



# Novel in-situ emulgel of acetazolamide for ocular drug delivery

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## ABSTRACT

In this investigation, a novel *in-situ* emulgel of acetazolamide was developed and evaluated. The gel was prepared using corn oil, pectin, and GellanGum at different concentrations of 10%–50% (*w/w*) and added to the homogenizer tube followed by the addition of the emulsifier (Tween 80 and span 80 in the ratio of 1:1) at 0.2% (*w/w*). The pH of all five formulations was between 5.58 and 5.75. The Fourier-transform infrared spectroscopy (FTIR) spectrum of all the formulations showed broadband in between the wavenumber range of 3,700  $\text{cm}^{-1}$  and 2,980  $\text{cm}^{-1}$ . The FTIR spectrum of all the formulations showed broadband in between the wavenumber range of 3,700  $\text{cm}^{-1}$  and 2,980  $\text{cm}^{-1}$ . The drug content in the samples was determined by a spectrophotometer at 267 nm. The drug release mechanism may be Fickian transport ( $0.45 \leq n$ ), anomalous (non-Fickian) transport ( $0.45 \leq n < 0.89$ ), and/or super case-II transport ( $n > 0.89$ ). The *n* values of all formulations were greater than 0.45. The *in-vivo* corneal tolerance experimentation of the prepared formulation eyes was assessed for 72 hours and found to be biocompatible based on visual inspection.

## INTRODUCTION

Glaucoma is the world's most prevalent eye disease and the leading cause of permanent blindness (Sun and Zhou, 2018). It is characterized by "tunnel vision," which is caused by the purposeful degeneration of retinal ganglion cells. High intraocular pressure is one of the most dangerous characteristics of glaucoma. Intra-ocular pressure (IOP) (Soltau and Zimmerman, 2002). Glaucoma affects more than 67 million people globally, according to the World Health Organization (Kaur *et al.*, 2011). Since the 1970s, there has been a concerted attempt to improve ocular therapeutic delivery. Various ways were tried at the beginning, which may be split into two categories, such as controlled-release medication administration and bioavailability enhancement

(Mohsen *et al.*, 2020). The bioavailability enhancement category includes viscosity enhancers (Patton and Robinson, 1975), gels (Zignani *et al.*, 1995), penetration enhancers (Kaur *et al.*, 2000), and prodrugs (Lee and Robinson, 1986). Controlled-release ocular medication delivery devices might replace traditional drug release strategies such as ocusert (Saettone and Salminen, 1995), *in-situ* gel (Cohen *et al.*, 1997), implant systems (Kunou *et al.*, 1995), and collagen shields (Friedberg *et al.*, 1991).

The traditional way of treating eye illness is no longer appropriate for the modern age of medicine (Calvo *et al.*, 1997; Grass and Robinson, 1988; Kreuter, 1993). Scientists and industry researchers have been more interested in pharmaceutical semisolid dosage forms, particularly emulgels systems, during the last decade. The main advantages of the drug delivery system were consistency and homogeneous behavior. The said drug delivery system is a biphasic dosage form, in the form of an *o/w* emulsion system in which an aqueous base is a gel, and called emulsion gel or emulgels. As the interior phase of colloidal systems contains an aqueous base and is mixed with a lipophilic material, it has explored the cosmeceutical industry for a long period of time

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(Marquardt and Sucker, 1998). In the modern era, emulgel has been investigated for controlled delivery, stability, and storage purposes in the pharmaceutical, dermatological, and food industries respectively. The invention of emulgels has been greatly aided in recent years by the discovery of new polymers with various functionalities such as increased gelling capacity, emulsifying agents, and thickening agents. This improved the viscosity of the aqueous phase and lowered surface or interfacial tension, resulting in the stability of emulsions and lotions (Gupta *et al.*, 2010). Finally, their rheological qualities may be readily modified by selecting the right gelling agent and an oily component.

An eye drop is an ideal alternative for topical treatment of ocular illnesses in conventional dose form, especially in certain circumstances when the drug's localized action (e.g., the cornea and/or anterior chamber) is required. However, a barrier such as lacrimal secretion and blinking reflex, along with corneal impermeability, make drug retention time so short that frequent administration is necessary for highly efficient therapy (Wei *et al.*, 2002). The drug retention period is so brief due to the self-protecting barrier, which causes frequent very effective treatment, which leads to several side effects with toxicity. To reduce the toxicity and overcome the situation the work was projected. This study's goal is to create acetazolamide (ACZ)-loaded *in-situ* emulgel systems using Corn Oil (CO), Pectin, Gellan Gum (GG), and surfactants (tween 80 & span 80).

