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Potential Use of Cyclodextrins for the Improvement of Ocular Bioavailability of Meloxicam

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Abstract: *Meloxicam (MX) is a nonsteroidal anti-inflammatory drug. Also, it is a more distinct COX-2 inhibitor than diclofenac with less secondary effects. Yet, its usage for the treatment of ophthalmic inflammations has retarded due to its low aqueous solubility and dissolution rate. The aim of this study was to prepare the inclusion complexes of MX with cyclodextrins to enhance its aqueous solubility, dissolution rate, and ocular bioavailability. Formulation of those complexes into different ocular delivery systems and also in-vitro and in-vivo assessments has not been accomplished in the earlier attempts. In this study, MX was complexed with each of hydroxyl propyl-β-cyclodextrin (HP-β-CyD) and β-cyclodextrin (β-CyD) utilizing kneading, co-evaporation, and freeze drying techniques. Moreover, the prepared complexes were discriminated in the solid state by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD). These complexes were incorporated into eye drops, eye gels, and ocuserts using hydroxypropyl methylcellulose (HPMC), sodium carboxy methylcellulose (Sod. CMC), carbopol 940 (CP_{940}), and sodium alginate (Sod. ALG). These formulae were characterized on the subject of drug content, pH, viscosity, in-vitro release characteristics. Kinetic analysis of the release data was done. Real inclusion complexes were obtained with co-evaporation method that confirmed by DSC investigations. The MX release was enhanced by complexation especially with HP-β-CyD. Ocular bioavailability of meloxicam from the selected formulations was investigated. In-vivo study proved that, ocuserts containing MX-HP-CyD complexes showed pronounced ocular bioavailability than MX alone which indicated by higher AUC, C_{max} and relative bioavailability values.*

Keywords: *cyclodextrins, inclusion complexes, meloxicam, ocular bioavailability.*

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I. Introduction

Topically applied nonsteroidal anti-inflammatory drugs (NSAIDs) are used in the control of post-operative ocular inflammation, as well as for the remedy of intraoperative miosis during cataract surgery **[1]**. The reduction of symptoms associated to seasonal allergic conjunctivitis and the relief of ocular discomfort after refractive surgery are the target for the use of NSAIDs **[2]**.

NSAIDs produce analgesic and anti-inflammatory effects by inhibiting prostaglandin synthesis by the cyclooxygenase (COX) enzyme. During the ocular injury, both COX-1 and COX-2 are expressed, but there is an evidence that, an increase in COX-2 expression by inflammation is occured **[3]**. So that, the use of a selective COX-2 inhibitor appears to be more preferable. Meloxicam is a NSAID derived from enolic acid that inhibits particularly, COX-2 enzyme with additional mechanisms of analgesia, such as activation of the nitric oxide-cyclic GMPK+ channel pathway in peripheral tissues **[4]**. It is a more specific COX-2 inhibitor than diclofenac with less secondary effects due to the constitutive COX-1 inhibition.

Meloxicam is named chemically; 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1- dioxide **[5]**. The impaired bioavailability of drugs from ocular dosage forms is mainly due to several protective mechanisms of the eye including; blinking, the rapid turnover of lachrymal fluid, short pre-corneal residence time, as well as the relative poor permeability of the corneal epithelial membrane. Among the several techniques that increasing the apparent aqueous solubility of poorly soluble drugs without lowering their lipophilicity for enhancing their absorption through biological membrane is the complexation with cyclodextrins **[6]**.

Cyclodextrins (CyDs) are cyclic oligosaccharides composed of D-glucopyranoside units linked by glycosidic bonds. They are obtained from biotechnological processes involving the enzymatic degradation of corn starch and offer greater yield with 6, 7 and 8 units of glucose, known as α-CyD, β-CyD and γ-CyD, respectively **[7]**. CyDs are characterized by their ability to modify the physicochemical characteristics of molecules that are accommodated within their internal cavity to form the so-called inclusion complexes **[8]**.

The cavity size is more appropriate for common pharmaceutical drugs with molecular weights between 200 and 800 g/mol **[9]**. Beta-cyclodextrin (β-CyD) was chosen to enhance the solubility of meloxicam because of its central cavity diameter (6-6.5 Aº) which is suitable to accommodate most aromatic rings, its efficiency in producing stable drug complexes, low toxicity and relatively low cost **[10]**. Hydroxyalkyl derivatives such as hydroxypropyl-β-cyclodextrin have high water solubility and low hygroscopicity compared to the original β-cyclodextrin **[11]**.

The main target of this study was to enhance aqueous solubility of MX by complexation with CyDs using different techniques. Formulation of MX-CyD complexes in different ophthalmic preparations was also of our goal. In addition, inspection of the influence of inclusion complexation of MX with β-CyD and HP-β-CyD on its solubility was of prime interest. Characterization of the complexes will be accomplished by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). Moreover, all the formulations were examined for their physical properties and *in-vitro* release characteristics. In addition, the ocular bioavailability of MX from the selected formulations were studied.

II. Materials And Methods

Meloxicam (MX) was provided by Zydus Pharms, USA. Hydroxypropyl methylcellulose (4000 cP) was provided by Dow chemical company, USA. Potassium dihydrogen orthophosphate, disodium hydrogen phosphate, propylene glycol, ammonia solution 25%, methanol and sodium carboxymethylcellulose (Sod. CMC) were supplied from Adwic, El Nasr, Pharmaceutical Chemicals Co., Egypt. Sodium alginate (Sod. ALG), beta-cyclodextrin (β-CyD) (M.W = 1135.12), hydroxypropyl-β-cyclodextrin (HP-β-CyD) (M.W = 1375.35) were provided from Saito Trading Co., Ltd, Japan. Triethanolamine was purchased by Provizer Pharma, India. Propyl paraben and methyl paraben were supplied by AppliChem GmbH, Germany. Acetonitril and methanol (HPLC grade) were supplied from Fisher scientific, Fair Lawn, New Jersy, UK. Piroxicam was supplied from Sigma-Aldrich Co. LLC, St. Louis, Missouri, USA.

Methodology

2.1. Phase solubility study

The solubility assessments were performed according to the method described by **Higuchi and Connors, [12]**. An excess amount of MX (10 mg) was added to aqueous solutions of β-CyD or HP-β-CyD in separate screw capped glass bottles. Solutions were shaken on thermostatically controlled water bath (Grant Instrument Cambridge Ltd., Barrington Cambridge (B2, 5002, England) at 37±0.5^{o}C at 50 rpm. After equilibrium was reached (72 hrs), the solutions were filtered through membrane filter (with 10 mm diameter and membrane filter Corporation, Bedford, MA 01730 of pore size 0.22 µm and 0.45µm, Berlin, Germany) and then one ml from each filtrate was diluted and analyzed spectrophotometrically at 362 nm for total drug content. The experiments were done in triplicate, the mean and SD were calculated. Then, the determination of an apparent 1:1 stability constant of the complexes ($K_{1:1}$) from the initial straight-line portion of the phase solubility diagrams were performed using the following equation:

$$K_{1:1} = \text{Slope} / S_o (1 - \text{slope}) \quad (1)$$

Where; S_o is the free MX aqueous solubility

2. 2. Formulation of solid meloxicam-cyclodextrin complexes

Inclusion complexes of 1:1 molar ratio of MX with β-CyD / HP-β-CyD were performed by the kneading, co-evaporation, and freeze drying methods **[13]**. For comparative study, physical mixtures (PMs) of MX with either CyDs were prepared by simple thoroughly mixing in a morter then, seived. The three complexation methods are further discussed as follows:

2.2.1. Kneading method (Kn)

Triturate a physical mixture of drug and CyDs in 1:1 molar ratio in a mortar with a small amount of water-methanol (1:1) solution **[13]**. Then the formed thick slurry was kneaded for 45 mins. after that, dried then sieved through (75-150 µm) seive and stored in desiccators, until use.

2.2.2. Co-evaporation method (Co)

An aqueous solution of either CyDs was added to the equivalent weight of the drug dissolved in (1 N) liquid ammonia (25%), then stirred for 1 hr and evaporated at 45^{o}C until dried after that, sieved through (75-150 µm) seive and stored in airtight vessels.

2.2.3. Freeze- drying method (FD)

In this method, dissolve stoichiometric amount of drug and CyDs in distilled water and shaken for 24 hrs. (1 N) ammonia solution (25% v/v) was added to the mixture drop wise till a clear solution was obtained. The solution was frozen overnight in stainless steel dishes then lyophilized in freeze dryer (FD8-8 with T

controller, Model " FD8-8B ", SIM-USA) at -45^{o}c for 48 hrs **[14]**.The obtained product was kept at room temperature for one day. Finally, the lyophilized powder was sieved and kept in a sealed vial.

