—Regular Articles—

Formulation and *In Vitro* Evaluation of *In Situ* Gels Containing Secnidazole for Vaginitis

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Gel dosage forms are successfully used as drug delivery systems considering their ability to prolong the drug release. The main objective is to formulate and evaluate *in situ* vaginal gels of secnidazole, based on ion activated systems. The system utilizes polymers that exhibit sol-to-gel phase transition due to change in specific physico chemical parameters. Ion triggered system using gellan gum (0.1–0.75% w/v) along with sodium carboxymethylcelluose was used to prolong the release of secnidazole (1% w/v). Formulations were evaluated for gelling capacity, viscosity, gel strength, mucoadhesive force, spreadability, microbiological studies and *in vitro* release studies. The transformation of sols occur in the presence of monovalent/divalent cations in the dissolution medium. Effect of calcium carbonate and other process parameters were optimized and found that increase in calcium ions produce stronger gels. The drug content, clarity, and pH of formulation were found to be satisfactory. The viscosity was found to be in the range of 0.005 to 0.085 for sols, whereas for the gels 16 Pa·s. Formulation showed pseudoplastic flow with thixotrophy. The gel strength (using texture analyzer) and mucoadhesion was found to be up to 6.5 g and 4 g respectively. The optimized formulations were able to release the drug for 360 min. The gels are expected to improve the administration at the site of infection and decrease frequency.

Key Words——mucoadhesive *in situ* gels; prolonged release; gellan gum; sodium carboxy methylcelluose; secnidazole

INTRODUCTION

Anaerobic bacteria are ubiquitous, they are commonly found component of the indigenous microbial flora of animals. In human they can outnumber aerobic organisms. Anaerobes are commonly found on the mucosal surfaces of the gastrointestinal tract, the gastro-urinary tract, the vaginal cavity and the upper respiratory tract. Under normal condition these organisms do not cause disease. However, the heavily colonized surfaces are portals of entry into tissues and the blood stream. When anaerobic bacteria gain access to sterile body sites, they can become opportunistic pathogens and cause serious, sometimes fatal infection.[1] The reason for incomplete eradication of these anaerobic infections in most cases may be due to the short residence time of antibacterial agents in the vaginal cavity. The other reason may be degradation of antibacterial agents in vaginal fluid. One way to improve the efficacy in eradicating the infection is to deliver the drug locally. Better stability and longer residence time will allow more of the antibacterial agents to penetrate through the vaginal mucous layer to act for longer duration of time. Therefore some researchers[2,3] had prepared and reported new formulation such as gels, mucoadhesive tablets, pH sensitive excipients composition, mucoadhesive microspheres, *etc.*, which were able to reside in vaginal cavity for an extended period of time. Jung Yun Chang *et al.*[3] developed mucoadhesive thermo sensitive gels and the results showed that *in vivo* antifungal activity of clotrimazole was significantly prolonged. Saracoglu F. *et al.*[4] evaluated the clinical efficiency and tolerance of vaginal ornidazole, secnidazole and metronidazole or their combinations for the treatment of bacterial vaginosis and found that there is 100% cure rate in oral and vaginal infection. Using suitable carriers which can effectively administer the drug for an extended period of time will reduce the systemic side effects but also improve the therapeutic efficacy and patient compliance.

The aim of the present work was to develop ion triggered system using gellan gum for local release of secnidazole, used in the vaginitis caused by *Trichomonas vaginalis*. A combination of gellan gum-sodium carboxymethylcellulose (NaCMC) was investigated as vehicle for formulation.

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MATERIALS AND METHODS

Materials Secnidazole was a gift sample from Astra Zeneca Pharma India Ltd, Bangalore. Sodium carboxymethylcellulose (NaCMC) was obtained as gift samples from Goodrich and Colorcon Asia Pvt. Ltd. Gellan gum was provided by Hi Media laboratories Ltd, Mumbai. All the other materials used were of analytical grade.

