CROSSLINKED CHITOSAN GEL FOR LOCAL DRUG DELIVERY OF CLOTRIMAZOLE

Bhupendra G. Prajapati and Dr. Madhabhai. M. Patel

S.K. Patel College of Pharmaceutical Education & Research, Ganpat Vidyanagar, Ganpat University, Kherva. PIN: 382711 City: Mehsana, State: Gujarat, Country: India. **E-mail: bhupen_27@yahoo.co.in, bhupen27@gmail.com**

Corresponding author: Bhupendra G Prajapati
S.K. Patel College of Pharmaceutical Education & Research,
Ganpat University, Ganpat Vidhyanagar, Kherva -382711, Mehsana, North Gujarat, India. Contact: bhupen27@gmail.com Phone: (O) 91-02762-286082

INTRODUCTION

The use of natural or synthetic biodegradable polymers in last decade was extensive as they offer many advantages over non-biodegradable polymers for topical drug delivery applications. The study of hyaluronic acid biopolymer was shown to penetrate and disseminate through all layers of intact skin in mice and humans, reaching the dermis within 30 minutes of application in mice.[1] Natural or synthetic polymers that are biodegradable may offer advantages over non-biodegradable polymers for topical drug delivery applications. In the present study we used chitosan [a (1-4) 2-amino-2-deoxy-β-D-glucan] is obtained by the alkaline deacetylation of chitin. Chitosan molecule is a copolymer of *N*-acetyl-D- glucosamine and D-glucosamine [2, 3]. The sugar backbone consists of β-1, 4-linked D-glucosamine with a high degree of *N*-acetylation, a structure very similar to that of cellulose, except that the acetylamino group replaces the hydroxyl group on the C-2 position. Thus, chitosan is poly (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose), where the *N*- acetyl-2-amino-2-deoxy-D-glucopyranose (or Glu-NH2) units are linked by (1□4)- β-glycosidic bonds[4,5]. Chitin is the second most abundant polysaccharide in nature, cellulose being the most abundant. Chitin is found in the exoskeleton of crustacea, insects, and some fungi. [4, 6]. Chitosan in solution exists in the form of quasi- globular conformation stabilized by extensive intra and inter-molecular hydrogen bonding. The hydrogen bonding in chitosan chains due to the presence of amine and hydroxyl groups causes the high viscosity of chitosan solutions. According to Chen et al.[7], the degree of deacetylation of chitosan, which will determine the number of intermolecular hydrogen bonds, was found to affect the rigidity of the polymer film. For chitosan hydrogels, the extent of dissociation of the hydrogen bonding may affect the swelling kinetics of the gels. At low pH, the hydrogen bonding dissociates due to the protonation of the amine groups leading to faster swelling.
The chitosan has favorable biological properties such as biodegradability [8] and biocompatibility [9, 10]; it has attracted a lot of attention in the pharmaceutical and medical fields. Due to its natural abundance and specific biological properties, chitosan is an attractive material for multiple applications including the following: ophthalmic delivery [11, 12] 23,24, nasal delivery [13,14], buccal delivery [15, 16], periodontal delivery [17,18], floating drug delivery [19] , peroral drug delivery [20, 21] intestinal drug

delivery [22, 23] , colon delivery [24, 25] vaginal delivery [26, 27], transdermal delivery [28, 29] and vaccine delivery [30].
Clotrimazole (CLZ) is an antifungal medication, which prevents fungus from growing on your skin. CLZ topical is used to treat skin infections such as athlete's foot, jock itch, ringworm, and yeast infections. Blending of chitosan with other polymers [31], [32, 33] and crosslinking are both convenient and effective methods of improving the physical and mechanical properties of chitosan for practical applications. Immunization studies carried out on rats using glutaraldehyde crosslinked chitosan spheres [34] showed promising tolerance by the living tissues of the rat muscles.
The objective of present study was to determine the effect of different grade of chitosan as a topical gel formulation crosslinked and non crosslinked with glutaraldehyde to form hydrogel as a controlled release vehicle containing CLZ by in-vitro drug release evaluation.

