Original Research Article

2-Hydroxypropyl-ß-Cyclodextrin Complex with Ketotifen Fumerate for Eye Drops Preparations

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Abstract

Ketotifen fumerate ophthalmic solution is an antihistaminic drug. The aim of this study was to develop a novel aqueous ketotifen eye drop formulation containin ketotifen/2-Hydroxypropyl-β-cyclodextrin complex, which has the ability to enhance the solubility of the drug at the physiological pH (7.4), consequently decreasing the irritation to the eye. Moreover, increaseing the fraction of the unionized drug leads to enhancing the permeability of the drug through the cornea. The stability constant was determined from phase-solubility diagrams and found to be higher with KF/HP-ß-CD complex (29.47 M^{-1}) than KF/ß-CD complex (10.36 M^{-1}). Differential scanning calorimetry (DSC), fourier transform-infrared (FT-IR) spectroscopy and X-ray analysis (X-ray) showed the formation and the physiochemical proporties of the complex. The effect of different additives including; antioxidants, isotonicity adjusting agents and preservatives on the stability of ketotifen fumerate solution were studied. Sodium ascorbate (0.1%w/v) showed a significant increase in the stability of the KF solutoion. Fluid thioglycolate medium and Tryptic Soy Broth were used as a medium to test of sterility of selected formulations to detect aerobic, anaerobic and fungal contamination and verified that no turbidity or surface growth was observed during the incubation period of all tested formulae. Finally, the chemical stability using the stability indicating HPLC assay showed that the stability of the selected formulation were significantly higher in comparison to that of a commercial product (Zaditen® eye drops Novartis Pharma-Egypt) (p>0.05). These results indicate that KF/HP-ß-CD eye drops formulation is a promising (formulation) resulting in enhancing the solability, stability and permeability of the drug.

Keywords: Ketotifen Fumerate; 2-Hydroxypropyl-ß-cyclodextrin; Inclusion complex; Solubility; Stability.

Introduction

The human eye has always been a challenging subject for topical administration of drugs. Many attempts have been made to deliver ophthalmic drugs to the eye by means of different drug delivery systems [1]. To achieve effective ophthalmic therapy, most drugs must penetrate through the eye tissue barriers (e.g., cornea, and conjunctiva) to reach the therapeutic targets. Furthermore, an adequate drug concentration must be maintained at the site of action within the eye for an extended time up to several hours [2, 3]. The principle disadvantage of ophthalmic solution is the relatively short contact time between the drug and absorbing surfaces which may be increased to some extent by addition of

viscosity inducing agents. Solutions to be applied to mucous membrane tissues are liable to cause irritation if their pH is greatly (removed) from the normal pH of the relevant body fluid optimum as patient comfort is usually found at the pH of the tear fluid, about 7.4[4].

Ketotifen is a relatively selective, non-competitive histamine antagonist (H1-receptor) and mast cell stabilizer. Ketotifen inhibits the release of mediators from cells involved in hypersensitivity reactions [5]. However, its commercial preparation (Zaditen® eye drops Novartis Pharma-Egypt) has an acidic pH of 4.4 to 5.6 that affects patient compliance by causing irritation to the eye.

Cyclodextrins (CDs) are shown to be nontoxic and well tolerated in eye drop formulations. CDs are water-soluble complexing agents that are able to solubilize poorly soluble drugs, and enhance their permeation through biological membranes, (through formation of water-soluble complexes)[6-8]. In ophthalmic preparations, drug/CD complexes have been shown to increase the drug permeation through the cornea[8, 9]. Moreover, the chemical stability of many active substances has been improved by (complexation) with different cyclodextrins.

The main object of this study is to develop and evaluate KF/HP-ß-CD inclusion complex and to prepare a novel eye drop solution of KF suitable for ophthalmic use.

Materials and methods
Materials
Ketotifen fumarate (Wuhan Yuanvheng Co, China, generously gifted by Memphis Co., Egypt). 2Hydroxy propyl- ß-cyclodextrin and ß-cyclodextrin (chemical Co., Milwaukee, WI, USA). Polyvinyl alcohol (PVA 1500), sodium ascorbate, benzalkonium chloride, methyl paraben, propylen glycol (EL-Nasr pharmaceutical chemical company, Egypt). Triethaylamine, methanol (HPLC grade ,Romil chemical, England). Disodium hydrogen phosphate, potassium dihydrogen phosphate (E.Merck, Darmstadt, Germany). All other ingredients were of analytical grade.

Methods
Saturated solubility study
The saturated solubilities of KF in distilled water and in phosphate buffers of pHs (4.5-7.8) were carried out. An excess amount of the drug was shaken with each solution at room temperature (25±1ºC) until equilibrium. The solutions were then filtered through a Millipore filter (pore size 0.45 μm, Millipore, Milford, USA.), and the concentration was determined spectrophotometrically at λ_{max} 300 nm.

Partition coefficient study
The partition coefficients of KF between n-octanol (represents organic phase) while, distilled water and phosphate buffers of pHs ranging from 4.5-7.8 (represent the aqueous phase) were determined according to the McDaid and Deasy method [10]. Equal volumes (50 ml) of n-octanol and each of the aqueous solutions were mixed and shaken for six hours, at (25±1ºC). The two phases were left to separate overnight. A known concentration of the drug was added and the solutions were shaken for 24 hours. The two phases were then separated using a separating funnel. The separated aqueous phases were assayed spectrophotometrically at λ_{max} 300 nm to determine their concentrations; consequently, the amount partitioned into n-octanol phase was calculated. The partition coefficient was expressed as the ratio between the drug concentration in the n-octanol phase and the drug concentration in the aqueous phase (% w/v).

Phase solubility studies
Excess amounts of ketotifen fumerate were added to phosphate buffer solutions containing increasing concentrations of the ß-CD and HP-ß-CD (ranging from 3 to 100 mM) in a series of glass stoppered bottles, the pH was adjusted at 7.4±0.2. run on sentence. The obtained suspensions were shaken at 37±0.5°C for 7 days.

