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**Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: Design, characterisation, toxicity and transcorneal permeation studies**

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**Abstract**

This study was aimed at preparing, characterising and evaluating in situ gel formulations based on a blend of two hydrophilic polymers i.e. poloxamer 407 (P407) and poloxamer 188 (P188) for a sustained ocular delivery of ketorolac tromethamine (KT). Drug-polymer interaction studies were performed using DSC and FT-IR. The gelation temperature ( $T_{\text{sol-gel}}$ ), gelation time, rheological behaviour, mucoadhesive characteristics of these gels, transcorneal permeation and ocular irritation as well as toxicity was investigated. DSC and FT-IR studies revealed that there may be electrostatic interactions between the drug and the polymers used. P188 modified the  $T_{\text{sol-gel}}$  of P407 bringing it close to eye temperature ( $35^{\circ}\text{C}$ ) compared with the formulation containing P407 alone. Moreover, gels that comprised P407 and P188 exhibited a pseudoplastic behaviour at different concentrations. Furthermore, mucoadhesion study using mucin discs showed that in situ gel formulations have good mucoadhesive characteristics upon increasing the concentration of P407. When comparing formulations PP11 and PP12, the work of adhesion decreased significantly ( $P < 0.001$ ) from  $377.9 \pm 7.79$  mN.mm to  $272.3 \pm 6.11$  mN.mm. *In vitro* release and *ex vivo* permeation experiments indicated that the in situ gels were able to prolong and control KT release as only 48% of the KT released within 12 h. In addition, the HET-CAM and BCOP tests confirmed the non-irritancy of KT loaded in situ gels, and HET-CAM test demonstrated the ability of ocular protection against strongly irritant substances. MTT assay on primary corneal epithelial cells revealed that in situ gel formulations loaded with KT showed reasonable and acceptable percent cell viability compared with control samples.

**Key words:** In situ gel, poloxamer, mucoadhesion, ocular delivery, ketorolac, physico-chemical characterisation, toxicity studies, *in vitro* release, *ex vivo* permeation, HET-CAM test, BCOP test.

## 1. Introduction

Conventional ophthalmic dosage forms, such as solutions and suspensions have many drawbacks, including - rapid precorneal elimination of the drug mainly due to nasolacrimal drainage (Abdelkader et al., 2011; Abdelkader et al., 2010), the need for frequent application, and pulse release from solutions in particular (Almeida et al., 2014). On the other hand, ophthalmic ointments, although provide a prolonged contact with the eye, they may trigger foreign body sensation, blurred vision and cause inconvenience to the patient (Abdelkader & Alany, 2012; Sintzel et al., 1996).

A relatively novel strategy in increasing the contact time of ocular formulations is through the formation of in situ gels using environment responsive polymers (Rupenthal et al., 2011; T.R. Thrimawithana et al., 2012). These polymer-based systems are liquid at room temperature, but undergo sol-gel transition on the ocular surface hence prolonging ocular residence time (Almeida, et al., 2014). Stimuli that may trigger sol-gel phase transition of the polymer network on the ocular surface could be owing to physical (temperature, light) or chemical (ions, pH). Amongst the natural polysaccharides that are considered as ion-activated polymers, the popular ones include gellan gum, kappa-carrageenan, alginates and xanthan gum. These polymers are able to interact with different cations that can be used to form ion-activated in situ gelling systems.(Fernández-Ferreiro et al., 2015).

Gellan gum and xanthan gum are the constituents of the commercially available products Timoptic® XE (Merck) and Timoptic® GFS (Alcon) respectively. These exhibit phase transition with increased ionic strength (Abdelkader & Mansour, 2015). Also, it has been found that the extent of gel-formation of gellan gum increases proportionally with the amount of mono- or divalent cations present in the tear fluid. Hence, the main triggering effect

inducing phase transition is the adequate availability of mono- and divalent cations associated with reflex tearing (Rupenthal et al., 2011; Thrimawithanaa et al., 2011).

On the other hand, carbopol is a polyacrylic acid (PAA) polymer, which exhibits sol-gel transition in an aqueous solution upon raising the pH above its pKa of 5.5. However, with the increase in the concentration of carbopol in the vehicle, in order to improve its rheological properties, the acidity of the vehicle increases. This increase in the acidity of the vehicle could induce ocular tissue irritation and induce lacrimation (Nanjawade et al., 2007) .

Also among commonly used in situ gel polymers is poloxamer 407 (P407), a thermoresponsive polymer that exists in a liquid state at low temperature i.e. between 4–5°C at a concentration range of 20-30% w/w, while converting into a gel upon increasing the temperature of the medium. P407 is a non-ionic polymer (polyoxyethylene-polyoxypropylene-polyoxyethylene; PEO<sub>n</sub>-PPO<sub>n</sub>-PEO<sub>n</sub>), consisting of a central hydrophobic block of polypropylene glycol in addition to hydrophilic blocks of polyoxyethylene (Baeyens et al., 1997). P407 has been widely used in nasal (Li et al., 2014), ophthalmic (Hao et al., 2014), vaginal (Rossi et al., 2014), and topical (Heilmann et al., 2013) formulations. However, one of the limitations of P407 is its weak mechanical strength leading to a rapid erosion of the polymer. Furthermore, it was previously reported that P407 at a concentration of 18% (w/v) or higher, has the ability to transform from a low viscosity solution into a gel under the ambient temperature (Kim et al., 2002; Qi et al., 2007). However, at this concentration the solution will lose its gelation ability after being diluted by lacrimal fluid upon instillation into the eye. Hence, 25% (w/w) P407 can be used in order to ensure the completion of the phase transition process of the polymer under ocular physiological condition. But, under these circumstances, the gelation temperature will be lower than room temperature and P407 solution have to be stored in the fridge, which makes

it inconvenient for use (Wei et al., 2002). Therefore, P188, which is an analog of P407, may be added to P407 solution as a gelation temperature modifying substance (Kim, et al., 2002; Qi, et al., 2007).

Moreover, previous reports have revealed that higher concentrations of P407 are required in a formulation when used on its own; such concentrations were found to be irritant to the eye. In order to overcome this challenge, researchers adopted the approach of blending P407 with other polymers like methyl cellulose, chitosan, and P188 in order to decrease the total concentration of P407 used, improve its gelling characteristics as well as mechanical properties of P407 and reduce its ocular irritation potential (Almeida, et al., 2014).

Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug that has potent analgesic activity; it is used for treating post-operative eye inflammation and reducing conjunctivitis (Gupta et al., 2000; Sunil et al., 2013). There are other potent NSAIDs that are currently used for ophthalmic conditions e.g. nepafenac 0.1% w/v (Nevanac®) which is a prodrug that is rapidly converted into amfenac after passing through the cornea. Also, bromfenac 0.09% w/v (or 0.1% w/v when labelled as the salt form) (Xibrom® and Bromday®) is available as ophthalmic solution. A previous animal study demonstrated that bromfenac 0.09% w/v, pH 8.3 readily penetrates ocular tissues. Both drugs are used for the treatment of pain and inflammation associated with cataract surgery (Baklayan & Muñoz, 2014; Kim et al., 2010).

It has been reported that the ocular bioavailability was up to 4% for the KT ophthalmic solution (0.5% w/v) following topical ocular administration in the form of conventional eye drops in anesthetized rabbits. The drug concentrations in the aqueous humour were compared after topical application with those obtained after intracameral injection of an equivalent dose of 0.25 mg of ketorolac tromethamine per eye using <sup>14</sup>C (Ling & Combas, 1987; Walters et

al., 2007). Such factors have prompted research for the development of a more safe and effective KT formulations (Schoenwald, 1985; Sinha et al., 2009). Various in situ ophthalmic preparations have been formulated incorporating KT in order to sustain its release, thus improving its ocular bioavailability as well as reducing the frequency of administration. Nanjwade et al. prepared pH-triggered in situ gel for sustained ophthalmic delivery of ketorolac tromethamine, using carbopol 934 and hydroxypropylmethylcellulose (Nanjwade et al., 2009). Also, KT has been used in form of hydrogel for nasal delivery using poloxamer 407 and carrageenan (Li, et al., 2014)

More recently, ofloxacin loaded Pluronic F127 and Pluronic F68 (20% w/v) in situ gelling formulation have been prepared and characterised. These deemed promising ocular formulation due to prolonged pre-corneal retention and good ocular tolerability using slug mucosal irritation assay and bovine corneal erosion study (Al Khateb et al., 2016).

The present study focuses on investigating into different polymer combination of P407 and P188 for ocular delivery of the non-steroidal anti-inflammatory drug KT. Physicochemical characterisation of drug/polymer interactions were investigated with DSC and FT-IR studies. The gelling properties and rheological characteristics for these formulations were studied. The mucoadhesive characteristics, in vitro release as well as *ex vivo* corneal permeation of KT were investigated. Furthermore, the ocular irritation potential of the KT-loaded in situ gel formulations was determined using a combination of the HET-CAM and BCOP tests. Finally the MTT cytotoxicity assay was carried out using a corneal epithelial cell line in order to elucidate the toxicity of the developed KT-loaded in situ gel formulations.

## **2. Materials and methods**

### **2.1. Materials**

Ketorolac tromethamine (KT), poloxamer 407 (P407, culture tested), and poloxamer 188 (F-168) were purchased from Sigma Aldrich chemical Co., Gillingham, UK. Whole fresh porcine eyes were purchased from C.D Jennings & Sons abattoir, Surrey, UK. All other chemicals and solvents were of analytical grade and used as received from the supplier. Fertilised white Leghorn eggs were purchased from Henry Stewart & Co. Ltd. Fakenham, Norfolk, UK. Bovine eyes were purchased from ABP Guildford Slyfield Industrial Estate, Guildford, UK. Primary Corneal Epithelial Cells; Normal, Human (ATCC® PCS-700-010™) were acquired from ATCC company, Manassas, VA, USA.

### **2.2. Determination of drug – polymer interactions**

#### **2.2.1. Differential scanning calorimetry (DSC) study**

DSC study was carried out on Mettler Toledo DSC 822e0, Switzerland. The drug, the polymers (P407 and P188) as well as their physical mixtures (PM) with KT was weighed separately in aluminium pans, covered with aluminium lids and hermitically sealed using a pan press (Thermal Science, USA). Once in the calorimeter, the temperature of the pan was gradually increased from 25°C to 300°C at a rate of 10°C/min. Nitrogen was purged at a flow rate of 45 mL/min. The data generated was consolidated using Mettler STARe software version 8.10.

#### **2.2.2. Fourier transform infrared spectroscopy (FT-IR)**

FT-IR spectrometer (Thermo Scientific Nicolet iS5, Thermo fisher, USA) was used to record the FT-IR spectra of KT, P407, and P188 and their PM. Sufficient amount (2 - 4 mg) of the



sample was placed to form a thin film covering the diamond window. The FT-IR spectra were recorded at a resolution of  $2\text{ cm}^{-1}$  with an average of 120 scans. The data was acquired and analysed using Omnic software (Omnic version 8.2, USA).

### **2.3. Preparation of in situ gel formulations**

Different amounts of P407 and P188 were dissolved in cold deionised water that has been equilibrated at  $4 - 8^{\circ}\text{C}$  before use to prepare various concentrations of P407: P188 (% w/v). The solutions were stirred for 2 h and within this period 5 mg/mL KT is added to the preparation and then kept in a refrigerator for at least 24 h to ensure complete dissolution.

### **2.4. Determination of physical properties of in situ gels**

Before performing characterisation studies, all preparations were subjected to visual examination for clarity, prior to and after gelation, then the gelation temperature and time were determined as follows.

