

Formulation and physical characterization of a novel sustained-release ophthalmic delivery system for sparfloxacin: the effect of the biological environment

Abd EL – Gawad H. Abd EL – Gawad¹, Osama A. Soliman¹, Susan A. Barker², and Germeen N.S. Girgis^{1*}

Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, 35516, Mansoura, Egypt.

²School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UK

ABSTRACT

Aims: The purpose of this study was to prepare and evaluate ocular thermosetting gels containing sparfloxacin-HP- β -CyD. The formulations based on pluronic F127 alone in concentration of 20 % or pluronic 15% in addition of other polymeric such as carbopol 980 (0.2%), chitosan (0.3%), and sodium alginate (0.1%).

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 HP- β -CyD spray dried complex using Buchi mini spray dryer. The resultant 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 in concentration of 20 % or pluronic 15% in addition of other polymeric additives such as carbopol 980 (0.2%), chitosan (0.3%), and sodium alginate (0.1%). All placebo and medicated formulations were characterized physically to determine, colour, clarity, pH, and its drug contents. In addition, rheological tests, texture analysis were performed in both non physiological conditions, and after dilution with the simulated tear fluid, in order to estimate the effect of the biological environment on the properties of these thermosetting gels.

Results: sparfloxacin-HP- β -CyD spray dried complex was successfully prepared and characterized by several techniques which 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 sparfloxacin-HP- β -CyD physical mixture. Ocular thermosetting gels containing sparfloxacin-HP- β -CyD complex were prepared simply by cold method using 20 % pluronic F127 alone or only 15 % pluronic F127 combined with other polymers. All prepared placebo and medicated gels were found to be colourless or yellow in colour and with a satisfactory clarity. In addition, the pH values of all formulations were within the acceptable range, which the eye can tolerate, without any irritation. Also, the drug contents of all formulations were found to be in the acceptable range.

The rheology and texture analysis study results revealed that, the prepared formulations are of suitable gelling temperatures, gelling times, and of a good strength to be used in ocular dosage forms. The biological environment has different influences on the phase transition temperature, and gel strength according to the polymer composition of each formulation.

Conclusions: ophthalmic *in-situ* gelling formulations containing the sparfloxacin-HP- β -CyD complex is considered a novel and promising ophthalmic delivery system for sparfloxacin. It showed a good rheological and texture properties by using either pluronic F127 alone (20%) or in low concentration (only 15%), in addition to other polymers. This results confirmed the possibility of lowering the percentage of pluronic F127 by addition of small concentration of other polymers.

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. 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 liquid to 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; it is converted to the gel phase, and thus increasing the precorneal residence time of the delivery system and enhancing ocular bioavailability. According to the mechanism of gelation, *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 25 °C to eye temp 34 °C polymers such as pluronic acid is working by this mechanism. The second is the pH-induced *in-situ* gel systems, in which the gels are formed increasing pH to 7.4, such as: carbopol 980, 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 like sodium and calcium that found in tear fluids polymers working by this mechanism includes sodium alginate and gelrite (Basavaraj et al., 2007).

Nowadays, *in-situ* gel forming systems are of great importance, having the combined advantage being patient convenient with favorable residence time for enhancing ocular bioavailability and for reducing systemic side effects. In addition it could be easily subconjunctivally injected as a method for periocular delivery system to prolong the drug release to the posterior eye segment tissues such as retina, and vitreous in order to treat the ocular diseases of these tissues.

Poloxamer 407 gives a colourless and transparent gel and is widely employed, but they suffer from a major drawback of having weak mechanical strength, which leads to rapid erosion (El Kamel, 2002). As a result, it requires higher concentration of about 25 to 30 % (W/V) to exhibit sol-gel phase transition at 37 °C when used alone. Gelation temperature can be adjusted within the required temperature range of 33-36 °C 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 like 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 (Figure 1) is a new generation fluoroquinolone antibiotic, which effectively has been used to treat eye infections, it provides improved efficacy against important ocular pathogens like *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*. It is reported to be more active *in vitro* than ciprofloxacin against mycobacteria and gram-positive bacteria (Sweetman, 2009). 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.

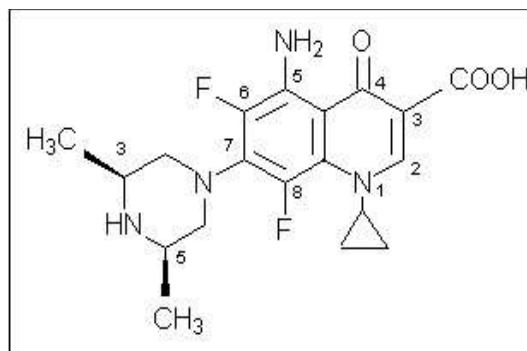


Figure (1): Chemical structure of sparfloxacin

Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity, as indicated in Figure 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 Chao *et al.*, 2003.

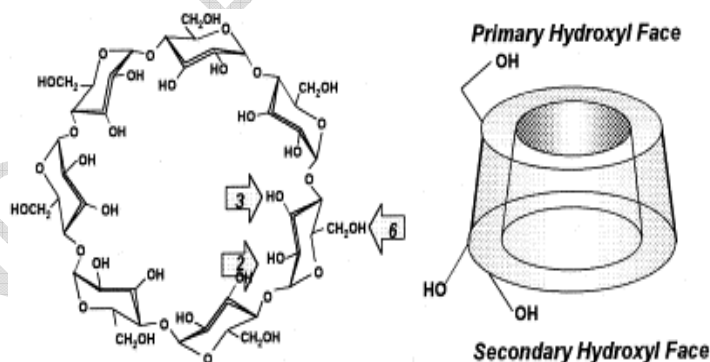


Figure (2): The structure of HP- β -CyD

Hydrophilic cyclodextrins, especially HP- β -CyD, have been shown to be non-toxic and well tolerated in the eye (Loftsson, and Ja'rvinen, 1999).

