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Bio Responsive In Situ Gel of Clindamycin for Vaginal Application

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ABSTRACT

The aim of present research is to investigate the effect of various bioresponsive polymers on drug release from Clindamycin gel formulating system developed for vaginal application. Bacterial infections are the major cause of cervical cancers. To achieve a better therapeutic efficacy and patient compliance in the treatment for vaginal diseases and infections. The problems like multiple days of dosing, dripping, leakage and messiness, causing discomfort to users were major attraction for research. Thus bioresponsive system has been formulated in a gel using the thermo sensitive, pH responsive and ion activated polymer together with alternative mucoadhesive polymers to enhance the effect for longer duration which in result decrease the dosing frequency. The present study will show the various effect of polymeric concentration on formulation and its release behavior. The developed formulation was characterized for various in-vitro parameters e.g. pH, viscosity, drug release profile, bioadhesive force, spreadability and stability studies. To simulate vaginal conditions, simulated vaginal fluid was used as diffusion media to observe release. The developed formulation is alternative to conventional vaginal dosage forms.

INTRODUCTION

Bacterial vaginosis (BV) is the most common cause of vaginitis, accounting for 50% of cases. Bacterial vaginosis is caused by a change or imbalance in the types of the bacteria normally found in the vagina and causes an overgrowth of organisms. Vaginal preparations, although generally perceived as safer most, still they are associated with a number of problems, including multiple days of dosing, dripping, leakage and messiness, causing discomfort to users and expulsion due to the self-cleansing action of the vaginal tract [1, 2]. These limitations lead to poor patient compliance and failure of the desired therapeutic effects. For effective vaginal delivery of antimicrobial agents, the drug delivery system should reside at the site of infection for a prolonged period of time. The vagina, unlike other systems is highly dynamic with respect to absorption of drugs, their metabolism and their elimination [3]. The vaginal defense i.e. epithelium, flora, immune cells and pH make it favorable site for local and systemic delivery of drugs that are used specifically for the treatment of female-related conditions [16]. The conventional dosage forms i.e preformed gel and solutions have a number of lacunas, which has limited their use in vaginal drug delivery. Direct application of gels onto the infected sites of the vagina might be difficult, inconvenient as well as have frequent dosing because the conventional gels do not remain for long time at the site of application. A new and recent approach is to try to combine advantages of both gels and solution so that an accurate dose can be administered with ease of administration i.e. in-situ gel system. These formulations remain to a solution state before administration but however transforms to gel after administration in to vaginal cavity [4].

The numbers of polymer those are liquid at room temperature become solid at body temperature. Poloxamer is a typical polymer in this regards. In addition to temperature there are other environmental triggers of gelation, such as calcium and pH. Such phase change

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polymer which solidify in the vaginal cavity, might indeed have a long contact time but will probably interfere with sexual intercourse. However there is polymer or polymer blends that soften but do not become solid. The great advantage of these polymers is that they can be inserted as liquid, suspension, gel. Moreover although the polymer do prolong contact time and have some ability to modulate drug release from gels, water -soluble drugs particular, release from such system quickly [1]. The *in situ* gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing cost [12].

MATERIAL AND METHODS

Material

Clindamycin was obtained as a gift sample from Royal pharmaceuticals, Mumbai. Chitosan, Poloxamer F127, Xanthan gum, Sodium alginate, Carbopol, Carrageenan obtained as a gift sample from Signet chemicals.

Methods

Preparation of Simulated Vaginal Fluid (SVF)

The SVF where, 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 [3].

Formulation of Clindamycin Hydrochloride

Preparation of placebo gel system- Different concentration of Gellan gum, Chitosan, Carrageenan, Poloxamer were prepared and evaluated n their gelling capacity in order to identify the compositions suitable for use in the in-situ gelling system all preparation where prepared in the phosphate buffer saline solutions pH 5.0 the gelling capacity was determine by placing the drop of formulation in the vial containing 2 ml of freshly prepared SVF, noting the time for gel formation and dissolve.

