

SISTEMA NANOESTRUTURADO CATIÔNICO PARA OBTENÇÃO DE PRODUTOS OFTÁLMICOS INOVADORES



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Tópicos

- Preparações oftálmicas convencionais: mercado;
- Doenças oculares: prevalência;
- Aspectos fisiológicos do órgão da visão: tratamento
- Desafios e oportunidades: preparações inovadoras
- Estratégias para a obtenção de sistemas nanoestruturados catiônicos:
 - Polímero catiônico
 - Tensoativo catiônico

Mercado Global: produtos oftálmicos

- Cerca de US \$ 5 bilhões
- Crescimento de 15%: 2008-2013
- Envelhecimento da população
- Crescente incidência e prevalência de alergias
- Exposição a telas digitais
- Poluição
- Doenças de estilo de vida
- Efeitos adversos de medicamentos
- G20: responsáveis por 90% dos cuidados com a visão e saúde ocular
- Síndrome do olho seco : ≥ 50 anos, 10 a 30 %

Prevalência

- 285 milhões de pessoas apresentam perda da acuidade visual;
- 39 milhões, cegueira permanente;
- 246 milhões, redução da acuidade visual;
- 60% das crianças com perda de acuidade visual morrem durante o ano seguinte a essa perda (OMS, 2012).

Causas evitáveis

- catarata (51%): 7 milhões → 20 milhões/ano
- glaucoma (8%);
- degeneração macular (5%) relacionada à idade.

As infecções como causa de perda de acuidade visual foram significativamente reduzidas nos últimos 20 anos.



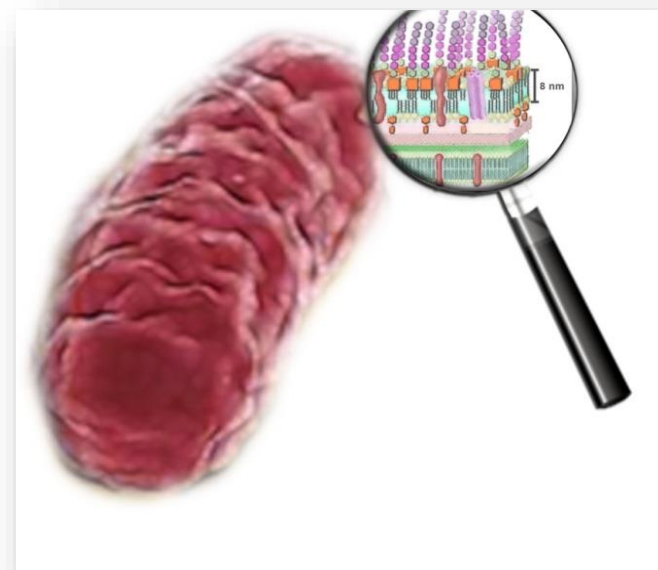
Fonte: OMS, 2014

Ilustração: Paes, O (2014)

Infecções oculares

Pseudomonas aeruginosa

- Uso de lente de contato;
- Endoftalmite após cirurgia de catarata.



P. aeruginosa produz enzima proteoglicanolítica, responsável pela destruição da córnea (Brown, Bloomfield, Tarn, 1974).

Uso de lente de contato

Pseudomonas aeruginosa foi isolada com maior frequência, nos casos de ceratite infecciosa e de úlcera corneana (Konda *et al.*, 2014)

Endoftalmite após cirurgia de catarata

- isolated from vitreous humor in all **12** patients after cataract surgery, in **Greece** (Maltezou et al., 2012).
- **11** patients developed acute postoperative endophthalmitis caused by *P. aeruginosa* after cataract surgery with intraocular lens implantation, in **South India** (Ramappa et al., 2012).
- In **Brazil**, **45** patients lost their visual acuity, 13% of them had no light perception, after the cataract surgery procedure as a result of endophthalmitis caused by this organism (Guerra et al., 2012).

Conventional preparations

- The formulation approach for ophthalmic preparations changed little since the nineteen fifties decade when, for the first time, the sterility was required for the liquid preparations intended for ophthalmic use (USP, 1955);
- Conventional ophthalmic solutions, ointments and suspensions account for 90% of the front-of-eye drug delivery (Manish and Kulkarni, 2012).

Órgão da visão: aspectos fisiológicos suas implicações na terapêutica

- Mecanismos de defesa: movimento involuntário da pálpebra, ato de lacrimejar, renovação do filme lacrimal
- Barreiras naturais: córnea e a conjuntiva
- Drenagem naso-lacrimal
- Absorção sistêmica indesejável: capilares saco conjuntival



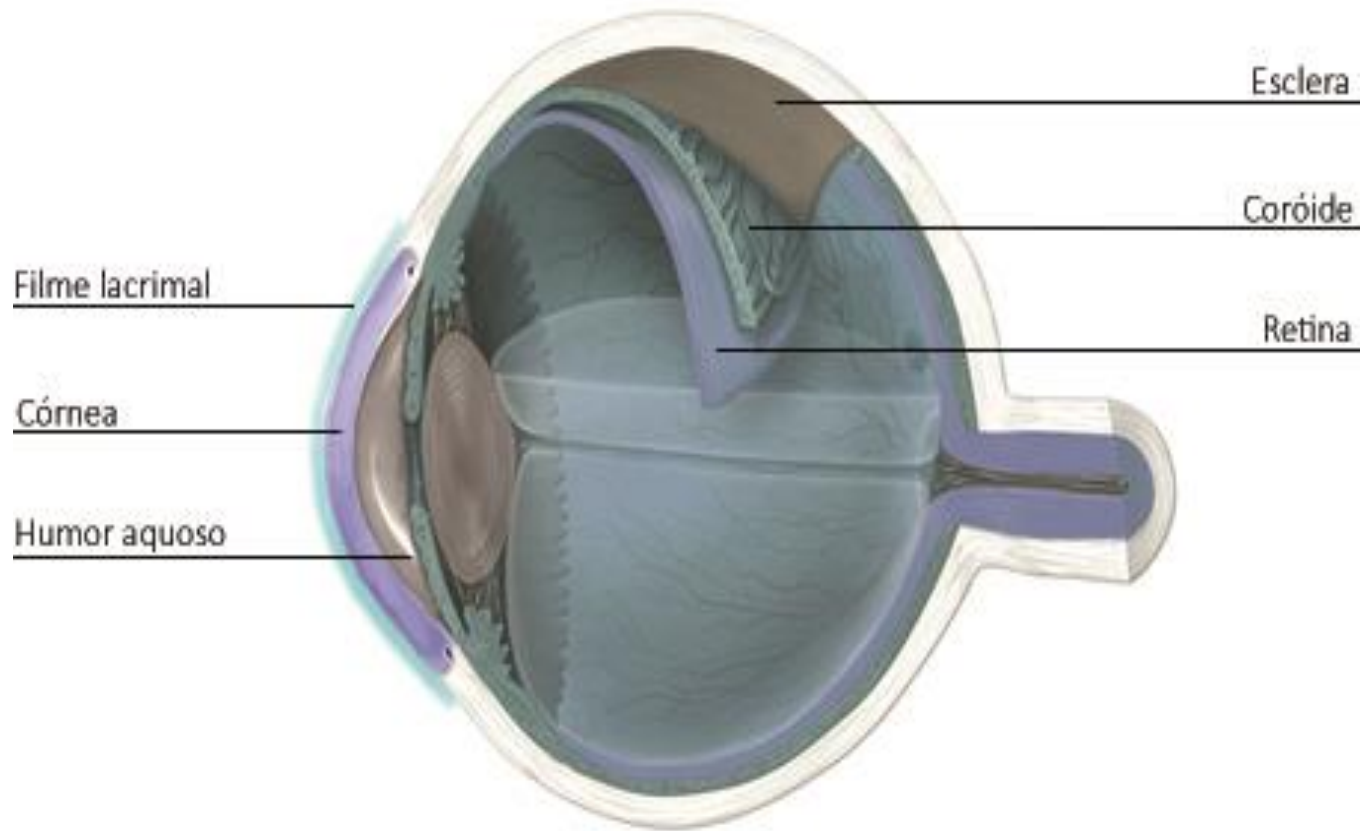


FIGURA 1. ESTRUTURA DO OLHO: FILME LACRIMAL E CórNEA (ADAPTADO DE NAKHLBAND; BARAR, 2011)

