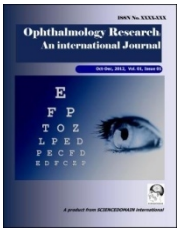

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Formulation and Physical Characterization of a Novel Sustained-Release Ophthalmic Delivery System for Sparfloxacin: the Effect of the Biological Environment

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The purpose of this study was to prepare and evaluate ocular thermosetting gels containing sparfloxacin-HP-β-CyD. The formulations based mainly on Pluronic F127 alone or combined with other polymers.
Study Design: Experimental study.
Place and Duration of Study: School of Pharmacy, University of East Anglia, Norwich, Norfolk, UK, between July 2010 and January 2011.
Methodology: Sparfloxacin, the more effective fluoroquinolone antibiotic used in ocular infection was prepared in form of 1:2 drug: HP-β-CyD spray dried complex. The complex was characterized by several techniques like DSC, FTIR and SEM. In this study, the possibility of incorporation of this complex into thermosetting gels was investigated using pluronic F127 alone or combined with other polymers. All placebo and medicated formulations were characterized physically (colour, clarity, pH, and drug contents). Rheological tests and texture analysis were performed in both non physiological conditions, and after dilution with the simulated tear fluid, to estimate the effect of the

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biological environment effect on thermosetting gels properties.
Results: Sparfloxacin-HP-β-CyD complex was successfully prepared and characterized by several techniques, all confirmed complete inclusion between sparfloxacin and HP-β-CyD. The prepared complex showed a significant improvement in the aqueous solubility of Sparfloxacin compared to untreated drug or the physical mixture. Ocular thermosetting gels containing sparfloxacin-HP-β-CyD complex were prepared simply by cold method using 20% Pluronic F127 alone or 15% Pluronic F127 combined with other polymers. All preparations were found colourless or yellow, clear, of acceptable pH, and drug content.
The rheologicaly and texture analysis studies revealed that, the formulations exhibited suitable gelling temperatures, gelling times, and appropriate strength to be used in ocular dosage forms. The biological environment highly affected gel properties according to the polymer type.
Conclusions: Ophthalmic *in-situ* gelling formulations containing sparfloxacin-HP-β-CyD complex are considered promising delivery systems. They showed good rheological and texturale properties. The results confirmed the possibility of lowering Pluronic F127 concentration by using other polymers in small concentrations. The effect of the biological environment must be considered in developing such formulations.

Keywords: Ocular; thermosetting gels; sparfloxacin; HP-β-CyD; Pluronic F127; sodium alginate; chitosan; Carbopol 980.

1. INTRODUCTION

Topical administration of eye drops is the ideal treatment for ocular diseases, especially when the drug must display a localized action (e.g., the cornea and/or anterior chamber). Unfortunately, in several cases, this treatment is not effective due to protective mechanisms of the human eye, short pre-corneal contact time, and corneal impermeability. And as a result, frequent dosing is usually needed leading to many side effects. Various ocular formulations have been developed in an effort to overcome the issues with conventional eye drop formulations.

Among them, the *in-situ* gel-forming formulations, which undergo phase transition from a liquid to a semisolid gel upon exposure to physiological environments, seem to be a promising tool. These formulations should be a free-flowing liquid at room temperature to allow easily reproducible administration into the eye as a drop, and then on exposure to physiological conditions; be converted to the gel phase, thus increasing the precorneal residence time of the delivery system and enhancing ocular bioavailability. According to the mechanism of gelatin, *in-situ* forming gels are classified into three different types: the first type is temperature induced *in-situ* gel system (thermosetting gels), in which the gel is formed by increasing the temperature from room temperature (circa 25 ºC) to ocular surface temperature (circa 34 ºC) polymers such as poloxamers (e.g. Pluronic ^{TM}) work by this mechanism. The second type is the pH-induced *in-situ* gel systems, in which the gels are formed by increasing the pH to 7.4, examples are Carbopol 980 ^{TM}, and chitosan. And finally, the third type is osmotically induced *in-situ* gel system in which the gels are formed by the presence of mono or divalent cations such as sodium and calcium that are found in tear fluids polymers working by this mechanism includes sodium alginate and gellan gum (e.g. gelrite ^{TM}) [1].

Nowadays, *in-situ* gel forming systems are of great importance, having the combined advantages of being patient convenient with favorable residence time for enhancing ocular

bioavailability and for reducing systemic side effects. In addition, they could be easily injected subconjunctivally as a method for periocular drug delivery to prolong the drug release to the posterior eye segment tissues such as the retina, and the vitreous in order to treat the ocular diseases of these tissues.

Poloxamer 407 gives a colourless and transparent gel and is widely employed, but these suffer from a major drawback of having weak mechanical strength, which leads to rapid erosion [2]. As a result, it requires a high concentration of about 25 to 30% (W/V) to exhibit sol-gel phase transition at 37ºC when used alone. Gelation temperature can be adjusted within the required temperature range of 33 to 36ºC by modifying cross-linking agents, by mixing the different series of poloxamer, and by mixing with other polymers. An interesting approach focuses on using mixture of poloxamers with other polymers such as Carbopol, alginate, and chitosan to prepare *in-situ* gels of suitable characters. Carbopol, a mucoadhesive polymer, increases the formulation's mechanical strength, and also increases surface interaction with the ocular tissue and, consequently, contact time. Chitosan is a biodegradable polymer that has showed excellent ocular compatibility. It presents positively charged amine groups in its chemical structure that could interact with the negatively charged mucous layer, conferring a mucoadhesive characteristic.

Therefore, a combination of these polymers with poloxamer would be very promising for ocular administration, as the *in- situ* mechanical strength of the formulation would be higher than that of both polymers alone.

Sparfloxacin (Fig. 1) is a new generation fluoroquinolone antibiotic, which has been used effectively to treat eye infections, it provides improved efficacy against important ocular pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Staphylococcus epidermis*. It is reported to be more active *in vitro* than ciprofloxacin against mycobacteria and gram-positive bacteria [3]. However, its main drawbacks as a therapeutic molecule are its very low water solubility and its photosensitivity. In this study, we have investigated the potential of a sparfloxacin-HP-β-CyD inclusion complex to overcome these issues.

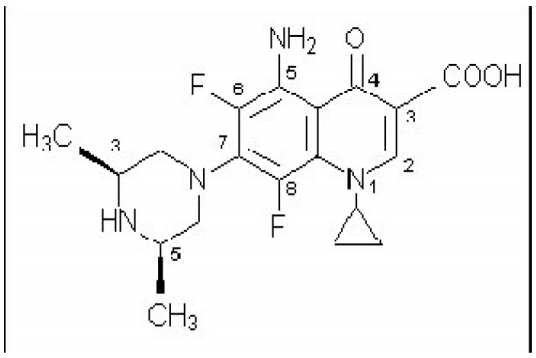

Fig.1. Chemical structure of sparfloxacin

Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity, as indicated in Fig. 2. They have potential as carriers in drug delivery systems, for example improving the apparent solubility of poorly water-soluble drugs and reducing stability issues associated with photo-sensitive compounds [4].