#### **2.4.1. Determination of gelation time and temperature**

The gelation time was determined using an aluminium pan which was placed on a hot plate equilibrated at  $(35^{\circ}\text{C})$ . A few drops of each test solution were instilled onto the pan using a Pasteur pipette. The pan was then tilted at  $90^{\circ}$  to examine the gelation. The final gelation time is recorded when the free flowing solution transforms into a thick textured gel and ceases to flow (no change in meniscus) upon tilting by up to  $90^{\circ}$ . The gelation temperature or the sol-gel transition temperature ( $T_{\text{sol-gel}}$ ) was determined by the tube inversion method. Briefly, the test solution was placed in a test tube which was dipped in a water bath maintained at a

temperature of  $40.0 \pm 1^\circ\text{C}$  for 5 min. The temperature at which the test solution was converted to gel ceases to flow with no change in meniscus upon tilting up to  $90^\circ$ , was recorded.

### **2.5. Determination of rheological properties of in situ gel formulations**

The viscosity of different P407 and P188 based formulations were measured using a Brookfield viscometer (DV-II+Pro), Brookfield Engineering LABS. Inc. MiddleBoro, MA, USA. Spindle type 62 was used at different shear rates (10, 20, 50, and 100 rpm) at  $4^\circ\text{C}$  and matching spindle rate to get the working range of the torque. Measurements were carried out where 25 mL sample was placed in a glass tube and the rotation speed was kept constant for at least 60 s before a reading was taken to ensure consistency among different preparations which were done in triplicate and the mean values  $\pm$  SD were calculated.

### **2.6. Mucoadhesion of in situ gel formulations**

The mucoadhesion ability of the formulated in situ gel systems was determined using a TA-XT plus texture analyser (Stable Micro Systems, UK) in a special adhesive mode. The in situ gel formulations PP3, PP7, PP11 and PP12 were selected based on their predetermined gelation time,  $T_{\text{sol-gel}}$ , and rheological properties. Mucin discs were prepared by compression of porcine stomach mucin Type II (250 mg) using a ring press (10 mm in diameter) at a compression force of 10 tonnes, that was applied for 30 s. The discs were attached horizontally to the lower end of a TPA probe using double sided adhesive tape. About 35 g of each sample was placed in 50 mL glass cylinder and brought to  $35^\circ\text{C}$  using a thermostatic water bath. Then, the mucin discs that were previously attached to the probe was brought in contact with the surface of the gel sample using a downward force of 5 g which was applied for 180 s to ensure adequate contact between the mucin disc and the gel surface. The probe

was then raised at a speed of 0.5 mm/s to a predetermined distance of 5 mm and the established force–distance curve was recorded for each formulation. The force required to detach the mucin disc from the gel was recorded and denoted as the adhesion force (mN); the area under the force–distance curve was estimated as the work of adhesion (mN.mm) (Xu et al., 2014).

### **2.7. In vitro release study**

An aliquot (1 mL) of the formulation equivalent to 5 mg/mL KT was transferred to a donor chamber in Franz-diffusion cell; it was then occluded with parafilm. The receptor chamber (12 mL volume) was filled with PBS pH 7.4 and stirred constantly using small magnetic bar. Donor and receptor chambers were separated by means of a dialysis (cellophane) membrane with a molecular weight cut-off of 12,000 - 14,000 Da. pre-soaked in the receptor medium overnight prior to the experiment. The temperature was set at  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . Samples (1 mL each) were withdrawn at predetermined time points for up to 12 h, and replaced with an equal volume of the receptor medium. The experiments were carried out in triplicate and the samples containing KT were determined by an in-house developed HPLC method (Fathalla et al., 2015).

### ***Ex vivo* permeation study**

Corneas used in the *ex vivo* experiments were obtained from freshly collected porcine eyes. The eyes were examined for any visual defects and were stored in normal saline solution. Porcine cornea (0.67 mm) mimic human cornea (0.52 mm) anatomically although the former is slightly thicker than the latter (Faber et al., 2008; Worakul & Robinson, 1997). They were directly transported to the laboratory and used within few hours of enucleation. Franz

diffusion cell was used and the tissue was placed between the donor and the receptor compartments with the endothelial side facing the receptor compartment; temperature was maintained at 35°C and for each formulation, three corneas were used (n = 3). Then the same steps carried out in the *in vitro* release study were followed in this study. The amount of KT that permeated across porcine corneas was quantified by HPLC method.

### 2.7.1. *Ex vivo* data analysis

The apparent permeability coefficient ( $P_{app}$ , cm/s) was calculated according to the following equation 1 (Abdelkader, et al., 2011).

$$P_{app} = \frac{\Delta Q}{\Delta t(3600)AC_0} \quad \text{Equation 1}$$

Where  $\Delta Q/\Delta t$  is the permeability rate constant of KT across the excised porcine corneas. It was calculated from the gradient of the plot of the amount of KT permeated (Q) versus time (t);  $C_0$  is the initial drug concentration ( $\mu\text{g/mL}$ ), A is the corneal surface area ( $\text{cm}^2$ ) in contact with the formulation from the epithelial side and the release media from the endothelial side and 3600 is a factor used for the conversion of hours to seconds (s). The lag time ( $t_L$ ) was determined by extrapolating the linear plot to the x-axis.

### 2.8. The Hen's egg test on chorioallantoic membrane (HET-CAM)

Freshly collected fertilised hen's eggs (White Leghorn) were incubated at  $37.5^\circ\text{C} \pm 0.5^\circ\text{C}$  and  $66 \pm 5\%$  relative humidity (RH) for 3 days according to the HET-CAM procedure previously described by (Luepke, 1985). The eggs were kept horizontally in their trays and rotated gently every day to make sure that the embryo was positioned properly. After 72 h of incubation, the egg shells were opened by cracking the underside of the egg, then the eggs

were poured into a Petri dish, according to the modified HET-CAM method (Abdelkader et al., 2012; Alany et al., 2006; Rupenthala, et al., 2011). Once in the growing dish, the egg was examined for the viability of the embryo (intact CAM and yolk). Only viable embryos with an intact CAM and yolk sac were further incubated at previously mentioned conditions. On day ten, 200  $\mu$ L of each test formulation was placed on the membrane. For each test substance three eggs were used. NaOH (0.5 M) was used as a positive control for a strong irritant effect, propylene glycol as a slight irritant, and normal saline as a negative control (Alany, et al., 2006). The blood vessels were examined for irritant effects like hyperaemia, haemorrhage and clotting after application for different time intervals (0.5 min, 2 min and 5 min). The sum of the time-dependent numerical scores for all three irritant responses gave a single numerical value. This value interpreted the irritation potential of the test substance. The mean score value of the test allowed the assessment by a classification scheme analogous to the well-known Draize test (Luepke, 1985). A cumulative score of  $< \text{or} = 0.9$  was considered as non-irritant;  $1 < \text{cumulative score} < 4.9$  was slight irritant;  $5 < \text{cumulative score} < 8.9$  was moderately irritant and  $9 < \text{cumulative score} < 21$  was severe irritant (Abdelkader et al., 2014).

### **2.9. Bovine corneal opacity and permeability (BCOP) test**

Bovine eyes were obtained from a local slaughterhouse (Guilford Meat Processors, UK). The eyes were freshly collected and transported to the laboratory in cold saline and used on the same day. The eyes were examined for epithelium detachment, corneal opacity and corneal vascularisation. Eyes with corneal damage were discarded. Three different controls were used for validation purposes; 0.5 M NaOH was used as a strong irritant control (positive control), propylene glycol as a slight irritant, and normal saline as a negative control. The same components and formulations described in the HET-CAM test were investigated. Small

plastic cups were used to hold the eyes (cornea upwards), which were then placed in a humid atmosphere of a closed water bath at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for 10 min (Weterings & Vanerp, 1987). A silicon O-ring (thickness 1.78 mm, an internal diameter 7.6 mm) was carefully placed on the central part of the cornea, to identify and localise the application site and ensure easy and reproducible test material application. One drop of saline was instilled inside the O-ring and the eyes were equilibrated in the closed water bath for 5 min. The test substance was applied to the cornea inside the ring at a volume of 0.1 mL test substance. After 30s, the eyes were rinsed with saline (approximately 10 mL), followed by further incubation in the closed water bath for an additional 10 min. Then, the extent of corneal injury was assessed by evaluating the opacity visually, followed by application of sodium fluorescein solution (2% w/v, pH 7.4) to examine the integrity of the corneal epithelium. The fluorescence was visualised using an examination lamp with a cobalt blue filter (Leica, GmbH, Germany). The observations were graded according to individual numerical scores for opacity, epithelial integrity (degree of staining) and epithelial detachment (Weterings & Vanerp, 1987). The scoring system of bovine cornea is based on assessing the disruption that a test substance can make to the epithelium barrier and consequently, corneal opacity and permeability induced. Corneal opacity which is determined by measuring the amount of light transmitted through the cornea, and corneal permeability is determined by measuring the amount of fluorescein dye that penetrates to the corneal stroma (Verstraelen et al., 2013). The sum score was calculated and the mean scores for each of the 3 exposed eyes were used to interpret the corneal irritation potential of the tested formulations.

#### **2.10. MTT reduction cytotoxicity test**

The MTT cytotoxicity test was conducted according to Mosmann's procedure (Mosmann, 1983) to assess mitochondrial function and cell viability of the corneal epithelial cells.

Primary human corneal epithelial cells (ATCC pcs-700-010) were prepared and seeded out at approximately  $2 \times 10^4$  cells/well into 96 well plates (Nunc) in Corneal Epithelial Cell Basal Medium containing (final concentration) the following supplements (LGC standards); apo-transferrin (5 mg/mL), epinephrine (1.0 mM), Extract P (0.4%), hydrocortisone hemisuccinate (100 ng/mL), L-glutamine (6 mM), rh insulin (5mg/mL) and CE Growth Factor (1 mL, proprietary formulation).

Cells were allowed to establish for 48 hours prior to treatment in the 96-well culture plate. Media were subsequently removed and fresh media containing treatments (4 wells used per condition) added. The treatments were in situ gel formulations both plain and loaded with 5 mg/mL KT includes (PP7 and PP11) in addition to KT solution (5 mg/mL). These formulations were prepared in culture media and all the solutions were prepared under aseptic conditions. The untreated media was used as a negative control; meanwhile hydrogen peroxide ( $H_2O_2$ ) (100 mg/mL) and benzalkonium chloride (BKC) at a concentration of 0.01% w/v were used as positive controls. After 4 h and 24 h of treatment, the media was aspirated carefully and the cells washed twice with 37°C sterile PBS. Cells were then incubated with 200 $\mu$ l per well of 0.5 mg/mL 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution in 37°C Corneal Epithelial Cell Basal Medium (LGC standards) with no additions for 4 h at 37°C. After incubation, the MTT solution was carefully removed and the wells washed twice with sterile PBS. Finally, 200  $\mu$ L of dimethyl sulfoxide (DMSO) was added to each well to lyse the cells. The cells were then gently agitated to mix the lysate and analysed on a TECAN Infinite M200 pro plate reader at a wavelength of 540 nm. Experiments were performed in triplicate and mean values were calculated. Results were expressed as a percentage of control cultures.

## 2.11. Statistical Analysis

All experiments were performed on three independent preparations and a mean value was determined  $\pm$  SD. Statistical analysis was performed using Graphpad Prism 6 (2014) software. A one-way analysis of variance (ANOVA) or a non-parametric Kruskal-Wallis test was performed on the data where appropriate. Dunnett's post hoc test was performed and a P value of  $< 0.05$  and  $< 0.001$  was considered to be statistically significant.

## 3. Results and discussion

Previous reports suggest that P407 and P188 solutions individually cannot undergo sol-to-gel transition at temperatures appropriate for ocular application (Abdelkader & Alany, 2012; Abdelkader & Mansour, 2015). For example, preformulation studies showed that P407, at a concentration of 10% and 30% w/w, has gelling temperatures which are higher than 40°C and lower than 25°C, respectively. However, by tailoring poloxamer mixtures of specific concentrations, it is possible to modulate the gelation temperature and to obtain a sol-gel transition temperature ( $T_{\text{sol-gel}}$ ) suitable for ocular application, i.e. near to corneal temperature (35°C) (Mayol et al., 2008).