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 to prepare and characterize an inclusion complex of sparfloxacin and hydroxypropyl- β -cyclodextrin (HP- β -CyD), to develop ophthalmic *in-situ* gelling

formulations containing the sparfloxacin-(HP-β-CyD) complex by using poloxamer either alone or combined with other polymers. And 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-cyclodextrin, poloxamer (pluronic F127), chitosan medium molecular weight, and alginic acid sodium salt from brown algae (All for analytical grades reagents) were purchased from Sigma Aldrich, Co, UK. Sodium chloride, calcium chloride dihydrates and sodium bicarbonate were obtained from Fisher Scientific, UK limited. 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 Phase solubility study

Phase solubility studies were carried out in water according to the method described by **Higuchi and Connors, (1965)**. Excess amount of drug (20 mg) was added into amber screw capped glass bottles or vials to 5 ml of aqueous HP-β-CyD solutions containing gradual increasing concentrations of the complexing agent (from 0 to 15 mM).

Then, the suspensions were shaken using magnetic bars at 25 ° C for 7 days. After equilibrium was achieved, the samples were filtered through 0.45 μm membrane filter and appropriately diluted.

The concentration of sparfloxacin was determined spectrophotometrically at 293 nm. The apparent stability constant K_s of sparfloxacin-cyclodextrin complex was calculated from the slope of the initial straight portion of the phase solubility diagram with the assumption of 1:1 stoichiometry, and according to Higuchi and Connor, (1965) equation 1.

$$K_s = \text{slope} / S_0 (1 - \text{slope}) \quad \text{equation 1}$$

S_0 is the solubility of drug in absence of CyDs.

2.2.1.2 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 85°C, atomized air pressure 3 kg/cm²; process yield being circa 50 %.

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

The physical mixture was prepared by the same method described by **Williams et al., 1998**. This is simply by mixing both the drug and HP-β-CyD, using mortar and pestle in the same molar ratio (1:2) that has been used to prepare the spray dried complex.

146
147 **2.2.1.4 Characterization of sparfloxacin, HP- β -CyD, the physical mixture and the**
148 **inclusion complex**

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

151

- **Differential scanning calorimetry (DSC)**

152 For the thermal analysis of sparfloxacin, HP- β -CyD, physical mixture and the prepared
153 inclusion complex, a Q1000 MTDSC system (TA Instruments, Newcastle, DE) was used.
154 Samples of circa 5 mg in aluminium pans were heated from 25°C to 300°C at 10°C/minute
155 (Chao *et al.*, 2003).

156

- **Fourier transform infrared spectroscopy (FT-IR)**

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

160

- **Scanning electron microscopy (SEM)**

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

166 **2.2.1.5 Dissolution rate study of inclusion complex compared to untreated drug and**
167 **physical mixture**

168 Dissolution studies were carried out for sparfloxacin, physical mixture, and inclusion
169 complexes by the same method described by Aleem *et al.*, (2008) with some modifications.
170 The experiment has been performed by using USP paddle type dissolution apparatus
171 adjusted at 37 °C $\pm$ 1 °C and 100 rpm. The dissolution medium used was 500 ml of freshly
172 prepared simulated tear fluid which was maintained at pH 7.4. The large volume of
173 dissolution medium was used in order to maintain the sink conditions as the methods
174 described by Shikhar *et al.*, 2011.

175 Ten mg of the drug or its equivalent weights of the physical mixture, or the inclusion complex
176 was added to the dissolution medium, samples of 5 ml were withdrawn at time intervals of 5,
177 10, 20, 30, 40, 50, 60, 90, and 120 min. The volume of dissolution medium was adjusted to
178 500 ml by replacing the sample withdrawn with 5 ml of fresh simulated tear fluid (STF) pH
179 7.4. The solutions were immediately filtered through 0.45 μ m membrane filter, suitably
180 diluted and the concentrations of sparfloxacin in samples were determined
181 spectrophotometrically at 290 nm.

182 The results of dissolution are analyzed statistically using one way analysis of variance
183 (ANOVA) followed by Tukey-Kramer multiple comparisons test (Petrie, 1987; Gad and Weil,
184 1989) at the time required for the release of 50% of drug (T_{50%}). Statistical calculations were
185 carried out using Instate - 2 computer programs (Graphpad software Inc., V2. 04 San Diego,
186 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 giving 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

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 4°C using an ice bath, over a magnetic stirrer. Then pluronic F127 was slowly added portion wise with continuous stirring for one hour keeping the temperature at 4 °C. 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 get a clear transparent solution (Gratieri et al., 2010).

For FP2, the carbopol 980 solution was prepared by dispersing the calculated weight in double distilled water with continuous stirring until completely dissolved. Then carbopol pluronic solution was prepared by dispersing the weighed amount of pluronic F127 in the previously prepared carbopol 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 (Lin and Sung, 2000).

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 4 °C. The solution was kept in refrigerator for at least 24 hours to ensure the complete dissolution (Varshosaz et al., 2008).

For FP4, The sodium alginate solution was prepared by dispersing the required weight in water, with continuous stirring till dissolved, and then was cooled to 4 °C; then pluronic was added with stirring for 1 hour using ice bath, kept the partially dissolved solution in refrigerator for at least 24 h till complete formation of *in-situ* gel (Liu et al., 2006).

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 which equivalent to 0.3% concentration of sparfloxacin in all formulations, was slowly added to the plain solution with continuous agitation in ice bath at 4°C till homogenous distribution then stored in screw capped amber glass vials for further studies at 4 °C 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 in the same concentration that equivalent to 0.3% sparfloxacin which has been used to prepare all other formulations (Varshosaz et al., 2008).

Table 1: Composition of thermosetting gels

FORMULATIONS	INGREDIENTS				
	PLURONIC F127	CARBOPOL 980	CHITOSAN	SODIUM ALGINATE	SPARFLOXACIN-HP-B-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 %

241

242 **2.2.3 Physical characterization of thermosetting gels**

243

244 The formulations were evaluated physically for colour, clarity, pH and drug content. The drug
 245 content was measured by taking 1 ml of each formulation diluting to 100 ml with distilled
 246 water in measuring flask. Then 5 ml was diluted to 25 ml with distilled water. The drug
 247 concentration was measured by the spectrophotometer. In addition both the rheological
 248 property and the texture profile of all formulation were estimated in details as in the following
 249 techniques.