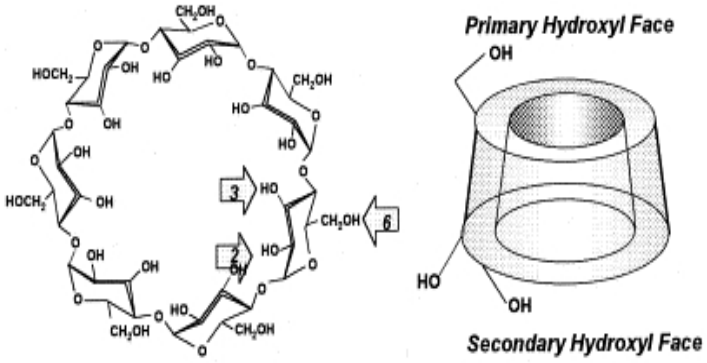

Fig.2. The structure of HP-β-CyD

Hydrophilic cyclodextrins, especially HP-β-CyD, have been shown to be non-toxic and well tolerated in the eye [5].

In this study, we have incorporated the prepared sparfloxacin-HP-β-CD complex into a range of thermo-setting gels and assessed their physical performance, both in the "as prepared" state and in a simulated ocular bio-environment.

The aims of this work are: 1) to prepare and characterize an inclusion complex of sparfloxacin and hydroxypropyl-β-cyclodextrin (HP-β-CyD), 2) to develop ophthalmic *in-situ* gelling formulations containing the sparfloxacin-(HP-β-CyD) complex using poloxamer either alone or combined with other polymers. 3) to study the effect of the biological environment on the physical properties of the different prepared gel formulations.

2. METHODOLOGY

2.1 Materials

Sparfloxacin of purity 98% for HPLC, 2-Hydroxypropyl-β-yclodextrin, poloxamer (Pluronic F127), chitosan medium molecular weight, and alginic acid sodium salt from brown algae (all analytical grades reagents) were purchased from Sigma Aldrich, UK. Sodium chloride, calcium chloride dihydrates and sodium bicarbonate were obtained from Fisher Scientific Ltd., UK. Carbomer 980 (Noveon) was obtained from Surfachem, UK. Deionised water was produced from an Elga still, Elga, UK. Syringe filters of 0.45 µm pore size were obtained from Sartorius stedim biotech GmbH, Germany.

2.2 Experimental

2.2.1 Sparfloxacin and HP-β-CyD inclusion complex

2.2.1.1 Preparation sparfloxacin-HP-β-CyD inclusion complex

The sparfloxacin-HP-β-CyD inclusion complex was prepared by spray-drying from a hydro-alcoholic solution containing both sparfloxacin and HP-β-CyD (1:2 molar ratio). Spray-drying was performed using a Mini Spray Dryer Büchi 290 (Büchi, Switzerland) with flow rate 5

ml/minute, inlet temperature 85ºC, atomized air pressure 3 kg/cm^2; process yield being circa 50%.

2.2.1.2 Preparation of sparfloxacin-HP-β-CyD physical mixture (PM)

The physical mixture was prepared by the same method described by[6].This is simply by mixing both the drug and HP-β-CyD, using a mortar and pestle in the same molar ratio (1:2) that has been used to prepare the spray dried complex.

2.2.1.3 Characterization of sparfloxacin, HP-β-CyD, the physical mixture and the inclusion complex

The drug alone, HP-β-CyD, drug-HP-β-CyD complex and physical mixture were characterized and evaluated by using the following methods:

2.2.1.3.1 Differential scanning calorimetry (DSC)

For the thermal analysis of sparfloxacin, HP-β-CyD, physical mixture and the prepared inclusion complex, a Q1000 MTDSC system (TA Instruments, Newcastle, DE) was used. Samples of circa 5 mg in aluminium pans were heated from 25 ºC to 300 ºC at 10 ºC /minute [4].

2.2.1.3.2 Fourier transforms infrared spectroscopy (FT-IR)

FT-IR studies were carried on a Bruker Optics IFS66/S spectrometer (Coventry, UK) that is equipped with a heated Golden Gate MkII ATR accessory (Specac Limited, Orpington, UK). Spectra were acquired over the range 4000 to 500 cm^{-1}.

2.2.1.3.3 Scanning electron microscopy (SEM)

Scanning electron microscopy was performed using a JEOL JSM 5900 LV (JEOL, Japan), mounted with a tungsten filament with an acceleration voltage of 5–20kV. The samples were mounted onto stubs using double-sided tape and were gold coated to a thickness of circa 15 nm by a Polaron SC7640 sputter gold coater (Quorum Technologies). The imaging process was performed in a high vacuum environment.

2.2.1.4 Dissolution rate study of inclusion complex compared to untreated drug and physical mixture

Dissolution studies were carried out for sparfloxacin, physical mixture, and inclusion complexes using the method described by [7] with some modifications. The experiment was performed by using a USP paddle type dissolution apparatus adjusted at 37ºC ± 1ºC and 100 rpm. The dissolution medium used was 500 ml of freshly prepared simulated tear fluid which was maintained at pH 7.4. Simulated tear fluid (STF) was prepared by Na Cl (0.67 g), Na HCO_3 (0.2 g), Ca Cl_2.2H2O (0.008 g), and purified water to 100 g, pH was adjusted to 7.4. The large volume of dissolution medium was used in order to maintain the sink conditions as in the methods described by [8].

Ten mg of the drug or its equivalent weights of the physical mixture, or the inclusion complex was added to the dissolution medium, samples of 5 ml were withdrawn at time intervals of 5, 10, 20, 30, 40, 50, 60, 90, and 120 min. The volume of dissolution medium was adjusted to

500 ml by replacing the sample withdrawn with 5 ml of fresh simulated tear fluid (STF) pH 7.4. The solutions were immediately filtered through 0.45 µm membrane filter, suitably diluted and the concentrations of sparfloxacin in samples were determined spectrophotometrically at 290 nm.

The results of dissolution were analyzed statistically using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test [9,10].at the time required for the release of 50% of drug ($T_{50\%}$). Statistical calculations were carried out using Instate - 2 computer programs (Graphpad software Inc., V2. 04 San Diego, CA, USA).

2.2.2 Preparation of ophthalmic thermosetting gels containing sparfloxacin-HP-β-CyD inclusion complex

Different concentrations of Pluronic F127 with or without other polymers were used for the preparation of four plain gels given the names FP1, FP2, FP3, and FP4. For the medicated gels, the sparfloxacin-HP-β-CyD inclusion complex was added to give a final drug loading equivalent to 0.3%w/w sparfloxacin. The four medicated gels were coded by FM1, FM2, FM3, and FM4, the composition of all plain and medicated formulations are represented in Table 1.

