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# OCULAR BIOAVAILABILITY OF TRIAMCINOLONE ACETONIDE COMPLEX FROM DIFFERENT OPHTHALMIC PREPARATIONS IN RABBITS' EYES

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## ABSTRACT

The effect of beta-cyclodextrin (β-CyD) on the solubility, release characteristics and ocular bioavailability of triamcinolone acetonide (TA) from ophthalmic gels and ocuserts were investigated. Triamcinolone acetonide-β-CyD complex was prepared by kneading method in a molar ratio of 1:1. The ophthalmic gels [carbopol 934, hydroxy propyl methyl cellulose (HPMC), sodium alginate] and ocuserts (HPMC and carbopol 934 combination, sodium alginate and carbopol 934 combination) were prepared using 0.1% of the drug or its equivalent amount of complex with β-CyD. The *in vivo* study was performed on male albino rabbits. The drug concentrations were determined in different eye tissues and aqueous humor of the rabbits after 1, 2, 4, and 6 hr of the application by HPLC method.

The obtained results revealed that, the solubility of TA was increased linearly as a function of β-CyD concentration following $A_L$ type phase solubility diagram. The percent released of the drug from the prepared formulations containing free TA could be arranged in the following order: HPMC and carbopol 934 combination ocuserts > sod. alginate and carbopol 934 combination ocuserts > HPMC gel > carbopol 934 gel > sod. alginate gel. Moreover, at all time intervals, the percent released of the drug from ophthalmic gels and ocuserts containing the drug complex with β-CyD was significantly higher than that of the free drug. Concerning the ocular bioavailability, all tested formulations provided the highest $C_{max}$ of the drug in conjunctiva followed by cornea, iris-ciliary body, then the aqueous humor, except that after 6 hr, it was high in cornea followed by iris-ciliary body, conjunctiva and then the aqueous humor. The peak time of maximum drug concentration ($T_{max}$) in rabbits' eye tissues and aqueous humor was 4 hr for all ophthalmic gels and ocuserts. The total ocular bioavailability of triamcinolone acetonide was improved when the drug was complexed with β-CyD.

## INTRODUCTION

Triamcinolone acetonide is a synthetic corticosteroid. It is a safe and effective drug used for the treatment of diseases requiring long term ocular steroid application such as uveitis, macular edema, and intraocular proliferations [1].

The anti-inflammatory mechanism of corticosteroids has been proposed to be through the induction of lipocortin synthesis. Prostaglandins and leukotrienes, mediators of inflammation, have a common precursor, arachadonic acid which is released from membrane phospholipids by phospholipase $A_2$ whose activity is inhibited by lipocortins [2, 3]. The relative anti-inflammatory strength of various corticosteroids is as follows:
desoxycorticosterone 0.0, cortisone 0.8, hydrocortisone 1.0, prednisone 4.0, methylprednisolone 5.0, triamcinolone acetonide 5.0, fludrocortisone 10.0, betamethasone 25.0 and dexamethasone 25.0 [3].

Mora *et al.*,[4], reported that, the human sclera is permeable to triamcinolone acetonide and has a scleral permeability coefficient of $1.47 \pm 0.17 \times 10^{-5}$ cm/s. The drug has a relatively small molecular weight (434.5 Da), high lipophilicity, and thus, it can easily penetrate the sclera.

Most ocular diseases are treated by topical drug application in the form of aqueous eye drops. Recent studies have shown that, cyclodextrins are useful additives in ophthalmic formulations for increasing the aqueous solubility, aqueous stability and bioavailability of ophthalmic drugs and to decrease drug irritation [5,6,7]. Cyclodextrins are cyclic oligosaccharides, with hydrophilic outer surface and a somewhat lipophilic central cavity. Cyclodextrins are able to form water soluble inclusion complexes with many lipophilic poorly soluble compounds [8,9]. The ability of cyclodextrins (CyDs) to form inclusion complexes, by taking up a whole drug molecule or non-polar part into the hydrophobic cavity, may considerably alters drug solubility, size of diffusive molecules and the release behavior of polymeric systems [5, 6].

Drug release from the CyD complex is mainly caused by dissociation due to dilution in fluids. In the case of topical applications, such as ocular, nasal, or dermal, with minimal dilution mechanism, the potential mechanism of drug release from CyD complex is preferential drug uptake by tissue [10]. They reported that, if the drug substances possess physicochemical properties that allow it to penetrate into or through biological membranes (skin, mucosa, or cornea), the tissue acts as a sink causing dissociation of the complex. Only a free fraction of drug that is in equilibrium with the complexed fraction may be available for absorption, thus CyDs are able to increase bioavailability rather by deliver the drug substance to absorption site and by minimization of drug hydrophobicity than permeation by itself.

The current research study aimed to investigate the effect of inclusion complexation of triamcinolone acetonide with β-CyD on the solubility. Formulation of this complex in different ophthalmic preparations was of prime interest. Moreover, the suggested formulations containing the drug or its complex were subjected to *in-vitro* release evaluation. In addition, ocular bioavailability of TA from selected ophthalmic

formulations in different tissues of rabbits' eyes was determined by HPLC technique after its application.

## EXPERIMENTAL

### 1. Materials

Triamcinolone acetonide was supplied by Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Methanol (HPLC grade) was purchased from Sigma (Buchs, Switzerland). Beta-cyclodextrin (Molecular weight = 1135) (Nippon Shokuhin Kako Co., Tokyo, Japan). Benzalkonium chloride, sodium metabisulphite, disodium edetate and prednisolone (Memphis Co., Egypt). Carbopol 934, hydroxy propyl methyl cellulose and sodium alginate (BDH Chemical Ltd, GB, Liverpool, England). Disodium hydrogen phosphate and n-butanol were supplied by Prolabo, Chemicals, Paris, France. Potassium dihydrogen orthophosphate and propylene glycol (Adwic, El Nasr, Pharmaceutical Chemicals Co., Egypt). Cellophane membrane, Spectrapor: M.W. Cutoff: 12000-14000 was supplied by Fischer Sci. Co., Pittsburgh, USA. Albino rabbits of 1.8-2 kg.

### 2. Equipment

Ultraviolet spectrophotometer (Shimadzu, UV-150-02, Sersakusho, Ltd. Kyoto, Japan). pH-meter (Beckman Instruments fullerton, CA 92634). MSE Minor Centrifuge (MSE Scientific Instruments, Manor Royal, Crawley RH/0200 sussex, England). Thermostatically controlled shaking water bath (Grant Instrument Cambridge Ltd., Barrington Cambridge (B2, 5002, England). Membrane filters (Millipore Corporation, Bedford, MA 01730, Germany) of pore size 0.2 µm. Modified diffusion cell, Rikugu, model Dmax 2500 PC X-ray diffractometer USA. Shimadzu FTIR-8300 spectrometer USA. Rotary viscometer (Haake Inc., Germany). Vortex mixer (Snijders Scientific Tilburg-Holland). High performance liquid chromatography (HPLC) equipment (Perkin Elner, USA).