EXPERIMENTAL

Materials

Chitosan HW and LW (SiberHegner India, Mumbai) with a viscosity ranging from 500 to 100 cps and 100 to 20 cps (1% solutions in 2% lactic acid as measured on a Brookfield LVT viscometer, Spindle No.2, 60 RPM at RT); 95 % and 90% of degree of deactivation respectivey. Lactic Acid and Ethanol were obtained from S. D. Fine Chem, Boisar, Mumbai, India. CLZ was gift from Helios Pharmaceutical, Kadi, India. All the other reagents were analytical grade.

Development of Gel Formulation

Accurately 1% CLZ was dissolved in 15% ethanol. Solutions of %2 and 4 % chitosan were prepared by dissolving chitosan in 2% lactic acid. Higher chitosan concentrations were obtained by first heating the chitosan slurry to 90ºC followed by addition of 2% lactic acid. To the each batch 5 % glycerin was added as a plasticizer. The CLZ solution was then mixed with the chitosan gel and stored in well-closed container.
To get crosslinked hydrogel of chitosan prepared gel containing CLZ were allow to crosslinked with 1% aqueous solution of glutaraldehyde. The mixture was stirred for 30 min at room temperature until it became increasingly viscous. The viscous gel was poured into a petridish and dried to room temperature overnight to remove bubble. The hydrogels were further dried under vacuum at 50 ºC to completely remove the residual solvent results in a hydrogel film. Typical composition showed in table 1.

Evaluation of Prepared Gel

pH Measurement

pH measurement of the gels were done by using a digital type pH meter (Systronic, 361-micro pH meter), by dropping the glass electrode and reference electrode completely into the gel system ,so as to cover the electrode.

Extrudability

The extrudability of the formulations from the aluminium collapsible tubes was determined using a universal testing machine, equipped with a 10 kg load cell (LF Plus, Lloyd Instruments, Hampshire, UK). Aluminium collapsible tubes filled with 10 g gels were held between two clamps positioned at a distance of 5 cm. The tube was compressed by the compression probe of 25 mm diameter, at a rate of 10 mm/s. The force required to extrude the gel from the collapsible tube was recorded. Measurements were run in six replicates, for each formulation.

Viscosity

The viscosity of the prepared gels was measured using a Brookfield viscometer (Model No: LV II +Pro), at a controlled temperature of 25±2° at 50 rpm.

Swelling Characteristics

The swelling behaviour of the crosslinked and non-crosslinked hydrogel films was measured deionized water at room temperature. Pre-weighed dry hydrogel films were immersed in acetate buffer and phosphate buffer solutions pH 5.0 and 7.2 respectively. The films were withdrawn from the solutions at different time intervals and their wet weight were determined after first blotting with a filter paper followed by blowing with a stream of air to remove the surface water and immediately weighing the films. The swelling ratio was calculated using the equation

$$\text{Esr}\,(\%) = \frac{(\text{Ws} - \text{Wd})}{\text{Wd}} \times 100 \qquad (1)$$

where *Esr* is the water absorption (%wt) of the films,
Wd and *Ws* are the weights of the samples in the dry and swollen states respectively.
The equilibrium water content (*EWC*) was calculated from the following equation:

$$\text{EWC}\,(\%) = \frac{(\text{We} - \text{Wd})}{\text{We}} \times 100 \qquad (2)$$

where *We* represents the weights of the swollen state at equilibrium

Spreadability

The spreadability [35] of the gel formulations was determined 48h after preparation, by measuring the spreading diameter of 1g of the gel between two glass plates after 1min. The mass of the upper plate was standardized at 125 g. The spreadability was calculated by using the formula S = m. l/t, where S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide, and t is the time taken. Homogeneity of various gel formulations was tested by visual observations.

Gel Characteristics

Gel strength of the formulations was measured using the Universal testing machine, as described by Lee *et al* . [36] and Chung *et al.* [37], with slight modification. Gel hardness was measured at room temperature using a 6 mm diameter probe, on the universal testing machine (Model, LF Plus, Lloyd Instruments, Hampshire, UK).