ACZ, a carbonic anhydrase inhibitor, is used to treat open-angle glaucoma symptoms and postpone the development of blindness, and lower IOP before surgery (Khaw and Cordeiro, 2000). Among the presently available treatments for treating open-angle glaucoma, ACZ remains the most effective and commonly used Carbonic anhydrase inhibitors (CAIs) (CAIs) (Kaur *et al.*, 2002). ACZ works by blocking the carbonic anhydrase enzyme (CAE), which is involved in the transfer of CO<sub>2</sub> from the tissues to the lungs and stimulates the generation of aqueous humor through ciliary mechanisms. To achieve the desired drop in IOP, high oral dosages of ACZ should be taken due to the widespread diffusion of CAE throughout the body (Kaur *et al.*, 2002). The high ACZ dosages cause systemic negative effects (Epstein and Graant, 1977; Gamm, 1984) such as diuresis, renal failure, vomiting, metabolic acidosis, and central nervous system depression. As a result, oral ACZ is despised, and several researchers have proposed that oral ACZ be replaced by topical ACZ to avoid systemic adverse effects (Kaur *et al.*, 2002).

The requirement of polysaccharides in the development of pharmaceutical preparation has been explored for the last two decades. GG is one of such anionic polysaccharides (Prajapati *et al.*, 2013) which brings a special attraction to the development of *in-situ* formulations (Balasubramaniam *et al.*, 2003; Carlfors *et al.*, 1998; Fernández-Ferreiro *et al.*, 2015; Paulsson *et al.*, 1999; Rozier *et al.*, 1997; Rupenthal *et al.*, 2011a, 2011b). The GG is water-soluble and obtained from the *Pseudomonas elodea*, and it shows anionic properties due to the presence of the D-glucose and one of each residue of D-glucuronic acid and L-rhamnose. Researchers use GG for its heat and acid resistance capacity and with a wide range of gel textures (Chen and Chen, 2007). GG is transparent and can bind with synthetic and natural polymers (Zia *et al.*, 2018).

Pectin an effective polysaccharide is a byproduct material of agriculture and food industries, used for the production of an edible film, because not only of its biocompatibility and biodegradability but also its non-toxicity nature (Lin *et al.*, 2020). Pectin draws attention toward the food and pharmaceutical industry because of its semi-soluble nature, that is, it retains some water (Marcus, 2013).

From ancient days, edible oil is used for cooking purposes and CO draws attention to the drug delivery system in the last few years. The main aim of chosen CO is due to the presence of essential fatty acids and vitamin E, and the pleasant sensory properties, which make it a suitable ingredient for delivery carriers (Krstonošić *et al.*, 2009).

In this work, a series of emulgels were developed using various amounts of an aqueous solution of GG and pectin with CO to achieve the goal of combining the beneficial qualities of all the ingredients. The produced emulgel will have a better texture, drug solubility, and drug absorption over caprine (goat) ocular tissue. The formulations were characterized using FTIR, gelation temperature, gelation time, hemocompatibility, and pH. The release study was done with the use of a semipermeable membrane barrier, and ACZ from the synthesized emulgels was evaluated *in vitro*. To know the efficacy of the emulgel as ocular delivery, it will be tested *in vivo* eye irritation test and *ex vivo* corneal permeation test.

## MATERIALS AND METHODS

### Materials

CO was purchased from Grainotch Industries Limited (Maharashtra, India). Pectin and GG were purchased from Suvividhinath Lab (Baroda, Gujarat). ACZ was gifted from Nakoda Chemicals Ltd, (Hyderabad, India, 99.91% pure), Tween 80 and span 80 (Hi-media Pvt. Ltd, Mumbai), and other chemical reagents including NaOH, K<sub>2</sub>HPO<sub>4</sub> were purchased from Suvividhinath Laboratories Pvt. Ltd. (Vadodara, India). Double distilled water and preservative water were used during the experimental study.

### Preparation of *in-situ* gel

#### Preparation of pectin solution

About 500 mg of pectin was weighed and slowly added to the 99.5 g of the double distilled water, which contain 0.1% of methylparaben. The mixture was homogenized using a magnetic stirrer (500 rpm) to form a pectin sol.

#### Preparation GG solution

About 500 mg of GG was weighed and slowly added to the 99.5 g of the double distilled water, which contain 0.1% of methylparaben. The mixture was homogenized using a magnetic stirrer (500 rpm) to form a GG sol.

#### Preparation of *in-situ* emulsion

*In-situ* emulsions of different compositions were prepared by using CO containing dissolved ACZ. The composition of the CO within the *in-situ* emulsion varied from 10%–50% (w/w). For the preparation of *in-situ* emulsion pectin, GG was weighed and added to the homogenizer tube. This was followed

by the addition of the emulsifier (Tween 80 and span 80 in the ratio of 1:1). In all the formulations, the concentration of the emulsifier was kept constant at 0.2% (w/w). At last calculated amount of the CO containing ACZ was added to the homogenizer tube. The mixture was then homogenized at 3,000 rpm for 15 minutes at room temperature. The formulations were then transferred into falcon tubes and kept under refrigeration (5°C); the composition of the prepared formulations has been tabulated in Table 1.

### Characterization of the prepared acz *in-situ* gels

#### Visual appearance, homogeneity, and clarity

The prepared *in-situ* gel formulation was evaluated visually. For the color and homogeneity study, we take the help of a white and dark background (Neeraja *et al.*, 2014).

