The Potential Use of Atorvastatin Calcium/Hydroxypropyl-β-Cyclodextrin complex loaded Hydrogel as an Ocular Delivery System.

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Abstract: **Objectives:** The aim was to prepare and evaluate ophthalmic hydrogels containing atorvastatin calcium/hydroxypropyl-β-cyclodextrin complex (ATSCa/HP-β-CyD). ATS used for atherosclerosis, was proved to have therapeutic effects on intraocular inflammation and uveoretinitis but its poor water solubility hindered its ocular application. **Methods:** First, complexes were prepared and characterized by different techniques. Different polymers (sodium alginate, carbopol934, and methylcellulose) were selected to formulate hydrogels containing either free or complexed drug. Physical and in-vitro release characteristics were studied. **Key findings:** The complexes showed improved dissolution rates compared to untreated drug; highest dissolution rate was observed in co-solvent and common solvent methods. Hydrogels were, clear with acceptable pH and drug content except sodium alginate was slight turbid. The results showed that there was increase in the drug percent released from carbopol934 or methylcellulose loaded with the complexed drug compared to the corresponding ones with free drug. On the other hand, percentages released from sodium alginate gels were decreased by complexation. **Conclusions:** Ophthalmic carbopol934 gels containing ATS Ca/HP-β-CyD complex are considered promising delivery systems with the highest in-vitro drug release profile.

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Keywords: Atorvastatin calcium, HP-β-CyD, ophthalmic hydrogels, polymer.

1. Introduction

Eye is considered one of the most sensitive organs in human body. Treatment of different ocular diseases is still a great challenge in pharmaceutical sciences. Uveitis is inflammation of the uvea, which the form eye middle layer that consists of the iris, ciliary body and choroid. According to the inflamed tissue, there are different types as anterior, intermediate, posterior, or diffuse uveitis. Symptoms of eyelids inflammation include crusting, swelling, redness, and itching. Uveitis is chronic disease and can produce numerous dangerous complications, such as clouding of the cornea, cataracts, glaucoma, retinal detachment. Also these complications may lead to permanent vision loss.

At the present time, ophthalmic anti-inflammatory steroids have serious side effects and some of them may take months to exert an effect. Generally, their anti-inflammatory action is associated with a significant local intolerance risk and allergy. Furthermore, antibiotic usage such as oral tetracyclines can converse drug-resistance and have severe systemic side effects and in turn restricting the duration of use of this medication. Hence, it is urgently needed to use other alternative therapies.

Basic actions of statins are their capability to lower cholesterol and reduce inflammation. So that, they are used as medications for the controlling of hyper-cholesterolaemia as well as the prevention of coronary artery disease and stroke. They selectively work by inhibiting a coenzyme called3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which is an integral protein of endoplasmic reticulum membranes and the rate-controlling enzyme of the sole metabolic pathway for cholesterol biosynthesis (1).

Among statins, atorvastatin calcium (ATS Ca) is a synthetic monocarboxylic lipid-lowering agent with a pKa of 4.46. Furthermore, ATS lipophilicity facilitates its passive diffusion across the phospholipid bilayer of cell plasma membranes. It is currently used as calcium salt for the treatment of hypercholesterolemia. ATS Ca is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and pH 7.4 phosphate buffer. For improving the drug solubility and dissolution rate, complexation with different derivatives of cyclodextrins was achieved using various techniques (2).

Away from its lipid-lowering action, it was found that topical application of ATS showed promising safe novel tear film stabilization and anti-inflammatory action. It was reported that topical ATS effectively targets T cells through an inhibition of proliferation and a reduction in T helper cell 1-induced inflammatory cytokines (3). ATS may also be able to restore the corneal and conjunctival epithelial barrier structure and function. It therefore potentially have an ocular anti-

inflammatory effects such as in case of local ocular surface blepharitis, Sjögren's syndrome, or auto-immune conditions such as ocular rosacea, atopic keratoconjunctivitis and episcleritis (1).

Hydrogels are smart drug delivery system due to their bio adhesive soft characters with high water content and permeability. For the specific hydrogels network constructions, they have been reported to retain the poorly water-soluble drugs within their network-structure improving their solubility and bioavailability (2).

The objective of the study was to develop anti-inflammatory hydrogel of ATS Ca with improved solubility and dissolution rate. To fulfil this target, inclusions complexes of ATS Ca and HP-β-CyD using different methods were prepared. The most promising complex was incorporated into hydrogels formulated using different types of bioadhesive polymers.

2. Materials and Methods

Materials:

ATS Ca was obtained from EPICO Co., Egypt. HP-β-CyD was purchased from Nippon Shokuhin Kako Co., Tokyo, Japan. Methanol, propylene glycol, sodium chloride, calcium chloride dihydrate, sodium bicarbonate, all of analytical reagents grade were supplied by Adwic, EL Nasr Pharmaceutical chemicals, Co., Egypt. Carbopol 934, sodium alginate, methylcellulose were purchased from B.D.H. Chemicals Ltd, GB, Liverpool - England. Cellulose membrane, Spectrapore, MW Cutoff: 12000-14000 (Fisher Sci. Co., Pittsburgh, USA). Millipore filter with 0.45μm pore size (Berlin, Germany).