### 3.1 Evaluation of drug -polymer interactions

#### 3.1.1. Differential scanning calorimetry (DSC) study

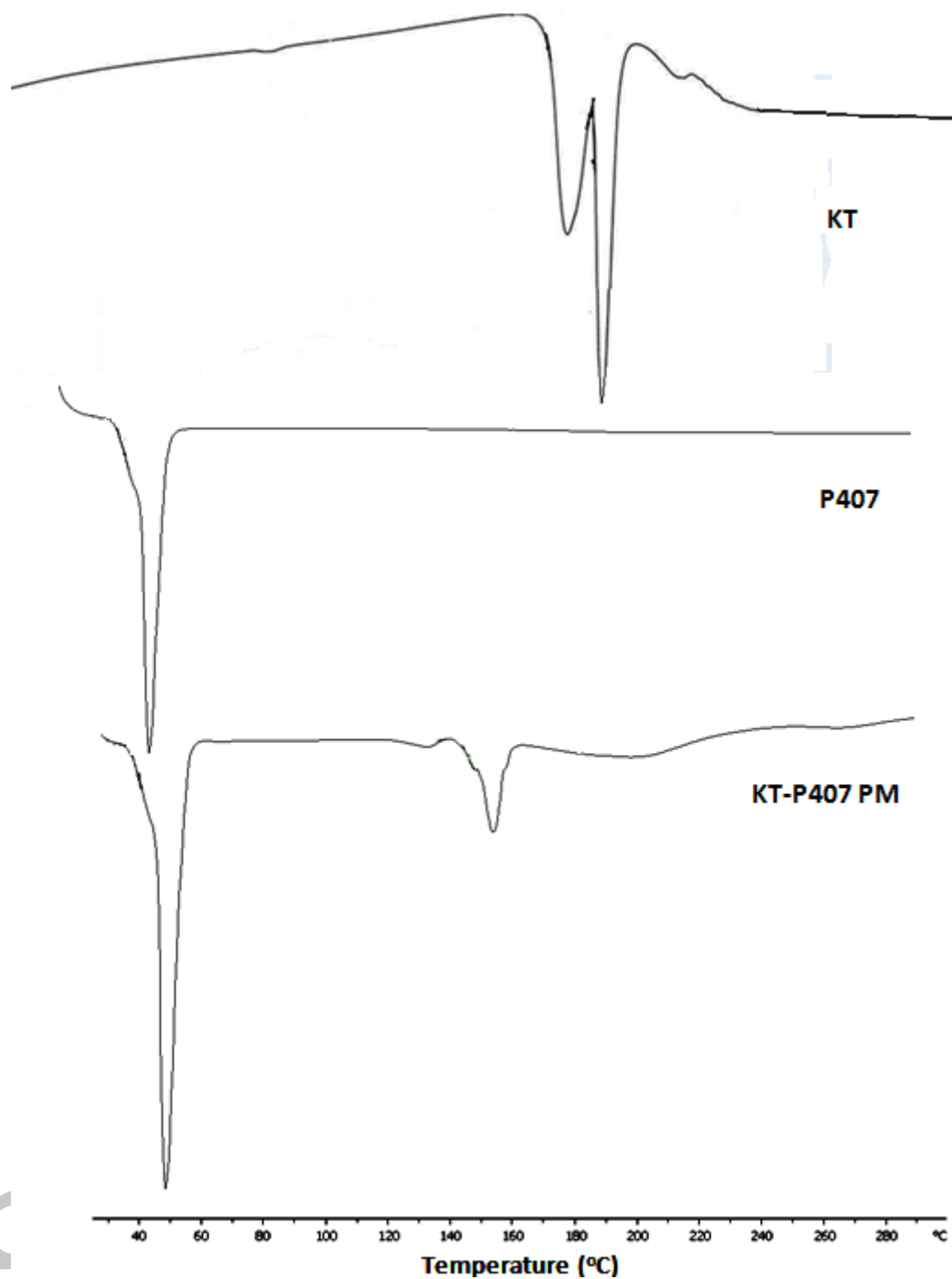
Figure 1A shows the thermal traces of KT, P407 and their PM (1:1 w/w). The thermal behaviour recorded for KT comprised two endothermic peaks at 168.9°C, and 167.5°C. These peaks related to the melting of KT. This is typical to the thermal melting behaviour of tromethamine salt (KT salt) (Abdelkader et al., 2007)

On the other hand, P407 showed endothermic peak at 50°C due to its melting point and this result complies with the data reported elsewhere (Garg et al., 2013). The PM of KT and

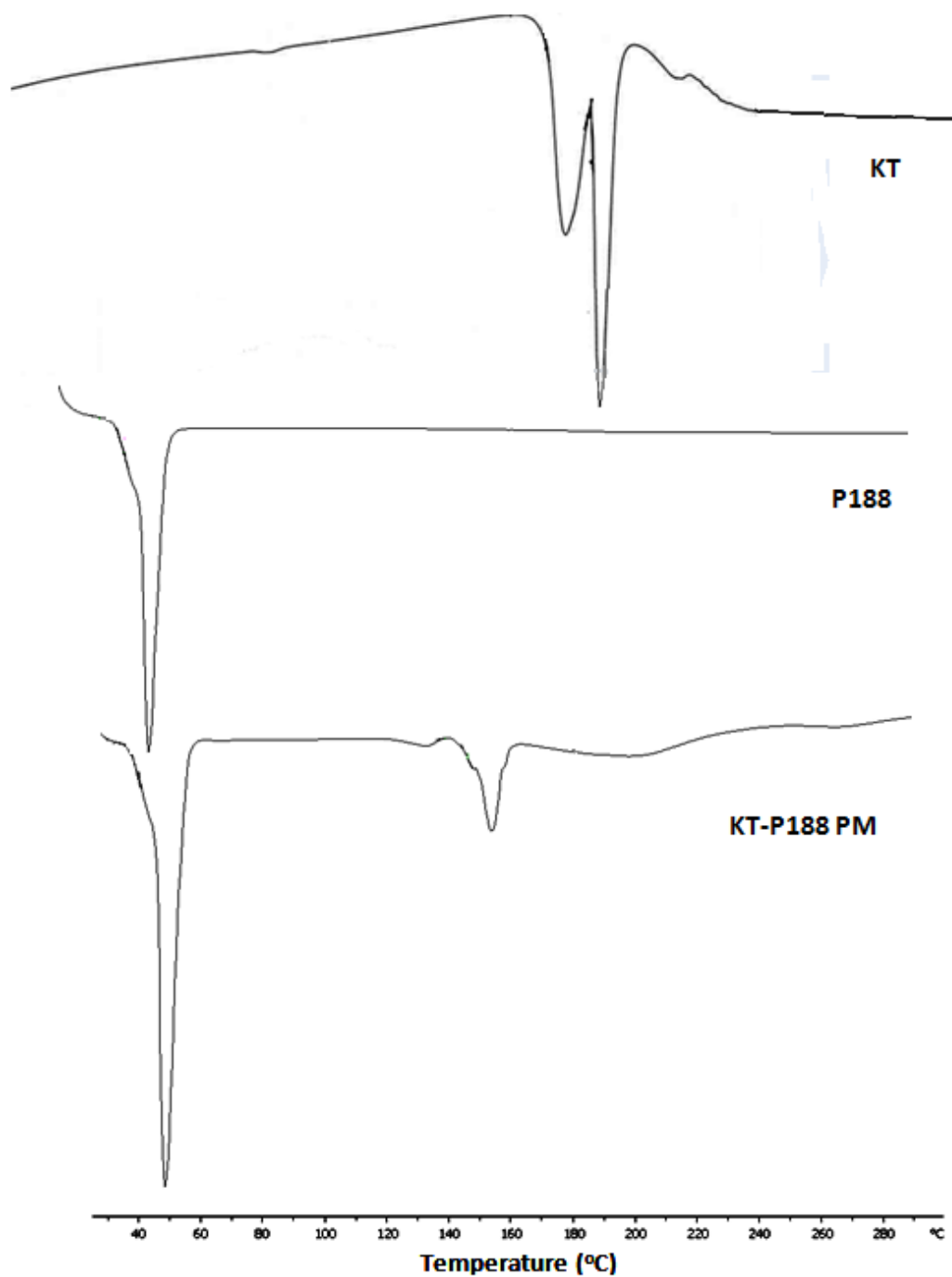


P407 showed two endothermic peaks at 158°C and 51°C, corresponding to the melting points of both the polymer (up) and that of drug which showed slight shift to a lower single peak (down) respectively, which suggested that there may be a weak interaction between drug and P407. This could be due to possible electrostatic interaction between the drug and the polymer.

Figure 1B indicates that there was no difference in the DSC traces of P407-KT and P188-KT. The DSC traces for KT, P188 and their PM showed the same thermal behavior as that obtained with P407-KT traces.



**Figure 1A: DSC traces for ketorolac tromethamine (KT), poloxamer 407 (P407), and their physical mixture (PM).**



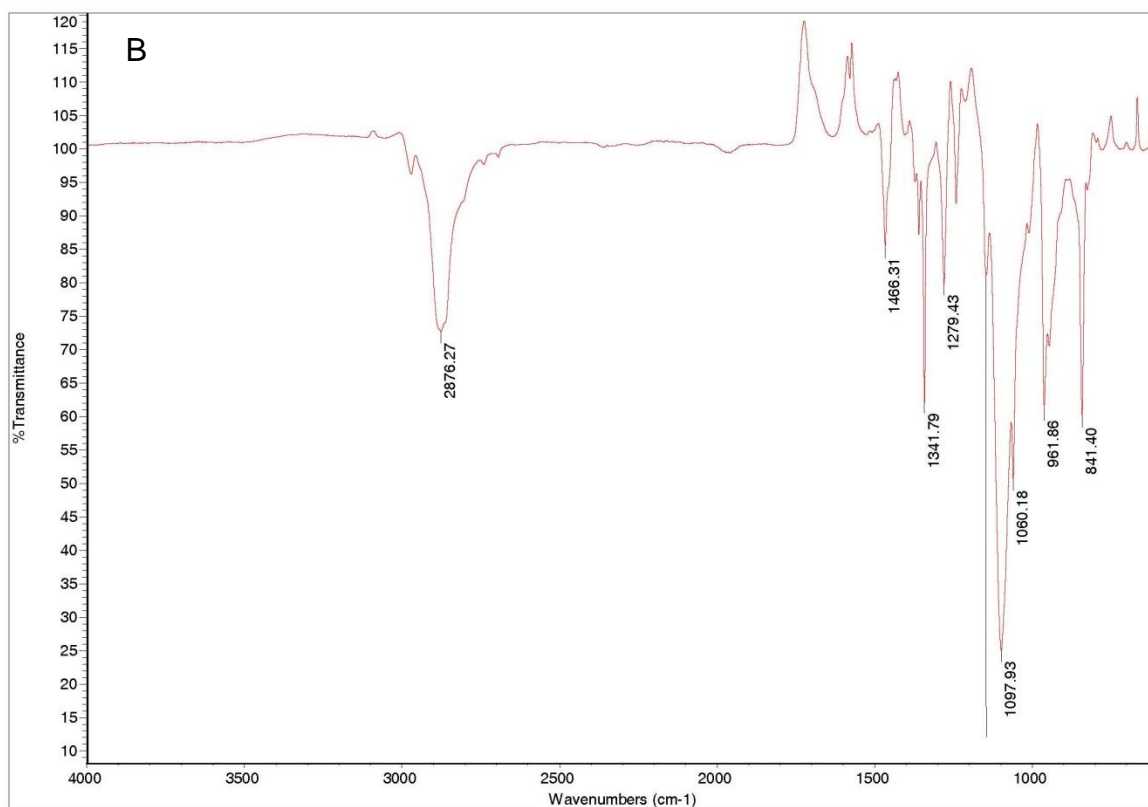
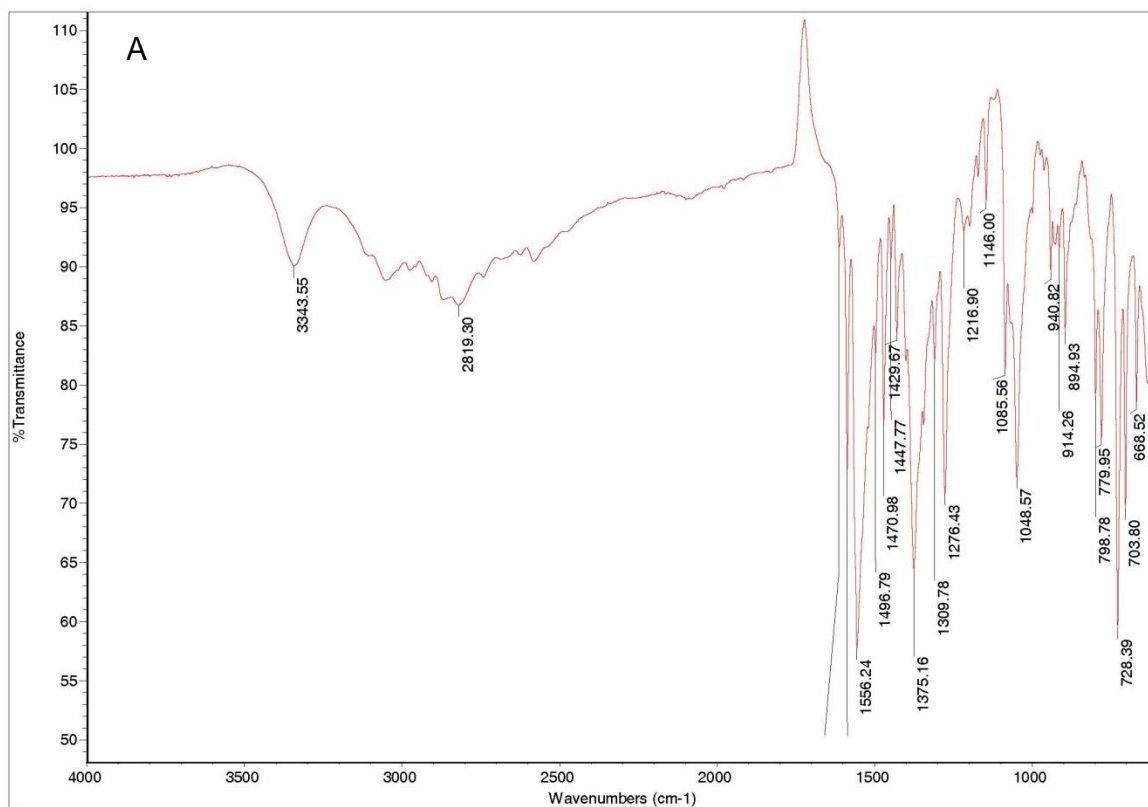
**Figure 1B: DSC traces for ketorolac tromethamine (KT), poloxamer 188 (P188), and their physical mixture (PM).**