#### Determination of gelation time and gelling capacity

A drop of the formulation was put into a watch glass holding 2 ml of newly manufactured artificial tear fluid at a temperature of 37°C (680 mg NaCl, 200 mg NaHCO<sub>3</sub>, 8 mg sodium chloride dehydrate, and volume make with water to 1 l); the gelation time and gelling capacity of the prepared *in-situ* forming emulsion were determined. The time it took to produce the gel and the time it took to dissolve it was observed to estimate the gelling capacity grade which was determined as follows:

- There was no gelation
- + The gel formed after a few minutes and quickly dissolved
- ++ The gel formed instantly and lasted for a few hours
- +++ The gel formed quickly and remained stable for a long period.

#### pH and isotonicity of prepared formulations

At room temperature, the pH of the produced formulations was measured with a calibrated pH meter (L1617 Elico Instrument Mumbai, India). Placing the pH meter probe in contact with the samples was used to determine the pH of the *in-situ* gel (about 1 cm deep). The measurements were done three times to compute the mean ± SD (Maru *et al.*, 2019). The hemolytic technique is used to determine isotonicity. The produced formulations were administered to a few blood drops, which were then compared to hypotonic, hypertonic, and normal saline solutions under a 45× optical microscope (Wo and Zhai, 2021).

#### Microscopic studies

On a glass slide, the *in-situ* emulsion gel drop was inserted, then covered with a coverslip. An upright bright-field compound microscope was used to view the microstructure organization of the produced emulgel (Leica Microsystems, model: DM750, GmbH, Wetzlar, Germany).

#### Fourier transform infrared spectroscopy analysis

Prepared *in-situ* gels were analyzed by attenuated total reflection infrared (ATR-IR) spectrometer (AlphaE ATR-FTIR, Bruker, USA) in the range of 4,000–4,500 cm<sup>-1</sup>. This instrument was used to examine the chemical interaction between the *in-situ* gels (Behera *et al.*, 2013).

#### Hemocompatibility study

The *in-situ* gel samples were subjected to a hemocompatibility test to determine the degree of hemolysis (Pal *et al.*, 2007a, 2007b). About 50 ml of normal saline (0.9% w/v; 37°C, 30 minutes) was used to equilibrate *in-situ* gels. The final volume was made up to 10 ml by adding normal saline after 0.5 ml of the equilibrated sample was diluted with 0.5 ml of diluted blood (produced by diluting 4 ml of citrated blood with 5 ml of normal saline). Positive control was made by mixing 0.5 ml diluted blood with 0.5 ml 0.1N HCl and 9 ml normal saline, whereas negative control was made by mixing 0.5 ml diluted blood with 9.5 ml normal saline. The samples were incubated for 1 hour and then centrifuged at 3,000 rpm for 10 minutes (Model: Remi-R-8C). A spectrophotometer (Shimadzu 1800, Japan) was used to determine the optical density (OD) of the supernatant at 545 nm. The percentage of hemolysis was calculated as per the following formula:

$$\% \text{ Hemolysis} = \frac{\text{OD test} - \text{OD negative}}{-\text{OD positive} - \text{OD negative}} \times 100 \quad (1)$$

#### Ex vivo corneal permeation study

Franz's diffusion cell was used to test the *ex-vivo* drug permeation of ACZ from the formulations. In the donor compartment, 1 ml of the formulation was deposited, while the receptor compartment was filled with 12 ml of artificial tear solution (ATF, pH 7.4). Corneas were taken from the goat eyeball and cleaned extensively in normal saline. The cornea was then visually checked to see if there were any wrinkles or pores. Between the donor and receptor compounds, the cornea was mounted. The receptor fluid was constantly stirred (300 rpm) at 37°C and the diffusion area was 0.64 cm<sup>2</sup>. At regular intervals, 1.5 ml of receptor fluid was removed from the receptor compartment and replaced with STF (STF). A spectrophotometer (UV-1900i, Shimadzu Corporation, Japan) at 267 nm was used to evaluate the drug content in the samples (Quereshi *et al.*, 2020).

#### In vivo glaucoma study

The rabbits were divided into three groups, each with three rabbits, to investigate the effects of topically given ACZ *in-situ* emulsions. For IOP measurements, the rabbits were confined

Table 1. Composition of prepared formulations.

| Formulations | CO | Pectin solution (0.5%) | Gellan solution (0.5%) | Tween 80 | Span 80 |
|--------------|----|------------------------|------------------------|----------|---------|
| PG1          | 10 | 45                     | 45                     | 0.27     | 0.23    |
| PG2          | 20 | 40                     | 40                     | 0.27     | 0.23    |
| PG3          | 30 | 35                     | 35                     | 0.27     | 0.23    |
| PG4          | 40 | 30                     | 30                     | 0.27     | 0.23    |
| PG5          | 50 | 25                     | 25                     | 0.27     | 0.23    |

in a restricted cage. Rabbits were anesthetized with 4% lignocaine HCl, 30 minutes before the experiment began, and initial IOP was measured. Then, a 5% glucose solution was injected through the marginal vein and IOP was recorded. After that the prepared *in-situ* gel solution was injected into the inferior conjunctival sac in the center, then the lid was closed. The IOP was measured on the cornea using a Schiottz Tonometer (Riester, Germany). The IOP in mmHg units is calculated by converting the scale value into the table value given by the manufacturer.

