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Development and Evaluation In-situ Gel Formulation of Clindamycin HCl for Vaginal Application

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ABSTRACT

The vagina has been studied as favorable site for the local and systemic delivery of drugs, for female associated conditions. Vaginal preparations, although generally perceived as safer most still associated with number of problems including frequent dosing and escape causing discomfort to patient. For efficient vaginal delivery of drugs, the delivery system should reside at the site of infection for a prolonged period of time. Present dosage form includes thermosensitive polymers (Poloxamer 127and Poloxamer 188) and pH activated polymers (Chitosan, Carbopol 974 p) for in-situ gel formulation which combines advantages of both gels and solution so that an accurate dose can be administered with ease. These formulations remain in solution state before administration and transforms to gel after administration in to vaginal cavity. Preliminary studies were carried out using different concentrations of various polymers evaluated for their gelling capacity & viscosity in order to identify the compositions suitable for use in the in situ gelling system. Clindamycin HCl was used with varying concentration of P127 and P188 in combination and found that ratio of concentration in %w/v of 23/7 of P127 / P188 is effective for sol-gel transformation at body temperature (37˚C). NaCl (0.9%) was added as isotonic agent and HPMC (0.1%) as bioadhesive polymer to provide strength to formulation. Formulations have satisfactory appearance, clarity & drug content in the range 98.1%- 101%. Performed studies and obtained results prove the efficacy of Poloxamer 127/188 combination as Bio-responsive polymer in the formulation of Clindamycin in situ gel system for vaginal application.

Keywords: In- Situ Gel**,** Poloxamer, Clindamycin HCl, Chitosan and Carbopol 974 P

INTRODUCTION

The vagina has been premeditated as a favorable site for the local and systemic delivery of drugs, for female associated conditions. For years dosage forms given by this route are tablets, creams, injections ointments, suppositories etc. Vaginal preparations, though typically thought to be safe, still associated with a number of problems, together with several days of dosing, dripping, leakage and messiness, causing discomfort to users. These limitations give reduced patient compliance and failure of the desired therapeutic effects. For efficient vaginal delivery of drugs, the delivery system should reside at the site of infection for a prolonged period of time [1-3]. The vagina, due to its epithelium, flora, immune cells and pH is the most suitable site for local and systemic delivery of drugs and shows adequate absorption of drugs by this route. The conventional dosage forms such as preformed gel and solutions have limitations that they do not remain for long time at the site of application and needs frequent dosing. Recent approach, in-situ gel system using bioresponsive polymers combines advantages of both gels and solution so that an accurate dose can be administered with ease of administration. These formulations remain in solution state before administration and transforms to gel after administration in to vaginal cavity [4].

Bioresponsive polymers show a change in swelling behavior upon the exposure to an external stimulus such as change in the pH, temperature and ionic strength. Thermo sensitive Polymers form gel at body temperature whereas ion activated polymers gels when comes in contact with ions in the vaginal fluid. In addition to temperature there are other environmental triggers of gelation, such as calcium and pH. The great advantage of these polymers is that they can be inserted as liquid, suspension and gel. Moreover though the polymer achieve prolong contact time and have some ability to modulate drug release from gels, water-soluble drugs particular release from such system quickly [5-7]. The present investigation deals with development and evaluation of combination of bioresponsive polymers for the formulation of vaginal gel of clindamycin. Present dosage form includes thermosensitive polymers and pH activated polymers for in-situ gel formulation. The prepared dosage regimens provided ease in administration along with good bioadhesion and retention properties.

MATERIALS AND METHODS

Clindamycin HCL was obtained as gift sample from Watson Pharmaceutical, Poloxamer407 and Poloxamer188, Chitosan, Carbopol 974 p, were obtained as gift sample from Signet Chemical. All other required reagents were of analytical grade are the highest grade commercially available.

