

Formulation and evaluation of pluronic F127 thermosetting gels containing Atorvastatin Calcium as novel anti-inflammatory Ophthalmic Delivery Systems.

ABSTRACT

Aims: The aim of this study is to formulate and evaluate ophthalmic thermo-sensitive gels containing atorvastatin calcium. The formulations based mainly on Pluronic F127 alone or combined with other viscosity increasing polymers.

Study Design: Experimental study.

Place and Duration of Study: Faculty of Pharmacy, Mansoura University, Egypt.(6 months)

Methodology:

Atorvastatin calcium the proved effective anti-inflammatory agent used in ocular diseases was prepared and characterized in form of hydroxypropyl beta cyclodextrin complex. In this study, the possibility to formulate thermosetting gels containing either the free drug, drug cyclodextrin physical mixture or complex was investigated by using heat sensitive polymers pluronic F127 alone or combined with other viscosity increasing polymers such as methyl cellulose, PVP K25.

All formulations were characterized physically for its color, clarity, viscosity, pH, and drug contents. In addition the effect of different polymeric composition on the in-vitro release profiles of Atorvastatin calcium from different preparations were investigated.

Results:

Ocular thermosetting gels containing Atorvastatin calcium -HP- β -CyD complex were prepared simply by cold method using 20% Pluronic F127 alone or combined with different polymers. All preparations were found colorless, clear, acceptable pH, rheological properties and drug content. In vitro release studies of different formulations in Simulated tear fluid indicated the possibility of altering the % drug release from thermosetting gels containing Atorvastatin calcium -HP- β -CyD complex relative to free drug by modification the formulae prepared. Methyl cellulose in 2 % was found to enhance the drug release profile of drug from Atorvastatin calcium -HP- β -CyD complex gels, while PVP K25 in 5 % caused retardation of drug released from the prepared thermosetting gels.

Conclusions: Ophthalmic thermosetting gels formulations containing Atorvastatin calcium -HP- β -CyD complex are considered promising delivery systems. They showed good physical properties. Depending on the type and concentration of the viscosity increasing polymer incorporated into the formulations, it can be applied either for improvement drug solubility and bioavailability or for retardation of Atorvastatin calcium release profile.

Keywords: Ocular; thermosetting gels; Atorvastatin calcium; HP- β -CyD; Pluronic F127; Methyl cellulose; PVPK25.

1. INTRODUCTION

Eye is one of the most important and sensitive organ in our body. Ocular Drug delivery systems are still challenging problem due to physiological constrains imposed by the unique eye structure and efficient protective mechanism [1].

Eye drops are considered the most commonly used traditional ocular dosage form [2]. (However, the poor drug bioavailability caused by short pre-corneal residence time and rapid turnover of lacrimal fluid is considered a great problem [3]. Thus, frequent instillation of eye drops is required to achieve therapeutic effect which may lead to undesirable systemic drug absorption and harmful side effects

[4]. Different approaches have been developed such as in-situ forming gel [5], Nan carrier systems [6], inserts [7] and vesicular systems [8].

In-situ gelling system is one of simplest, effective ocular delivery systems. It should be a low viscous, free flowing liquid that allow administration as eye drops, and the gel formed following phase transition should be strong enough to with stand the shear forces in the cul de sac and provided long residence times in the eye. Depending on the method employed to cause sol to gel phase transition on the ocular surface, there are different systems either pH-triggered systems [9], temperature triggered in-situ gel [10] or Ion activated in-situ gels [11]. Over the last decades, an impressive number of novel temperature, pH, and ion induced in-situ forming solutions have been developed each system has its own advantages and drawbacks. In temperature triggered in-situ gel, the system is designed mainly by using poloxamer 407 (pluronic F127) as a vehicle for ophthalmic drug delivery. It possesses many properties make it particularly suitable for ophthalmic use such as its low toxicity, muco mimetic properties, and optical clarity [12].



Statins, which are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are approved for cholesterol reduction and are commonly used to treat atherosclerosis and coronary artery disease. Statins may also be potent as immunomodulatory agents and may be effective in the treatment of autoimmune diseases and this prompted the novel idea of utilizing them as a topical drug for ocular therapy.