2.3. Characterization of inclusion complexes

2.3.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of MX, β-CyD, HP-β-CyD, PMs and the prepared complexes by different methods were determined using Fourier transform infrared spectroscopy (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Each sample was mixed with potassium bromide (KBr). These mixtures were ground into fine powder and then compressed into KBr discs using a hydraulic press. Each KBr disc was scanned over a wavenumber region of 400-4000 cm^{-1} at a resolution of 1 cm^{-1}. Characteristic bands were determined for all samples.

2.3.2. Differential scanning calorimetry (DSC)

The thermograms of MX, β-CyD, HP-β-CyD, PMs and the prepared complexes were analyzed using differential scanning calorimeter (Perkin Elmer DSC 7, USA). The powdered sample (10 mg) was sealed in aluminum pans and an empty aluminum pan was used as a reference, then covered by lids and scanned under nitrogen flow rate of 4^{o}C/min over the temperature range of 30-300^{o}C.

2.3.3. Powder X-ray diffraction (PXRD)

Powder X-ray diffraction patterns of MX, β-CyD, HP-β-CyD, PMs and the prepared complexes by different methods were performed using X-ray diffractometer (Rigaku Denki, Rint-2500 VL, Tokyo, Japan) over the range of 10-30^{o} (2θ) angles and operated at the conditions of Ni-filtered Cu-kα radiation. The voltage of 40 KV and a current of 40 mA, were used. The time constant was 1.25 sec, and a scanning speed was adjusted to 0.02^{o}C / min.

2.4. Preparation of different formulations

2.4.1. Preparation of eye drops

Sodium carboxy methylcellulose (Sod. CMC), hydroxylpropyl methylcellulose (HPMC) and sodium alginate (Sod. ALG) in concentrations of 1, 0.5 and 0.7% w/w respectively, were dissolved in distilled water containing 10% propylene glycol at which , MX 0.03% w/w or its equivalent weights of MX-β-CyD or MX-HP-β-CyD complexes were previously dissolved in it **"Table 1"**. 0.05% w/w methyl paraben, 0.01% w/w propyl paraben were added as preservatives **[15]**. The mixtures were completed to final weight, and then filled in clean, dry and sterile glass containers for further studies.

2.4.2. Preparation of eye gels

MX (0.03% w/w) or its equivalent weights of MX-β-CyD or MX-HP-β-CyD complexes were dissolved separately in 10% propylene glycol and added to aqueous solution of different polymers namely Sod. CMC, HPMC and Sod. ALG in concentrations of 3.5, 3 and 3% w/w, respectively **"Table 1"**. Methyl and propyl parabens were used as preservatives in concentration of 0.05% and 0.01% w/w, respectively **[15]**. The dispersion was mixed until a clear transparent gel free from air bubbles was obtained. The weight of gel was adjusted and then packaged in clean, dry and sterile glass containers until used.

2.4.3. Preparation of ocuserts

Ocuserts containing MX or its complexes were prepared according to the film casting method **[16]**. Carbopol 940 (CP_{940}) (0.1% w/w) was used in combination with Sod. CMC, HPMC and Sod. ALG to enhance the elasticity, film properties, and bioadhesion. Meloxicam (0.03%) or its equivalent weights of MX-β-CyD or MX-HP-β-CyD complexes were dissolved in distilled water containing 10% propylene glycol which acted as a plasticizer to aid the formation of flexible films as well as to guard the polymeric inserts against being brittle upon storage **[17]**. Then, the solution was added to the different polymeric solutions as illustrated in **"Table 1"**. Methyl and propyl parabens were used as preservatives in concentrations of 0.05% and 0.01% (w/w), respectively **[15]**. Triethanolamine (TEA) was added to allow CP_{940} gelation since this polymer is a polyacrylic acid which undergoes a sol to gel transition in aqueous solution at pH above its pKa (5.5) **[18]**. Sonication for 1 hr for all the prepared polymeric solutions in an ultrasonic water bath (Saris- USA) were done to remove entrapped air and then, stored for 24 hrs at ambient temperature to allow total hydration of the used polymers. Then, equal volumes of the prepared solutions were transferred into teflon plates. The solvent was allowed to evaporate for 3 days at ambient temperature. The formed films were weighed and transferred to a desiccator containing silica gel, where it was stored for another 24 hrs before use. The prepared ocuserts were cut in the form of circular discs having thickness of 0.4-0.5 mm and diameter of 5 mm. Then, individually sealed in foil sachets until use.

Table 1: Composition of MX-CyDs complexes ophthalmic formulations

Type of Formulation	Type of polymer Concentration% (w/w)	Type of complex	Formulation code
Control			CT
Eye drops	1% Sod.CMC	MX-β-CyD	Dβ 1a
		MX-HP-β-CyD	DH-β11a
	0.5% HPMC	MX-β-CyD	Dβ 2a
		MX-HP-β-CyD	DH-β12a
	0.7% Sod. ALG	MX-β-CyD	Dβ 3a
		MX-HP-β-CD	DH-β13a
Eye gels	3.5% Sod. CMC	MX-β-CyD	Gβ 4a
		MX-HP-β-CyD	GH-β14a
	3% HPMC	MX-β-CyD	Gβ 5a
		MX-HP-β-CyD	GH-β15a
	3% Sod. ALG	MX-β-CyD	Gβ 6a
		MX-HP-β-CyD	GH-β16a
Ocuserts	1.5% Sod. CMC + 0.1% CP_{940}	MX-β-CyD	Oβ 7a
		MX-HP-β-CyD	OH-β17a
	1% HPMC + 0.1% CP_{940}	MX-β-CyD	Oβ 8a
		MX-HP-β-CyD	OH-β18a
	2% Sod. ALG + 0.1% CP_{940}	MX-β-CyD	Oβ 9a
		MX-HP-β-CyD	OH-β19a

Where; All formulae contain 0.03% w/w meloxicam or its equivalent weights of complexes, 10% propylene glycol, 0.05% w/w methyl paraben and 0.01% w/w propyl paraben.
CT: Control, Sod. CMC: sodium carboxy methylcellulose, HPMC: hydroxypropyl methylcellulose, Sod. ALG: sodium alginate, β-CyD: β-cyclodextrin, HP-β-CyD: hydroxypropyl β-cyclodextrin, MX: meloxicam and a: is Kn (kneading method), C_o (co-evaporation method) or FD (freeze-drying method).

2.5. Physicochemical characterization of the formulations

2.5.1. The drug content

Drug content was determined by weighing one gram from each formulation accurately and dissolved in 50 ml phosphate buffer pH 7.4 and shaken in thermostatically controlled water bath at 37±0.5^{o}C for 1 hr then by using membrane filters of 0.45 µm the solutions were filtered and assayed at 362 nm.

2.5.2. The formulations pH

One gram of each formula was dissolved in 20 ml of distilled water, and then the pH was measured using pH-meter (Beckman Instruments Fullerton, CA 92634, Germany) **[19]**.

2.5.3. The formulations viscosity

The viscosity measurement was performed by the use of a cone and plate rotary viscometer (Haake Inc., Germany). One gram of each formulation was spreaded on the stationary plate of the viscometer and permitted to equilibrate for 5 min. to reach the running temperature before each measurement. The rotary viscometer was thermostatically adjusted to 37±0.5^{o}C. Viscosity values were calculated using the following equation;

$$\eta = G.\ S / N \qquad (2)$$

Where:

η = Viscosity in mPa.s (mPa. S = 1 centipoise, cP).
G = Instrumental factor = 14200 (mPa.s / scalagrad.min).
S = Torque (scale grad).
N =Speed (256 rpm).

2.5.4. *In-vitro* release study

The drug release from different formulations, in phosphate buffer solution of pH 7.4 was established according to the method assumed by **Shastri *et al*., [20]**. The *in- vitro* release of MX from different formulations was performed using the dialysis method. Dialysis membrane (12000–14000 molecular weight Cutoffs) was

soaked in phosphate buffer pH 7.4 for 24 hrs before the experiment. The membrane was stretched over the open end of 3 cm diameter glass tube and was made watertight by rubber bands. One gram of each formulation or one ocusert were accurately weighed and thoroughly spreaded on the membrane. To each tube, 1.5 ml of the buffer solution was added. The tube was then immersed upside-down in a beaker containing 50 ml phosphate buffer pH 7.4, which was maintained at 37±0.5^{o}C using thermostatically controlled shaking water bath. The tube height was adjusted, so that the cellulose membrane was just below the surface of the release medium. The rotary shaker was adjusted to a rate of 50 strokes / min. At predetermined time intervals of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 hrs. Aliquots of 2 ml were withdrawn from the release medium and replaced by an equivalent volume of the buffer solution. The released amounts of the drug were analyzed spectrophotometrically at 362 nm. The experiments were repeated three times and the results were calculated as mean ± SD. The data were represented as plots of cumulative percent drug released versus time.

