Development And Evaluation Of a Mucoadhesive Chitosan-Coated Liposome-Based Formulation For Vaginal Infection

*Authors:Farheen khan ,Dr.Dharmendra Kumar Ojha**

**Rajarshi Rananjay Singh Collage of Pharmacy ,UttarPradesh*

Abstract

Vaginal administration is useful for many medications, especially for treating gynecological conditions including stomach infections. The proficiency of current medication dosage regimen constrained by their brief retention times at the site of the treatment despite the fact that numerous alternative pharmacological formulations have been created for vaginal delivery. When dealing with formulation development, the vaginal self-cleansing process and physiological changes in the vaginal mucous membranes remain a challenge. The objective of this study was to create and assess mucoadhesive liposomes that can enhance local, vaginal clotrimazole treatment. The so-called "mechanical dispersion" method was used to incorporate clotrimazole into liposomes, and the suspension incorporated liposomes was then put at sonication to produce the necessary vesicle size. The liposomes' dimensions, polydispersity, and level of drug incorporation were assessed. To enhance the mucoadhesive characteristics of the liposomes, a coating made of polymer chitosan was applied to their surface. After the free drug was removed, chitosan coatings (0.1% w/v and 0.6% w/v) were put to liposomes. When the drug release liposomes coated by chitosan was contrasted in vitro with that from liposomes without chitosan and from free drug, chitosan-coated liposomes displayed delayed drug release to a greater extent. The formulation's adherence to the mucosa and tissue penetrationwasinvestigatedincowvaginaltissue.Accordingtopreliminaryevidence,clotrimazoledoesn't actually penetrate vaginal tissue; instead, it stays in the tissue. The results of the trials suggested that chitosan-coated phospholipid vesicles may be used for therapies to treat vaginal conditions locally.

1. Introduction

Currently, the scientific community and the pharmaceutical industry are interested in employing vaginal delivery as a drug administration method. The two main advantages of vaginal drug delivery over traditional routes of administration are the avoidance of hepatic first-pass metabolism and the relatively high permeability for many drugs. The vagina not only has a large surface area, an ample blood supply, and few side effects, but it also presents a possible site for local and systemic therapy. Since the vaginal system spontaneously cleanses itself, conventional vaginal dose forms—such as creams, foams, pessaries, and jellies—are believed to only persist briefly at the targeted region, which negatively affects the effectiveness of treatment.Therefore, it is necessary to create efficient drug delivery methods that should extend the drug's interaction with the mucosal surface and permit prolonged drug release, resulting in enhanced medication therapy.

When appropriate, extended stay at the site of administration is interesting for both systemic drug bio availability and local therapy. Since the early1980s, pharmaceutical technology has been interested in the function and potential of mucoadhesion in drug delivery as a means of extending the residence time for numerous drug formulations. The adhesion between two materials in this example the vaginal mucosa and at least one of which has a mucosal surface is known as mucoadhesion.

In the case of polymers utilized in the creation of delivery systems, hydrogen bonding ability, pH, molecularweight, charge, and polymer concentration were identified to be the dominant determinants. Mucoadhesive polymers have a number of benefits when used in drug delivery. The potential for longer residence time in situ and intimate adherence with mucus, which results in improved and sustained drug distribution, are the main benefits of vaginal administration. medication carrier systems that have the capability of controlling the release of linked drugs is an extra proin better medication therapy.

Especially indermatology, liposomal medication has been extensively exploited semii cation carriers in topical therapies for local disorders. They may incorporate a wide range of hydrophilic and hydrophobic medications, and because they can perform controlled and/or continuous release of the entrapped medication, they are also thought to be appropriate for application in vaginal abnormalities. But utilizing liposomes topically has significant drawbacks, including their liquid nature, which results in short-live dretention at the administration site. This can be avoided by incorporating them into a suitable vessel that nevertheless preserves the original vesicle structure. Another strategy for extending the liposomal formulation's residence time is to change the liposomal surface by covering it with mucoadhesive polymers. Dependingon the desired polymer qualities, a mucoadhesive polymer will be selected

as the coating material. Chitosan is one of the best polymers in terms of safety and mucoadhesive ness. For the vaginal administration of medications, the well-known natural-origin mucoadhesivepolymerchitosanisadvisedasastableandacceptablecarrier.Inourongoingstudy, we have chosentouse this polymer as a coating material in an effortto improve the mucoadhesive delivery method.