Preparation of medicated gel system- Base on the above studies the various combination of temperature sensitive + ion activated and temperature sensitive and pH sensitive polymer combination where selected. For antibacterial activity Clindamycin Hydrochloride was available as 2% cream hence a dose of 2% was used in present formulation.

- The weighed quantity of drug dissolves in saline phosphate buffer in aseptic condition.
- Preservative was added in the above step.
- The polymeric solution was prepared separately kept undisturbed for 24 hours to ensure proper mixing
- Mix the drug and polymeric solution properly and add calculated amount of the osmotic agent to it.

Physicochemical characterization

The developed formulation was evaluated for various physicochemical properties.

pH evaluation: The pH of the formulation recorded with the glass microelectrode and allowing it to equilibrate for 1 min. Experiment where perform in triplicate and average where calculated.

Determination of Viscosity of prepared formulation: Viscosity measurements where performed by the Brookfield Model DVIII+ Digital Viscometer. All measurements where conducted using SC-4 spindle using 5ml of sample, at various rpm. The tests were performed in triplicate with 5% coefficient of variation.

Gel Persistent Capacity (GPC): Place drop of prepared formulation in vial containing 2ml of SVF and observe the gel till it's completely erode.

Bio - adhesion Measurements: The method is 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 is sandwiched between two model membrane and fixed on the flexible support in the assemblies for 10 sec. after adhesive bond is form the force required to separate the bond was measured and calculated as bioadhesive force.

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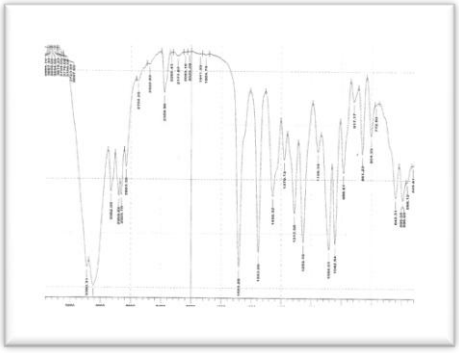
In- Vitro Drug release behavior: The In-Vitro drug release was performed in sink condition, by means of Franz Diffusion cell, with water jacketed receptor chamber (20 ml) and donor chamber thermo stated at 37 ^{0} C. the receptor chamber were separated by membrane and each formulation was spread on the circular portion of the membrane. The drug release was measured by UV analysis method.

Stability: Stability studies were carried out on optimized formulation as per ICH guidelines.

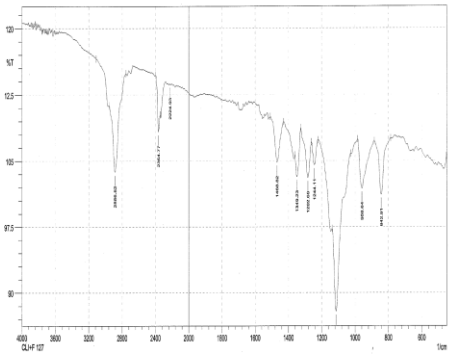
RESULTS AND DISSCUSSION

Clindamycin HCl was scanned for its absorbance at 213nm for generation of standard graph. The IR absorption spectra of the pure drug and drug-excipient mixture were taken in the range of 400 – 4600 cm^{-1} using KBR disc method.

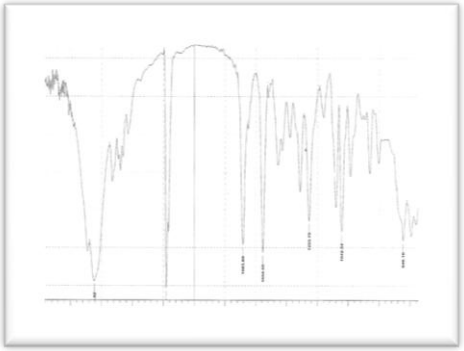
Figure 1: IR spectra of Drug and Excipient