Preliminary studies.
Preliminary studies were carried out using different concentrations of various polymers evaluated for their gelling capacity & viscosity in order to identify the compositions suitable for use in the in situ gelling system. Various concentrations of individual polymers & combination of thermo sensitive polymers (Poloxamer 127and Poloxamer 188) with pH activated polymers (carbopol and chitosan) were studied for the above mentioned parameters [5,6,8]

Gelation temperature was determined by taking 10 ml polymer solution in transparent vial & gradually heated by electric heater with magnetic bid immersed in it. The magnetic rotor was set at 100 rpm, as temperature rise at gelling point bid stop rotating, corresponding temperature was noted down as gelation temperature. Gelation temperature was determined with & without Clindamycin 2% w/w (CLM).

Viscosity determination was carried out using Brookfield DVII programmable viscometer. At selected concentration polymer have viscosity less than 80 cp. i.e. Newtonian flow that is required for in situ gel preparation.

Effect of poloxamer on drug was studied by varying polymer concentration & concluded that as the concentration of poloxamer in formulation increase , the gradual decrease in gelling temperature occurred & then the combination of 23% P-127 & P-188 was finalized for development of clindamycin HCL in –situ gel.

Chitosan (0.3%w/w) as pH activated polymer was found effective in formulation. Addition of drug showed increase in the gelation temperature to the body temperature. CLM 2%w/w affects the gelling temperature of the Poloxamer alone and in combination with other additives. An in-situ gel must possess the mucoadhesive property so hydroxyl propyl methyl cellulose (HPMC 0.1%) was also included in the formulation. Benzalkoniamchloride and sodium chloride (0.9%) were used as preservatives and isotonic agent. All preparation was prepared in phosphate buffer saline solution pH 5.0 with and without drug. Combination of thermo sensitive polymer with pH activated polymer in various concentrations showed gelation temperature ranging from 36 ± 0.20˚c & with HPMC, Benzalkonium chloride & NaCl showed between 35 ˚c to 37±0.30˚c. In situ gel system was developed with 23%w/w P- 127 & 7% P-188, 0.3 %w /w chitosan & 0.3w/w carbopol. Formulation in physiological condition pH 7 at body temp 37˚c & in presence of ions they show pseudo plastic flow.

Table 1. Preliminary studies for polymers and excipients in formulation.

Polymer	Gelling Temp	Gelling Temp with CLM 2%w/w	Viscosity in cp.
P-127/188(23/7)	20±0.30	37±0.33	48.5
Chitosan 0.3%	21±0.28	36±0.31	48
Carbopol 0.3 %	22±0.31	37±0.27	45
HPMC 0.1%	20±0.25	35±0.41	61
Nacl 0.9 %		36.8±0.29	59 cp
Dextrose 5%		33.4±0.31	89 cp
Manitol 50%		30.4±0.32	65 cp

Preparation of Simulated Vaginal fluid [2]
Simulated vaginal fluid was prepared from 3.51 g/l NaCl, 1.40g/l KOH, 0.222g/l $Ca(OH)_2$, 0.018g/l bovine serum albumin, 2g/l Lactic acid, 1g/l acetic acid, 0.16 g/l glycerol, 0.4 g/l urea and 5 g/l glucose. The pH of the mixture adjusted to 4.5±0.02 using 0.1 N HCl.

Preparation of thermo sensitive gel

The "Cold Method" was used for preparation with slight modifications [9]. The composition of each formulation was listed in table 2. The weighed quantity of drug was dissolved in saline phosphate buffer in aseptic condition. Benzalkonium chloride as preservative was added at the same time. Individually the polymeric solution was prepared and kept undisturbed for 24 hours for proper mixing. Further the drug and polymeric solution was mixed properly and intended quantity of the isotonic agent was also added to it.

Table 2. Formulation of Clindamycin HCl in-situ gel.

