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OCULAR BIOAVAILABILITY OF ENROFLOXACIN-CYCLODEXTRIN COMPLEXES FROM CERTAIN OPHTHALMIC PREPARATIONS IN RABBITS EYES

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ABSTRACT

The effect of inclusion complexes of enrofloxacin (Enr) with cyclodextrins (CyDs); hydroxypropyl beta-cyclodextrin (HP-β-CyD) and beta-cyclodextrin (β-CyD) was studied. These complexes were prepared in a molar ratio (1:2). The ophthalmic gels [sodium carboxymethyl cellulose (sod. CMC), sodium alginate] and ointments (emulsion, absorption and water soluble bases) were prepared using 0.5% of the drug or equivalent amounts of its complexes with HP-β-CyD or β-CyD. The ocular disposition of the drug in rabbit's eyes has been studied.

The obtained results revealed that, all tested formulations provided the highest C_{max} of the drug in conjunctiva followed by cornea, iris-ciliary body, and then aqueous humor. The peak time of maximum drug concentration in rabbit's eye tissues and aqueous humor was two hours for various ophthalmic ointments and gels. The total availability of enrofloxacin was improved when the drug was complexed with HP-β-CyD.

INTRODUCTION

Topical application of drugs to the eye is the most frequently employed route of administration for treatment of eye diseases in order to restrict the site of drug action to the eye, reduce the quantity of drug employed and to avoid the possibility of unwanted systemic effects[1].

Enrofloxacin is a synthetic broad spectrum antibacterial agent. It is a fluoroquinolone derivative. Its chemical structure is closely related to that of ciprofloxacin. Enrofloxacin belongs to the new quinolone carboxylic acid group of antimicrobials with an extended antibacterial spectrum. In the tissues, enrofloxacin is de-ethylated to ciprofloxacin (the primary metabolite), which is detectable in urine and serum after oral administration. It was investigated as to its usefulness in the treatment of chronic bacterial prostatitis[2].

The antibacterial activity of fluoroquinolones results from interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA. So they act by inhibiting gyrase-mediated DNA supercoiling, not permitting DNA replication at concentrations that correlate well with those required to inhibit bacterial growth (0.1 to 10 µg/ml)[3].

The quantitative determination of microbiologically active drug in plasma and urine was reported by Brogard et al.[4], through agar diffusion assay. Also, they compared between the high-pressure liquid chromatography (HPLC) and the microbiological assay for the determination of plasma level and urinary elimination of ciprofloxacin in human. They found that, the average serum concentration-time curves of ciprofloxacin determined by HPLC did not significantly differ from those determined by microbiological assay, also the mean concentrations of ciprofloxacin found in urine by the two analysis methods did not differ significantly. According to the previous investigation, the microbiological assay was used in this part to study the ocular bioavailability of enrofloxacin from certain ophthalmic preparations in various eye tissues of rabbits.

This study aims to evaluate enrofloxacin in certain ophthalmic preparations from different tissues of rabbit eyes. Also, it was of great interest to study the effect of vehicle type on the uptake of enrofloxacin by eye tissues and fluid of rabbits eyes at various time intervals after application.

EXPERIMENTAL

Materials:

Benzalkonium chloride, polyethylene glycol 4000, sodium alginate, cetyl alcohol, sodium metabisulphite, disodium edetate (BDH Chemical Ltd, G.B. Liverpool, England). Disodium hydrogen phosphate, yellow soft petrolatum and n-octanol (Prolabo, Chemicals, Paris, France). Enrofloxacin (Cenavisa S. A. Laboratories. Reus, Spain). Polyethylene glycol 400 (Fluka AG, CH-9470 Buchs, Switzerland). Acetone, potassium dihydrogen orthophosphate, sodium carboxymethylcellulose, tween 40 and acetonitrile (Adwic, El Nasr Pharmaceutical Chemicals Co., Egypt). HP-β-CyD (Molecular weight = 1413.4) and β-CyD (Molecular weight = 1135) (Nippon Shokuhin Kako Co., Tokyo, Japan). Cellulose membrane (Fisher Sci. Co., Pittsburgh, U.S.A). Nutrient agar medium (Oxoid LTD., Basingestoke, Hampshire, England). Orthophosphoric acid (Merck, Darmstadt, G.F.R.). Organism: Bacillus subtilis ATCC 6633 (obtained from the department of microbiology, Faculty of pharmacy, Mansoura university). Experimental animals: albino rabbits of 1.8-2 kg.

