Original Article

Natural polymer Effect on gelation and rheology of ketotifen-loaded pH-sensitive in situ ocular gel (Carbapol)

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ABSTRACT

In situ gel can be defined as a polymer solution administered as a liquid and when exposed to some physiologic condition such as the pH, ionic, temperature modulation or solvent and UV induced gelation undergo to phase transition to a semisolid gel. Ketotifen fumarate belongs to the histamine H1 receptor antagonists, and Ketotifen fumarate is used in the treatment of allergic conditions like conjunctivitis and rhinitis. This work aims to study the natural polymer effects (xanthan gum,gellan gum) on the properties of pH-trigger in situ ocular gel, then compared the drug-releasing rate of optimized formula with the market ketotifen eye drop. Eight formulations (F1-F8) were prepared using different concentrations of xanthan gum, gellan gum with carpbapol based gel. The best formulation F5 prepared by using0.75% carbapol940 as a gelling agent with 0.3% xanthan gum as a viscosity builder and 0.02% methyl hydroxyl benzoate as a preservative with drug content 99.74%±1.31, pH value, 5.2±0.31, Gel strength 46.6±0.1sec with sustained drug release over period 8h.

Keywords: Ketotifen fumarate, Carbapol940, Xanthan gum, Ophthalmic in-situ gel

Introduction

The topical ophthalmic pharmaceutical preparation is applied to eyelids or eyes for surface or intraocular conditional treatment such as allergic or viral, fungal, and bacterial infection [1]. Conventional ophthalmic preparations available in the market such as ointment, suspension, solution have many disadvantages like poor solution bioavailability produced by drainage or dilution of pharmaceutical solution from eyes, high variability inefficiency, rapid precorneal drug elimination, and blurred vision so that many ocular drug delivery system like gels, hydrogels, in situ gelling system and ocular insert are developed to overcome the above problems [2]. In situ mean in place which is a Latin phrase, and when exposed to some physiologic condition such as the pH, ionic, temperature modulation or solvent and UV induced gelation undergo to phase transition to a semisolid gel. In situ gel can be defined as a polymer solution administered as liquid and when exposure to the physiological condition such as the pH, ionic, temperature modulation or solvent and UV induced gelation undergo to phase transition to a semisolid gel [3]. This system has many merits such as reducing the frequency of drug administration so that patient compliance will be improved and comfortable, providing accurate and reproducible drug quantity when compared to the already formed gel preparation, and providing sustain drug release by remaining the drug in contact with eye cornea for extended time [1]. Polyelectrolyte is a polymer containing either alkaline or acidic functional group which release or accept the protons in responsive to pH environmental changes so that when the change of pH occurred leading to gelling of the polymer solution [4]. Ketotifen fumarate belongs to the histamine H1 receptor antagonists, Ketotifen fumarate is used in the treatment of allergic conditions like conjunctivitis, rhinitis, and the prophylactic management of asthma [5]. It is found in white or light yellowish-white crystallize powders and it was sparingly soluble in acetic acid and methanol, slightly soluble in ethanol, water, and acetic anhydride with pKa: 8.75

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and Log P 4.99 [6, 7]. This work aims to study the natural polymer effect on the properties of pH-trigger in situ ocular gel, including; gelation capacity and rheological properties in terms of viscosity determination before and after gelling. then compared the drug-releasing rate of the optimized formula with the market ketotifen eye drop.

Materials and Methods

Materials

ketotifen fumarate was Supplied as a gift sample from the Al-Sharq al- Awsat factory (Iraq). Carbapol 940 and Xanthan gum was obtained from Hangzhou Hyper Chemicals limited. Gellan gum from (Himedia India). Methyl hydroxyl benzoate was purchased from the Gain Land chemical community.Sodium Chloride from Riedel-de Haën® AG Seelze Hannover (Germany). Sodium bicarbonate from samara (Iraq), Calcium chloride dihydrate obtained from MERCK (Germany), the reagents and chemicals used were analytically graded.