Ingredients	F1	F2	F3	F4	F5	F6
CLM	2%	2%	2%	2%	2%	2%
P127/P188	23%/7%	23%/7%	23%/7%	23%/7%		23%/7%
Chitosan		0.3%		0.3%	0.3%	0.3%
Carbopol			0.3%	0.3%	0.3%	0.3%
HPMC						0.1%
Benzalkonium chloride	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Sodium chloride	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Note: All the formulations prepared in %w/w basis

Physicochemical Characterization of in-situ gel [10- 13]

Vagina is capable of self -cleaning & regularly secretes vaginal fluid with slowly flush to wash out unwanted waste & foreign material. One of the most challenging tests for vaginal drug delivery is bioadhesive property which helps to prolong the residence of formulation.

Gelling temperature ($T_{sol\text{-}gel}$) lowering effect of mucoadhesive polymer could be explained by its ability to bind to PEO chain present in P127 molecules. Promoting dehydration & causing an increase in the entangalment of adjacent molecules with more extensive intermolecular hydrogen bonding.

pH Evaluation

The pH of the formulation was determined using glass microelectrode (Mettler Instrument, Germany), and allowing to equilibrate for 1 min. Experiment was performed in triplicate.

Viscosity Measurements and Gelling Temperature (GT)

Viscosity measurements and gelling temperature for all the formulations was determined as per the method described in preformulation section**.** For all the formulations determination was carried out in triplicate.

Gel Persistent capacity (GPC) and Spreadability

GPC was determined by placing drop of prepared formulation in vial containing 2ml of SVF and observed till it completely erodes. Spreadability was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time were noted for upper slide (movable) to separate completely from the fixed slides.

Bioadhesion measurement

The method was based on the measurements of the tensile strength or shear stress required to break the adhesive bond between the model membrane and the test formulation. The test formulation was sandwiched between two model membranes and fixed on the flexible support in the assemblies for 10 sec. after adhesive bond was formed, the force required to separate the bond was measured and calculated as bioadhesive force.

Drug Release

The in –vitro drug release study was performed in sink condition, using Franz diffusion cell with water jacketed receptor chamber (20ml) and donor chamber thermostated at 37^0 C. The receptor chamber was separated by egg membrane and each formulation was spread on the circular portion of the membrane. The drug release was measured by UV analysis method.

RESULTS AND DISCUSSION

Clindamycin HCl was used with varying concentration of P127 and P188 in combination and found that ratio of concentration in %w/v of 23/7 of P127 / P188 is effective for sol-gel transformation at body temperature (37˚C). At high ratio i.e, of 23%/15% it showed decrease in GT than body temperature. pH activated polymers i.e, chitosan (0.1% &0.3%)and carbopol 974P (0.3%) in presence of Clindamycin showed gelation near to body temp and so

chitosan in 0.1%& 0.3% & carbopol 974 P 0.5% were used in thermo sensitive polymer in combination . At the same time effect of isotonic agent was also determined in gelling temperature of in situ system.

As compared to mannitol (5.0%) and dextrose (0.5%), NaCl (0.9%) showed gelling temp 36.8^{0}C and viscosity 59 cps near to the viscosity of thermosensitive system. So NaCl (0.9%) was added as isotonic agent in the formulation. HPMC (0.1%) as bioadhesive polymer was also added to provide strength to formulation, addition of it increased bioadhesion strength of the formulation.

Physico- chemical evaluation of the prepared formulation:

The physico-chemical properties such as gelation temperature, clarity, appearance, spread ability and GPC (gel persistent capacity) of the formulations are given in table 3.pH of all the formulations were found to be in the range 5.3-5.5 i.e. as per the pH of vagina. Formulations have satisfactory appearance, clarity & drug content in the range 98.1%- 101%.

Table No 3: Physicochemical evaluation of formulations.