Table 1. Composition of thermosetting gels

Formulations	Ingredients				
	Pluronic F127	**Carbopol 980**	**Chitosan**	**Sodium alginate**	**Sparfloxacin-HP-β-CYD complex equivalent to sparfloxacin**
FP1	20 %	---	---	---	---
FP2	15 %	0.2 %	---	---	---
FP3	15 %	---	0.3 %	---	---
FP4	15 %	---	---	0.1 %	---
FM1	20 %	---	---	---	0.3 %
FM2	15 %	0.2 %	---	---	0.3 %
FM3	15 %	---	0.3 %	---	0.3 %
FM4	15 %	---	---	0.1%	0.3 %
CONTROL	----	---	---	---	0.3 %

2.2.2.1 Preparation of plain in-situ forming gels

All the plain *in-situ* gels were prepared by the cold method with some modification in each formula according to the different polymer used.

For FP1, a certain volume of double distilled water was cooled to 4ºC using an ice bath, over a magnetic stirrer. Pluronic F127 was slowly added portion wise with continuous stirring for one hour keeping the temperature at 4ºC. Then the volume was completed by water, the preparation was kept in refrigerator for at least 24 hours to ensure the complete dissolution of the polymer and obtain a clear transparent solution [11].

For FP2, the Carbopol 980 solution was prepared by dispersing the calculated weight of Carbopol 980 in double distilled water with continuous stirring until it had completely dissolved. Then Carbopol Pluronic F127 solution was prepared by dispersing the weighed

amount of pluronic F127 in the previously prepared Carbopol solution after cooling to 4ºC, 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 [12].

For FP3, the chitosan solution was initially prepared by dissolving the required weight in a solution of acetic acid 0.5% v/w with continuous stirring until completely dissolved. Then the chitosan solution was refrigerated and used as a solvent for the poloxamer dispersion for 1 hour at 4ºC. The solution was kept in refrigerator for at least 24 hours to ensure the complete dissolution [13].

For FP4, the sodium alginate solution was prepared by dispersing the required weight in double distilled water, with continuous stirring until dissolved, and then was cooled to 4ºC; then Pluronic F127 was then added with stirring for 1 hour over an ice bath. The partially dissolved solution was kept in the refrigerator for at least 24 hours until complete formation of *in-situ* gel [14].

2.2.2.2 Preparation of medicated in-situ gels

Medicated *in-situ* gels were prepared with the same plain *in-situ* gels compositions in addition to the incorporation of sparfloxacin-HP-β-CyD complex which was previously prepared by the spray drying technique. The four medicated gels are coded by FM1, FM2, FM3, and FM4.

In order to prepare the medicated formulations FM1, FM2, FM3, and FM4, the accurately weighted amount of the sparfloxacin-HP-β-CyD complex equivalent to 0.3%w/w final concentration of sparfloxacin in all formulations, was slowly added to the plain solution with continuous agitation in ice bath at 4ºC until a homogenous distribution was obtained, then stored in screw capped amber glass vials for further studies at 4ºC in the refrigerator. In addition, for comparison, a control solution was prepared by the dissolution of the drug-HP-β-CD spray dried complex in double distilled water to give the same concentration equivalent to 0.3% w/w sparfloxacin which used to prepare all other formulations [13].

2.2.3 Physical characterization of thermosetting gels

The formulations were evaluated physically for colour, clarity, pH and drug content. The drug content was measured by taking 1 ml of each formulation and diluting to 100 ml with distilled water. Then 5 ml was further diluted to 25 ml with distilled water. The drug concentration was measured spectrophotometerically at λ = 293 nm using standard calibration curve within the range of (1 to 30 µg/ml).

In addition both the rheological property and the texture profile of all formulations were estimated in details as in the following techniques.

2.2.4 Rheological studies

Rheological properties were measured using an AR-1000 controlled stress oscillatory rheometer (TA Instruments, UK). Four different rheological tests were performed in order to evaluate all the prepared *in-situ* gel properties and each test was repeated at least three times.

Data are presented as G' (the solid component) and G'' (the liquid component), with the transition (or gelling) point being the point at which the two components become equal in magnitude. Both the temperature at which the systems gelled ($T_{sol/gel}$) and the time the systems took to gel at 37ºC were measured.

2.2.4.1 Flow test

Flow tests were conducted to determine the type of flow of all gel samples, i.e. to assess whether it is Newtonian or non-Newtonian. The sample was equilibrated at 35ºC (temperature of eye surface) for at least 5 minutes before the continuous flow test was started, the flow test is destructive, so the sample must be discarded after the measurment.

2.2.4.2 Strain sweep (LVR determination test)

It is very important test used to determine LVR (linear viscoelastic region) which is defined as the region where the stress was directly proportional to strain while the storage modulus G', and loss modulus G'' remain constant. It is used to know the maximum strain for the gel and to indicate that any strain above this LVR will cause destruction of three dimensional network of the gel .So any further tests applied for measurement of elastic properties should be made in this LVR. This test was conducted at 35ºC, sample being equilibrated at this temperature for at least 5 minutes before the test began. The torque range studied was 0.1 to 1000 µN.m. at a frequency of 1 Hz.

2.2.4.3 Temperature sweep

This was used to determine the temperature of the phase transition for the gel formulations. The phase transition temperature (also called $T_{sol/gel}$) is defined as the temperature at which the gel is converted from the liquid state to the solid state. The test was performed by first setting the initial temp at 10ºC for 5 min before the test was began, then the temp ramp was performed to be increased from 10 to 45ºC at a rate 1ºC per min with an applied frequency of 1 Hz and torque of 10 µN.m for FP1 or 1 µN.m for FP2, FP3, and FP4. Each sample was performed at least three times. The phase transition temperature is called $T_{sol/gel}$ and was determined from the obtained curves as the temperature at which the two moduli G', G'' crossover or intersected each other.

2.2.4.4 Time sweep

This was used to determine the gelation time which is defined as the time after which the gel was formed. The initial temperature was set at 10ºC for 5 minutes before the test began, then the temp was raised to 35ºC and the test was made for 10 min with a frequency of 1 Hz and torque of 10 µN.m for FP1 or 1 µN.m for FP2,FP3, and FP4.

2.2.5 Texture profile

Texture profile analysis of the pre-gelled formulations was conducted using a TA.XT2 Texture Analyser (Stable Microsystems, UK) and the strength of the gel was taken to be the hardness in compression. The test was performed by placing 5 gm of each sample in plastic sample tube and holding at 35ºC overnight, then put under probe in a manner that the probe penetrate the gel into its centre 11.5 mm diameter Perspex cylinder probe was used for the test with pre-test speed 0.5 mm/s, test speed 1 mm/s, post test speed 5 mm/s, force 0.1 g, penetration distance 3 mm, trigger force 0.5 g, and the data acquisition rate 400 pps.

2.2.6 Effect of simulated *in- vivo* conditions

In order to evaluate the formulation performance after ocular instillation, the gels were mixed with simulated tear fluid at a ratio of 40:7 [15]. These mixtures were subjected to the rheological studies and the texture analysis. For comparison, samples diluted with purified water in the same ratio were also studied.