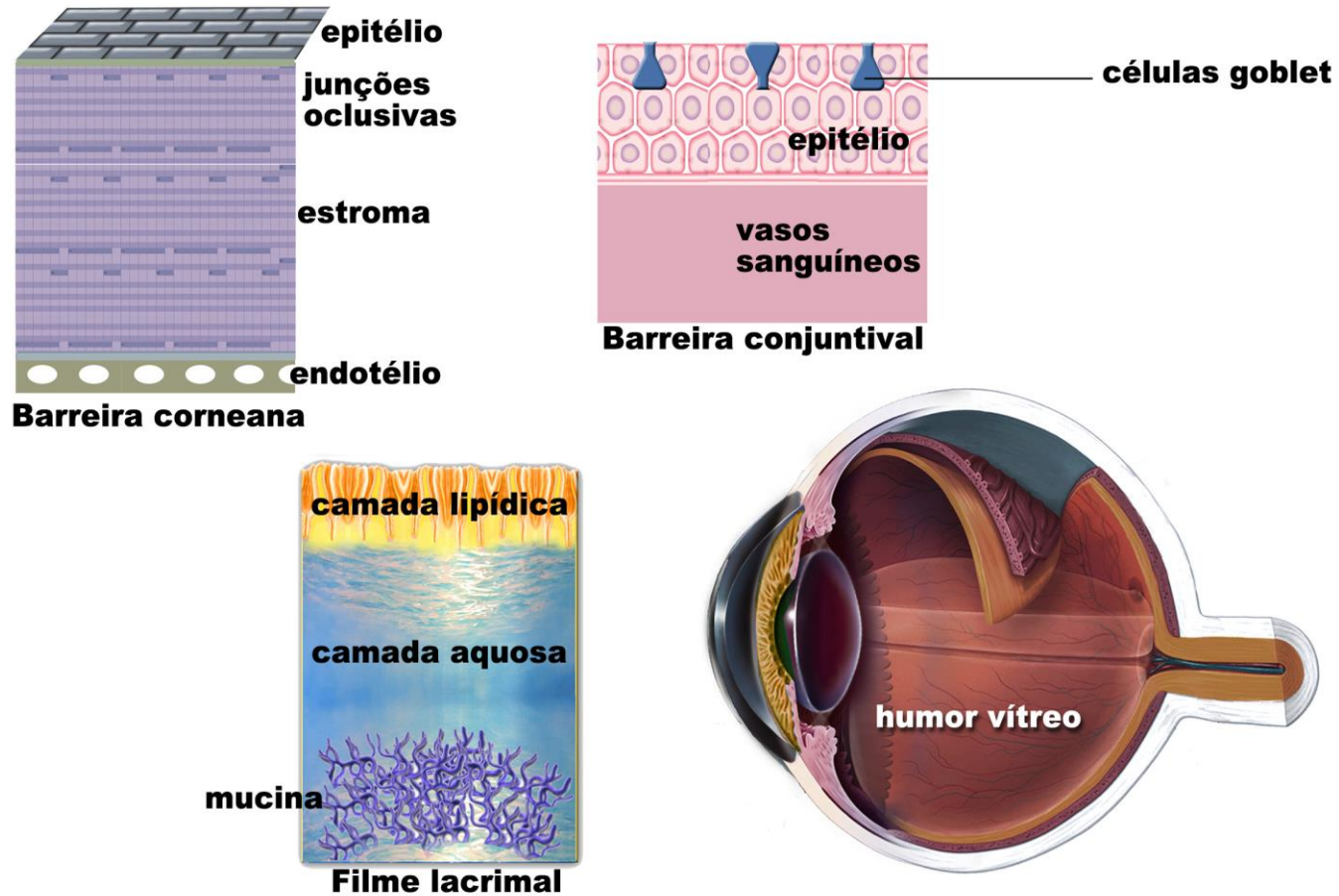


FIGURA 2. ESQUEMA DAS BARREIRAS BIOLÓGICAS CRÍTICAS PARA A ADMINISTRAÇÃO DE FÁRMACOS VIA OCULAR (ADAPTADO DE FUENTE ET AL., 2010)

Properties of the tear film

- conjunctiva and the cornea are protected by this film, a multi-layered structure, comprised of a buffered solution (pH 7.4) containing electrolytes and:
- **1543** proteins reported so far (lysoenzyme, albumin and glycoproteins like mucin) (Zhou et al., 2012);
- more than **600 lipid species** from 17 major lipid classes (Lam et al., 2014).

Properties of the tear film

- The third layer of this film, closest to the cornea: aqueous-mucin gel layer containing water, salts, proteins and carbohydrates besides **mucins**, which are high molecular weight glycoproteins heavily glycosylated (50 to 80%) (Gipson et al, 2004).
- The goblet cells of the conjunctiva mainly secrete this component.

Mucin

- **negatively charged** molecule due to its associated sialic acid and sulfate residues (Royle et al., 2008).
- Additionally, it modifies the hydrophobic corneal surface to a hydrophilic surface by adhering to the glycocalyx on the corneal microvilli allowing the hydration of the tissues.
- **can play an important role in ocular bioavailability** depending on the extent of its behavior as barrier or retention site (Ruponen, Urtti, 2015).