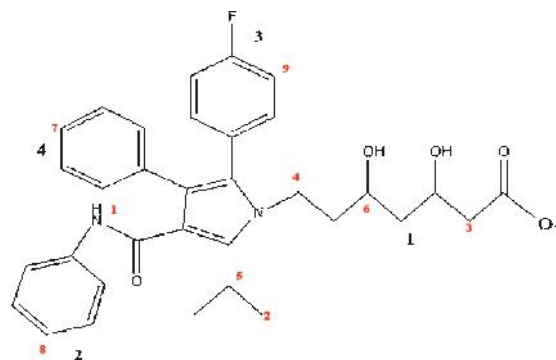
Moreover, topical atorvastatin Calcium (ATC) Fig (1) is proved to have a potential therapeutic effects for patients with dry eye and blepharitis (DEB) [13]. Lipid abnormalities in meibomian gland dysfunction, cluding an excess of free cholesterol in tears, result in the evaporative dry eye and chronic DEB [14]. Red, painful, irritated and itchy eyes, along with blurred vision, result from these disorders. Tear evaporation can lead to an increase in osmolality, which produces an inflammatory response [15]. One of demerits of atorvastatin calcium is its low water solubility, several techniques have been developed in order to increase solubility as particle size reduction [16], nanoparticles [17] or using cyclodextrin as complexing agent.

Hydrophilic cyclodextrins, especially hydroxypropyl beta cyclodextrin (HP- β -CyD) Fig (2) is oligosaccharide often used as medium for the encapsulation of active substances for medicines that will improve the water solubility, stability and bioavailability of the drug in the body by forming active substance-cyclodextrin complex [18].

In this study, we have prepared and characterized the ATC as a complex with HP- β -CyD in 1:1 molar ratio using common solvent evaporation technique. Then the prepared complex was incorporated into a poloxamer 407 based thermosetting gels. Also gels were prepared using ATC alone and ATC- HP- β -CyD physical mixture in same molar ratio for comparison. All prepared thermosetting gels were evaluated for their physical characters, as clarity, pH, viscosity, drug contents and in- vitro release profiles in simulated tear fluid as dissolution medium.

The aims of this work are: 1) to investigate the possibility to formulate ophthalmic poloxamer 407 in-situ gelling formulations containing the ATC-(HP- β -CyD) complex 2) to evaluate in-situ gelling formulations containing ATC-(HP- β -CyD) complex, and compare that with gels prepared by the ATC-HP- β -CyD physical mixture or ATC alone.

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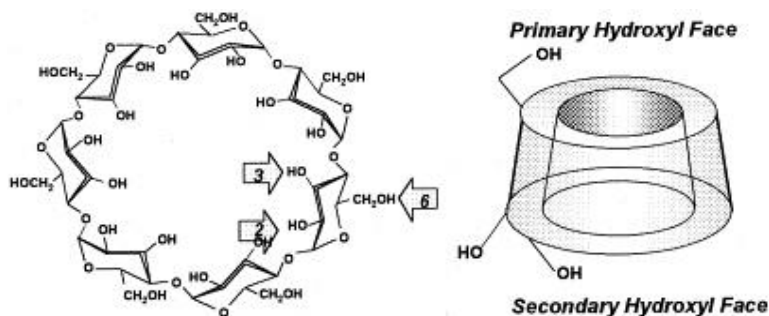
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Fig (1): Chemical structure of atorvastatin



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Fig.2. The structure of HP-β-CyD

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2. METHODOLOGY

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2.1 Materials

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Atorvastatin calcium, 2-Hydroxypropyl-β-cyclodextrin (Epico Co., Egypt), methanol, propylene glycol, sodium chloride, calcium chloride dihydrate, sodium bicarbonate all of analytical reagents grade (Adwic, EL Nasr Pharmaceutical chemicals, Co., Egypt). Pluronic F127, PVP K25, methylcellulose (B.D.H. Chemicals, Liverpool - England). Cellulose membrane, spectrapore, M-W. cutoff: 12000-14000 (Fisher Sci. Co., Pittsburgh, U.S.A.)

