In vitro and *in vivo* ophthalmic bioadhesion and ocular safety characterization of cyclodextrin based-solutions.

Díaz-Tomé Victoria[1,2 †], García-Otero Xurxo[1,3 †], Varela-Fernández Ruben[1,4], Blanco-Fernández Guillermo[1,5], Gómez-Lado Noemí[3], Fernández-Ferreiro Anxo[2], Otero-Espinar Francisco J.[1,5]

1. Pharmacology, Pharmacy and Pharmaceutical Technology. University of Santiago de Compostela (USC). Spain. victoriadiaztome@gmail.com
2. Clinical Pharmacology Group. Health Research Institute of Santiago de Compostela (IDIS). Spain.
3. Molecular Imaging Group. Health Research Institute of Santiago de Compostela (IDIS). Spain.
4. Clinical Neurosciences Group. University Clinical Hospital, Health Research Institute of Santiago de Compostela (IDIS). Spain.
5. Paraquasil Group. University Clinical Hospital, Health Research Institute of Santiago de Compostela (IDIS). Santiago de Compostela. Spain.

† The authors contributed equally: Victoria Díaz-Tomé and Xurxo García-Otero

INTRODUCTION AND OBJECTIVES

The use of cyclodextrins in ophthalmic formulations is highly expanded due to their ability to improve the drug solubility by forming inclusion complexes, increasing the drug bioavailability and stability. Different safety levels have been described for ophthalmic route in some types of cyclodextrins [1]. The aim of this work was to compare the corneal alterations as a consequence of the aqueous solutions contact of different cyclodextrins (αCD 15%, HPαCD 20%, βCD 2%, HPβCD 20%, γCD 20%, HPγCD 20%, SBECD 20%, MβCD 20%) by Bovine Corneal Opacity and Permeability (BCOP) and Hen´s Egg Test Chorioallantoic Membrane (HET-CAM) assays. In addition, as the bioadhesive ability of some cyclodextrins has already been described [2], their ocular mucoadhesive ability was also compared by *ex vivo* assays (fresh bovine cornea) and *in vivo* assays (instillation in rats eyes in order to obtain Positron Emission Tomography (PET) images).

METHODS

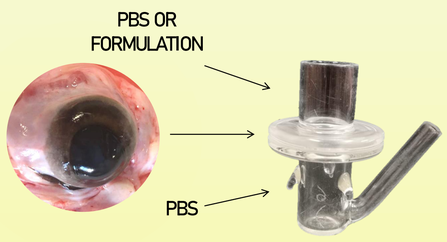
IRRITATION STUDIES

BCOP

A. Excised corneas were placed on Franz Cells.

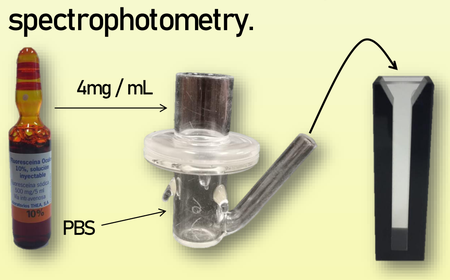
B. Transmitance and Opacity were measured before and after corneal formulation contact.

C. Fluorescein permeability was measured at 90 min. by UV-Vis spectrophotometry.



Spectrophotometer Agilent Cary UV Vis

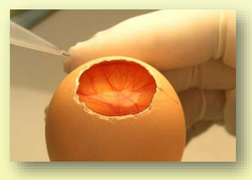
Luxmeter Gossen Mavolux 5032C



HET-CAM

Lysis, hemorrhage and coagulation processes were assessed in the chorioallantoideal membrane (CAM) of fertilized chicken eggs after 5 minutes application of each formulation.

