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Preparation and Evaluation of Clotrimazole Loaded Nanosponges Containing Vaginal Gels

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ABSTRACT

The rationale of the present work is to design a vaginal gel formulation with mucoadhesive properties to ensure longer residence at the infection site, providing a favourable release profile for the antifungal drug clotrimazole with the help of β-cyclodextrin nanosponges. Hence efforts were made to prepare clotrimazole loaded nanosponges containing vaginal gels using polymers like hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (NaCMC), methyl cellulose (MC) and carbopol. The gel formulations were prepared with a view to improve permeability of drug. The prepared gel were evaluated for pH, Viscosity, Spreadability, Extrudability, Mucoadhesive time and In vitro diffusion study. The gel formulations can be graded in the following order with respect to the rates of diffusion of drug from them: (HPMC) < (MC) <(NaCMC < (Carbopol). The correlation coefficient values (r) revealed that the diffusion profiles follows zero order kinetics and the mechanism of drug release was governed by peppas model. The diffusion exponent of release profiles (slope) has a value of ($n \geq 0.5$), which indicates non fickian diffusion. It was found that the clotrimazole loaded nano sponges containing gels prepared with HPMC showed good extrudability, homogeneity, spreadability and required diffusion rate in comparison with other formulations and was selected as suitable candidate to be delivered through vaginal route at controlled rate.

Keywords: Clotrimazole, β-cyclodextrin, nanosponges, diffusion rate.

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INTRODUCTION

Conventional formulations for intravaginal delivery comprises of creams, foams, pessaries and jellies that are designed to disperse drug throughout the vaginal tract. Long residence time is often required for the activity of vaginal administration of antimicrobial drugs. Vaginal formulations are prone to leakage and their efficacy is limited by a poor retention at the site of action. Patients discontinued the treatment because of inconvenience for usage. This is also a major reason for poor acceptability and usefulness of intravaginal medications[1]. These vaginal formulations are associated with limitations such as poor retention, leakage and messiness, there by causing inconvenience for users. To overcome these limitations, formulations that adhere to vaginal mucosa for a sufficient period of time need to be developed. Bioadhesion and prolonged retention are desirable characteristics can be built in vaginal formulation by the use of bioadhesive polymers. Hence, the vaginal route of administration offers a promising option for local and systemic delivery of drugs with the use of bioadhesive polymers[2].Vaginal gels has advantages wide acceptability, feasibility and low cost. Out of the various semisolids dosage forms, the gels are becoming more popular due to ease of application and better percutaneous absorption, than other semisolids preparations. Gels can resist the physiological stress caused by skin flexion, blinking and mucociliary movement, adopting the shape of the applied area, and controlling drug release. Mucoadhesive polymers of natural, semisynthetic or synthetic origin are able to form hydrogels. In the simplest case the drug is dispersed in a mucoadhesive polymer which swells in the presence of biological fluid and exhibits bioadhesive properties. [3]Vaginal gels are known to possess a higher biocompatibility and bioadhesivity and can be rapidly eliminated through normal catabolic pathways, decreasing the risk for irritative or allergic host reaction at the application site. In the present study clotrimazole loaded nano sponges containing vaginal gels in the form of different gels using various bioadhesive polymers Carbopol 934 P, cellulose derivative hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (NaCMC) and carbopol and methyl cellulose (MC) were prepared and evaluating them for different parameters including bioadhesive strength and *in vitro* release of clotrimazole.

MATERIALS AND METHOD

Clotrimazole was obtained as a gift sample from Aurobindo Pharma Ltd, Hyderabad. Sodium CMC (200-300cPs, S. D. fine-chem Ltd.; Mumbai), Carbopol 934(Arihanth traders; Mumbai) Hydroxy Propyl Methyl cellulose (50cPs S. D. fine-chem Ltd.; Mumbai) and Methyl cellulose

(28-32%, S. D. fine-chem Ltd.; Mumbai) obtained commercially. All other materials used were of analytical grade.

Synthesis of β-cyclodextrin nanosponges

β-cyclodextrin based nanosponges was prepared using Di phenyl carbonate as a cross-linker. Nanosponges were prepared using different ratios of β-cyclodextrin and Di phenyl carbonate [1:0.25, 1:0.5.1:0.75 and 1:1]. Finely homogenized anhydrous β-cyclodextrin and Di phenyl carbonate were placed in a 100 ml conical flask. The system was gradually heated to 100 ^{0}C under magnetic stirring, and left to react for 5 h. During the reaction crystals of phenol appeared at the neck of the flask. The reaction mixture was left to cool and product obtained was broken up roughly. The solid was repeatedly washed with distilled water to remove unreacted β-cyclodextrin and then with acetone, to remove the unreacted Di phenyl carbonate and the phenol present as by-product of the reaction. After purification, nanosponges were stored at 25 ^{0}C until further use[4].