Preparation of *In Situ* Gelling Systems Gellan gum solutions of various concentrations were prepared by adding the gum to deionized water containing 0.17% w/v sodium citrate and heating up to 90° C while stirring. After cooling to below 40° C, appropriate amounts of calcium chloride (0.05% w/v) was added into the sol. Low level of cations present in the solution was sufficient to hold the molecular chains together and inhibit hydration.[5] Secnidazole was dissolved in ethanol and a concentration of 2% w/w was added into the resulting solution. The mixture was shaken by using a magnetic stirrer to ensure thorough mixing.

Determination of pH The pH of the gel (1 g) was determined using a calibrated pH meter. The readings were taken for average of 3 samples.

Determination of Mucoadhesive Force The mucoadhesive forces of *in situ* gels were determined by means of the mucoadhesive force measuring apparatus (Fig. 1) using tissue specimen obtained from the mucosal side of the goat vagina. The pieces of tissue were stored frozen in acetate buffer at pH 4.5, and thawed to the room temperature before use. At the time of testing, a section of tissue was secured (keeping the mucosal side out) to the upper side of a glass vial (C) using a cyanoacrylate adhesive. The diameter of each exposed mucosal membrane was 1.5 cm. The vials were equilibrated and maintained at 37° C for 10 min. One vial with a section of tissue (E) was connected to the balance (A) and the other vial was fixed on a height adjustable pan (F). To the exposed surface of the tissue attached on the vial, a constant amount of 0.1 g gel (D) was applied. Before applying the gel, 150 μl of simulated vaginal solution, (pH 4.5) was evenly spread on the surface of the test membrane. The height of the vial was adjusted so that the gel could adhere to the mucosal surface of both vials. Immediately, a constant force of 0.5 N was applied for 2 min to ensure intimate contact between the tissues and the samples. The upper vial was then moved upwards at a constant force, while it was connected to the balance. Weights were added at a constant rate to the pan on the other side of the modified balance until the two vials were separated.[5] The mucoadhesive force, expressed as the detachment stress in dyn/cm^2, was determined from the minimal weights needed to detach the tissues from the surface of each formulation, using the following equation.[6]

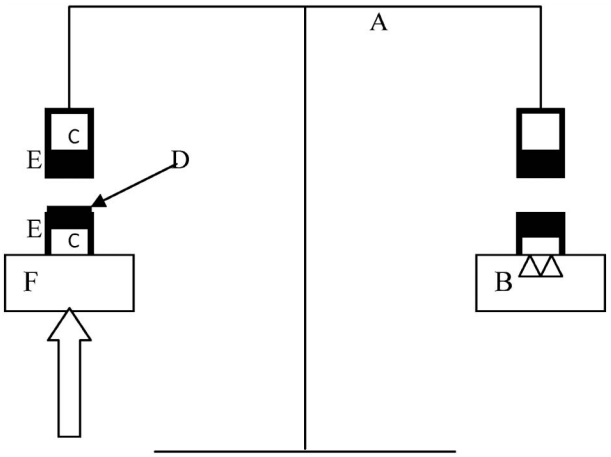

Fig. 1. Mucoadhesive Force-measuring Device
(A) modified balance; (B) weights; (C) glass vial; (D) secnidazole gel; (E) mucosal tissue; (F) height-adjustable pan.

$$\text{Detachment stress (dyn/cm}^2) = \frac{mg}{A}$$

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2; and A is the area of tissue exposed. Effect of varying contact time (1, 2, 3, 5 and 10 min) was investigated for some of the gel preparations to optimize initial contact time. In brief, formulations were allowed to be in contact with mucosa for carrying contact time (1, 2, 3, 5, and 10 min), and the mucoadhesive force was determined as discussed above.[7] Contact time that resulted in maximum bioadhesive strength was selected as optimum contact time required for adequate adhesion. All the above mentioned experiments were carried out in triplicates.

Measurement of Viscosity Viscosity determinations of the prepared *in situ* gels as well as sols were carried out on a cone and plate geometry viscometer (Brookfield Viscometer, USA) using spindle no. 40. Viscosity of *in situ* gelling solutions was measured at different angular velocities at a temperature of 37° C. A typical run comprised changing of the angular velocity from 0.0 to 100 RPM. The average of two readings was used to calculate the viscosity. Evalua-

tions were conducted in triplicate.