Drug content

The Drug content was determined in each gel by spectrophotometrically at 270 nm in pH 7.2-phosphate buffer.

Invitro Drug Diffusion

In vitro diffusion profile of the hydrogel was determined by using Franz diffusion cells. A semi permeable membrane with 2.2 cm^2 diffusion area was used as a barrier. The diffusion studies were carried out at 37±0.5 ºC using pH 7.2 phosphate buffers as (receptor phase) diffusion medium. 1 gram of the gel of each formulation was exposed to diffusion study and samples were withdrawn at different time intervals such as 1,2,4,6 and 8 h with addition of appropriate amount of same fresh buffer solution to keep the volume constant. The samples were analyzed by UV spectrophotometer at 270nm and the amount of drug released was determined from a previously calculated standard curve, each data was repeated in triplicate.

***Invitro* drug permeation through rat skin**

The university animal ethical committee approved the experimental protocol. Swiss albino mouse aged between 6 to 8 w were taken and sacrificed by cervical dislocation, and the epidermal skin was carefully removed and rinsed with normal saline to remove any loose materials. The epidermal skin was cut into 5 cm length. The epidermal skin was stored in cold (5-8°) normal saline [38]. Before the experiment, the epidermal skin was taken out and thawed until it reached room temperature, and was kept soaked in citrate phosphate buffer (pH 7.2) for 1 h. It was gently blotted dry with a filter paper. For delipidization of the epidermal skin, adherent fatty materials were removed carefully by using the fine forceps. The integrity of the skin was tested microscopically, before use, to detect any histological change [39]. No significant histological changes were observed.

The epidermal thickness was measured microscopically. The thickness was found to be 200 ± 12 µm from three measurements. Skin permeation studies of the prepared gels, were carried out using a modified Franz diffusion cell [40]. Pre-treated abdominal skin of albino mouse was fixed onto the Franz diffusion cell. Accurately weighed 1 g gel was spread uniformly on an area of 1cm ^{2} of the skin, previously fixed in between the donor and receptor compartment of the Franz diffusion cell. The receptor compartment contained 12 ml of phosphate buffer, pH 7.2. The temperature of the elution medium was thermostatically controlled at 37 $\pm$ 1° by a surrounding water jacket, and the medium was stirred with a bar magnet at slow speed (500 rpm), using a magnetic stirrer [41]. Aliquots of 1 ml withdrawn at predetermined intervals for 8 h, and an equal volume of pre-warmed buffer, was replaced. The samples were analyzed, after appropriate dilution, for CLZ content spectrophotometrically at 270 nm. Blanks were run for each set as described above, using placebo gel.

Skin irritation test

The skin irritation test was performed on guinea pig, by applying 1 g gel formulation on 3 cm^2 sub area, saturated drug solution (1ml) soaked in 3 cm ^{2} cotton wool. An aqueous solution of 0.5ml, containing 0.8% formalin soaked in 3 cm ^{2} cotton wool as a standard irritant was placed in the back of the guinea pig. The cotton wool was secured firmly in place with adhesive plaster. The animals were observed for 7 d for any sign of edema and erythrema. [42]

RESULTS AND DISCUSSION

Non crosslinked gel formulations were clear compared to crosslinked which showed slightly brownish colour. Both the gel formulations were found smooth, pliable, homogenous and elegant in appearance when applied on the skin with the finger. The DSC studies gave conformation of crosslinking of chitosan and glutaraldehyde. There is no interaction was found between drug and any excipients.

Viscosity of prepared gel was found to be significantly affected by crosslinking on positive side, even more than increase in concentration or increase in molecular weight, which also shown increased in viscosity (Table 2 and figure 1). The result of batch G4 with highest viscosity 13.216 x 103 cps viscosity, indicated extensive effect of crosslinking in higher molecular weight chitosan, as well as high concentration of 4%. The viscosity is too high to remove easily from container compared to the viscosity of batch G3 with 7.892 x 103 cps viscosity, with low concentration of high molecular weight chitosan and crosslinked with glutaraldehyde. The effect of

crosslinking can be explain with example of batch G1 and G3 with HW chitosan with viscosity of 2.567 x 103 cps and 7.892 x 103 cps viscosity; indicated drastic increase in viscosity. The same results were also supported with the viscosity data of other batches. Overall in lower concentration of chitosan with sustained drug release and good extrudability can be a useful formulation for topical drug delivery of antimicrobial agents.