Equipment:

U.V. spectrophotometer (Shimadzu, UV-150-02, sersakusho, Ltd, Kyoto, Japan) pH-meter (Beckman Instruments fullerton, CA-92634). Thermostatically controlled shaking water bath (Grant instrument Cambridge Ltd. Barrington Cambridge, B2, 5002, England). Membrane filter (Millipore corporation, Bedford, MA 01730), modified diffusion cell, rotary viscometer (Haake Inc., Germany). Controlled environment incubator (Manufactured by New

Brunswick Scientific Co., Inc, Edison, N.J., U.S.A). MSE minor centrifuge (MSE scientific instruments, Manor Royal, Crawley RH/0200 sussex, England). Reusable petri dishes.

Methods:

1- Preparation of enrofloxacin - CyDs complexes, enrofloxacin eye gels and ointments, determination of enrofloxacin content, viscosity and pH of prepared formulations (Table 1), were performed and reported earlier by Ramadan et al.[5].

Table (1): The composition of enrofloxacin ophthalmic preparations

Formulations / Ingredients	Ointments (% W/W)			Gels (% W/W)	
	Absorption base	Emulsion base	W.S.B.	Sodium alginate	Sodium CMC
Enrofloxacin	00.50	00.50	00.50	00.50	00.50
Liquid paraffin	29.85	15			
Yellow soft petrolatum	59.7	15			
Cetyl alcohol	9.95	15			
Distilled water		49.5	9.5	81.5	90.5
Tween 40		5			
PEG 4000			20		
PEG 400			70		
Sodium alginate				13	
Propylene glycol				5	5
Sodium CMC					4

Sodium CMC: Sodium carboxymethylcellulose
W.S.B.: Water soluble base
PEG: Polyethylene glycol

2- Determination of potency percent of enrofloxacin applying the microbiological method.

Procedure:

Antimicrobial assay studied by Kirby-Baur disc diffusion technique[6].

Microorganism and media:

Bacillus subtilis ATCC 6633 was the test organism used in the microbiological assay. Reference standard antimicrobial agent used was enrofloxacin.

Nutrient agar medium:

Pepton	5 gm
Beef extract	2 gm
Sodium chloride	2 gm
Agar	20 gm
Distilled water to	1000 ml

This medium was sterilized by autoclaving at 121 °C for 15 minutes.

Method:

Fifty ml melted nutrient agar at 50°C ± 0.5°C was seeded with 0.1 ml of 24 hr culture of Bacillus subtilis ATCC 6633. Accurately 25 ml of seeded agar was poured into 15 cm diameter petri-dishes and allowed to solidify. Wells of 7 mm diameter were made into the seeded agar by the aid of sterile Weatherman tube. Fifty microliters of each tested sample was applied into the corresponding well. In addition, 50 µl of the reference standard enrofloxacin was applied to the corresponding cup in each plate. All aliquots were allowed to diffuse at the room temperature for 30 min and plates were incubated at 37°C ± 0.5°C for 24 hr. Zones of inhibition in mm were measured using Verniar caliper to the nearest 0.5 mm.

The tested formulations :

Ointments	1- Absorption base + Drug
	2- Emulsion base + Drug
	3- W.S.B + Drug
	4- W.S.B.+ Drug-HP-β-CyD
	5- W.S.B.+ Drug-β- CyD
Gels	6- Sod. CMC + Drug
	7- Sod. alginate + Drug
	8- Sod. alginate + Drug-HP-β-CyD
	9- Sod. alginate + Drug-β-CyD

Preparation of standard solution:

The standard solution was prepared by dissolving an accurate weight of enrofloxacin (50 mg) in 100 ml of acetate buffer (pH 5.2). Three levels of dilutions were prepared from standard solution and designed as S1, S2 and S3. The concentration ratio between these three dilutions was 1: 2: 4 respectively. The same amount of constituents present in the tested ophthalmic solutions from each preparation was added to the standard solution to eliminate their interference, if any, with the assay.