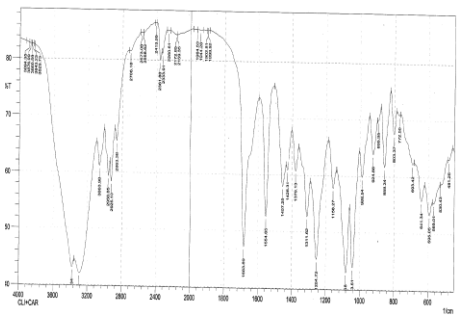
IR spectra of Clindamycin



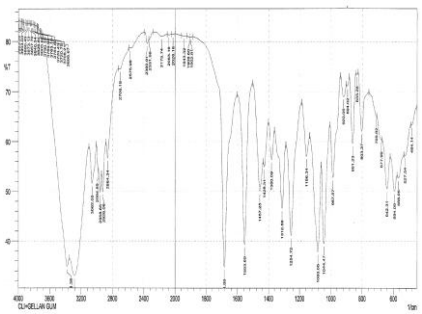
IR spectra for Drug + Poloxamer



IR spectra for Drug + Chitosan



IR spectra for Drug +Carrageenan



IR spectra for Drug + Gellan gum

The effect of polymers with different concentration was evaluated with respect to their biological response. Gelation temperature of Poloxamer at various concentrations, combination effect of temperature sensitive polymer with Ion responsive polymers, pH sensitive polymers, effect of various isotonic agents on bioresponsive polymers.[15]

The physicochemical properties of the prepared formulation are shown in below table. The drug content uniformity, Gelation temperature, appearance, spread ability, GPC and pH of the formulation were found to be satisfactory and the formulation was liquid at non-physiological condition. The formulation prepared found to be free flowing liquid, which helps in easy instillation in site of action.
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 polacrylic acid based have useful pK as (i.e. in range of 4-5 range) [1, 11]
Shows comparative observation of physical and chemical evaluation of formulation
+++ = Free Flowing Liquid; ++ = Viscous Liquid; + = Thick Liquid

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Table 1: Composition of formulation of Clindamycin Hydrochloride formulation prepared in % w/w basis

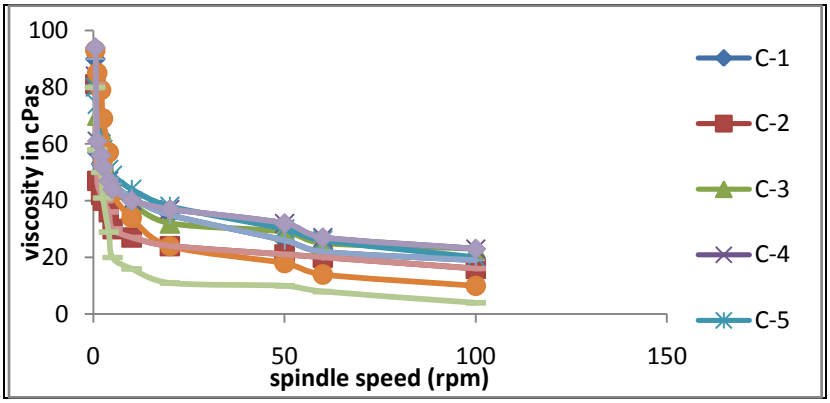
Ingredient	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Clindamycin	2	2	2	2	2	2	2	2	2	2
Poloxamer F127/F68	23 / 7	23 / 7	23 / 7	23 / 7	23 / 7	23 / 7	23 / 7	23 / 7	23 / 7	23 / 7
Carrageenan	0.1	–	0.1	0.2	–	0.2	–	–	–	–
Chitosan	–	–	–	–	–	–	0.3	–	0.3	0.3
Carbopol- 974P	–	–	–	–	–	–	–	0.3	0.3	0.3
Gellan gum	–	0.1	0.1	–	0.2	0.2	–	–	–	–
Hypromellose	–	–	–	–	–	–	–	–	–	0.1
Sodium Chloride	0.366	0.366	0.366	0.366	0.366	0.366	0.366	0.366	0.366	0.366
BKC	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2: Physical and chemical properties of developed formulation.