The results of extrudability, spreadability, gel strength, content uniformity and percentage drug diffusion were complied in table 2. The drug content was in the range of 94.66±0.13 to 98.75±0.31.The pH of all the formulations was between 6.03±0.09 and 6.34±0.20, which lies in the normal pH range of the skin, and did not produce any skin irritation.

The results of extrudability showed that the gel formulations from the collapsible tube, varies from 2.04±0.19 to 53.58±1.67 N where, as the results of spreadability varies from 4.08±0.32 to 50.19±2.09 g cm/sec. The formulations viscosity ranged from 0.389±0.093 to 13.216±0.517 cps, and they showed gel strength between 357±19 to 4683±98 N/g. Amount of chitosan shown positive effect on gel strength, which gives negative effect on drug release. The high gel strength in crosslinked formulation compared to non-crosslinked formulation might be due to lower degree of polymer network in the gel. The results of 8 hrs percentage drug release shown that formulation with high molecular weight chitosan, high percentage and crosslinked, slowest drug release of 42.40±2.06 (G4) and highest release was observed 83.26±3.28 with non-crosslinked G5 formulation.

The study of swelling study was important as it is the one of the main mechanism for drug release from gel formulation. As water penetrates to gel and allow the drug to dissolve in the water and thus by this mechanism drug is released. In present study the swelling index was found in the range of 10.35±0.5 to 17.2±0.81, which indicate good swelling in all formulations. There is positive effect observed of polymer concentration on swelling index. 24 hours studies suggest that as we increase amount of chitosan in formulation comparative swelling was slower but higher (Figure 2). The effect of crosslinking agent showed negative effect on the swelling index, may be due to the crosslinking of polar functional groups of polymer with crosslinking agent i.e. swelling index of batch G1 and G2 was found to be 16.14±0.76 to 12.72±0.62 respectively. The high molecular weight chitosan showed greater swelling index compare to the same amount of lower molecular weight chitosan, after 24 hour of swelling index of G1 and G5 respectively 16.14±0.76 and 13.310.66 (Figure 3).

Drug release study of prepared gel showed all the factors like polymer concentration, crosslinking and molecular weight showed negative effect, which can be utilized to get desired release pattern from the formulation. Increase in polymer concentration and molecular weight leads to increase in viscosity which may be the main cause of decrease in drug release. The crosslinking agent decease the number of polar groups and increase the viscosity, which may be the mechanism behind slower release. The in-vitro drug release after 8 hours was found to be in range of 42.40 ± 2.06 to 83.26 ± 3.28 for G4 and G5 respectively (table 2 and figure 3). The crosslinking of gel showed drastic increase in viscosity and decrease in drug release, supported by results of G1 and G3, 73.03±2.65 to 49.50±2.94 respectively. The figure 3 suggest order of drug release as, G5>G7>G5>G1>G8>G2>G3>G4.

The skin irritation test showed that all no one gel preparation showed any development of edema or erythrema on normal visualization.

From the study optimized chitosan gel containing CLZ was found to be G3, which is crosslinked and prepared using high molecular weight chitosan based on good spreadability (25.15±1.24 g cm/sec), extrudability (16.92±0.93 N) and sustained drug release (49.5±2.94 % in 8 hrs). G4 which showed most sustained drug release but poor in both extrudability (53.58±1.67 N) because of too high viscosity (13.216±0.52 13^3 cps.

CONCLUSIONS

The in vitro studies showed that chitosan gels act as sustained delivery vehicles for CLZ, the delivery rate being a function of the viscosity of the gel. The cumulative drug release of CLZ could be decreased approximately from 83.26±3.28% to 42.40±2.06 % by increasing the viscosity of the topical chitosan gel. Thus from present preliminary studies, it could be concluded that chitosan can be used as gelling agent for the development of gel formulations, because of its sustain release profile, water-soluble nature, physical stability and good spreadibility.