Formulation code	pH	GT (^{0}c)	Viscosity (cps)	Spredabiliy (mm)	GPC (Hrs)	Drug Content (%)	Mucoadhesive Strength dynes/cm^2
F1	5.3	36.8	50	22	8	94.3±0.53	3.556±0.174
F2	5.4	36.4	46	23	9	100.2±0.61	12.186±0.147
F3	5.4	35.5	44	22	>9	101.2±0.34	12.248±0.325
F4	5.5	36.9	58	19	>9	98.1±0.56	12.019±0.596
F5	5.3	36.8	52	15	>9	99.3±0.62	16.697±0.158
F6	5.3	37.1	60	21	>9	99.8±0.24	18.125±0.098

Comparative viscosity profile of the formulation

Rheological analysis is a powerful technique to comprehensively investigate the gelation process and viscoelastic properties of thermo sensitive gel. The prepared formulations showed Newtanian flow as their viscosity is in 40-80 cp range at formulation conditions. However the formulations showed increased viscosity in the SVF this behavior is desirable as it can promote good strength at various temperature and easy application. From the gelation temperature it was clear that the prepared formulation showed gellation at physiological condition .Addition of mucoadhesive polymer decrease the gellatioon temperature but it is near to body temperature.

Evaluation of Mucoadhesive, Gel Strength and Spreadability:

Bio adhesion and long retention are desirable characteristics of vaginal formulation. HPMC and Carbopols act as mucoadhesive as well as permeation enhancer, performed adhesion force studies proves the mucoadhesive nature of the HPMC and carbopols. For poloxamer based in situ gel the bioadhesive property of pH activated combination like chitosan, carbopol & HPMC as bioadhesive material showed better results. It has been proposed that carbopol itself possess certain bioadhesive property due to its abundant hydrogen bond forming groups.

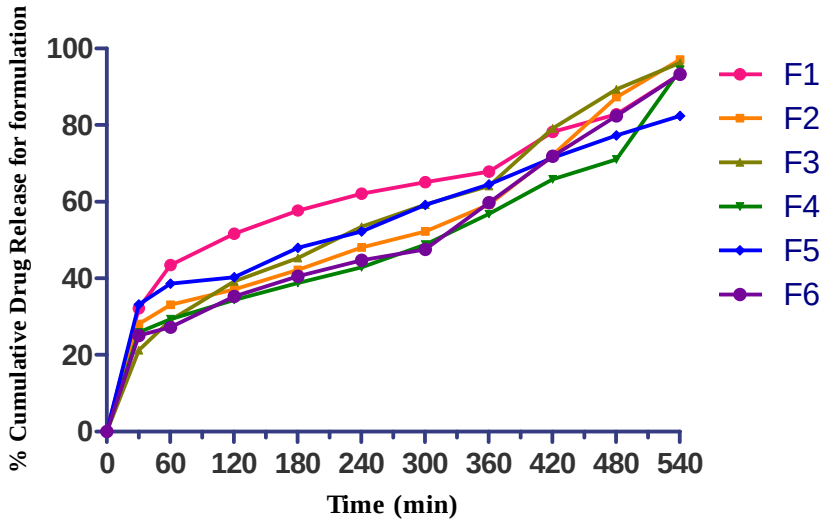

Fig No 1: Drug release profile of the formulations.

Accordingly synergistic effect of bioadhesive is predictable if carbopol can be used with the widely use bioadhesive material like HPMC.

Addition of mucoadhesive polymer improves the physical property of the formulation by mucoadhesive strength which was essential for the formulation to remain at the site of action for effective amount of time.

Comparative Drug Release Profile through Egg Membrane

In vitro drug release study was carried out as shown in fig 1 Chitosan, Carbopol and HPMC combination significantly decreased the rate of Clindamycin release. The drug release mechanism was also change by presence of chitosan and Carbopol due to predominance of diffusion in release mechanism was observed for Carbopol containing group, whereas gel composed of Poloxamer alone demonstrate the release mechanism mainly control by diffusion of drug. Such difference might be explained by the difference in erosion rate of the gel matrix caused by the presence of Chitosan, Carbopol and HPMC.

STABILITY STUDIES

The stability study was carried out on optimized formulation for 3 month. Based on visual identification the in –situ gel remained liquid for long period of months without incidence of turbidity or gelation. Samples were also analyzed for gelling temperature, pH, viscosity & drug release. Cumulative drug release was also satisfactory for various samples.