Formulation	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Gelation temp (^{0}c)	36.4	37.1	35.4	35.7	37.2	34.8	36.4	35.5	36.9	37.4
pH	5.4	5.4	5.5	5.6	5.7	5.7	5.4	5.4	5.5	5.6
Appearance	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Spreadability (mm)	19	18	20	22	18	17	17	16	19	20
CU (%)	107	96.4	97.5	95.8	98.3	97.8	100.2	101.2	98.1	95.8
GPC (Hrs.)	8	8	9	9	9	9	9	9	<9	<9

The spreadability plays an important role in patient compliance and helps in uniform application of gel to the skin. A good gel takes less time to spread and will have high spreadability [6]. The spreadability of formulated gels was decreased as the concentration of polymer increased.[15]. Viscosity measurements where performed by the Brookfield Model DVIII+ Digital Viscometer. All the formulation evaluated for Viscosity measurement was appropriate for insitu gelling system. Gels presented non-Newtonian, pseudoplastic, thixotropic behavior, with yield stress. Overall viscosities varied between 13500 Pas and approximately 80 Pas within a biologically relevant shear rate interval [4, 5].

Figure 2: Rheological properties of formulation.



At this viscosity a dose volume of about 3.0 gms 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 recognize that we are relying on bioadhesion, rather

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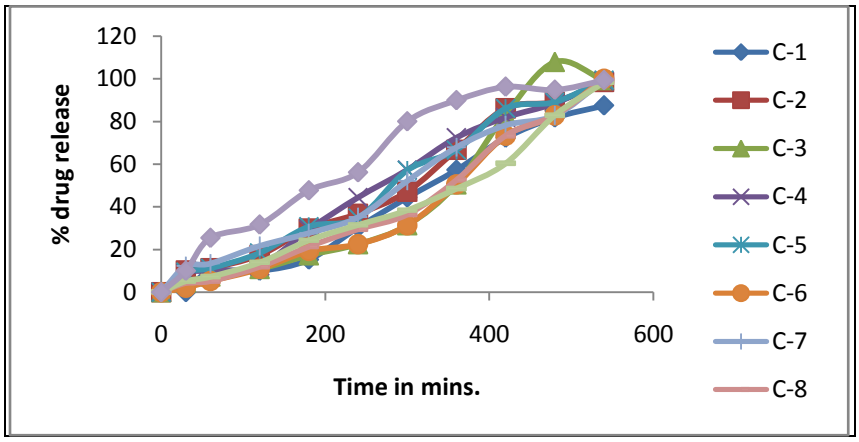
than viscosity, as the retentive mechanism. Viscosity should therefore be viewed more from the point of view of case of application and dose volume required[1]. In vitro drug release behavior: In vitro drug release study was carried out in KC-Diffusion cell using SVF as diffusion medium. The processed cellophane membrane was used, simulating the vaginal in vivo condition like vaginal epithelial barrier. The drug content in withdrawn sample was estimated by UV-Visible spectrophotometer at 213 nm. The rate of drug release was dependent upon the polymeric concentration used in the development of formulation. The drug release from the formulation was prolonged for 9hrs and release behavior was observed same.

Figure 3: Invitro release behavior for developed formulation



Determination of Bioadhesive: The vaginal bioadhesive property of formulation is in C-10>C-9>C-8 and respectively other. It was concluded that Carbopol 974 showed the highest bioadhesive property [6, 10, 13].

Table 3: Bioadhesive force of the formulation

Formulations	Bioadhesive force (dynes/ Cm2)
C-1	2.719
C-2	2.186
C-3	2.548
C-4	2.619
C-5	3.175
C-6	3.229
C-7	3.556
C-8	4.174
C-9	4.591
C-10	6.197

Stability Evaluation

Conditions: Based on visual identification the in situ gel has remained as liquid for a long period of 03 months without the occurrence of turbidity or gelation at 25^{0}c /65% RH. Sample were also kept at 30°C/ 65% RH, 2-8°C.