Preparation of test solutions:

An accurately weighed amount of each formula (1 gm) was dissolved in sterile acetate buffer (pH 5.2) to produce a final dilution of 40 ug/ml, which was designed as T3. Then two dilutions T2 and T1 were prepared so that, T1: T2: T3 equals 1: 2: 4, respectively.

Design of assay:

The six-point assay method was used. Six plates were used for each assay as previously described. Six cups were bored in each plate. In each cup, 50 µl of either standard or test solution was pipetted, (in each plate S_1, S_2, S_3, T_1, T_2, and T_3 are present). The present method comprising the three concentration levels for each of standard and test solutions in the same plate would make the comparison of the inhibition zones more reliable as well as avoiding the effect of plate-plate variation. The plates were left to prediffuse in the refrigerator for 2 hours, then incubated at 37°C ± 0.5°C for 24 hrs. The inhibition zones were measured

The log potency "M" was determined as the following[7]:

Ratio of doses	= 2.0
Log ratio of doses (I)	= 0.301
Effect difference due to doses (E)	= 1/4 [(T3-T1) + (S3-S1)]
Effect difference due to preparations (F)	= 1/3 [(T3+T2+T1) - (S3+S2+S1)]
Slope (b)	= E/I
Log potency ratio test/standard (M)	= F/b
Potency ratio	= anti-log of M
Percent potency	= anti-log Mx100

3. Ocular bioavailability of enrofloxacin from certain ophthalmic preparations

3.1. Tested formulations:

The following preparations were selected and used according to the drug release, stability and percent potency of enrofloxacin at different conditions as reported by Ramadan et al.[5].

1-Water soluble base containing enrofloxacin (0.5%).
2-Water soluble base containing enrofloxacin- HP-β-CyD complex (equivalent to 0.5%).
3-Sodium alginate gel containing enrofloxacin (0.5%).
4-Sodium alginate gel containing enrofloxacin-HP-β-CyD complex (equivalent to 0.5%).

3.2. The microbiological assay using agar diffusion method.

The ocular bioavailability of the drug was studied by microbiological assay using agar diffusion method. Firstly, the standard calibration curve of the drug was done by plotting the X 2 versus Log concentrations of the drug, using inoculated agar plate with Bacillus subtilis ATCC 6633. Then, this curve was used to determine the bioavailability of the drug in the different tissues of rabbits eyes at several time intervals after the application of the ophthalmic preparations containing the drug to rabbits eyes[8].

N. B.) X 2 = [(a-b)/2]2

a = Zone diameter.

b = Well diameter = 7 mm.

3.3. Construction of calibration curve of enrofloxacin in agar plate (using agar diffusion method).

One hundred mg of the drug was dissolved in one ml filtered extract of rabbits eyes (Solvent composed of 0.025 M ortho-phosphoric acid mixed with acetonitrile in a ratio of 87:13) to give a concentration of 1×10^5 ug/ml (Stock solution).

Serial dilutions were prepared from the stock solutions to give concentrations of 50000, 25000, 12500, 6250, 3125, 1562.5, 781.25, 390.6, 195, 97.7 and 48.8 ug/ml representing the tested samples. An exactly measured volume of 50 µl of each sample was transferred into each cup of inoculated-agar plates. Each sample was run in triplicate. Inhibition zones were measured after overnight incubation at 37°C. The photograph of Petri dish (figure 1) showed the inhibition zone resulted from different concentrations of enrofloxacin against Bacillus subtilis ATCC 6633.