2.2.7 *In-vitro* release study using membranless method

For the determination of the release profile of sparfloxacin from the inclusion complex incorporated in different thermosetting gel formulations, a membrane-free experimental set-up was used to study the drug release pattern of gels in the eyes. This method was similar to that described by [16,17].

The method was carried out with some modification in order to use a dialyzer diffusion cell chamber of volume 1500 µl (Harvard apparatus, USA) fitted within the vessels of a paddle USP II dissolution apparatus (Copley apparatus, UK).

The method was performed by filling 1 ml of the each *in-situ* gel formulation in the dialyzer body with care to avoid air bubbles inside the preparation. Then, it was placed at 35ºC inside an oven for 30 mins to ensure the complete gelation. Then, it was immediately transferred with care to the vessels of the dissolution bath containing 300 ml of simulated tear fluid (STF) as dissolution medium of pH 7.4. The temperature and stirring rate were maintained at 35ºC and 25 rpm, respectively. Aliquots of 5 ml were withdrawn from the release medium at predetermined time intervals and then replaced with fresh STF solution heated to 35°C. The samples were filtered through 0.45 µm syringe filters and subjected to spectrophotometric analysis to determine the sparfloxacin concentrations at λ_{max} 290 nm. Each experiment was repeated three times and the average was calculated.

3. RESULTS AND DISCUSSION

3.1 Preparation and Characterization of Sparfloxacin-HP-β-CyD Complex

Spray drying method is one of the most exciting technologies for the pharmaceutical industry. It is an ideal process for complex formation where the end-product must comply with precise quality standards regarding to the particle size distribution, residual moisture content, bulk density and morphology.

Previously, it was found that with spray drying technique one can co-precipitate the drug with a polymer like cyclodextrins in a stable amorphous solid dispersion.

The prepared sparfloxacin-HP-β-CyD complex was characterized by several techniques as DSC, FTIR, and SEM.

3.1.1 Differential scanning calorimetry (DSC)

Thermal analysis has been reported as an important method to recognize and characterize CyDs complexes. When guest molecules were included inside CyDs cavities or in the crystal lattice, the peak corresponding to their melting point is generally shifted to another temperature. Also the peak intensity may be decreased or disappeared [18].

The DSC curves of pure components and the prepared complex are illustrated in Fig. 3. It was observed that, the DSC curve of HP-β-CyD alone showed a very broad endothermic peak, between 38ºC and 118ºC, which attained a maximum at 86ºC which probably corresponding to the dehydration process of the cyclodextrin. This finding was in agreement with the results obtained by [19]. The authors concluded that in case of HP-β-CyD owing to its amorphous nature, it showed a broad endothermic peak.

For the sparfloxacin alone, it displayed one sharp endothermic peak at about 262ºC corresponding to the melting point of the crystalline form of the drug followed by decomposition phenomena at slight higher temperature. This is similar to the results obtained by [17].

In thermogram of the physical mixture of drug with HP-β-CyD, there is an unchanged broad band due to the HP-β-CyD dehydration, while the sharp endothermic drug peak was decreased in its intensity but did not completely disappeared, this may be due to partial inclusion of drug with cyclodextrin by the physical mixing process. On the other hand, there was no peak corresponding to the sparfloxacin observed in case of the sparfloxacin- HP-β-CyD spray dried complex. This may be explained by the complete inclusion of the drug inside the cyclodextrin cavity by this method.

Thus, these thermal behaviourl changes showed an interaction between the drug and HP-β-CyD and indicated the possibility of inclusion complex formation by these techniques. The thermal analysis of spray dried system revealed the disappearance of the endothermic peak of sparfloxacin at 262ºC. While, there was no change in the endothermic peak at 86ºC, this may be attributed to HP-β-CyD dehydration. This phenomenon may be indicative of complete inclusion complex formed [20].

3.1.2 Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of sparfloxacin, HP-β-CyD, sparfloxacin and HP-β-CyD physical mixture, sparfloxacin- HP-β-CyD inclusion complex are illustrated in Fig. 4. The characteristic peaks of sparfloxacin in FT-IR spectra showed, that is, C = O stretching around 1715 cm^{-1}, C = C at 1620 cm^{-1}, and –CH (1440 to 1500 cm^{-1}), Fig. 4a, while that of HP-β-CyD Fig. 4b 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 [21].
The FT-IR spectrum of the physical mixture Fig. 4c did not differ significantly from those of the individual components. However, the FT-IR spectrum of the sparfloxacin- HP-β-CyD inclusion complex shows no peaks similar to the drug alone. This can be probably due to the inclusion complexation of sparfloxacin into the HP-β-CyD cavity.

3.2 Scanning Electron Microscopy (SEM)

The surface morphology of the powders for sparfloxacin, HP-β-CyD, sparfloxacin and HP-β-CyD physical mixture, and sparfloxacin - HP-β-CyD complex was assessed by SEM. As illustrated in Fig. 5, sparfloxacin existed in plate-like crystal, whereas HP-β-CyD was observed as amorphous, cylindrical spheres. Regarding sparfloxacin and HP-β-CyD physical mixture, the characteristic sparfloxacin crystals were clearly observed, which were mixed with HP-β-CyD particles or adhered to their surface. In contrast, the sparfloxacin-HP-β-CyD spray dried complex appeared in the form of irregular particles in which the original morphology of both components disappeared and tiny aggregates of amorphous particles of irregular size were present. The comparison of these images revealed that, the inclusion

complex was structurally distinct from the isolated pure components and the physical mixture of sparfloxacin and HP-β-CyD. The sizes and shapes of particles of spray dried complex were different from those of the sparfloxacin or HP-β-CyD alone; this confirmed the formation of the inclusion complex of sparfloxacin and HP-β-CD by using the spray dried technique. This was similar to the finding of [19]; they proved that, the inclusion complex of trans-ferulic acid with HP-β-CyD was confirmed by SEM method.

A)

B)

C)

D)