Determination of Gel Strength The method by which the properties of polymeric system may be conveniently determined is texture profile analysis. A TA-TX2 Texture analyzer (The experiments were conducted at Digital Scientific Equipments, RK Puram, New Delhi). The experiment was done by placing the gels in standard beaker below the probe. In this, an analytical probe is then depressed into the sample. The Texture analyzer was set to the 'gelling strength test' mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per second and a trigger force of 5–3 g were selected. An aluminum probe of 7.6 cm diameter was used for all samples. The study was carried out at room temperature.[7] The force required to penetrate the gel was measured as gel strength in terms of grams.

Gelling Capacity The gelling capacity of the formed gel was determined visual inspection and the different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time.

Spreadability For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 min. Weight (50 g) was added to the pan. The time required to separate the two slides, *i.e.*, the time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability (S).[6]

$$S=\frac{ML}{T}$$

Where M=weight tide to upper slide
L=length moved on the glass slide
T=time taken.

***In Situ* Release Studies** For carrying out *in situ* release studies and determination of duration of mucoadhesion/erosion, a ''Flow-throu'' apparatus[4] was designed based on the modification of a ''Flow-throu'' device cell. The ''Flow-throu'' apparatus was made of glass and had a length of 10.5 cm and a diameter of 2.1 cm. It was closed at one end and open at the other. In the center of the lower base there was a cavity of 1.6 cm length and 1.5 cm depth for placement of the goat vaginal mucous membrane. Dissolution medium pH 4.5 (simulated vaginal pH) was pumped at a flow rate of 0.5 ml/min using flow regulators. Sample (2 ml) was removed at different time intervals from the reservoir till the gel completely eroded. The cumulative percent drug released was determined by measuring the absorbance at 260 nm.

Drug Release Kinetics To know the mechanism of drug release from various formulations, the data were treated according to first-order (log cumulative percentage of drug remaining *vs* time), Higuchi's[8] (cumulative percentage of drug released *vs* square root of time), zero order (cumulative amount of drug released *vs* time) and Korsmeyer et al.'s (log cumulative percentage of drug released *vs* log time) equation $M^t/M^\infty=Kt^n$, where, M^t/M^∞ is the fraction of drug released after time 't' and 'K' is kinetic constant and 'n' is release exponent which characterizes the drug transport mechanism.[9] If the values of 'n' are less than 0.45 then it is considered as fickian release mechanism, 0.45 to less than 0.89 it is considered as non-fickian, 0.89 for case II and more than 0.89 it is understood as super II release.

RESULTS AND DISCUSSION

The formulations of this study contained Ca^{++} ions in complexed form, the release of which in the slightly acidic conditions of the vaginal cavity ensured reproducible gelation of the gellan gum. The quantities of the complexing agents calcium chloride and sodium citrate must be such that there is no free calcium ionic form in the formulation, ensures that they are fluid before administration, but sufficient Ca^{++} ions are released when the complex is broken down in the stomach to cause gelation. Determination of the optimum amounts of these compounds for gellan gum sols (0.5%, w/v) (Table 1) showed that only those containing 0.05% (w/v) calcium chloride in combination with 0.17% (w/v) sodium citrate were satisfactory; all other formulations gelled before contact with simulated vaginal fluid. Low level of cations present in the solution was sufficient to hold the molecular chains together and inhibit hydration. All further experiments in this study were performed with formulations containing 0.05% (w/v) calcium chloride and 0.17% (w/v) sodium citrate.

Effect of pH Aqueous gellan gum 0.5% w/v sols exhibited pH values in the range of 5.0 to 7.5, at 25° C (Table 2). The pH of all formulations was adjusted to 4.0–4.5 with diluted acetic acid.