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2.2 Experimental

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2.2.1 Estimation of ATC by UV spectrophotometer

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A simple method is used for estimation of ATC based on UV spectrophotometric analysis using Spectro UV-VIS double beam spectrophotometer, Labomed Inc., USA.

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Depending on the free solubility of ATC in methanol. The technique performed by dissolving accurately weighed 20 mg of Atorvastatin calcium in pure methanol, the solution was scanned from 200-400 nm to determine maximum wavelength. The standard calibration curve was performed in Methanol / STF (1:9) by serial dilution of the previously prepared stock solution, different drug concentration were prepared, their absorbance were determined, calibration curve was drawn to determine slope.

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2.2.2 Preparation of ATC- HP-β-CyD inclusion complexes by common solvent evaporation method:

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ATC-and HP-β-CyD in equimolar ratio of 1:1 were dissolved in a common solvent as extra pure methanol to get a clear solution. The prepared solution was allowed to evaporate overnight at the

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room temperature. The collected drug-cyclodextrin complex was pulverized and passed through sieve number 80 [19].

2.2.3 Preparation of ATC- HP-β-CyD Physical mixture

In order to prepare the physical mixture, Atorvastatin Calcium and H-P-β-CD in equimolar proportion (1:1 molar concentrations) were mixed in a mortar for one hour without applying any pressure [20].

2.2.4 Characterization of Atorvastatin Calcium inclusion complexes:

2.2.4.1. Differential Scanning Calorimetry (DSC):

The thermal behavior of drug, H-P-β-cyclodextrin, Physical mixture 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.

2.2.4.2. Scanning electron microscopy (SEM):

The surface morphology of Atorvastatin Calcium was determined using scanning electron microscope (JSM- 6510LV, JEOL, Japan).

2.2.4.3. FT-IR studies:

All pure drug, cyclodextrin, Physical mixture and complexes were subjected to IR studies to check whether interaction occurs between pure atorvastatin drug and added ingredients. Instrument used was Fourier transform infra-red spectrophotometer (FT-IR), Thermo Fisher Scientific, Inc., Waltham, MA, USA [21].

2.2.5 Formulation of medicated in-situ gels

Different polymers were selected as a viscosity increasing agents to prepare the thermosetting gels based on pluronic F127 for ophthalmic application such as and methyl cellulose and PVP K25. All preparations compositions are indicated in table (1).

All the  in-situ gels were prepared by the cold method. For preparations containing methyl cellulose or PVP K25. The polymer solution was prepared by dispersing the calculated weight of methyl cellulose or PVP K25 in double distilled water with continuous stirring until it had completely dissolved. Then polymer pluronic F127 solution was prepared by dispersing the weighed amount of pluronic F127 in the previously prepared polymeric solution after cooling to 4°C, then stirring for 1 hour in an ice bath. The final preparation was kept in refrigerator for at least 24 hours to ensure the complete dissolution.

For medicated formulae, three preparations were made from each formula either by incorporating free atorvastatin calcium (F1A, F2A, F3A), or Atorvastatin Calcium/ H-P-β-cyclodextrin physical mixture (F1B, F2B, F3B), or Atorvastatin Calcium/ H-P-β-cyclodextrin complex (F1C, F2C, F3C).

In order to prepare the medicated ophthalmic gels, atorvastatin calcium 0.5% (W/W) or its equivalent weight of Atorvastatin Calcium/ H-P-β-cyclodextrin Physical mixture, and complex was dissolved firstly in propylene glycol and added to the previous mixture drop by drop by stirring using magnetic bars till complete dissolution. The weight of gel was adjusted to final prepared weight, and then packaged in clean, dry and sterile glass containers until use.