250

251 **2.2.4 Rheological studies**

252 Rheological properties were measured using oscillatory rheometry (TA Instruments,
 253 Newcastle, DE). Different four rheological tests were performed in order to evaluate all the
 254 prepared *in-situ* gel properties and each test was repeated at least three times.

255 Data were presented as G' (the solid component) and G'' (the liquid component), with the
 256 transition (or gelling) point being the point at which the two components become equal in

257 magnitude. Both the temperature at which the systems gelled (T_{trans}) and the time the
258 systems took to gel at 37°C was measured.

259

260 **2.2.4.1 Flow test**

261 It was conducted to determine the type of flow of all samples to know either it is Newtonian
262 or Non-Newtonian. The sample was equilibrated at 35 °C (temp of eye surface) for at least 5
263 minutes before the continuous flow test was started, the flow test is destructive one, and so
264 the sample must be discarded after each time.

265

266 **2.2.4.2 Strain sweep (LVR determination test)**

267 It is very important test used to determine LVR (linear viscoelastic region) which is defined
268 as the region where the stress was directly proportional to strain while the storage modulus
269 G' remained constant. It is used to know the maximum strain for the gel and to indicate that
270 any strain above this LVR will cause destruction of three dimensional network of the gel .So
271 any further tests applied for measurement of elastic properties should be made in this LVR.
272 This test was made at 35 °C, sample was equilibrated at this temperature for at least 5
273 minutes before the test began, and a torque sweep over the range of 0.1 and 1000 μ N.M. at
274 frequency of 1 HZ.

275

276 **2.2.4.3 Temperature sweep**

277 It is used to determine the temperature of the phase transition for the gel formulations. The
278 phase transition temperature is defined as the temperature at which the gel is converted
279 from liquid state to solid state. The test was performed by first setting the initial temp at 10
280 °C for 5 min before the test was began, then the temp ramp was performed to be increased
281 from 10 to 45 °C at a rate 1 °C per min with applied frequency of 1 HZ and torque of 10
282 μ N.M for FP1 or 1 μ N.M for FP2, FP3, and FP4. Each sample was performed at least three
283 times, the phase transition temp is called $T_{sol/gel}$ and is determined from the obtained
284 curves by the temperature at which the two moduli G' , G'' (Elastic, Viscous modulus)
285 crossover or intersected each other.

286

287 **2.2.4.4 Time sweep**

288 It is used to determine the gelation time which is defined as the time after which the gel was
289 formed. The initial temperature was set at 10 °C for 5 minutes before the test began, then
290 the temp was raised to 35 °C and the test was made for 10 min at frequency 1HZ and torque
291 of 10 μ N.M for Fp1 or 1 μ N.M for Fp2,Fp3, and Fp4.

292

293 **2.2.5 Texture profile**

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

301

302 Each minitabket was accurately weighed and transferred to a glass vial containing 1.00 mL of
303 STF pH 7.4. To avoid water evaporation, the vials were covered with caps or parafilm
304 membrane. Then all vials were placed in an oscillating incubator, thermostatically set at $32 \pm$
305 1° C and rotating at 25 rpm. During the experiment, aliquots of 100 μ L were withdrawn at
306 pre-determined time intervals. The samples were diluted with 10 % v/v ethanol/STF solutions
307 and the concentration of piroxicam determined spectrophotometrically at 355 nm against a
308 standard calibration curve previously constructed in the same solvent system. The

percentage released at each time interval was expressed as a fraction of the total amount released after 8 hours. The experiment was repeated three times for each formulation.

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 (Wei *et al.*, 2002). These mixtures were subjected to the rheological studies and the texture analysis. Simulated tear fluid (STF) was prepared by Na Cl 0.67 g, NaHCO₃ 0.2 g, CaCl₂.2H₂O 0.008 g, purified water to 100 g, pH 7.4 was adjusted. For comparison, samples diluted with purified water in the same ratio were also studied.

3. RESULTS AND DISCUSSION

3.1 Phase solubility study

The solubilization ability of CyDs can be quantitatively evaluated by the phase solubility method developed by Higuchi and Connors, (1965). It was observed visually that, at high concentrations of HP-β-CyD, the supernatant was less turbid with less drug settling into the bottom of the vial, this may be due to the enhanced wetting of excess sparfloxacin particles at the higher concentrations of HP-β-CyD. So the increase in the solubility of drug by forming higher order complex with respect to HP-β-CyD at higher concentration (Del Valle, 2004).

The phase solubility diagram of sparfloxacin with HP-β-CyD resulted in an A_p - type Higuchi phase solubility diagram (regression R₂ of 0.980 from the linear part of the curve) similar to that reported in case of the complexation of cholesterol and HP-β-CyD by Williams *et al.*, (1998).

Figure (1) is showing the phase solubility diagram of drug in the presence of various HP-β-CyD concentrations. The curve showed a positive deviation from the straight line of A_L-type, where complexes formed were of the first order with regards to the host molecule. Therefore, the formed complexes, in the A_p-type were presented in a higher order than one in the host molecule (i.e. HP-β-CyD). Additionally, this may indicate the formation of 1:1 and 1: 2 stoichiometric ratios of sparfloxacin-HP-β-CyD complexes.

At low HP-β-CyD concentration, a straight portion of the phase solubility diagram was observed with a slope of less than one, so 1:1 complex was predicted, but at the higher concentrations of HP-β-CyD, complexation between one drug molecule and more than one HP-β-CyD molecule was likely to have occurred (Loftsson *et al.*, 2002).

The apparent stability constant was calculated from the initial straight portion of the phase solubility diagram (Fig. 17) by using the following equation 2

$$K_{1:1} = \text{Slope} / \text{intercept} (1 - \text{slope}) \quad \text{equation 2}$$

The value of the stability constant was found to be 105.0228 M⁻¹ (Del Valle, 2004).