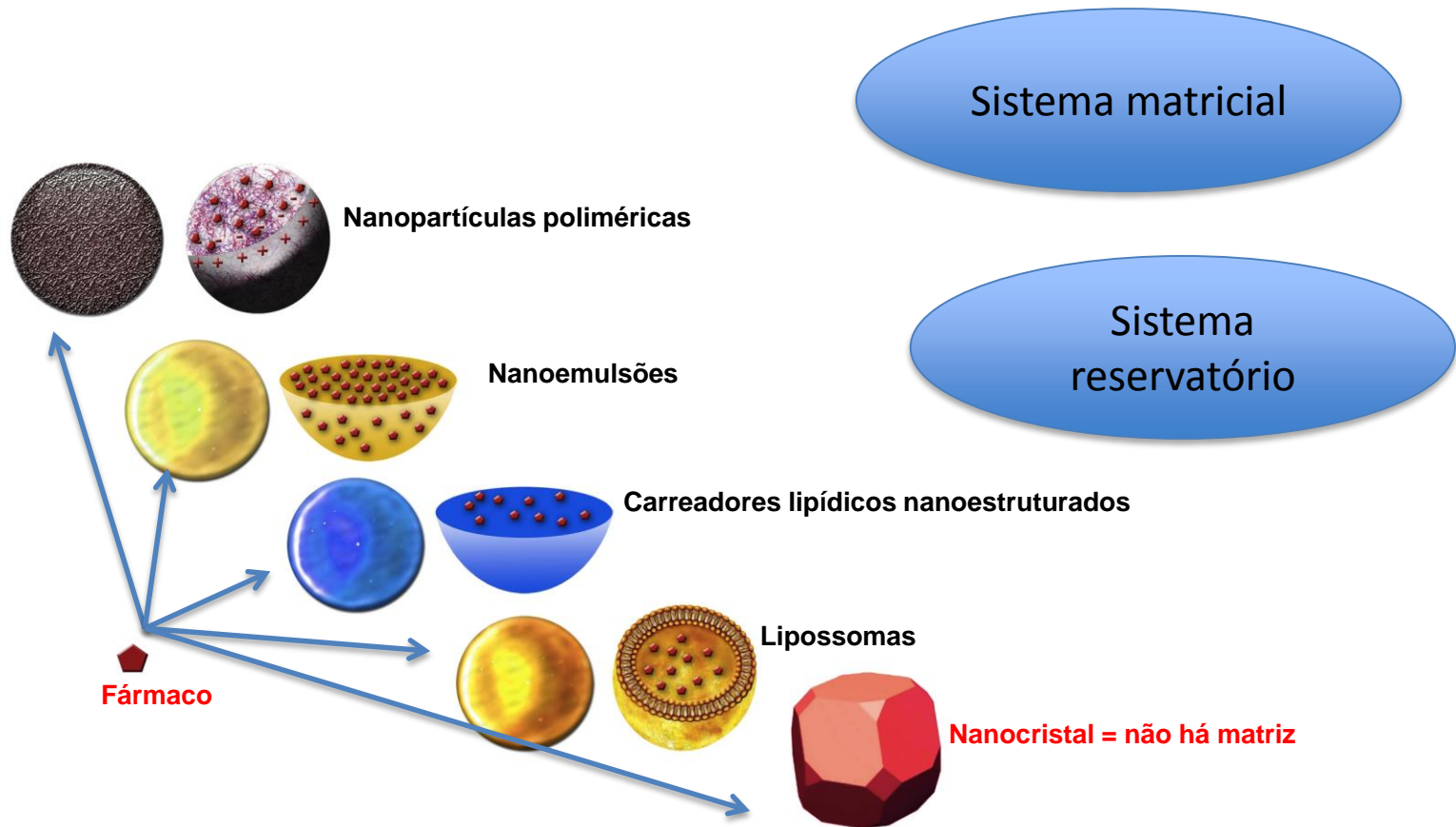
Desafios

- Maior tempo de residência na superfície ocular
- Liberação modificada
- Melhor biodisponibilidade
- Ação sítio-específico
- Redução dos efeitos adversos
- Maior adesão do paciente ao tratamento

Fonte: Achouri et al., *Drug Dev. Ind. Pharm.* (2013)

Sistema	Produto	Fármaco	Empresa	Indicações	Lançamento	Status/mercado
Emulsão	Restasis®	Ciclosporina A	Allergan	Síndrome severa do olho seco	Dezembro/2002	EUA
Emulsão	Refresh dry eye therapy®		Allergan	Síndrome do olho seco	Abril/ 2002	EUA
Emulsão	Durezol™	difluprednato	Alcon	inflamação	Junho/2008	EUA
Emulsão catiônica	Cationorm®		Novagali	Síndrome do olho seco	Outubro/2008	Europa
Emulsão	Lipimix®		Tubilux Pharma	Síndrome do olho seco	Setembro/ 2007	Europa
Emulsão	Sooth® XP Emollient		Bausch & Lomb	Síndrome do olho seco	Maiio/ 2010	EUA
Emulsão catiônica	Cyclokate®	Ciclosporina A	Novagali	Síndrome do olho seco		Fase III
Emulsão catiônica	Catioprost®	Latoprost	Novagali	Glaucoma		Fase III

Estratégias para a liberação modificada de fármacos



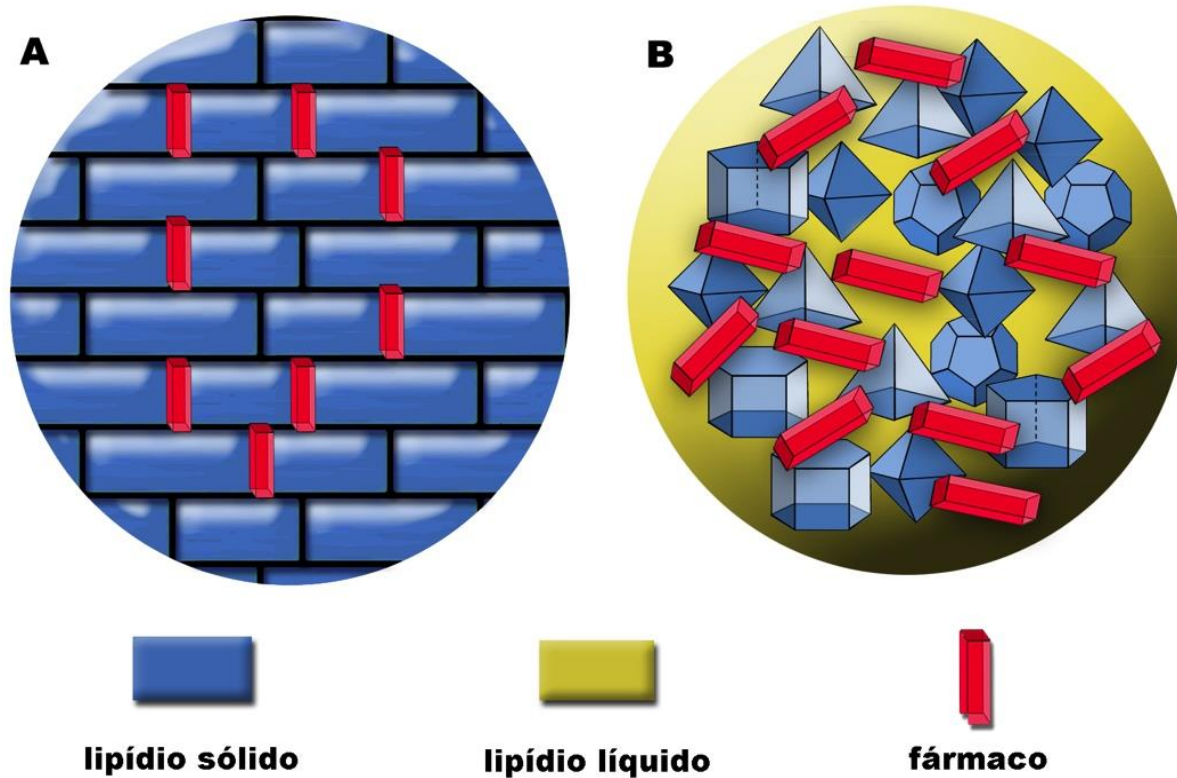
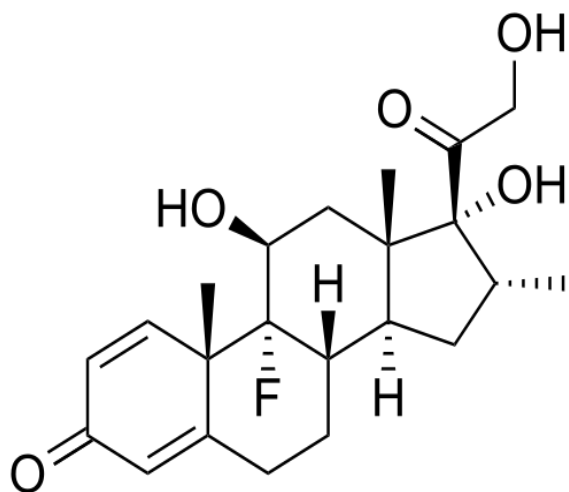


FIGURA 3. REPRESENTAÇÃO ESQUEMÁTICA DA DISPOSIÇÃO DO FÁRMACO NAS NANOPARTÍCULAS LIPÍDICAS SÓLIDAS (NLS) E DOS CARREADORES LIPÍDICOS NANOESTRUTURADOS (CLN) (ADAPTADO DE MUCHOW; MAINCENT; MÜLLER, 2008)

Objetivo

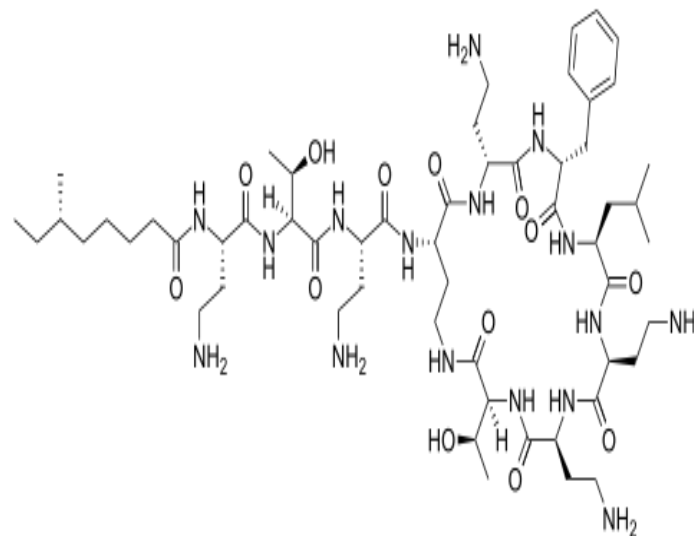
O desenvolvimento e a caracterização físico-química e microbiológica de carreador lipídico nanoestruturado, contendo fármaco hidrofóbico e hidrofílico, acetato de dexametasona e sulfato de polimixina B, respectivamente, com potencial aplicação no desenvolvimento de produtos oftálmicos inovadores, para o tratamento de infecções oculares.