Aliquots were withdrawn and the filtered through a Millipore filter. The filtered solutions were measured spectrophotometrically at λ_{max} 300 nm. The stability constants $K_{1:1}$ were estimated from the straight line of the phase solubility diagrams [11] according to equation (1)

$$K_{1:1}=slope/S_o(1\text{-slope}) \qquad \ldots\ldots\ldots(1)$$

Where S_o represents the drug solubility in absence of cyclodextrins (the intercept of the phase solubility diagram). The complexation efficiency (CE)[12] was calculated according to equation (2)

$$CE=S_0K_{1:1}=[drug\text{-}CD]/[CD]=slope/1\text{-slope} \ldots(2)$$

Where [drug-CD] is the concentration of the drug-CD complex and [CD] is the concentration of the free cyclodextrin.

Preparation of solid inclusion complex
Inclusion complexes were prepared by the coprecipitation method [13]. The clear solutions of HP-ß-Cyclodextrin in distilled water and KF in ethanol-water (80:20, v/v) solution were mixed at the molar ratios of (1:1). The obtained solution was dried under vacuum at room temperature using a rotary evaporator. Further drying of the prepared coprecipitate was carried out in a dessicator over anhydrous calcium chloride.

Characterization of prepared inclusion complex
Differential scanning calorimetry (DSC)
DSC studies were performed for pure drug, HP-ß-CD, the drug- cyclodextrins physical mixture (1:1) and the prepared inclusion complex. The samples (3-4mg) were placed in aluminum pans and the experiments ran in a calorimeter (Universal V2.3D TA Instruments) at a 10ºC/min heating rate over a temperature range of 25° to 300° C.

Fourier transform-infrared (FT-IR) spectroscopy

The FTIR spectra of pure drug, HP-ß-CD, the drug- cyclodextrins physical mixture (1:1) and the prepared inclusion were recorded using a
Formulation method
The weighed quantity of KF/2-HP-ß-CD complex, preservatives and antioxidant were dissolved in phosphate buffer (pH 7.4). The isotonicity was adjusted by addition of sodium chloride (0.9% w/v) or propylene glycol (2% v/v). The selected formulae were sterilized by filtration using 0.22 μm millipore membrane filters. Bruker FTIR spectrophotometer according to the KBr disc technique. The FTIR measurements were performed in the scanning range of 4000 - 400 cm^{-1} at ambient temperature.

X-ray powder diffractometry (X-ray)
The X-ray diffraction patterns were recorded at room temperature using Scintag XGEN-4000 diffractometer. The samples were irradiated with Ni filtered Cu K_{α} radiation, at a voltage of 45 Kv and a current of 40 mA. The scanning rate employed was 2°/minute over a diffraction angle (2 Θ) range of 3 - 70°.

Table 1. Factorial design for studying the effect of different additives on the eye drops.

Antioxidant (sodium ascorbate)	Isotonicity adjusting agent	Preservatives	
		Methyl paraben 0.2% w/v	(Benzalkonium chloride) 0.02%
0.05%w/v (Low level)	Propylene glycol (2%v/v)	F1*	F5*
	Sodium chloride (0.9 % w/v)	F2*	F6*
0.1%w/v (High level)	Propylene glycol (2%v/v)	F3*	F7*
	Sodium chloride (0.9 % w/v)	F4*	F8*

*: Represents the different formula number.

Effect of different additives on the stability of KF/2- HP-ß-CD complex in eye drops formulations
The effect of different additives including; antioxidants, isotonicity adjusting agents and preservatives on the stability of ketotifen fumerate solution were studied. The experiment

was designed according to 2^3 factorial design, as shown in table 1. The results were statistically analyzed using one way ANOVA at $p<0.05$ (using SPSS® software program).

Table 2. Saturated solubilities of KF in ifferent Solutions.

Vehicle	Saturated Solubility (mg/ml)
Distilled Water	17.471±2.741
pH 4.5 Phosphate Buffer	94.72±8.462
pH 5.5 Phosphate Buffer	85.91 ±4.790
pH 6 Phosphate Buffer	62.54±6.024
pH 6.8 Phosphate Buffer	11.37±1.273
pH 7.4 Phosphate Buffer	4.02±0.926
pH 7.8 Phosphate Buffer	2.91±0.518

Adjustment of the viscosity of the selected formulae

Polyvinyl alcohol (0.25% - 3%)[14] was used as a viscosity-increasing agent [15]. The different concentrations of PVA 1500 were prepared by melting them in the selected formulae using thermostatically controlled magnetic stirrer at 70ºC.

Accelerated stability testing
The sterilized eye drops were aseptically filled in volumetric flasks wrapped in aluminum foil and stored at 40ºC, 50º C and 60ºC in ovens for 13 weeks and examined physically for any changes and chemically using the HPLC stability indicating assay[16]. The HPLC apparatus consists of: Isocratic pump LC-10 AS and a UV/VIS detector SPD-10A connected to a C-R6A Integrator (Shimadzu, Koyoto, Japan). The analytical column was Ponapak C18 HPLC column, 4.6 × 250 I.D mm, particle size 125 ºA (Waters Associates, Ireland). The mobile phase composed of methanol:water in a ratio of 80:20 (V/V) containing triethaylamine (0.2%v/v), with a flow rate of 1 ml. min^{-1}. The system was operated at ambient temperature and the detection wavelength was 296 nm.

The rate constant of decomposition (K) was calculated according to the determined order at each of the three temperatures. The (K) values at different temperatures were plotted against the reciprocal of the corresponding temperature on logarithmic scale according to the Arrhenius plot for the determination of the expiration date[17]

Results & Discussion
Saturated solubility study
Table 2 shows the saturated solubilities of KF in distilled water and in phosphate buffer solutions of pHs (4.5-7.8). The solubility of the drug at acidic pH was significantly higher than in water; this is due to the alkaline nature of the drug as it will be in the ionized form in the acidic medium. Upon increasing pH, the unionized species predominated, which explain the sharp decrease in the solubility at basic pH (7.4 -7.8).