Preparation of clotrimazole loaded nanosponges

The clotrimazole loading into β-cyclodextrin nanosponges was carried out by solvent evaporation technique. In 100 ml of each solvent 4000 mg of Clotrimazole was dissolved separately to form solutions. To the each solution, prepared nanosponges were added and triturated until the solvent evaporated. While triturating the clumps of nanosponges will be segregated and absorbs the drug solubilized solvent. The solid dispersions were dried in an oven overnight (at 50 ^{0} C at atmospheric pressure) to remove any traces of solvents and were sieved through 60 # and used for further work[5].

Preparation of clotrimazole nanosuspension

The dried drug encapsulated nanosponges were collected and required quantities of drug equivalent nanosponges were transferred into 250 ml volumetric flask containing 100ml methanol in order to remove the free unencapsulated drug by solubilizing in the methanol. The drug encapsulated nanosponges were separated from the free drug by membrane filtration by using 0.22µ membrane filter. The residual drug loaded nanosponges were collected and dispersed in distilled water by using ultra sonication to form a nanosuspension[6].

Formulation of vaginal gels containing clotrimazole loaded nanosponges

Different drug reservoir gels were formulated as per the composition given in Table 2. The required quantities of polymer was weighed and transferred separately into a mortar. It was triturated with5 ml of water. The previously prepared required drug equivalent nano suspensions .methylparaben and propylparaben were incorporated into the polymer dispersion slowly with

continuous trituration to obtain a gel. In case of carbopol gel, specified amount of carbopol 940 was soaked in 5 ml of water over night. The previously prepared required drug equivalent nanosuspensions, methylparaben and propylparaben were incorporated into the polymer dispersion with stirring at 500 rpm, by a magnetic stirrer for 1 h. The P^H of above mixture was adjusted to 4.5 with tri ethanolamine (0.5%). The gel was transferred in to a measuring cylinder and the volume was made up to 10 ml with distilled water. The resulting gels were filled in collapsible tubes[7]

Table 1: Selected ratios of β-cyclodextrin (polymer) and Di phenyl carbonate (cross linking agent) for the synthesis of nanosponges

S. No	Batch code	B-CD (gms)	Di phenyl carbonate(gms)
1	NS1: 0.25	4	1
2	NS1: 0.5	4	2
3	NS1: 0.75	4	3
4	NS1: 1	4	4

Table 2: Composition of Clotrimazole Gels Containing Various Polymers

Ingredients	CG1	CG2	CG3	CG4
Clotrimazole Loaded nanosponges (mg)	3276.18	3276.18	3276.18	3276.18
Methyl cellulose (mg)	500			
Sodium carboxy methyl cellulose(mg)		500		
Carbopol 934 (mg)			500	
Hydroxy propyl methyl cellulose(mg)				500
Methylparaben(mg)	100	100	100	100
Propylparaben(mg)	50	50	50	50
Tri ethanolamine (0.5%)	---	---	q.s	---
Glycerin (ml)	1	1	1	1
Distilled water (ml) up to	10	10	10	10

Evaluation of Drug Reservoir Gels

Drug content

The gels (1gm containing 100 mg of drug) were dissolved in 50 ml of phosphate buffer having pH 4.5. The absorbance was measured after suitable dilution at 261 nm against the corresponding blank solution. The blank solutions were prepared with gels free from drug[8].

pH and viscosity

The pH of the dispersion was measured using pH meter (Systronics Digital –DI-707).The pH of the gels were measured before and after the incorporation of the drug. Viscosity of the gels was determined using a Brook field viscometer. In the present study ,gels were subjected to a shear rate ranging from 10 and 90% .The rheograms and viscosity were obtained with the software rheocal[9].

Extrudability

Closed collapsible tubes containing gel was pressed firmly at the crimped end. When the cap was removed gel extrudes until pressure is dissipated. The weight in grams that was required for extruding 0.5 cm of ribbon of gel in 10 seconds was determined. The results for all the formulations were recorded as extrusion pressure in grams[10].