### 3. Methodology

#### 3.1. Solubility study

The solubility of triamcinolone acetonide (TA) in distilled water alone, in 0.2% w/v aqueous solution of the polymers (HPMC or sodium alginate) and in 0.2% w/v aqueous solution of the polymers (HPMC or sodium alginate) and β-CyD was determined by the method of Higuchi and Connors. [10]. Excess TA (40 mg) was added to screw-capped vials containing the aqueous solutions mentioned before. The screw-capped vials were shaken for 7 days at 8 cycles/min in a thermostatically controlled water bath at 37±0.5°C. The concentration of TA in samples passed through 0.45 µm millipore filters was determined spectrophotometrically at 240 nm. The experiments were done without drug and served as a blank. All experiments were done in triplicate and the mean was recorded.

#### 3.2. Phase solubility study

The solubility of TA in distilled water in the presence of various concentrations of β-CyD from 0 to 17.62 mM, was determined by the method of Higuchi and Connors.[10] The stability constant ($K_{1:1}$) was calculated from the slope and $S_0$ using the following equation:

$$K_{1:1} = \text{Slope} / S_0 (1 - \text{Slope})$$

*Where;*

$S_0$ is the intrinsic solubility of the drug, i.e. the solubility when no cyclodextrin is present.

#### 3.3. Determination of partition coefficient

Ten ml of n-butanol were added to an equal volume of distilled water and placed in a screw capped bottles. Triamcinolone acetonide (20 mg) or its equivalent amount of the drug complex with β-CyD was added to the partitioning system. The tubes were shaken on thermostatically controlled water bath at 37±0.5°C. The aqueous phase and n- butanol phase were separated and assayed for drug content at 240 nm. When no difference was observed between repetitive sampling, the equilibrium was attained (after 3 days). The experiments were triplicated and the mean was calculated.

#### 3.4. Preparation of triamcinolone acetonide-β-CyD complex

The drug complex was prepared by kneading method [12] in a molar ratio of 1:1. Triamcinolone acetonide (0.1 gm) and β-CyD (0.26 gm) were mixed and kneaded in a mortar with an adequate amount of acetone-water mixture (3:1 V/V) for 45 min and kept over night in a dark place. The resulted masses were dried under reduced pressure at 25±0.5°C. The products were then sieved and the fractions of average particle size 10 µm were collected and stored in desiccators until use.

#### 3.5. Characterization of TA-β-CyD solid complex

##### 3.5.1. X-ray diffractometry (XRD)

The XRD measurements were performed for the drug alone, beta-cyclodextrin alone, TA-β-CyD physical mixture (1:1) and TA-β-CyD complex using X-ray diffractometer with a $2\theta$ range between 2 and 50 using Cu Kα radiation (λ = 1.5406 Å). The XRD patterns were recorded at room temperature (25 °C).

##### 3.5.2. Infrared spectroscopy (IR)

Infrared spectroscopy was performed on the same samples as X-ray diffractometry at ambient temperature, in the range of 400-4000 $cm^{-1}$, using KBr pellets in a Shimadzu FTIR-8300 spectrometer.

#### 3.6. Preparation of triamcinolone acetonide ophthalmic gels

The calculated amounts of carbopol 934 (2% w/w), HPMC (20% w/w) or sodium alginate (5% w/w) containing 0.01% benzalkonium chloride, 0.03% sodium metabisulphite and 0.1% EDTA were dissolved in distilled water. Then, TA 0.1% (w/w) or its

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equivalent amount of drug-β-CyD complex was dissolved in 20 ml propylene glycol and added to the previous mixtures. The mixtures were completed to 100 gm, and then filled in clean, dry and sterile glass containers.

### 3.7. Preparation of triamcinolone acetonide ophthalmic ocuserts

Triamcinolone acetonide 0.1% was dissolved in 20 ml propylene glycol. This solution was added to HPMC (6% w/w) and carbopol 934 (0.04% w/w) mixture, sodium alginate (1.5% w/w) and carbopol 934 (0.04% w/w) mixture, in distilled water containing 0.01% benzalkonium chloride, 0.03% sodium metabisulphite and 0.1% EDTA. Equal volumes from the prepared solutions were transferred into polytetrafluorethylene (PTFE) moulds. Each mould was covered with an inverted funnel (stem orifice diameter 6.9 mm) to control the solvent evaporation. The solvent was permitted to evaporate for 48 hr at ambient temperature. The formed films were transferred to desiccators containing silica gel, where it was stored for another 24 hr before use [13]. The prepared ocuserts (0.4 - 0.5 mm thickness) were cut in the form of circular discs, each of 6 mm diameters. The ocuserts were individually sealed in foil sachets until used. All ophthalmic preparations used (gels and ocuserts) were sterilized by autoclaving [14].

### 3.8. Determination of the viscosity

The ophthalmic gels were subjected to viscosity determination using rotary viscometer which has been calibrated before use. The temperature was maintained at 37±0.5 °C. One gram of each formulation containing TA (0.1% w/w) was placed on the plate of viscometer (with a diameter of 2.9 cm) and cone with 2.8 cm in diameter. The torque value "S" were determined for each "N" value (speed). viscosity is calculated using the following equation:

$$\eta = \frac{G.S}{N}$$

Where,

- η: Viscosity in mpa.s (mpa. S = 1 centipoise)
- G: Instrumental factor (14200 mpa.s/scalagrad. Min)
- S: Torque (scale grad.)
- N: Speed (rpm)

### 3.9. *In-vitro* drug release study from different formulations

The release of TA from ophthalmic gels and ocuserts in phosphate buffer solution of pH 7.4 was carried out according to the method adopted by Levy and Benita.[15]. Accurate weights (5 gm) of gels or (three ocuserts) containing 5 mg of the drug or its equivalent amount of complex were placed in dialysis glass tubes (diameter, 3 cm), whose lower end is closed by a semipermeable membrane which is made tight by rubber band and immersed in the release medium. To each tube, 1.5 ml of phosphate buffer was added. Fifty ml of phosphate buffer solution (pH 7.4) were placed in 150 ml capacity beaker, and the temperature of the medium was adjusted at 37±0.5°C using thermostatically controlled water bath with magnetic stirrer. At predetermined time intervals of 5, 10, 20, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min., aliquots of one ml were withdrawn and diluted with the dissolution medium, which is replaced with the same volume of fresh buffer to maintain it at a constant volume. The percent released of TA from each formula were analyzed spectrophotometrically at 240 nm. Blank experiments were carried out using non-medicated formulations and served as blank. The experiments were triplicated and the mean was calculated.