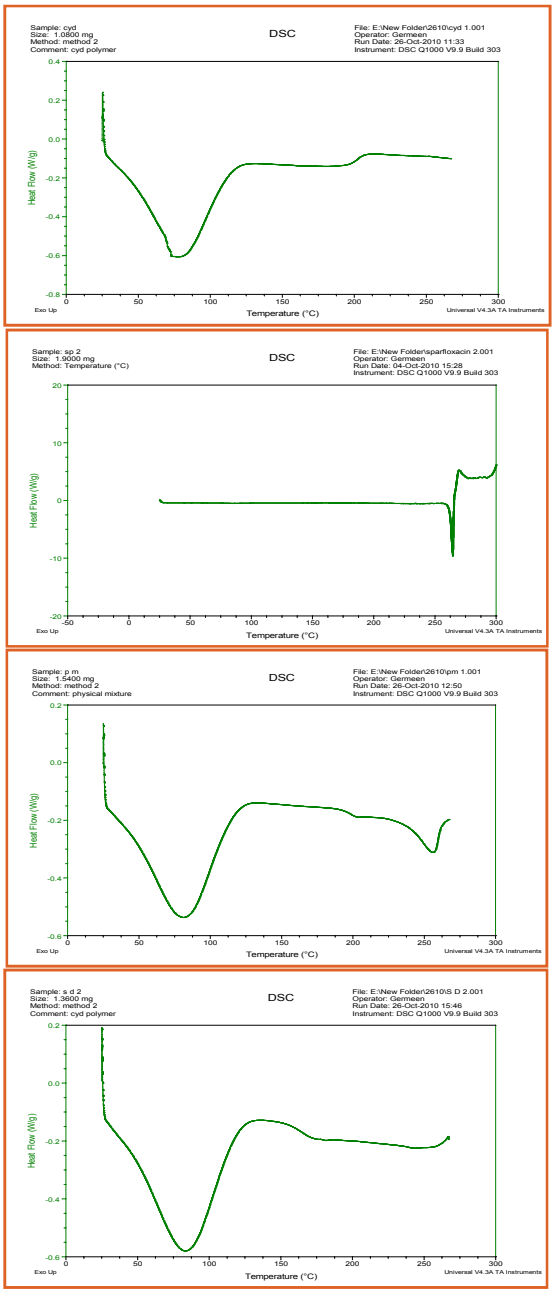

Fig.3. DSC thermograms of A) HP-β-CyD, B) sparfloxacin, C) physical mixture and D) Spray dried complex

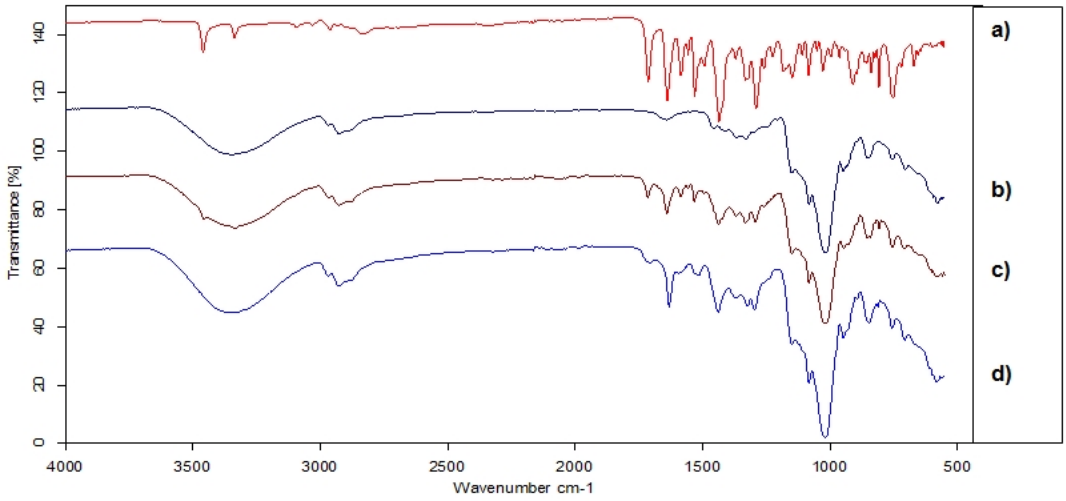

Fig. 4. FT-IR spectra of a) sparfloxacin, b) HP-β-CyD, c) physical mixture and d) spray dried complex

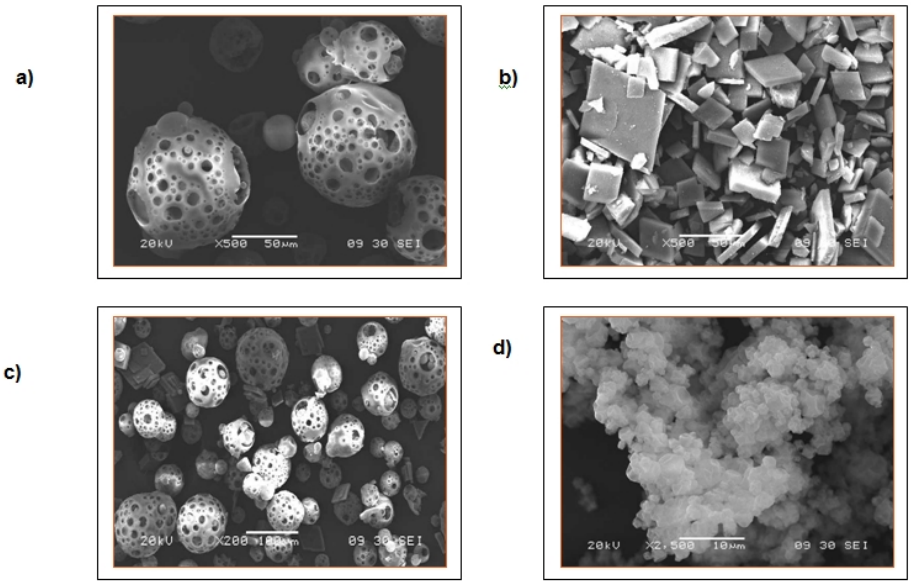

Fig. 5. SEM micrographs of a) HP-β-CyD, b) Sparfloxacin, c) Physical mixture and d) Spray dried complex

3.3 Dissolution Rate Study

In-vitro dissolution results for the untreated sparfloxacin, sparfloxacin - HP-β-CyD physical mixture, and sparfloxacin-HP-β-CyD spray dried complex were illustrated in Fig. 6. As a general trend, there was an increase in the sparfloxacin dissolution rate from the HP-β-CyD physical mixture and spray dried complex compared to the untreated drug. This may be due to the increase of the drug solubility by using HP-β-CyD.