Spreadability

Spreadability of formulation was determined with the apparatus proposed & fabricated by Multimer*et al.* It consists of wooden block provided with two glass slides. Lower slide was fixed on wooden block and upper slide with one end was tied to glass slide and other end tied to weight pan. A gel equivalent to 2.5 g was placed between two slides and 1000 g weight was placed over it for 5 minutes to press the sample to a uniform thickness. 100g of weight was added to pan. The time (in seconds) required to separate the two slides was taken as a measure of spreadability. Shorter time interval to cover the distance of 7.5 cm indicates better spreadability[11].

Preparation of goat vagina

Goat vagina was collected from slaughter house by cervical dislocation. 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°C) normal saline solution[12]. The prepared gels were evaluated by studying drug diffusion through goat vagina.

Drug Diffusion Study

Drug diffusion study was conducted using Franz diffusion cell[13]. The receptor compartment was filled with 15 ml of phosphate buffer having pH 4.5 as diffusion media. Rat skin was mounted on the donor compartment with the help of an adhesive. The gels (1 gm containing 100mg of clotrimazole) was placed into the donor compartment. Magnetic stirrer was set at 50 rpm and whole assembly was maintained at 32 $\pm$ 0.5 ^{0}C. The amount of drug released was determined by withdrawing 1 ml of sample at regular time intervals for 12 hours. The volume withdrawn was replaced with equal volume of fresh buffer solution. Samples were analyzed for drug content using a U V spectrophotometer at 261 nm. The rate and the mechanism of drug diffusion through the prepared gels were analyzed by fitting the diffusion data into[14], zero-order equation, $Q=Q_0-k_0t$, where Q is the amount of drug diffused at time t, and k_0 is the diffusion rate. First order equation, $\text{Ln } Q=\text{Ln } Q_0 - k_1t$, where k_1 is the diffusion rate constant and Higuchi's equation, $Q= k_2t^{1/2}$, where Q is the amount of the drug diffusion at time t and k_2 is the diffusion rate constant. The diffusion data was further analyzed to define the mechanism of diffusion by applying the

diffusion data following the empirical equation , $M_1/M_\alpha=Kt^n$, where $Mt/M_\alpha$is the fraction of drug diffused at time t, K is a constant and n characterizes the mechanism of drug diffusion from the formulations during diffusion process.

RESULTS AND DISCUSSION

β-Cyclodextrin based nanosponges were prepared by cross-linking β- Cyclodextrin with carbonate bonds of di phenyl carbonate in 1:1 ratio. Clotrimazole was incorporated into nanosponges by solvent evaporation method by dissolving the drug in chloroform. The formulated nanosponges were incorporated into various gels and evaluated for diffusion studies. In the present study efforts were made to prepare vaginal gels of clotrimazole using polymers like HPMC, NaCMC, M.C and Carbopol. Vaginal gels prepared with carbopol and hydroxy propyl methyl cellulose were found to be white, translucent and homogenous. But gels prepared with sodium carboxy methyl cellulose and methyl cellulose was found to be off white and homogenous. Drug content values of the formulations were well with in the range between 98.56-99.82% (Table 2).The pH of all formulations was around the skin pH 4.26 to4.61 reflecting no risk of skin irritation which was further confirmed by vaginal irritation testing. Viscosities of gels were presented in Table 3. All gels were found to exhibit plastic flow. It was observed that the gel formulations showed good extrudability, homogeneity and spreadability and the data was presented in Table 3. The gels prepared with the methyl cellulose, sodium carboxy methyl cellulose, carbopol and hydroxy propyl methyl cellulose shown drug diffusion for a period of 8.5 hours, 9 hours, 10.5 hours and 12 hours respectively. The *in vitro* diffusion profiles of gels across the mouse skin were showed in Figure1.To ascertain the mechanism of drug diffusion, the diffusion data was analyzed by zero order, first order, and Higuchi and Peppas equations. The correlation coefficient values (r) were reported in Table 4. Amount of drug diffused versus time curves exhibited straight line for the formulations and confirmed that the diffusion rate followed zero order release kinetics (Figure.2). Percentage of drug release versus square root of time curves shows linearity and proves that all the formulations followed Peppas model (Figure.3). These values revealed that the diffusion profiles followed zero order kinetics and the mechanism of drug release was governed by peppas model. The diffusion exponent of release profiles (slope) has a values of 0.9513-1.0797 ($n\geq1$), which indicates case II transport diffusion. Among all the formulations the gels prepared with hydroxy propyl methyl cellulose were found to be best formulation.