Anti fungal and Antibacterial medications have been given intravaginally for years to treat yeast and bacterial infections, respectively. One prevalent cause of vaginitis, Candida, is particularly intriguing. When properly diagnosed, simple candidiasis can be effectively treated with a range of azole anti candidal drugs, including short-course regimens. Amongthem,clotrimazole is well known for itsexceptionallocalefficacyandlackofsignificant side effects. An imidazole derivative called clotrimazole ($C_{22}H_{17}ClN_2$) is typically used to treat vaginalye as t infections. Althought here are medicines on the market that contain clotrimazole, the therapeutic results are frequently subpar and patient compliance is low, necessitating repeated treatments.

Since clotrimazole is a lipophilic chemical with low solubility, its in clusion into liposomes might cause it tobe comemore soluble and possibly have stronger anti-candidal effects .Chitosan coating on the liposomal surface is anticipated to prolong the system's stay at the vaginal location.

2. Result and Discussion

2.1 Liposome characterization

2.1.1 Uncoated vesicles-Particle Size Analysis

Using a scattering-intensity-weighted assessment of the particles in vesicle mode, the vesicle size and size distributions were ascertained. Liposomes' particle size and size distribution are crucial for their use as drug carriers in topical medication administration. The majority of research to date has been on how liposomal size affects how well liposomal transport to the skin works (Cevc, 2004). The impact of vesicle size on the distribution of medications meant for mucosal targeting is comparatively poorly understood. Takeuchi et al. discovered that when liposomes were shrunk to a size of roughly 100 nm, both uncoated and chitosan-coated liposomes had an increase in the quantity of liposomal particles that penetrated the mucous layer (Takeuchi et al., 2001).Our liposomes that were sonicated for four minutes fell within the desired size range, as indicated by the vesicle particle size displayed in Table 1.

Table1. Sonicated samples- Representativeparticlesizedistribution

Sonication time(Min)	**Vesicle size**				**PI***
	Peak1	Intensity(%)	Peak2	Intensity (%)	
1	320 ± 50	51.9	39±6	45.0	0.55
2	245 ± 29	49.8	38±6	44.8	0.41
4	116 ± 12	79.2	30±3	17.9	0.42

The values denote the average of three cycles of determination ±SD.

***PI- Poly dispersity Index**

The width of unimodal size distributions is measured by the polydispersity index (PI, Table 1) (Vermaet al., 2003). A polydispersity index with a value less than 0.70 is considered acceptable. The majority of our samples displayed particle distributions with two distinct peaks, indicating a bimodal distribution that leads to relatively high polydispersity (Table 1). While most liposomal formulations showed bimodal distributions, indicating that two distinct populations of vesicles were seen, certain liposomal samples sonicated for one or two minutes showed trimodal distributions. All samples had relatively significant polydispersity; however, the polydispersity index values dropped as the sonication period increased (Table 1). Since bimodal distributions are very prevalent and the sonication process's performance is affected by the probe's position, power, and duration, it is anticipated that sonication, as a vesicle size reduction technique, will produce vesicles with a relatively high polydispersity (New, 1990). We made an effort to employ the shortest possible duration for the liposomes to be exposed to the sonication force because prolonged sonication is known to cause the medication that was originally encapsulated to release (di Cagno et al., 2011). The amount of medicine coupled with liposomes must be sacrificed in order to get the required vesicle size.

