaméthasone afin d'améliorer la prise en charge thérapeutique des patients souffrant d'une inflammation ophtalmique. Le défi de formulation proposé était d'améliorer la solubilité apparente en milieu aqueux de l'acétate de dexaméthasone (DXMa) et d'augmenter son temps de résidence sur la surface oculaire, tout en lui conférant des propriétés de réservoir, afin d'accroître son efficacité et à terme de diminuer la fréquence d'instillation. Pour répondre à ces objectifs, nous avons mis au point trois formulations présentant des caractéristiques différentes en termes de profil toxicologique, de profil de libération, de temps de résidence oculaire ou de passage transcornéen. En outre, il apparait clairement que chacune de ces formulations permet d'obtenir des concentrations comprises dans l'index thérapeutique de la dexaméthasone et qu'elles pourraient être plus efficaces que les formulations commercialisées pour traiter les inflammations oculaires.

Chacune des trois formulations possède des intérêts spécifiques. Le Gel A présente le double avantage d'augmenter la solubilité de la DXMa, par un facteur 300 en milieu aqueux et tous ces constituants ont une monographie à la Pharmacopée Européenne. Le Gel A étant stérilisable et stable 12 mois à 25 °C, son utilisation en clinique pourrait donc être potentiellement plus rapide. Le Gel B, lui, présente le meilleur profil de tolérance tout en augmentant encore la solubilisation de la DXMa en milieu aqueux, par un facteur 900. Malheureusement l'HPγCD n'est pas encore inscrite à la Pharmacopée Européenne ce qui rendrait dans l'état actuel son utilisation en clinique moins aisée. Cependant, il faut signaler que des progrès réglementaires ont été enregistrés pour ce dérivé. En effet, la spécialité VOLTAREN OPHTA® Eye drop 0.1 % contenant l'HPγCD est commercialisée depuis 2017 en Nouvelle Zélande. Le Gel B étant également stérilisable et stable 12 mois à 25 °C, son utilisation en clinique pourrait donc être envisagée dans un second temps. Enfin, les nanoparticules chargées positivement associées au Gel B permettent d'augmenter la solubilisation de la DXMa, par un facteur 1000 en milieux aqueux, et présente le grand avantage de combiner mucoadhésion, système réservoir et polymères viscosants, permettant ainsi d'augmenter le passage transcornéen et le temps de résidence sur la cornée. Il faut cependant attendre les résultats de l'évaluation toxicologique *in vivo*. En effet, il est bien reconnu que des formulations colloïdales contenant des nanoparticules sont

soumises à exigences toxicologiques et réglementaires très strictes. Ainsi, bien que l'utilisation de nanoparticules pour la voie topique ophtalmique soit très prometteuse, aucun essai clinique n'était en cours en 2017 [30].

Pour toutes ces formulations, il conviendra avant toute utilisation en clinique, d'évaluer leur tolérance *in vivo* par un test de Draize, d'envisager des études pharmacocinétiques et d'efficacité. A l'issue de ces évaluations, un transfert des formulations peut être envisagé pour une exploitation dans un cadre hospitalier. En effet, les pharmacies à usage intérieur (PUI) sont autorisées à fabriquer et à dispenser des médicaments sans AMM, jugés indispensables pour les patients [11,31]. Un exemple fréquent est la préparation de collyres antibiotiques fortifiés dans les PUI. Dans ce cadre, les préparations à base de DXMa développées dans cette thèse pourraient être utilisées dans la prise en charge thérapeutique rapide de l'inflammation oculaire sévère afin de réduire le risque d'handicap visuel tout en limitant les effets indésirables.

La décision d'exécution de telles préparations est prise par le pharmacien selon des critères définis dans le chapitre 3 des Bonnes Pratiques de Préparation de 2007 :

- l'intérêt pharmaco-thérapeutique (choix du principe actif, évaluation de la tolérance et de l'efficacité),
- le bon usage de la préparation dont l'ajustement thérapeutique, l'amélioration de l'acceptabilité, le renforcement de l'observance et la diminution des risques en font partie,
- le risque sanitaire vis-à-vis du patient et du préparateur,
- la galénique et le contrôle en termes de réalisation (formulation, tolérance, stabilité physico-chimique et microbiologique, disponibilité du personnel, du matériel et des locaux),
- le respect des textes en vigueur.

Ainsi, un des intérêts de ce travail serait de transférer les résultats d'une recherche fondamentale et expérimentale conduite en laboratoire vers une application pratique en milieu hospitalier par la mise à disposition au personnel de santé et aux patients des préparations ophtalmiques originales et indispensables.

RESUME

Abstract: The therapeutic management of ophthalmic inflammation must be rapid and effective in order to avoid deleterious effects for the eye and vision. Generally, it includes steroidal (SAID) and non-steroidal (NSAID) anti-inflammatory drugs used by topical ocular administration. Unfortunately, their low bioavailability (1 to 5 % of the instilled dose) implies frequent instillations in patients. Among anti-inflammatory drugs, dexamethasone (DXM) is one of the most powerful and is considered as a reference molecule. DXM acetate (DXMa) is a lipophilic derivative, potentially interesting for topical ophthalmic use, but not marketed to date. This ester would be better absorbed without increasing the risk of ocular hypertension, a frequent adverse effect encountered with SAID. However, DXMa has a major disadvantage: a very low solubility in water (0.021 g / mL at 25 ° C).