### 3.10. Ocular bioavailability of triamcinolone acetonide in rabbits' eyes

#### 3.10.1. *In-vivo* study

Ocular bioavailability of the selected formulations on the basis of drug release was performed on male New Zealand albino rabbits weighed 1.8-2 kg. The following tested formulations were used: carbopol 934 gel containing free TA, carbopol 934 gel containing TA-β-CyD complex, HPMC gel containing TA-β-CyD complex, HPMC and carbopol 934 combination ocuserts containing TA-β-CyD complex, sod. alginate and carbopol 934 combination ocuserts containing TA-β-CyD complex. Animals were divided into five groups, each of 12 rabbits, and 100 mg of each formulation containing 0.1 mg/ml TA or its equivalent amount of complex, was placed in the cul-de-sac of albino rabbits. 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. Three rabbits were used for the determination of the amount of drug deposited in different eye tissues and aqueous humor at each time interval.

Triamcinolone acetonide ocular concentration was determined at 1, 2, 4, and 6 hr after dose application. Therefore, three eyes which receive the medicament in each tested formula were used for the determination of drug concentration. For each animal, one eye was loaded with the tested formulation, while, the other was loaded with the plain base and served as a control.

#### 3.10.2. Separation of eye tissues

At different time intervals (1, 2, 4 and 6 hr) after dose application, rabbits were sacrificed and the eyes were proptosed and rinsed with normal saline. The conjunctiva was separated, 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 anterior segment tissues: conjunctiva, cornea and iris-ciliray body was obtained in that order. The whole cornea and conjunctiva were subsequently dissected in- situ. Each tissue was rinsed with normal saline, blotted dry to remove any adhering drug and weighed. The excised tissue was minced with methanol to extract TA and methanol was then evaporated. Then another 500 µl of methanol was added, vortexed for 3 min, centrifuged and filtered

through 0.2 µm millipore filters. A portion (50 µl) was analyzed by HPLC. The surgical procedures on each eye were completed within 10 min of sacrificing the animal. Consequently, any errors due to redistribution of the drug during the time required to obtain 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 (this is performed by cooperating with an associated professor of ophthalmology, faculty of medicine, Mansoura University).

**3.10.3. Extraction of the drug from different tissues and fluid of rabbits' eyes**

The amount of TA was measured by HPLC technique using a C-18 reverse-phase column (Nucleosil C-18, 254 mm × 4mm × 5µm, Macherey Nagel, Switzerland) at a flow rate of 1 ml/min and detected with a UV spectrophotometer at 250 nm. The mobile phase was composed of a mixture of methanol (60%) and water (40%) v/v. Analysis was performed at room temperature. Prednisolone was used as an internal standard (IS) [16].

Aqueous humor samples (100 µl) or iris-ciliray body (100 mg) were added to 25 µl (5 µg $ml^{-1}$) of prednisolone by mixing with vortex-mixer for 30 s. Then 175 µl of methanol was added and mixed using vortex-mixer for 2 min. The samples were centrifuged at 5000 rpm for 10 min & filtered using 0.2 µm millipore filters, 150 µl of filterate was transferred into micro vial and 50 µl of the solution was injected into the column [16]. In case of conjunctiva and cornea, the excised tissue was minced with methanol to extract TA and the methanol was then evaporated. Then another 500 µl of methanol was added, vortexed for 3 min and centrifuged. Then filtered using 0.2 µm millipore filters, a portion (50 µl) of filterate was analyzed by HPLC [17].

**Data management and statistical analysis**

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-kramer multiple comparisons test [18]. 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. Solubility study**

The solubility of TA was increased in the presence of both polymers (0.2% w/v HPMC or 0.2% w/v sodium alginate) and also in the presence of polymers (0.2% w/v HPMC or 0.2% w/v sodium alginate) & β-CyD combination. This is may be due to the formation of water soluble complex [19, 20].

The obtained results revealed that, the solubility of the drug increased from 4.8±0.1 mg /100 ml (for drug alone) to 6.4±0.3 and 7.1±0.5 mg/100 ml by adding 0.2% w/v sod. alginate and 0.2% w/v HPMC, respectively. Also, the solubility of TA was greatly increased in presence of both sod. alginate and β-CyD or HPMC and β-CyD. The amount dissolved was 25.5±0.01 and 26.7±0.04 mg /100, respectively.

The obtained results were analyzed using T test which indicates a significant difference between the solubility values in water, HPMC or sod. alginate individually and HPMC or sod. alginate with β-CyD.

**2. Phase solubility study**

The phase solubility diagram of TA in aqueous solutions containing β-CyD are illustrated in Fig.1. The data revealed that, a soluble TA–β-CyD complex with a stability constant of 2720 $M^{-1}$ and complexation efficiency of 0.114 was formed. The phase solubility diagram was following an AL type. The solubility of TA increased linearly with β-CyD concentration. The solubility of the drug increased from 4.8 mg /100 ml (for drug alone) to 70.9 mg / 100 ml by adding β-CyD. Also, the stoichiometry of β-CyD complex was determined to be 1:1 (guest / host).

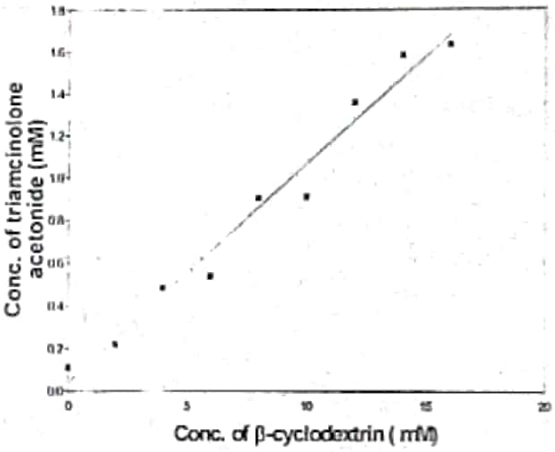


**Fig. 1.** Phase solubility diagram of TA in presence of β-CyD

**3. The partition coefficient of triamcinolone acetonide and its complex**

The partition coefficient values of the drug and its complex with β-CyD using n-butanol/water system after three days were 2.27 and 2.02, respectively. According to these results, the lipophilicity of free TA was higher than that of TA-β-CyD complex. These results are in agreement with that obtained by Nigner *et al.*,[21], who stated that, n-octanol / water partition coefficient of tenoxicam decreased and water solubility increased with the addition of cyclodextrins.