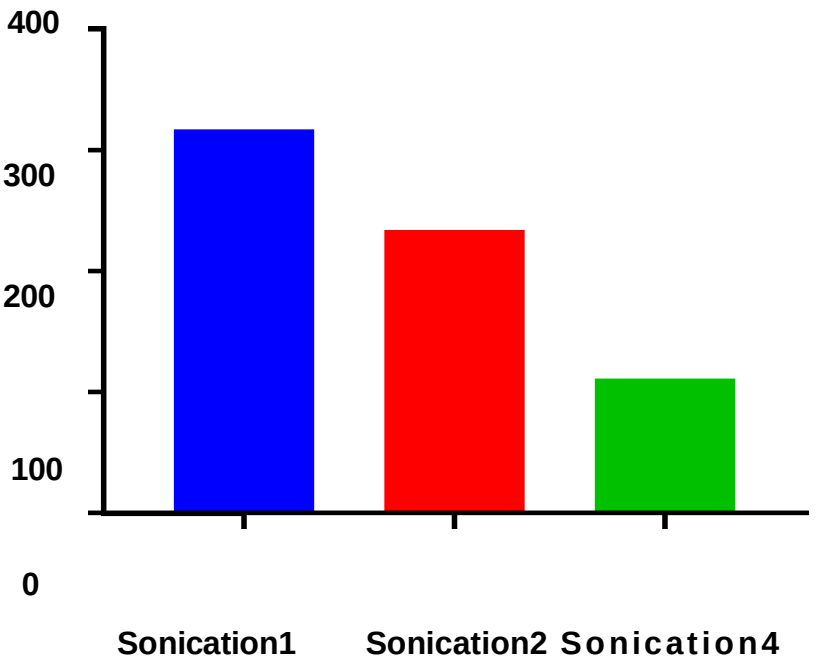

Fig.1 Sonicated liposomesMeandiameter.

It was clear that shorter vesicles were produced as predicted by longer sonication times when a mean diameter was computed in proportion to sonication time (Figure 1). It is important to note that the mean diameter in this instance is only an estimate because the samples' high polydispersity made it impossible to use a Gaussian distribution. For each of the three distinct formulations, the intensity % of vesicles grouped in two populations was used to calculate the mean diameter (Figure 1). Standard deviations, however, are not shown because this is only an estimate and have no real significance.

2.1.2 Clotrimazole entrapment efficiency

According to comparable quantities of the standard clotrimazole, the absorbance of the standard clotrimazole at 261 nm was used to create the calibration curve for clotrimazole in methanol (Fig. 2).

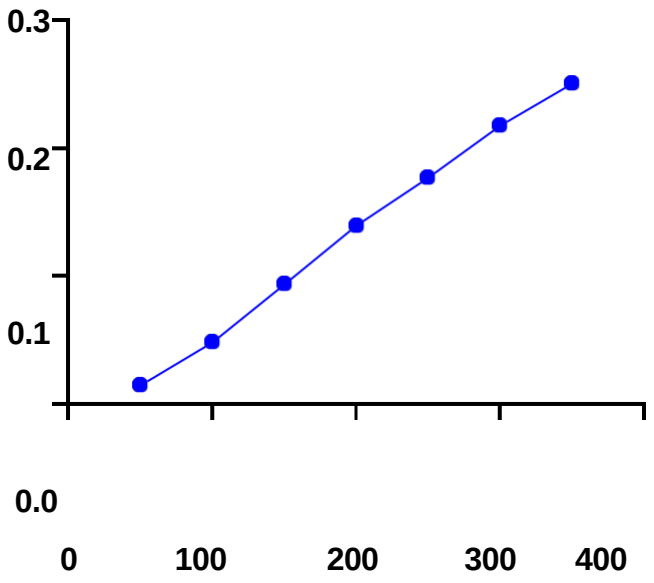

Fig.2. Clotrimazole calibration curveinMeOH.

It was discovered that the connection between drug concentration and absorbance was linear within the concentration range of 20–300 µg/ml. It was found that the correlation coefficient was 0.8994. Entrapment efficiency must be sufficient to deliver the intended therapeutic effect. The medication's physicochemical characteristics, particularly its solubility, will influence how well the drug is entrapped in liposomes (Mura et al., 2007). Clotrimazole was dissolved in the organic solvent with lipid during the liposomal synthesis since the medication is lipophilic (logP of 3.5; Wulff et al., 2001) and was anticipated to integrate into the lipid bilayers of liposomes.