3.4 Application of enrofloxacin formulations into rabbits eyes.

Individual doses of 100 mg of each sterile preparation were accurately weighed, then immediately and carefully transferred by micro-spatula into the center of the lower lid (cul-de-sac) of albino rabbits weighed 1.8-2 kg. The lower eyelid was gently moved to spread the dose on corneal surface during dosing (care should be taken not to irritate the eye or touch the corneal surface). All rabbits were kept in up-right position in restraining boxes. Four rabbits were used for the determination of the amount of drug disposed in different eye tissues and aqueous humor at each time interval. The enrofloxacin ocular concentration was determined at 1, 2, 3, and 5 hours after dose application. Therefore, four eyes which receive the medicament in each tested formula were used for determination of drug concentration. For each animal, one eye was loaded with the tested formulation, while, the other was loaded with the plain vehicles and served as a control.

3.5. Separation of eye tissues.

Following killing of the rabbits and separation of its conjunctival surface, one ml of aqueous humor was aspirated from the anterior chamber using micrometer syringe. Then, a single incision was made with a scalpel at the corneal margin and the entire cornea was excised. The whole cornea and conjunctival surface were rinsed with the least amount of normal saline. The anterior segment tissues; conjunctiva, aqueous humor, cornea and iris-ciliray body was obtained in that order. The surgical procedures on each eye were completed within 10 minutes of sacrificing the animal. So that, any errors due to redistribution of the drug during the time required obtaining ocular tissue samples were minimized. Each individual tissue was transferred into scintillation vial and the net weight of tissue was determined using an analytical balance.

3.6 Extraction of enrofloxacin from different tissues and fluid of rabbits eyes.

The concentration of enrofloxacin in different eye tissues and fluid was determined after 1, 2, 3 and 5 hours of application of each formulation. At each time interval, conjunctiva, cornea, and iris-ciliary body of each eye were separated immediately, rinsed with isotonic saline solution weighed and grinded with powdered glass. The grinded tissues were extracted with 8 ml of mixture composed of 0.025 M *ortho*-phosphoric acid mixed with acetonitrile in a ratio of 87:13 respectively. On the other hand, aqueous humor was mixed with 8 ml of the extracting solvent. These solutions were centrifuged at 9000 rpm for 30 minutes, then, filtered using 0.22 µm millipore filter. The determination of the drug concentrations in different tissues and fluid had been done by microbiological method[4]. A fifty ul sample of each

solution was dispensed into each cup of agar plates sealed with Bacillus subtilis ATCC 6633. Each sample was run in triplicate. Inhibition zones were measured after overnight incubation at 37°C. The drug concentration was calculated from the calibration curve.

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-kramer multiple comparisons test[9-11]. Statistical calculations were carried out using instate 2- computer program (Graphpad software Inc., V2.04, San Diego, CA, U.S.A).

RESULTS AND DISCUSSION

1- The percent potency of enrofloxacin by applying the microbiological method.

The percent potency of enrofloxacin in the prepared formulations was determined by applying agar diffusion method. The results obtained were demonstrated in table (2). The results revealed that, the percent potency of all formulations were in the range from 61.1 %, for emulsion base, to 93.13 %, for W.S.B + Drug-HP-β-CyD. It is clear that, HP-β-CyD produces the highest percent potency of enrofloxacin in both selected gel and ointment bases. This is may be attributed to the higher solublizing and penetrating power of HP-β-CyD compared with parent CyDs[12].

Table (2): The percent potency of enrofloxacin in different ophthalmic preparations.

	Formulae	Percent potency %
Ointments	Absorption base + Drug	86.75 ± 0.533
	Emulsion base + Drug	61.10 ± 0.551
	W.S.B + Drug	71.47 ± 0.259
	W.S.B.+ Drug-HP-β-CyD	93.13 ± 0.412
	W.S.B.+ Drug-β- CyD	66.75 ± 0.606
Gels	Sod. CMC + Drug	71.46 ± 0.460
	Sod. Alginate + Drug	68.04 ± 0.121
	Sod. alginate + Drug-HP-β-CyD	91.90 ± 0.410
	Sod. alginate + Drug-β-CyD	65.30 ± 0.131

W.S.B	Water soluble base
Sod. CMC	Sodium carboxymethylcellulose
HP-β-CyD	Hydroxypropyl beta-cyclodextrin.
β-CyD	Beta-cyclodextrin

2- Ocular bioavailability of enrofloxacin from certain ophthalmic preparations

The concentration of enrofloxacin uptaken in eye tissues and aqueous humor from ophthalmic gels and ointments as determined by microbiological assay are given in tables (3-6).