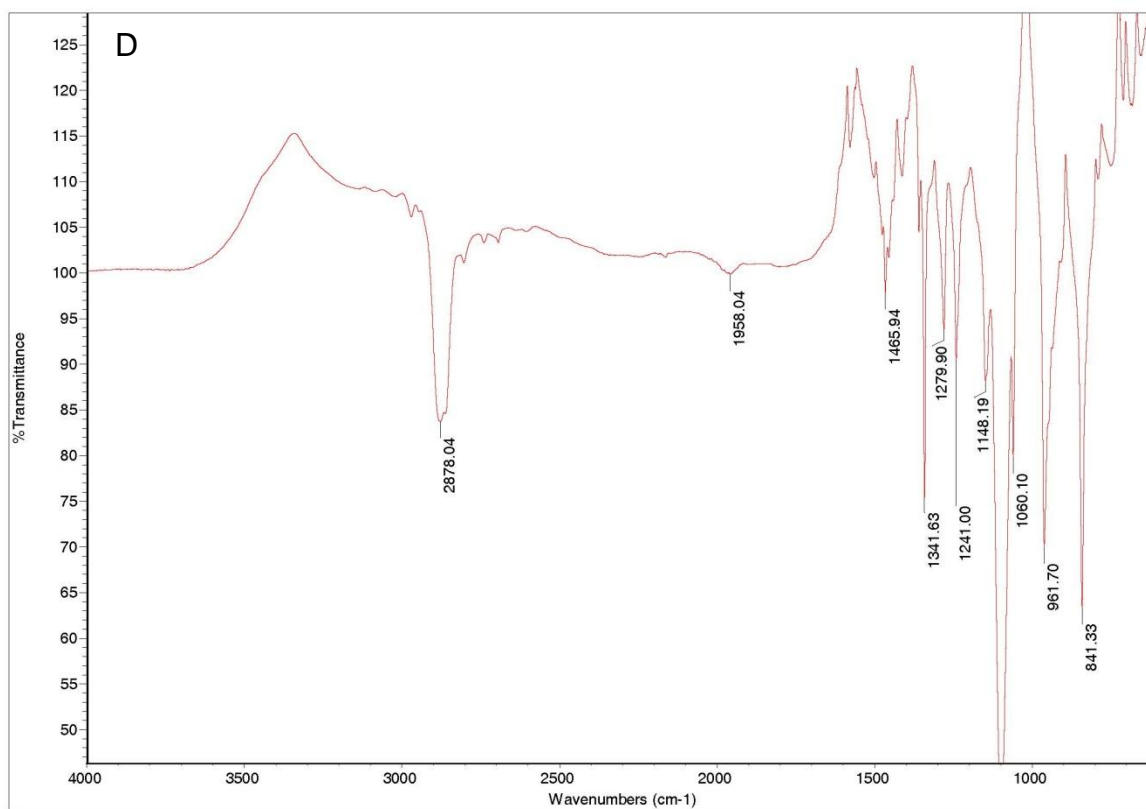
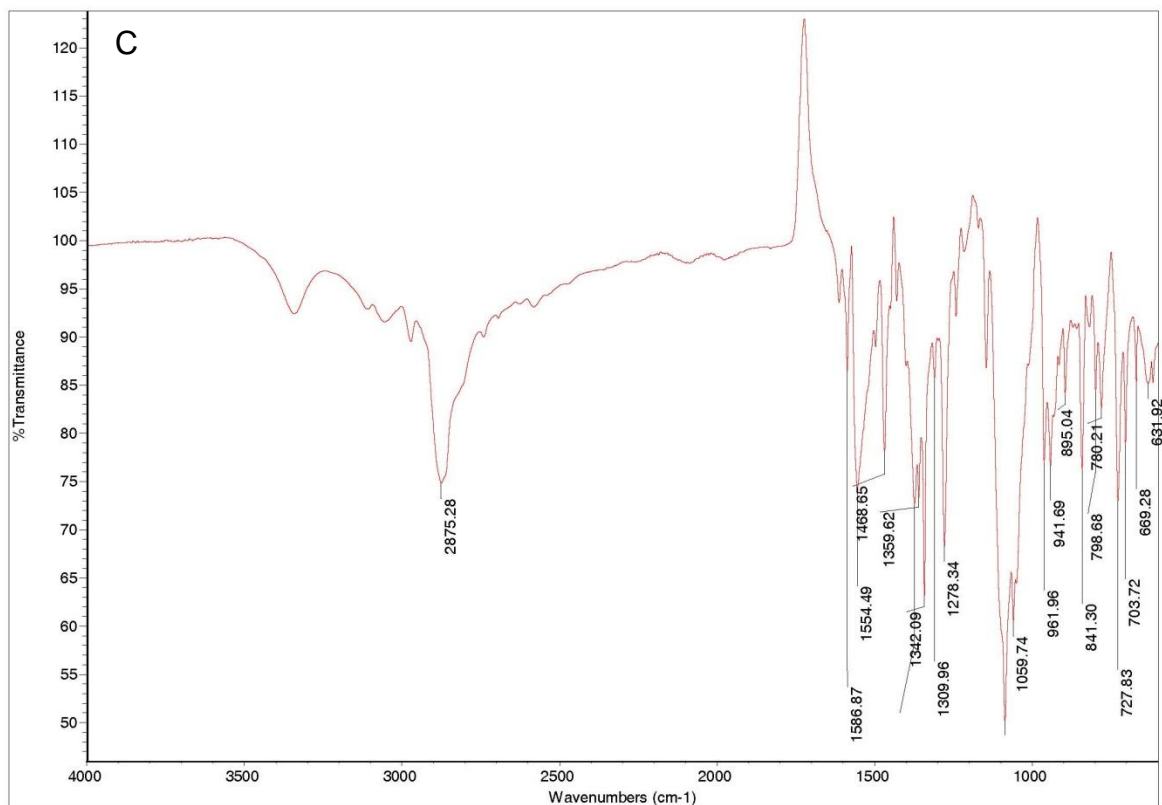
### 3.1.2. Fourier transform infrared spectroscopy (FT-IR) study

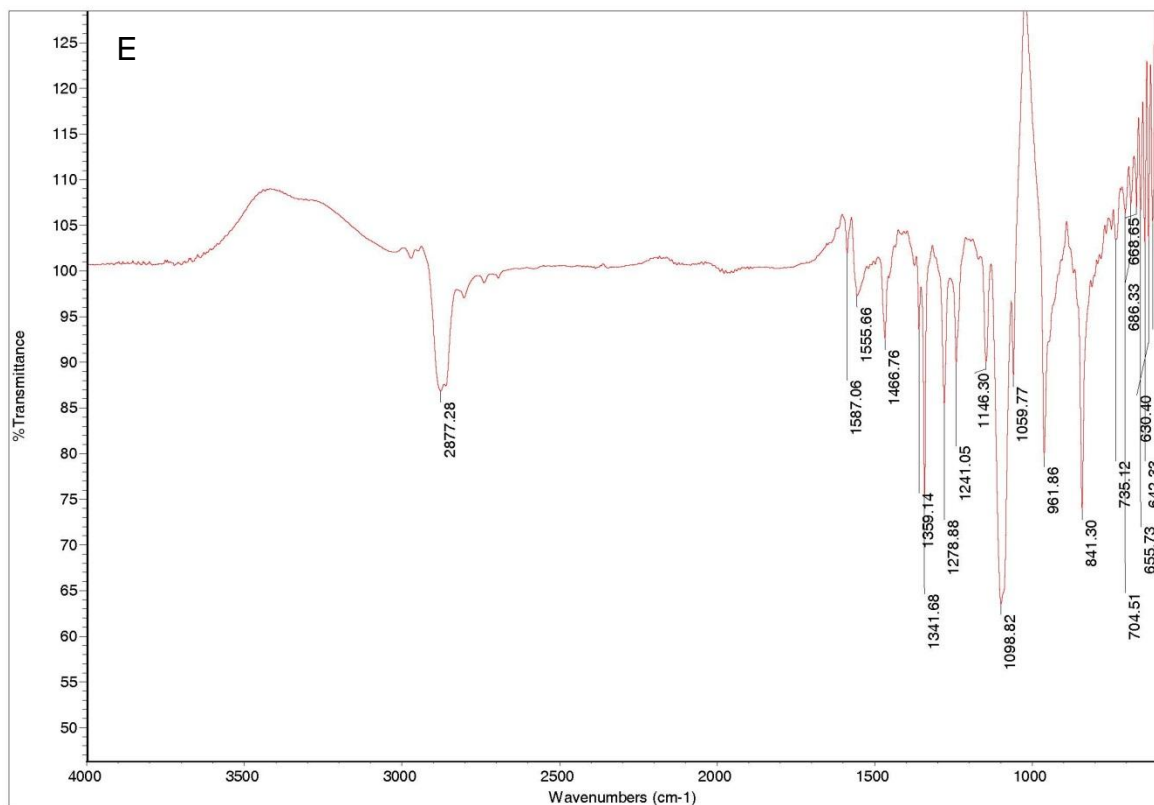
Figure 2 shows FT-IR spectra characteristic of KT (figure 2A), P407 (figure 2B), and their physical mixture (KT-P407 PM) (figure 2C) as well P188 (figure 2D) and its physical mixture with the drug (KT-P188 PM) (figure 2E) over the range 4000-500  $\text{cm}^{-1}$ . For KT spectrum, major peaks (3,343  $\text{cm}^{-1}$  [NH stretch]; 1,146  $\text{cm}^{-1}$  C = O stretch (diaryl ketone); and 1611  $\text{cm}^{-1}$  due to C-C aromatic stretching are seen. Also the peak observed at 728  $\text{cm}^{-1}$  which is related to aromatic C-H bending vibration; aliphatic C-H bending vibration is observed at 1375  $\text{cm}^{-1}$ . The peak at 1556  $\text{cm}^{-1}$  is due to carbonyl C=O stretching vibrations (Ajit P. et al., 2006).