#### Ocular irritation test

Visual evaluation research was carried out to evaluate the corneal tolerance of the generated emulsions to that of the corneal tissues. The rabbits used in this investigation were albino rabbits weighing between 1.5 and 2.0 kg. In a nutshell, 0.1 ml of the produced emulsions were administered into the rabbit's right eye's ocular sacs. About 0.1 ml saline was administered into the rabbit's left eye, which functioned as the control eye. Following that, the rabbit's eyes were checked for evidence of ocular chemosis after 0, 1, 24, 48, and 72 hours. The process was repeated three times (Quereshi *et al.*, 2020).

## RESULTS AND DISCUSSION

#### Preparation of *in-situ* emulgels

The pictographs of the *in-situ* gel formulations are shown in Figure 1. The prepared *in-situ* emulgel is milky white. With the addition of oil to the formulations, the shade of white was found to rise. Light scattering from the interface between the aqueous and oil phases can explain this phenomenon (Quereshi *et al.*, 2020). The drug was dissolved in the oil phase and the final concentration was made to 0.5% w/v.

#### Determination of gelation time

The viscosity and the gelling capacity are the most important requirement for *in-situ* forming gelling systems. Gelation time plays a vital role in the *in-situ* formulations because it affects the release of drugs at the site of action.

The gelation time of the formulations varies from 16 to 54 seconds (Table 2). The drug's water affinity could be a determining factor in the drug-loaded solution's sol dynamic rate. The presence of ACZ (a drug that is insoluble in water) had a considerable impact on the sol dynamics because it took longer to slide.

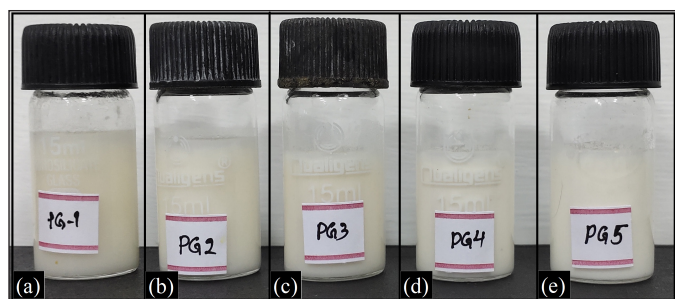


Figure 1. Pictographs of *in-situ* gel (a) PG1, (b) PG2, (c) PG3, (d) PG4, and (e) PG5.

#### Gelling capacity determination

The developed formulations have a “+++” gelling capability since they gelled quickly and remained in the same state for a long time, which is more acceptable to the other formulation because it could be assumed that the release of the drug is to the site of action is for a long period which can reduce the toxicity and the taking the medicaments doses (Table 2).

#### pH and isotonicity of the prepared gels

The pH of all formulations is measured and found within 5.58 to 5.75 which does not irritate the eye as confirmed by the Draize test.

From the isotonicity observation under a microscope, it was confirmed that the said formulations are isotonic with the blood or/and the lachrymal fluid. Because of the hemolytic test, it was confirmed that the presence of the said preparation in the blood does not change the shape or size of the red blood cell, which shows that the prepared formulation is isotonic to human use (Table 2).

#### Microscopic study

The microstructure of the *in-situ* emulsion gel was shown in Figure 2. It was visualized that PG1 shows a low globular size particle whereas the concentration of CO increased with the globular particle was also increased till PG3. After that in PG4, it has been seen that the oil particles were aggregated in a region, and when oil and the hydrophilic solution were the same at that time the particle was not clearly visualized. In all the formulations the particle size was the same and in PG3 it was found to be clumsy.