Partition coefficient study (P)
The partition coefficients (P) of KF between n-octanol and phosphate buffer solutions of pH values (4.5-7.8) are shown in table 3. There was an increase in (P) upon increasing the pH with a sharp rise after a pH of 6.0. This was expected at pH values above the pKa of the drug (6.7) where the drug will exist mainly in the unionized form.

Table 3. Partition coefficient of KF in different tested media.

System	Partition Coefficient
Octanol/ Distilled Water	0.676 ±0.0481
Octanol/pH 4.5 buffer	0.112± 0.062
Octanol/pH 5.5 buffer	0.131± 0.0658
Octanol/Ph 6 buffer	0.263±0.0501
Octanol/pH 6.8 buffer	0.752±0.0801
Octanol/pH 7.4 buffer	1.601±0.0316
Octanol/pH 7.8 buffer	1.924±0.0271

Phase solubility studies
The phase solubility diagrams of KF with β-CD and HP-β-CD in phosphate buffer (pH7.4) at 37 ± 0.5 °C are graphically illustrated in figure 1 and the parameters are shown in table 4.
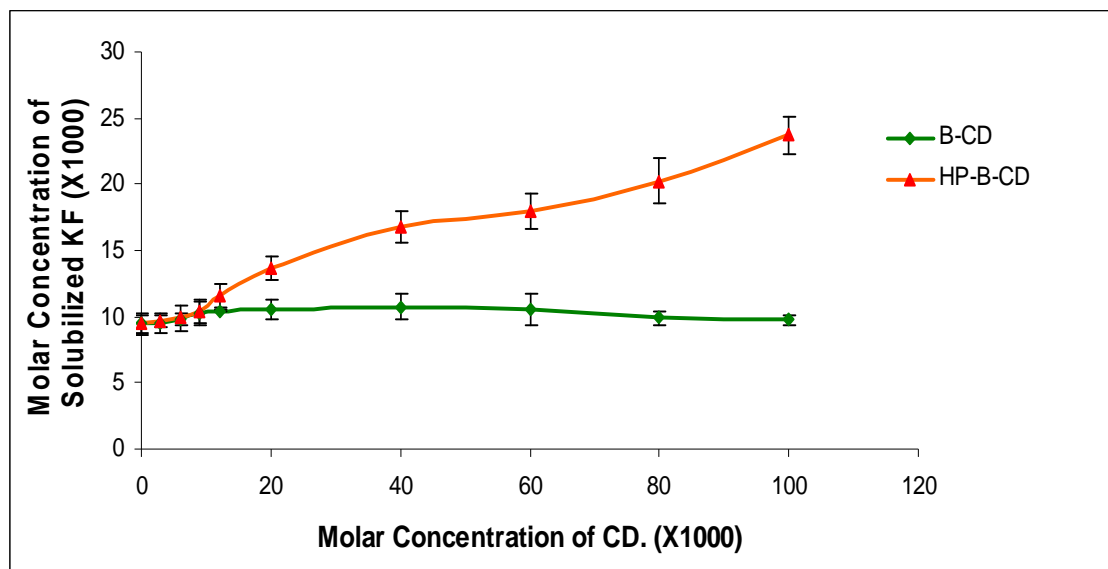
Figure 1. Phase solubility diagrams of KF with different cyclodextrins in phosphate buffer (pH 7.4) at 37 ± 0.5 ºC.

Table 4. **Stability constant ($K_{1:1}$), and the complexation**

Parameter	Inclusion complex of KF with cyclodextrins	
	B-CD	**HP- β-CD**
Complexation medium	phosphate buffer pH (7.4)	Phosphate buffer pH (7.4)
Curve Type	B_S	A_p
S_0 (M)	9.344×10^{-3}	9.4626×10^{-3}
Slope	0.0883	0.224
R2*	0.9128	0.9785
$K_{1:1}$**	10.364	29.474
CE*	0.09685	0.28866

efficiency (CE) of KF/cyclodextrins.
*R^2 (determination coefficient)
**$K_{1:1}$ (stability constant)
***CE (complexation efficiency

The solubility of KF increased linearly with the addition of HP-β-CD. The correlation coefficient value (R^2) was equal to 0.9785 indicating the formation of A_p type curve[18] . On the other hand, the phase solubility diagrams of KF with ß-CD exhibited B_S type[19]. The diagram began with an initial rising part followed by a plateau region. The observed decrease in the amount of KF dissolved could be attributed to the precipitation of microcrystalline complex at higher cyclodextrin concentration. The complexation efficiency (CE) was calculated form equation (2). Since the value of the complexation efficiency (CE) is dependent only on the slope of the phase solubility profile, less variation is usually observed in CE values compared to $K_{1:1}$ value which contains S_o of the drug. The CE values were computed and found to be 0.09685 and 0.28866 for KF/β-CD and KF/HP-β-CD respectively. Therefore KF had higher affinity for HP-β-CD and, thus, was selected for further investigation. The high affinity to HP-β-CD might be due to the hydrophilicity of HP-ßCD, which gave good adjustment of ketotifen fumarate to the cyclodextrin cavity.