2.5.5. Kinetic study of the release data

To inspect the release mechanism of the kinetic model that better characterizes the design of drug release, the *in-vitro* release data were analyzed according to zero-order, first order **[21]**, and simplified Higuchi model **[22]**, which considered as diffusion controlled release mechanism. To elucidate the release mechanism of MX from ophthalmic formulations, Koresmeyer-peppas model was also used **[23]**. The best fitting model with the highest correlation coefficient (r^2) was determined.

2.6. MX-CyD ocular bioavailability

For this study, three formulations were chosen in comparison with MX suspension in water as a control (CT). The selected formulae were DH-β12, GH-β15 and OH-β17 from **"Table 1"** depending on their acceptable physical and *in vitro* release characteristics.

2.6.1. Studies of ocular bioavailability

The ocular bioavailability was performed on the selected formulations using male New Zealand albino rabbits each weighing 2-2.5 kg. All needed rabbits should be healthy and free from any clinical observable abnormalities. Individually, rabbits were housed in standard cages, in a light controlled room (12 hrs light and 12 hrs dark cycles) at 20-24^{o}C, with no food or water restrictions. Conforming to the ethical principles of the scientific committee of the Pharmacy faculty, Mansoura University, Egypt, for the animal experimental procedures. The rabbits were divided into ten groups each group consisting of twelve rabbits. Each one was received one ocusert or drops or gels with equivalent dose of drug which were applied to the center of eye ball (cul-de-sac) of the right rabbits' eyes, while the left eyes were considered as control by application of the plain formulation. The lower lid was carefully moved to spread formulation on corneal surface during application. Each animal was kept in up-right position in restraining boxes. At each time interval 0.5, 1, 2, 3, and 5 hrs, six rabbits were sacrified for each formulation. Both eyes were enucleated and dissected while fresh to separate different eye tissues (conjunctiva, cornea, iris-ciliary body, and aqueous humor) which were kept frozen at -80^{o}C until subjected to further analysis. Using HPLC, the amount of MX deposited in different eye tissues and aqueous humor was determined.

2.6.2. HPLC assay

At each time interval, each eye tissue and aqueous humor were splitted, then each eye tissue was rinsed with isotonic normal saline solution, weighed, and grinded with powdered glass under aseptic conditions. The grinded tissues were extracted with 4 ml acetonitrile for 24 hrs at room temperature to insure drug extraction from different eye tissues and aqueous humor. These extracts were filtered using 0.22 µm nylon membrane filter. The tissue extracts were spiked with 220 µl of piroxicam (PI) as an internal standard (200 ng/ml). Each mixture was mixed using vortex mixer (Snijders Scientific Tilburg-Holland) for 1 min., then filtered through 0.22 µm nylon membrane filter and 20 µl of filtered extract was injected into HPLC system.

The concentration of MX in each tissue was measured by HPLC assay as reported by **Emara *et al* [24]**, with a slight modification. The quantitative analysis of MX was carried out by a reverse phase HPLC system constituting of a pump (LC-20 AD), CBM-20A interface, degasser (DGU-20A5), UV-VIS spectrophotometric detector (SPD-20A UV-VIS detector), and a reverse phase column (C-18 column, 5 µm, 4.6x250 mm, Waters, Ireland). The mobile phase consisting of 55% acetonitrile and 45% deionized water acidified with glacial acetic acid at pH 3, was filtered under vacuum through a 0.22 µm nylon membrane filter and was pumped at a flow rate 1 ml/min. The UV detector was adjusted at 360 nm. The retention time of PI and MX was 5.4 and 7.5 mins., respectively. The concentration of MX was expressed as micrograms of drug /gram of eye tissue.

6.3. Pharmacokinetic parameters

According to **Cheruvu *et al* [25]** method, the pharmacokinetic parameters were calculated. Direct estimation, from the eye tissue concentration-time curves, the maximum meloxicam concentration in eye tissues (C_{max}) and the time required to attain the maximum eye tissue concentration (t_{max}) were determined. As well, the elimination rate constant (K_e) was calculated from the linear portion of the plot by linear regression analysis. The biological half-life ($t_{1/2}$) was estimated as $0.693/K_e$. Additionally, by using the trapezoidal rule, the area under eye tissue concentration-time curve from 0 to 5 hrs (AUC_{0-5}) was calculated. The AUC was extrapolated to infinity ($AUC_{0-\infty}$) and calculated according to;

$$AUC_{0-\infty} = AUC_{0-5} + C_{last} / K_e \qquad (3)$$

Where; C_{last} was the last measurable drug concentration after 5 hrs

The ratio between $AUC_{0-\infty}$ of the tested formulation to that of the control was the relative bioavailability of MX.

7. Statistical analysis

The resulting data of *in-vitro* dissolution study were expressed as mean ± SD, while ocular bioavailability studies results were represented as mean ± SEM. Multiple groups comparisons were performed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparisons test. Statistical calculations were carried out by using Instate Graphpad prism software (version 6.00 Graphpad software, San Diego, CA, USA) **[26]**.

III. Results And Discussion

3.1. Phase solubility study

The phase solubility diagrams of MX and β-CyD or HP-β-CyD were illustrated in **"Fig. 1"**. The MX-CyDs phase diagrams were classified as A_L type according to **Higuchi and Connors, [12]**. A_L type of MX may be attributed to the formation of soluble 1:1 MX-CyD inclusion complexes.

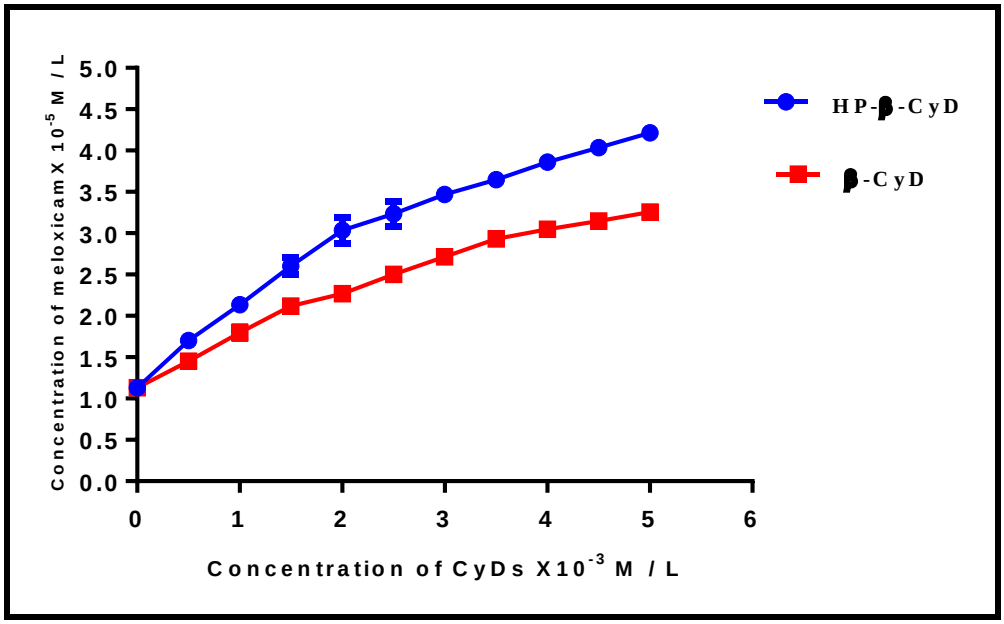

Figure 1: Phase solubility diagram of meloxicam in presence of β-CyD and HP-β-CyD

From the results, both HP-β-CyD and β-CyD had solubilization effect on the poorly soluble drug. Also, the solubilizing efficiency of HP-β-CyD was higher than β-CyD. These findings were in agreement with **Bhati *et al* [13]**, who, found that, the solubility of MX increased by the effect of HP-β-CyDs. In addition, the affinity of MX for HP-β-CyD is higher than for β-CyD. This may be due to the larger cavity size of HP-β-CyD as well as the presence of 2-hydroxypropylated substituents on the CyD molecule indicating that, MX interacted more strongly with HP-β-CyD which was optimal for entrapment of MX molecules, thus providing a greater solubilizing effect than β-CyD **[27]**. The stability constant ($K_{1:1}$) values for MX with β-CyD and HP-β-CyD were 960±1.15 M^{-1} and 1352±1.25 M^{-1}, respectively. This was confirmed by the results of **[28]**, who was reported that, when $K_{1:1}$ values ranged from 200 to 5000M^{-1}, there was an improvement in the solubility and dissolution rate of low aqueous solubility drugs.

3.2. Characterization of inclusion complexes

Meloxicam inclusion complexes with CyDs were prepared and characterized in the solid state using FT-IR, DSC, and PXRD.

