Research Article

Mucoadhesive oral *in situ* gel of itraconazole using pH-sensitive polymers: Preparation, and *in vitro* characterization, release and rheology study

Roaa A. Nief*, Manar Adnan Tamer, Shaimaa Nazar Abd Alhammid

ABSTRACT

Objective: The study was to design a mucoadhesive pH-triggered *in situ* gel of itraconazole, to prolong the residence time on oral mucosa, and to reduce the frequency of application and amount of drug administrated. **Materials and Methods:** Six formulations containing itraconazole 1% were prepared using different concentrations (0.5 and 0.7% w/w) of carbopol 934 (CB) and in combination with different viscosity-enhancing agents such as hydroxypropyl methylcellulose (HPMC) K4M (1 and 1.5%), xyloglucan (0.5%), and hyaluronic acid (1%), to formulate a pH-triggered system that undergoes sol-to-gel transition on change in pH to the physiological pH. The formulations were evaluated for physical appearance, PH, gelation capacity, rheological properties, spreadability, mucoadhesive force, and *in vitro* release of drug. **Results:** Formula F4 (0.7% CB, 1.5% HPMC K4M) showed acceptable pH and appearance, acceptable gelling capacity, and shear thinning rheological property, good mucoadhesive force, and provided 80% release over a 6-h period. **Conclusion:** The developed itraconazole mucoadhesive oral *in situ* gel was non-irritant, retarded the release for 6 h, and it is good alternative to conventional oral gel.

KEY WORDS: Carbopol 934, Hydroxypropylmethylcellulose, Itraconazole, Mucoadhesive *in situ* gel

INTRODUCTION

Mucoadhesive dosage forms have been used to target local disorders at the mucosal surface to reduce the overall dosage required and to minimize the side effects that may be caused by the systemic administration of the drugs. Mucoadhesive formulations use polymers as the adhesive component. These polymers attract water from the mucosal surface and this water transfer leads to a strong interaction. These polymers also form viscous layers when hydrated with water, which increases the retention time over the mucosal surfaces and leads to adhesive interactions.[1]

In situ is a Latin phrase which can be translated literally as "in process." *In situ* gels are drug delivery systems that are in solution forms before administration in the body, once administered they undergo gelation *in situ* to form a gel. It is basically a polymeric drug delivery system.

Advantages of *in situ* forming mucoadhesive polymeric delivery systems include ease of administration, improved local bioavailability, reduced dose concentration, reduced dosing frequency, and improved patient compliance and comfort. Furthermore, the formulation is less complex which lowers the manufacturing cost.[2] There are several possible mechanisms that lead to *in situ* gel formation: Solvent exchange, ultraviolet (UV) irradiation, ionic cross-linkage, pH change, and temperature modulation.[3]

pH-triggered *in situ* gelation shows sol-gel transformation when pH is raised. All the pH-sensitive polymers contain pendant acidic or basic groups that can either accept or release protons in response to changes in environmental pH. Polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of these polymers increases as the external pH increases in the case of weakly acidic (anionic) group-rich polymer but decreases if the polymer contains weakly basic (cationic) groups.[4]

Itraconazole is a triazole antifungal agent with a wide spectrum of activity. It is well tolerated in patients as compared to other triazoles such as fluconazole, ravuconazole, and posaconazole. Itraconazole being Class II drug, it is a highly hydrophobic weak base and it is used in the treatment of fungal infections.[5]

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The objective of this study was to develop mucoadhesive *in situ* gel formulation containing itraconazole for the treatment of oropharyngeal candidiasis. This was needed to reduce degradation of antifungal agents in salivary fluid and increase absorption of the drug, leading to an improvement in its bioavailability, to reduce its dosing frequency, and to achieve sustained release effect.

MATERIALS AND METHODS

Materials

Itraconazole powder supplied by Provizer Pharma (India), carbopol-934 (CB) supplied by HiMedia (India), hydroxypropyl methylcellulose (HPMC K4M), hyaluronic acid (HA), xyloglucan supplied by Hangzhou Hyper Chemicals limited, methylparaben supplied by Gainland Chemical Community, UK, Tween 80 supplied by Merck (Germany), and triethanolamine supplied by Hopkins and Williams Ltd., England. All other chemicals and solvent were of analytical grade.