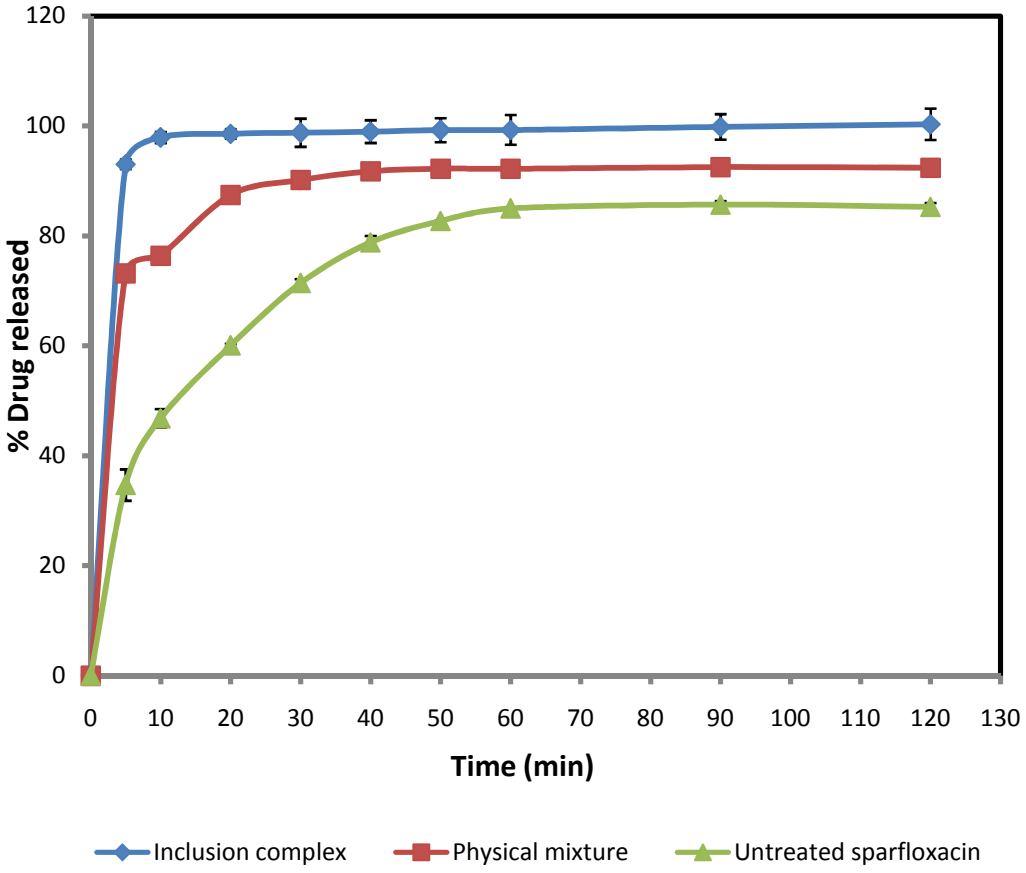

Fig. 6. *In-vitro* dissolution study of sparfloxacin in simulated tear fluid ± S.D.

The untreated sparfloxacin showed a low water dissolution profile due to its low water solubility. From the curve, it was found that, the percent released of sparfloxacin alone after 5 minutes was 34.69%.

Sparfloxacin dissolution rate was improved when it was mixed physically with HP-β-CyD. It showed an increase in the percent released after 5 minutes to be 73%. So, there was more than double increase in the percent by mixing the drug with HP-β-CyD physically. This may be explained on the bases of the partial inclusion of sparfloxacin in the cavity of HP-β-CyD by physical mixing. This was confirmed by DSC results. These results were in agreement with [22]. They proved that, the dissolution rate of tadalafil (which is a poor water soluble and effective phosphodiesterase-5 inhibitor) was enhanced when mixed with CyDs due to the *in-situ* inclusion process between the drug and CyD.

In case of sparfloxacin-HP-β-CyD spray dried complex, it was observed that, there was a highly significant increase in the drug dissolution rate compared to the drug alone. It showed a marked increase of the dissolved percentages during the initial intervals of the dissolution process, to be 93% after the first 5 minutes and 98% after 10 minutes. Finally it was reached

99.8% after 60 minutes. This may be attributed mainly to complete inclusion of the sparfloxacin in HP-β-CyD cavity by the spray drying technique. The complete inclusion was confirmed before by DSC, FTIR results. This was in agreement with [23], who found that there was a marked increase in the dissolution rate of glyburide (a poorly water soluble oral hypoglycemic agent) which might be due to the formation of solid complex as a result of the complete inclusion. Also, they mentioned that, particle size was reduced to the molecular size when the carrier brought the drug into the dissolution medium, leading to fast dissolution.

It was proved that, there was an improvement of amiodarone dissolution rate (water insoluble anti arrhythmic agent) by complexation with β-CyD using freeze drying and spray drying techniques [24].

The high dissolution rate of the former drug-complex was explained by changing the crystalline form of the drug into the amorphous form as indicated by SEM results. This finding was also proved by [7,25].

Also, it was concluded that, there was a marked enhancement of tadalafil dissolution after complexation with CyDs, which was more than 75 % after the first 5 minutes [22].

The results of two-way ANOVA statistical analysis revealed that, there was extremely significant difference between the tested prepared physical mixture and HP-β-CyD complex compared to the untreated sparfloxacin at $P < 0.001$.

Therefore, sparfloxacin-HP-β-CyD spray dried complex prepared in a molar ratio 1 : 2 could be chosen for further ocular formulations of the sparfloxacin. This is owing to an improved water solubility of the formed complex in comparison to the untreated drug.

3.4 Physical Evaluation of the *In-situ* Gels

The results are shown in Table 2. All indicated that, the formulations were colourless or yellow in colour and the clarity was satisfactory. In addition, the pH values of all formulations were within the acceptable range (4-9) which the eye can tolerate, without any irritation [26]. The ophthalmic products outside this range may cause irritation to the eye. And finally the drug contents of all formulations were found to be in the acceptable range.

Table 2. Physical characters of *in-situ* gels

Formulation	Colour	Clarity	pH	Drug content
FP1	colourless	clear	7.06	--
FP2	colourless	translucent	4.2	--
FP3	colourless	clear	4.05	--
FP4	colourless	clear	6.85	--
FM1	pale yellow	translucent	7.01	98 %
FM2	yellow	translucent	5.6	97.23 %
FM3	yellow	clear	4.38	103.8 %
FM4	pale yellow	translucent	6.85	95.09 %

3.5 Rheological Study in Non-Physiological Conditions

All the results of four rheological tests for plain and medicated formulations are represented in Table 3.

3.5.1 Flow test

All four placebo formulations exhibited non Newtonian behaviour with plastic flow for FP1, and pseudo plastic flow (shear thinning) for the other three *in-situ* gels, FP2, FP3, and FP4. According to [11], the administration of the ophthalmic preparation should have as little as possible of the pseudo plastic character of the pre-corneal formulae. The incorporation of the drug complex has limited effect on the type of the flow, so FM1, FM2, FM3, and FM4 have the same flow types as the plain ones.

3.5.2 Linear viscoelastic region (LVR)

It was found that FP1 has LVR of 1 to100 µ.N.m and all other plain preparations have LVR equal 1-10 µ.N.m so, FP1 is the most strong and stable formula this is due to the higher concentration of Pluronic F127 (20%) compared to others which contains only (15%). For FM1, FM3, FM4 the LVR was the same as FP1, FP3, FP4 but FM2, it was found to be 1 to 100 µ.N.m.

This may be explained by the presence of interaction between carbopol 980 and cyclodextrins group especially HP-β CyD leading to decomplexation of the free sparfloxacin from the cyclodextrin cavity. This was previously demonstrated in [27], authors studied the interactions between Carbopol and β-cyclodextrin or hydroxypropyl-β-cyclodextrin by differential scanning calorimetry (DSC) and FT-IR spectroscopy.

As a result of the separation of sparfloxacin from the complex which is less water soluble than the complex, leaving free H-bonding which make crosslinking between two polymers, three dimentional network was formed giving more stronger gel with higher LVR.

3.5.3 Temperature test

It is important to know that the ideal phase transition temperature ($T_{sol/gel}$) for the *in-situ* gel should be located between the average room temperature and 35ºC which is the eye temperature [11]. If it is higher than this temperature the gel will not be formed in the eye and it will be drained like a liquid.