3.2.1. Fourier transform infrared spectroscopy

The FT-IR spectra of MX, β-CyD, HP-β-CyD, the PMs and inclusion complexes prepared by different methods were analyzed using FT-IR spectrophotometer for characteristic bands as shown in **"Fig. 2"**. The characteristic absorption peaks of MX appeared at 3288 cm^{-1} denoting stretching vibration of –NH, 1549 cm^{-1} together with 1183 cm^{-1} denoting stretching vibration of the thiazole ring, 1346 cm^{-1} for the asymmetry stretching vibration of sulfone, 1526 cm^{-1} for amide II band of –CO–NH–C and 1263 $cm^{-1}$for amide III band of –CO–NH–C.

The FT-IR spectrum of β-CyD was characterized by intense bands at 3200-3600 cm^{-1} due to O-H stretching vibration bands. The vibration bands of the C-H and CH2 groups appear in 2800-3000 cm^{-1} region, a broad band appeared at 1649 cm^{-1} due to adsorbed water **[29]**. While, that of HP-β-CyD, showed prominent absorbance bands at 3417 cm^{-1} and 2930 cm^{-1} for O-H stretching vibrations. Also, two stretching vibration bands were obtained at 1421 cm^{-1} and 1083 cm^{-1} for C-H and C-O, respectively **[30]**. In both PMs spectra, the characteristic peaks of both MX and β-CyD or HP-β-CyD can be observed, and the spectra can be regarded as simple superimposition of the individual components.

However, obvious changes occurred in the feature and fingerprint region of the FT-IR spectra of MX-β-CyD and MX-HP-β-CyD inclusion complexes prepared either by kneading, co-evaporation or freeze drying methods. In the feature region, the band at 3288 cm^{-1} for –NH stretching vibration peak of MX disappeared in all inclusion complexes. It seemed that intermolecular hydrogen bond between–NH of MX and –OH of the carrier or inclusion complexes have been formed **[31 & 32**].

Similarly, almost all of the peaks of the functional groups containing electronegative atoms underwent changes: for the amide groups, the amide II (1526 cm^{-1}) and amide III (1263 cm^{-1}) bands were shifted to lower wavenumber band bands; (1516 and 1239 cm^{-1}), respectively. The asymmetry stretching vibration of sulfone at (1346 cm^{-1}) shifted to lower wavenumber band too (1326 cm^{-1}).

Interestingly, both of the two thiazole rings characteristic peaks simultaneously disappeared in the FT-IR spectra of all inclusion complexes. This is a direct evidence indicating a possible inclusion of the thiazole ring into CyD cavities as for the amide groups, which are adjacent to the thiazole ring, the disappearance and the shifting of the absorption peaks were also attributable to the inclusion into the CyDs cavity. All of the above evidences implied possible intermolecular interaction between MX and both cyclodextrins.

3.2.2 Differential scanning calorimetry

When guest molecules were included inside CyDs cavities, the peak corresponding to their melting point was generally shifted to another degree. Also, the peak intensity may be decreased or disappeared. Therefore, the thermal behavior of MX-β-CyD and MX-HP-β-CyD solid complexes and PMs were studied using DSC in order to characterize the complex formation process.

As shown in **"Fig. 3"**, the thermograms of MX and its PMs indicated a sharp endothermic peak at 254^{o}C attributable to the melting process of the anhydrous crystalline form of the drug, followed by its thermal decomposition **[13]**.

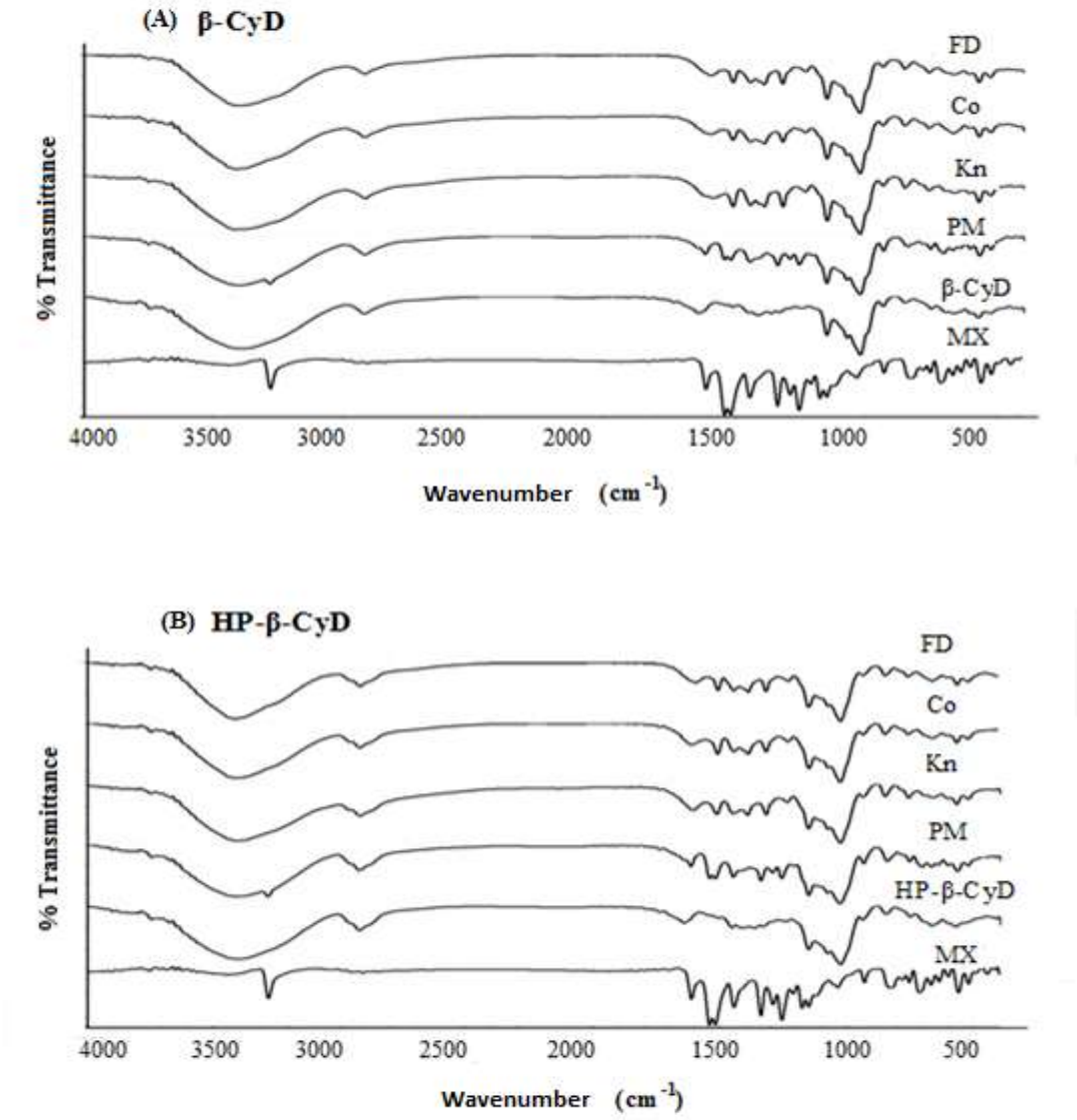

Figure 2: FT-IR spectra of MX, β-CyD or HP-β-CyD, physical mixtures (PMs), kneaded (Kn), co-evaporated (Co) and freeze dried (FD) complexes

The thermogram of β-CyD showed two peaks; one broad endothermic peak at (90-120)^{o}C, consistent to the release of water molecules from the structure lattice, and another less broad endothermic peak above 300^{o}C, corresponding to the decomposition of β-CyD. Similar results were obtained previously by **Ainurofiq *et al.*, [33]**. While, the thermogram of HP-β-CyD showed regular broad endothermic peak between 58^{o}C and 110^{o}C, which attained a maximum at 66.33^{o}C that might be corresponding to dehydration process of CyD **[34]**. The thermograms of PMs of MX with β-CyD or HP-β-CyD were found to be the summation of those the drug and CyDs.

In case of MX-β-CyD complexes which prepared by different methods, the endothermic peak of MX was shifted, whereas the melting temperature of the drug was lowered to 247^{o}C which may be due to dehydration during evaporation process related to the increase in solubility.

In case of MX-HP-β-CyD complexes which prepared by kneading, co-evaporation or freeze drying methods, there was more reduction in the endothermic peak of MX to 229, 115, 223^{o}C, respectively. As well as, the intensity of the endothermic peak of the drug was decreased in all complexes. Particularly, in MX-HP-β-CyD complexes prepared by co-evaporation methods, the peak intensity was very diminished and nearly disappeared due to maximal complete complex formation. These results suggested the increase in the dissolution rate of MX-CyDs complexes which could be correlated with the reduction in the melting point of the drug.