KF/cyclodextrins
Characterization of prepared inclusion complex
Differential scanning calorimetry (DSC)
Figure 2. Shows the DSC thermograms of pure KF, (KF/ HP-ßCD), 1:1 physical mixtures and the inclusion complex. It was clear that the sharp endothermic peak of the drug, around 200ºC, became shorter in the thermograph of the physical mixtures which may be attributed to the reduced purity of samples after mixing[20].
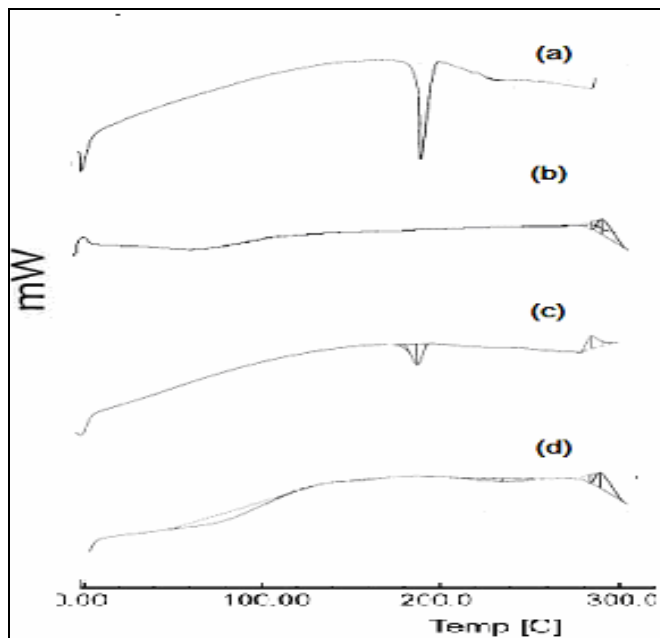
Figure 2. DSC thermograms of KF-H-β-cyclodextrin solid systems.. (a) Pure Ketotifen fumerate; (b) Pure HP-β-CD; (c) Physical mixture and (d) Coprecipitation.

For HP-ß-CD, it was also observed that a peak around 60ºC corresponds to water loss. On the other hand, the characteristic peak of the drug totally disappeared in the complexes thermograph. This result proved formation of inclusion complex characteristic to cyclodextrins. A similar effect was reported by Liu and Zhu [21] for inclusion complexes of prazosin hydrochloride with HP-β-CD.

Fourier-transform infrared spectroscopy
FTIR spectroscopy was used to confirm the interaction between KF and HP-ß-CD as changes or shifts in the drug's absorption spectrum occur upon complexation[22]. The IR spectra are illustrated in figure 2. KF shows its characteristic peaks at 3097.1 cm^{-1} assigned to –CH thiophene stretching, 3000-2840 cm^{-1} assigned to aromatic stretching, 1650 cm^{-1} for carbonyl group, while, the spectra of HP-ß-CD illustrate an intense broad absorption bands at 3500-3300 cm^{-1} corresponding to the free –OH stretching vibration [23].
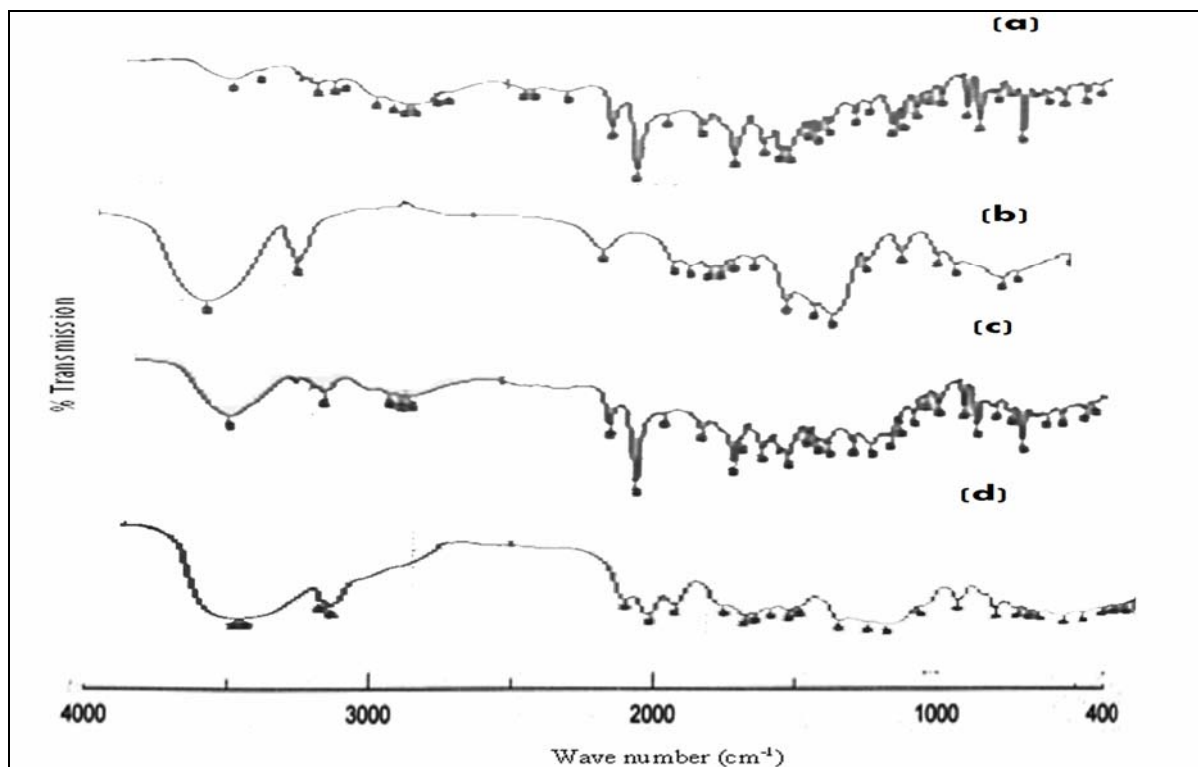

Figure 3. FTIR patterns of KF-H-β-cyclodextrin solid systems. (a) Pure Ketotifen fumerate; (b) Pure HP-β-CD; (c) Physical mixture and (d) Coprecipitation.