Acetato de dexametasona



anti-inflamatório esteroide
adrenocortical, PM 392,47 Da,
praticamente insolúvel em água,
solúvel em clorofórmio e pouco
solúvel em álcool e acetona.

Sulfato de polimixina B



tensoativo catiônico, de
natureza peptídica de caráter
básico, 1.000 Da, solubilidade
em água 50 mg/mL

TABELA 1. FÓRMULA DO CARREADOR LIPÍDICO NANOESTRUTURADO (CLN)

Fase	Componentes	% (p/p)
Oleosa	Triésteres de glicerol dos ácidos cáprico e caprílico	4,00
	Palmitato de cetila	6,00
	Fosfatidilcolina	0,60
Aquosa	Tween 80 [®]	1,50
	Pluronic [®] F68	1,50
	Água ultrapura Milli-Q [®]	q.s.p. 100

TABELA 2. DIÂMETRO HIDRODINÂMICO MÉDIO DA PARTÍCULA (DHM), ÍNDICE DE POLIDISPERSIVIDADE (IP) E POTENCIAL ZETA (PZ) MV DAS PREPARAÇÕES DE CLN-DEXA+POLI

Fórmula	SP UI/mL	Tempo (horas)	DHM ±DP (nm)	IP ±DP	PZ ±DP (mV)
1	7462,9	6	235,6 ±4,2	0,204 ±0,04	+3,59 ±0,16
2	5.000	8	244,1 ±2,3	0,247 ±0,06	-0,124 ±0,12
3	10.000	8	255,7 ±4,1	0,192 ±0,04	+2,47 ±0,56
4	11.000	6	250,3 ±2,4	0,295 ±0,03	+3,45 ±0,49
5	5.000	4	251,4 ±1,3	0,223 ±0,01	-2,06 ±0,17
6	10.000	4	230,9 ±2,8	0,214 ±0,07	+2,55 ±0,44
7	7462,9	6	246,9 ±3,8	0,297 ±0,02	+2,28 ±0,53
9	7462,9	3h 10min	235,0 ±2,0	0,201 ±0,01	-0,929 ±1,27
10	7462,9	8h 50min	244,9 ±3,7	0,216 ±0,02	+2,87 ±0,15
12	3964,5	6	243,4 ±1,9	0,148 ±0,03	-1,34 ±0,52
13*	-	-	220,2 ±3,9	0,147 ±0,04	-45,75 ±01,18

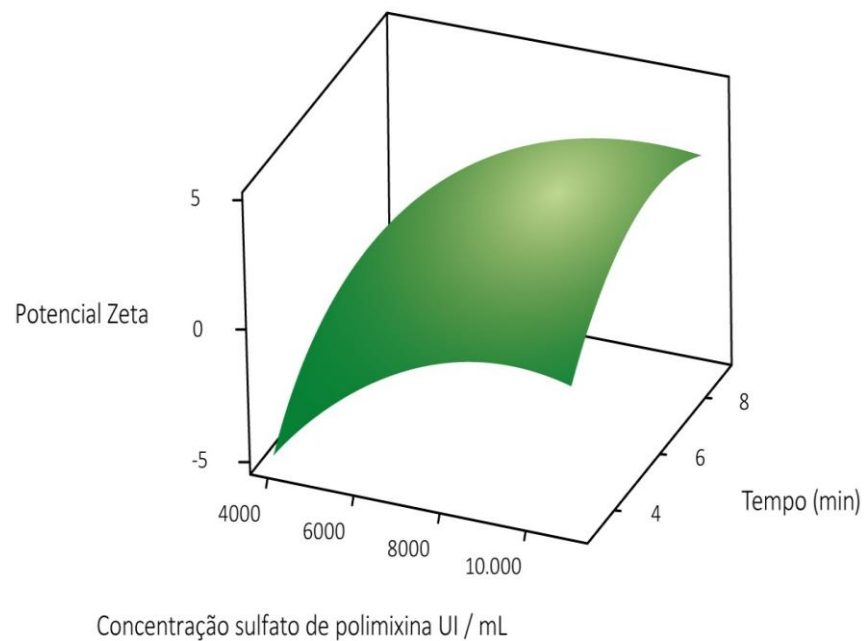
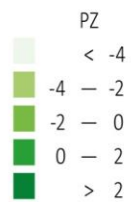
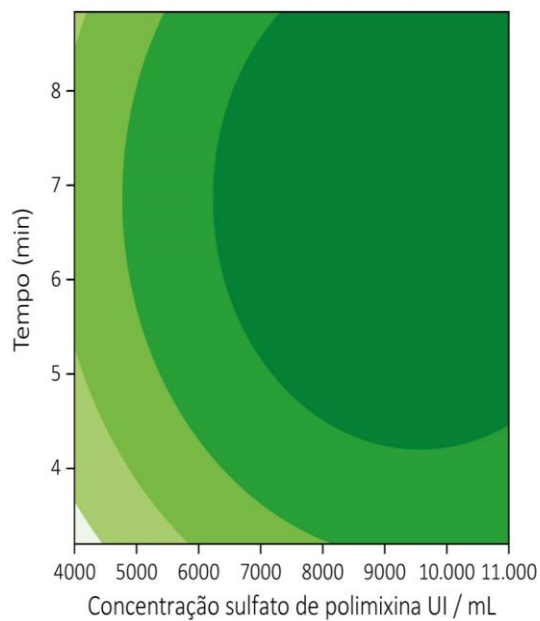


FIGURA 4. GRÁFICO DE CONTORNO E SUPERFÍCIE: POTENCIAL ZETA (MV) EM FUNÇÃO DAS VARIÁVEIS: TEMPO DE AGITAÇÃO (HORAS) E CONCENTRAÇÃO DE SULFATO DE POLIMIXINA B (UI/ML)

Tabela 3. Potencial zeta em mV (PZ) dos CLN-Dexa + Poli por período de 56 dia mantidos em geladeira (5° C).