Figure 2B, shows P407 characteristic peaks at around 3000  $\text{cm}^{-1}$  and around 1100  $\text{cm}^{-1}$ . The principal absorption bands (stretching) at 3463  $\text{cm}^{-1}$  correspond to the following functional groups; (O-H), 2874  $\text{cm}^{-1}$  (CH), 1096.9  $\text{cm}^{-1}$  (C-O). Our spectra for P407 was comparable with the previously recorded FT-IR spectra of P407 (Newa et al., 2008). There was no significant change in the absorption spectra for the drug when incorporated into PM (figure 2C). The incorporation of drug into poloxamer did not modify the position of the major peaks for KT (3,343  $\text{cm}^{-1}$  [NH stretch]; 1146  $\text{cm}^{-1}$  C = O stretch (diaryl ketone); and 1611  $\text{cm}^{-1}$  due to C-C aromatic stretching; 1556  $\text{cm}^{-1}$  due to carbonyl C=O stretching vibrations showed a minor shift to 1554  $\text{cm}^{-1}$ . On the other hand, the FT-IR spectrum for P188 (figure 2D) shows that P188 has a characteristic spectrum with peaks at around 3000 and 1100  $\text{cm}^{-1}$ . P188 exhibited characteristic peaks at 3449  $\text{cm}^{-1}$ , 2876  $\text{cm}^{-1}$ , and 1096.8  $\text{cm}^{-1}$  due to stretching of O-H, C-H, and C-O groups respectively. The FT-IR spectrum for the physical mixture of KT and P188 (figure 2E) did not show any shifts in the peaks of both the drug and the polymer but the intensity of the peak slightly decreased which may be due to the dilution of the drug with the polymer compared with KT alone. Thus, it can be concluded that based on FT-IR

spectra there is no interaction between the drug and polymer in the PM.







**Figure 2: FT-IR spectra for ketorolac tromethamine (KT) (A), poloxamer 407 (P407) (B), and their physical mixture (KT-P407 PM) (C), poloxamer 188 (P188) (D), and their physical mixture (KT-P188 PM) (E).**

### 3.2. Characterisation of in situ gel formulations

Different combinations of P407 and P188 were trialed to prepare the in situ gel formulations. Visual observation showed that poloxamer-containing in situ gel formulations were clear, colourless and transparent. Clarity is a highly desirable characteristic in ophthalmic formulations as non-transparent formulations may blur the vision and are not acceptable by the patient (Thakor et al., 2012). The physicochemical characteristics of KT loaded in situ gel formulations were found to be affected by the polymer compositions. For example, increment of P407 content decreased  $T_{\text{sol-gel}}$  of the formulation while increase in P188 concentration tended to increase  $T_{\text{sol-gel}}$ , as presented in Table 1. These results are in a good

agreement with those obtained by Asasutjarit et al. who investigated the effectiveness of diclofenac sodium loaded in situ gel formulations (Asasutjarit et al., 2011). All samples prepared at various concentrations presented in Table 1 were clear and transparent in both liquid and gel state.

The pH of P407/P188 in situ gel formulations were measured using a pre-calibrated pH-meter. All the formulations were found to have neutral pH, ranging between  $6.43 \pm 0.1$  to  $7.06 \pm 0.01$  (Table 1).

The gelation time measured for all prepared formulations is presented in Table 1. It is clear from the data shown that at constant P188 concentration, the gelation time of different formulations decreased as the concentration of P407 increased. For instance, there was a significant difference ( $P < 0.001$ ) between formulations PP10 and PP11 where their gelation time was  $53.0 \pm 16.9$  s and  $16.3 \pm 5.03$  s respectively. Also, upon comparing PP5 and PP6 formulations, there was a significant ( $P < 0.001$ ) decrease in the gelation time from  $256.0 \pm 29.50$  s to  $43.3 \pm 20.80$  s. On the other hand, comparing formulations PP11 and PP12, it was noted that there was no significant difference ( $P > 0.05$ ) in the time required for their gelation and the same is also true for formulations PP2 and PP3. However, some samples did not experience gelation at all despite being exposed to physiological temperature and higher, e.g. PP1 formulation did not undergo gelation even after 5 min and its  $T_{\text{sol-gel}}$  was over  $40^{\circ}\text{C}$ . This was ascribed to the lower concentrations of both polymers used which would make the formulation relatively more hydrophilic even at a higher temperature. Hence, samples PP1 and PP9 were excluded from further studies.

On the other hand, it is obvious from the data shown in Table 1 that an increase in P188 concentration leads to increase in  $T_{\text{sol-gel}}$  of the resultant gel. According to Dumortier et al.  $T_{\text{sol-gel}}$  transition temperature of any in situ gel formulation increases when P407



concentration decreases (Dumortier et al., 2006). In our preformulation study (data not shown), when formulations were prepared using P407 alone, the gelation temperature decreased with increasing P407 concentration. Increasing the concentrations of P188 in the P407 polymer based in situ gels an increase in  $T_{\text{sol-gel}}$  of these formulations was noted, which was within the physiological range, compared to the gels containing P407 alone. For example, in Table 1 the comparison of the sol-gel transition temperatures of PP4, PP8 and PP12 suggested that the formulation that contained more P188 exhibited sol-gel transition at a higher temperature. This may be due to the fact that the more hydrophilic P188 possesses a lower ratio of PPO units/PEO units per mole (0.19), compared to P407 (0.32), and could disrupt the hydration layers surrounding the hydrophobic part of P407 molecules. It caused higher order of water molecules around the hydrophobic PPO units. When gelation occurred, these ordered water molecules had to be squeezed out into the bulk solution. Therefore, the increase in the temperature was required for the system to promote the hydrophobic interactions between the formed micelles (Asasutjarit, et al., 2011; Vadnere et al., 1984). In another study, it was noted that P188 concentration could not be increased over 15% w/v as this would render the formulation poorly flowable at room temperature which is not a desirable property for an in situ gel that is intended for topical ocular application; such a formulation should exhibit solution characteristics (easy to instil) at room temperature thus enabling easier ocular administration (Asasutjarit, et al., 2011). Also, Wei et al. reported the effect of high P188 concentration on sol-gel transition temperature of the formulations as he stated that an optimized formulation containing 21% F127 and 10% F68 increased the phase transition temperature by 9°C (Wei, et al., 2002).

### 3.3. Determination of rheological properties and viscosity of in situ gel formulations

As shown in Table 1, all gel formulations exhibited a characteristic pseudoplastic (shear thinning) flow behaviour at room temperature. The viscosity increased significantly ( $P < 0.001$ ) with increasing concentration of P188 as well as P407 for all tested formulations. This may be explained by the fact that any incremental increase in the shear rate, results in the alignment of the polymer chains parallel to each other along their axes in the direction of a flow, thus reducing the internal resistance of the material and lowering viscosity (Abou El-ela & El-khatib, 2014). The viscosity of the formulations decreases with increasing shear rate in the presence of hydrophilic additives (Table 1). For example, the viscosity of PP11 in situ gel formulation at 10 rpm was  $574 \pm 17.99$  mPas, however, further increase in the shear force to 100 rpm resulted in a significant decrease ( $P < 0.001$ ) in the viscosity of the formulation ( $451.1 \pm 16.34$  mPas, Table 1). Also, the formulations acquire more fluidity, which improves their flow properties. These results are in a good agreement with those obtained by Ricci et al. who reported that P407 gels were pseudoplastic ; therefore, when shear deformed, their viscosity decreases (Riccia et al., 2002).

These results are in a good agreement with those obtained by El-Kamel et al. who explored the properties of different pluronic F-127 formulations comprising methylcellulose, hydroxypropylmethyl cellulose, and sodium carboxymethyl cellulose (El-Kamel, 2002). Also, the pseudoplastic behaviour of F127 formulations loaded with ciprofloxacin hydrochloride was observed previously by (Mansour et al., 2008).

**Table 1: Properties of different combination of poloxamer 407 (P407) and poloxamer 188 (P188) in situ gel systems and the effect of shear force on the rheological properties of P407/ P188 based in situ gel formulations. Results are expressed as mean values  $\pm$  SD, (n=3).**

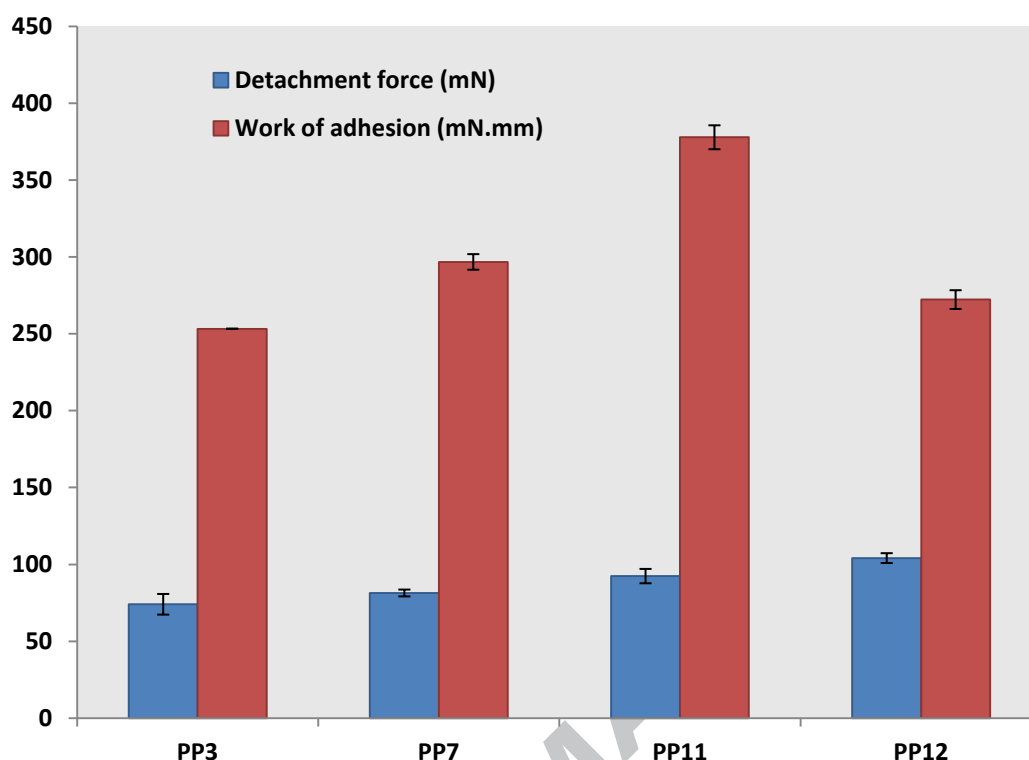
Formulation code	P407 (%w/v)	P188 (%w/v)	pH	Gelation temperature ( $^{\circ}$ C)	Gelation time (s)	Viscosity (mPas)			
						10rpm	20rpm	50rpm	100rpm
PP1	15	5	7.06 $\pm$ 0.01	Over 40	*	-	-	-	-
PP2	20	5	6.81 $\pm$ 0.02	29.5 $\pm$ 0.5	30.7 $\pm$ 4.51	460.5 $\pm$ 2.1	420.9 $\pm$ 2.8	338.0 $\pm$ 4.2	265.0 $\pm$ 3.2
PP3	23	5	6.87 $\pm$ 0.10	32.5 $\pm$ 0.5	17.7 $\pm$ 1.52	456.8 $\pm$ 19.4	448.0 $\pm$ 4.4	407.6 $\pm$ 3.6	379.0 $\pm$ 11.3
PP4	25	5	6.85 $\pm$ 0.003	30.0 $\pm$ 0.5	13.5 $\pm$ 3.00	507.8 $\pm$ 10.1	490.1 $\pm$ 8.1	46.5 $\pm$ 2.8	421.4 $\pm$ 5.5
PP5	15	10	6.71 $\pm$ 0.01	Over 40	256.0 $\pm$ 29.50	335.3 $\pm$ 18.8	325.6 $\pm$ 7.9	308.0 $\pm$ 8.5	301.5 $\pm$ 9.2
PP6	20	10	6.43 $\pm$ 0.10	34.0 $\pm$ 1.0	43.3 $\pm$ 20.80	403.9 $\pm$ 2.8	397.9 $\pm$ 8.7	374.5 $\pm$ 19.1	346.5 $\pm$ 13.4
PP7	23	10	6.88 $\pm$ 0.02	33.5 $\pm$ 0.5	21.0 $\pm$ 2.50	573.1 $\pm$ 7.9	560.4 $\pm$ 10.5	520.0 $\pm$ 1.4	490.5 $\pm$ 4.1
PP8	25	10	6.95 $\pm$ 0.001	31.5 $\pm$ 0.5	20.0 $\pm$ 1.25	627.7 $\pm$ 3.4	609.0 $\pm$ 4.8	588.1 $\pm$ 11.5	552.0 $\pm$ 6.5
PP9	15	15	6.89 $\pm$ 0.03	Over 40	105.0 $\pm$ 13.23	-	-	-	-
PP10	20	15	6.93 $\pm$ 0.01	28.5 $\pm$ 1.5	53.0 $\pm$ 16.09	438.0 $\pm$ 21.8	431.8 $\pm$ 1.8	3279.0 $\pm$ 5.1	351.9 $\pm$ 8.0
PP11	23	15	6.99 $\pm$ 0.02	33.2 $\pm$ 1.0	16.0 $\pm$ 5.03	574.0 $\pm$ 17.9	561.8 $\pm$ 11.0	501.7 $\pm$ 14.0	451.1 $\pm$ 16.3
PP12	25	15	7.00 $\pm$ 0.01	32.5 $\pm$ 0.5	12.5 $\pm$ 1.60	733.0 $\pm$ 15.2	711.0 $\pm$ 9.5	681.9 $\pm$ 7.1	655.0 $\pm$ 4.3