Preparation of in-situ gelling system

Carbapol940 in concentration 0.5%- 0.75% as a gelling agent and Xanthan gum-. Gellan gum of different proportions as thickening agents were used as shown in **Table 1** to prepare eight formulations. Formula 1 and 2 were prepared by dispersing the required amount of carbapol insufficient quantity of D.W. Following the addition of the drug solution (ketotifen and Methyl hydroxyl benzoate as preservative) to the polymer solution at a constant stirring rate so that a homogenous dispersion is formed, the required volume was obtained by adding the D.W and allowed to hydrate overnight. Other six formulations were prepared by adding the thickening agents (Xanthan gum-. Gellan gum) to 0.75% carbapol solution following the addition of the drug solution to the polymer solution with a constant stirring rate until a homogenous dispersion will be formed. Finally, the required volume was obtained by adding the D.W and allowed to hydrate overnight.

Table 1. Formulation of ketotifen ocular in situ gel (expressed as % w/v)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
ketotifen	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Carbapol940	0.5	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Xanthan gum			0.1	0.2	0.3			
Gellan gum						0.1	0.2	0.3
Methylhydroxyl benzoate	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
DW	100	100	100	100	100	100	100	100

Evaluations of the prepared ocular in-situ gel

Clarity

In ophthalmic preparations, one of the important characteristic features is clarity. The clarity was performed for color, turbidity, and the presence of suspending particles. The clarity of all formulations was checked under good light by using white and black background areas [8].

pH measurement

All prepared formulations were pH evaluated by utilizing a calibrated pH meter in which all readings were taken by immersed the probe of the pH meter for 5 min and the measurement was replicated three times [9].

Drug content

The content of ketotifen for all formulations was calculated by dissolving 1ml of each formulation in pH 7.4 simulated tear fluid (STF) then analyzed spectrophotometrically to determine the amount of ketotifen in each formulation [10].

Gelling capacity

The gelling capacity of all prepared formulations was evaluated by measuring both times for the gelation and for dissolving the gel, which was determined by placing each formulation in a tube containing simulated tear fluid (STF) at the proportion of 25:7 at 37 °C [11].

Gel strength

A Five-gram sample of ketotifen in situ gel was put in a 10 ml cylinder. A mass of 3.5g was put on the surface of the gel. The gel strength was determined by the time required of mass to 0.5 cm down penetration into the gel [12].

Spreadability test

The spreadability of the ketotifen formulas was measured by placing one gram of the formula after complete gelation on a glass plate center. Another glass plate of the same size was used above the glass plate containing the gel. 1 kg scale weight was put for five min on the plate upper side, the diameters of the spreading circle were calculated in cm and were considered as a comparative spreadability value (the spread circle diameter– the initial diameter) [13].

Rheological studies

The viscosity of preparation in the ocular in-situ gel is considered an important indicator for sustaining drug release and easing drug administration. The samples' viscosity in mPa was recorded before and after gelation using a digital

viscometer with spindle no.3 at different rotational speeds (6, 12, 30, and 60 rpm) [14].

In-vitro dissolution study

The dissolution of ketotifen in-situ gel was done by the method of diffusion in which the glass tube with open-ended used. In this method, the membrane of cellophane was pre-soaked overnight with the media of dissolution then fixed on a glass tube with one open end. After that, put inside beaker containing 50ml of STF, under the magnetic stirrer 1ml of the in-situ gel was allowed for diffusion throughout the membrane of cellophane into the receptor compartment. After that, 5ml of the sample was withdrawn at a regular time and replaced with equal volume for 8 hrs. then analyzed by UV spectrophotometry apparatus at 300 nm to detect the ketotifen concentration [15].

Differential scanning calorimetric DSC

DSC was used to determine the thermal behavior and compatibility between the ketotifen and the polymer used. Therefore DSC-60 plus shimdzu, Japan of pure ketotifen and the selected formula of the ketotifen in-situ gel were carried out [16].

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectrum was analyzed in the range between 4000-400 $cm^{-1}$for pure ketotifen and selected formula by using pressed-disk technique [17].