3.2 Preparation and characterization of sparfloxacin-HP-β-CyD complex

Spray drying method is presently 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. As a result, greatly improving the dissolution rate of many drug substances, such as tolbutamide, indomethacin

and ibuprofen (Gonnissen *et al.*, 2008). Also complexes of paracetamol and diazepam have been prepared with cyclodextrin using the same method. In addition, Esclusa-Diaz *et al.*, (1996) prepared ketoconazole- β -cyclodextrin multicomponent complex using the spray drying technique.

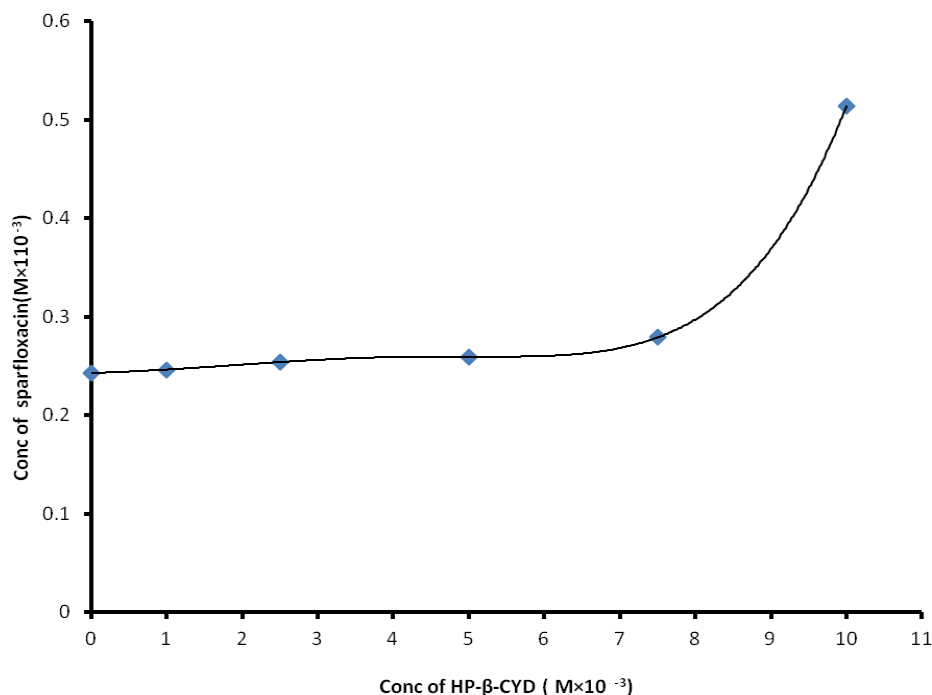


Fig. (3): Phase solubility diagram of sparfloxacin.

360
361

362

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

365

366 **3.2.1 Differential scanning calorimetry (DSC)**

367 The thermal analysis has been reported as an important method to recognize and
368 characterize CyDs complexes. When guest molecules were included inside CyDs cavities or
369 in the crystal lattice, the peak corresponding to their melting point is generally shifted to
370 another temperature. Also the peak intensity may be decreased or disappeared (Liu and
371 Zhu 2006).

372 The DSC curves of pure components and the prepared complex are illustrated in Fig. (2). It
373 was observed that, the DSC curve of HP- β -CyD alone showed a very broad endothermic
374 peak, between 38 °C and 118 °C, which attained a maximum at 86 °C which might be
375 corresponding to dehydration process of cyclodextrin. This finding was in agreement with the
376 results obtained by Wang *et al.*, (2011). The authors concluded that in case of HP- β -CyD
377 owing to its amorphous nature, it showed a broad endothermic peak.

378 For the sparfloxacin alone, it displayed one sharp endothermic peak at about 262 °C
379 corresponding to the melting point of the crystalline form of the drug followed by
380 decomposition phenomena at slight higher temperature. This is similar to the results
381 obtained by Gupta *et al.*, (2010).

In thermogram of the physical mixture of drug with HP- β -CyD, unchanged 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 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 behaviour 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 262 °C. While, there was no change in the endothermic peak at 86 °C, this may be attributed to HP- β -CyD dehydration. This phenomenon may be indicative of complete inclusion complex formed (Jun *et al.*, 2007).

3.2.2 Fourier transform infrared spectroscopy (FT-IR)

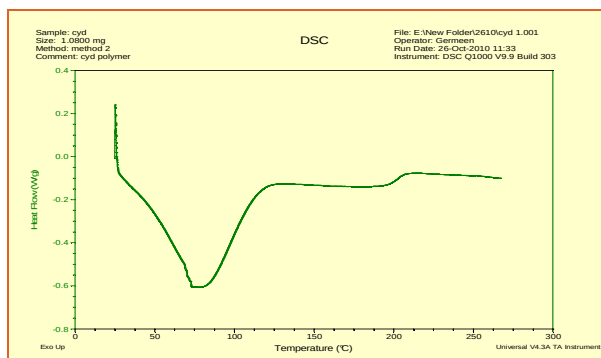
The FT-IR spectra of sparfloxacin, HP- β -CyD, sparfloxacin and HP- β -CyD physical mixture, sparfloxacin- HP- β -CyD inclusion complex are illustrated in Fig. (3). The characteristic peaks of sparfloxacin in FT-IR spectra showed, that is, C = O stretching around 1715 cm⁻¹, C = C at 1620 cm⁻¹, and -CH (1440 to 1500 cm⁻¹), (Fig. 19-a), while that of HP- β -CyD (Fig. 19-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 (Al Omaria *et al.*, 2009).

The FT-IR spectrum of the physical mixture (Fig. 3- c) 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.

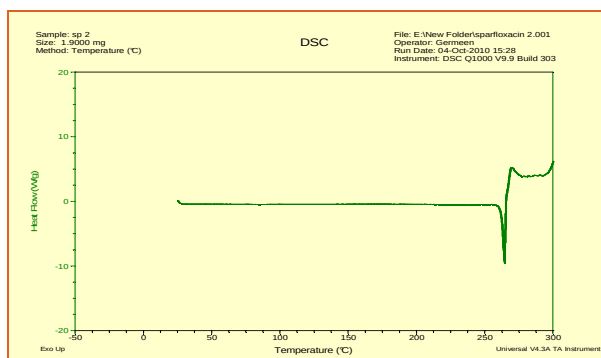
3.2.3 Scanning electron microscopy (SEM)

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. (4), 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 Wang *et al.*, (2011); they proved that, the inclusion complex of trans-ferulic acid with HP- β -CyD was confirmed by SEM method.