#### Fourier transform infrared spectroscopy analysis

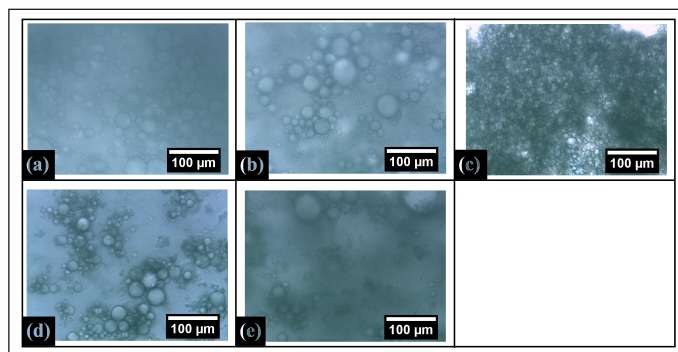
The FTIR analysis was carried out to evaluate the interactions between the CO, pectin, GG, and ACZ within the *in-situ* gel. FTIR spectra of all the formulations (PG1–PG5) are shown in Figure 3. The FTIR spectrum of all the formulations showed broadband in between the wavenumber range of 3,700  $\text{cm}^{-1}$  and 2,980  $\text{cm}^{-1}$ . The peak of the broadband was located at the wavenumber of 3,327  $\text{cm}^{-1}$ . This peak can be related to hydrogen bonding and –OH stretching vibration. The presence of hydroxyl groups in the glucopyranose ring of GG as well as in pectin could form both inter and intramolecular hydrogen bonds (Gnanasambandam and Proctor, 2000; Narkar *et al.*, 2010). In addition, the different signals were observed at 2,923  $\text{cm}^{-1}$ , 2,855  $\text{cm}^{-1}$ , 1,739  $\text{cm}^{-1}$ , 1,636  $\text{cm}^{-1}$ , 1,455  $\text{cm}^{-1}$ , and 1,158  $\text{cm}^{-1}$ . The sharp peak at 2,923  $\text{cm}^{-1}$  and 2,855  $\text{cm}^{-1}$  was attributed to C–H stretching vibrations of methyl groups present in the pectin and GG (Ezati and Rhim, 2020; Narkar *et al.*, 2010). A common peak found at 1,457  $\text{cm}^{-1}$  and 1,162  $\text{cm}^{-1}$  (except PG5) was found in all formulations due to the bending vibration of  $\text{CH}_2$  and the stretching vibration of C–O–C (Zhang *et al.*, 2021). The C=O stretching of the methyl ester (which is generally employed to detect the degree of esterification of pectin) was responsible for the band at 1,739  $\text{cm}^{-1}$  (Shivangi *et al.*, 2021).

#### Hemocompatibility study

In the presence of the sample leachant, the % hemolysis of chelated human blood (with EDTA) was determined and

**Table 2.** Physicochemical properties of formulations.

| Formulations | Gelation time | pH          | % Hemolysis | Gelation capacity |
|--------------|---------------|-------------|-------------|-------------------|
| PG1          | 16.00 ± 2.00  | 5.58 ± 0.10 | 0.176       | +++               |
| PG2          | 20.33 ± 1.52  | 5.75 ± 0.12 | 0.149       | +++               |
| PG3          | 25.33 ± 1.15  | 5.69 ± 0.18 | 0.339       | +++               |
| PG4          | 32.00 ± 2.00  | 5.75 ± 0.14 | 0.309       | +++               |
| PG5          | 53.33 ± 1.15  | 5.62 ± 0.21 | 0.334       | +++               |

**Figure 2.** Micrographs of *in-situ* gel (a) PG1, (b) PG2, (c) PG3, (d) PG4, and (e) PG5.

summarized in Table 2. The result recommended that all the *in-situ* gel samples were highly hemocompatible (% hemolysis <5%) and may be considered safe and biocompatible (Pal *et al.*, 2007a).

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

Drug permeation is the transport of drug molecules from the carrier through the biological membrane to the releasing medium. The carrier type(s), its partition coefficient, and its concentration within the delivery system affect the drug permeation. The figure depicts the cumulative percentage drug release (CPDR) profiles obtained from the prepared *in-situ* forming emulsion (Fig. 4). The permeation of the drug was evaluated for 180 minutes. It was observed that PG1 had the lowest CPDR (1.17% ± 0.05%) and PG5 showed the highest CPDR (1.97% ± 0.11%). The CPDR of the formulations was found in the order of PG5 (1.97% ± 0.11%) > PG4 (1.59% ± 0.09%) > PG3 (1.38% ± 0.07%) > PG2 (1.24% ± 0.03%) and PG1 (1.17% ± 0.05%). The results showed that the CPDR from the formulations was increased with the increase of oil content.

The mechanism of drug diffusion from the films was fitted to the Korsmeyer-Peppas model (KP model) (Qureshi *et al.*, 2021). The diffusion coefficient “*K*” and the release exponent “*n*” in the KP model were calculated using Equation (2) and the results are tabulated in Table 3. An increased *K* value was observed in the increment of oil content within the formulation in PG1 to PG3. The *K* value of PG4 was the lowest among all formulations. Further, increase in oil content in the formulation, the *K* value was increased compared to PG4, but lower than PG3. This suggested the fact that the rate of diffusion of the drug was composition-dependent. The diffusional exponent (*n*) further explained the release mechanism of the drug from a gel base. The drug release

mechanism may be Fickian transport ( $0.45 \leq n$ ), anomalous (non-Fickian) transport ( $0.45 \leq n < 0.89$ ), and/or super case-II transport ( $n > 0.89$ ) (Dash *et al.*, 2010). The *n* values of all formulations were greater than 0.45. This suggested the fact that the drug release from all formulations followed anomalous transport. Thus, the KP model explained the drug release from the polymeric gel base is composition-dependent and follows anomalous diffusion. Further, the CPDR profile was fitted to the Peppas-Shalin model (PS model) to examine the influence of both Fickian diffusion and the relaxation of polymers in releasing ACZ from the gel base. The Fickian diffusion constant ( $k_d$ ) and the polymer relaxation constant ( $k_r$ ) were calculated using Equation (2) and tabulated in Table 3. The results of the PS model parameter designated that the mechanism of diffusion from the emulgel was influenced by the polymer relaxation.

