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FORMULATION AND OPTIMISATION OF CIMETIDINE PRONIOSOMES: AN IN VITRO AND EX VIVO STUDY

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ABSTRACT

Objective: The objective of the study was to develop a proniosomal system for cimetidine, a potent H_2 receptor antagonist that could efficiently deliver entrapped drug over a prolonged period. **Methods:** The proniosomal system was formulated by selecting various ratios of span 40 and cholesterol using a coacervation- phase separation method. The formulated system were characterized for vesicle size determination by the particle size analyser, % drug encapsulation, drug-release profiles, drug content and vesicular stability at different storage conditions. **Results:** By using this method, the % drug loading that resulted by the encapsulation of proniosome was found to be 68%-78%. Increase in cholesterol and surfactant concentration increases encapsulation efficiency, but further increment decreases encapsulation. *In vitro* drug release studies showed prolonged release of entrapped Cimetidine. The *ex vivo* data on the release of Cimetidine from proniosomal formulations have shown significantly increased per cent release and flux in comparison to the same dose of marketed preparation of Cimetidine. Stability studies were carried out in refrigerated conditions, and higher drug retention was observed. **Conclusion:** It is evident from this study that proniosomes are a promising prolonged delivery system for Cimetidine and have reasonably good stability characteristics.

KEYWORDS: Proniosomes, Cimetidine Proniosomes, *in vitro* release, Prolonged Release, *Ex Vivo* Permeation Studies.

INTRODUCTION

Novel drug delivery systems have emerged embracing various routes of administration, to attain targeted and controlled drug delivery. Drug encapsulation in the vesicles is one such

system which helps to prolong drug duration in systemic circulation and decreases the toxicity by selective uptake. Based on this technique, a number of vesicular drug delivery systems such as liposomes, niosomes and provesicular systems like proliposomes and proniosomes have been developed.[1]

An alternative approach that overcomes several of these problems associated with liposomes involves the formation of liposome-like vesicles (niosomal dispersions) from nonionic surfactants, commonly referred to as niosomes. Niosomes are capable of entrapping hydrophilic and hydrophobic solutes. However, stability is a prime concern in the development of any formulation, and even though, niosomes have shown advantages as drug carriers, such as being low cost and chemically stable as compared to liposomes.[2] Proniosomal gels and solutions are of great stability due to very little water content. Gels or alcoholic solutions of non-ionic surfactants can transform into niosomal vesicles immediately upon hydration, hence, called proniosomes.[3] Proniosomes are semisolid liquid crystal (gel) products of non-ionic surfactants easily prepared by dissolving the surfactant in a minimal amount of an acceptable solvent namely ethanol and the least amount of water could solve the problem.[4] The proniosomal structure is aliquid crystalline-compact niosomes hybrid that gets converted into niosomes immediately upon hydration.[5] Proniosomes minimizes problems of noisome physical stability, such as aggregation, fusion, and leaking, and provide additional convenience in transportation, distribution, storage and dosing; this makes proniosomes a versatile delivery system with potential for use with a wide range of active compounds. In release studies, proniosomes appear to be equivalent to conventional niosomes. Size distributions of proniosome-derived niosomes are somewhat better than those of conventional niosomes.[6]

Cimetidine is a histamine congener; it competitively inhibits histamine binding to histamine H2 receptors. Cimetidine has a range of pharmacological actions it inhibits gastric acid secretion, as well as pepsin and gastrin output. It also blocks the activity of cytochrome P-450 which might explain proposals for use in neoadjuvant therapy. Cimetidine has a bioavailability of 70% and drug release from proniosome derived niosome is controlled than from the conventional niosomes.

MATERIALS AND METHODS

Materials

Cimetidine was obtained from Micro labs, Hosur, India. Cholesterol and Span 40 was purchased from Himedia and Merck, respectively. All other chemicals were of analytical grade.

Methods

The proniosomal system was formulated with various ratios of cholesterol and surfactant due to effect in encapsulation efficiency and drug release of the formulation system. It was carried out by trial and error method.

Table 1: Comparison of the various proniosomal system.