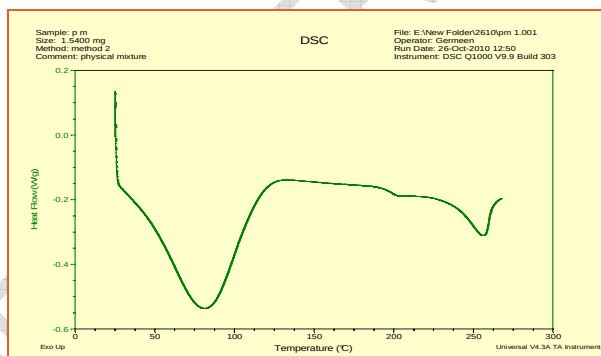
A)



B)



C)



D)

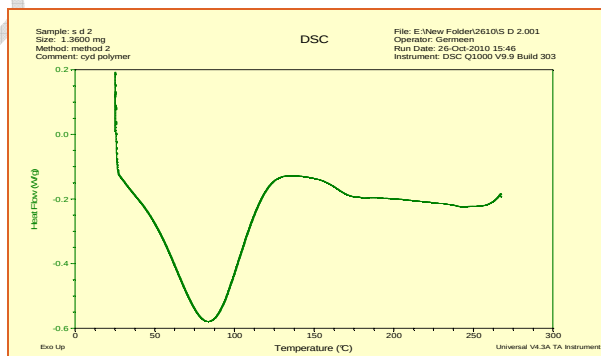


Figure 4: DSC thermograms of A) HP- β -CyD, B) sparfloxacin, C) physical mixture and D) Spray dried complex

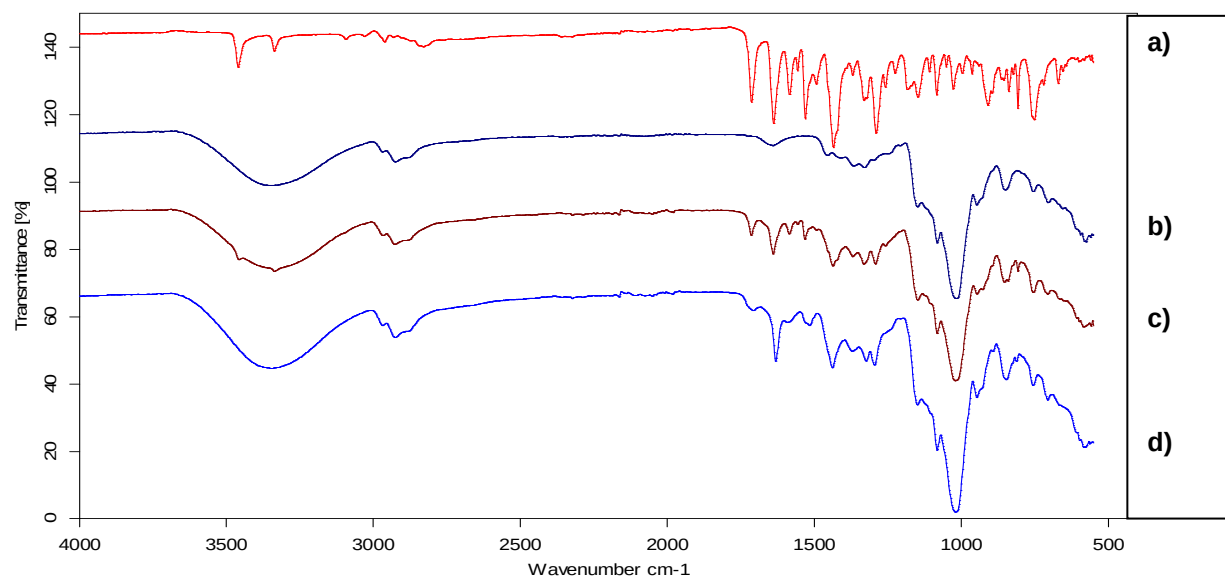


Figure (5): FT-IR spectra of a) sparfloxacin, b) HP- β -CyD, c) physical mixture and d) spray dried complex