The spectra of the physical mixtures were the superposition of pure components spectra, indicating the absence of interaction between KF and HP-ßCD in the physical mixture. This result was in agreement with the work of Fernandes et al [24]. The IR spectra of (KF/ HP-ßCD) complex corresponding to thiophene was strongly stretched, explained by the dissociation of the intermolecular hydrogen bonds associated with crystalline drug molecules. The broadening and the decrease in the intensity of the drug's aromatic stretching band observable in these systems might be due to its restriction within the cyclodextrin cavity [25].
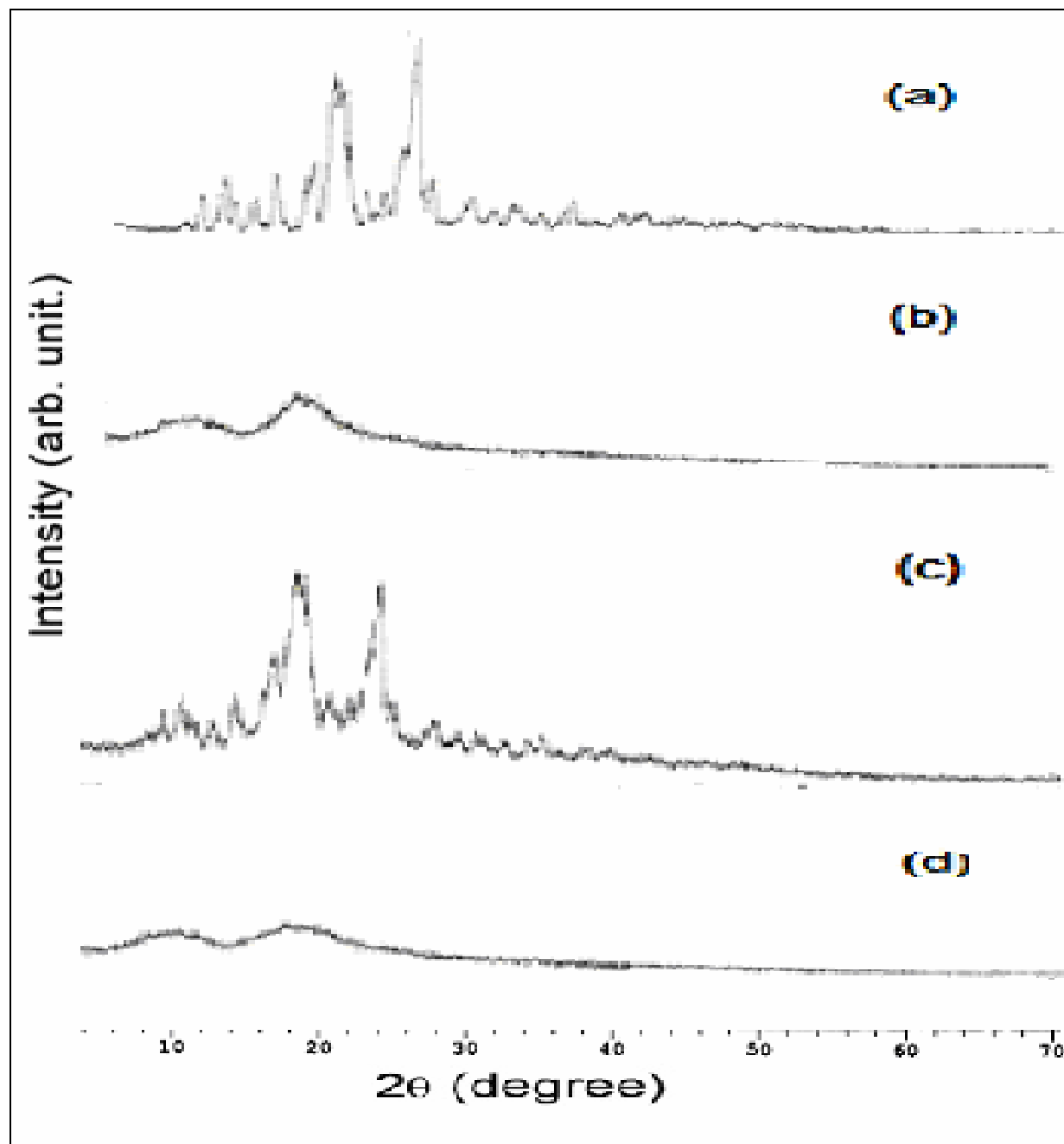