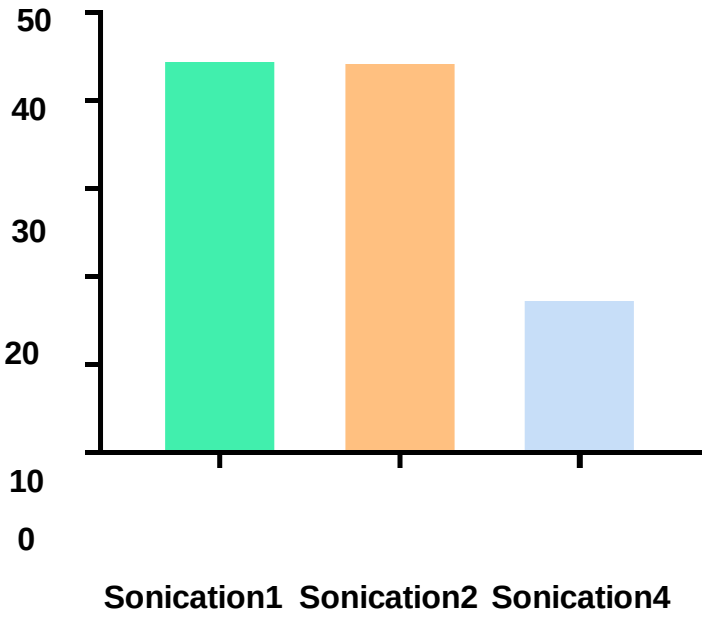

Fig3. Entrapmenteffectiveness of Clotrimazole.The values show the mean ± SD of three different experiments.

Entrapment efficiencies are shown in Fig. 3 as a function of sonication time. Given that sonication is associated with the loss of the drug that was originally integrated, it makes sense to anticipate that shorter sonication times would lead to higher drug entrapment values than longer sonication times. The limitation of ultracentrifugation as a separation method can be ascribed to the fact that we observed nearly identical entrapment efficiency (25–35%) for liposomes sonicated for different times, or even higher entrapment for smaller liposomes. To be more precise, liposomes that are larger than 200 nm will also precipitate down with an unentrapped drug; thus, it appears that the values for entrapment in larger liposomes are underestimated. Table 2's particle size distributions, both prior to and following ultracentrifugation, attest to the larger liposomes' precipitation.

Table2:Ultracentrifugation-Particle sizedistribution

SonicationTime(min)	Ultrasonication	Vesiclesize				Polydispersity Index
		Peak1 (nm)	Intensity (%)	Peak2 (nm)	Intensity (%)	
2	Before	222± 29	43.9	26±5	46.8	**0.45**
	After	69± 8	61.6	28±3	38.4	**0.35**
4	Before	110± 12	93.1	30±4	26.9	**0.49**
	After	80± 6	76.8	35±3	43.2	**0.36**

We did not substitute the ultracentrifugation procedure with any other means of separation because our focus was on creating smaller vesicles that were sonicated for longer periods of time (2 and 4 minutes). Although gel filtering of Sephadex or Sepharose columns would have required more time and money, we may have used those alternatives instead. Furthermore, we wanted to employ the same techniques for the duration of the project, and those techniques could not be used to separate coated liposomes.

The trapping was reliable and appropriate for coating to move further with. Our entrapment was found to be lower than data from the literature; however, none of the available articles described the use of the identical phospholipid composition and liposome manufacturing procedure. Using the thin-film approach, Ning et al. (2005a) reported a very high entrapment of clotrimazole (above 90%); however, they employed dialysis to separate the liposomal drug from the unentrapped drug. Because clotrimazole is essentially insoluble in water and there is no information available regarding whether or not the sink conditions were guaranteed, it is probable that some of the medication collected in the dialysis tube as precipitates, which artificially raised the entrapment values.Clotrimazole entrapment was shown to be relatively high in liposomes made using the proliposome and polyol dilution methods (Pavelić et al., 1999). Nevertheless, the vesicle size was larger than in our instance and the preparation techniques were different. For bigger liposomes, Pavelić et al. (2005) similarly reported an entrapment efficiency of 64–71%. In their instance, liposomally encapsulated clotrimazole was released via gel chromatography.

2.2 Coatingofliposomes

2.2.1 Size characteristics

It is anticipated that coating liposomes with different mucoadhesive polymers will extend their residence time once they come into touch with mucosal tissues, whether in the vaginal, intestinal, buccal, or colon. The drug's properties and the targeted mucosal region will determine which polymer is employed to coat the liposomes (Karn et al., 2011). Because of its superior mucoadhesiveness and safety profile, we chose chitosan as our model coating polymer (Perioli et al., 2009; Karn et al., 2011). The effectiveness of the coating can be determined by a number of markers, including changes in the zeta potential on the surface of the vesicles and an increase in vesicle size (Karn et al., 2011). The increase in initial vesicle size is one of the simplest and most widely utilized markers; we have employed this measure in formulation development. Our tests supported the hypothesis, and Table 3's findings demonstrate that liposome coating really occurred.