The obtained results showed that, the peak time of maximum drug concentration from W.S.B and sod. alginate gel for Enr and Enr complexed with HP-β-CyD was two hours in all tissues after application of these formulations table (8).

The total ocular availability of the drug in eye tissues of rabbits after two hours, was presented in table (9) and illustrated in figure (2), were 1266.6, 1554.5, 1722.6 and 1973.1 ug /gm for sod. alginate containing Enr, W.S.B. containing Enr, sod. alginate containing Enr-HP-β-CyD complex, and W.S.B containing Enr-HP-β-CyD complex respectively.

The C_{max} of enrofloxacin was, 376.6, 515.6, 527.1 and 588.5 ug/gm in conjunctiva, 340.7, 417.2, 463.4 and 494.1 ug/gm in cornea, 255.9, 340.7, 417.2 and 473.3 ug/gm in iris-ciliary body, and 221.7, 280.8, 314.9 and 417.2, ug/ml in aqueous humor for the same formulations, respectively (table 8). These obtained results showed that, the higher concentration of the drug was uptaken in conjunctiva followed by cornea, iris-ciliary body and then aqueous humor from all tested preparations. This is may be due to the direct contact of these tissues with the tear pool which houses the drugs. These results were similar to those obtained by Shaker[13], who found that, ciprofloxacin hydrochloride was deposited in conjunctiva in a higher concentration than cornea then iris-ciliary body followed by aqueous humor. W.S.B. containing drug complexed with HP-β-CyD provided the highest C_{max} in all tissues of eye at the peak time 2 hr followed by sod. alginate gel containing drug complexed with HP-β-CyD. While, sod. alginate gel containing drug alone provided the least C_{max} in all tissues.

Area under the curve (AUC) was carried out using pharmacologic calculation computer program Version 3, (New York). It was found to be 1134.8, 1821.4, 1730.9 and 2115.3 ug hr/gm in conjunctiva, 1266.6, 1496.2, 1555.1 and 1834.4 ug hr/gm in cornea, 956.2, 1126.2, 1362.4 and 1576.2 ug/gm in iris-ciliary body and 712.9, 999.3, 1123.3 and 1395.8 ug hr/ml in aqueous humor for sod. alginate containing Enr, W.S.B containing Enr, sod. alginate containing Enr-HP-β-CyD complex, W.S.B containing Enr-HP-β-CyD complex respectively (table 7).

The AUC_{0-t} was calculated and found to be 4193.4, 5445.6, 5771.9 and 6921.3 ug hr/gm for the same formulations, respectively (table 9). In this study also, the total availability of the drug from preparations was in the following order: W.S.B containing Enr-HP-β-CyD complex > sod. alginate containing Enr-HP-β-CyD complex > W.S.B containing Enr alone > sod. alginate containing Enr alone. This finding was in agreement with the results

obtained by shaker, 2000, who found that, the total availability of ciprofloxacin-HCl was in the following order: HPMC gel > MC gel > sod. alginate gel.

The results of statistical analysis were illustrated in table (9) and showed that, there is an extremely significant difference between the tested formulations.

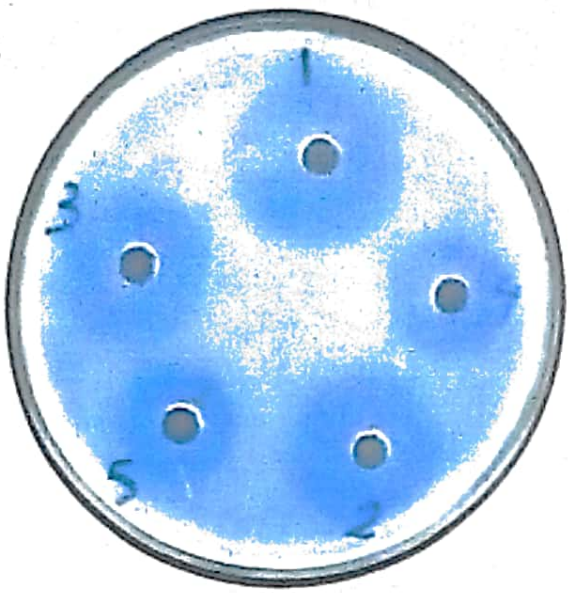

Fig. (1): Photograph of Petri- dish showing the inhibition zones resulted from different concentrations of enrofloxacin against Bacillus subtilis ATCC 6633.