Figure 4. X-ray diffraction patterns of KF-H-β-cyclodextrin solid systems. (a) Pure Ketotifen fumerate; (b) Pure HP-β-CD; (c) Physical mixture and(d) Coprecipitation.
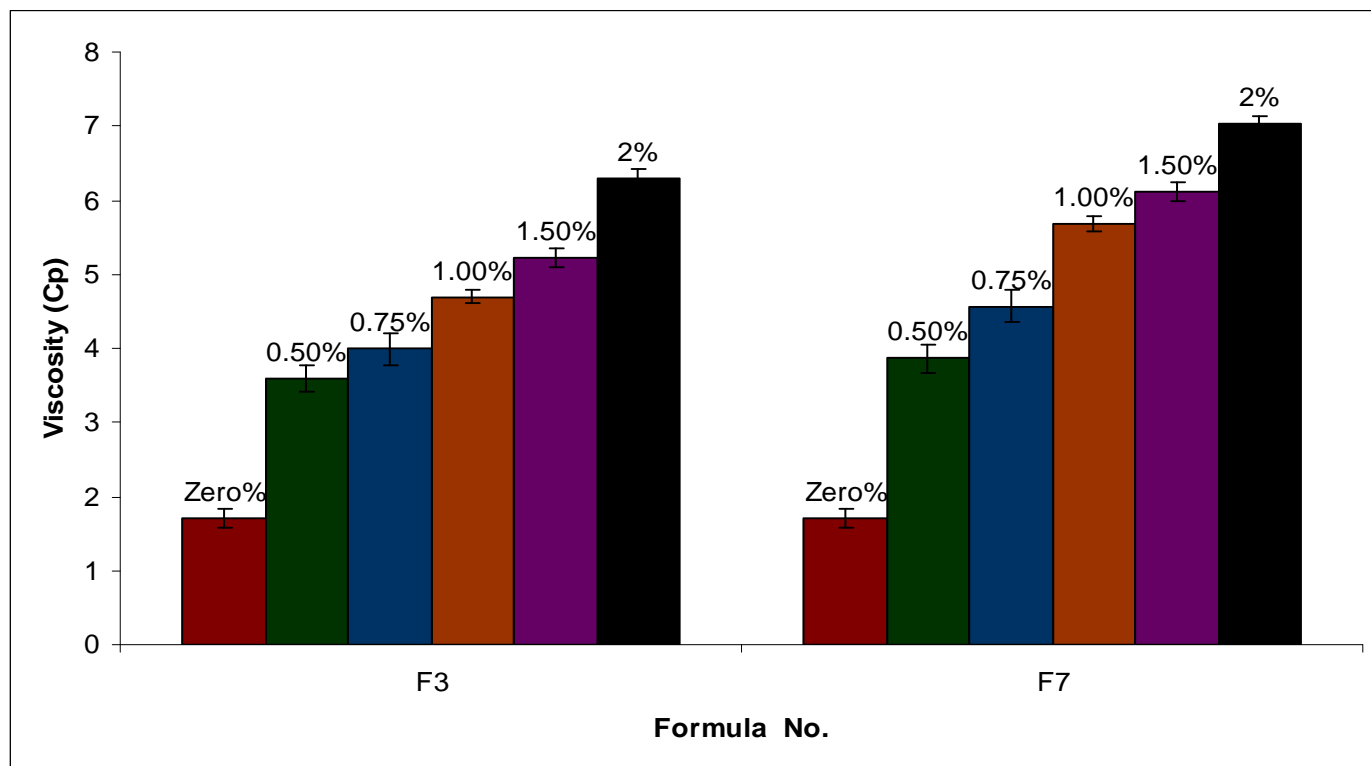
Figure 5. Viscosity of the prepared eye drops (F3 and F7) containing different concentrations of PVA 1500.

X-ray powder diffractometry studies

X-ray diffractograms confirmed the crystalline nature of KF, while HP-ß-CD was presented as an amorphous structure. The diffractogram of the physical mixture could be considered the superposition of the patterns of the pure components with some variations in the shapes and intensities of the characteristic diffraction peaks, (shown in figure 5) while the inclusion complex showed that the crystalline KF pattern disappeared. However, lower intensities of the diffraction peaks were observed due to particle size reduction during mixing and dilution of the pure crystalline components. These results are in strong agreement with the work of Bayomi et al. [26] who studied the inclusion complexation of nifedipine with cyclodextrins using the coprecipitation method.

Stability of the different Formulations

Eight formulations of eye drops were investigated. Table 5 shows the percent of KF remaining after different time intervals. Statistical analysis was preformed to detect the significant differences between the different additives. The results showed the stability of the eye drop formulations (F3, F4, F7 and F8) which contained sodium ascorbate (0.1%w/v) were significantly higher than the formulations (F1, F2, F5 and F6) which contained sodium ascorbate (0.05%w/v) The other different types of isotonicity adjusting agents and preservatives showed no significant difference ($p > 0.05$) with the stability of the eye drops. From these results F3 and F7 were chosen for further tests, due to their greatest stability.

Adjustment of the viscosity of the selected formulae

Figure 5 shows that PVA 1500 in concentration of 1.5% (w/v) was found to have a suitable viscosity with F5 (5.23 cP)[27], while PVA 1500 in concentration of 1.00% (w/v) exhibited a suitable viscosity of all the tested solutions of F7 (5.49cP).

Table 5. **Stability of different eye formulae during the storage at 60°C for 45 days.**

| Formula no.	Percent of KF remaining after storage at 60°C for the following time intervals (in days)							
	0	2	7	14	21	28	36	45
F1	100.4±0.52	99.12 ±1.02	98.33 ±1.25	96.63 ±0.94	94.95 ±0.72	93.24 ±1.05	92.51 ±0.69	91.42 ±0.893
F2	99.73±0.40	97.69 ±0.59	96.21 ±0.47	93.04 ±0.69	91.62 ±0.89	89.39 ±0.74	87.18 ±0.72	84.96 ±0.685
F3	99.64±0.89	99.37 ±0.74	99.05 0.592	98.75 ±0.92	98.18 ±0.46	97.26 ±0.61	96.02 0.980	95.35 ±0.148
F4	99.52±0.81	96.72 ±1.35	95.3 ±1.50	94.27 ±0.95	92.19 ±0.82	91.05 ±1.16	90.49 ±0.79	88.92 0.991±
F5	100.1±0.87	99.03 ±1.12	97.75 ±1.0	96.46 ±0.95	95.87 ±1.24	94.02 ±1.19	93.30 ±0.85	89.83 ±0.926
F6	99.18±0.58	98.78 ±0.73	97.77 ±0.91	95.81 ±0.96	92.06 ±1.14	90.36 ±1.08	88.90 ±1.37	86.94 ±0.981
F7	100.0±0.47	100.1 ±0.85	99.91 ±0.91	99.86 ±1.11	99.75 ±0.87	99.42 ±0.92	98.87 ±0.71	98.17 ±0.126
F8	99.68±0.38	97.01 ±0.69	96.78 ±0.91	94.13 ±1.06	93.24 ±0.72	92.55 ±0.96	91.18 ±1.26	90.08 ±1.380
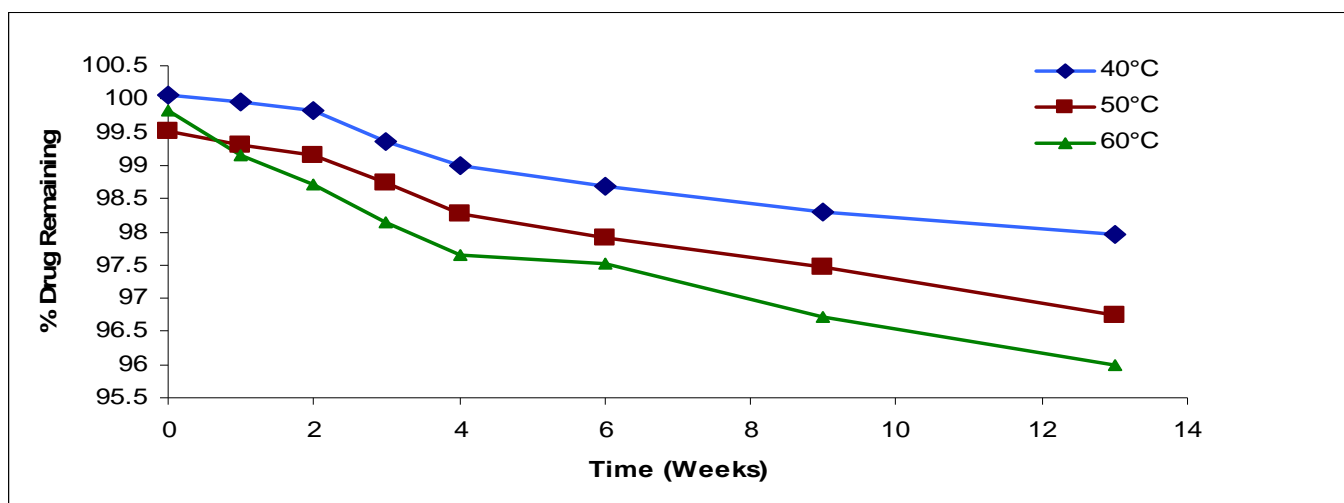
Figure 6. Chemical stability of KF in the tested eye drops of F3.