\* = No gelation observed even after several minutes.

### 3.4. Evaluation of mucoadhesive properties of in situ gel formulations

The data presented in Figure 3 show that increasing either P407 or P188 concentration leads to a significant increase ( $P < 0.001$ ) in the detachment force for all tested formulations. For example, the detachment force of PP3 and PP12 was found to be  $74.05 \pm 6.72$  mN and  $104.1 \pm 6.11$  mN, respectively. On the other hand, the work of adhesion of the tested formulation increased with increasing concentration of P188. However, upon increasing the concentration of P407 when comparing formulations P11 and PP12, the work of adhesion decreased significantly ( $P < 0.001$ ) from  $377.9 \pm 7.79$  mN.mm to  $272.3 \pm 6.11$  mN.mm. This may be attributed to the mucoadhesive characteristics of these polymers upon gelation as well as the relatively high molecular weight of the two polymers (P407 and P188) as both polymers have an average molecular weight (MW) of around 8400 Da. As with the increase in the molecular weight of the polymer chain there is an increase in the mucoadhesiveness of a polymer (Mythri .G et al., 2011).

It has been shown previously that the stronger the mucoadhesive force, the greater is the amount of formulation that is retained on the ocular surface (Baloglu et al., 2011). However, if the mucoadhesive force is excessive, the gel might damage the mucous membrane present on the ocular surface (Abdelkader & Mansour, 2015).



**Figure 3: Detachment force and work of adhesion of P407-P188 in situ gel formulations. Results are expressed as mean values  $\pm$  SD, (n=3).**

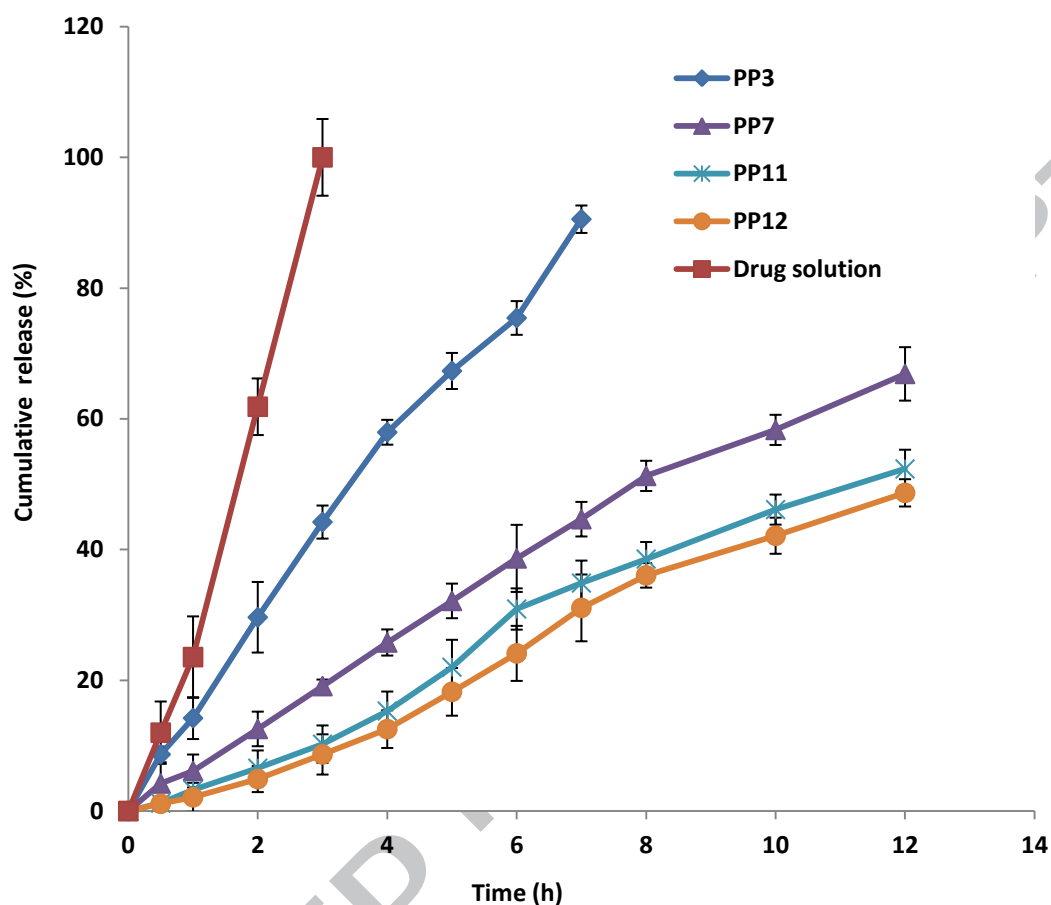
### 3.5. In vitro release study

Figure 4 shows the release profile of different in situ gel formulations. P188 and P407 retarded the dissolution of the drug in a concentration-dependent manner as the release rate of KT decreased with increasing P407/P188 ratio. PP3 formulation gave the highest drug release rate among all tested ratios since nearly 90% of the total amount of KT released within 7 h, but still exhibited a sustained drug release compared with KT solution. Also, a significant difference ( $P < 0.001$ ) was noted in the release profiles of all tested in situ gel formulations when compared with KT solution. Furthermore, increasing P407 concentration while keeping

P188 concentration constant has resulted in 50% drug released within a period of 12 h (Figure 4).

This effect on the release may be due to the presence of P188 in high concentration which increases the polymer chain entanglement as well as the viscosity of P407 solutions. Both polymers together yielded thick gel at 35°C that hardly released KT. Also, as shown in Table 1, the addition of P188 into different concentrations of P407 (20, 23 and 25% w/v) raised the sol-gel transition temperature of in situ gel formulations.

It can be concluded from the release data that P407 molecules form tight gel structures via hydrogen bonding in the aqueous solution. Moreover, as KT is water-soluble it interacts with poloxamer thus resulting in a delay in the drug release pattern. Thus, the rate of release of KT is decreased due to the delay in both gel dissolution and drug diffusion.



**Figure 4:** Release profile of KT from different P188 and P407 (% w/v) in situ gel formulations in PBS (pH 7.4). Results are expressed as mean values  $\pm$  SD, (n = 3).

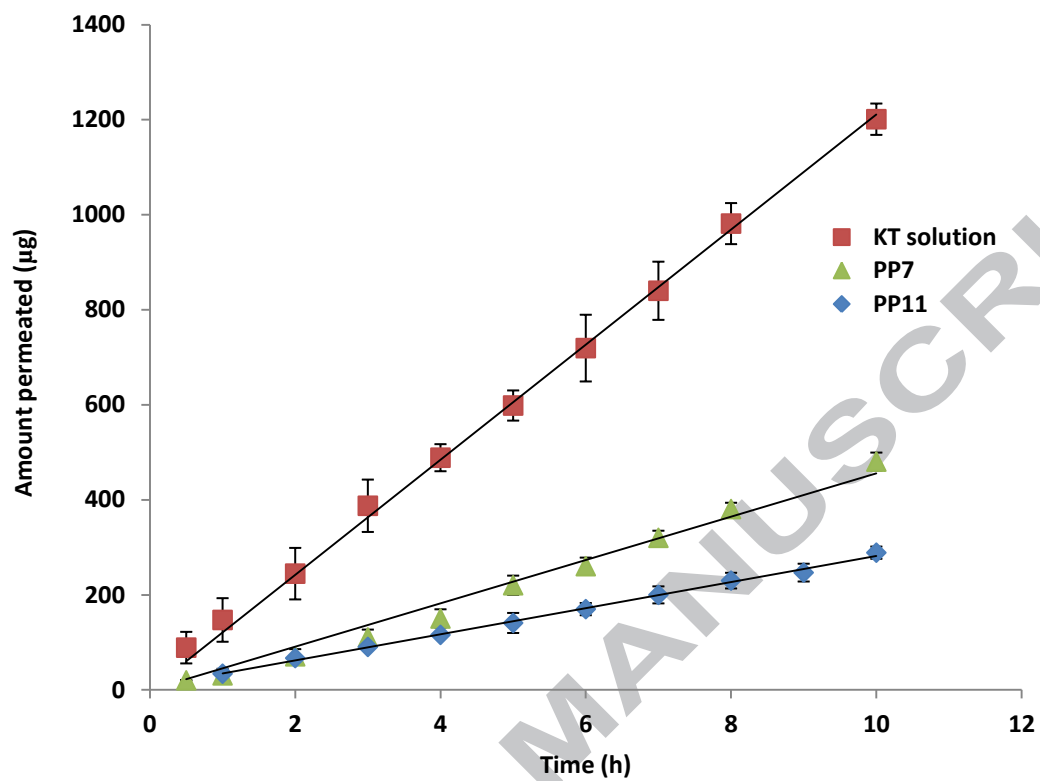
### 3.6. *Ex vivo* permeation study

Two in situ gel samples have been selected for this study (PP7 and PP11) depending on their mucoadhesive characteristics as well as their *in vitro* release profile. Table 2 shows a comparison of the *ex vivo* data of these two formulations with that of KT solution. The steady state flux for KT solution was significantly ( $P < 0.05$ ) higher than PP7 and PP11 as it was 2.37 and 4.28 folds faster than that of PP7 and PP11 formulation, respectively. While the

value of  $P_{app}$  for these in situ gel samples were lower by 2.4 and 4.3 folds for PP7 and PP11 respectively compared with the  $P_{app}$  value for KT solution, Table 2. KT took longer time to partition through gel matrix and furthermore time to diffuse through poloxamer-based matrix compared to free KT using the static Franz diffusion cells model. This can be explained on the ground that while the drug solution showed better permeation rates compared with the gel systems on the static receptor compartment of the Franz diffusion model used in this study, there is an extensive body of research to suggest that this pattern can be reversed in vivo. Similar results were reported with methyl cellulose and carrageenan-based in situ gels using excised bovine sclera (Thrimawithanaa, et al., 2011) and other vesicular delivery systems, such as liposomes, niosomes and solid lipid nanoparticles (SLNs), using excised rabbit, excised bovine and bioengineered human corneal sample (Abdelkader, et al., 2011; Attama et al., 2009; Law et al., 2000).