Physical Evaluation of the formulation:

Table 4: Physical evaluation of formulation on stability

Sr. No.	Formulation code	Gelation temperature	pH	Clarity	Appearance
01	C-3	36.4^{0}c	5.6	***	+++
02	C-4	35.8^{0}c	5.7	***	++
03	C-7	36.8^{0}c	5.9	***	+++
04	C-10	36.2^{0}c	5.5	***	+++

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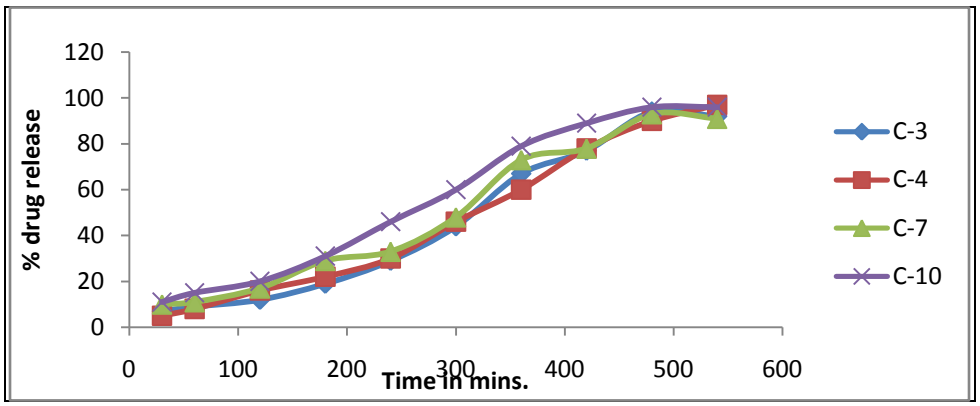
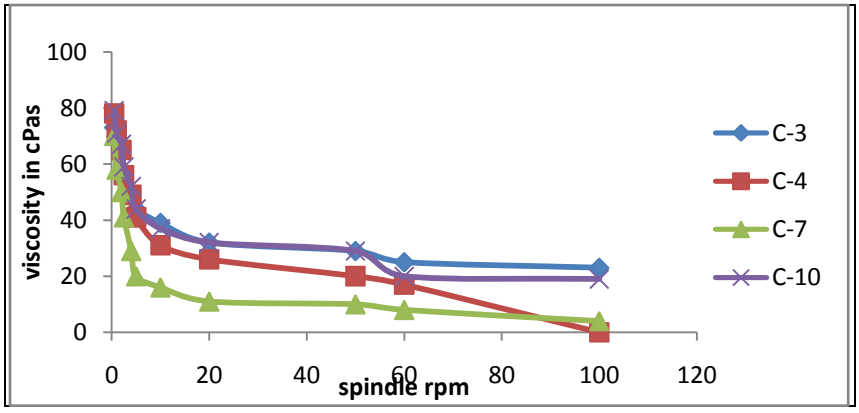
Comparative drug release profile of the formulation

Figure 4: Percentage drug release for samples kept on stability at 25ºc /65% RH



Comparative Viscosity profile of the formulations

Figure 5: Viscosity for formulation kept on 3months stability



Physical evaluation of the formulation:

Table 5: Evaluation of bioadhesive force and spreadability

Sr. No.	Formulation	Bioadhesive Force	Spreadability
1	C-3	2.548	22
2	C-4	18.625	21
3	C-7	12.248	22
4	C-10	18.125	21

CONCLUSION

The vaginal route has been traditionally used for the conventional delivery of several locally acting drugs like antimicrobial agents [7]. Bioadhesive polymer Carbopol presumed to provide better vaginal bioadhesion [8]. A low viscosity product may leak out of the vaginal cavity and too high viscous may interact with sexual intercourse. A gel in the range of 50,000-80,000 cps is deemed suitable for the both ease of administration and retention. From present investigation it can be concluded that Clindamycin gel formulating system can be successfully formulated by using combination of Carbopol, Poloxamer, Carrageenan and other polymers. Selection of polymer and content is the major key factor in deciding viscosity and release from gel. Therefore combination effect of polymers was evaluated for prolong release of drug from formulation.

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