2.2.6 Physical evaluation of in- situ thermosetting systems

2.2.6.1 Determination of the viscosity of prepared formulations

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

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

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)

Table (1): Compositions of ATS Ca ophthalmic thermosetting gels

Ingredients	Formulations								
	F1A	F1B	F1C	F2A	F2B	F2C	F3A	F3B	F3C
Atorvastatin calcium	0.5 %	---	---	0.5 %	----	----	0.5 %	----	----
Atorvastatin/ HP- β -CyD physical mixture equivalent to Atorvastatin	----	0.5 %	---	---	0.5 %	----	----	0.5 %	----
Atorvastatin/ HP- β -CyD complex equivalent to Atorvastatin	----	---	0.5 %	---	---	0.5 %	----	----	0.5 %
Pluronic F127	20%	20%	20%	20%	20%	20%	20%	20%	20%
Methyl cellulose	----	-----	---	2%	2%	2%			
PVP K25	-----	----	---	---	----	----	5 %	5 %	5 %
Propylene glycol	20%	20%	20%	20%	20%	20%	20%	20%	20%
Distilled water	Q S	Q S	Q S	Q S	Q S	Q S	Q S	Q S	Q S

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2.2.6.2 Determination of the pH of prepared formulations

One gram of each formulation was dispersed in 25 ml of double distilled water, and the pH was measured using pH-meter (U.S.P., 2004) [22].

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2.2.6.3 Determination of drug content in the prepared formulations

One gram from each formulation containing the drug was accurately weighed and placed in tightly closed 100 ml volumetric flask containing 50 ml methanol.

The content of each flask was shaken for 2 hours and then, 10 ml were centrifuged. The supernatant was filtered through 0.45 m membrane filter and measured spectrophotometrically at 242 nm for drug content [23].

2.2.7 In-vitro release study of in- situ gelling systems

The method was performed by filling 1 ml of the cold in-situ gel formulation into the dialysis tube with care to avoid air bubbles inside the preparation. Standard cellophane membrane (previously immersed in STF, pH 7.4, for 24 hrs.) was stretched over the dialyzer tube. Then, it was placed at 35°C inside an oven for 30 mins to ensure the complete gelation. whole dialysis unit was then immersed in a 100 ml beaker containing 50 ml of the STF (pH=7.4) in such a manner that the membrane was located just below the surface of the sink solution. (Simulated tear fluid, STF, was prepared by Na Cl (0.67 g), Na HCO₃ (0.2 g), Ca Cl₂.2H₂O (0.008 g), and purified water to 100 g, pH was adjusted to 7.4) [24].

The beaker was placed in a thermostatically controlled incubator system (GFL Germany shaking incubator type 3033 with orbital motion incubator) adjusted at 37± 0.5 °C with a constant stirring at 100 rpm to avoid any development of a concentration gradient.

At appropriate sampling intervals an aliquot of 1 ml, was collected and replaced by equal volume of the STF at the same temperature to keep the volume of the sink solution constant during the experiment. Samples were then assayed spectrophotometrically at $\lambda_{\max}$ 242 nm. Each experiment was repeated three times and the average was calculated. The concentration of ATC in each sample was determined from the standard curve previously constructed after suitable dilution. Blank gel samples were carried out simultaneously to check for any interference [25].

2.2.8 Kinetic analysis of In vitro drug 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) [26].

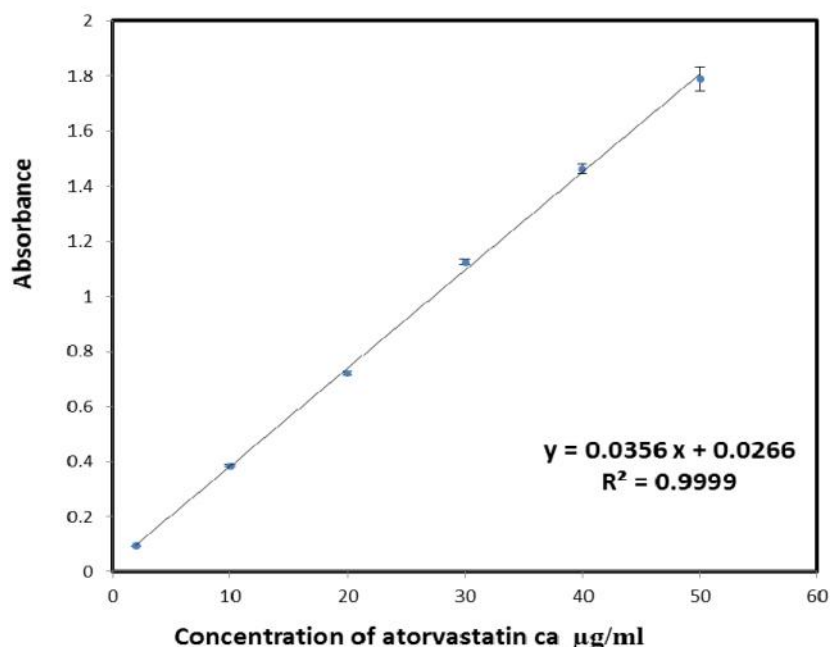
3. RESULTS AND DISCUSSION

3.1 Estimation of ATC by UV spectrophotometer

The absorption maximum was found to be at 242 nm. A linear relationship between the absorbance and the drug concentration is shown in figure (3).