Methodology:

Phase solubility diagram studies

Phase solubility diagram was performed according to the method applied by Higuchi and Connors (4). An excess amounts of ATS Ca nearly 50 mg were added to test tubes containing increasing serial concentrations of HP-β-CyD aqueous solution from1 to 10 mM. The tubes were stirred for 7 days at 25 ºC until equilibrium then samples were withdrawn, filtered using Millipore filter (0.45 μ) and measured after suitable dilution spectrophotometerically at 242 nm. The phase solubility diagram was drawn, then the K value which is stability constant was calculated from the initial straight-line of the curve using the equation.

$$K_{1:1} = \frac{\text{Slope}}{\text{Intercept}\,(1 - \text{Slope})}$$

Preparation of ATS Ca / HP-β-Cy D inclusion complexes

Different methods were used to prepare ATS complex (5).

1-Physical mixing method:

ATS Ca and HP-β-CyD in equimolar proportion (1:1 molar concentrations) were mixed in a mortar for one hour without applying any pressure.

2-Kneading method:

ATS Ca and HP-β-CyD mixture in the molar ratio of 1:1 were kneaded in a mortar for at least one hour with small quantities of methanol / distilled water solvent mixture in ratio 1:1 v/v. The formed paste was dried at 45˚C for 24 hours by using an oven. Dried complex was pulverized into fine powder and sifted using sieve number 80.

3- Co- Solvent evaporation method:

ATS Ca, HP-β-CyD were dissolved in pure methanol and distilled water respectively until clear solutions were attained. Both solutions were mixed at constant stirring on magnetic stirrer. The resulting solution was evaporated at 45˚C for 24 hours. Dried drug complex was pulverized into fine powder and sifted with sieve number 80.

4- Common Solvent evaporation Method:

ATS Ca and HP-β-CyD in equimolar ratio of 1:1 were dissolved in extra pure methanol as common solvent to get a clear solution. The prepared solution was allowed to evaporate overnight at room temperature. The collected complex was pulverized and passed through sieve number 80.

Characterization of ATS Ca inclusion complexes:

Differential Scanning Calorimetry (DSC)

The thermal behavior of drug, HP-β-CyD, PM and the complex was studied in order to confirm the formation of complex. Thermal analysis was carried out by using Differential scanning calorimeter, Pyris 6 DSC, Perkin Elmer, USA. The samples were heated from 20 -300˚C at 10 ˚C heating rate.

Scanning Electron Microscopy (SEM)

The surface morphology of ATS Ca was determined using scanning electron microscope (JSM-6510LV, JEOL, Japan)

Fourier Transform Infra-red Spectrophotometer (FT-IR)

Pure ATS Ca, HP-β-CyD, PM and complexes were subjected to IR studies to check whether any interaction occurred between pure ATS Ca drug and employed ingredients. This was performed by using FT-IR, Thermo Fisher Scientific, Inc., Waltham, MA, USA.

Percentage yield

The efficiency of the different methods used for ATS Ca/HP-β-CyD complexation is determined by calculating the yield % using following equation.

$$\text{The yield \%} = \frac{\text{Practical yield}}{\text{Theoritical yield (drug} + \text{HP} - \beta - \text{CyD)}} * 100$$

Drug content

The concentration of drug present in 10 mg equivalent amount of complex powder was determined

by dissolving the powder mixture in 10 ml of methanol, then suitable dilution with methanol and measurement at 242 nm by UV spectrophotometer. Drug concentration was determined from predetermined standard calibration curve of the ATS Ca.

Dissolution studies

Dissolution studies on the pure drug, PM, and the inclusion complexes prepared by different methods were performed using 500 ml simulated tear fluid (pH 7.4) as a dissolution medium. The experiment was performed in USP six station dissolution test apparatus (Dissolution apparatus, Labo America, Abbotta High Performance of 8 Cup System, USA) with a paddle stirrer. Powdered samples of each preparation equivalent to 10 mg of ATS Ca were placed in the dissolution medium. The experiment was performed at50 rpm, and 37ºC ± 0.5ºC. Samples of 3 ml were taken and analyzed spectrophotometerically at different predetermined time intervals.

Formulation of ATS Ca ocular hydrogel

Different polymers were selected to prepare the gels for ophthalmic application such as sodium alginate, carbopol 934, and methylcellulose as indicated in table (1).

For each formulae, three preparations were prepared by incorporating free ATS Ca **(F1A, F2A, F3A**) or ATS Ca/ HP-β-CyDPM **(F1B, F2B, F3B**) or ATS Ca/ HP-β-CyD complex **(F1C, F2C, F3C**).