Where;

Enrofloxacin concentrations;
1 = 781.25 µg/ml
2 = 390.6 µg/ml
3 = 195 µg/ml
4 = 97.7 µg/ml
5 = 48.8 µg/ml

Table (3): Availability of enrofloxacin in conjunctiva from ophthalmic preparations.

Formulae / Time (hrs)	Concentration of enrofloxacin (ug /gm) ± S.D			
	W.S.B + Drug	W.S.B + Drug-HP-β-CyD	Sod. alginate + Drug	Sod. Alginate +Drug-HP-β-CyD
1	417.2 ± 10.1	515.8 ± 5.5	347.5 ± 8.6	494.1 ± 5.6
2	515.8 ± 5.0	588.5 ± 8.1	376.6 ± 7.1	527.1 ± 7.5
3	400.9 ± 7.8	425.2 ± 5.1	280.6 ± 12.1	285.5 ± 7.1
5	196.3 ± 5.1	340.7 ± 5.4	166.4 ± 7.0	196.3 ± 5.5

W.S.B = water soluble base.
HP-β-CyD = hydroxypropyl beta-cyclodextrin.

Table (4): Availability of enrofloxacin in cornea from ophthalmic preparations.

Formulae / Time (hrs)	Concentration of enrofloxacin (µg /gm) ± S.D			
	W.S.B + Drug	W.S.B + Drug-HP-β-CyD	Sod. alginate +Drug	Sod. alginate +Drug-HP-β-CyD
1	354.5 ± 12.6	444.2 ± 4.0	265.5 ± 7.5	417.2 ± 7.5
2	417.2 ± 3.5	494.1 ± 6.0	340.7 ± 7.7	463.4 ± 5.5
3	340.7 ± 7.6	410.9 ± 5.0	260.8 ± 5.6	246.7 ± 7.6
5	169.1 ± 6.3	206.6 ± 6.5	163.8 ± 4.5	190.7 ± 6.5

Table (5): Availability of enrofloxacin in Iris-ciliary body from ophthalmic preparations.

Formulae / Time (hrs)	Concentration of enrofloxacin (µg /gm) ± S.D			
	W.S.B + Drug	W.S.B + Drug-HP-β-CyD	Sod. alginate +Drug	Sod. alginate +Drug-HP-β-CyD
1	280.8 ± 7.6	392.2 ± 5.1	233.7 ± 5.1	347.5 ± 5.6
2	340.7 ± 5.5	473.3 ± 5.3	255.9 ± 6.5	417.2 ± 6.5
3	198.3 ± 5.1	297.3 ± 6.3	174.7 ± 5.1	237.9 ± 5.3
5	166.4 ± 7.5	196.3 ± 5.5	160.4 ± 6.0	174.7 ± 7.8

Table (6): Availability of enrofloxacin in aqueous humor from ophthalmic preparations.

Formulae / Time (hrs.)	Concentration of enrofloxacin (µg /gm) ± S.D			
	W.S.B.+ Drug	W.S.B.+ Drug-HP-β-CyD	Sod. alginate +Drug	Sod. alginate +Drug-HP-β-CyD
1	246.7 ± 5.5	340.7 ± 10.5	166.4 ± 6.1	291.7 ± 5.1
2	280.8 ± 8.5	417.2 ± 10.3	233.7 ± 4.5	314.9 ± 5.0
3	196.3 ± 5.5	265.5 ± 10.2	123.1 ± 5.4	213.9 ± 5.5
5	151.4 ± 6.2	186.6 ± 6.0	94.52 ± 5.0	156.2 ± 5.4

Table (7): Area under the curve of enrofloxacin ophthalmic preparations for each tissue.