**4. Characterization of TA-β-CyD complex**

**4.1. X-ray diffractometry**

Figure 2. illustrates X-ray diffraction patterns obtained from samples containing different powders. By comparing the patterns of TA-β-CyD physical mixture, 1:1 (c) and TA-β-CyD complex (d) with the patterns from β-CyD (a) and TA (b), it was found that the scattering peaks found in patterns (c) and (d) are similar to each other, while different from that of β-CyD (a) or TA (b). This is an indication that a new entity is being observed in patterns of TA-β-CyD physical mixture (c) and TA-β-CyD complex (d)

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relative to patterns of β-CyD (a) and TA (b), and thus corresponds to the inclusion complex. The absence of peaks from either β-CyD or TA on the pattern of TA-β-CyD (d) means that, the amounts of these substances (if exist) are beyond detection and this arises because β-CyD remains in a too small fraction for detection due to a high inclusion yield. By observing the scattering pattern of TA-β-CyD physical mixture (c), one finds that all the peaks found in the pattern of TA-β-CyD complex (d) correspond to the inclusion complex and extra peaks that were registered along with peaks from β-CyD (pattern a) corresponding to the free β-CyD. The absence of peaks from non-complexed TA is caused by a low percentage of this component in the powder [22].

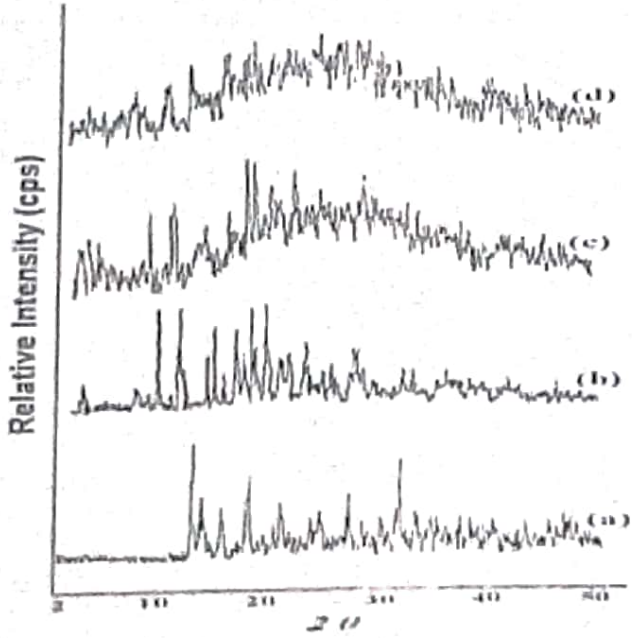


Fig. 2. X-ray diffraction patterns of:
a- TA
b- β-CyD
c- TA-β-CyD physical mixture (1:1)
d- TA-β-CyD complex

### 4.2. Infrared spectroscopy (IR)

The infrared spectroscopy (IR) analysis was performed to confirm the results from X-ray diffractometry. The IR spectra for all samples are shown in Fig. 3. The characteristic bands observed from the IR data of TA included the OH group in the range 3390–3462 $cm^{-1}$, C=O bands at 1708 $cm^{-1}$, C=C bands in the range 1663–1600 $cm^{-1}$ and C–H stretching of sp3 and sp2 carbons in the range of 3000 and 2900 $cm^{-1}$. The analysis of the spectra of TA-β-CyD complex shows OH group and C=O band shifted to a lower wave number (3380 and 1700 $cm^{-1}$, respectively) and the absence of peaks correspond to free β-CyD. The shift of a band on the corresponding carbonyl group proved the presence of the solid complex formation suggested this group was not fully included within the CyD cavity [23]. The spectra from TA-β-CyD physical mixture shows the OH group band broadening which shifted to a lower wave number (3384 $cm^{-1}$). This broadening is an evident for the presence of free β-CyD. Also the carbonyl group was shifted to a lower wave number (1703 $cm^{-1}$), and the characteristic band for β-CyD at 1160 $cm^{-1}$ was found [23].

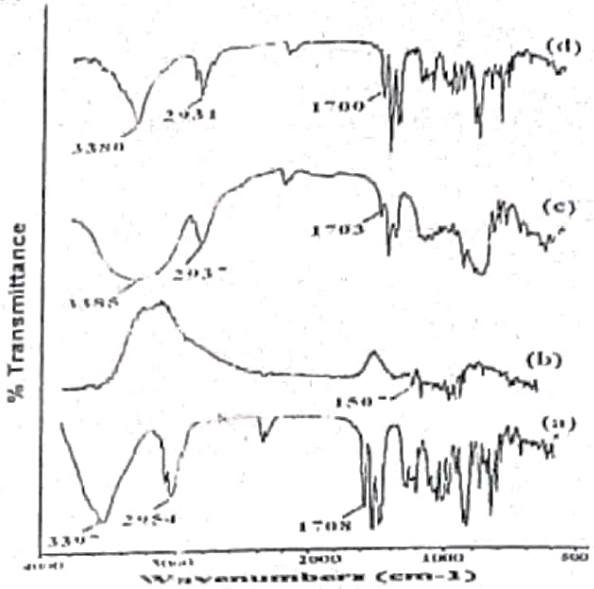


Fig. 3. Infrared spectra of:
a- TA
b- β-CyD
c- TA-β-CyD physical mixture (1:1)
d- TA-β-CyD complex

### 5. The viscosity of the prepared formulations

The viscosity values of the prepared ophthalmic gels were determined and it was 1498, 1387, 1276, 1165, 1051 and 943 m Pa.s for HPMC gel, HPMC gel containing TA-β-CyD complex, carbopol 934 gel, carbopol 934 gel containing TA-β-CyD complex, sod. alginate gel, sod. alginate gel containing TA-β-CyD complex, respectively. The tested ophthalmic formulations can be arranged as follows according to their viscosity: HPMC gel > carbopol 934 gel > sod. alginate gel. Generally, the gel containing TA-β-CyD complex has low viscosity relative to gel containing free TA, this may be due to hydrophobic interaction between polymer chains and CyD cavity, thus polymer swelling properties decreased [24].

### 6. *In-vitro* drug release from the tested formulations

The release characteristics of triamcinolone acetonide and its complex from different ophthalmic gels and ocuserts were studied and illustrated in Fig. 4-8. The percent drug released after 8 hr of the free drug from HPMC and carbopol 934 combination ocuserts, sod. alginate and carbopol 934 combination ocuserts, HPMC gel, carbopol 934 gel and sod. alginate gel were 94.5, 36.2, 27.0, 25.8 and 22.6, respectively, while the percent released after 8 hr of the drug complex from HPMC and carbopol 934 combination ocuserts, sod. alginate and carbopol 934 combination ocuserts, HPMC gel, carbopol 934 gel and sod. alginate gel were 99.5, 56.2, 53.3, 46.4 and 30.9. The obtained results clarified that, triamcinolone acetonide released from its complex with β-CyD in a rate significantly higher than

the free drug. This indicates a higher solubilizing effect of β-CyD. Furthermore, the maximum percent released after 8 hr increased two-fold when formulated with β-CyD compared to the free drug. The tested ophthalmic formulations containing TA-β-CyD complex can be arranged as follows according to the release rate: HPMC and carbopol combination 934 ocuserts > sod. alginate and carbopol 934 combination ocuserts > HPMC gel > carbopol 934 gel > sod. alginate gel.