Concentração de Sulfato de Polimixina B (UI/mL)	PZ (mV)								
	1º Dia	7º Dia	14º Dia	21º Dia	28º Dia	35º Dia	42º Dia	49º Dia	56º Dia
-	-62,8±4,90	-61,3±1,80	-58,7±3,52	-61,3±1,80	-58,7±3,52	-60,1±5,99	-53,8±1,12	-54,7±3,82	-53,4±2,05
1.000	-51,4±1,63	-48,3±5,41	-53,3±1,63	-50,8±1,28	-50,6±1,30	-51,2±1,75	-49,1±3,66	-44,7±0,58	-53,0±2,29
2.000	-44,3±0,95	-48,6±6,00	-45,0±1,78	-48,5±2,29	-47,5±3,77	-53,3±5,58	-42,0±0,74	-43,4±3,29	-
3.000	-39,0±2,21	-38,3±3,35	-45,8±3,69	-33,7±4,03	-39,5±1,53	-40,9±6,03	-39,9±0,85	-24,2±1,40	-44,5±2,16
4.000	-22,3±4,78	-31,0±1,48	-29,9±3,64	-21,2±4,33	-27,8±4,04	-28,6±2,35	-24,3±3,40	-22,9±0,59	-33,7±2,35
7.500	-1,7±0,62	-6,2±0,15	-2,1±0,08	-3,9±0,46	-1,2±0,17	-3,2±0,55	-4,8±0,29	-4,2±0,33	-2,9±0,49
10.000	-0,9±0,24	-2,8±0,29	-0,2±0,27	-0,6±0,70	+0,9±0,14	-2,5±0,41	-1,2±0,06	-1,5±0,284	-1,0±0,15

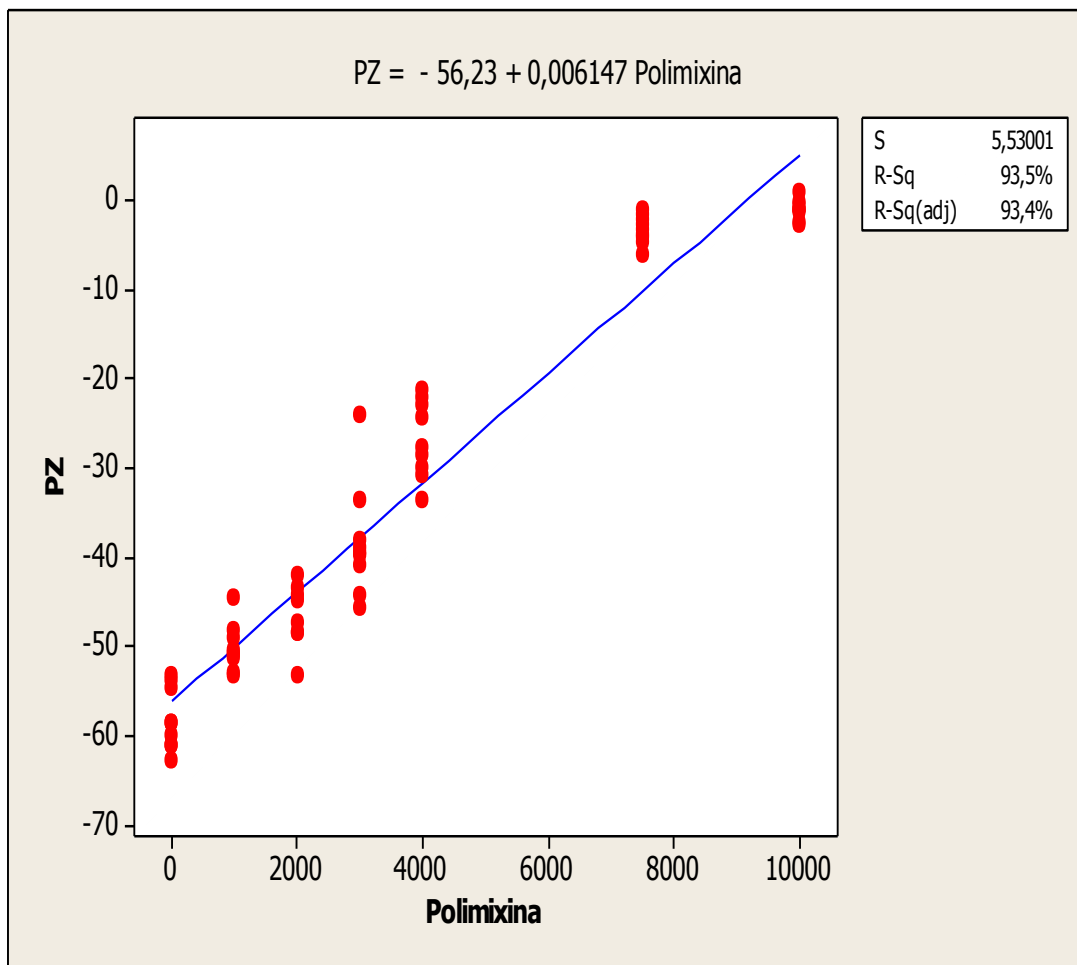


FIGURA 5. ANÁLISE DA REGRESSÃO SIMPLES DO POTENCIAL ZETA (PZ) VERSUS CONCENTRAÇÃO DE SULFATO DE POLIMIXINA B EM UI/ML (POLIMIXINA)

Tabela 4. Eficiência de encapsulação estimada do sulfato de polimixina B pela determinação da Concentração Mínima Inibitória (CMI)

CLN-Dexa + Poli (UI/mL)	Polimixina B Livre (UI/mL)	Polimixina B Livre (% p/v)	Eficiência de Encapsulação (%p/v)	Polimixina B (UI/mL)
5.000	<31,25	0,63	>99,38	4.969
7.500	62,5	0,83	99,17	7.438
10.000	125	1,25	98,75	9.875

Tabela 5. Concentração mínima inibitória (CMI) dos CLN-Dexa+Poli diluídos em tampão fosfato 10% (p/v) pH 6,0 e em água ultrapura Milli-Q® frente a *Bordetella bronchiseptica* ATCC 4617

CLN-Dexa + Poli (UI/mL)	Concentração Mínima Inibitória			
	tampão fosfato 10% (p/v) pH 6,0		água ultrapura Milli-Q®	
	UI/mL	µg/mL	UI/mL	µg/mL
5.000	0,50	0,06	0,50	0,06
7.500	0,50	0,06	0,50	0,06
10.000	0,50	0,06	0,50	0,06

CMI do sulfato de polimixina B em tampão 10% e água ultrapura Milli-Q®: 1,25 UI/mL ou 0,15 µg/mL

CMI CLN-Dexa+Poli: 2,5 vezes maior, quando comparada ao sulfato de polimixina B livre.

PROTASAN UP CL 113:

- based on a chitosan where between 75-90 percent of the acetyl groups are removed;
- a highly purified and well-characterized water-soluble chloride salt;
- the functional properties are described by the molecular weight and the degree of deacetylation:
 - 50000-150000 g/mol range (measured as a chitosan acetate). The ultra low levels of endotoxins and proteins allow for a big variety of in vitro and in vivo applications.

Fonte:

<http://www.novamatrix.biz/Products/PROTASANUltrapureChitosans/tabid/4460/List/1/CategoryID/4/Level/1/Default.aspx?SortField=ProductName,ProductName>

Tabela 6. Diâmetro hidrodinâmico médio em nm (DHM) e PZ em mV dos CLN-Dexa+Poli+Protasan[®] por período de 28 dias mantidos em geladeira (5° C)

Polimixina B (UI/mL)	1º Dia		7º Dia		14º Dia		21º Dia		28º Dia	
	DM (nm)	PZ (mV)	DM (nm)	PZ (mV)	DM (nm)	PZ (mV)	DM (nm)	PZ (mV)	DM (nm)	PZ (mV)
-	395,2±26,98	+44,7±0,99	370,5±0,78	+44,7±0,93	423,0±11,2	+50,7±6,93	408,3±7,62	+44,1±0,57	457,1±20,6	+49,1±0,42
1.000	382,7±15,42	+43,9±0,50	325,5±5,80	+40,2±0,99	348,6±9,02	+44,5±0,71	383,8±11,2	+40,8±1,34	410,5±4,54	+51,3±0,71
2.000	318,8±9,25	+43,3±0,86	310,5±1,63	+41,3±1,95	337,6±9,59	+45,7±1,48	314,9±5,41	+39,0 ±1,20	350,8±6,65	+48,5±1,13
3.000	283,7±5,71	+45,0±3,32	275,8±7,64	+41,3±0,71	307,6±7,71	+47,2±1,13	286,5±4,17	+44,4±1,41	304,5±7,61	+49,4±2,40
4.000	255,0±0,21	+42,3±0,30	248,9±2,23	+42,2±2,33	257,2±4,25	+46,3±3,04	250,3±1,33	+38,9±2,55	259,1±3,51	+46,4±0,00
7.500	219,1±2,04	+33,0±2,52	214,1±1,63	+27,5±2,90	221,1±3,40	+30,8±3,11	225,3±2,75	+30,8±0,77	234,6±3,47	+38,4±4,24
10.000	216,7±2,69	+27,8±3,02	219,1±4,38	+24,5±0,63	215,9±4,92	+27,3±0,85	209,0±2,56	+24,3±0,50	218,0±2,36	+30,4±2,40