The gels were prepared simply by dispersion of the selected polymers in the recommended concentrations in double distilled water. The different dispersions were mixed until clear transparent gels free from air bubbles were obtained. The plain gels were kept overnight at the refrigerator. In order to prepare the medicated ophthalmic gels, ATS Ca 0.5% (W/W) or its equivalent weight of ATS Ca/ HP-β-CyD PM or complex was dissolved in propylene glycol and added to the previous mixture drop by drop by stirring till complete dispersion. The weights of gels were adjusted to final prepared weight, and then packaged in clean, dry and sterile glass containers until use.

Evaluation of prepared hydrogels

Physical evaluation

All the prepared formulations were inspected for color, clarity, and transparency.

Measurement of pH

The pH was measured with the help of pH meter (Consort, Belgium, Model P901-pH/mV/c meter). One gram of each formula was dissolved or dispersed in 30 ml of distilled water, and the pH was measured using pH-meter (6).

The pH of ophthalmic formulation should not be irritation to the patient upon instillation into the eye (7).

Drug content

Drug content of each ophthalmic gel was determined by dissolving an accurately weighed quantity of formulation (1g) in 50 ml of methanol in tightly closed 100 ml volumetric flask. The solution was stirred for at least 2 hours then filtered by using 0.45 m membrane filter and measured using spectrophotometer at 242 nm.

Measurement of viscosity

Viscosity of each formulation is very important factor in determining residence time of drug in the eye. The viscosity of the prepared formulations was measured at 37 ± 0.5ºusingrotary viscometer which has been calibrated before its application (Rotary viscometer, Haake Inc., Germany). One gram the formulation was placed on the plate of viscometer (diameter of 2.9 cm) and cone (diameter of 2.8 cm). The torque value "S" was determined for each "N" value (speed), viscosity is calculated using the following equation:

$$Y = \frac{G.S}{N} (mpa.S)$$

Where; Y: Viscosity in mpa.s (mpa.s = 1 centipoise)

G: Instrumental factor (14200 mpa.s/scalagrad. min)

S: Torque (scale grad.)

N: Speed (rpm)

In vitro drug release from gel formulations.

The release of ATS Ca from ophthalmic formulae in freshly prepared simulated tear fluid (STF) of pH 7.4 was carried out according to the method adopted by Levy and Benita (8). The in vitro release of the drug from different formulations was performed using modified diffusion cell. Semipermeable cellophane membrane which was previously soaked in STF overnight was stretched over the open end of a tube. One gram of ophthalmic gel was accurately weighed and spread on the membrane. The tubes were then immersed upside-down in a 100 ml beaker containing 50 ml STF of pH 7.4 which is preheated and maintained at 37 ± 0.5ºC using thermostatically controlled incubator system (GFL Germany Shaking Incubator Type 3033 with orbital motion incubator). All beakers were shaken at 100 rpm for 24 hours. Samples of 1 ml were withdrawn at predetermined time intervals of 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 minutes and suitably diluted with STF. The dissolution medium was kept constant in volume by replacing sample withdrawn by the same volume of fresh STF. The released amounts of the drug from each formula were analyzed spectrophotometrically at 242 nm. The experiments were done three times and the average was recorded.

Kinetics of ATS release

In order to determine the drug release kinetics, the in vitro release data were analyzed according to different kinetic models, Zero-order kinetics, First-order kinetics and Higuchi diffusion mechanism (Fickian and non Fickian) (9).

3. Results and Discussion

Phase solubility diagram

The phase solubility diagram of ATS Ca is indicated in Fig 1. The obtained results revealed that, ATS Ca solubility increases by increase HP-β-CyD concentration giving phase solubility diagram of A_L type. The stoichiometry constant $K_{1:1}$ was calculated from the initial straight line of phase solubility diagram, it was found to be 3666.667 M^{-1} (10).

Characterization of ATS Ca inclusion complexes:

Differential Scanning Calorimetry (DSC)

DSC thermograms of ATS Ca, HP-β-CyD PM, and complexes prepared by different three methods are shown in Fig. (2). The DSC thermogram of ATS Ca showed a sharp endothermic peak at about 157°C corresponding to its melting point. It was observed that, the DSC curve of HP-β-CyD alone showed a broad endothermic peak, between 38 °C and 118 °C, which attained a maximum at 86 °C which might be corresponding to dehydration process of cyclodextrin and water release. This finding was in accordance with the results obtained by Lv et al. (11).

The thermograms of ATS Ca with HP-β-CyD (1:1 M) prepared either by physical mixing method or kneading method showed broad band due to the HP-β-CyD dehydration, while the sharp endothermic drug peak was decreased in its intensity but not completely disappeared, this may be due to partial inclusion of drug with cyclodextrin by the physical mixing as well as the kneading technique. On the other hand, there was a very little peak corresponding to the drug was observed in case of ATS Ca with HP-β-CyD complexes prepared either by co solvent evaporation or common solvent evaporation. This may be explained by the nearly complete inclusion of the drug inside the cyclodextrin cavity by these two methods (12).