There was a linear relationship between the percent drug released from various ophthalmic gels and ocuserts and the square root of time except for carbopol 934 gel containing free drug, sod. alginate gel containing TA-β-CyD complex, (sod. alginate & carbopol 934) ocuserts containing free drug, (HPMC & carbopol 934) ocuserts containing TA-β-CyD complex and (sod. alginate & carbopol 934) ocuserts containing TA-β-CyD complex which show a linear relationship between log percent of drug released per unit surface area and log time. The correlation coefficient (r) ranged from 0.9697 to 0.9902 and from 0.9521 to 0.9853 for first and second plot, respectively. The first plot indicates a typical-root of time release pattern according to Higuchi equation while the second plot indicates Fickian-diffusion mechanism.

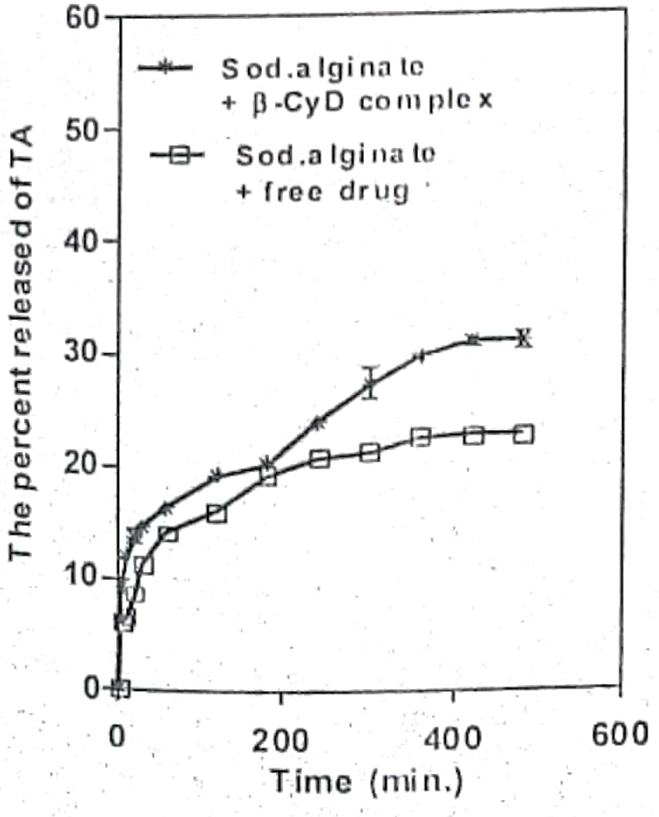


Fig. 4. *In-vitro* release profiles of TA from sod. alginate gels.

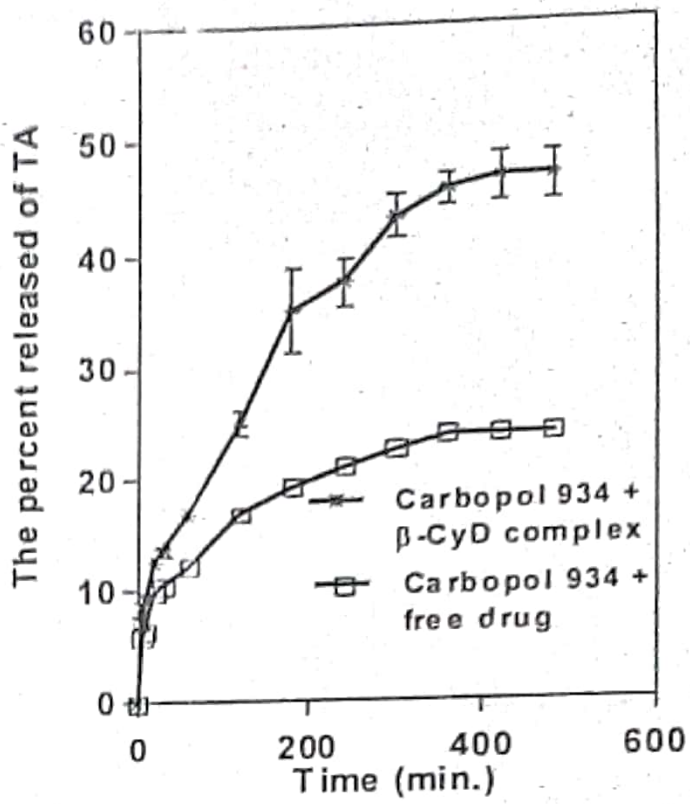


Fig. 5. *In-vitro* release profiles of TA from carbopol 934 gels

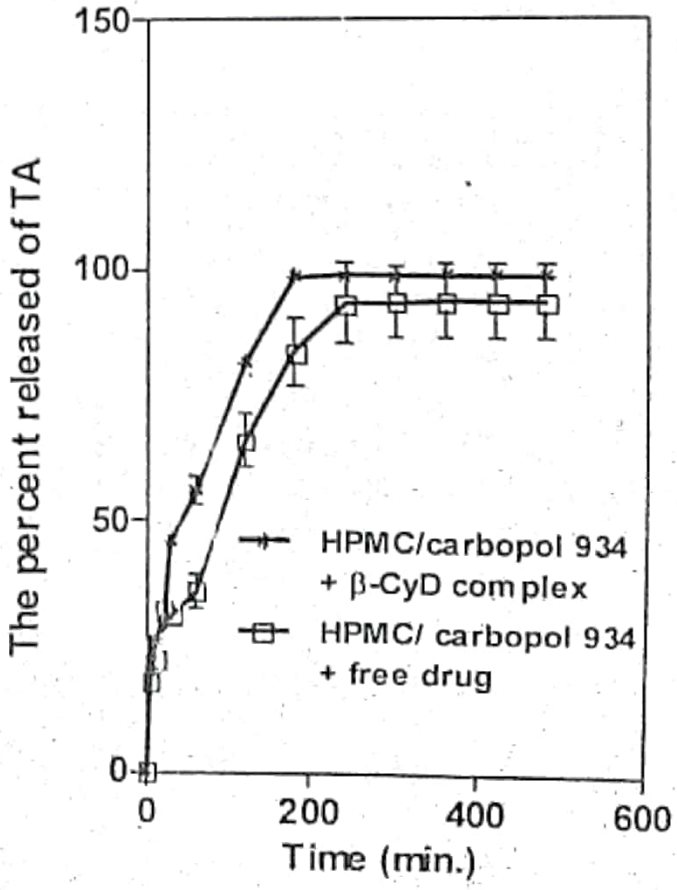


Fig. 7. *In-vitro* release profiles of TA from HPMC/carbopol 934 ocuserts.