Sterility testing of the selected eye drops formulations

Sterility testing of the prepared eye drops was performed according to the procedure described by USP[28]. Fluid thioglycolate medium was used to detect both aerobic and anaerobic bacterial contaminants, while Tryptic Soy Broth was used to detect both bacterial and fungal contamination. All the samples tested passed the sterility test, where no turbidity or surface growth was observed during the incubation period of all tested samples.

Physical stability

None of the selected formula solutions stored at the three elevated temperatures for three months

showed any change in color, appearance or clarity throughout the storage period.

Chemical stability

The selected formulae F3 and F7 were subjected to accelerated stability study to clarify the expiration date of the formula under normal storage conditions in comparison with the commercially available Zaditen® eye drops. The results of the accelerated stability testing of the drug in the prepared eye drops were presented in

figure 7, 8 and 9. All the tested formulae were within the range permitted by the USP (90-120%).

Kinetic analysis of the stability data reveals that KF degradation followed first-order kinetics. The K values (decomposition rate constant) of the prepared formula at different temperatures were plotted graphically on a logarithmic scale, as illustrated in figure 9.
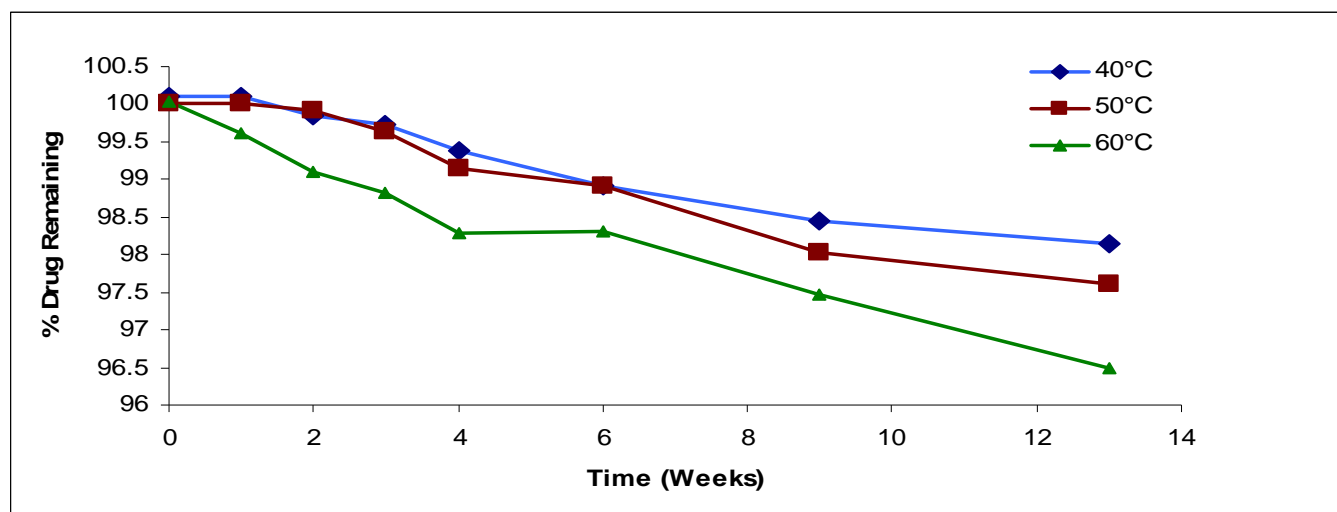