Statistical analysis

The obtained results were analyzed statistically by a one-way analysis of variance. When the differences of $P < 0.05$, was considered as significant statistically.

Evaluations of the clarity, drug content, pH, and gelling capacity

The results of, drug content, pH, and gelling capacity are seen in **Table 2**. These results showed that all prepared formulations have a transparent dispersion with an acceptable drug content and pH. Also, it was seen that the pH of formulations decreases as the concentration of carbapol940 increases, due to its acidic nature. Concerning the gelling capacity, it was found upon increasing the concentration of xanthan gum and gellan gum the gelling capacity was significantly increased, with formulation F5 in which the gelation occurs immediately and persists for an extended long time. This is because these polymers contain hydroxyl and carboxyl groups which undergo cross-linking as a result of the increase in the concentration of the polymer, leading to generating a stronger bridge that forms a rigid matrix, there for the gelling will strength [18].

Gel strength

The gel strength is depended on the strength of the chemical or physical bonds that produce the polymer interplaying network, long chains of internal structure, and the polymer type used [19]. The gel strength for all formulations **(Table 2)** indicates that the addition of the second polymer to carbapol940 increased the strength of gel significantly ($p < 0.05$). This increased because of the hydrogen bond formation between the carbapol 940 and secondary polymer within the ocular gel [20].

Spreadability test

The diameters of spreading in situ gel are found to be between 2.7 ± 0.3 and 1.5 ± 0.1 cm **(Table 2)**, which is indicated as the polymer concentration increase, and the spreadability decrease. This is because the spreadability was inversely related to the degree of the polymer networks cross-link which may be returned to the high strength and viscosity when the in situ gel is prepared at a high concentration [21].

Results and Discussion

Table 2. Results of % drug content, clarity, pH, , gelling capacity, gel strength and spreadability of ketotifen Formulas

Formula	Drug content (%)	pH	Gel strength(sec)	Spreadability(cm)	Gelling capacity
F1	97.13±1.13	4.7±0.04	15.2 ±0.12	2.7±0.3	-
F2	99.5±1.07	4.5±0.14	20.12±0.08	2.5±0.11	+
F3	97.11±1.19	4.9±0.01	35.04±0.3	2.0±0.31	+
F4	98.04±0.02	5.0±0.07	43.11±0.3	1.8±0.6	++
F5	99.74±1.31	5.2±0.31	46.6±0.1	1.5±0.1	+++
F6	98.1±0.74	4.6±0.31	26.0±0.17	2.3±0.2	-
F7	97.03±1.55	4.8±0.05	30.21±0.22	2.0±0.33	+
F8	99.11±1.63	5.1±0.18	34.3±0.4	1.8±0.05	++

Where - No gelation,+ Gelation within a few minutes and dissolve rapidly,++ Immediate gelation and remaining within a few min,+++ Immediate gelation with a few extended periods, the results represent mean± SD, n=3

Rheological studies

Figure 1 showed the values of viscosity obtained for all formulations at different angular velocities. The formulations exhibited pseudoplastic rheology in which it exhibit high viscosity at a decease shear rate with low viscosity at increasing shear rates. This is due to the polymer molecules normally disarranging and as the shear stress was increasing. The normal disarrangement molecules are aligned in their direction so that these alignments reduce the gelling agents' internal resistance and hence reduce the viscosity [22, 23]. In addition, as the concentration of the polymer increased, a significant increase ($P < 0.05$) in viscosity was observed this is because the high degree of cross-linking occurred when the polymer concentrations increased [24].