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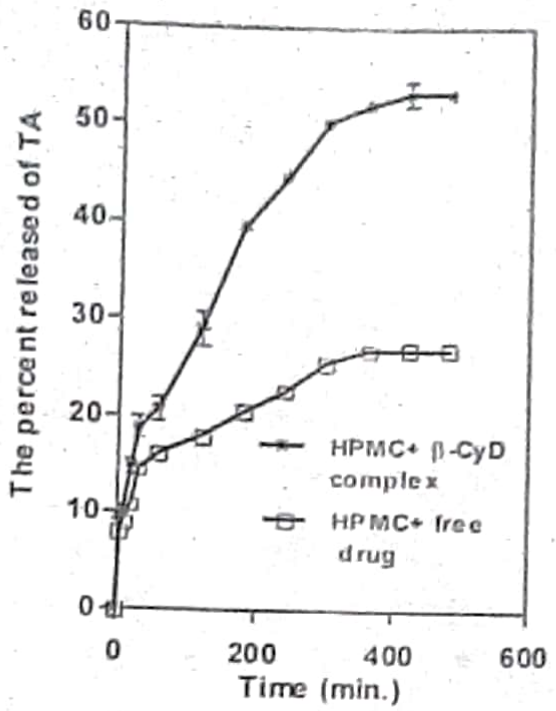


Fig.6. *In-vitro* release profiles of TA from HPMC gels

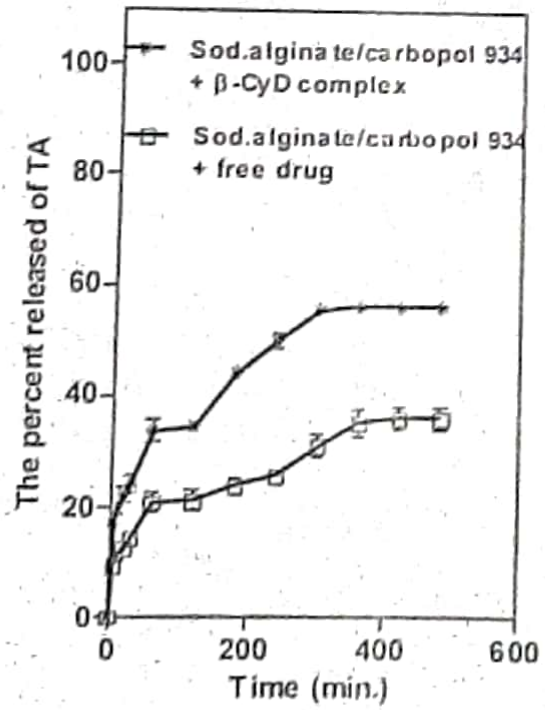


Fig. 8. *In-vitro* release profiles of TA from sod. alginate/carbopol 934 ocuserts

**7. Ocular bioavailability of the drug in rabbits' eyes**

The concentration of triamcinolone acetonide uptaken by eye tissues and aqueous humor from ophthalmic gels and ocuserts which determined by HPLC method, is illustrated in tables 1-4.

Under these experimental conditions, retention time of TA was 5.1 min. The obtained results showed that, the peak time of maximum drug concentration from carbopol 934 gel containing free TA, carbopol 934 gel, HPMC gel, HPMC and carbopol 934 combination ocuserts, sod. alginate and carbopol 934 combination ocuserts, containing TA-β-CyD complex was 4 hr in all tissues after application of these formulations (table 6).

The total ocular bioavailability of the drug in rabbits' eye tissues after 4 hr, was presented in table 7 and illustrated in Fig. 4, were; 686.8, 2151.3, 2402.9, 2871.3 and 3236.5 ng/gm for carbopol 934 gel containing free TA, carbopol 934 gel containing TA-β-CyD, HPMC gel containing TA-β-CyD, sod. alginate and carbopol 934 combination ocuserts containing TA-β-CyD, HPMC and carbopol 934 combination ocuserts containing TA-β-CyD, respectively.

The $C_{max}$ of TA was: 259.9, 785.1, 909, 1150 and 1062.2 ng/gm in conjunctiva, 236.5, 687.3, 811.8, 1066.6 and 927.9 ng/gm in cornea, 177.2, 610.4, 690.4, 922.2 and 798.5 ng/gm in iris-ciliary body, and 13.2, 68.5, 71.7, 97.7 and 82.7 ng/ml in aqueous humor for the same formulations, respectively (table 6). These obtained results showed that, the higher concentration of the drug uptaken was in conjunctiva followed by cornea, iris-ciliary body and then aqueous humor from all tested preparations except after 6 hr, where it was in cornea followed by iris-ciliary body, conjunctiva and then aqueous humor. These results were similar to those obtained by Gan *et al.*[17], who found that, cyclosporine A levels in the conjunctiva decreased much faster than in the cornea after 6 hr. This may be attributed to the uptake of the drug by Langerhans cells and macrophages[125] or to the diffusion of the drug into the blood and lymphatic vessels underlying the fine and leaky conjunctival epithelium.

Carbopol 934 and HPMC combination ocuserts containing TA complex provided the highest $C_{max}$ in all tissues of eye at the peak time 4 hr followed by sod. alginate and carbopol 934 combination ocuserts containing TA-β-CyD complex., while, carbopol 934 gel containing free drug provided the least $C_{max}$ in all tissues.

Area under the curve (AUC) was calculated using GraphPad Prism 4 computer program Version 4, (New York). It was found to be 789.7, 2472, 2969, 3445 and 3887 ng.hr/gm in conjunctiva, 745.3, 2466, 3016, 3512 and 4063 ng.hr/gm in cornea, 553.7, 2072, 2479, 2864 and 3248 ng.hr/gm in iris-ciliary body and 47.7, 272.4, 296.7, 345.1 and 398 ng.hr/ml in aqueous humor for carbopol 934 gel containing free TA, carbopol 934 gel containing TA-β-CyD, HPMC gel containing TA-β-CyD, sod. alginate and carbopol 934 combination ocuserts containing TA-β-CyD, HPMC and carbopol 934 combination ocuserts containing TA-β-CyD, respectively. Table 5 represents the drug bioavailability to each tissue from the ophthalmic preparations.

The $AUC_{0-\infty}$ was calculated and found to be 2136.6, 7232.1, 8730.7, 11596 and 10166.1 ng.hr/gm for the same formulations, respectively (table 7). In this

study also, the total bioavailability of TA from preparations was in the following order HPMC and carbopol 934 ocuserts containing TA-β-CyD > sod. alginate and carbopol 934 ocuserts containing TA-β-CyD > HPMC gel containing TA-β-CyD > carbopol 934 gel containing TA-β-CyD > carbopol 934 gel containing free TA. This finding was in agreement with the results obtained by Shaker, [26], who found that, the total bioavailability of Ciprofloxacin-HCl was in the following order: HPMC gel > MC gel > sod. alginate gel.

The results of statistical analysis were illustrated in table 7 and showed that, there is an extremely significant difference between the tested formulations.