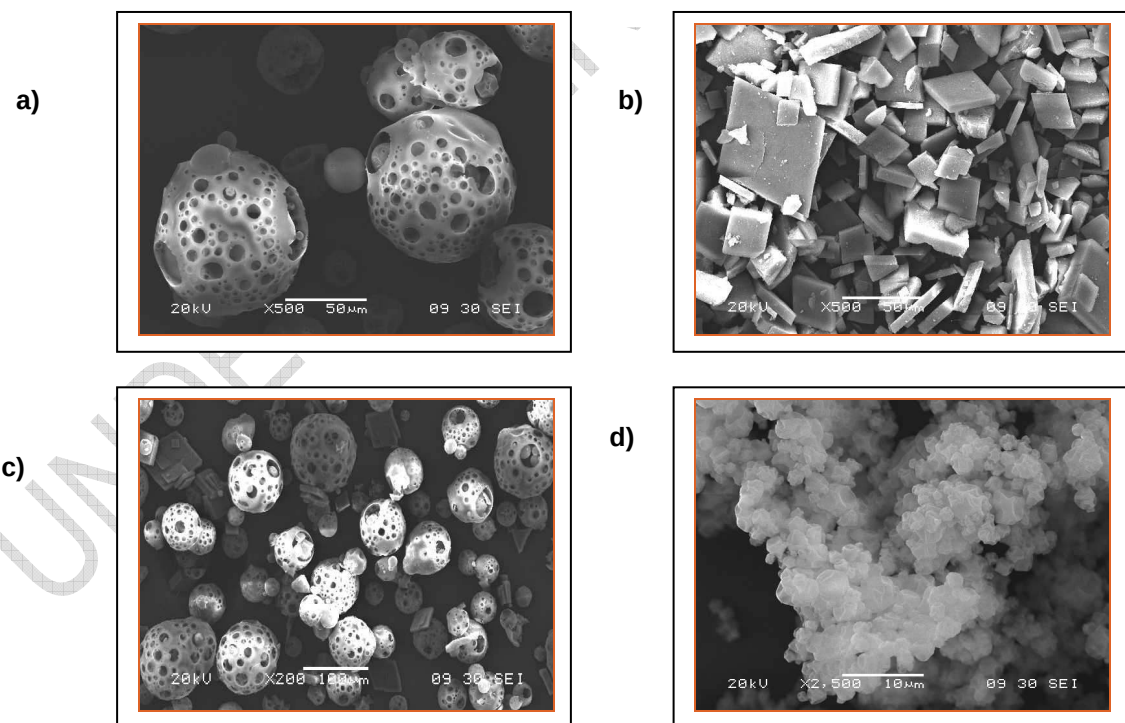


Fig. (6): SEM micrographs of a) HP- β -CyD, b) sparfloxacin, c) physical mixture and d) spray dried complex

3.3 Dissolution rate study

In-vitro dissolution studies for the untreated sparfloxacin, sparfloxacin - HP- β -CyD physical mixture, and sparfloxacin-HP- β -CyD spray dried complex were illustrated in Fig. (5). 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.

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 **Badr-Eldin et al, (2008)**. 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 high 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 min., 98% after 10 min. Finally it was reached 99.8 % after 60 min. 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 **Betageri and Makarla, (1995)**, 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.

Riekes et al., 2010 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.

The high dissolution rate of the formed 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 **Dollo et al., (1999)**, and **Aleem et al., (2008)**.

Also, **Badr-Eldin et al, (2008)**, concluded that, there was a marked enhancement of tadalafil dissolution after complexation with CyDs, which was more than 75 % after the first 5 min.

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 (**Gupta et al., 1986**). 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.

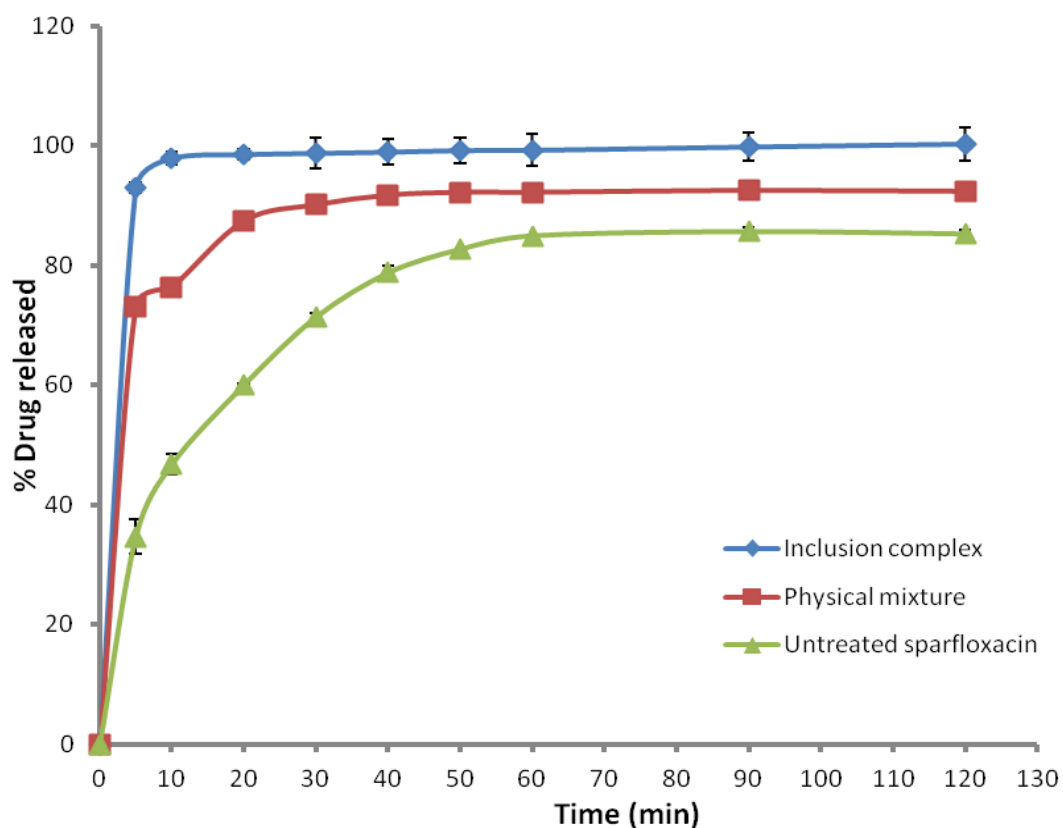


Fig. (7): *In-vitro* dissolution study of sparfloxacin in simulated tear fluid $\pm$ S.D.

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 (9).

3.5.1 Flow test

All the 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 Gratieri et al., (2010), 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 plain ones.

3.5.2 Linear viscoelastic region (LVR).

It was found that FP1 has LVR of 1 to 100 $\mu\text{N.m}$ and all other plain preparations have LVR equal 1-10 $\mu\text{N.m}$ 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 $\mu\text{N.m}$.

3.5.3 Temperature test

It is important to know that the ideal phase transition temperature ($T_{\text{sol/gel}}$) for the in-situ gel should be located between the average room temperature and 35 °C which is the eye temperature (Gratieri et al., 2010). 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 $T_{\text{sol/gel}}$ for FP1 was 21.3 °C, and for the other three plain gels (FP2, FP3, and FP4) was around 26 °C. This indicates that in non physiological conditions $T_{\text{sol/gel}}$ is dependent mainly on the pluronic F127 concentration. The presence of other polymers like carbopol 980, chitosan or sodium alginate has no effect on $T_{\text{sol/gel}}$. This is in agreement with Gratieri et al., (2010), they proved that, the presence of chitosan in different concentrations did not significantly interfere with the formulations $T_{\text{sol/gel}}$. Also this was confirmed by many authors who reported that, $T_{\text{sol/gel}}$ is dependent on the poloxamer concentrations; there is an inverse relationship between the $T_{\text{sol/gel}}$ and the poloxamer concentration (Talasaz et al., 2008).