Methods

Preparation of pH-induced in situ gel formulations

pH-sensitive *in situ* gel formulations were prepared according to Table 1.

Formulas 1 and 2 were prepared by dispersing CB in a concentration (0.5%, 0.7% w/v) in a beaker containing purified water. Itraconazole was dissolved in a mixture of 0.1 N HCl and 1% w/v Tween 80. Methylparaben was added as a preservative to the resulting drug solution. The drug solution was then added to polymer solution with constant stirring using magnetic stirrer until a uniform solution was obtained and the volume completed with distilled water (D.W).

Formulas 3 and 4 were prepared using CB in a concentration (0.7% w/v) in combination with HPMC K4M (1%, 1.5% w/v) using hot method, by heating 70 ml of water until it boils. Then slow addition of the desired amount of HPMC part wise with stirring on a hot plate, after complete addition of HPMC, the solution was allowed to cool to obtain a clear colorless viscous dispersion. Then, in another beaker, CB was weighted and added to the HPMC dispersion with continuous stirring on the magnetic stirrer with heating to approximately 70°C. The drug solution was then added to polymer solution with constant stirring and the volume completed with D.W.

Formula 5 was prepared with xyloglucan (0.5%w/v) dispersed in water, this dispersion was added to CB with continuous mixing; then, the itraconazole solution was added and the volume completed with D.W.

While formula 6 with HA (1% w/v) was dispersed in cold water using a high-speed mixer, this dispersion was added to CB with continuous mixing; then, the itraconazole solution was added and the volume completed with D.W.

Evaluation Parameter

Appearance

The formulations were observed for general appearance, i.e., color, and for the presence of suspended particulate matter. The clarity of the preparation was checked using against black and white background.[6]

pH of the formulation

The developed formulations were evaluated for pH using a digital pH meter. The pH meter probe was immersed in the formulation for 5 min and then the readings were taken.[6]

Gelling capacity (sol-to-gel transition/in vitro)

The gelling capacity test was implemented by placing a drop of each formula in a test tube containing 5-ml phosphate buffer (pH 6.8) and equilibrated at 37°C. Visual assessment of the gel as it forms time for gelation as well as time taken for the gel formed to dissolve was monitored during this test.[7]

Rheological studies

Viscosity and rheological properties of *in situ* forming drug delivery systems are an important factor in determining residence time of drug.[8] Viscosity determination was carried out using digital viscometer under different shear rates (6, 12, 30, and 60 rpm) with spindle no.3. It was determined for in situ gel at non-physiological pH and room temperature (As a solution) and at physiological pH 6.8 and 37°C (As a gel) conditions, respectively. The viscosity of the

Table 1: Formula composition of itraconazole mucoadhesive *in situ* oral gel (expressed as % w/v)

Formulation	Itraconazole	Methylparaben	Carbopol 934	HPMC K4M	Xyloglucan	Hyaluronic acid	D.W
F1	1	0.02	0.5	-	-	-	100
F2	1	0.02	0.7	-	-	-	100
F3	1	0.02	0.7	1	-	-	100
F4	1	0.02	0.7	1.5	-	-	100
F5	1	0.02	0.7	-	0.5	-	100
F6	1	0.02	0.7	-	-	1	100

HPMC: Hydroxypropyl methylcellulose, D.W: Distilled water

samples was recorded before and after gelation. The viscosity of the formulations was measured in mPa.s.

Spreadability test

A sample of 0.1 g of each gel was pressed between two slides with 500 g weights and left for about 5 min where no more spreading was expected. Diameters of spread circles were measured in cm and were taken as comparative values for spreadability (diameter of the spread circle– initial diameter).[9]

Mucoadhesive force

The mucoadhesive forces of the formulas determined using modified physical balance method. This equipment comprised a two-arm balance, one side of which contained two glass plates and the other side contained a beaker.