Formulae / Tissue	AUC (ug.hr / gm)			
	W.S.B.+ Drug	W.S.B.+ Drug-HP-β-CyD	Sod. alginate +Drug	Sod. alginate+ Drug-HP-β-CyD
Conjunctiva	1821.4 ± 5.2	2115.3 ± 6.3	1334.9 ± 5.4	1730.9 ± 8.7
Cornea	1496.2 ± 6.3	1834.4 ± 5.6	1211.1 ± 4.2	1555.1 ± 5.5
Iris-ciliary body	1126.2 ± 7.5	1576.2 ± 3.5	934.2 ± 8.1	1362.4 ± 6.3
Aqueous humor	999.3 ± 4.5	1395.8 ± 5.1	712.9 ± 9.5	1123.3 ± 5.5

Table (8): Peak concentration and peak time of enrofloxacin ophthalmic preparations.

Formulae / Tissue	Peak concentration and peak time of enrofloxacin ophthalmic formulations							
	W.S.B + Drug		W.S.B + Drug HP-β-CyD		Sod. alginate + Drug		Sod. alginate + Drug-HP-β-CyD	
	C_{max} µg/gm	T_{max} (hr)	C_{max} µg/gm	T_{max} (hr)	C_{max} µg/gm	T_{max} (hr)	C_{max} µg/gm	T_{max} (hr)
Conjunctiva	515.8	2	588.5	2	376.6	2	527.1	2
Cornea	417.2	2	494.1	2	340.7	2	463.4	2
Iris-ciliary body	340.7	2	473.3	2	255.9	2	417.2	2
Aqueous humor	280.8	2	417.2	2	233.7	2	314.9	2

Table (9): Total ocular availability of enrofloxacin from various ophthalmic preparations in rabbits eyes.

Formulae / Time (hr.)	Total ocular availability of enrofloxacin in µg/gm after hours			
	W.S.B + Drug	W.S.B + Drug-HP-β-CyD	Sod. alginate + Drug	Sod. alginate + Drug-HP-β-CyD
1	1299.2 ± 7.30	1692.9[a] ± 4.83	1013.05[ab] ± 3.81	1550.5[abc] ± 5.21
2	1554.5 ± 5.27	1973.1[a] ± 5.35	1206.6[ab] ± 5.49	1722.6[abc] ± 4.05
3	1136.2 ± 6.58	1398.9[a] ± 7.85	839.2[ab] ± 6.26	984[abc] ± 5.15
5	683.2 ± 9.85	930.2[a] ± 5.27	585.1[ab] ± 5.56	717.9[abc] ± 6.55
AUC_{0-5}	5445.6 ± 8.55	6921.3[a] ± 7.68	4193.4[ab] ± 7.10	5771.9[abc] ± 6.69

a- Significantly different at P< 0.0001 compared with W.S.B + drug alone

b- Significantly different at P< 0.0001 compared with W.S. B +drug- HP-β-CyD

c- Significantly different at P< 0.0001 compared with sod. alginate. + drug alone

In all above-mentioned statistical comparisons, one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple comparisons test was adapted.