Egg shell removal

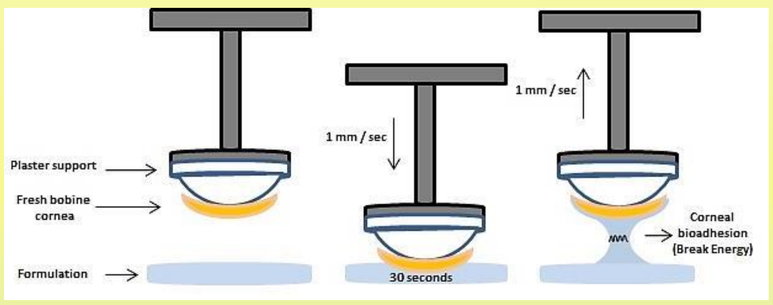


Lysis
Hemorrhage
Coagulation

MUCOADHESION STUDIES

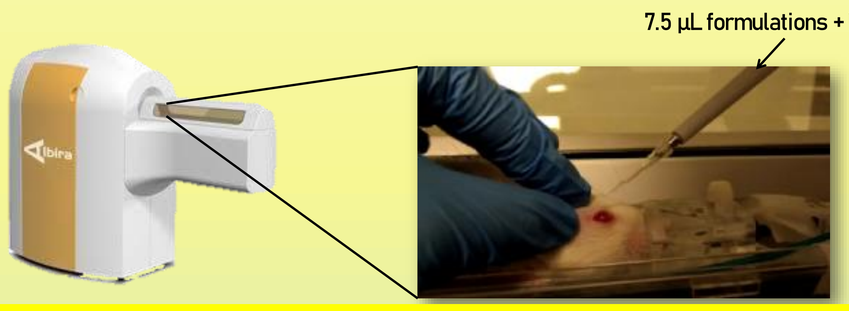
Ex vivo

A SHIMADZU® texturometer



In vivo

Albira PET/CT Preclinical Imaging System



RESULTS

IRRITATION STUDIES

BCOP

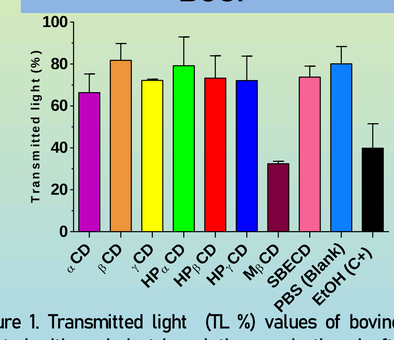


Figure 1. Transmitted light (TL %) values of bovine corneas treated with cyclodextrin solutions and ethanol after 10 min drug treatment and 120 min PBS treatment. 100% corresponds to the total light transmitted through bovine corneas incubated in PBS. *ETOH (C+): Ethanol (positive control).

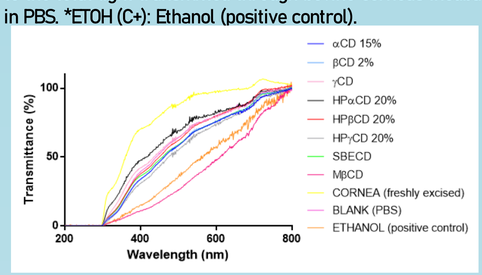


Figure 2. Transmittance representation obtained after the instillation of differents cyclodextrins solutions.

→ The fluorescein permeability has been affected to a greater extent by the solution of αCD and HPγCD.

HET-CAM

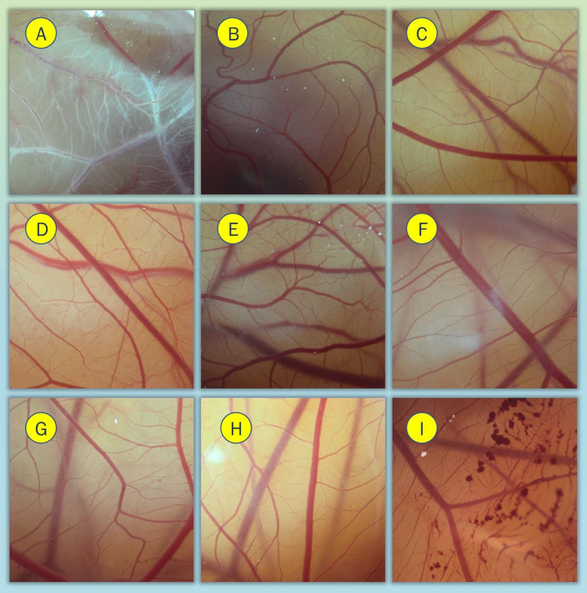


Figure 3. HET-CAM images 5 minutes post-instillation for the differents cyclodextrins solutions: A) αCD, B) βCD, C) γCD, D) HPαCD, E) HPβCD, F) HPγCD, G) SBECD, H) MβCD, I) Ethanol (positive control).

MUCOADHESION STUDIES

In vivo

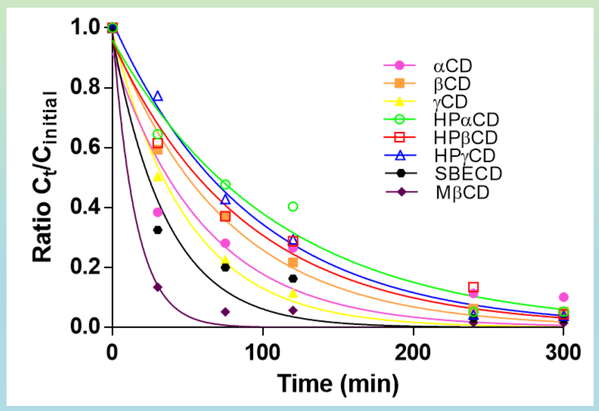


Figure 4. Clearance ratio from the ocular surface determined by PET. Ct/Cinitial radioactivity ratio remaining on the ocular surface over time was calculated assuming Cinitial value recorded in the ROI (ocular globe) equaled 1.