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Table 1: Composition of chitosan gels containing CLZ

Ingredients	G1	G2	G3	G4	G5	G6	G7	G8
CLZ (%)	1	1	1	1	1	1	1	1
Chitosan HW (%)	2	4	2	4	-	-	-	-
Chitosan LW (%)	-	-	-	-	2	4	2	4
Glutaraldehyde (%)	-	-	0.1	0.1	-	-	0.1	0.1
Glycerine (%)	5	5	5	5	5	5	5	5

Table 2: Summery of evaluated parameters.

Batch Code	Drug content	Viscosity (10^3cps)±S.D.	Extrudability (N)	Spreadability (g cm/sec)	pH	Gel Strength (N/g)	Percentage drug release (8 hrs)
G1	94.66±0.13	2.567±0.14	5.09±0.26	8.63±0.45	6.03±0.09	680±35	73.03±2.65
G2	98.75±0.22	6.288±0.27	14.52±0.72	20.97±0.98	6.11±0.11	1275±73	55.23±2.47
G3	97.50±0.56	7.892±0.32	16.92±0.93	25.15±1.24	6.23±0.14	1480±82	49.5±2.94
G4	96.61±0.21	13.216±0.52	53.58±1.67	50.19±2.09	6.18±0.08	4683±98	42.40±2.06
G5	98.22±0.21	0.389±0.09	2.04±0.19	4.08±0.32	6.25±0.10	357±19	83.26±3.28
G6	98.75±0.31	1.247±0.10	3.24±0.22	6.16±0.31	6.34±0.20	428±25	77.79±3.82
G7	97.50±0.62	0.935±0.13	2.91±0.15	5.37±0.28	6.19±0.17	394±21	80.51±4.05
G8	96.61±0.19	4.028±0.19	9.13±0.46	13.48±0.67	6.16±0.12	1083±43	68.71±3.21

Note: 1) +: good; + +: very good; + + +: excellent.
2) All measurements were made in triplicate.