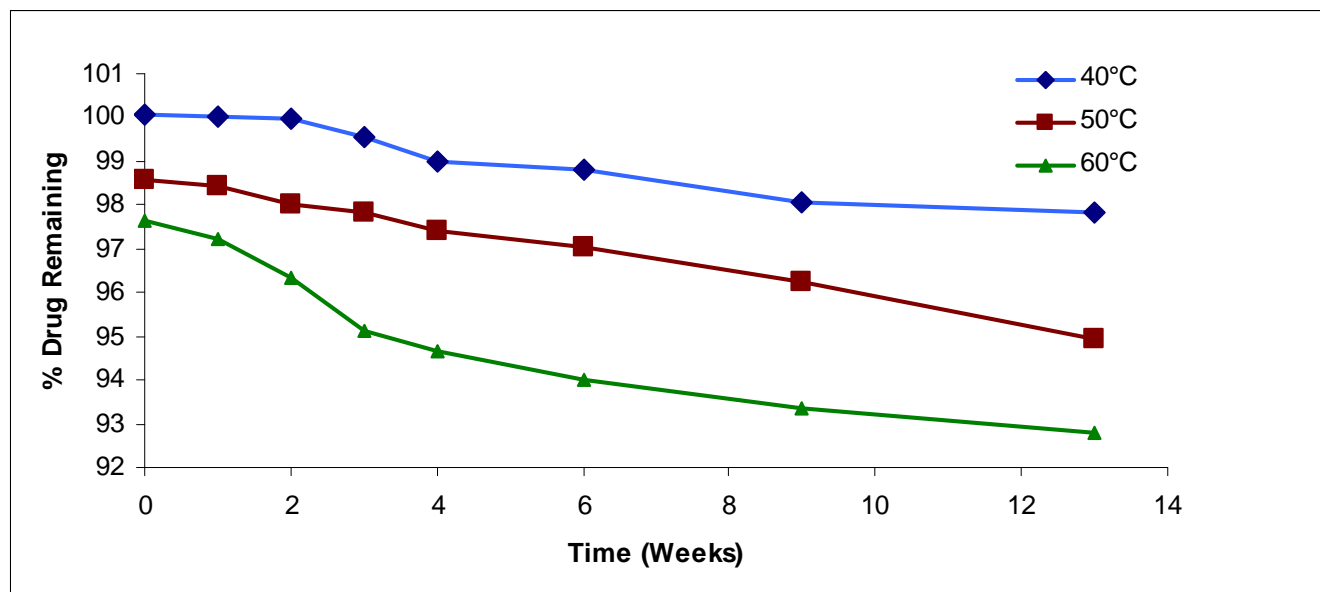**Figure7. Chemical stability of KF in the tested eye drops of F7.**

Figure 8. Chemical stability of KF in the tested eye drops of Zaditen® eye drops.
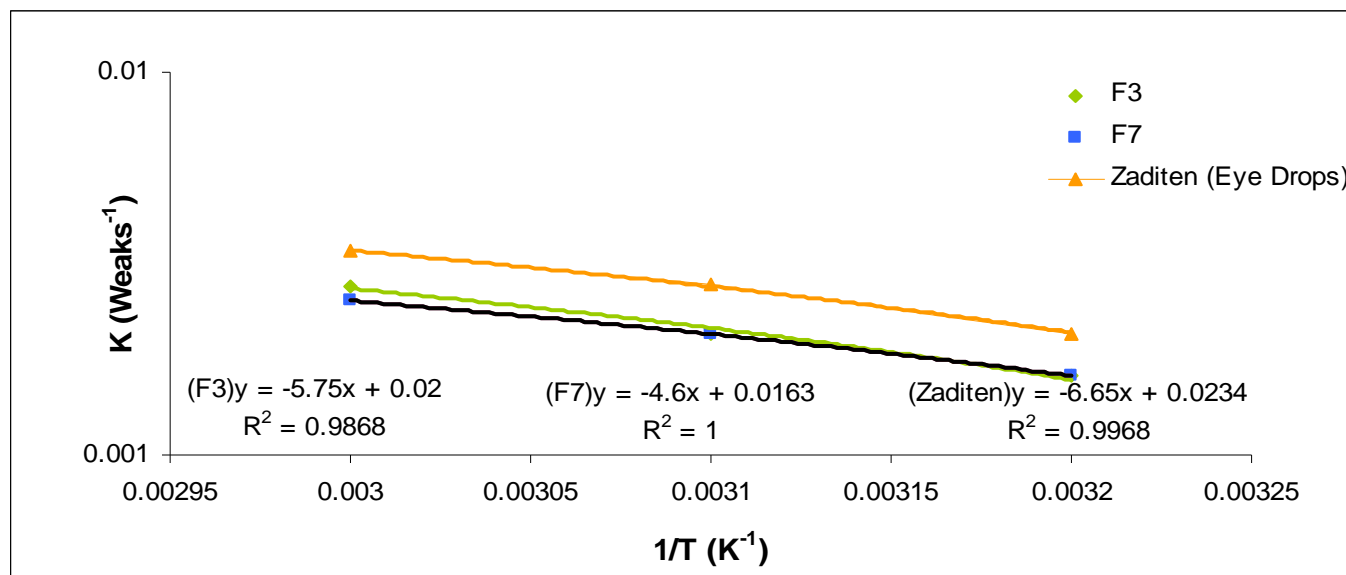
Figure 9. A Plot of K Values of KF eye drops formulations F3, F7 and the commercial available Zaditen® after three months of storage on logarithmic scale.

The predictive shelf life was calculated [4], according to the equation (3).

$$t_{90} = 0.105 / K_{25} \qquad (3)$$

Where t_{90} is the time at which the percent drug remained is 90%. The tested formula F3, F7 and Zaditor eye drops possessed a predictive shelf life of 2.89, 2.52 and 1.85 years respectively, as shown in table 6.

Table 6. Kinetic data of accelerated stability testing of KF prepared eye drops

Formula no.	K Values at the Following Temperatures (°K) on Logarithmic Scale			K_{25} (weeks^{-1})	$t_{1/2}$ at 25°C (years)	t_{90} at 25°C (years)
	313 °K	323 °K	333 °K			
F3	0.00161	0.00207	0.00276	0.00070	19.038	2.885
F7	0.0016	0.00207	0.00253	0.00082	16.659	2.524
Zaditen® E.D	0.0021	0.0028	0.0034	0.00108	12.339	1.8457

Conclusions

This study demonstrated that the inclusion complex of KF-HP-ßCD in eye drop preparations is a promising formulation resulting in enhancing the solubility, permeability and stability of KF eye drops prepared at the physiological pH.

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