The results were the average of three values. The curve indicates that Atorvastatin calcium obeys Beer's law on the tested concentration range with slope equals 0.035

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188 **Fig (3): Standard calibration curve of ATC in STF.**

189 **3.2 Characterization of Atorvastatin Calcium inclusion complexes**

190 The different methods DSC, FT-IR, and SEM used to estimate the complex formation indicated the
191 formation of ATC- HP-β-CyD inclusion complexes of distinctive characteristics compared with that of
192 the pure drug or HP-β-CyD. This was explained with details in our previously published paper [27].

193 **3.3 Physical evaluation of in- situ thermosetting systems**

194 The obtained results are illustrated in table (2). It was observed that, the prepared ocular gels were
195 colourless and with good clarity. In addition, the pH values of all formulations were within the
196 acceptable range (4-9) which the eye can tolerate, without any irritation.

197 Finally the viscosity values and drug contents of all formulations were found to be in the acceptable
198 range.

199 **Table (2): Physical characters of ophthalmic gels**

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Formula	Colour	Clarity	pH	Viscosity (m pa.s)	drug content
F1A	colourless	clear	6.6	1002.5	106%
F1B	colourless	clear	6. 8	1000.2	94%
F1C	colourless	clear	6.53	1005	96%
F2A	colourless	clear	4.65	1181.25	102%
F2B	colourless	clear	4.55	1181.25	88%
F2C	colourless	clear	4.85	1200.25	94%
F3A	colourless	clear	4.79	1195.25	104%

F3B	colourless	clear	4.8	1212.5	108%
F3C	colourless	clear	5.02	1406.25	92%

3.4 In- vitro drug release from different formulations:

Percentages released of ATC from Pluronic F127 different thermosetting gels containing free drug (F1A), drug- HP- β -CD physical mixture (F1B), and drug- HP- β -CD complex (F1C) through semipermeable cellophane membrane were shown in figure (4). Hydrophilic membrane was used in order to simulate the release towards a hydrophilic surface such as the ocular mucosa.

From the results, it was found that percent released of ATC from F1C gel was 95.6 % after 6 hours while that from F1A, F1B were 71.1% and 72.6 % respectively. This improvement in the drug release may be due to the ability of the incorporated cyclodextrin in the complex to enhance the aqueous solubility and hence the bioavailability of drug. This is in accordance with Guo and Cooklock [28] 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 % released of ATC from Pluronic F127 and 2 % MC gels were represented in figure (4). Both thermosetting gels containing drug- HP- β -CD physical mixture (F2B), and drug- HP- β -CD complex (F1C) also showed high increase in % drug release compared with thermosetting gels containing free drug (F2A) from 46.5 % to nearly 60 % after 6 hours.

On the other hand, drug release profiles of third group of thermosetting gels of different polymers composition (containing Pluronic F127 and 5 % PVPK25) representing in figure (6) showed some differences. There was an observed reduction in % drug release of formulae containing ATC- HP- β -CD complex (F3C) to 67 % compared with F3A, F3B with 80 % after 5 hours release period. This may be explained on the basis of the thermosetting gel composition and also measured viscosity values on these formulations.

Moreover, another explanation is that the capability of HP- β -CD o complex with the thermosetting gel polymer itself (PVPK25). The physical presence of polymers capable of inclusion within the HP- β -CD cavity would be expected to hinder the ability of HP- β -CD to complex with drug itself, which resulting in de-complexation free drug and hence a decrease in % release [29].