$$F = K.t^n \quad (2)$$

where, *F* is the solute fraction released; *K* is the release rate constant; *t* sampling time and *n* signifies the diffusion exponent.

$$F = k_d.t^m + k_r.t^{2m} \quad (3)$$

Where  $k_d$ ,  $k_r$ , and *m* represent the Fickian diffusion constant, polymer relaxation constant (case II), and the diffusion exponent, respectively.

#### **In vivo corneal tolerance**

The Draize rabbit eye test technique was used to conduct the ocular irritation investigation. Institutional animal ethical approval was obtained for this investigation by license # 49/IAEC/IPT/19, dated 16/03/2019. The left eye served as the control, and regular saline was administered to it.

The prepared gel sample (0.04 ml) was applied to the right eye. Chemosis, conjunctival redness, and corneal opacity were all seen in the ophthalmic health of both eyes. The eyes were assessed after 0, 1, 24, 48, and 72 hours (Fig. 5). All formulations appeared to be biocompatible based on visual inspection.

#### **Intraocular pressure result**

After giving the 5% glucose solution the IOP increases to  $18.7 \pm 0.5$  mm of Hg. When the *in-situ* emulsion gel was inserted, the IOP was decreased to  $9.5 \pm 0.15$  mm of Hg within 2 hours. From these results, it can be concluded that the prepared formulation was used in ocular drug delivery. The studies were done in triplicate and data are tabulated in Table 4 (Fig. 6).

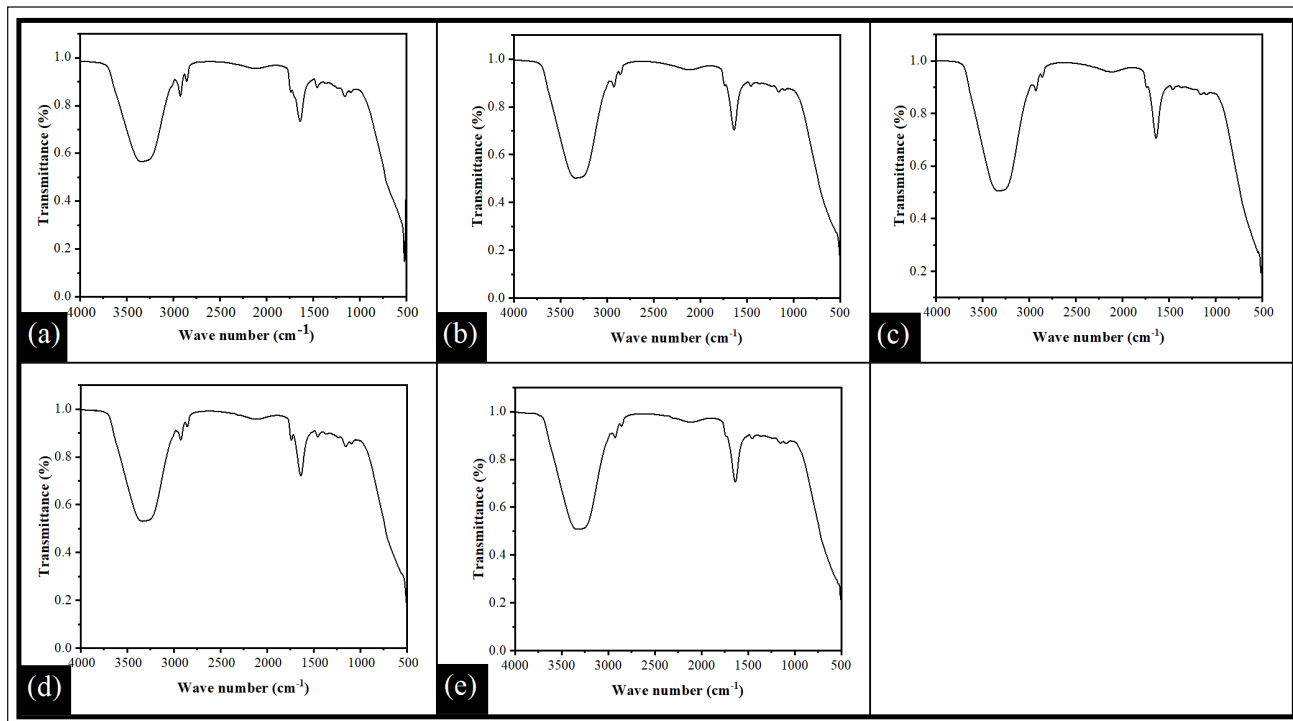


Figure 3. FTIR spectra of *in-situ* gel (a) PG1, (b) PG2, (c) PG3, (d) PG4, and (e) PG5.