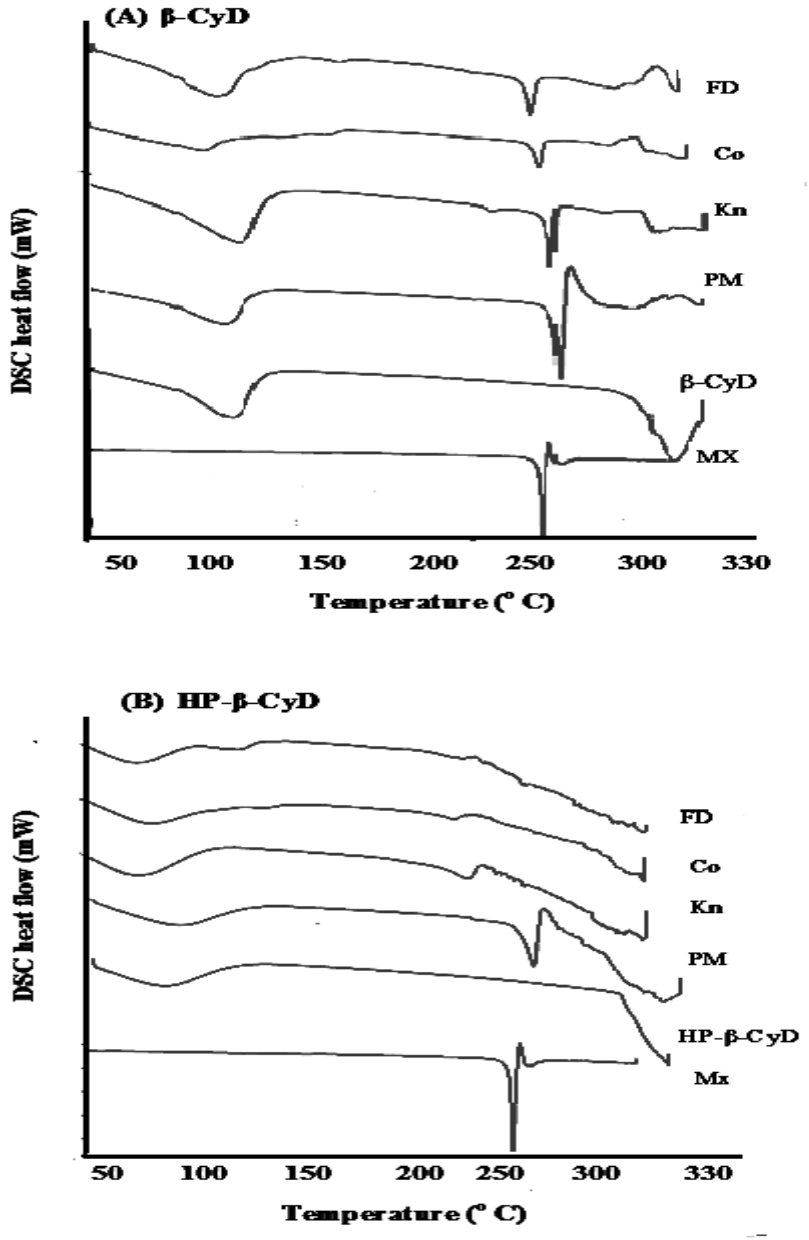

Figure 3: DSC thermograms of MX, β-CyD or HP-β-CyD, physical mixtures (PMs), kneaded (Kn), co-evaporated (Co) and freeze dried (FD) complexes

3.2.3. Powder X-ray diffraction analysis (PXRD) studies

The X-ray diffraction patterns of MX, β-CyD, HP-β-CyD, their corresponding PMs and inclusion complexes of MX-β-CyD and MX-HP-β-CyD were illustrated in "**Fig. 4**". The diffraction pattern of MX showed that, the drug is crystalline in nature as demonstrated by numerous distinct peaks notably at 2θ angles 11.3°, 13.03°, 14.87°, 18.54°, 21.9°, 25.78°, 26.3° and 29.45° **[13, 31]**.

Characteristic peaks of β-CyD showed more complicated diffractogram with characteristic peaks appeared at 2θ equal to 10.6°, 17.8°, 22.8°, 29.44°, 36.59° and 39.13°, while, the diffraction pattern of amorphous HP-β-CyD showed one broad peak at 2θ of 19.2°. The X-ray diffraction patterns of PMs exhibited the identifiable peaks of MX in their spectra, indicating no interaction occurred between the drug and CyDs.

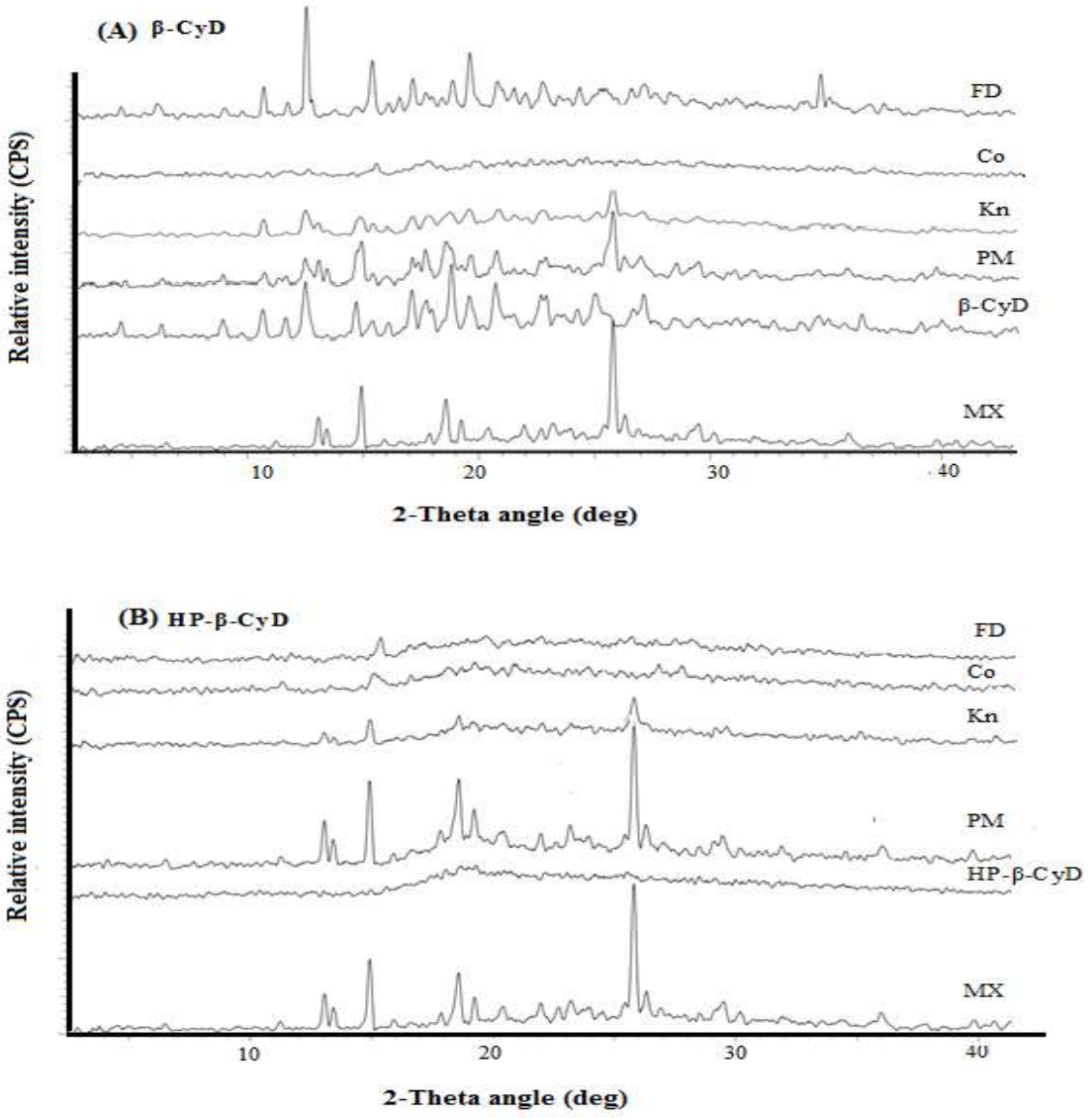

Figure 4: Powder X – ray diffraction patterns of MX, β-CyD or HP- β-CyD, Physical mixtures (PMs), kneaded (Kn), co-evaporated (Co) and freeze dried (FD) complexes

The X-ray diffraction patterns of MX-β-CyD complex showed diffuse peaks with low intensities, indicating that, the crystallinity of the drug was remarkably reduced leading to the formation of a new solid state due to inclusion complex formation between MX and β-CyD prepared by different techniques **[13]**.