The membrane used for mucoadhesive testing was fresh sheep buccal mucosa. Fresh sheep buccal mucosa was sprinkled by phosphate buffer (PH 6.8), then fixed using rubber band or glue to the upper side of the lower plate and another was glued to the lower side of the upper plate using rubber band. The *in situ* gel was placed on the mucosal membrane fixed to the upper side of the lower plate. Then, the upper plate was placed over the lower plate and 5 g preload force (or contact pressure) was applied for 2 min (preload time). After removal of the preload force, the water was added slowly to previously weighted beaker placed on the right hand pan until vial gets detach. The mucoadhesion force expressed as the detachment stress in dyne\cm^2 was determined from the minimal weight that detaches the tissue from the surface of each formula using the following equation:[10,11]

$$\text{Detachment stress dyne/cm}^2 = \text{m.g/A}$$

Where,
m: The weight added to the balance in g,
g: The acceleration gravity 980 (cm/s^2) and
A: Area of tissue exposed.

In vitro release study

The *in vitro* release study was carried out using a modified dissolution apparatus type II (paddle type). 1 ml of each formula was placed in a dialysis membrane (0.08 µm pore size) which was previously soaked in phosphate buffer pH 6.8 overnight. The dialysis membrane is tied to the paddle shaft and immersed in 150 ml phosphate buffer pH 6.8 as a dissolution media rotated at 50 rpm and maintained at 37 ± 1°C.[12]

Samples of 5 ml were withdrawn at specific time interval and replaced with equal volume of fresh media. The samples were analyzed for drug concentration using UV-visible spectrophotometer at 263 nm.

Statistical Analysis

The results obtained statistically analyzed using one-way analysis of variance. Differences of $P < 0.05$ considered statistically significant.[13]

RESULTS AND DISCUSSION

Evaluation Parameter

Visual appearance and pH

All the prepared *in situ* gelling systems were evaluated for visual appearance, clarity, and pH as shown in Table 2. The prepared formulas were white dispersions retained liquid state (free flow) at pH range (4.2–5.1) and at room temperature except F6 shown very thick gel at formulation, so F6 was excluded because it is not considered within *in situ* gelling systems. It was observed that, as CB concentration increases, the pH of the formulation decreases due to the acidic nature of the polymer.

Gelling capacity (sol-to-gel transition/in vitro)

The gelling capacity of the formulations was evaluated for gelling property to identify the formulations suitable for use as *in situ* gelling systems. The time taken for gel to form and the time taken for it to dissolve were noted. The grading for gelling capacity is shown in Table 3. Increasing the concentration of CB improved the gelation time. Formulation F4 containing HPMC K4M (1.5%) showed excellent gelation as compared to the F3 containing HPMC K4M (1%) due to increasing the concentration of HPMC K4M for the same CB concentration.[14]

Rheological study

Rheology is the study of the deformation and flow of matter that includes the measurement of viscosity, which indicates resistance of a fluid to flow. Viscosity

Table 2: PH values and physical appearance of the prepared *in situ* gel formulations

Formulas	pH	Physical appearance
F1	5.1	White thin dispersion
F2	4.8	White dispersion
F3	4.7	Opaque dispersion
F4	4.9	Opaque pourable dispersion
F5	5	White very thin dispersion
F6	4.2	Very thick gel

Table 3: *In situ* sol-gel transition time and gelation capacity of the prepared *in situ* gel formulations

Formulas	Gelation time (min)	Gelling capacity*
F1	0.8	−
F2	1	−
F3	7	+
F4	16	+++
F5	8	++

*Where: No gelation, +Gel after few minutes, dissolve rapidly, ++immediate gelation, remain for few min, +++immediate gelation but for few extended periods

is the measure of the internal friction of a system, the greater the friction requires the greater the amount of force to cause this movement that is called shear.[15] Figures 1 and 2 show the rheological profile of the formulations at physiological and non-physiological pH, respectively.