Effect of Viscosity The viscosity of sols and gels of various formulations was determined at various shear rates shown in Figs. 2 (a) and (b). As the shear rate increased the viscosity of gel decreased. Also, the

Table 1. Composition of Various Formulations Used in the Prepared *In Situ Gels*

Formulation	Secnidazole	Gellan gum	Na-CMC	Sodium citrate	Calcium chloride	Deionized water Upto (ml)
	Concentration (% w/v)					
GF1	1	0.1	0.5	0.17	0.05	10
GF2	1	0.2	0.5	0.17	0.05	10
GF3	1	0.3	0.5	0.17	0.05	10
GF4	1	0.4	0.5	0.17	0.05	10
GF5	1	0.5	0.5	0.17	0.05	10
GF6	1	0.75	0.5	0.17	0.05	10

Table 2. Characteristics of Various Secnidazole Gel Formulation

Formu lations	pH	Viscosity (Pa·s)	Spreadibility (g·cm/s)	Drug Content (% w/w)	Muco-adhesive force (dyn/cm^2)	Gelling Capacity	Gel strength (g)
GF1	4.2	11	25.2	98.7	52.2±2.40	−	0.5
GF2	4.1	11.2	25.8	98.6	53.5±1.09	+	2.0
GF3	4.2	12.02	26.0	98.8	54.8±3.41	+	3.5
GF4	4.3	12.54	27.5	98.5	55.2±5.37	++	4.0
GF5	4.2	14	28.5	90.4	55.8±7.55	+++	6.5
GF6	4.5	16	30.7	98.5	56.3±5.67	+++	7.2

−, No gelation; +, Gel after few minutes, dissolved rapidly; ++, Gelation immediately, remains for few hours; +++, Gelation immediately, remains for extended period. (n=6).

increase in gellan gum concentration from 0.1% w/v to 0.75% w/v showed increase in viscosity from 11 Pa·s to 16 Pa·s respectively. Findings indicated that marked differences were noted in viscosity of gels with respect to there corresponding sols. Formulation GF6 having the maximum concentration of gellan gum showed maximum viscosity for both sols (0.085 Pa·s) as well as its corresponding gel (16 Pa·s). It is also understood that formulation having higher content of polymer (GF6) were poor candidate for *in situ* formulation since the exhibited higher viscosity for sols, hence were not pourable. All formulations were exhibited shear thinning pseudoplastic behavior with thixotrophy. Formulations GF4 and GF5 showed the optimum variation in viscosity. Formulation viscosity contributed to product adhesiveness, reflecting the importance of product rheology on this parameter.

Mucoadhesion Force The mucoadhesive force is an important physicochemical parameter for topical application in vaginal cavity used for vaginitis. The effect of different concentrations of secnidazole gel formulation on mucoadhesive force is shown in Table 2. The mucoadhesive force was significantly increased as the concentration of mucoadhesive polymer increased over the range of 0.6–1.25% ($p<$ 0.05). Formulation GF6 (containing the maximum polymer ratio 0.75 : 0.5) exhibited maximum mucoadhesive strength. The results also showed that the presence of secondary polymer (NaCMC) significantly increased the viscosity as well as the mucoadhesive property.

***In Vitro* Release Studies** The *in vitro* dissolution profile of secnidazole from the gels containing different concentration of gellan gum is shown in Fig. 3. The release of drug from these gels was characterized by an initial phase of high release (burst effect) and as the gelation proceeded, the remaining drug was released at a slower rate followed by second phase of moderate release. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration. The cumulative percent of drug released as a function of time is shown in Fig. 3. Formulations GF1 and GF2 which had the lower polymer ratio (0.1 : 0.5 and 0.2: 0.5) showed the release profile only up to 240 min, whereas formulation having higher polymer ratio *i.e.*, GF6 showed only 50 % release at the end of 360 min, since we had an objective to formulate an *in situ* gel showing 80 % release profile up to 360 min. Hence we chose GF4 and GF5 as optimum formulations for *in situ* gels for vaginitis.