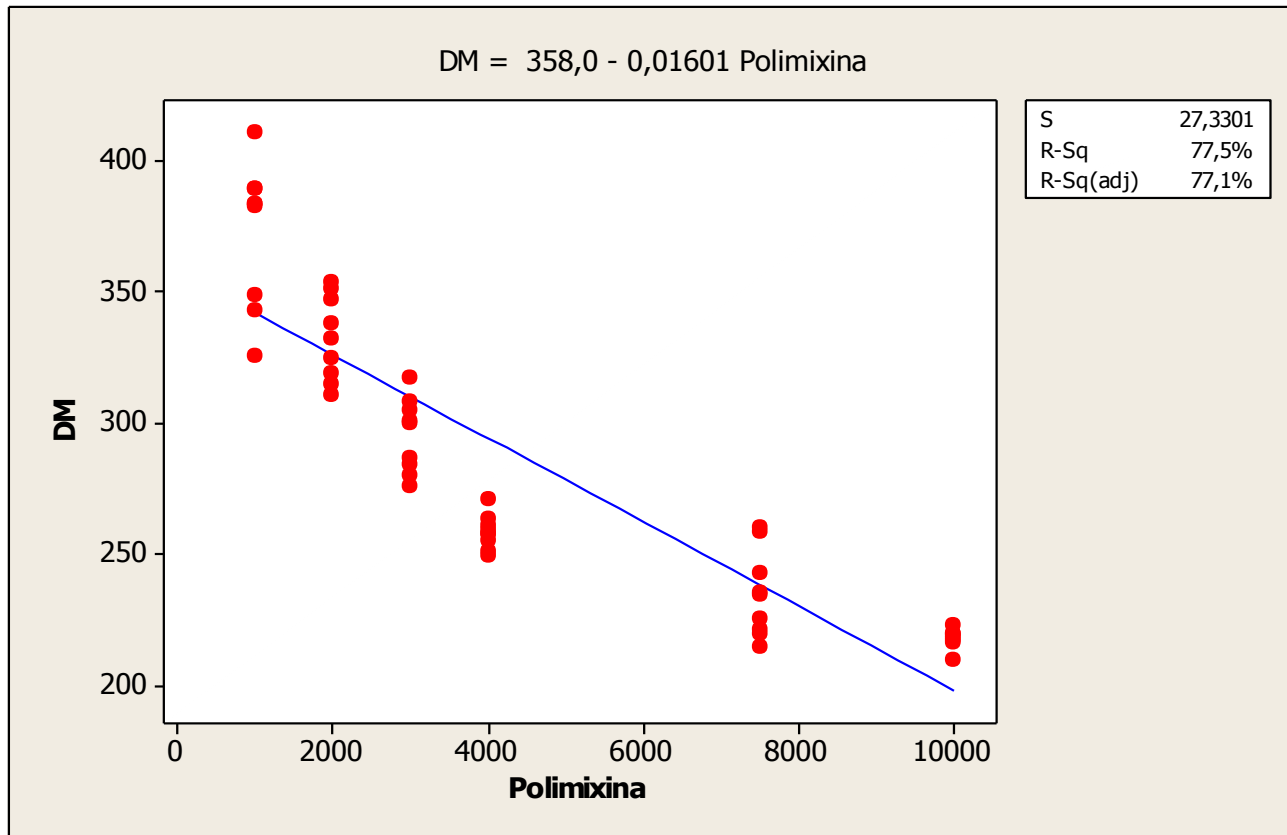


Figura 6. Análise da regressão simples do diâmetro hidrodinâmico médio (DM) versus concentração de sulfato de polimixina B em UI/mL (polimixina)

Novasorb[®] versus Ophthino[®]

	Novasorb [®]	Ophthino [®]
Fase Oleosa	Triglicerídeo de cadeia média	Triglicerídeo de cadeia média
	Cloreto de cetalcônio	
		Palmitato de Cetila
	Tylopaxol	Lipoid 100
Fase Aquosa	Poloxamer 188	Poloxamer 188
	Glicerol	Glicerol
	Hidróxido de Sódio	
		Polissorbato 80
		Cloreto de quitosana
	Água para injeção	Água Ultrapura MilliQ [®]

Patente

**PROCESSO DE OBTENÇÃO DE UM SISTEMA NANOESTRUTURADO
CATIÔNICO, SISTEMA NANOESTRUTURADO CATIÔNICO E SEU USO**

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**Mucoadhesive Dexamethasone acetate-
Polymyxin B sulfate cationic ocular
nanoemulsion – novel combinatorial
formulation concept**

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Table 1

Composition of DEX-loaded nanoemulsion applied in formulation A produced by HPH at 600 bar for three cycles (make up to 95% with Milli-Q water) and PCS data (z-average (nm), PI as well as LD data (d(v)50 %, d(v)90 %, d(v)95 %) after production.

Code	DEX (%w/w)	Eutanol G (w/w)	Poloxamer 407 (%w/w)	PCS		LD		
				z-average (nm)	PI	d(v)50% (μm)	d(v)90% (μm)	d(v)95% (μm)
A	0.05	20.00	4.00	128 ± 2	0.071 ± 0.031	0.134	0.192	0.203

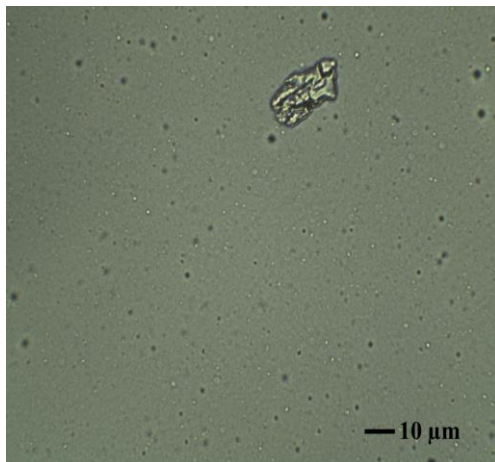


Fig. 1. Light microscopy picture of formulation A on 2 day after production (magnifications: 600 \times , scale bar: 10 μm).

- Eutanol G (2-octyldodecan-1-ol): widely used in topical pharmaceutical formulations, nontoxic and nonirritant at the levels employed as an excipient (Rowe et al., 2009).
- Poloxamer[®] 407, an amphiphilic synthetic copolymer consisting of a hydrophobic poly(oxypropylene) (POP) block between two hydrophilic poly(oxyethylene) (POE) blocks (molecular weight 9840 to 14600).

Poloxamer[®]407

- inhibited approximately 75% of the bacterial adherence to epithelial cells from rabbit corneas ($p < 0.05$), using *Pseudomonas aeruginosa*;
- the antiadherent surfactant in eye drops could act as a prophylactic agent to infections caused by the Gram-negative organism (Portolés et al., 1995).

- drug crystallization occurred during short-term storage possibly due to the limited DEX solubility in the eutanol G.
- The drug, by diffusion, reached the aqueous medium precipitating as free crystals as a result of its low water solubility, immediately after nanoparticle preparation (Beck et al., 2003).