Filipović-Grčić et al. [30] also stated that drug release from polymer matrices containing drug inclusion complexes may be retarded. They verified a reduced release of the poorly water-soluble nifedipine complexed with HP- β -CD in case of chitosan microspheres, compared to those contained only free drug. It was explained on the basis that during the release, drug rapidly dissociated from the complex, resulting in an increased concentration of free HP- β -CD within the polymer matrix. A more hydrophilic, polymer: HP- β -CD matrix was created which in turn decreased permeability and slowed the release of drug.

So, hydrophilic Cyclodextrins are useful to attain either high increase or decrease in release profile depending on the selected thermosetting gel formulated. They have been used extensively to enhance the bioavailability of poorly water soluble drug [31].

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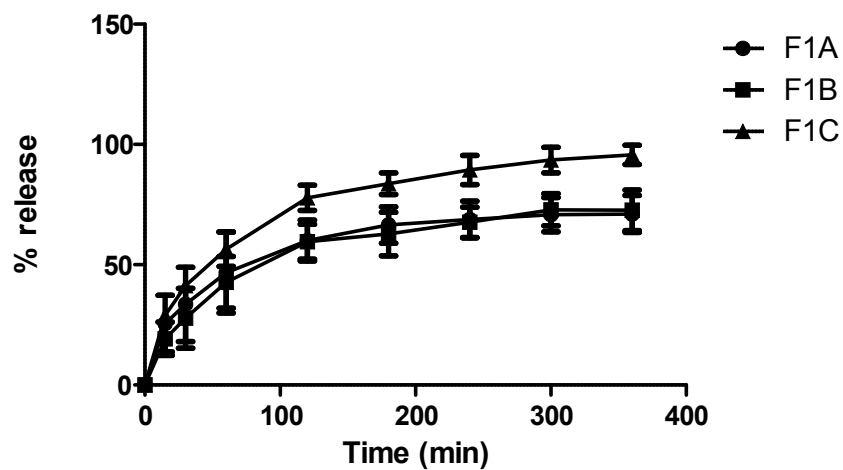


Fig (4): In- vitro release profiles of ATC from Pluronic F127 thermosetting gel in STF.

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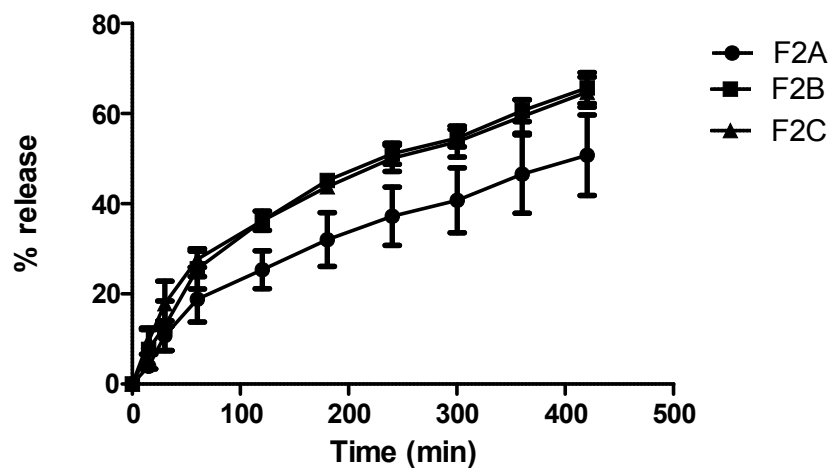


Fig (5): In- vitro release profiles of ATC from Pluronic F127 and MC thermosetting gel in STF.

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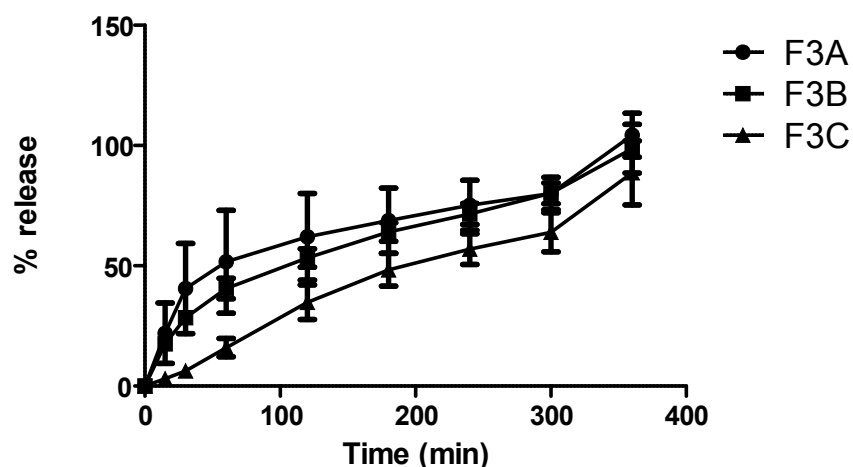