It was found that, as the shear rate increased, the viscosity of gel decreased indicating shear thinning pseudoplastic flow property.[16] The formulations in a liquid state (at pH of the preparation) were exhibited low viscosity, an increase in the pH to 6.8 caused the solutions to transform into gels with high viscosity.

The viscosity of the formulations was found to be influenced by the concentration of polymers used; hence, a significant increase ($P < 0.05$) in viscosity was observed with increasing polymer concentrations as showed in F1 and F2 using CB in increasing concentrations and in F3 and F4 when using combination of CB and HPMC in increasing concentration. Using different polymer combinations resulted in a significant increase in viscosity ($P < 0.05$). Formulation F5 that was containing 0.7% CB and 1.5% HPMC exhibited higher viscosity than other formulations containing CB 934 (F1-F4) at the same concentration. This may be due to higher degree of cross-linking at higher concentrations of polymers.[17]

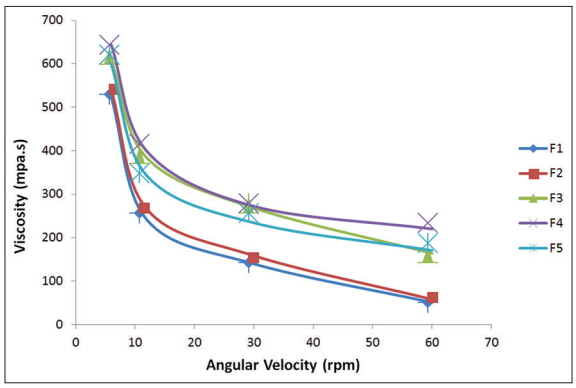

Figure 1: The viscosity of *in situ* gel at non-physiological pH (before gelation)

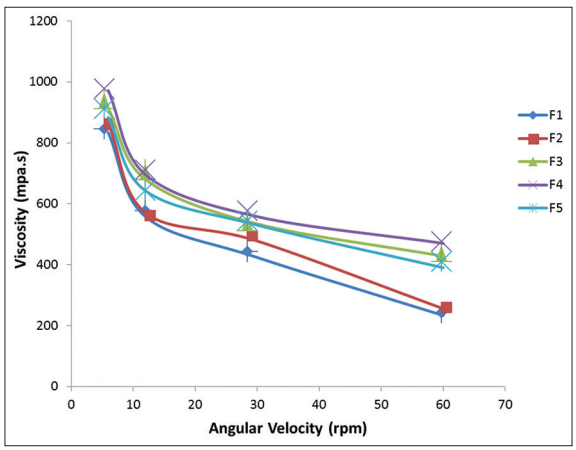

Figure 2: The viscosity of *in situ* gel at physiological pH (after gelation)

Spreadability test

Spreadability is a significant property within the improvement of semisolid preparations designed for topical and mucosal systems, due to the fact it is responsible for the overall performance of a formula.[18] It was observed when an increase the polymeric concentration has significant effect on spreadability, due to viscosity of the gel been increased, at the same time, spreadability of the formulation was reduced.

It seen that F4 (0.7% CB and 1.5% HPMC) gave minimum spreadability area (1.3 cm) compared with other formulas 2.5, 2, 1.5, 1.3, and 1.8 cm, respectively. The spreadability is inverse relationship to the degree of cross-linking in the polymer networks when increase.[19] The latter effect may be returned to the higher viscosity and strength of prepared gel at higher concentration used.[20]

Mucoadhesive force

The mucoadhesion strength is one of the most important physicochemical parameters for prolonging mucoadhesive retention time and thereby better therapeutic effects of the mucoadhesive polymer. The degree of mucoadhesion depends on type and concentration of polymer, excipients used in the dosage form, degree of hydration, polymer chain length, and molecular weight of the polymer.[21] The mucoadhesion properties of the formulations of varying ratio of polymers are shown in Table 4.