Table3:The effectofchitosancoatingonparticle size distribution.

Time of Sonication (min)	Coating (%w/v)	Vesiclesize				Polydispersity Index(PI)
		Peak1 (nm)	Intensity (%)	Peak2 (nm)	Intensity (%)	
2	-	97± 9	72.3	20±3	47.2	0.44
	0.1	138± 24	51.3	44±7	65.4	0.37
	0.6	149±16	53.3	61±5	45.8	0.35
4	-	95± 12	62.5	21±1	57.5	0.32
	0.1	122±13	39.2	41±4	50.1	0.35
	0.6	178±16	61.2	61±5	57.4	0.39

The initial size of the vesicles was in fact enhanced by coating, and larger vesicles were formed when the coating included a higher concentration of chitosan (Table 3). Even though coating causes vesicles to grow larger, the polydispersity of these populations was reduced and the vesicles' sizes were more uniform. The larger, coated vesicles exhibit a more homogeneous size distribution than the uncoated vesicles, which may come as a surprise. Nevertheless, the findings can be explained by the NICOMP size evaluation systems' determination of the specificity of the vesicle size distributions. It was discovered that the polydispersities of coated vesicles were adequate, especially

for those coated with 0.1% (w/v) chitosan. Since the vesicle size given is an estimate based on the intensity of the particle subpopulations, we have not computed the degrees of significance.

Our coated liposomes' size closely matched Takeuchi et al.'s recommended optimal size for coated liposomes, which makes sense given the drug's actual therapeutic potential and ability to pass through the mucous layer (2001). According to their suggestion, the size range of 100 nm for nanoparticles has the highest potential for deeper penetration into the mucosal layer and inducing a therapeutic response to the medicine coupled with the nanoparticles.

Submicron-sized chitosan-coated liposomes were found to be more effective than liposomes of the same composition but larger size. The same group conducted several experiments on the mucoadhesiveness of coated liposomes and confirmed that it is dependent on the particle size (Takeuchi et al., 2005a). Nevertheless, oral administration of liposomes entrapping the medication calcitonin and fluorescent dye produced these results in the intestines of rats.

2.2.2 Liposome coating with Clotrimazole

When liposomally unentrapped medicines are present, chitosan coating may improve the effectiveness of entrapment for particular pharmaceuticals (Filipović-Grčić et al., 2001; Karn et al., 2011). Centrifugation caused a semi-gel to form in the centrifugation tube, which made it extremely difficult to separate the liposomes from the medication when we attempted to coat them in the presence of unentrapped clotrimazole. We opted to perform coating in the absence of unentrapped clotrimazole instead of using dialysis due to the low solubility of clotrimazole. After examining if the entrapped clotrimazole could still be detected in coated liposomes and calculating the entrapment efficiency, we discovered that the values were precisely within the range that was established for the entrapment yield in non-coated liposomes, which is 34.4 ± 13.9%. The sole distinction was the somewhat higher SD found for coated liposomes in comparison to uncoated ones.

2.2.3 Invitro clotrimazole release

According to Siewert et al. (2003) and das Neves and Bahia (2006), the Franz cell diffusion system is widely regarded as the most suitable in vitro technique for assessing drug release from topical formulations, including those for vaginal application. According to Ning et al. (2005a), the pH of a healthy human vagina is reported to range from 4.0 to 5.0. For this reason, a pH 4.6 acetate buffer was used as the receptor media. According to Ning et al. (2005b), clotrimazole released from proliposomes at a higher rate in acetate buffer (pH 4.5) compared to phosphate buffer (pH 7.4).

This result was expected as clotrimazole is a weak base (pKa 4.7) (Ning et al., 2005c). To verify if the delayed release is indeed related to the impact of liposomes as a carrier system, clotrimazole dissolved in 2 mg/ml propylene glycol was used as a control.