Scanning Electron Microscopy (SEM)

The SEM is used to examine the microscopic aspects of the drug as well as the ATS Ca /HP-β-CyD complexes. Fig (3) shows SEM microphotographes of ATS Ca, HP-β-CyD, complexes prepared by PM, kneading, co-solvent and common solvent complex. ATS Ca existed in plate-like crystal, whereas HP-β-CyD was observed as amorphous. Regarding drug and HP-β-CyD physical mixture, the characteristic crystals of both drug and HP-β-CyD were observed. On the other hand, the ATS Ca/HP-β-CyD complex appeared in the form of irregular particles in which the original morphology of their components did not appear but tiny aggregates of irregular sized amorphous particles were present. The comparison of these images indicated that, the inclusion complex was structurally different from the isolated pure components and the PM of ATS and HP-β-CyD. The sizes and shapes of particles of complex were different from those of the drug or HP-β-CyD alone; this confirmed the formation of the inclusion complex of ATS Ca and HP-β-CyD by using the different technique (13).

Fourier Transform Infrared Spectrophotometry (FT-IR)

All pure ATS Ca, HP-β-CyD, PM and complexes were subjected to FT-IR to check whether interaction occurs between pure ATS and HP-β-CyD (14). The FTIR spectra are indicated in Fig. (4). The characteristic peaks of HP-β-CyD (Fig. 4-b) showed prominent absorption bands at 3418 cm^{-1} for O–H stretching vibrations, 2930 cm-1 for C–H stretching vibrations and 1154, 1085 and 1036 cm^{-1} for C–H, C–O stretching vibration (15). In addition, for pure ATS Ca, the absorption observed at 3408 cm^{-1} and between 1700–1400 cm^{-1} could be due to the stretching vibrations of the N- H and the C- C bond of the aromatic ring. The peaks observed in this spectrum at 1400–1100 cm^{-1} reflects vibrations of C- O, C- F groups and 690–850 cm^{-1} reflects C-H bond bending vibrations of the benzene ring. It can be seen that the FTIR spectra of the PM and the inclusion complexes prepared by different methods varied with that pure ATS, for the three absorption bands (1700–1400 cm^{-1}, 1400–1100 cm^{-1} and 690–850 cm^{-1}). In addition, the FT-IR spectra of the ATS Ca /HP-β-CyD complexes show no peaks similar to the drug alone. This can be probably due to the inclusion complexation of drug into the HP-β-CyD cavity.

Percentage yield

The percent yield for all preparations were calculated and shown in table (II). The maximum % yield was found with physical mixing method followed by common solvent method, co-evaporation method and then kneading method with the lowest percent.

Drug content:

The percentages of drug content present in the prepared complexes are mentioned in table (2). The drug content of various products ranges from 101.1%to 103%. Thus the drug content was found to be within the accepted range.

Dissolution studies

The drug dissolution rate of untreated ATS Ca and that with HP-β-CyD in different preparations indicated in Fig. (5). As a general trend, there was an increase in the ATS Ca dissolution rate from the HP-β-CyD PM and complex compared to the untreated drug. This may be due to the increase of the drug solubility by using HP-β-CyD. The untreated drug showed a low water dissolution profile due to its low water solubility. From the curve, it was found that, the percent dissolved of ATS Ca alone after 120 minutes was 54.8%. While drug dissolution rate was improved by physical mixing or kneading with HP-β-CyD. It showed an increase in the percent dissolved after 120 minutes to be 76.2% 74.2% respectively. This may be due to the partial inclusion of drug in the cavity of HP-β-CyD by physical mixing or

kneading technique. This was confirmed by DSC results. These results were in agreement with Badr-Eldin et al. **(16),** who proved that, the dissolution rate of tadalafil (a poor water soluble drug) was enhanced when mixed with HP-β-CyD due to the in-situ inclusion process between the drug and HP-β-CyD.

In case of ATS Ca/HP-β-CyD complexes prepared by both co-solvent and common solvent techniques, it was observed that, there was a high increase in the drug dissolution rate to be 93.2% after 120 minutes. Moreover, after 180 minutes, the percent of drug release from the pure drug of ATS Ca exhibited only 54.8%. While that from all inclusion complexes was found in the range from 74.2% (physical mixing or kneading) to 101.7% (co solvent and common solvent techniques). The rate of dissolution is higher in complexes prepared with co-solvent and common solvent evaporation methods compared with PM or kneading method as shown in the Fig. (5). This may be attributed mainly to complete inclusion of the drug in HP-β-CyD cavity by these technique which was confirmed before by DSC, FT-IR results (17).

Evaluation of prepared ophthalmic hydrogels

Physical evaluation

The obtained results are illustrated in table (3). It was observed that, the prepared ocular gels were colourless or pale yellow in colour and with good clarity. In addition, the pH values of all formulations were within the acceptable range (4-9) which the eye can tolerate, without any irritation (18). Finally the viscosity values and drug contents of all formulations were found to be within the acceptable range.