The X-ray diffraction patterns of MX-HP-β-CyD complex showed broad and diffuse peaks with low intensities, indicating an amorphous solid state was performed may be due to inclusion complex formation between MX and HP-β-CyD prepared by different techniques. Similar findings have been reported by other authors **[35]**.

Based on these findings, a decrease in the drug crystallinity with subsequent increase in the surface area of the drug exposed to the dissolution medium might be responsible for the improved dissolution of MX.

3.3. Physicochemical characterization of different formulations

The superiority of co-evaporation technique was confirmed from the results of FT-IR, DSC and PXRD due to the combined effect of complexation and crystallinity reduction; hence, the formulations prepared by this method were further investigated.

3.2.1. Drug content of the formulations

The drug content was calculated for each formulation. The results are presented in **"Table 3"**. It was obvious that, the percentage of MX content in all prepared formulations ranged from 96.71±1.56% to 100.1±1.55%. These results complying with the official requirements within the range of 90-110% **[36]**.

3.2.2. pH of the formulations

The pH values of the prepared formulations were determined and the results were showed in **"Table 3"**. The pH of eye tears is 7.4, the eye can tolerate the ophthalmic formulations with wide pH range (3.5 – 8.5). Due to the ideal ophthalmic dose is only one drop, the tear film can be rapidly regained neutral pH **[37]**. The pH values of the prepared formulations ranged from 6.78±0.05 to 7.32±0.03, which are suitable values that can be easily tolerated by the eye without irritation or discomfort.

3.2.3. The viscosity of the formulations

The viscosity values of the prepared eye drops and eye gels were measured and the results were represented in **"Table 3"**. The values were extended from 443.75±25 to 536±57 mPa.s for eye drops and from 1250±85 to 1410.18±130 mPa.s for eye gels. It was found that, the viscosity of eye gels was higher than that of eye drops, hence prolonged their contact with the eye surface and retarded the rapid drainage of the formulations from the eye which in turn improved their bioavailability **[38]**.

Table 3: Physical evaluation of different ophthalmic formulations

Formula		pH	Drug content %(w/w)	Viscosity (mPa.s)
Eye drops	**Dβ 1co**	7.10±0.02	99.00±2.87	443.75±29
	Dβ 2co	6.90±0.15	99.45±1.36	536±57
	Dβ 3co	7.00±0.01	98.99±1.12	443.75±41
	DH-β11co	7.19±0.13	99.78±1.05	443.75±25
	DH-β12co	6.88±0.04	99.76±0.54	506±33
	DH-β13co	7.21±0. 11	100.1±1.55	489±26
Eye gels	**Gβ 4co**	7.32±0.03	99.33±1.51	1250±85
	Gβ 5co	6.80±0.16	99.76±2.15	1410.18±89
	Gβ 6co	7.10±0. 20	97.54±3.57	1331.25±77
	GH-β14co	7.24±0.03	98. 82±2.07	1250±116
	GH-β15co	6.78±0.05	99.44±3.52	1410.18±130
	GH-β16co	7.09±0.03	96.71±1.56	1410.18±95
Ocuserts	**Oβ 7co**	7.26±0.10	98.76±2.51	---
	Oβ 8co	6.99±0.08	99.32±3.25	---
	Oβ 9co	7.31±0.06	99.00±2.72	---
	OH-β17co	7.20±0.02	98.99±1.83	---
	OH-β18co	6.80±0.04	97.55±2.33	---
	OH-β19co	7.12±0.04	99.42±1.53	---

Where;

All values were expressed as means ± SD (n=3)

3. 4. *In-vitro* drug release

The release results of MX in phosphate buffer pH 7.4 from eye drops, eye gels and ocuserts containing MX complexes prepared by different methods indicated the superiority of co-evaporation technique. Thus, only release data for the prepared formulations containing MX-β-CyD or MX-HP-β-CyD 1:1 co-evaporated complex were represented in **"Fig. 5"**. It was found that, the cumulative percent released of the untreated drug after 8 hrs was only, 58.60%. The tested ophthalmic preparations containing MX-β-CyD or MX-HP-β-CyD 1:1 complexes showed significantly higher release than that observed with corresponding formula of control. This may be referred to the hydrophobic nature of MX which retards its contact with the release medium and consequently hindering its dissolution.

The extent of the dissolution rate enhancement was dependent on the preparation technique, since co-evaporated complexes of both β-CyD and HP-β-CyD presented higher dissolution rate than other methods indicating a better inclusion of the drug with CyD by this technique and formation of soluble complexes in the solid form with highly diminishing of MX crystallinity **[39]**, as confirmed by FT-IR, DSC, and PXRD studies.

Additionally, the HP-β-CyD co-evaporated complex showed high dissolution rate than that of β-CyD, this was related to the higher solubilizing effect of HP-β-CyD than β-CyD. These findings were in agreement with the results obtained by **Abou-Taleb *et al* [40]**, as they found that, HP-β-CyD have higher solubilizing effect with rofecoxib than that β-CyD.

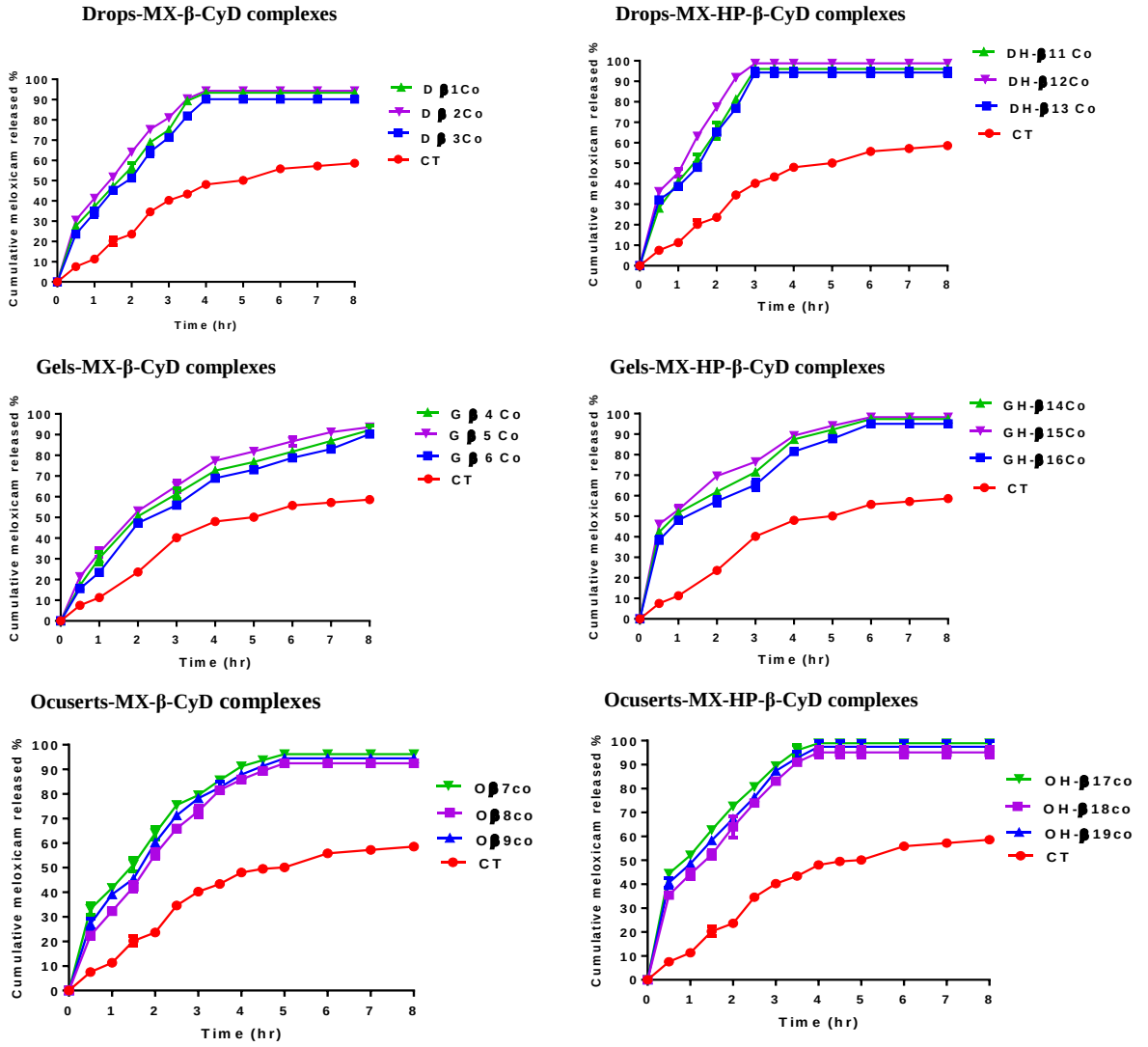

Figure 5: *In-vitro* dissolution profiles of MX-CyDs inclusion complexes prepared by co-evaporation method from different formulations in phosphate buffer (pH 7.4) at 37°C

It was found that, the incorporation of complexed drug into different vehicle resulted in a significant ($p < 0.05$) higher drug release compared with control. From the obtained results **"Fig. 5"**, the vehicles played an important role in controlling the drug release rate, as we found that, the release rates from the formulations were in the following order: eye drops > ocuserts > eye gels. This might be due to the difference in their viscosities upon exposure to the release conditions, as the higher the viscosity the slower drug diffusion and subsequent slower release rate **[41]**. The higher release of MX from ocuserts might be due to the enhancing effect of triethanolamine on CyDs solubilizing power for the poorly water soluble drugs **[42]**.