It was found that the T sol/gel for FP1 was 21.3ºC, and for the other three plain gels (FP2, FP3, and FP4) was around 26ºC. This indicates that in non physiological conditions T sol/gel is dependent mainly on the pluronic F127 concentration. The presence of other polymers such as Carbopol 980, chitosan or sodium alginate has no effect on $T_{sol/gel}$. This is in agreement with [11], they proved that, the presence of chitosan in different concentrations did not significantly interfere with the formulations T sol/gel. Also this was confirmed by many authors who reported that, Tsol/gel is dependent on the poloxamer concentrations; there is an inverse relationship between the $T_{sol/gel}$ and the poloxamer concentration [28].

For the medicated preparations, it was found that, the incorporation of sparfloxacin-HP-β-CyD complex in all medicated formulations causes an increase in T sol/gel either a slight increase for FM1and FM2 or a large one in case of FM3 and FM4. This is in accordance with

the results obtained by [29], who found that the addition of human epithelial growth factor-CyD complexes (rhEGF-HP-β-CyD) increased the gelation temperature of poloxamer gel used for ophthalmic delivery system. The author explained this result by a possible mechanism, that the binding force (H-bonding) of cross-linked reticular poloxamer gel became weaker by replacing rhEGF/HP-β-CyD complexes in the gel matrix. In addition, it was observed that, the effect of incorporation of sparfloxacin-HP-β-CyD complex into the gel on the phase transition temperature was found to be greater in case of gels containing law concentration of Pluronic F127 (FM2, FM3, FM4) than that containing higher Pluronic F127 concentration (FM1).

3.5.4 Time test

It is important to know the gelling time of all the prepared formulations, because if the gel takes a long time to form in the eye, the drug may be drained out of the eye before gel formation occurs, leading to deceasing ocular bioavailability.

From the obtained rheograms, the gelling time was found to be in the order of FP2 < FP1< FP4 < FP3.

In addition, the presence of Carbopol 980 lowers the gelling time to great extent more than sodium alginate. On the other hand, the presence of chitosan increases the gelling time of the preparation.

In general, it was found that, all the prepared formulations either plain or medicated will form *in-situ* gels within the first three minutes of their instillation.

3.6 Rheological Studies in Physiological Condition

3.6.1 Linear viscoelastic region (LVR)

From the obtained rheograms after the dilution, it was found that FP1and FM1 showed the same strength and the same LVR range after dilution either by water or STF, indicating their stability. This may be due to higher concentration of Pluronic F127 compared to all other preparations.

3.6.2 Temperature test

From the results shown in Table 3, it was observed that, after dilution with the distilled water all the eight formulations showed an increase in $T_{sol/gel}$. This may be explained by dilution effect which resulted in reduction of polymer concentration in the formulae leading to an increase in $T_{sol/gel}$.

Regarding dilution with STF, FM1, FP1, showed the same $T_{sol/gel}$ after dilution either by STF or distilled water (27.5, 24.4 ºC). This indicating that, effect is due only to the decrease in Pluronic F127 concentration by dilution and that there was no effect of STF composition on $T_{sol/gel}$.

On the other hand, all other 6 formulations containing other polymer in combination with pluronic F127, showed decrease in $T_{sol/gel}$ after dilution with STF compared to the dilution with water, in the same used ratio of dilution and the same method. This is an indication of

the effective composition of STF in case of these preparations. This may be due to the effect of presence of other polymers presence in combination with Pluronic F127 in these preparations. Sodium alginate forms a stable hydrogel in presence of divalent cations of STF (Ca^{+2}) by the interaction between carboxylic functional group located on polymer chain and cation [30].

Table 3. Results of rheology tests in both physiological and non-physiological conditions

Formulation	LVR (µ.N.m)	Breakdown stress (µ.N.m)	Phase Transition Temp. (ºC)	Phase Transition Time (seconds)	Phase Transition Temp. (ºC) after dilution with STF	Phase Transition Temp. (ºC) after dilution with water
FP1	1 to 100	630.96	21.3	39	24.4	24.4
FP2	1 to 10	158.49	26.5	26.5	28.6	32.7
FP3	1 to 10	31.62	26.3	177	27.5	29.6
FP4	1 to 10	25.15	25.8	79	29.6	34.7
FM1	1 to 100	630.96	22.25	44	27.5	27.5
FM2	1 to 100	158.49	26.9	56	24.4	30.6
FM3	1 to 10	31.62	29.8	180	24.4	31.6
FM4	1 to 10	25.15	28.3	44	30.7	36.8

3.7 Texture Analysis Results

From the results represented in Table 4, it was found that among the prepared gels FP1, FM1 have the highest value of hardness compared with the others, this finding was in agreement with the obtained of rheology study as they contain higher pluronic F127 concentration. So the order of hardness for plain gels was FP1 > FP2 > FP4 > FP3. Incorporation of the sparfloxacin-complex in all formulations causes an increase in their hardness except FM3 which became weaker after the incorporation of drug complex within the clear gel matrix (decrease from 2.9 to 1.2 g). This may be explained by that, FM3 is a transparent preparation due to the complete dissolution of sparfloxacin in acetic acid. Also, this could be explained by the drug trapping between the gel network structure leading to loss of the texture of the medicated formulation in case of chitosan-pluronic F127 in-situ forming gel [13].

Table 4. Results of texture analysis test represented in g.

Formulation	Hardness (Without dilution)	Hardness (Diluted by STF)	Hardness (Diluted by water)
FP1	33.9±1.1	20.58±1.2	21.7±1.1
FP2	32.4±2.06	1.1±0.1	1.2±0.1
FP3	2.9±0.1	0.3±0.05	0.4±0.05
FP4	26.36±1.06	0.9±0.06	0.9±0.09
FM1	36.2±1.8	16.58±1.4	17.2±1.5
FM2	35.03±0.5	0.4±0.09	0.3±0.05
FM3	1.2±0.1	1.1±0.1	0.9±0.08
FM4	29.5±2.5	1.1±0.09	1±0.12

The gel textures were decreased after dilution either by STF or water, while FP1 and FM1 were observed to be the highest in resistance to dilution effect compared with others, for example FP1 decreased in texture from 33.9 g to 20.5 g when mixed with STF and to 21.7 g when mixed with water. Also, FM1 decreased from 36.2 g to 16.5 g when diluted with STF and to 17.2 g when diluted with water. On the other hand FP2 texture was extensively decreased from 32.4 g to 1.1 g (STF) and to 1.2 g (water). This may be due to the higher concentration of the pluronic acid (20%) in both FP1, FM1 preparations.

This may be attributed to the fact that poloxamer is non-ionic polyoxythylene–polyoxypropylene–polyoxyethylene triblock copolymer molecules that aggregate into micelles at 34ºC due to the dehydration of the polymer blocks with temperature. The gel formation is a result of micellar enlargements, and the gel is more rigid at higher poloxamer concentrations [31].