In-vitro drug release from gel formulations

Percentages released of ATS Ca from sodium alginate gels through semipermeable cellophane membrane were shown in Fig. (6). Hydrophilic membranes were used to simulate the release towards a hydrophilic surface (e.g. ocular mucosa). The percentage of ATS Ca released were 62.74, 76.12 and 21.55% for F1A, F1B and F1C respectively. It was found that the percent released of ATS Ca from F1B gel was the greatest followed by F1A which was higher than F1C. It was observed that the release profiles were linear as a function of time in all the tested formulations. Hence, atorvastatin diffusion was not restricted by the capacity of the membranes where ATS Ca molecules diffuse by passive diffusion. However, HP-β-CyD has a great influence on drug solubility. As complexation increases the molecular weight of 3-25 folds, the diffusivity of drug molecules within a polymer matrix may also be reduced(19). Therefore, diffusion of the drug complex is not possible and retardation of drug release may occur.

Moreover, the reduced release of atorvastatin from F1C gel could be attributed to that the solubility study of atorvastatin in HP-β-CyD solutions was performed without alginate and the formed inclusion complexes might be dependent on the solvent only. In case of F1C gel, the system becomes even more complicated by addition of macromolecules (alginate). Consequently, it is challenging to predict the stoichiometry of ATS Ca/HP-β-CyD complexes in the actual formulations which contain alginate in addition to HP- β-CyD. Some studies suggested that ATS Ca/ HP-β-CyD complexes can self-associate to form additional water-soluble non-inclusion complexes (20).

In addition, HP-β-CyD are known to form a complex with the polymers used for hydrogel formulations. The presence of polymers capable of being included within the HP-β-CyD cavity and hindering the ability of HP-β-CyD to complex with suitable drugs (19).

Filipović-Grčić et al. (21) also proved that drug release from polymer matrices containing drug inclusion complexes may be retarded. They observed a reduced release of the poorly water-soluble nifedipine complexed with HP-β-CyD in the form of chitosan microspheres, compared to those contained free drug. This reduction occurred regardless a near two fold increase in the aqueous solubility of nifedipine in the presence of HP-β-CyD. It was suggested that during the release studies, the drug rapidly dissociated from the complex, resulting in great increase of HP-β-CyD concentration within the polymer matrix. A more hydrophilic, polymer: HP-β-CyD matrix was created which in turn decreased permeability and slowed the release of drug.

On the other hand, percentages released of ATS Ca from carbopol 934 gels through cellophane membrane were shown in Fig. (7). It was found that the cumulative percent ATS Ca increased in case of gel containing the drug HP-β-CyD physical mix and complex compared with that containing the free drug. There was a significant high increase in cumulative % drug released from 15.33% (in case of F2A) to 45.4%, 50.5% (in case of F2B, F2C respectively). It is clear that the incorporation of cyclodextrins into polymeric drug delivery systems has a great effect on drug release mechanism. Physically mixed or complexed cyclodextrins/drug for example can modify drug solubility, diffusivity, and hence improve hydration of the polymer matrix and promote its erosion.

This is in accordance with Guo and Cooklock (22) who proved that cyclodextrins have the potential to enhance drug release from polymeric systems by increasing the concentration of diffusible species within the matrix. In addition, the improved of drug release is due to the ability of the incorporated cyclodextrin to enhance the aqueous solubility of drug, that was previously proved by dissolution results of solid complex compared with that of pure drug.

In addition, in case of preparations which containing methylcellulose polymer (F3A, F3B, F3C), the results indicated by Fig. (8) Showing an increase in cumulative % drug released in the order F3C > F3B > F3A. This also emphasis the fact that incorporation of cyclodextrins have the ability to enhance ATS Ca release from these polymeric systems. Various hydrophilic cyclodextrins are useful to attain immediate release formulations. They have been used extensively to enhance the bioavailability of poorly water soluble drug (23).

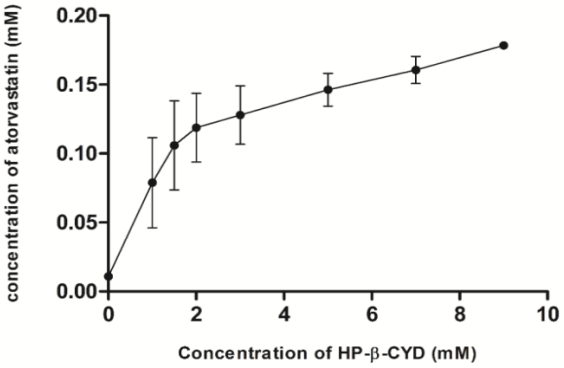

Fig (1): Phase solubility diagram of atorvastatin calcium.