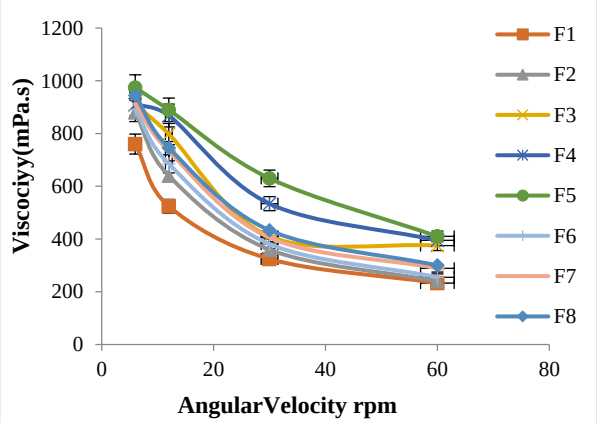


Figure 1. The viscosity of in situ gel at physiological pH (after gelation), (mean± standard deviation, n=3)

In-vitro dissolution study

The release of ketotifen was affected by the concentration and type of the polymer used. The result shows the in vitro release percent of ketotifen from in situ ocular gelfor F1 was 83.88% and for F2 was 79.06% after 8 h. showed the effect of polymer concentration through using carbapol 940 in 0.5% and 0.75% w/w, respectively. The result indicate that as increasing the carbapol 940 concentration, the drug release will decrease because increasing the viscosity of the gel layer and a long diffusion path length will create retardation of the drug release from the gel [25]. F3-F8 give percent ketotifen release after 8 h was 70.55%,66.77%, 61.58%, 76.15%, 72.12%, and 67.83%, repectively. The results shows the effect of polymer combination, it was seen the percent of drug release decreased as the concentration of xanthan gum or gellun gum increased. This is because of increasing the gel strength and decrease in dimension and number of the gel structure channel so that reduce the dissolution media penetration and limit the movement of the drug molecule, thus the release of drug delayed [26]. **Figure 2** show a comparison of selected formula F5 with a marketed ketotifen eye drop, it was seen that there is a statistically significant decrease ($p < 0.05$) of drug release from the selected formula when compared with marketed ketotifen eye drop this is because of the incorporation of xanthan gum to carbapol enhanced the polymer consistency and sustained the drug release.

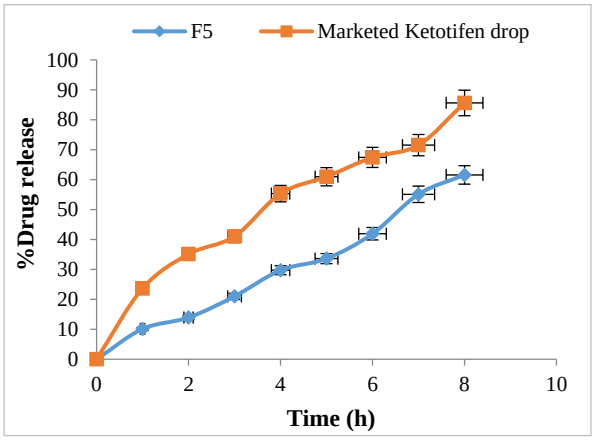
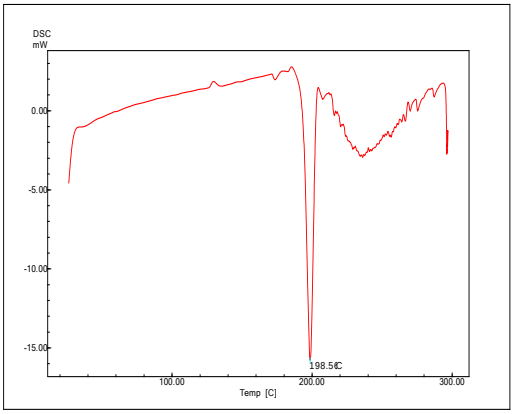


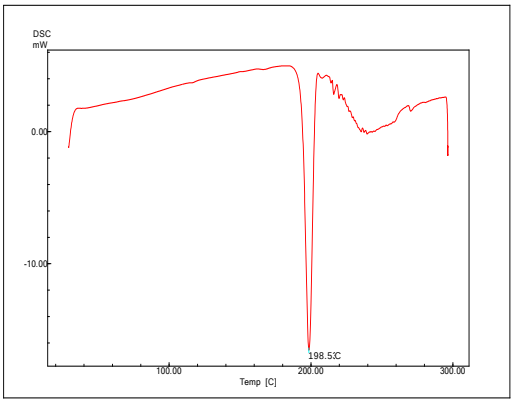
Figure 2. Comparison of selected formula with marketed ketotifen eye drop on the release of in pH7.4 STF at 37°C