Table 3: Characteristics of clotrimazole gels formulated with different polymers

Formulation	%Drug content	pH	Viscosity (cps)	Spreadability (gm.cm/sec)	Extrudability (N)	Mucoadhesive time
CG_1	98.52±0.32	4.43±0.09	3523±22	32.23±1.36	90.35±0.03	> 12
CG_2	99.84±0.04	4.51±0.07	3734±15	33.62±1.83	91.39±0.04	> 12
CG_3	99.28±0.16	4.47±0.07	3986±23	34.62±2.34	92.42±0.06	> 12
CG_4	99.42±0.15	4.40±0.17	4432±17	35.11±1.47	92.84±0.05	> 12

Table 4: *In vitro* drug release kinetic data of clotrimazole gels containing nanosponges prepared with β -cyclodextrin and Di phenyl carbonate in 1:1 ratios and by using different polymers

Formulation Code	Correlation Coefficient Values (R^2)				Diffusion Rate Constant (mg/hr) Ko	$t_{50\%}$	$t_{90\%}$	N value
	Zero Order	First Order	Higuchi Model	Peppas Model				
CG_1	0.9992	0.8671	0.9324	0.9984	11.26	4.44	8	0.9513
CG_2	0.9995	0.8248	0.9246	0.9996	11.01	4.54	8.18	0.9837
CG_3	0.9998	0.8127	0.9225	0.9991	9.50	5.26	9.42	1.0043
CG_4	0.9997	0.7791	0.9176	0.9986	8.37	5.97	10.74	1.0797

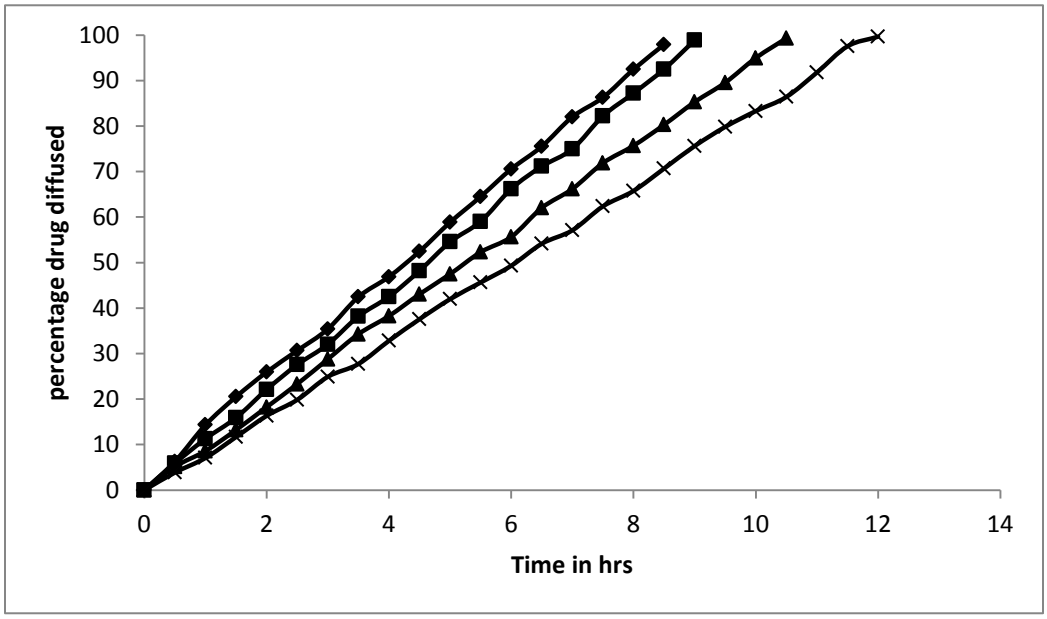

Figure 1: Comparative *in vitro* drug release profiles of clotrimazole gels prepared with different polymers.