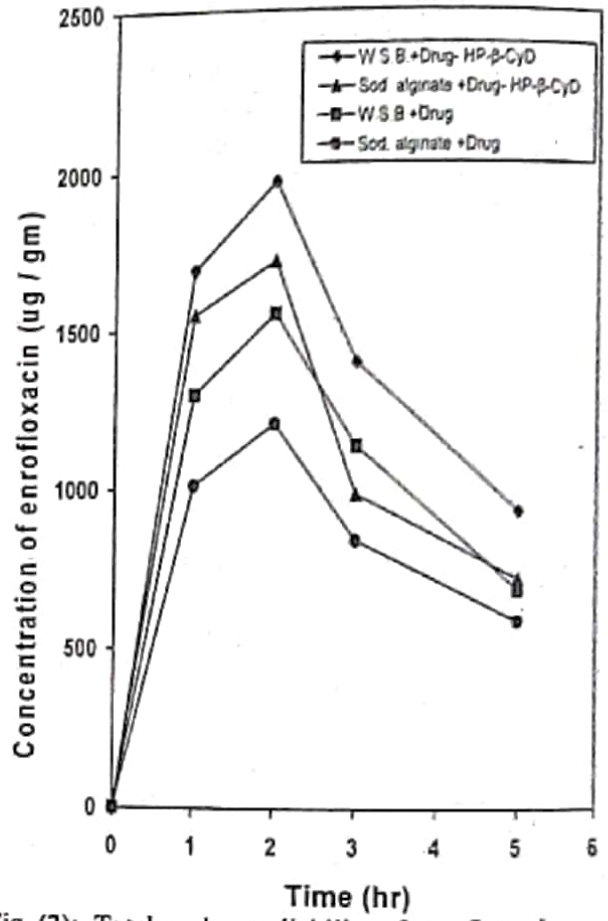

Fig. (2): Total ocular availability of enrofloxacin from various ophthalmic preparations in rabbits eyes.

Conclusion

From the obtained results, it could be concluded that:

- Regarding the percent potency of the drug in the different preparations after the microbiological assay, the tested formulae can be arranged in the following order; Enr-HP-β-CyD complex in W.S.B > Enr-HP-β-CyD complex in sod. alginate > Enr in absorption base > Enr in W.S.B > Enr in sod. CMC > Enr in sod. alginate > Enr-β-CyD complex in W.S.B > Enr-β-CyD complex in sod. alginate > Enr in emulsion base.
- The peak time of maximum drug concentration in rabbit's eye tissues and aqueous humor was two hours for various ophthalmic ointments and gels.
- All tested formulations provided the C_{max} of the drug in conjunctiva followed by cornea, iris-ciliary body then aqueous humor.
- The total availability of enrofloxacin was improved when the drug complexed with HP-β-CyD.
- Regarding the total availability of the drug from the tested formulations, it was found to be in the following order; W.S.B + Enr-HP-β-CyD > sod. alginate + Enr-HP-β-CyD > W.S.B+ Enr alone > sod. alginate + Enr alone.

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التوافر الحيوى لمتراكب الإنروفلوكساسين مع السيكلودكسترينات في أنسجة عيون الأرانب من بعض صواغات العين

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تناولت هذه الدراسة تحضير متراكب الإنروفلوكساسين مع السيكلودكسترينات (البيتاسيكلودكسترين والهيدروكسى بروبيل بيتاسيكلودكسترين) بطريقة العجن بنسبة ١:٢ ثم أضيف العقار أو متراكباته بنسبة ٠,٥ فى المائة الى صواغات العيون المختلفة وتشمل المراهم (القاعدة قابلة الذوبان فى الماء ،قاعدة الامتصاص وقاعدة المستحلب) والمستحضرات الهلامية (الجيبات الصوديوم وكربوكسى ميثيل سيليولوز الصوديوم).

وقد تضمن هذا البحث أيضا دراسة تأثير نوع الصواغات على توافر الإنروفلوكساسين فى الأنسجة المتباينة والسائل المائي لعيون الأرانب خلال فترات زمنية محددة بعد استخدام صواغات العين المحضرة المحتوية على تركيز ٠,٥% من الدواء.

كما أظهرت نتائج هذه الدراسة أن الوقت اللازم للحصول على أعلى تركيز يصل إليه الدواء في الأنسجة والسائل المائي لعين الأرانب هو ساعتين من مراهم العين ومستحضراتها الهلامية المحتوية على العقار. كما وجد أنه مع جميع الصواغات المختبرة كانت نسبة الإنروفلوكساسين في الملتحمة أعلى منها في القرنية بينما احتوت القزحية والسائل المائي على نسبة أقل من العقار. وأثبتت التجارب أيضا أن استخدام الإنروفلوكساسين في صورة متراكب مع هيدروكسى بروبيل بيتاسيكلودكسترين يعطى زيادة في التوافر الحيوي للعقار في جميع أنسجة العين أكثر من العقار وحده.