**Table 1: Concentration of triamcinolone acetonide in conjunctiva from ophthalmic preparations**

| Formula / Time (hr) | Concentration of triamcinolone acetonide (ng /gm) ± S.D | | | | |
|---|---|---|---|---|---|
| | Gels | | | Ocuserts | |
| | Carbopol 934 + free drug | Carbopol 934 + Drug-β-CyD | HPMC + Drug-β-CyD | HPMC + carbopol 934 + Drug- β-CyD | Sod. alginate+ carbopol 934 + Drug- β-CyD |
| 1 | 43.6 ± 3.1 | 209.0± 22.7 | 242. 7 ± 24.6 | 328.0 ± 17.3 | 282.5 ± 14.8 |
| 2 | 127.4 ± 8.8 | 303.0± 15.5 | 397.3 ± 32.7 | 523.3 ± 23.5 | 464.2 ± 21.1 |
| 4 | 259.9 ± 26.0 | 785.1± 70. 6 | 909.0 ± 20.8 | 1150.0 ± 55.0 | 1062.2 ± 7.9 |
| 6 | 35.2 ± 3.2 | 238. 6 ± 20.8 | 312.2 ± 32.3 | 474.1 ± 31.1 | 342.4 ± 33.5 |

**Table 2: Concentration of triamcinolone acetonide in cornea from ophthalmic preparations**

| Formula / Time (hr) | Concentration of triamcinolone acetonide (ng /gm) ± S.D | | | | |
|---|---|---|---|---|---|
| | Gels | | | Ocuserts | |
| | Carbopol 934 + free drug | Carbopol 934 + Drug-β-CyD | HPMC + Drug-β-CyD | HPMC + carbopol 934 + Drug- β-CyD | Sod. alginate+ carbopol 934 + Drug- β-CyD |
| 1 | 35.3 ± 8.1 | 174.5 ± 3.5 | 208.2 ± 1.9 | 286.5 ± 9.1 | 243.9 ± 31.8 |
| 2 | 112.9± 33.0 | 253.8 ± 39.4 | 357.6 ± 22.4 | 493.8 ± 13.7 | 416.2 ± 31.2 |
| 4 | 236.5 ± 41.8 | 687.3 ± 84.1 | 811.8 ± 2.2 | 1066.6 ± 117.1 | 927.9 ± 39.2 |
| 6 | 67.7 ± 21.4 | 536.3 ± 4.3 | 677.4 ± 25.7 | 962.8 ± 43.2 | 788.4 ± 69.9 |

**Table 3: Concentration of triamcinolone acetonide in iris-ciliary body from ophthalmic preparations**

| Formula / Time (hr) | Concentration of triamcinolone acetonide (ng /gm) ± S.D | | | | |
|---|---|---|---|---|---|
| | Gels | | | Ocuserts | |
| | Carbopol 934 + free drug | Carbopol 934 + Drug-β-CyD | HPMC +Drug- β-CyD | HPMC + carbopol 934 + Drug- β-CyD | Sod. alginate+ carbopol 934 + Drug- β-CyD |
| 1 | 24.3 ± 2.3 | 131.9 ± 5.7 | 159.8 ± 14.6 | 203.9 ± 9.3 | 185.8 ± 24.6 |
| 2 | 88.1 ± 20.6 | 216.1 ± 17.2 | 263.3 ±10.4 | 352.8 ±13.3 | 309.3 ± 42.8 |
| 4 | 177.2 ± 15.3 | 610.4 ± 17.0 | 690.4 ± 36.6 | 922.2 ±18.2 | 798.5 ± 66.7 |
| 6 | 42.8 ± 2.9 | 394.6 ±14.7 | 543.3 ± 23.4 | 670.4 ± 21.1 | 617.5 ± 53.1 |

**Table 4: Concentration of triamcinolone acetonide in aqueous humor from ophthalmic preparations**

| Formula / Time (hr) | Concentration of triamcinolone acetonide (ng /ml) ± S.D | | | | |
|---|---|---|---|---|---|
| | Gels | | | Ocuserts | |
| | Carbopol 934 + free drug | Carbopol 934 + Drug-β-CyD | HPMC + Drug-β-CyD | HPMC + carbopol 934 + Drug- β-CyD | Sod. alginate+ carbopol 934 + Drug- β-CyD |
| 1 | 4.0 ± 0.3 | 16.9 ± 1.7 | 23.4± 1.7 | 31.0 ± 2.7 | 28.4 ± 2.7 |
| 2 | 6.4 ± 0.5 | 46.1 ± 3.6 | 52.1 ± 4.0 | 69.5 ± 4.9 | 61.1 ± 8.2 |
| 4 | 13.2 ± 2.3 | 68.5± 4.9 | 71.7± 2.1 | 97.7 ± 9.7 | 82.7 ± 8.6 |
| 6 | 7.6 ± 0.9 | 49.3 ± 3.9 | 51.6± 3.0 | 67.3 ± 4.6 | 59.7 ± 6 |

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**Table 5: Area under the curve (AUC) of triamcinolone acetonide ophthalmic preparations for different eye tissues**

| Formula / Tissue | $AUC_{0-6}$ (ng.hr/gm) | | | | |
|---|---|---|---|---|---|
| | Gels | | | Ocuserts | |
| | Carbopol 934 + free drug | Carbopol 934 + Drug-β-CyD | HPMC + Drug-β-CyD | HPMC + carbopol 934 + Drug- β-CyD | Sod. alginate+ carbopol 934 + Drug- β-CyD |
| Conjunctiva | 789.7 | 2472.0 | 2969.0 | 3887.0 | 3445.0 |
| Cornea | 745.5 | 2466.0 | 3046.0 | 4063.0 | 3512.0 |
| Iris-ciliary body | 553.7 | 2072.0 | 2479.0 | 3248.0 | 2864.0 |
| Aqueous humor | 47.7 | 272.4 | 296.7 | 398.0 | 345.1 |

**Table 6: Peak concentration ($C_{max}$) and peak time ($T_{max}$) of triamcinolone acetonide for different eye tissues**

| Formula / Tissue | Peak concentration ($C_{max}$) and peak time($T_{max}$) of triamcinolone acetonide for different eye tissues | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gels | | | | | | Ocuserts | | | |
| | Carbopol 934 + free drug | | Carbopol 934 + Drug-β-CyD | | HPMC + Drug-β-CyD | | HPMC + carbopol 934 + Drug-β-CyD | | Sod. alginate+ carbopol 934 + Drug-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) | $C_{max}$ µg/gm | $T_{max}$ (hr) |
| Conjunctiva | 259.9 | 4 | 785.1 | 4 | 909.0 | 4 | 1150.0 | 4 | 1062.2 | 4 |
| Cornea | 236.51 | 4 | 687.3 | 4 | 811.8 | 4 | 1066.6 | 4 | 927.9 | 4 |
| Iris-ciliary body | 177.2 | 4 | 610.4 | 4 | 690.4 | 4 | 922.2 | 4 | 798.5 | 4 |
| Aqueous humor | 13.17 | 4 | 68.5 | 4 | 71.7 | 4 | 97.7 | 4 | 82.7 | 4 |