On the other hand, the  $t_L$  calculated for the selected in situ gel samples used in this study are shown in Table 2. It was found that PP7 formulation gave the longer  $t_L$  compared with PP11 and also longer time compared with KT solution. As the  $t_L$  values for PP7, and PP11 was  $0.526 \pm 0.66$  h, and  $0.268 \pm 0.38$  h. respectively. This may be due to the fact that the in situ gel at  $35^\circ\text{C}$  (experiment temperature) form a viscous gel that would delay KT permeation through the cornea compared with solution. In addition to the mucoadhesive properties of the in situ gel is likely to retain the drug on the corneal surface for a prolonged time, furthermore, these formulations spread and adhered to the hydrophobic surface of the cornea better than the aqueous drug solution.





**Figure 5: Transcorneal permeation profiles of ketorolac tromethamine (KT) from KT solution PP7 and PP11 in situ gel formulations using excised porcine corneas. Results are expressed as mean values  $\pm$  SD, (n = 3).**

**Table 2: Steady-State Flux, Apparent Permeability Coefficient ( $P_{app}$ ), and Lag Time ( $t_L$ ) of KT after *ex vivo* corneal permeation study. Results are expressed as mean values  $\pm$  SD, (n = 3).**

<b>Formulation Code</b>	<b>Steady-State Flux (<math>\mu\text{g/h}</math>)</b>	<b><math>P_{app} \times 10^{-6}</math> (cm/sec)</b>	<b><math>t_L</math> (h)</b>
<b>KT- solution</b>	117.65 $\pm$ 0.96	5.31 $\pm$ 0.13	0.198 $\pm$ 0.34
<b>PP7</b>	49.58 $\pm$ 1.23	2.24 $\pm$ 0.22	0.526 $\pm$ 0.66
<b>PP11</b>	27.46 $\pm$ 0.67	1.24 $\pm$ 0.73	0.268 $\pm$ 0.38

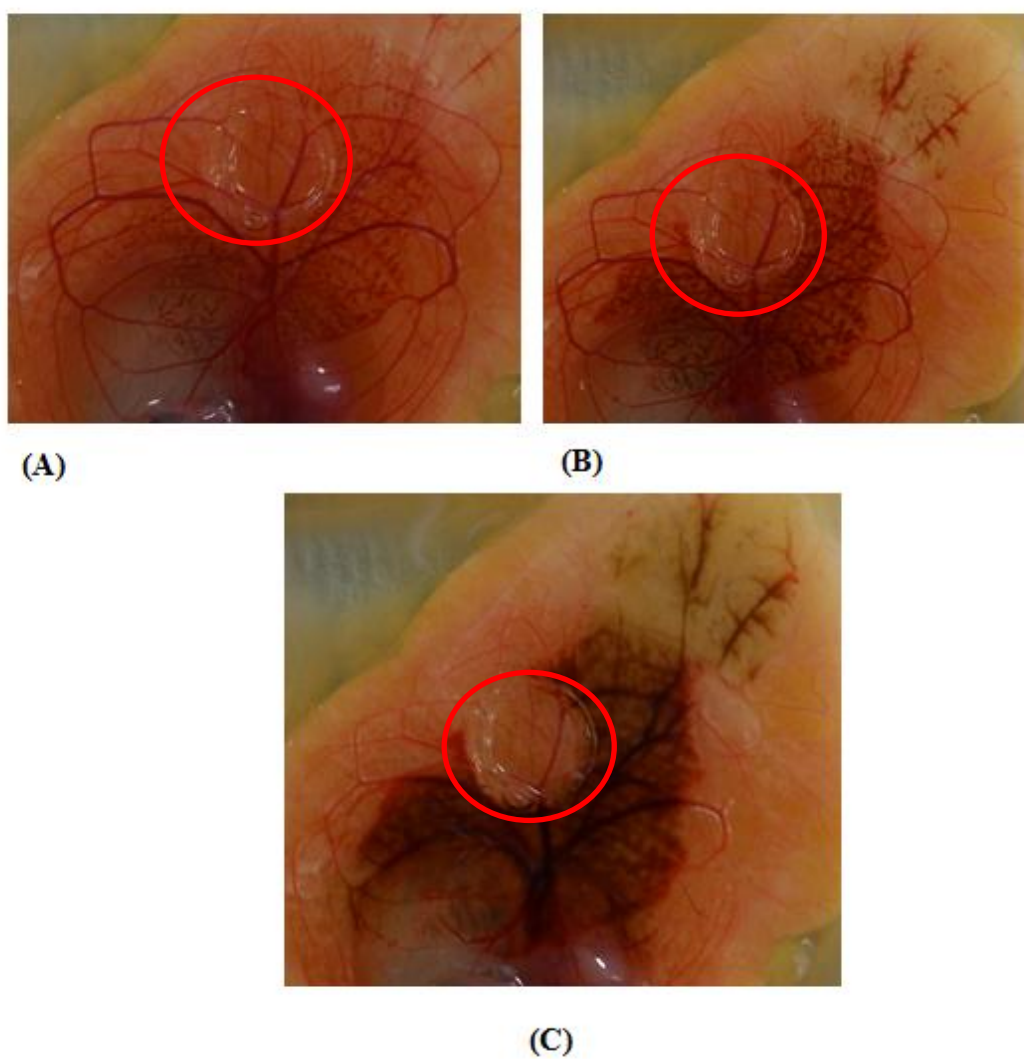
### 3.7. HET-CAM (Hen's Egg Test on Chorioallantoic Membrane)

This test has been used for evaluation of conjunctival response to test material, and it has been recently reported as a well-accepted in vitro conjunctival membrane model (Abdelkader et al., 2015). A modified method of the conventional one was reported in the literature, where the embryo was grown in a Petri dish from day 3 onwards to allow ready access to the entire CAM surface for better visibility and convenience (Auerbach et al., 1974; Dohle et al., 2009).

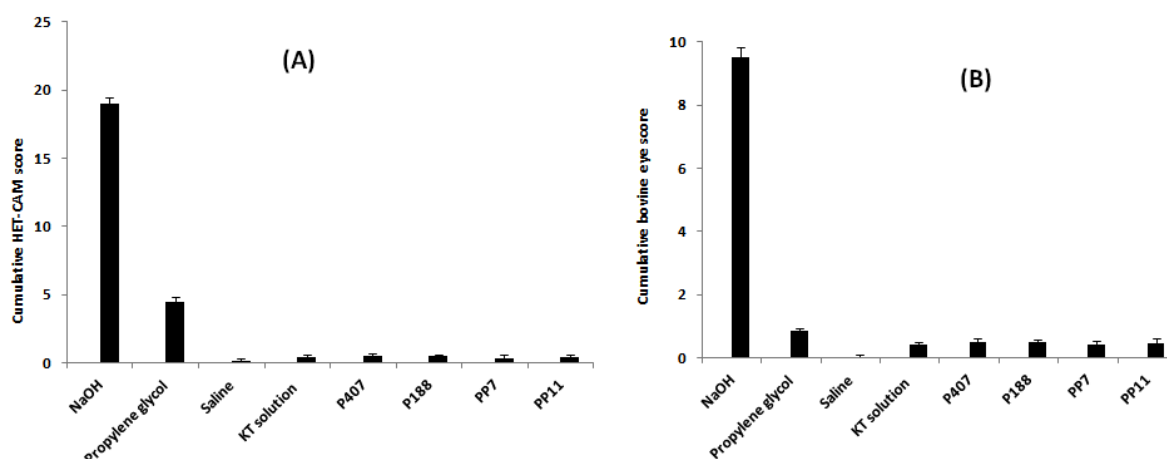
The selected PP7 and PP11 in situ gel formulation demonstrated no irritation potential and no signs of inflammation on the CAM during the test period. Furthermore, the gel formulation loaded with KT (0.5% w/v) imparted a protective effect through the formation of a diffusional barrier against the caustic effect of NAOH on the CAM, Figure 6.

On a time dependent numerical score for three irritant responses, a single cumulative value is estimated to interpret the irritation potential of different formulations. Figure (7A) shows the cumulative HET-CAM scores for the controls; KT solution (5 mg/mL) in PBS, the test polymers used in the formulation process and the KT loaded in situ gel formulation.

The cumulative scores for KT solution, P407 and P188 were  $0.43 \pm 0.10$ ,  $0.53 \pm 0.15$  and  $0.48 \pm 0.11$  respectively (Figure 7A). This is an indication that the drug and the polymers used in the formulation process were not irritant to the CAM. Also, Chena et al. confirmed that P407 is a non-irritant substance in their study involving liquid crystalline nanoparticles. These formulations exhibited excellent ocular tolerance according the ocular irritation testing method used (Chena et al., 2012). Also, Rooks et al. reported that KT solution when instilled into the eyes of rat and rabbit models of ocular inflammation, inhibited the inflammatory response and were seen to be a non-irritant (Rooks W.H. 2nd et al., 1985).



**Figure 6:** Images showing the protective effect of KT loaded in situ gel (PP11) formulation on 10 day old CAM after treatment with NaOH at different time intervals (A) 0.5 min, (B) 2 min, and (C) 5 min.



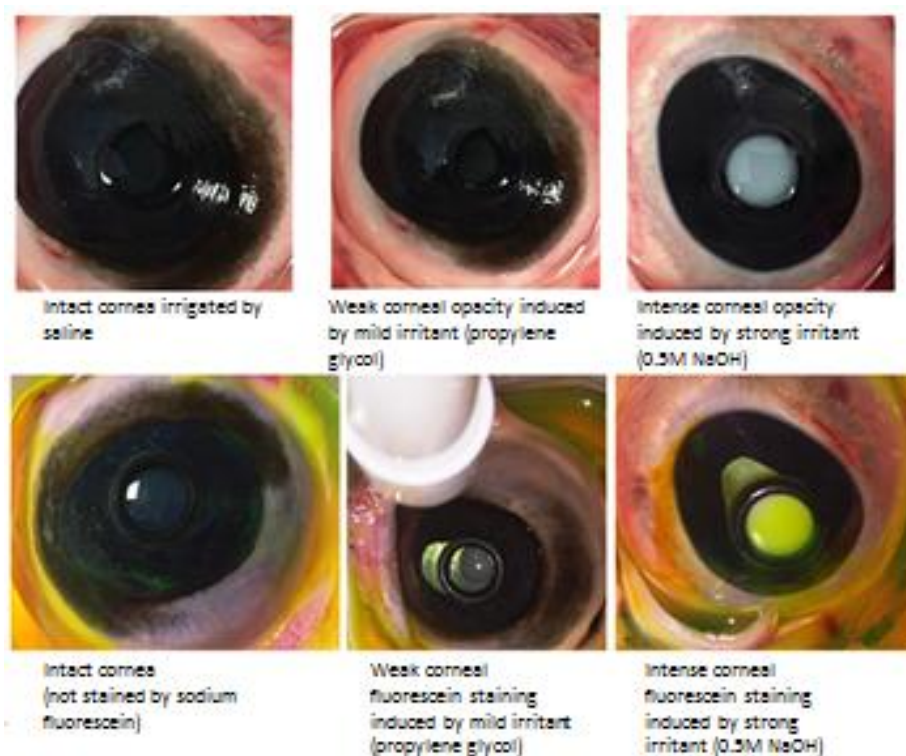
**Figure 7: Cumulative HET-CAM scores (A) and cumulative BCOP scores (B) for the controls, KT solution, used polymers, and formulations PP7 and PP11. Results are expressed as mean values  $\pm$  SD, (n = 3).**

### 3.8. The BCOP (Bovine corneal opacity and permeability) assay

Figure 8 represents the degree of corneal opacity and fluorescein permeability used to score the test substances. These substances included mild irritant (propylene glycol) and strong irritant (NaOH 0.5 M).