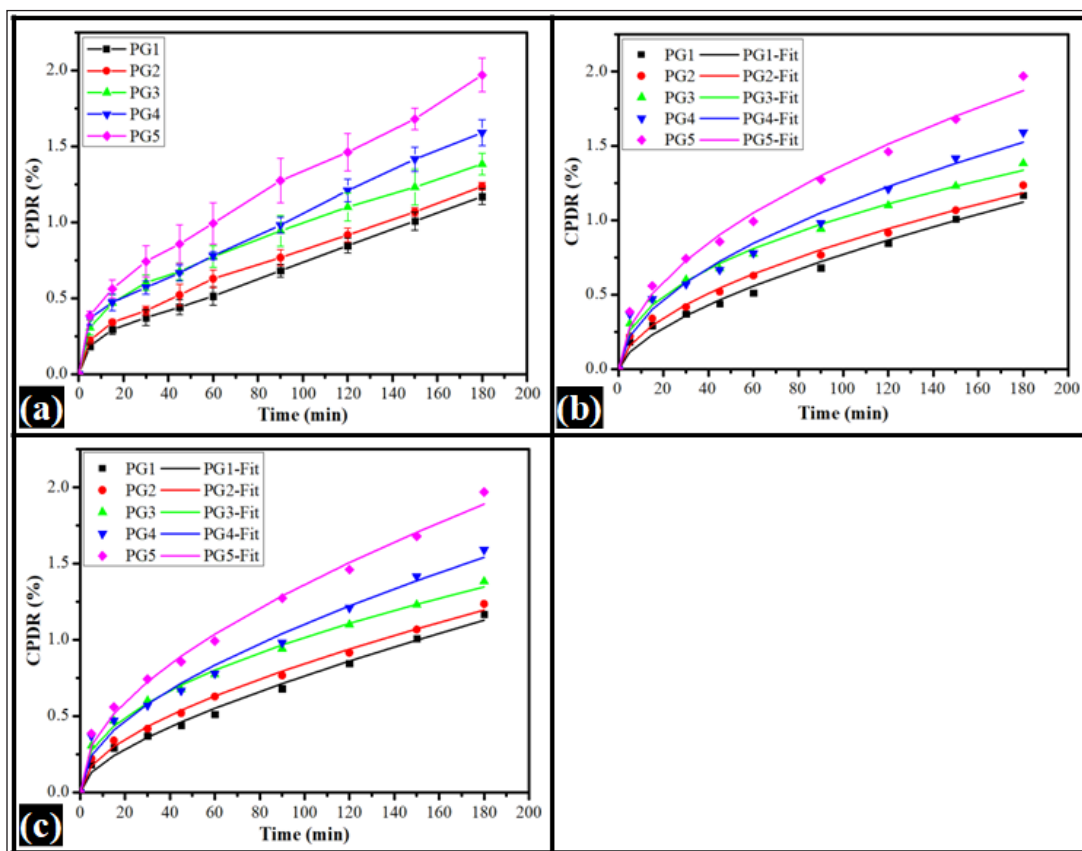
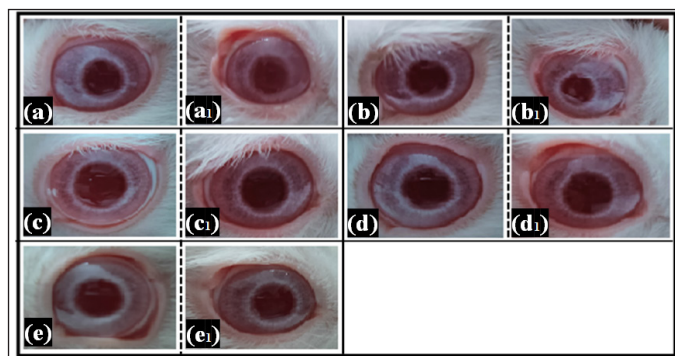


Figure 4. Drug permeation profile of *in-situ* emulgels (a) CPDR, (b) Korsmeyer-Peppas model, and (c) PS model.