For the medicated preparations, it was found that, the incorporation of sparfloxacin-HP- β -CyD complex in all medicated formulations causes an increase in $T_{\text{sol/gel}}$ either a slight increase for FM1 and FM2 or a large one in case of FM3 and FM4. This is in accordance with the results obtained by Kim et al., (2002), they 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 low concentration of pluronic F127 (FM3, FM4) than that containing higher pluronic F127 concentration (FM1, FM2).

3.5.4 Time test

It is important to know the gelling time of all the prepared formulations, as if the gel takes long time to be formed in the eye, it may be drained out of the eye before its formation, so decreased ocular bioavailability. From the obtained rheograms, the gelling time was found to

be in the order of FP2 < FP1 < FP4 < FP3. 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 FP1 and 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 (9), 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 °C (27.5, 24.4. This indicating that, effect is only due to decrease in pluronic F127 concentration by dilution and there was no effect of STF composition $T_{sol/gel}$.

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 (Cohen et al., 1997).

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. (°C)	Phase Transition Time (seconds)	Phase Transition Temp. (° C) after dilution with STF	Phase Transition Temp. (° C) after dilution with water
FP1	10 to 100	630.96	21.3	39	24.4	24.4
FP2	1 - 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 (10), it was found that among the prepared gels FP1, FM1 have the highest texture or hardness compared with the others, this finding was in agreement with the obtained of rheology study as they contain higher pluronic F 127 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). 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 (Varshosaz *et al.*, 2008).

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 polyoxyethylene–polyoxypropylene–polyoxyethylene triblock copolymer molecules that aggregate into micelles at 34°C 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 (Mansour *et al.*, 2008).

Table (4): Results of texture analysis test represented in gm.

Formulation	Hardness	Hardness	Hardness
	(Without dilution)	(Diluted by STF)	(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

4. CONCLUSION

The sparfloxacin-HP-β-CyD inclusion complex could be 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.

The phase solubility diagram indicated a A_p type according to Higuchi and Connors classifications. The stoichiometric ratio was assumed to be 1: 2 (sparfloxacin : HP-β-CyD) with a stability constant equals to 105.0228 M^{-1} . It was proved 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, the more water soluble sparfloxacin-HP- β -CyD complex can be incorporated into ophthalmic thermosetting gels based mainly on pluronic F127 alone or combined with other polymers.

The rheological and textural studies showed that, for placebo preparations, a solution to gel phase transition temperature ($T_{sol/gel}$) is $26^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, but varying gelling times (from 27 to 177 sec.) and gel strengths (2.9 to 33.9 gm). 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 STF showed striking differences for the formulations containing 0.3 % w/w chitosan or 0.2 %w/w carbopol 980, in that $T_{sol/gel}$ was decreased compared to the respective undiluted formulations.

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 can not be explained simply by dilution, but shows a complex relationship between the gel composition, the sparfloxacin-HP- β -CyD complex, and the biological environment.

AKNOWLEDGEMENTS

The authors wish to thank the Egyptian Education Mission Sector for funding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al Omaria, M. M., Daraghmeha, N. H., El-Barghouthib, M. I., Zughulc, M. B., Chowdhryd, B. Z., Leharded, S. A., and Badwana, A. A. (2009). Novel inclusion complex of ibuprofen tromethamine with cyclodextrins: Physico-chemical characterization, Journal of Pharmaceutical and Biomedical Analysis 50, 449–458.
2. Aleem, O., Kuchekar, B., and Pore, Y., Late S. Effect of β -cyclodextrin and hydroxypropyl β -cyclodextrin complexation on physicochemical properties and antimicrobial activity of cefdinir. Journal of Pharmaceutical and Biomedical Analysis, 47(3): 535-40.
3. Badr-Eldin, S. M., Elkheshen, S. A., and Ghorab, M. M. (2008). Inclusion complexes of tadalafil with natural and chemically modified β -cyclodextrins. I: Preparation and in-vitro evaluation, European Journal of Pharmaceutics and Biopharmaceutics 70, 819–827
4. Basavaraj, K. Nanjawade, F. V. Manvi, A. S., Manjappa. (2007). In situ-forming hydrogels for sustained ophthalmic drug delivery, Journal of Controlled Release 122, 119–134.