Physical Evaluation of the formulation:

Table No 4: Physicochemical evaluation of formulations

Sr. No.	Formulation code	Gelation temperature	pH	Viscosity	Appearance	% Drug Release
02	F3	35.8^{0}c	5.7	65 cp	++	92±0.21
03	F4	36.8^{0}c	5.9	65 cp	+++	96±0.41
04	F6	36.2^{0}c	5.5	50 cp	+++	96±0.12

Viscosity – A low viscosity product may leak out of the vaginal cavity and too high viscous may interact with sexual intercourse. A gel with Newtonian flow is suitable for the both ease of administration and retention. At this viscosity a dose volume of about 3.0 gm was found suitable for the premenopausal women, and a dose of about 2.2gm was suitable for the postmenopausal subjects to avoid leakage. It is important to make out that we are relying on bioadhesion, rather than viscosity, as the retentive mechanism. Viscosity should therefore be viewed more from the point of view of case of application.

PH- It is desirable to maintain acidic environment in the vaginal cavity to mimic normal physiological condition in the healthy premenopausal women. Addition of a typical acidic buffer will probably have only a transitory effect on resident pH, whereas employing a bioadhesive polymer with an acidic pKa builds the acidifying component into the polymer, sustained the pH. A number of existing polymers most notably the carbopols, which are polyacrylic acid based have useful pKa (i.e. in range of 4-5 range) [1].

CONCLUSION

Performed studies and obtained results prove the efficacy of Poloxamer 127/188 combination as Bio-responsive polymer in the formulation of Clindamycin in situ gel system for vaginal application. The formulation is isotonic, easy to administer along with good bioadhesion and retention property. This formulation has potential for better patient compliance as vaginal formulation. The efficacy of the formulation can further be studied by In vivo and clinical experiments.

REFERANCES

[1] R .William, Vaginal Microbicide Formulation Workshop, Contraceptive Research and Development Program Arlington, Virginia, **1998**.
[2] S .Hillier, Vaginal Bacterial Flora in Vaginal Health, Department of Obstetrics and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvanian, **1998**.
[3] G. Bonacucina, S. Martelli, G Palmieri, *Int J of Pharm*., **2004**, 282, 155-130.
[4] V. Claudia, *Adv Drug Delivery Reviews*., **2005**, 57,1692-1712.
[5] L. Bromberg, *Adv Drug Delivery Reviews*., **1998**, 31, 197-221.
[6] J.Y. Chang, Y. K. Oh, H. Choi, Y. B. Kim, C. K. Kim , *Int J of Pharm*., **2002**, 241, 155-163.

[7] J.Y. Chang, V.K Oh., H.S Kong., E.J Kim., D.D., Jang, K.T Nan, C.K Kim, *J of Control Release*., **2002**, 82, 39-50.
[8] C. Coggins, K. Blanchard, F. Alvarez, V. Brache, E. Weisberg, P. Kilmarx, M. Lacarra, R. Massai, D. Mishell, A. Salvatierra, P. Witwatwongwana, C. Elias, C. Ellertson, *Sex Transm Infect*., **2000**, 76, 480-483.
[9] Y. Liu, W. Lu, J. Wang, X. Zhang, H. Zhang, X. Wang, T. Zhou, Q. Zhang, *J of Control Release*., **2007**, 117, 387-395.
[10] S.Garg, R. Kandarapu, K .Vermani, K. Tambwekar, A. Garg, D. P. Waller, L. J. D. Zaneveld, *Aids Patient Care and STDS*., **2003**,17, 377-399.
[11] Y. Mao, M.J. Thompson, Q.X. Wang, E.W.Tsai, *J of Pharm and Bio Ana*, **2004**, 35, 1127-1142.
[12] J .Siepmann, N.Peppas, *Adv Drug Delivery Reviews*., **2001**, 48, 139-159.
[13] M. E. Perotti, A. Pirovano, D. M. Phillips, *Biology of Reproduction*., **2003**, 69, 933-939.