**Table 3.** Drug permeation parameters.

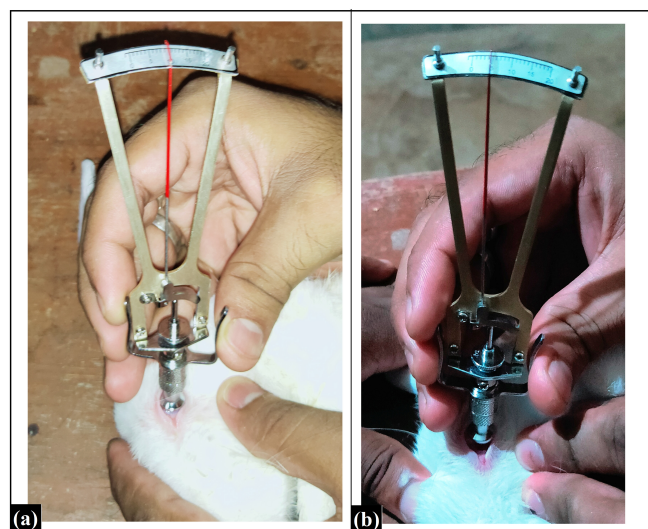
| Study     | Model            | Parameter | Formulations       |                   |                   |                   |                   |
|-----------|------------------|-----------|--------------------|-------------------|-------------------|-------------------|-------------------|
|           |                  |           | PG1                | PG2               | PG3               | PG4               | PG5               |
| Diffusion | Korsmeyer-Peppas | $k$       | $0.042 \pm 0.013$  | $0.065 \pm 0.017$ | $0.126 \pm 0.025$ | $0.095 \pm 0.021$ | $0.123 \pm 0.037$ |
|           |                  | $n$       | $0.639 \pm 0.064$  | $0.562 \pm 0.043$ | $0.457 \pm 0.032$ | $0.538 \pm 0.041$ | $0.530 \pm 0.053$ |
|           |                  | $R^2$     | $0.994 \pm 0.000$  | $0.995 \pm 0.003$ | $0.996 \pm 0.001$ | $0.990 \pm 0.002$ | $0.995 \pm 0.003$ |
|           |                  | $kd$      | $0.053 \pm 0.013$  | $0.076 \pm 0.015$ | $0.130 \pm 0.021$ | $0.106 \pm 0.018$ | $0.137 \pm 0.035$ |
|           |                  | $kr$      | $0.004 \pm 0.002$  | $0.008 \pm 0.003$ | $0.018 \pm 0.006$ | $0.012 \pm 0.004$ | $0.015 \pm 0.005$ |
|           | PS               | $m$       | $0.468 \pm 0.043$  | $0.413 \pm 0.030$ | $0.340 \pm 0.026$ | $0.395 \pm 0.029$ | $0.392 \pm 0.037$ |
|           |                  | $kd/kr$   | $12.484 \pm 2.015$ | $9.828 \pm 1.077$ | $7.746 \pm 1.241$ | $8.793 \pm 1.079$ | $9.170 \pm 1.235$ |
|           |                  | $R^2$     | $0.996 \pm 0.000$  | $0.997 \pm 0.002$ | $0.998 \pm 0.001$ | $0.993 \pm 0.002$ | $0.996 \pm 0.002$ |



**Figure 5.** Draize test conducted in rabbit model as per SOP. Above picture of the eye administered with PG5: (a) Left eye administered with normal saline at 0 hours, (a1) Right eye administered with PG5 at 0 hours, (b) Left eye administered with normal saline at 1 hour, (b1) Right eye administered with normal saline at 1 hour, (c) Left eye administered with normal saline at 24 hours, (c1) Right eye administered with PG5 at 24 hours, (d) Left eye administered with normal saline at 48 hours, (d1) Right eye administered with PG5 at 48 hours, (e) Left eye administered with normal saline at 72 hours, (e1) Right eye administered with PG5 at 72 hours.

**Table 4.** Intro ocular pressure result.

| Formulation           | PG5 (mm of Hg) |
|-----------------------|----------------|
| Before administration | $18.9 \pm 0.5$ |
| After administration  | $9.5 \pm 0.15$ |



**Figure 6.** Measuring of IOP by using Schiottz tonometer (a) After giving the emulgel formulation. (b) After giving the 5% glucose solution.

## CONCLUSION

In this study, the results and discussions proved the potential effects of ACZ-incorporated emulgel for ocular disease. The selection of excipients and methodologies adopted to manufacture *in-situ* emulgel were found to be result-oriented as evidenced by various investigations of this research work. Currently, the regularly used ocular formulations are associated with many unwanted complications. Henceforth, the developed formulation of *in-situ* emulgel warrants further investigations on the mechanism of action of the drug at the molecular level in various ocular diseases. Furthermore, extensive research work is needed to develop different dosage forms of ACZ incorporated *in-situ* gel with reduced toxicity and value-added clinical utility.

## AUTHOR CONTRIBUTIONS

F.A.: Methodology, Investigation, Formal analysis, Writing—original draft. S.H.: Formal analysis, Data curation, assisted during the *in vivo*, and *ex vivo* studies. B.M.: Conceptualization, methodology, supervision, review, editing, and approval of the manuscript. A.B.: Review, editing, and Data curation., Y.G., Formal analysis, Data curation, assisted during the *in vivo*, and *ex vivo* studies. B.S.N.: Methodology, formal analysis, data curation. All authors have read and agreed to publish the manuscript.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## ETHICAL APPROVAL

Institutional animal ethical approval was obtained for this investigation by license # 49/IAEC/IPT/19, dated 16/03/2019.

## DATA AVAILABILITY

The data presented in this study are available from the corresponding author upon request.

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