It was seen that formula F4 which contains CB 0.7% w/w and 1.5%w/w HPMC gave best result among the other formula, this may be attributed to the effect of hydrophilic properties of CB, that resulted in a hydration of polymeric chains which involve glycoprotein chain of mucin in the oral mucous membranes,[22] in addition the 1.5% HPMC, appeared to have maximum mucoadhesive force compared with other viscosity enhancer used. The latter result is referred to in an increasing the number of penetrating hydrophilic chain to glycoprotein with concentrations of polymer increase.[23]

Table 4: Mucoadhesive force of itraconazole mucoadhesive oral *in situ* gel formulas

Formulas	Mucoadhesive force (dyne/cm^2)
F1	4274.12
F2	4360
F3	5047.6
F4	6219
F5	5013.3

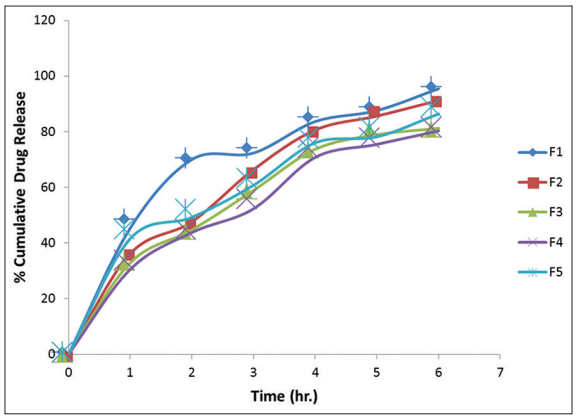

Figure 3: Variables affecting the release profile of *in situ* gel formulas in phosphate buffer pH 6.8 at 37°C

In vitro release study

The release of drug from the dosage form plays an important role in the drug delivery systems and in determining the therapeutic effect of the drug. An *in vitro* drug release study is indeed as a prerequisite to obtaining correct predictions to design and test the *in vivo* activity of drug delivery systems.[24]

The prepared itraconazole oral *in situ* gels using CB934 as pH-triggered polymer were subjected to dissolution study in phosphate buffer (pH 6.8) to study the variables that affecting the percentage of drug release [Figure 3].

Effect of polymer concentration is shown in F1 and F2 using CB 934 in 0.5% and 0.7% w/w, respectively. The results indicated that, as the concentration of CB increases, the release of drug decreases. An increase in polymer concentration will increase the viscosity of gel layer as well as cause gel layer with longer diffusional path length creating greater retardation of drug release.[25]

Effect of polymer combination was shown in the formulas (F3, F4, and F5) that containing different viscosity enhancer polymer in combination with CB. CB is usually used in combination with other viscosity enhancer polymers to achieve the desired consistency so as to facilitate sustained drug release, also to improve mucoadhesion.[26]

It was seen that F4 with 0.7% CB + 1.5% HPMC has significant ($P < 0.05$) longer dissolution profile in comparison with other formulas. It shown that the presence of a highly water-soluble component such as HPMC generates an additional osmotic gradient, thus resulting in a faster rate of polymer swelling and a large increase in gel thickness.[27]

This effect of drug release rate and extent is inversely proportional to the thickness of this gel layer because it takes time for drug molecules to travel across the gel layer and reach the dissolution media.[28] The results indicated that the formula F4 was considered as an optimized formulation among all formulations. This may be due to the presence of higher concentration of CB along with HPMC K4M.

CONCLUSION

pH-sensitive *in situ* gel of itraconazole was successfully prepared for controlled release of drugs that provide a number of advantages over conventional dosage forms.

Formula F4 with 0.7% CB and 1.5% HPMC showed excellent physical property, pH-triggered *in situ* gelation time (that mean it will give longest resident time in the oral cavity), good viscosity and mucoadhesive force, and sustain the release of itraconazole in test time period.

In situ gel formulation of itraconazole with mucoadhesive properties was found to be promising for prolonging buccal residence time and thereby better therapeutic effects. In addition, they provide intimate contact between a dosage form and the absorbing tissue which may result in high drug concentration in local area. The *in situ* formulation may improve the patient acceptability, as the formulation is applied in the form of sols, which on contact forms the corresponding gels causing less irritation or pain.

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