Table 2

Solubility of DEX in different ratios of Eutanol G : lecithin (mg/g) (n=2)

Lecithin	Eutanol G: lecithin (%w/w : %w/w)				
	90:10	80:20	70:30	60:40	50:50
Lipoid S100	5	5	10	10	creamy
Lipoid S75	<5	5	5	10	creamy
Lipoid E 80	5	5	10	10	creamy
Phospholipon®80	5	5	10	10	creamy

Lipoid S100: phosphatidylcholine from soybean - pharma parenteral

Lipoid S75: fat free soybean phospholipids with 70% phosphatidylcholine, pharma parenteral

Lipoid E 80: egg phospholipids with 80% phosphatidylcholine, pharma parenteral

Phospholipon®80: 76±3% phosphatidylcholine

- As a penetration enhancer, lecithin benefits increasing the drug entrapment efficiency avoids drug leaking from lipid matrix (Li et al., 2014).
- Eutanol G: Lipoid S 100 or Lipoid E80 or Phospholipon[®]80 (ratio of 70:30) increased DEX solubility to 10 mg/g of oil phase.

Code	DEX (%w/w)	Eutanol G : Lipoid S 100 (70% : 30%)	Poloxamer 407 (%w/w)	Tween 80 (%w/w)	PCS data		LD data		
					z-average (nm) PI	d(v)50 % (μm)	d(v)90% (μm)	d(v)95 % (μm)	
B	0.05	20.00	1.00	---	500 ± 18 0.409 ± 0.130	0.573	1.498	2.495	
C	0.05	20.00	2.00	---	166 ± 3 0.097 ± 0.035	0.153	0.236	0.264	
D	0.05	20.00	3.00	---	151 ± 3 0.119 ± 0.045	0.149	0.212	0.232	
E	0.05	20.00	4.00	---	122 ± 2 0.115 ± 0.019	0.136	0.195	0.213	
F	0.05	20.00	---	1.00	1029 ± 131 0.969 ± 0.052	1.445	4.062	17.335	
G	0.05	20.00	---	2.00	312 ± 10 0.349 ± 0.045	0.425	0.844	1.051	
H	0.05	20.00	---	3.00	302 ± 8 0.168 ± 0.051	0.400	0.839	1.071	
I	0.05	20.00	---	4.00	282 ± 3 0.294 ± 0.033	0.395	0.684	0.795	
J	0.05	15.00	---	2.00	187 ± 5 0.103 ± 0.046	0.206	0.371	0.427	
K	0.05	15.00	---	4.00	139 ± 2 0.113 ± 0.043	0.142	0.208	0.229	

Cationic agents

- Polymyxin B
- Alkylbenzyltrimethylammonium chloride (BAC)
- Benzyltrimethylhexadecylammonium chloride (CAC)
- Cetylpyridinium chloride (CPC)

Code	Cationic components	Zeta potential (mV)	
		original medium	Water (50 μ s/cm)
C		-5.2 ± 0.2	-20.3 ± 0.1
C-1	Polymyxin B + CPC	3.1 ± 0.2	13.8 ± 1.5
C-2	Polymyxin B + BAC	3.9 ± 0.3	7.7 ± 0.6
C-3	Polymyxin B + CAC	3.2 ± 0.3	6.5 ± 0.6
H		-4.14 ± 0.4	-17.2 ± 0.5
H-1	Polymyxin B + CPC	22.3 ± 1.1	11.2 ± 0.7
H-2	Polymyxin B + BAC	20.8 ± 0.5	7.9 ± 0.4
H-3	Polymyxin B + CAC	18.0 ± 0.4	4.7 ± 0.2
J		-4.7 ± 0.2	-10.3 ± 0.2
J-1	Polymyxin B + CPC	21.0 ± 0.6	10.6 ± 0.5
J-2	Polymyxin B + BAC	21.9 ± 0.7	5.4 ± 0.0
J-3	Polymyxin B + CAC	21.9 ± 0.6	4.0 ± 1.0



Fig. 2. Photograph of DEX and polymyxin B loaded cationic nanoemulsion after 180 days storage at different temperatures (left: 4 °C, middle: RT, right: 40 °C).

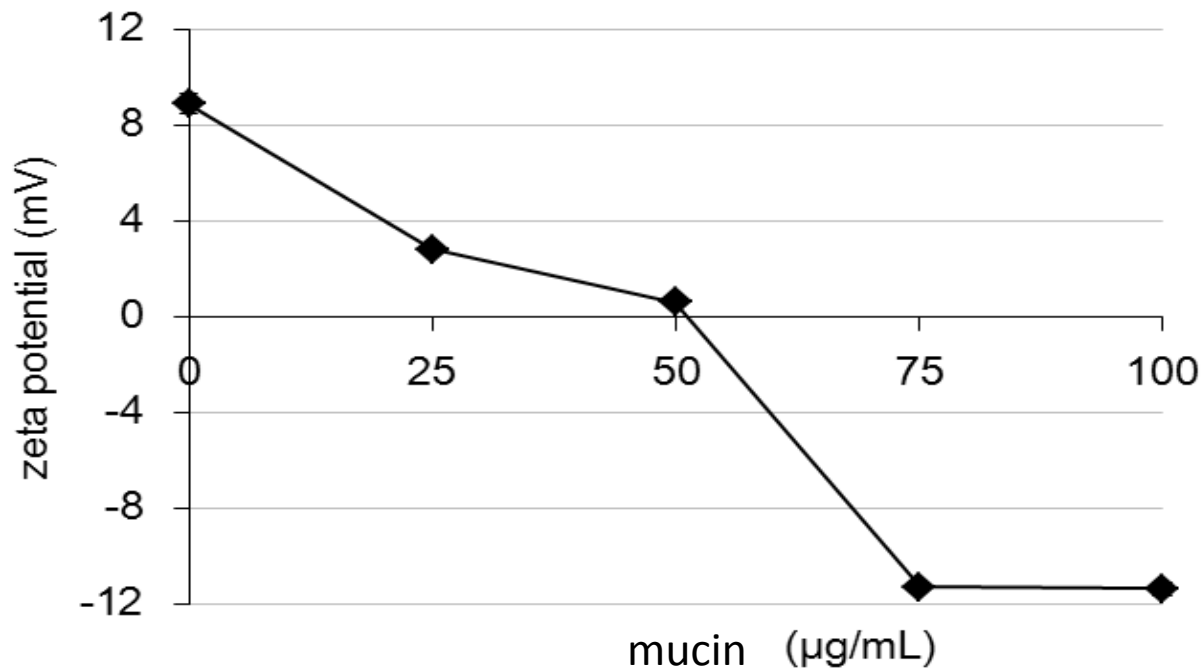


Fig. 3. Zeta potential of the cationic nanoemulsion when mixed with a series of concentrations of mucin (measured in conductivity adjusted water (50 $\mu\text{S}/\text{cm}$) ($n=3$) and light microscopy picture of cationic nanoemulsion when mixed with 50 $\mu\text{g}/\text{mL}$ of mucin solution.

Table 6

Zeta potential data measured in original medium and in conductivity adjusted water ($\mu\text{s}/\text{cm}$) and assay of DEX and Polymyxin B loaded cationic nanoemulsion at different temperatures: 4 °C, room temperature (RT) and 40 °C over a period of 90 days.