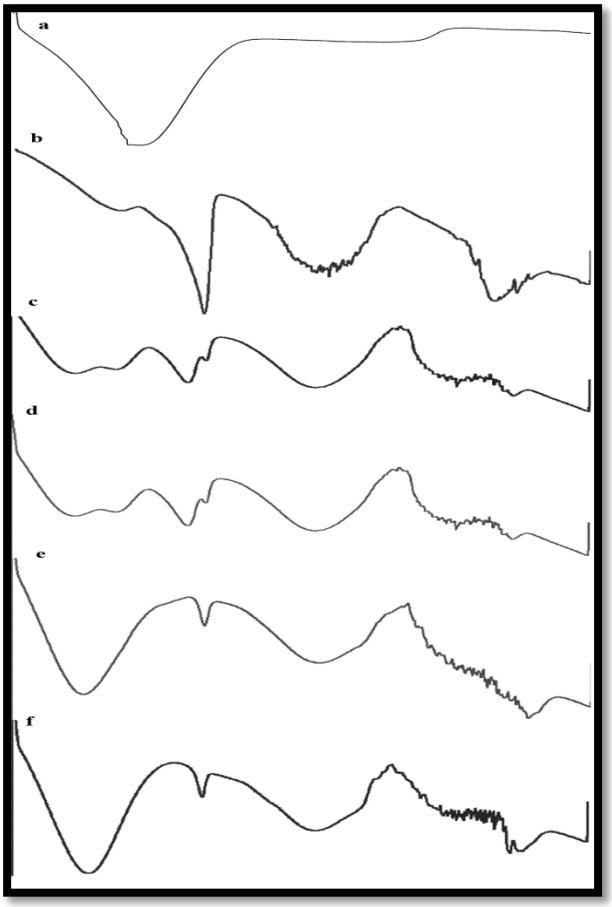

Fig (2): DSC thermograms of HP-β-CyD (a), ATS Ca (b), physical mixture (c), complexes prepared by kneading (d), co-solvent (e) and common solvent complex (f).

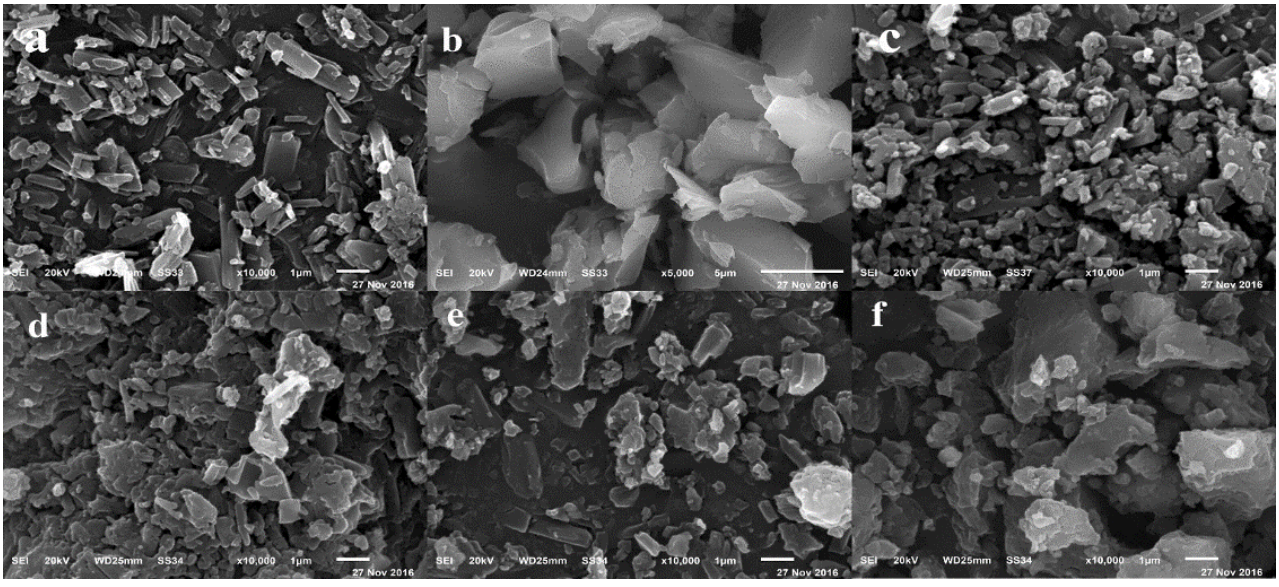

Fig (3): SEM microphotographs of ATS Ca (a), HP-β-CyD (b), physical mixture (c), complexes prepared by kneading (d), co-solvent (e) and common solvent complex (f).