**Table 7: Total ocular bioavailability of triamcinolone acetonide from various ophthalmic preparations in rabbits'eyes**

| Formula / Time (hr) | Total ocular bioavailability of triamcinolone acetonide (ng. hr /gm). | | | | |
|---|---|---|---|---|---|
| | Gels | | | Ocuserts | |
| | Carbopol 934 + free drug | Carbopol 934 + Drug-β-CyD | HPMC + Drug-β-CyD | HPMC + carbopol 934 + Drug-β-CyD | Sod. alginate+ carbopol 934 + Drug-β-CyD |
| 1 | 107.2 | 532.3[a] | 634.1[ab] | 849.4[abc] | 740.6[abcd] |
| 2 | 334.8 | 819.0[a] | 1070.3[ab] | 1439.4[abc] | 1250.8[abcd] |
| 4 | 686.8 | 2151.3[a] | 2482.9[ab] | 3236.5[abc] | 2871.3[abcd] |
| 6 | 153.3 | 1218.8[a] | 1534.5[ab] | 2114.6[abc] | 1808.0[abcd] |
| $AUC_{0-6}$ | 2136.6 | 7232.4[a] | 8730.7[ab] | 11596[abc] | 10166.1[abcd] |

Data are expressed as mean

-a- Significantly different at $P< 0.0001$ compared with carbopol 934 gel + free drug.
b- Significantly different at $P< 0.0001$ compared with carbopol 934 gel +drug-β-CyD.
c- Significantly different at $P< 0.0001$ compared with HPMC gel + drug-β-CyD.
d- Significantly different at $P< 0.0001$ compared with HPMC and carbopol 934 ocuserts + drug-β-CyD

(ANOVA) followed by Tukey-Kramer Multiple comparisons test was adapted.

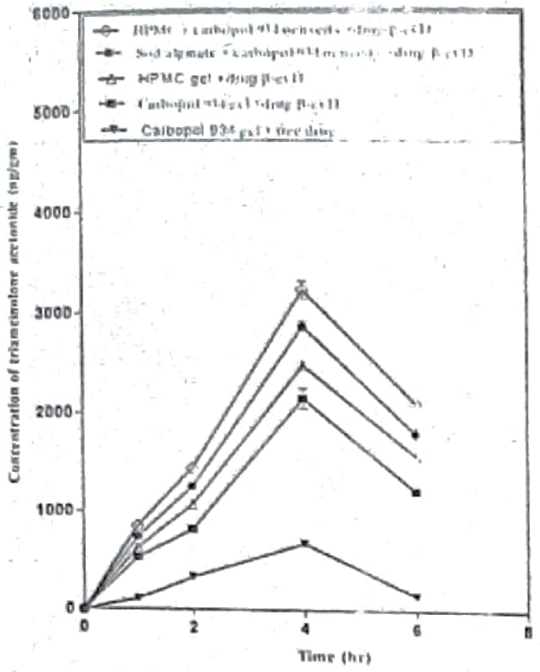


Fig. 9. Ocular bioavailability of triamcinolone acetonide in total eye tissues from different ophthalmic preparations

## CONCLUSION

From the obtained results, it could be concluded that:

- The solubility of triamcinolone acetonide had been greatly improved by the complexation with β-CyD. Also, the rate of drug release from the ophthalmic gels and ocuserts was improved.
- The peak time of maximum drug concentration in rabbits' eye tissues and aqueous humor was 4 hr for various ophthalmic gels and ocuserts.
- All tested formulations provided the $C_{max}$ of the drug in conjunctiva followed by cornea, iris-ciliary body then aqueous humor except after 6 hr where it was as follow, cornea, iris-ciliary body, conjunctiva then aqueous humor.
- The total ocular bioavailability of triamcinolone acetonide was improved when the drug was complexed with β-CyD.
- The total ocular bioavailability of the drug from the tested formulations, was found to be in the following order: HPMC and carbopol 934 ocuserts containing drug- β-CyD > sod. alginate and carbopol 934 ocuserts containing drug- β-CyD > HPMC gel containing drug- β-CyD > carbopol 934 gel containing drug-β-CyD > carbopol 934 gel containing free drug.

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# التوافر الحيوي لمتراكب الترای أمسينولون أسيتونيد في أنسجة عيون الأرانب من مستحضرات العين المختلفة

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قسم الصيدلانيات - كلية الصيدلة- جامعة المنصورة- المنصورة- مصر

تناولت هذه الدراسة تحضير متراكب الترای أمسينولون أسيتونيد مع البيتاسيكلودكسترين بطريقة العجن بنسبة 1:1 ثم أضيف العقار أو متراكبه بنسبه0.1 فى المائه الى مستحضرات العين المختلفة وتشمل المستحضرات الهلامية (كاربوبول 934 و الجينات الصوديوم و هيدروكسى بروبيل سيليل سلبولوز ) ورقائق العين (خليط من كاربوبول 934 و هيدروكسى بروبيل ميثيل سليولوز ، خليط من كاربوبول 934 و الجينات الصوديوم). تم دراسة تأثير البيتاسيكلودكسترين على الذوبانية وخواص انطلاق الترای أمسينولون أسيتونيد من مستحضرات العين المختلفة. وقد تضمن هذا البحث أيضا دراسة تأثير نوع المستحضرات على توافر الترای أمسينولون أسيتونيد فى الانسجة المتباينة والسائل المائى لعيون الارانب خلال فترات زمنية محددة بعد استخدام مستحضرات العين المحضرة المحتوية على تركيز 0.1 من الدواء. كما أظهرت نتائج هذه الدراسة أن الوقت اللازم للحصول على أعلى تركيز يصل إليه الدواء في الأنسجة والسائل المائي لعين الأرانب هو أربع ساعات من رقائق العين ومستحضراتها الهلامية المحتوية على العقار. كما وجد أنه مع جميع المستحضرات المختبرة كانت نسبة الترای أمسينولون أسيتونيد في الملتحمة أعلى من القرنية بينما احتوت القزحية والسائل المائي على نسبة أقل من العقارباستثناء بعد ست ساعات حيث كانت نسبة العقار فى القرنية أعلى من القزحية بينما احتوت الملتحمة والسائل المائي على نسبة أقل من العقار. اثبتت التجارب أيضا أن استخدام الترای أمسينولون أسيتونيد في صورة متراكب مع البيتاسيكلودكسترين يعطى زيادة في التوافر الحيوي للعقار في انسجة العين أكثر من العقارمنفردا.