Fig (6): In- vitro release profiles of ATC from Pluronic F127 and PVPK 25 thermosetting gel in STF.

3.5 Kinetics of drug release

The kinetics studies of in-vitro drug release of different types of prepared thermosetting gels were demonstrated in [table \(3\)](#).

It was found that the release of atorvastatin from both F2A, F2B and F2C showed Higuchi diffusion kinetics model with r^2 of 0.992, 0.988 and 0.989 respectively. On the other hand, F1C exhibited controlled first order release kinetics with r^2 of 0.991. It was reported that the incorporation of HP- β -CD into polymeric drug delivery systems can influence the mechanisms by which drug is released. Additionally, drug-HP- β -CD complex can modify drug solubility, drug diffusivity and hydration of the polymer matrix [\[32\]](#).

In addition, the release from all other different formulations showed a good fit to Korsmeyer-Peppas models. The analyzed results indicated n values either located between 0.5, 1 for F2A, F2B, F2C, and F3C or some other formulations with n values less than 0.5.

This indicating that, the atorvastatin release mechanism from these formulations was non-Fickian diffusion which suggested both diffusion and erosion mechanisms controlling the drug release.

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270 **Table (3): Kinetic Analysis of the Release Data of Atorvastatin from different**
 271 **thermosetting gel formulations**

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Formulation		F1A	F1B	F1C	F2A	F2B	F2C	F3A	F3B	F3C
Parameter										
Zero- order	k (min ⁻¹)	7.48	8.81	10.85	6.7	8.8	7.9	11.2	12.4	14.74
	r^2	0.798	0.82	0.83	0.94	0.92	0.93	0.91	0.96	0.98
First- order	k (min ⁻¹)	0.161	0.18	0.46	0.09	0.138	0.115	0.529	0.529	0.368
	r^2	0.86	0.90	<u>0.99</u>	0.97	0.97	0.974	0.7	0.73	0.82
Higuchi Model	k (min ⁻¹)	23.8	27.8	34.2	20.6	27.1	24.5	34.06	37.4	43.7
	r^2	0.916	0.93	0.94	<u>0.992</u>	<u>0.988</u>	<u>0.989</u>	0.93	0.98	0.972
Korsmeyer-Peppas	k (min ⁻¹)	39.8	31.6	50.1	14.4	19.9	19.9	39.8	31.6	12.5
	r^2	<u>0.96</u>	<u>0.96</u>	0.97	0.953	0.97	0.976	<u>0.94</u>	<u>0.989</u>	<u>0.989</u>
	n	0.33	0.42	0.37	0.70	0.64	0.54	0.40	0.49	1.04
	Transport mechanism	Fickian	Fickian	Fickian	Non-Fickian	Non-Fickian	Non-Fickian	Fickian	Fickian	Non-Fickian

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274 4. CONCLUSION

275 So, Percentages released of ATC from thermosetting gels through cellophane membrane
 276 were found to be dependent mainly on the composition of the formulae itself. HP-β-CD succeeded to
 277 increase the cumulative ATC release % in case of thermosetting gel containing only 20% pluronic F
 278 127 or 20% pluronic F 127 in addition to low concentration of additional polymer (2 %) MC. On the

other hand, in case formulation containing 20% pluronic F 127 and a high concentration (5%) PVPK 25, incorporation of ATC-HP- β -CD complex in the gel caused a marked retardation of drug release.

We can conclude that the type and percentages of polymer used in thermosetting gel formulation is considered the main effective factor controlling the % released of ATC from thermosetting gels incorporated with Atorvastatin calcium -HP- β -CyD complex.

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