5. Betageri, G. V., and Makarla, K. R. (1995). Enhancement of dissolution of glyburide by solid dispersion and lyophilization techniques, *Int. J. Pharm.* 126, 155–160
6. Chao J, Li J, Meng D, Huang S. (2003). Preparation and study on the solid inclusion complex of sparflaxacin with HP-[beta]-cyclodextrin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*;59(4):705-11.
7. Cohen, S., Lobel, E., Trevoda, A., and Peled, Y. (1997). A novel in-situ forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. *Journal of Controlled Release*, 44(2-3), 201-8
8. Del Valle, E. M. M., Cyclodextrins and their uses: a review. *Process Biochemistry*, 39(9):1033-46 (2004).
9. Dollo, G., Corre, P., Chollet, M., Chevanne, F., Bertault, M., Burgot, J., and Verge, R. (1999). Improvement in solubility and dissolution rate of 1,2-dithiole-3-thiones upon complexation with β -cyclodextrin and its hydroxypropyl and sulfobutyl ether-7 derivatives, *J. Pharm. Sci.* 88, 889–895
10. El-Kamel A.H. (2002). In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. *International Journal of Pharmaceutics*; 241(1):47-55.
11. Esclusa-Diaz, M. T., Guimaraens-Méndez, M., Pérez-Marcos, M. B., Vila-Jato, J. L., and Torres-Labandeira, J. J. (1996). Characterization and in-vitro dissolution behaviour of ketoconazole/ β - and 2-hydroxypropyl- β -cyclodextrin inclusion compounds. *International Journal of Pharmaceutics*. 143(2):203-10.
12. Gad, S. C., and Weil, C. S. (1989). "Statistics for Toxicologists", in: "Principles and Methods of Toxicology", 2nd Ed., Hayes, A. W., ed., Raven press Ltd., New York, PP. 435-483
13. Gonnissen, Y., Verhoeven, E., Peeters, E., Remon, J. P., and Vervaet, C. (2008). Coprocessing via spray drying as a formulation platform to improve the compactability of various drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, 69(1):320-34.
14. Gratieri, T., Gelfuso, G. M., Rocha, E. M., Sarmiento, V. H., de Freitas, O., and Lopez, R. F. V. (2010). A poloxamer/chitosan in-situ forming gel with prolonged retention time for ocular delivery. *European Journal of Pharmaceutics and Biopharmaceutics*. 75(2), 186-93
15. Gupta SK, Uppal RK, Agnihotri S, Jain N. (1986). Need for monitoring strict quality control measures in ophthalmic preparations. *East Pharmacist*., 43–8.
16. Gupta, H., Aqil, M., Khar, R. K., Ali, A., Bhatnagar, A., and Mittal, G. (2010). Sparflaxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 6(2): 324-33.
17. Higuchi, T., and Connors, K. A. (1965). Phase solubility techniques. *Adv. Anal. Chem. Instrum.*, 4: 117-212.
18. Jun, S. W., Kim, M. S., Kim J. S., Park, H. J., Lee, S., Woo J. S., and Wang, H. (2007). Preparation and characterization of simvastatin/hydroxypropyl- β -cyclodextrin

749

- 759 inclusion complex using supercritical antisolvent (SAS) process. European Journal
760 of Pharmaceutics and Biopharmaceutics, 66(3):413-21
- 761
- 762
- 763 19. Lin, H., and Sung, K. C. (2000). Carbopol/pluronic phase change solutions for
764 ophthalmic drug delivery. J. Control. Release, 69, 379–388
- 765
- 766 20. Liu, L. and Zhu, S., (2006). Preparation and characterization of inclusion complexes
767 of prazosin hydrochloride with β -cyclodextrin and hydroxypropyl- β -cyclodextrin. J.
768 Pharm. Biomed. Anal. 40: 122–127
- 769
- 770 21. Liu, L. and Zhu, S. (2006). Preparation and characterization of inclusion complexes
771 of prazosin hydrochloride with β -cyclodextrin and hydroxypropyl- β -cyclodextrin. J.
772 Pharm. Biomed. Anal. 40: 122–127.
- 773
- 774 22. Loftsson, T., Magnúsdóttir, A., Másson, M., Sigurjónsdóttir, J. F. (2002). Self-
775 association and cyclodextrin solubilization of drugs, J. Pharm. Sci., Nov,
776 91(11):2307-16.
- 777
- 778 23. Loftssona, T., Jaärvinen , T., (1999). Advanced Drug Delivery Reviews 36, 59 –79
- 779
- 780 24. Mansour, M., Mansour, S., Mortada, N. D., and ElHady, S. S. A. (2008). Ocular
781 poloxamer-based ciprofloxacin hydrochloride in-situ forming gels. Drug Development
782 and Industrial Pharmacy, 34(7):744-52
- 783
- 784 25. Petrie, A., (1987).Lecture notes on Medical statistics, 2nd Ed., Blackwell scientific
publications, Oxford, U.K.
- 785
- 786
- 787 26. Riekes, M. K., Tagliari M. P., Granada, A., Kuminek, G., Silva, M. A. S., and Stulzer,
788 H. K. (2010).Enhanced solubility and dissolution rate of amiodarone by complexation
789 with β -cyclodextrin through different methods. Materials Science and Engineering:
790 C. 30(7):1008-13
- 791
- 792 27. Shikhar, A., Bommana, M. M., Gupta, S. S., and Squillante, E. (2011).Formulation
793 development of Carbamazepine–Nicotinamide co-crystals complexed with β -
794 cyclodextrin using supercritical fluid process, J. of Supercritical Fluids 55, 1070–
795 1078
- 796
- 797 28. Sweetman, S. (2009). Editor. Martindale: the complete drug reference. London:
798 Pharmaceutical Press;
- 799
- 800 29. Talasaz, A. H. H., Ghahremankhani, A. A., Moghadam, S. H., Malekshahi, M. R.,
801 Atyabi, F., and Dinarvand, R. (2008). In situ gel forming systems of poloxamer 407
802 and hydroxypropyl cellulose or hydroxypropyl methyl cellulose mixtures for
803 controlled delivery of vancomycin, J. Appl. Polym. Sci. 109, 2369–2374
- 804
- 805 30. Van Ooteghem, M.M. (1993), In: Edman, P. (Ed), Biopharmaceutics of Ocular Drug
Delivery, CRC Press, Boca Raton pp. 27–41.
- 806
- 807 31. Varshosaz, J., Tabbakhian, M., and Salmani, Z. (2008). Designing of a
808 Thermosensitive Chitosan/Poloxamer in-situ gel for ocular delivery of ciprofloxacin,
The Open Drug Delivery Journal, 2, 61-70

- 809 32. Wang J, Cao Y, Sun B, Wang C. (2011). Characterisation of inclusion complex of
810 trans-ferulic acid and hydroxypropyl- β -cyclodextrin. Food Chemistry. 124(3):1069-
811 75.
- 812 33. Wei, G., Xu, H., Ding, P. T., Li, S. M., and Zheng, J. M. (2002). Thermosetting gels
813 with modulated gelation temperature for ophthalmic use: The rheological and
814 gamma scintigraphic studies. Journal of Controlled Release, 83(1), 65-74
815
- 816 34. Williams, R. O., Mahaguna, V., and Sriwongjanya, M. (1998). characterization of an
817 inclusion complex of cholesterol and hydroxypropyl- β -cyclodextrin. European
818 Journal of Pharmaceutics and Biopharmaceutics., 46(3): 355-60