The release of MX from eye drops and eye gels was in the following order; HPMC > Sod. CMC > Sod. ALG. The lower release of MX from Sod. ALG compared to HPMC and Sod. CMC might be due to the slow ALG matrices dissolution due to the slightly basic pH **[43]**. While, the release of MX from ocuserts was in the following order; Sod. CMC + CP_{940} > Sod. ALG + CP_{940} > HPMC + CP_{940}.

The enhancement of the drug dissolution rate from CyDs complexes may be attributed to the formation of inclusion complexes in the solid state and reduction in the crystallinity of the product, particle size and accordingly increase in the surface area of the complexed drug exposed to the dissolution medium **[44]**.

3.5. Kinetics of drug release

The release kinetic parameters and correlation coefficients (r^2) were calculated **"Table 4".** The kinetic analysis showed that, the release of MX from its co-evaporated complexes with both β-CyD and HP-β-CyD formulations was most fitted to diffusion-controlled mechanism (Higuchi model) except eye gels of β-CyD complexes followed first order kinetic. Further examination using Koresmeyer-Peppas equation exhibited that, the release exponents (n) values for all eye formulations were below 0.45 which suggesting Fickian mechanism except (n) values of CT, $O\beta8_{co}$ and eye gels of β-CyD complexes were situated between 0.45 and 0.73, which indicated that, they exhibited a non-Fickian (anomalous diffusion).

Table 4: Kinetic analysis of the drug release data from different formulations prepared by co-evaporation method

Formulations		Correlation coefficient (r^2)			Release order	Koresmeyer peppas		Main transport mechanism
		Zero-order	First-order	Higuchi model		r^2	n	
Eye drops	CT	0.8854	0.9513	0.9374	First-order	0.9481	0.7327	Non-Fickian
	Dβ1co	0.7425	0.8294	0.9146	Diffusion	0.9296	0.4401	Fickian
	Dβ2co	0.7026	0.8362	0.9021	Diffusion	0.9183	0.3948	Fickian
	Dβ3co	0.7666	0.8536	0.9248	Diffusion	0.9378	0.4779	Non-Fickian
	DH-β11co	0.6480	0.7049	0.8551	Diffusion	0.8722	0.4065	Fickian
	DH-β12co	0.5911	0.6639	0.8360	Diffusion	0.8424	0.3277	Fickian
	DH-β13co	0.6555	0.6952	0.8279	Diffusion	0.8696	0.3850	Fickian
Eye gels	Gβ4co	0.8925	0.9806	0.9711	First-order	0.9840	0.5509	Non-Fickian
	Gβ5co	0.8767	0.9896	0.9789	First-order	0.9813	0.5082	Non-Fickian
	Gβ6co	0.9136	0.9835	0.9755	First-order	0.9512	0.6007	Non-Fickian
	GH-β14co	0.7891	0.9493	0.9514	Diffusion	0.9762	0.3066	Fickian
	GH-β15co	0.7481	0.9322	0.9329	Diffusion	0.9780	0.2814	Fickian
	GH-β16co	0.8259	0.9524	0.9652	Diffusion	0.9740	0.3298	Fickian
Ocuserts	Oβ7co	0.7382	0.9034	0.9261	Diffusion	0.9380	0.3936	Fickian
	Oβ8co	0.7846	0.9031	0.9344	Diffusion	0.9421	0.5138	Non-Fickian
	Oβ9co	0.7614	0.9029	0.9324	Diffusion	0.9400	0.4473	Fickian
	OH-β17co	0.6495	0.8379	0.8796	Diffusion	0.9185	0.2956	Fickian
	OH-β18co	0.6993	0.7957	0.9000	Diffusion	0.9183	0.3651	Fickian
	OH-β19co	0.6797	0.6482	0.8940	Diffusion	0.9217	0.3266	Fickian

Where; (n) is release exponent.

3.6. Ocular bioavailability of MX-CyDs

The gross examination of the rabbit eyes showed no signs of redness, irritation, allergic complications, increased blinking, abnormal blinking or ocular damage to eye tissues during the *in-vivo* study. Previous studies for determining the acute toxicity of natural CyDs on rats gave very high LD_{50} values in several administration routes. **Saarinen-Savolainen *et al* [45]** found that, topically applied HP-β-CyD seem to be relatively safe on the corneal epithelium.

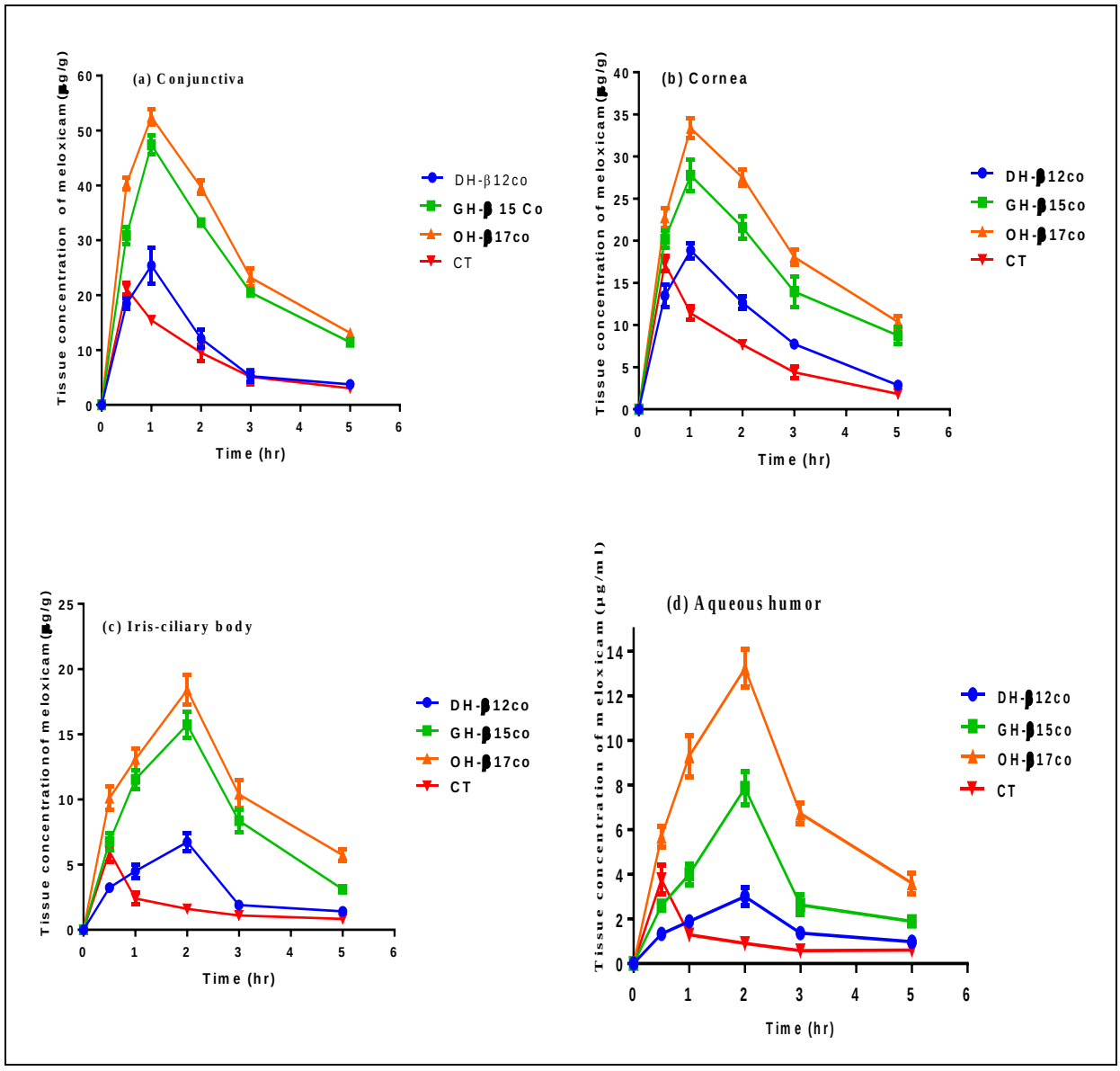

Figure 6: The eye tissue concentration-time profiles of MX-HP-β-CyD inclusion complexes following topical application of the selected formulations

"Fig. 6" showed that, the concentration of MX after a single application of the selected formulations or CT in the eye tissues and the aqueous humor. The pharmacokinetic parameters of MX were shown in **"Table 5"**. It was clear that, MX bioavailability in eye tissues and aqueous humor were arranged in the order of; conjunctiva > cornea > iris-ciliary body > aqueous humor which confirmed by the C_{max}, AUC_{0-5}, $AUC_{0-\infty}$ and the relative bioavailability values. The higher MX bioavailability in conjunctiva and cornea may be related to the direct contact of these tissues with the tear film which containing the drug. These results were in agreement with those adopted by **Soliman *et al* [46]**, as they reported that, there is a higher concentration of loteprednol etabonate in cornea than in aqueous humor. The ocular bioavailability of the prepared formulations were arranged in the following order; ocuserts > eye gels > eye drops **[47]**, who found that, the total bioavailability of ciprofloxacin hydrochloride from the tested formulations was in the same order.