3.8 *In- vitro* Drug Release Study with Membraneless Method

The cumulative percentage of sparfloxacin released from all medicated *in-situ* gels and the control were illustrated in Fig. 7. All preparations contained sparfloxacin-HP-β-CyD complex equivalent to 0.3%w/w sparfloxacin. In the case of the control, it was found that the whole sparfloxacin was released into the dissolution medium very quickly after the start of release experiment (it released over 95% after only 15 minutes). However, the incorporation of the sparfloxacin-HP-β-CyD complex in the pluronic F 127 based thermosetting gels caused slowing of the drug release compared to the control at each time interval. This finding was in agreement with that obtained by [32].

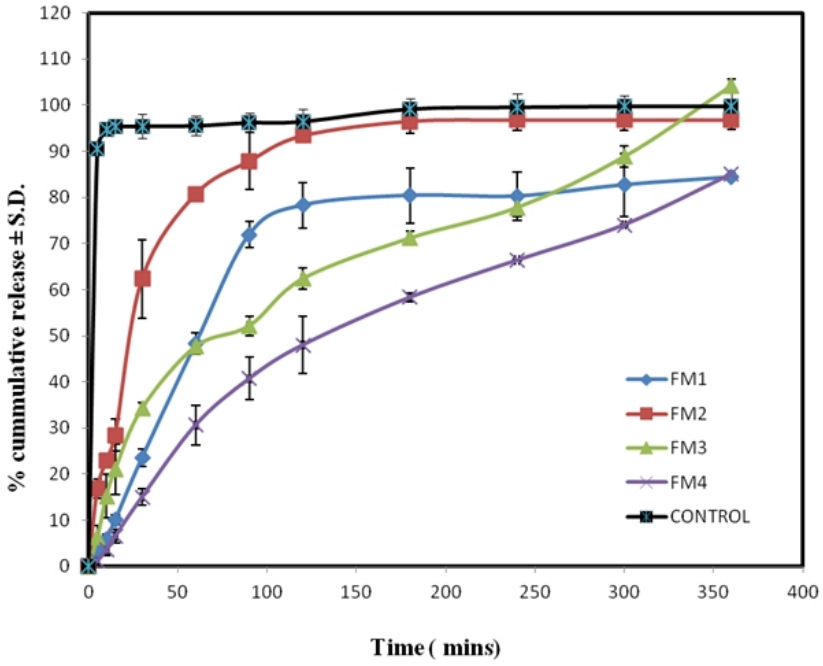

Fig. 7. *In- vitro* release of sparfloxacin from *in-situ* gels containing sparfloxacin-HP-β-CyD complex by membraneless method

The results showed that, there were significant differences in release rate between the four *in-situ* gels containing sparfloxacin-HP-β-CyD complex. It indicated that, FM2 which contains carbopol 980-Pluronic F127 *in-situ* gel showed the fastest drug release profile. The sparfloxacin released was about 80% after only 1 hr, and reached 96% after 6 hrs. While FM1 which containing Pluronic F127 alone released sparfloxacin in slower rate with 48% after 1 hr, and 84% after 6 hrs.

The obtained results may be explained by the presence of HP-β CyD in the formulation. There may be an interaction between carbopol 980 and HP-β-CyD leading to depletion of Carbopol 980 from the preparation, and replacement of the sparfloxacin from the cyclodextrin cavity. These results were in agreement with [27], who confirmed the interactions between carbopol and β-CyD or HP-β-CyD by differential scanning calorimetry (DSC) and FTIR spectroscopy.

In case of FM3, which contain chitosan-pluronic F127 and sparfloxacin-HP-β-CyD complex, it showed a different profile, drug release curve follow biphasic pattern compared to FM1. With a higher rate during first hour, then nearly the same as FM1 which was about 48% after 1hr., and then followed by slower rate. This may be related to the drug trapped between the tortuous ways of the gels which takes longer time with a slower slop to be released [13].

For FM4 (sodium alginate-Pluronic F127 *in-situ* gel containing sparfloxacin-HP-β-CyD complex), it showed a decrease in the drug release rate. There was only 30% drug released after 1hr. and approximately 85% after 6 hrs. This may be due to presence of sodium alginate polymer along with Pluronic F127, it instantaneously formed gels upon its addition to simulated tear fluid as described above, leading to slower drug release.
This was in agreement with [30], who concluded that *in- situ* gelling alginate system is a promising drug carrier for prolonged ophthalmic delivery of pilocarpine.

We acknowledge several limitations to our study. Ideally, each component should be separately treated with the spray-dry technique and assessed with DSC, IR and SEM to confirm complex formation and suitability of the analysis techniques. Ideally, the dissolution process should also be performed using physical mixture prepared from spray-dried drug and CyD. As it is possible that gel formulations in the present work can be achieved by another procedure without using spray drying, it would be best to also prepare the same formulas (FM1, FM2, FM3 and FM4) using non-treated components (Drug and CyD) and then compare with the current results.

4. CONCLUSION

The sparfloxacin-HP-β-CyD inclusion complex was successively prepared by spray-drying method and its characterization was investigated by different analytical techniques. The utilized techniques confirmed the complete formation of the complex in case of spray drying technique, while the partial or incomplete inclusion with the physical mixing method.

It was demonstrated from the *in-vitro* dissolution study of that there was a marked enhancement in the dissolution rate of the sparfloxacin in case of the spray dried complex. Also, there was an improvement in drug released from the physical mixture compared to the untreated sparfloxacin. As a result, it can be suggested that, the more water soluble sparfloxacin-HP-β-CyD complex could be incorporated into ophthalmic thermosetting gels based mainly on Pluronic F127 alone or combined with other polymers in order to improve bioavailability and drug utilization.

The rheological and textural studies showed that, for placebo preparations, a solution to gel phase transition temperature ($T_{sol/gel}$) is 26ºC ± 0.5ºC, but varying gelling times (from 27 to 177 sec.) and gel strengths (2.9 to 33.9 g).

Incorporation of the drug complex increased $T_{sol/gel}$, but had variable effects on the gelling temperature and gel strength.

Dilution of placebo with water resulted in a significant increase in $T_{sol/gel}$, but dilution with STF showed only an intermediate effect. Gel strengths decreased dramatically when diluted, irrespective to the dilution medium. While for medicated gels, after dilution with water, showed the same results as for placebo gels, but dilution with STFshowed striking differences.

It could be concluded that the physical evaluation of the *in-situ* gels indicates that, the biological environment has a great influence on the performance of the formulation. This particular interaction cannot be explained simply by dilution, but shows a complex relationship between the gel composition, the sparfloxacin-HP-β-CyD complex, and the biological environment.

In-vitro release results showed that, the four medicated formulations succeeded to prolong the drug release from the Pluronic F127 based thermosetting gels compared to control. It was found that, FM2 has the fastest release and FM4 the slowest release profile.

The results revealed the possibility of obtaining a sustained release profile of sparfloxacin from a novel formulation approach using thermosetting gels containing sparfloxacin-HP-β-CyD inclusion complex. These results indicate that, this formulation approach could be developed and trialed in the treatment of intra-ocular infections with a reduced dosing frequency.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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