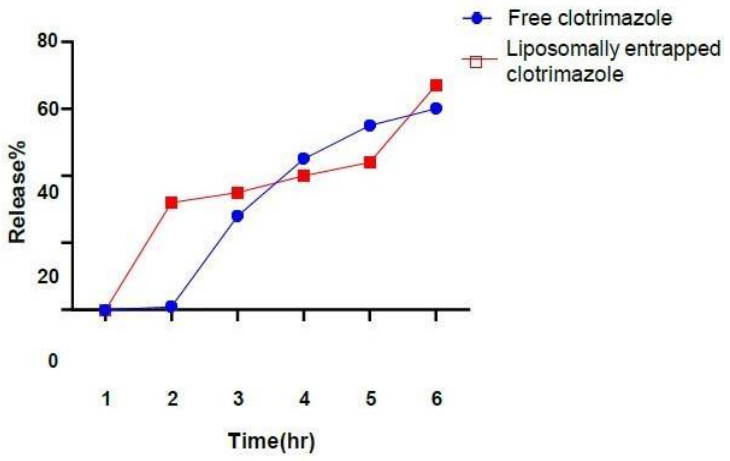

Fig.4 Clotrimazolerelease paradigm

Free clotrimazole (clotrimazole in propylene glycol) released slowly for the first 1.5 hours, but after that time, it started to release more quickly. But after four hours, the outflow became flat (Figure 11). Almost half of the medication is released in 4 hours. Initially, non-coated liposomes showed a faster release of medication as compared to free clotrimazole. This may come as a surprise because liposomes are supposed to slow down the release of medication. It is important to remember that liposomes act as clotrimazole solubilizers, and that the release of clotrimazole from our bilayers is what caused the initial burst. The release from liposomes slowed after two hours, reflecting an anticipated delay in the drug's release. Additionally, we collected samples following a 24-hour release period, and discovered that the drug recovery exceeded 130%. For in vitro release experiments, the drug recovery in the first eight hours ranged from 87% to 96%. The fact that menstruation, vaginal discharge, or the presence of semen is known to aid in the quick removal of formulation from the vaginal site was another factor in our decision to concentrate our experimental setup on the first eight hours (das Neves et al., 2011).

To ascertain the drug recovery, the remaining samples were taken from the donor chambers after eight hours. Remarkably, samples containing clotrimazole in propylene glycol were found to have a higher volume. This suggests that some acetate buffer had diffused through the membrane from the acceptor chamber, which could have an impact on the reported equilibrium and interfere with the levels. The experiments were conducted using the same experimental setupperformedwithcoatedliposomes.

Only liposomes coated with 0.6% w/v chitosan were examined for drug release since it was anticipated that they would have greater mucoadhesiveness. Although the two parallels initially exhibit identical release, the amount of medication released after 24 hours differed.

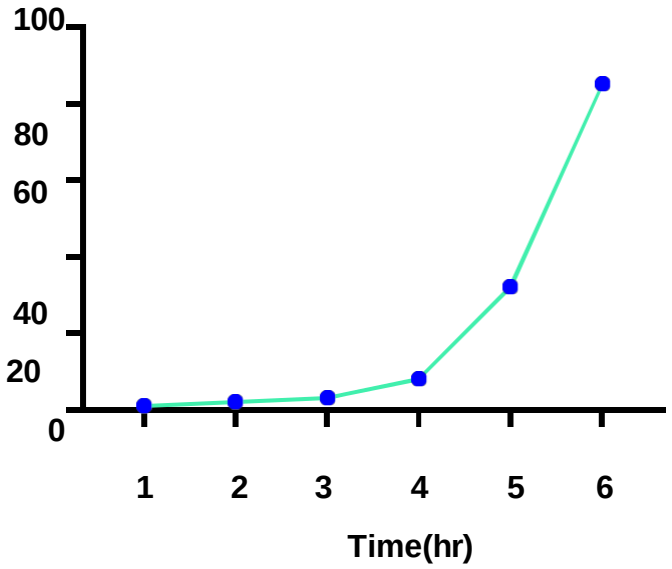

Fig.4*In-vitro*clotrimazolereleasefromcoated(0.6%,w/v)liposomes

In contrast to liposomes without a coating, clotrimazole released from chitosan-coated liposomes was postponed during the first four hours. Figure 12 shows that liposomes coated with chitosan can extend the release of clotrimazole more than liposomes without coating.