Figure 1: Viscosity data of chitosan gels containing CLZ

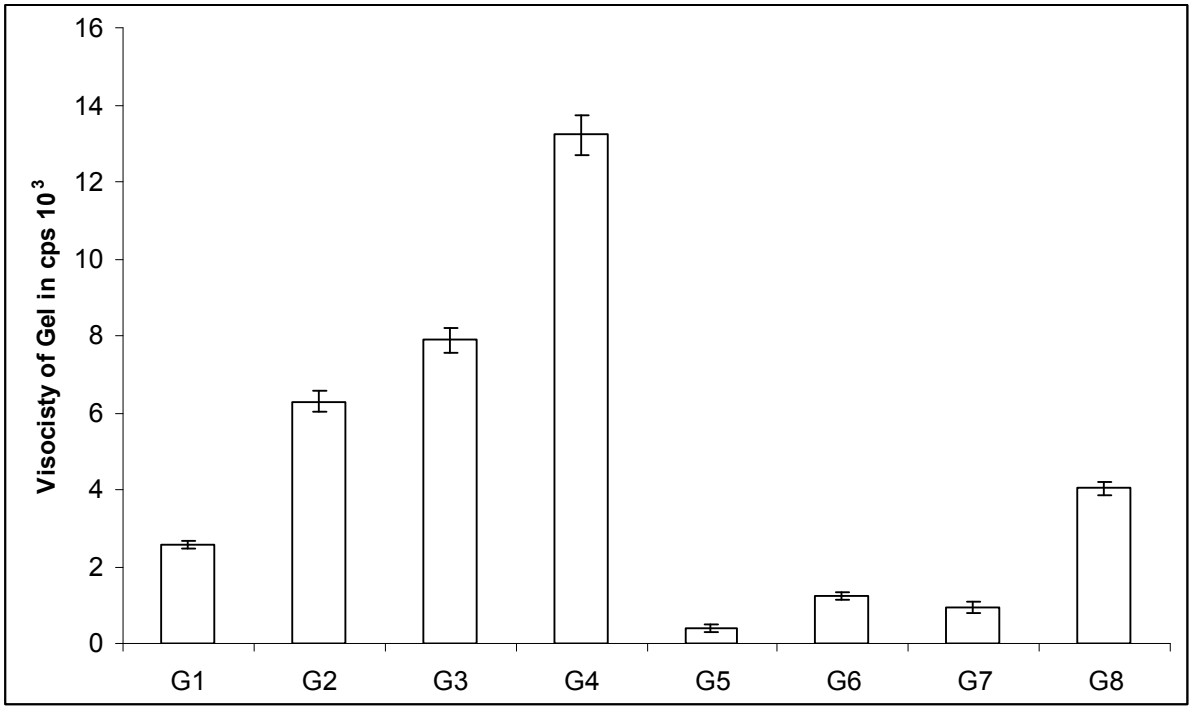

Figure 2: swelling behaviour of chitosan gel

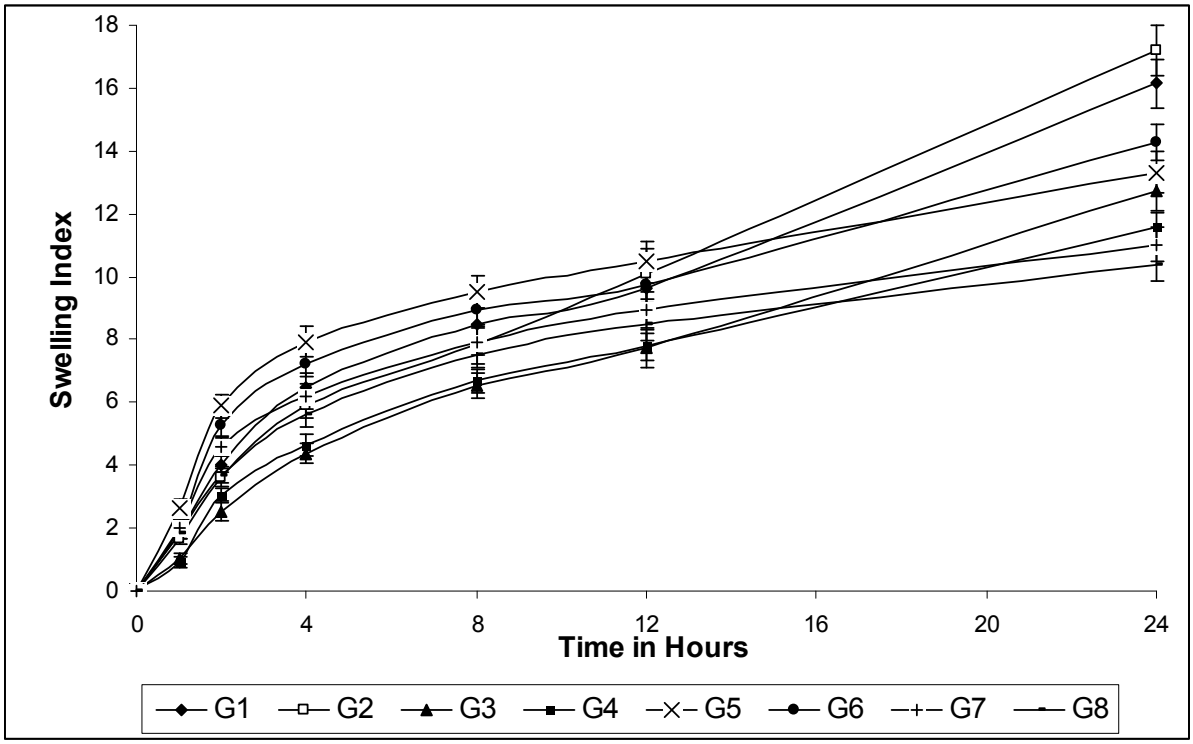

Figure 3: Cumulative percentage drug release from CLZ gel