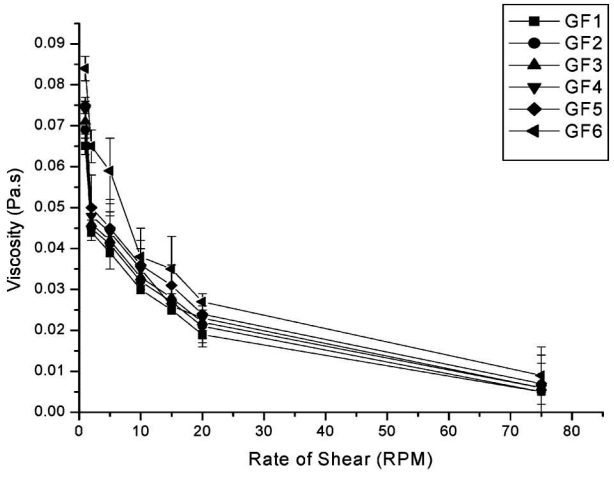

Fig. 2. Viscosity of Sols and Gels of the Various Formulations (a) Sols and (b) Gels
Each point represents mean±SD; $n=3$.

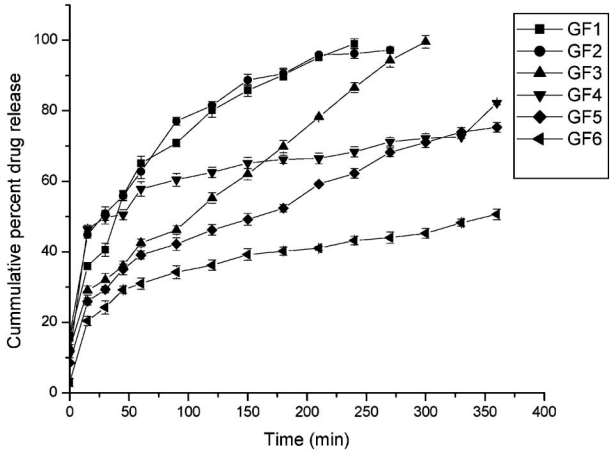

Fig. 3. The *In Vitro* Release Profiles of Secnidazole Gels Using Gellan Gum
Each point represents mean±SD; $n=3$.

Release Kinetics The examination of the correlation coefficient (R^2) indicated that the drug release followed diffusion controlled mechanism from the *in situ* gels, as the values for first order (ranged from 0.9183 to 0.9891) are always higher in comparison to zero order (ranged from 0.6629 to 0.9333) and Higuchi's square root of time (ranged from 0.6558 to 0.9875). It was understood to be predominant first order release.[8] The most possible mechanism of drug release from the *in situ* formulations appeared to by diffusion dominated. This may be due the swelling nature of hydrophilic polymer gellan gum and NaCMC. The results of the release kinetics elucidated from the power equation are given in Table 3. The values 'n' were in the range of 0.3224–0.428, which was indicative of the drug release following fickian diffusion release mechanism. The amount of secnidazole released from GF1, GF2 and GF3 showed a linear relationship with the square root of time (correlation coefficient 0.9875–0.9442); therefore, the release rate of secnidazole was expressed following the theoretical model by Higuchi for those formulations containing lower concentration of gellan gum. Formulation consisting of higher concentration of gellan gum GF4, GF5 and GF6 did not show linear relationship (correlation coefficient=0.622–0.875) with time. This may be due to increase in swelling property of the gel with increase in polymer concentration.

Statistical Analysis The results obtained from the experiments of mucoadhesive strength and release studies were analyzed statistically using multivariate tests. A statistically significance difference was conducted using SPSS (SPSS v.4.0, Chicago, IL). A

Table 3. Release Exponent Values and Release Rate Constant Values for Different Formulation

Formulation Code	Kinetic Models				
	Zero Order	First Order	Higuchi	Korsmeyer *et al.*	
	R^2	R^2	R^2	n	R^2
GF1	0.4426	0.9874	0.9875	0.3275	0.9865
GF2	0.8273	0.9654	0.9442	0.3224	0.8942
GF3	0.8451	0.9354	0.9708	0.428	0.9537
GF4	0.6629	0.9183	0.622	0.3588	0.9912
GF5	0.8333	0.9816	0.875	0.3915	0.9152
GF6	0.7825	0.9891	0.6558	0.418	0.9239

statistically significant difference was considered when $p<0.05$.

CONCLUSION

Gel formulation of secnidazole with mucoadhesive properties is 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 will have better patient acceptability since formulation will be applied in the form of sols which upon contact will form the corresponding gels causing less irritation or pain.

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