Sl. No	Formulation code	Surfactant: cholesterol ratio (µmol)	Drug content	Encapsulation efficiency in %
1	CIM1	125:25	68.07mg	68 ± 0.71
2	CIM2	100:50	65.08mg	65 ± 0.80
3	CIM3	75:75	58.05mg	58± 0.51
4	CIM4	50:100	56.73mg	56.73 ± 1.15
5	CIM5	25:125	60mg	60 ± 0.61

Preparation of Proniosomes

Proniosomes were prepared by an optimized method.[5] The accurate amount of surfactants and cholesterol were taken in glass vials, cimetidine and absolute methanol were added to the mixtures and then vials were tightly dealt and warmed in the water bath at 50℃ with frequent shaking of the solution. Hot distilled water was added while warming in the water bath. It was then allowed to cool at room temperature and the obtained formulation was kept in glass vials in dark place.

Formation of Niosomes

Niosomes were prepared by hydration of the gels prepared as described above. About 7 ml of warm distilled water was added into each vial followed by heating it at 60C in a water bath. Vortexing of the formulations was done two to three times with 10 min heating. The final volume was adjusted to 10 ml using distilled water.

Lyophilization

The formulated proniosomal systems were lyophilized at 20^0C for 1 hour and then frozen at 70^0C for 2 hours. Vacuum manifolds were closed and the temperature was brought down to

40^0C. A vacuum was applied to 0.01 M Pascal. The frozen samples were attached to the vacuum manifold and the process was continued for 8 hours. The freeze-dried samples were collected at the end of the operation.

Characterization

Measurement of vesicle size

The vesicle dispersions were diluted about 100 times in the same medium, which was used for their preparation. Vesicle size was measured on a particle size analyzer. The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5Mw using a Fourier lens (R-5) to a point at the center of the multi-element detector and a small volume sample holding cell. The samples were stirred with a stirrer before determining the vesicle size.[11]

Drug content and entrapment efficiency

Proniosomes equivalent to 100 mg was taken in a standard volumetric flask. They were lysed with 50 ml methanol by shaking for 15 min. The solution was diluted to 100 ml with methanol. Then 10 ml of this solution was diluted to 100 ml with saline phosphate buffer at certain pH. Aliquots were withdrawn and absorbance was measured at a certain wavelength and drug content was further calculated from the calibration curve.[12]

Separation of the unentrapped drug from the niosomal suspension was carried out by exhaustive dialysis method and centrifugation method. The niosomal suspension was taken into a dialysis tube to which osmotic cellulose membrane was taken into a dialysis tube to which osmotic cellulose membrane was securely attached to one side, the dialysis tube was suspended in 100 ml saline buffer at certain pH, which was stirred on a magnetic stirrer. The niosomal suspension and the unentrapped d drug were separated into the medium through osmotic cellulose membrane. After 6 h of exhaustive dialysis, optical density values were noted and the estimation of the entrapped drug was carried out by UV spectrophotometric method. Entrapment Efficiency was calculated using the formula.

$$\text{Entrapment efficiency} = \frac{\text{Amount of entrapped drug} * 100}{\text{Total amount of drug}}$$

***In vitro* dissolution study:** The drug-release rate study was carried out by comparing formulated proniosomes with the marketed tablet formulation of some dose using USP

dissolution apparatus II (Paddle Method). A weighed amount of proniosomes corresponding to the 20-mg drug was filled into a hard gelatin capsule. 900 ml Dissolution media of 0.1N HCl maintained at 37^0 C and stirred at 100 rpm. 5-ml sample was withdrawn at suitable time intervals for (0.5, 1, 2, 3, 4, 5, 10, 15, 20 and 30 hours) and 5-ml fresh dissolution medium was replaced after each withdrawal. These samples were analyzed for the drug spectrophotometrically at 216 nm. Dissolution experiments were conducted in triplicate. In order to study the kinetics and mechanism of drug release, the result of *in vitro* drug release study, were fitted into various kinetic equations like zero order (cumulative % release versus time), first order (log % drug remaining versus time) and the Higuchi model (cumulative % drug release versus square root of time).