Table 5: Pharmacokinetic parameters of selected formulations containing MX-HP-β-CyD complex

Tissues	Pharmacokinetic parameters	DH-β12$_{co}$	GH-β15$_{co}$	OH-β17$_{co}$	CT
Conjunctiva	**C_{max} (µg/g)**	25.39±3.33	47.40±1.76*	52.46±1.39*	21.20±1.10
	t_{max}(hr)	1*	1*	1*	0.5
	K_e (hr^{-1})	0.468±0.035	0.359±0.014*	0.356±0.014*	0.432±0.03
	$t_{1/2}$(hr)	1.48±0.11	1.93±0.08*	1.94±0.08*	1.60±0.09
	AUC_{0-5} (µg.hr/g)	51.93±2.46*	126.37±2.34^{*,a}	147.1±2.52^{*,a}	42.36±2.36
	$AUC_{0-\infty}$ (µg.hr/g)	60.02±3.55	158.18±5.52^{*,a}	184.12±4.20^{*,a}	49.44±3.46
	Relative Bioavailability	1.22±0.05	3.21±0.17^{a}	3.74±0.32^{a}	----------
Cornea	**C_{max} (µg/g)**	18.85±0.9	27.77±1.85^{*,a}	33.37±1.17^{*,a}	17.3±0.92
	t_{max}(hr)	1*	1*	1*	0.5
	K_e (hr^{-1})	0.479±0.03	0.295±0.03*	0.302±0.02*	0.483±0.05
	$t_{1/2}$(hr)	1.45±0.09	2.36±0.24*	2.29±0.13*	1.44±0.14
	AUC_{0-5} (µg.hr/g)	48.03±1.34*	82.18±5.8^{*,a}	101.38±2.71^{*,a}	33.30±1.14
	$AUC_{0-\infty}$ (µg.hr/g)	54.01±2.22	112.24±11.57^{*,a}	135.87±6.94*	37.15±1.90
	Relative Bioavailability	1.46±0.04	3.02±0.16^{a}	3.66±0.30^{a}	----------
Iris-ciliary body	**C_{max} (µg/g)**	6.73±0.67	15.73±1.01*	18.40±1.14*	6.00±0.8
	t_{max}(hr)	2*	2*	2*	0.5
	K_e (hr^{-1})	0.458±0.01	0.531±0.043*	0.375±0.014	0.38±0.049
	$t_{1/2}$(hr)	1.51±0.02	1.31±0.11*	1.85±0.07	1.84±0.22
	AUC_{0-5} (µg.hr/g)	15.99±1.33*	43.42±0.87^{*,a}	54.58±1.78^{*,a}	8.89±0.39
	$AUC_{0-\infty}$ (µg.hr/g)	19.09±1.53*	49.33±1.86^{*,a}	69.85±2.30^{*,a}	11.14±0.36
	Relative Bioavailability	1.71±0.10	4.43±0.05^{a}	6.28±0.38^{a}	----------
Aqueous humor	**C_{max} (µg/g)**	3.00±0.40	7.87±0.75*	13.23±0.85*	3.77±0.65
	t_{max}(hr)	2*	2*	2*	0.5
	K_e (hr^{-1})	0.33±0.045	0.43±0.07	0.42±0.043	0.34±0.04
	$t_{1/2}$(hr)	2.06±0.26	1.64±0.26	1.67±0.18	2.04±0.24
	AUC_{0-5} (µg.hr/g)	8.11±0.23*	18.02±1.0^{*,a}	36.76±0.39^{*,a}	5.23±0.23
	$AUC_{0-\infty}$ (µg.hr/g)	11.04±0.81*	22.57±0.20^{*,a}	45.53±1.72^{*,a}	7.00±0.12
	Relative Bioavailability	1.58±0.11	3.22±0.08^{a}	6.50±0.14^{a}	----------

All values are expressed as means ± SEM (n=6)

C_{max} the maximum concentration of drug in eye tissues, t_{max} time required to reach the maximum eye tissue concentration, K_e the elimination rate constant, $t_{1/2}$ the biological half-life, AUC_{0-5} the area under eye tissue concentration-time curve from 0 to 5 hrs, $AUC_{0-\infty}$ the area under eye tissue concentration-time curve from 0 to infinity.

$^{(*)}$ considered significant compared to control ($p < 0.05$) and (^{a}) considered significant compared to DH-β12$_{co}$

The time required to reach maximum concentration values t_{max}, for all the investigated formulations, gave extended values which reached up to 2 hrs in iris- ciliary body and aqueous humor, while in conjunctiva and cornea, t_{max} reached up to 1 hr. It was clear that, the t_{max} of all formulations were significantly ($p < 0.05$) superior to that of control in different eye tissues. Also, $t_{1/2}$ values for all tested formulations had prolonged $t_{1/2}$

that ranged from 1.31±0.11 hrs to 2.36±0.24 hrs in different eye tissues compared to 1.44±0.14 hrs to 2.04±0.24 hrs for the control.

From C_{max} and t_{max} results, the data showed that, after single administration of 0.03% MX ophthalmic formulae, permeated poorly into aqueous humor, iris and ciliary body, whereas it permeated well into the cornea and the conjunctiva. These results were parallel to that revealed by **[48]**, who study the ophthalmic composition containing fourth generation flouroquinolones for the treatment of eye infections and with **[49]**, who studied the distribution of amefenac to the posterior segment of the eye. The concentration gradient between the tear film and the drug concentration in the cornea and conjunctival epithelium was the driving force for passive diffusion across these tissues **[50]**.

Furthermore, the relative bioavailability was generally increased from 1.22 to 6.50 folds vs. control for all tested formulations in different eye tissues and aqueous humor. The higher bioavailability of MX from ocuserts that contain CP $_{940}$ can be explained by the enhancing effect of triethanolamine on CyDs solubilizing power for the poorly water soluble drugs by forming drug-CyD-TEA multicomponent system improving the ocular delivery of the drug **[42]**.

The enhanced bioavailability of the eye gels and ocuserts containing MX-CyDs complexes than eye drops may be due to the high viscosity and bioadhesiveness properties of the polymers which forms the eye gels and ocuserts. These properties prevented the rapid drainage of the formulations from the eye and so increased their contact time with the eye surface which in turn improved their bioavailability. These results are in line with **Budai *et al* [51]**, as they reported that, the high viscosity and the bioadhesive properties of the gel formulations improved the ocular bioavailability of ciprofloxacin.

It was obvious that, the C_{max}, AUC_{0-5} and $AUC_{0-\infty}$ of all formulations were significantly ($p < 0.05$) superior to that of CT in cornea, conjunctiva and iris-ciliary body. While, in aqueous humor, all formulations were significantly different at ($p < 0.05$) versus that of CT except eye drops were non-significant. This might be due to rapid turnover of the eye drops from the eye surface **[19]**, as they found that, gels containing celecoxib nanoparticles showed a higher ocular bioavailability which indicated by higher AUC and C_{max} values.

IV. Conclusion

Inclusion complexes of MX with β-CyD or HP-β-CyD have been successfully prepared. The higher degree of amorphous entities has been yielded by the co-evaporation method ensuring the formation of true MX-β-CyD/MX-HP-β-CyD inclusion complexes. Thus, the complex preparation methods played an important role in improving the dissolution rate. Also, the MX ocular bioavailability was improved by complexation of it with HP-β-CyD more than β-CyD. Ocuserts and eye gels containing MX-CyD have a higher bioavailability and more extended action than eye drops and control. Accordingly, the complexation of MX with CyDs provided a promising mean for enhancement the dissolution rate and the ocular bioavailability of MX.

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