(-■-) CG1_ Clotrimazole gels prepared with Methyl cellulose

(-●-) CG2_Clotrimazole gels prepared with Sodium Carboxy Methyl cellulose

(-▲-)CG3_Clotrimazole gels prepared with Carbopol

(-×-)CG4_Clotrimazole gels prepared with HPMC

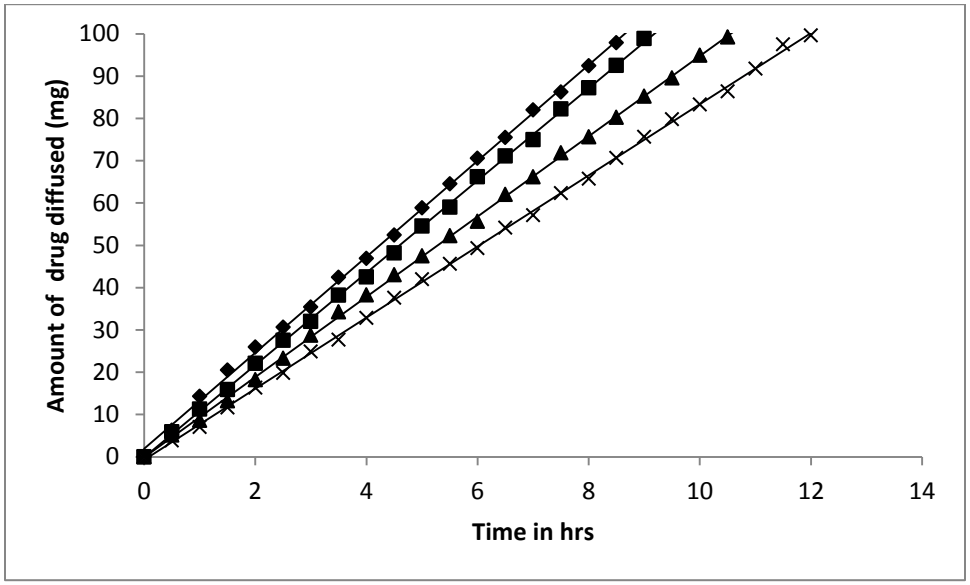

Figure 2: Comparative zero order profiles of clotrimazole gels prepared with different polymers

(-■-) CG1_ Clotrimazole gels prepared with Methyl cellulose

(-●-) CG2_ Clotrimazole gels prepared with Sodium Carboxy Methyl cellulose

(-▲-)CG3_Clotrimazole gels prepared with Carbopol

(-×-) CG4_Clotrimazole gels prepared with HPMC

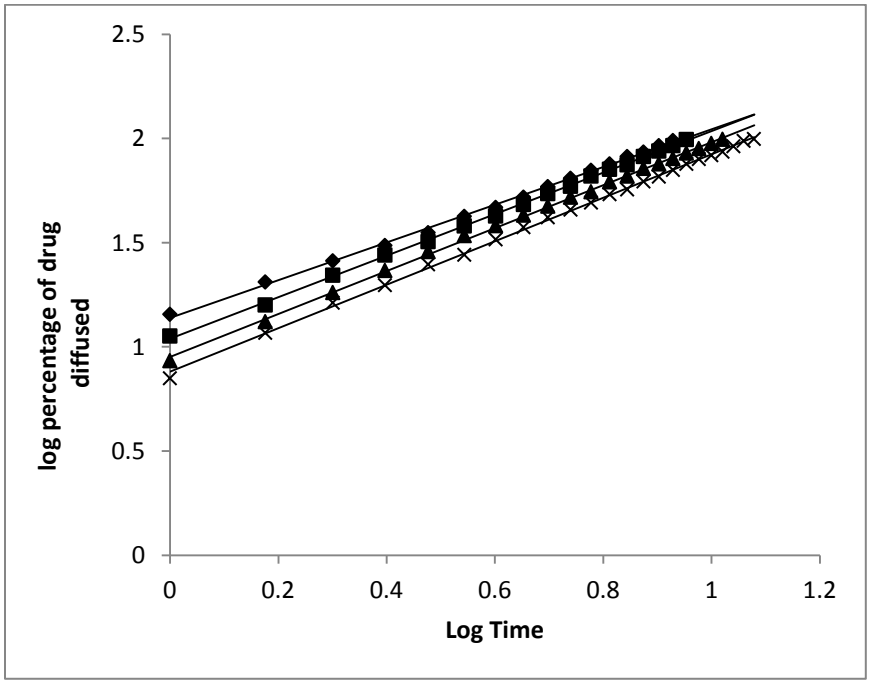

Figure 3: Comparative peppas plots of clotrimazole gels prepared with different polymers

(-■-) CG1_ Clotrimazole gels prepared with Methyl cellulose

(-●-) CG2_ Clotrimazole gels prepared with Sodium Carboxy Methyl cellulose

(-▲-)CG3_Clotrimazole gels prepared with Carbopol

(-×-) CG4_Clotrimazole gels prepared with HPMC

CONCLUSION

It was found that the clotrimazole loaded nano sponges containing gels prepared with HPMC showed good extrudability, homogeneity, spreadability and required diffusion rate in comparison with other formulations and was selected as suitable candidate to be delivered through vaginal route at controlled rate.

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