Figure 8 shows photographs for normal cornea before and after staining as well as the effect of different types of controls used in this experiment. Also, data presented in Figure 7B indicates the cumulative scores for different in situ gel formulations, KT solution (5 mg/mL) in PBS along with polymers to make the in situ gelling formulation. It is clear from the data that KT solution did not show any signs of irritation and the same is also true for the in situ gel formulations as the cumulative score was not more than 0.5. Similar results have been reported by Kadam et al. where the in situ gel formulations for ocular delivery of ketorolac tromethamine containing combination with hydroxypropylmethyl cellulose and P407 were stable and non-irritant (Kadam et al., 2010).

On the other hand, the cumulative scores for P407, and P188 and KT solution alone indicate a mild irritation potential (Figure 7B). These results for the BCOP test are consistent with those obtained using the HET-CAM test, as both the experiments revealed that the tested formulations were non-irritant to the models employed in the study.



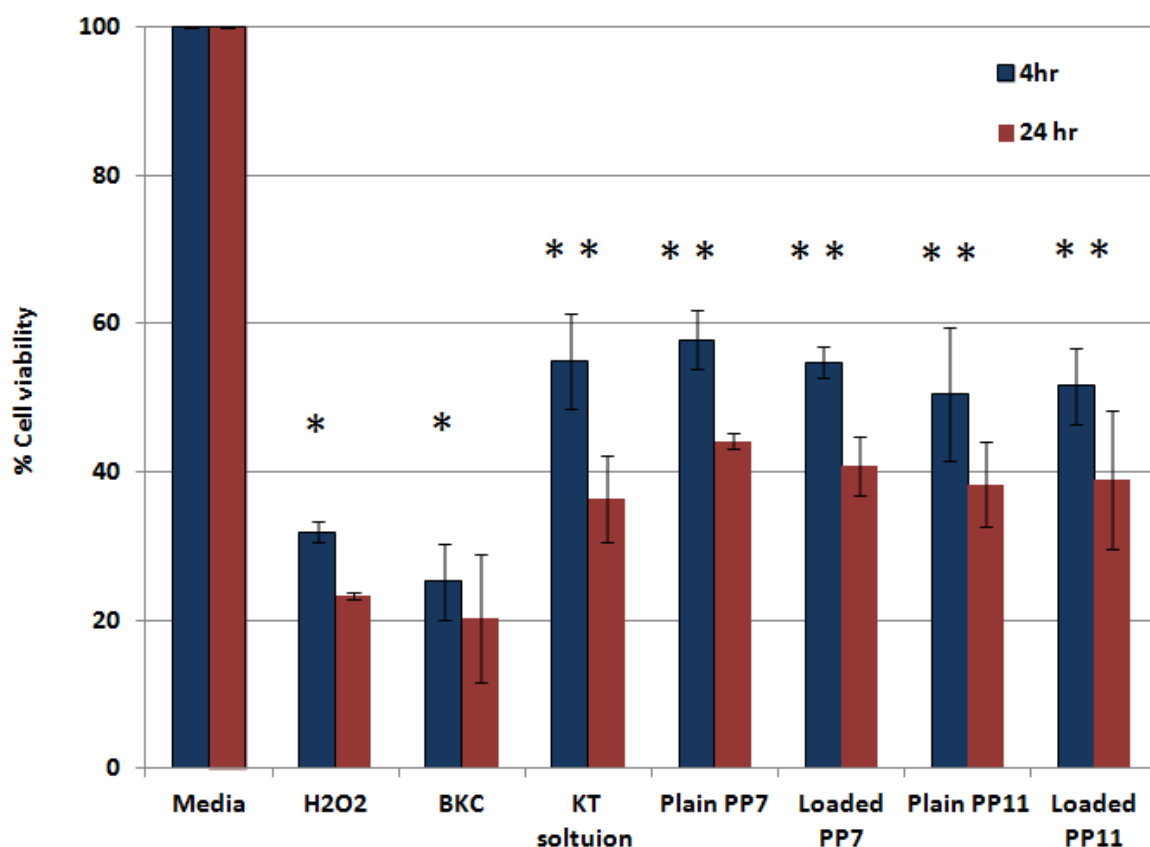
**Figure 8: Degree of corneal opacity (upper) and fluorescein permeability (lower) used to score the test substances [mild irritant (propylene glycol) and strong irritant (NaOH 0.5 M) models].**

### 3.9. MTT cytotoxicity assay

Selected in situ gel formulations (PP7 and PP11) both drug free and loaded with KT (5mg/mL) were used in this experiment. As stated before the MTT assay is a quantitative colorimetric assay that measures the activity of the mitochondrial cells as an index of their

viability and proliferation. This test only detects living cells, and the signal generated is directly proportional to the number of live cells (Angius & Floris 2015; Yang et al., 2015).

Figure 9 shows estimated cell viability after a 4 h and 24 h exposure to different treatments. The positive control (BKC 0.01%) has been shown to be cytotoxic at the duration tested in this study. Whilst there were a noticeable decrease in cell viability after exposure to the different in situ gel formulations and KT solution, these were statistically significant compared to the negative control ( $P < 0.001$ ). But the difference between % cell viability were not remarkable ( $P > 0.05$ ) when comparing KT solution with plain formulation but the difference was significant ( $P < 0.05$ ) when comparing KT loaded formulations with KT solution. Meanwhile there was significant ( $P < 0.01$ ) difference between positive controls and KT solution and tested formulations. It is worth nothing that BKC (which for durations comparable to this study is cytotoxic) is used routinely as a preservative in commercial ophthalmic eye drops at the tested concentration of 0.01% w/v ; the duration of exposure of the eye when BKS is applied in eye drops is considerably shorter. These findings indicate that KT loaded in situ gel formulations exhibit acceptable percent cell viability according to the regulatory requirements for ophthalmic pharmaceuticals which should be tolerable to the ocular surface compared with control samples as shown in Figure 9. Thus, the tested formulations are considered suitable for ocular application taking into consideration the tear turnover and the time these formulations are likely to remain in the eye.



**Figure 9:** MTT assay results on primary corneal epithelial cells after 4h and 24h treatment with plain and loaded in situ gel formulations. Results are expressed as mean values  $\pm$  SD, (n=3). \* Significant difference; \*\* non-significant difference.

### Conclusion

This study demonstrated the favourable rheological and gelling properties of the investigated poloxamer formulations. The KT-loaded formulations exhibited desirable rheological properties as well as mucoadhesive characteristics. Furthermore, *in vitro* and *ex vivo* permeation study revealed that the prepared in situ gels sustained drug release pattern when compared with KT solution. While the drug solution showed better permeation rates compared with the prepared PP7 and PP11 systems on the static receptor compartment of the Franz diffusion model



used in this study, there is an ample body of research to suggest that this pattern can be reversed *in vivo*. In an *in vivo* setting there will be many factors affecting the drug permeation, such as tear turnover and blinking. This is a static model and any delay in the flux rate can be compensated by prolonging the precorneal residence time and this is clear from the results obtained from mucoadhesion and viscosity tests. Additionally, the gels did not show any signs of conjunctival or corneal irritation as revealed by the HET-CAM or BCOP tests respectively. MTT cytotoxicity assay data demonstrated that the selected *in situ* gel formulations showed reasonable and acceptable percent of cell viability when compared with control samples.

Thus, due to the strong concentration dependence of the sol-gel transition temperature combined with acceptable corneal epithelial cell viability, it can be concluded that KT loaded thermoresponsive *in situ* gel formulations (PP7 and PP11) with concentration of P407:P188 (23:10 w/v%) and (23:15 w/v%), respectively appeared to be a promising ocular delivery system for KT delivered across the cornea.

#### **Declaration of interest and acknowledgments**

The authors would like to report that they have no conflicts of interest relevant to the contents of this article. This work is undertaken with the financial support from the Culture Affairs and Mission Department, Ministry of Higher Education, Cairo, Egypt. Authors would like to thank Dr Hamdy Abdelkader for helping with setting up HET-CAM and BCOP tests.

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Graphical abstract

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## Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: Design, characterisation, toxicity and transcorneal permeation studies

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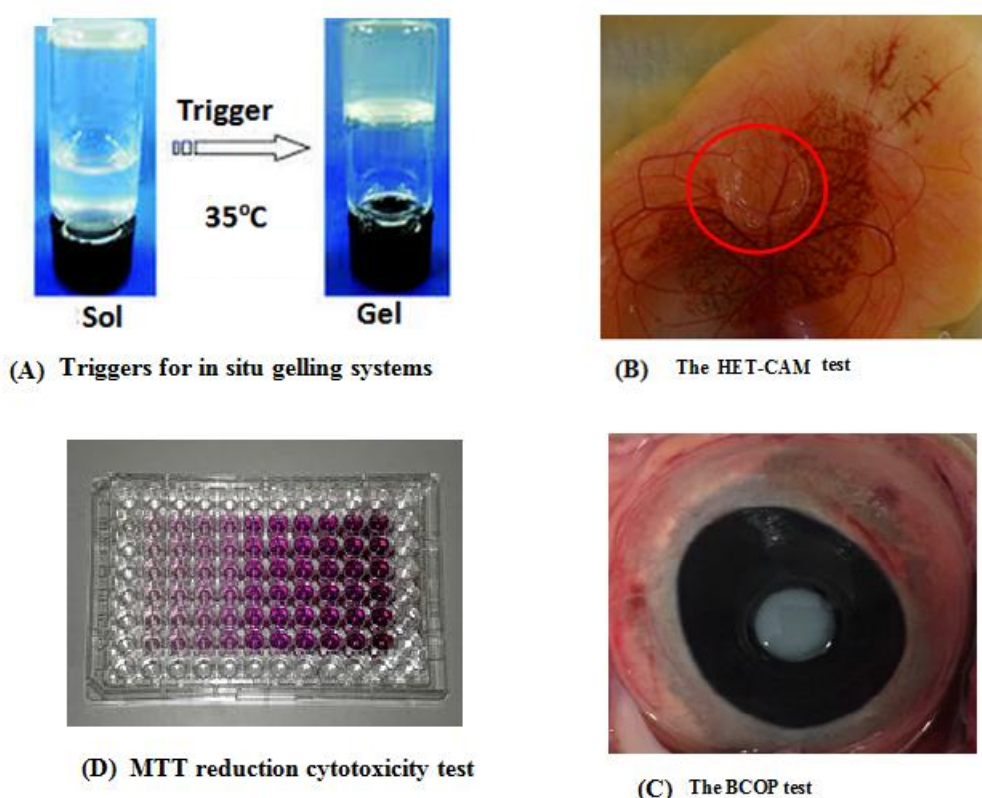


Figure 1: In this study, the thermoresponsive behaviour of poloxamers was employed as a trigger for the formation of in situ gel systems incorporating ketorolac tromethamine (KT) (A). The protective effect of KT loaded in situ gel preparation on 10 day old chorioallantoic membrane (CAM) has been investigated after treating the CAM with a strong irritant, NaOH (B). The BCOP test revealed the corneal opacity and permeability of the prepared in situ gel system (C). The MTT cytotoxicity assay demonstrated that the cell viability when treated with the selected in situ gel preparations was at an acceptable level compared to the control samples (D).