In this work, we developed a formulation strategy combining the means of improving apparent solubility and prolonging the drug's residence time at the ocular surface. We used water-soluble cyclodextrins (HPβCD and HPγCD), polysaccharides (CELLUVISC® - Carmellose sodium and VISMED®- sodium hyaluronate) and cationic nanoparticles. Three main formulations emerged from our pharmaceutical development: Gel A (HPβCD / DXMa / VISMED®), Gel B (HPγCD / DXMa / VISMED® / CELLUVISC®) and Nano-Gel B (DXMa-γCD-C10 / MAP 103a nanoparticles / HPγCD / DXMa / VISMED® / CELLUVISC®). They achieved the following objectives: i) a significant increase in the apparent solubility of DXMa 300 times in Gel A, 950 in Gel B and 1000 in Nano-Gel B, ii) an increase in the time residence of the formulations on the ocular surface, 2.9 times for Gel A, 4.9 for Gel B and 5.9 for cationic nanoparticles, iii) an enhancement in DXMa transcorneal penetration, 1.8 -3.8 and 2.5-5.2 times higher than DEXAFREE® and MAXIDEX®. Taking into account the route of administration, the pH, osmolality and viscosity values of the various formulations were found compatible with the eye.

In addition, Gels A and B were stable over a period of 12 months at 25 ° C. Although the cytotoxicity and tolerance results would need to be completed by *in vivo* eye irritation tests, this work sets solid milestones for considering a topical ocular form of DXMa.

Keywords : dexamethasone acetate; ocular inflammation; topical ophthalmic administration; hydrogel; cationic nanoparticles; cyclodextrins

Résumé : L'inflammation ophtalmique nécessite un traitement rapide et efficace afin de limiter ses effets néfastes pour l'œil et la vision. La prise en charge thérapeutique consiste dans 90 % des cas en l'administration d'anti-inflammatoires (AI) par voie topique. Leur pénétration oculaire et leur biodisponibilité étant faibles, 1 à 5 % de la dose instillée traverse la cornée, la fréquence des instillations est importante pour les patients. Parmi les AIS et AINS disponibles dans l'arsenal thérapeutique, la dexaméthasone (DXM) est une des molécules les plus puissantes et efficaces, apparaissant comme une référence. L'acétate de DXM (DXMa) est un dérivé lipophile, potentiellement intéressant pour une utilisation topique ophtalmique, mais non commercialisée à ce jour. Cet ester serait mieux absorbé et moins enclin à provoquer une hypertension oculaire que les autres formes de DXM. Cependant, l'acétate de DXM présente un inconvénient majeur, sa très faible solubilité dans l'eau (0,021 g/mL à 25 °C). Dans ce travail de thèse, nous avons développé une stratégie de formulation combinant d'une part les moyens d'amélioration de la solubilité de la DXMa et d'autre part les moyens de prolonger le temps de résidence du médicament sur le site d'administration. Pour y parvenir, nous avons mis en œuvre des cyclodextrines hydrosolubles (HPβCD et HPγCD), des polysaccharides (CELLUVISC® - Carmellose sodique et VISMED®- Hyaluronate de sodium) et des nanoparticules cationiques. Trois formulations principales sont ressorties de notre développement galénique : Gel A (HPβCD/DXMa/VISMED®), Gel B (HPγCD/DXMa/VISMED®/ CELLUVISC®) et Nano-Gel B (DXMa-γCD-C_{10}/MAP 103a nanoparticles/HPγCD/DXMa/VISMED®/ CELLUVISC®). Elles ont permis d'atteindre les objectifs suivants : **i)** une augmentation significative de la solubilité apparente de la DXMa de 300 fois dans le Gel A, 950 dans le Gel B et 1000 dans le Nano-Gel B, **ii)** une augmentation du temps de résidence des formulations à la surface oculaire par rapport au BSS, de 2,9 fois pour le Gel A, 4,9 pour le Gel B et 5,9 pour les nanoparticules cationiques, **iii)** un passage transcornéen la DXMa de 1,8-3,8 et 2,5-5,2 fois plus élevée que DEXAFREE® et MAXIDEX®. Tenant compte de la voie d'administration, trois paramètres le pH, l'osmolalité et la viscosité des différentes formulations ont été étudiées et démontrées comme étant compatibles avec l'œil.

En outre, la stabilité des gels A et B a été démontrée sur une durée de 12 mois à 25°C. Bien que les résultats de cytotoxicité et de tolérance présentés dans ce travail demanderaient à être complétés par des tests *in vivo* d'irritation oculaire, l'ensemble de ce travail pose de solides jalons permettant d'envisager une forme topique oculaire de DXMa.

Mots clés : Acétate de dexaméthasone, inflammation oculaire, voie topique ophtalmique, hydrogel, nanoparticules cationiques, cyclodextrines