Differential scanning calorimetry (DSC)

For the DSC study, **Figure 3** (left) shows a sharp endothermic peak near the ketotifen melting point that is equal to 198.56 C indicating the ketotifen used within the pure state [26]. Also, the endothermic peak in **Figure 3** (right) of the physical mixture (1:1:1) ketotifen, carbapol, xanthan gum of selected formula F5 was observed at 198.53 C giving indication there is no interaction between the drug and polymer used.



a)



b)

Figure 3. DSC thermo gram of pure ketotifen (left) and physical mixture of selected formula (right)

FTIR spectroscopic analysis

The infrared spectra for pure ketotifen powder. The pure drug exhibits characteristic peaks at 2979.48 cm^{-1} of C-H thiophene stretching, 3448.1 cm^{-1} of aromatic C-H stretching, 1648 $cm^{-1}$of carbonyl C=O and 1257.36/1317.14 cm^{-1} of in-plane C-Hstreching [27]. Meanwhile, the physical mixture of ketotifen, carbapol, and xanthan gum powder in the ratio (1:1:1) revealed appearance of ketotifen characteristic peaks at 2676.71,3423.03, 1648.84 and 1259.29/1317.14 cm^{-1}, respectively as shown in **Figure 4** The result indicates no interaction between the drug and the polymers used in the formulation [28].

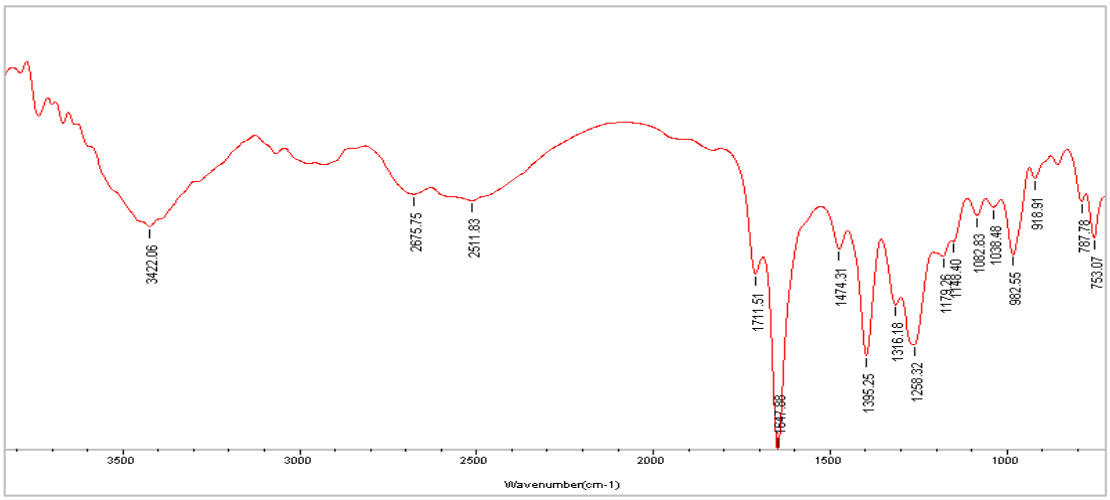


Figure 4. FTIR spectrum of physical mixture of ketotifen,carbapol, and xanthan gum at ratio (1:1:1)

Conclusion

ketotifen fumarate can be successfully formulated as pH-sensitive in situ ocular gel. The best results were obtained from a combination of 0.75% carbapol 940 as a gelling agent with 0.3% xanthan gum as a viscosity builder. The drug release was sustained from the ocular in situ gel compared with a conventional eye drop. Hence, pH-sensitive in situ ocular gel is useful as a promising approach for sustaining the drug release.

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Conflict of interest: None

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Ethics statement: None

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