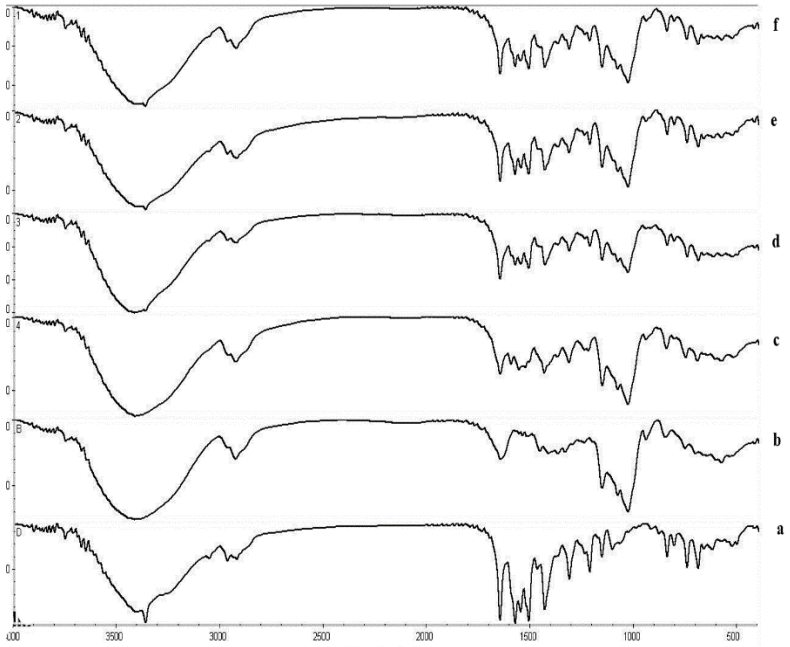

Fig (4): FT-IR spectra of ATS Ca (a), HP-β-CyD (b), physical mixture (c), complexes prepared by kneading (d), co-solvent (e), common solvent complex (f).

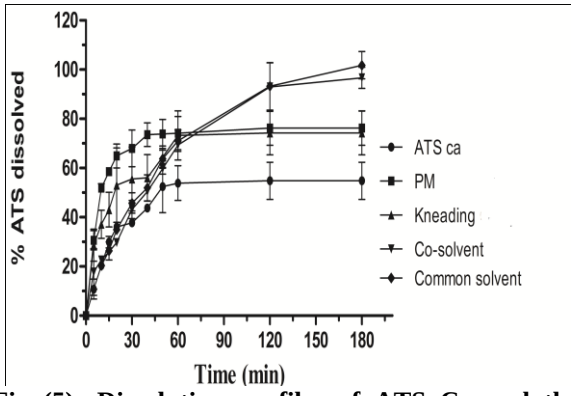

Fig (5): Dissolution profiles of ATS Ca and the prepared complexes in STF.

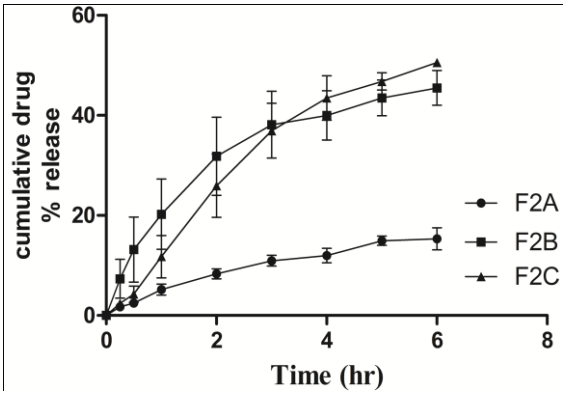

Fig (7): In vitro release profiles of ATS Ca from carbopol 934 gel in STF.

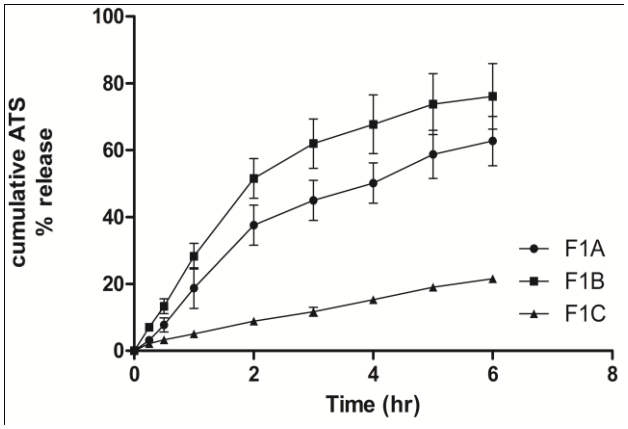

Fig (6): In vitro release profiles of ATS Ca from sodium alginate gel in STF.