It is anticipated that mucoadhesive liposomes will stick to the vaginal surface and stay there for a longer amount of time. The release pattern from chitosan-coated vesicles that we observed in our studies matches quite well with the clotrimazole release from liposomes included in Carbopol® hydrogels that Pavelić et al. reported (Pavelić et al., 2005). This suggests that our coated liposomes have the same capacity as liposomal hydrogels to postpone the release of clotrimazole. Reports regarding the release of clotrimazole from hydrogels based on chitosan are not currently accessible.

2.2.4 Preliminarymucoadhesiontesting

The vaginal environment has a major impact on the persistence of contact when a mucoadhesive formulation is applied vaginally. It is known that interaction with low-pH vaginal fluid causes viscosity loss and erosion, for example, of gels (Geonnotti et al., 2005). For various formulations, alterations in the rheological characteristics of gels have been noted when simulated vaginal fluid is present (Chang et al., 2002; Geonnotti et al., 2005; Lai et al., 2008; Andrews et al., 2009). Therefore, the formulation should be exposed to real or simulated vaginal fluid in order to assess its mucoadhesive qualities (das Neves and Bahia, 2006; das Neves et al., 2008).

Accordingly, mucoadhesion of the chitosan-coated liposomes was examined in this investigation using a vaginal fluid simulant (pH 4.5; Owen and Katz, 1999). Furthermore, research findings indicate that notable variations in mucoadhesion have been noted when formulations have been tested at room temperature (20 °C) and at body temperature (37 °C). The significance of temperature and experimental setup was elucidated by Das Neves et al. (2008).

In our trial, which was conducted at room temperature (24 °C), we assessed the mucoadhesiveness of liposomes coated with chitosan in comparison to liposomes that were not coated and free medication. Samples of non-coated liposomes, 0.1% chitosan-coated liposomes, 0.6% chitosan-coated liposomes, and free clotrimazole (2 mg/ml in propylene glycol) were applied to tissue and exposed to vaginal fluid simulant.

It was anticipated that the entrapped medication would remain within the liposomes and that the coated liposomes would have better contact than the non-coated liposomes. The amount of medication that was initially entrapped and was still present in the donor chamber of liposomes could not be quantified due to an alteration in the absorbance at 261 nm. Measurements at 261 nm were affected by the extremely high absorption at 209 nm. This is believed to be the result of mucosal tissue-resident proteins and other molecules interfering, as demonstrated by the increased absorbance at 209 nm that we saw in the ex vivo penetration experiment. The samples were filtered before being determined to remove the disturbance, but interference remained. This provides more evidence that the HPLC method should be used to determine the amount of clotrimazole.

Based on our first research, it appears that clotrimazole applied to vaginal tissue tends to enter the tissue rather than exit it, which is better given the goal of treating vaginal infections locally. We need to use more sensitive drug detection methods, such HPLC, to be able to corroborate these intriguing discoveries.

3. Conclusion

Phospholipid vesicles can be applied topically to treat local vaginal infections and may enhance the administration of integrated drugs. It is anticipated that liposomal surface modification with mucoadhesive polymers may lengthen their retention duration at the vaginal location.

In addition to treating local vaginal infections topically, phospholipid vesicles may improve the way integrated medications are administered. Mucoadhesive polymers are expected to modify the liposomal surface, thereby increasing the period of retention at the vaginal site.

Clotrimazole was given prolonged release characteristics by chitosan-coated liposomes, which were also demonstrated to enter vaginal tissue but not pass through it. While clotrimazole's systemic absorption is undesirable, it is proven to be beneficial when used locally to treat vaginal infections. As a clotrimazole delivery method, chitosan-coated phospholipid vesicles seem promising;

nevertheless, further investigations on penetration and mucoadhesion are needed to maximize this innovative delivery method. is the treatment of vaginal infections locally. We need to use more sensitive drug detection methods, such HPLC, in order to be able to corroborate these intriguing discoveries.

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Authors sharenoconflictofInterest

Correspondingauthor:

Dr.DharmendraKumarOjha, Assistant

professor

RajarshiRananjaySinghCollageofPharmacy,Uttar Pradesh