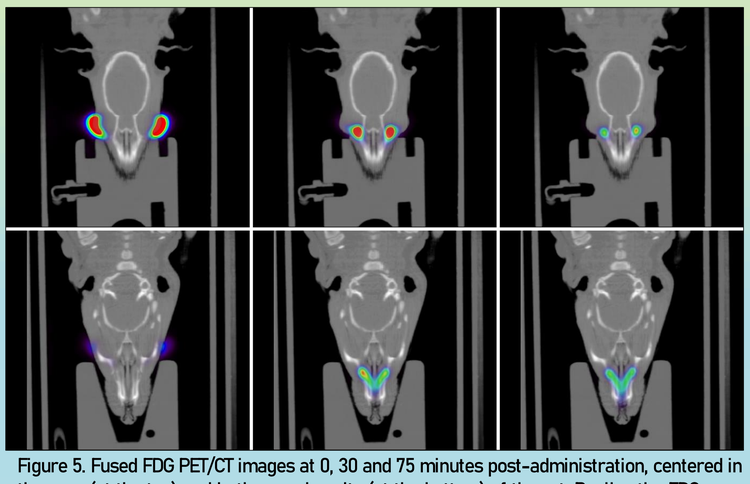


Figure 5. Fused FDG PET/CT images at 0, 30 and 75 minutes post-administration, centered in the eyes (at the top) and in the nasal cavity (at the bottom) of the rat. Realize the FDG goes from the eyes to the nasal cavity and pharynx with time.

	HPαCD		HPβCD		HPγCD		SBECD		MβCD		βCD		αCD		γCD	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
K (min^{-1})	0.0115	0.0063	0.0144	0.007	0.0111	0.0021	0.0318	0.022	0.0658	0.0078	0.0141	0.0038	0.0166	0.0027	0.0221	0.0067
$t_{1/2}$ (min)	72.04	29.44	59.85	32.39	64.31	11.84	27.98	12.07	10.65	1.22	51.66	12.58	42.64	6.25	33.56	9.73
AUC_0^{300}	10024.75	3052.24	8945.5	2953.29	9252.25	1514.83	5323.75	1792.24	2901	471.41	7911.75	990.02	7728.5	430.9	5837.25	1153.98

Table1. Parameters obtained by the fitting of the % formulation remaining on the ocular surface obtained by PET imaging to a mono-compartimental model.

Ex vivo

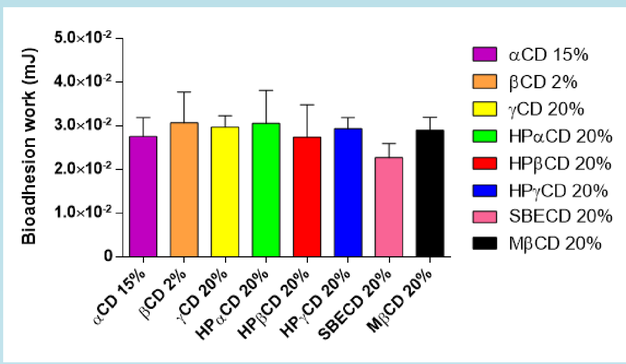


Figure 6. Maximum breaking strength and bioadhesion work obtained using bovine cornea as a substrate.

CONCLUSIONS

All tested cyclodextrins are safe for ophthalmic administration with the exception of:

→ MβCD, due to the fact that significantly modifies corneal transparence . This fact is supported by transmittance and opacity values (Figures 1 and 2).

→ HPγCD and αCD, because of the alterations on corneal permeability.

→ αCD modified the vessels's appearance and color as presented on HET-CAM assay.

Additional studies are required for the determination of their toxicity.

Ex vivo mucoadhesive assay shows that all cyclodextrin solutions have similar bioadhesive properties, except for SBECD that presents a significantly lower bioadhesion work-value than the rest of cyclodextrins.

In vivo mucoadhesive results show hydroxypropylic cyclodextrins have a better $t_{1/2}$ on the ocular surface than the rest of tested cyclodextrins. MβCD is associated with a lower $t_{1/2}$, probably due to an increase on animal´s blinking observed during the study.

REFERENCES

[1] EMA, Background Review for Cyclodextrins Used as Excipients, pp. 1–17, 2014.

[2] Marc Francois, Eric Snoeckx, Peter Putteman, Fons Wouters, Eddy De Proost, Urbain Delaet, Jef Peeters, and Marcus E. Brewster. A mucoadhesive, cyclodextrin-based vaginal cream formulation of itraconazole. AAPS PharmSci. 2003;5(1):E5.

ACKNOWLEDGEMENTS

García-Otero Xurxo and Varela-Fernández Rubén acknowledges the financial support of the Health Research Institute Foundation of Santiago de Compostela (FIDIS) (Predoctoral research fellowship).