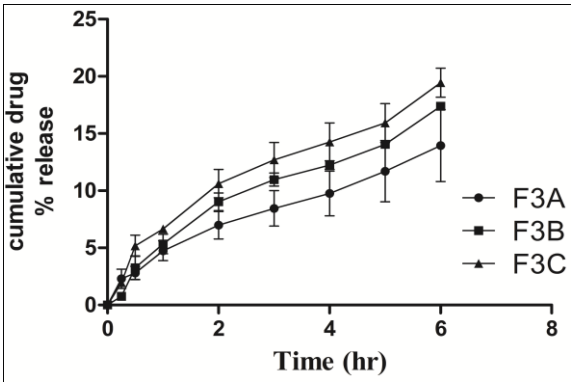

Fig (8): In vitro release profiles of ATS Ca from methyl cellulose gel in STF

Table (1): Compositions of ATS Ca ophthalmic gels

Ingredients	Formulations								
	F1A	F1B	F1C	F2A	F2B	F2C	F3A	F3B	F3C
ATS Ca	0.5 %	---	---	0.5 %	----	----	0.5 %	----	----
ATS/HP-β-CyD PM equivalent to ATS	----	0.5 %	---	---	0.5 %	----	----	0.5 %	----
ATS/HP-β-CyD complex equivalent to ATS	----	---	0.5 %	---	---	0.5 %	----	----	0.5 %
Sodium alginate	5%	5%	5%	----	---	---	----	----	----
Carbopol 934	----	----	--	1%	1%	1%	----	----	----
Methyl cellulose	----	-----	---	---	----	----	0.5 %	0.5 %	0.5 %
Sodium chloride	-----	----	---	---	----	----	5 %	5 %	5 %
Propylene glycol	20%								
Distilled water	QS								

Table (2): Percentage yield and drug content of prepared complexes by different methods

Preparation	Theoretical yield	Practical yield	% yield	drug content percent
Physical Mixing	908	900	99.011	103
Kneading	908	650	71.59	101.1
Co-evaporation	908	670	73.79	101.1
Common solvent method	908	680	74.89	101.1

Table (3): Physical characters of ophthalmic gels

Formula	Colour	Clarity	pH	Viscosity (mpa.s)	drug content percent
F1A	pale yellow	clear	5.6	1012.5	79.28%
F1B	pale yellow	translucent	5.48	900	83.40%
F1C	pale yellow	translucent	5.53	1125	92.72%
F2A	colourless	clear	4.65	1181.25	100.6%
F2B	colourless	clear	4.75	1181.25	104%
F2C	colourless	clear	4.85	900	102 %
F3A	colourless	clear	4.9	956.25	102 %
F3B	colourless	clear	4.8	1012.5	104 %
F3C	colourless	clear	5.02	1406.25	106%

Table (4): Kinetic analysis of the release data of ATS Ca from different gel formulations

Parameter \ Formulation		F1A	F1B	F1C	F2A	F2B	F2C	F3A	F3B	F3C
Zero- order	k (min^{-1})	10.8	13.02	3.4	2.433	6.38	8.84	1.93	2.58	2.68
	r^2	0.84	0.815	**0.9681**	0.957	0.884	0.943	0.986	0.956	0.956
First- order	k (min^{-1})	0.18	0.28	0.039	0.026	0.089	0.124	0.02	0.027	0.03
	r^2	0.83	0.775	0.9680	0.963	0.919	0.971	**0.988**	0.963	0.964
Higuchi Model	k (min^{-1})	28.79	35.48	9.008	7.37	19.89	26.9	5.7	7.8	8.13
	r^2	**0.8791**	**0.8823**	0.9357	**0.992**	**0.968**	**0.986**	0.985	**0.987**	**0.985**
Korsmeyer-Peppas	k (min^{-1})	13.83	23.36	5.46	4.57	18.62	10.23	4.57	4.07	6.16
	r^2	0.9745	0.9673	0.9943	0.989	0.974	0.98	0.99	0.928	0.957
	N	0.95	0.7655	0.7343	0.721	0.56	1.01	0.57	0.86	0.644
	Transport mechanism	Non-Fickian								

Kinetics of drug release

The kinetics studies of in-vitro drug release of different types of prepared gels were demonstrated in table (4). It was found that the release of ATS Ca from both F1A and F1B showed Higuchi diffusion kinetics model with r2 of 0.8791 and 0.8823 respectively. On the other hand, F1C exhibited controlled zero-order release kinetics with r^2 of 0.9681. It was reported that the incorporation of HP-β-CyD into polymeric drug delivery systems can affect the mechanisms by which drug is released. Additionally, ATS Ca/HP-β-CyD complex can modify drug solubility, drug diffusivity and hydration of the polymer matrix (19). In addition, the release from other different formulations showed a good fit to first and Higuchi diffusion models. The Higuchi diffusion model was found to be the most predominant model for F2A, F2B, F2C, F3B and F3C. While, first order was the most fitted to only F3A.

When the results were analysed by Korsmeyer-Peppas model, n values were between 0.5, 1 for all formulations indicating that, the ATS Ca release mechanism from these formulations was non-Fickian diffusion which suggested both diffusion and erosion mechanisms controlling the drug release.

Conclusion

There was an increase in the dissolution rate of ATS Ca upon complexation with HP-β-CyD. The higher rate was observed with ATS Ca/HP-β-CyD complexes prepared by co-solvent and common solvent evaporation techniques. In- vitro studies indicated high increase in percentage drug released from carbopol934 or methylcellulose polymeric hydrogels containing complexed drugrelative to free drug. While, percentages released from sodium alginate gels were decreased by complex. It could be concluded, polymer type used in hydrogel formulation can affect complexation and consequently drug release profile. Ophthalmic ATS Ca/HP-β-CyD complex hydrogels formulated by either carbopol 934 or methylcellulose are considered promising delivery systems for treatment ocular inflammation.

Competing Interests

The authors declare that they have no competing interests.

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