Day	Condition	Zeta potential (mV)		Assay of DEX (%w/w)
		original medium	Water (50 $\mu\text{s}/\text{cm}$)	
0		19.9 ± 1.0	8.9 ± 0.9	95.7
90	4 °C	21.9 ± 0.4	11.5 ± 0.0	95.6
90	RT	20.7 ± 0.6	5.3 ± 0.2	92.7
90	40 °C	18.1 ± 0.1	-1.0 ± 0.3	48.7

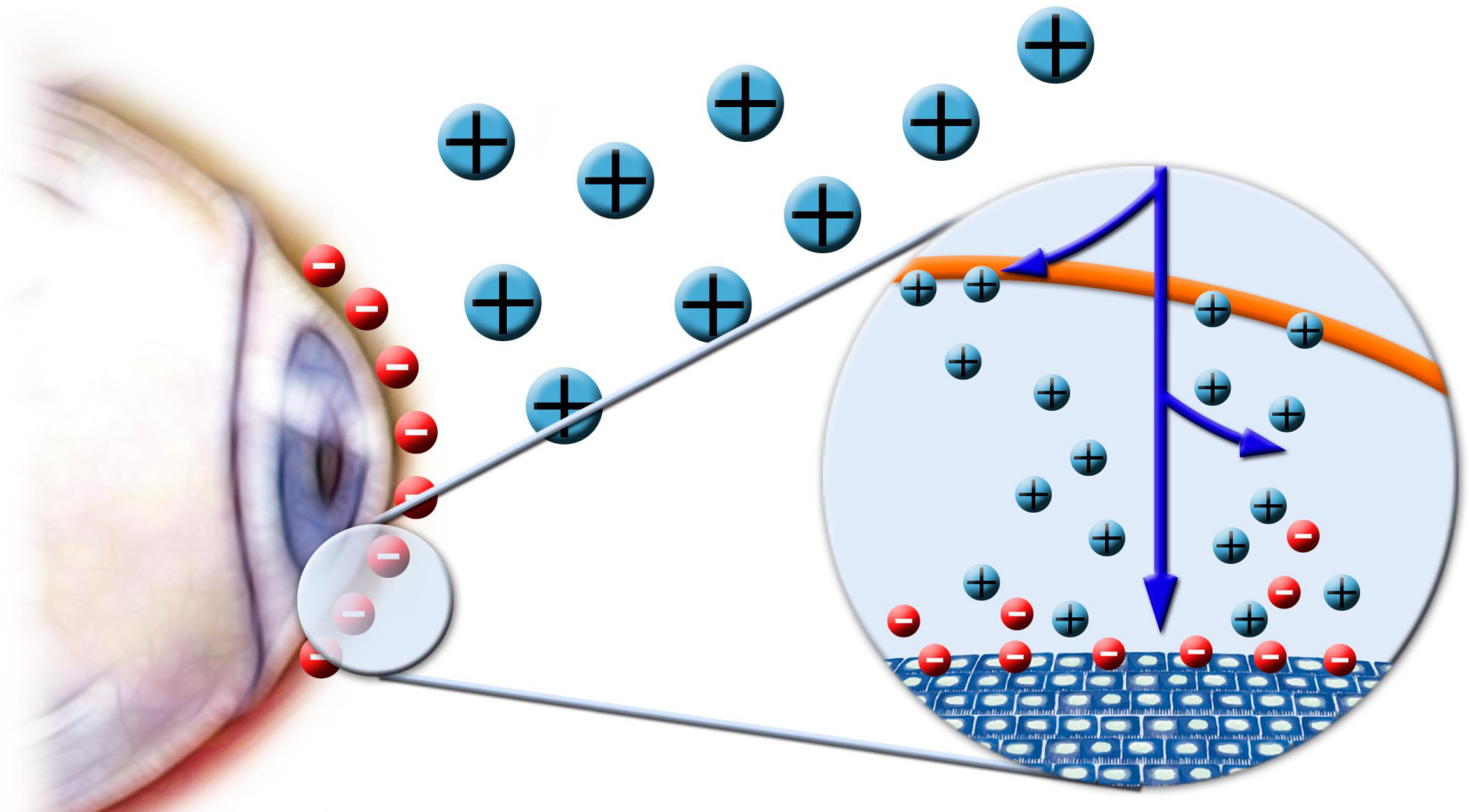


Ilustração: Bou-chacra, N.

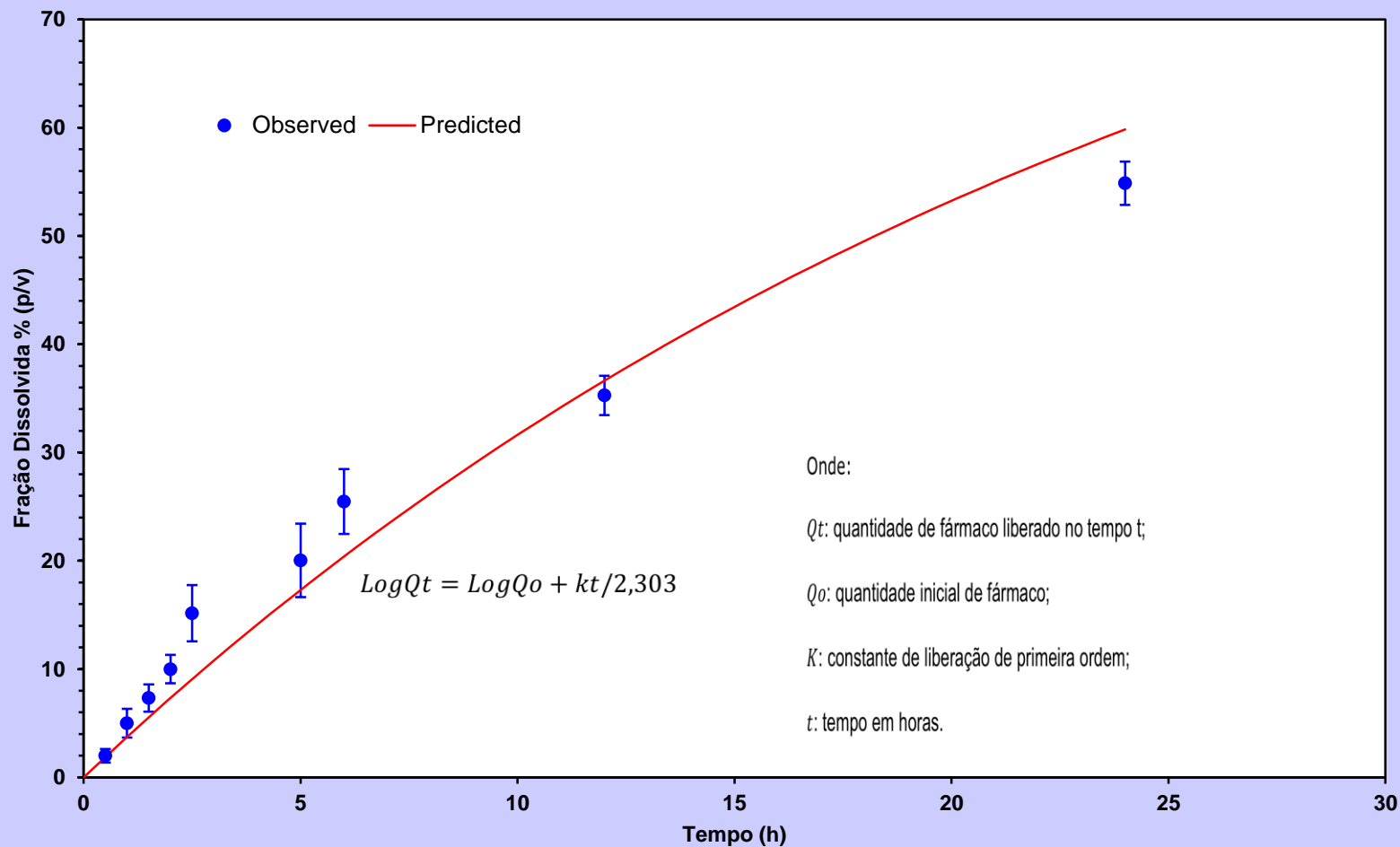


Figura 4. Curva para valores observados e previstos relativos à fração liberada acumulada de acetato de dexametasona a partir da nanoestrutura para modelo de cinética de primeira ordem ($K: 0,038 \pm 0,002 \text{ h}^{-1}$).

Conclusion

The developed product is a viable alternative to the commercial ophthalmic suspensions. Moreover, this concept of generating the positive charge by preservative addition can be transferred to other ophthalmic products.

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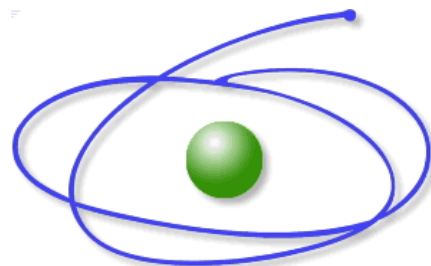
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