***Ex vivo* permeability studies:** The permeation studies were performed using a goat stomach mucosa mounted on the Franz diffusion cell. Then this mucosa was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. The stomach mucosa was mounted between the donor and receptor compartment. The receptor compartment was filled with 12.0 ml of 0.1N HCl maintained at 37^0 C and was constantly stirred by a magnetic stirrer at 600 rpm. The proniosomes gel formulation was placed on the tissue and the top of the deficiencies was covered with a paraffin paper. Samples (1 ml) were withdrawn through the sampling port of the diffusion cell at pre-determined time intervals (1, 2, 4, 6, 8 and 24 hours) and analyzed for drug content by UVis spectrophotometer at a wavelength of 256 nm, keeping the respective blank. The receptor medium was immediately replenished with an equal volume of fresh diffusion solution. Sink conditions were maintained throughout all the experiment. Triplicate experiments were conducted for each study Proniosome formulation CIM2 was selected for an ex vivo permeation study based on the entrapment efficiency, as it showed the highest entrapment efficiency within the prepared formulations with a good release profile. The release from marketed formulation (F) was also performed by using the above-mentioned procedure.

Stability studies

The stability study was carried out to study the ability of proniosomes to retain the drug by keeping the proniosomal system at room (25 ± 2^0C), oven (32 ±2^0C) and refrigeration temperatures (4^0C-8^0C). In a definite time interval, samples were withdrawn over a period of eight weeks and drug content was analyzed spectrophotometrically and % drug retained was calculated.

RESULTS

Drug content and Encapsulation efficiency

As shown in Table 1, the drug content and encapsulation efficiency were found to be 68.07% with the formation of CIM1. Encapsulation efficiency was found to be 65.08,58.05,56.73,60.0 for the formation of CIM2, CIM3, CIM4, CIM5. It shows that encapsulation efficiency decreases with increase in a ratio of cholesterol in cholesterol. This could be due to the fact that cholesterol beyond a certain level disrupts regular bilayer structure.

In vitro dissolution study

The dissolution profile of proniosomes from capsules is shown in Figure 1. The release study shows that most of the formulation provides about 80% release in 24 hours Kinetic analysis of drug-release profiles. The correlation coefficient value (r 2) was determined by kinetic analysis and was found to be 0.9059, 0.9646, 0.9761, 0.9643, 0.9441 and 0.8789 for CIM1, CIM2, CIM3, CIM4, CIM5, and C, respectively.

Exvivo permeability studies

The drug diffused at a faster rate from the proniosome formulations than from marketed preparation. The total percentage diffusion was much higher for the proniosome formulations than from marketed preparation. The data of the release of cimetidine from proniosomal formulations have shown significantly increased percent release and flux with the comparison to the same dose of marketed preparation of cimetidine. It was found that the value of the correlation coefficient of the Higuchi equation is nearer to 1.0 for all formulations, and hence it can be predicted that the release from proniosome follows the Higuchi kinetics.

Stability study

To determine stability, residual drug content was determined by keeping the initial drug concentration 100% for a sample kept at room, oven and refrigeration temperatures for a period of eight weeks. The result showed that the proniosomal system was stable at room temperature and refrigerated temperature, but was more stable at refrigerated temperature than room temperature (Figure 3).

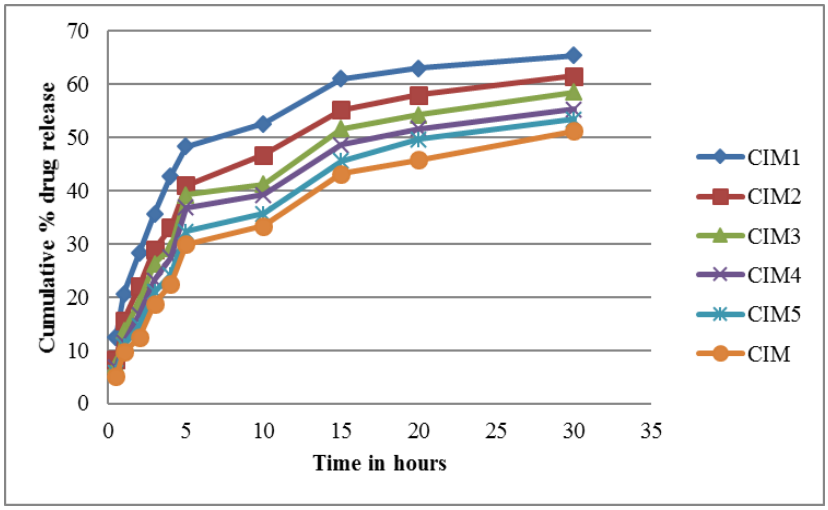

Fig. 1: *In vitro* release of all formulation.

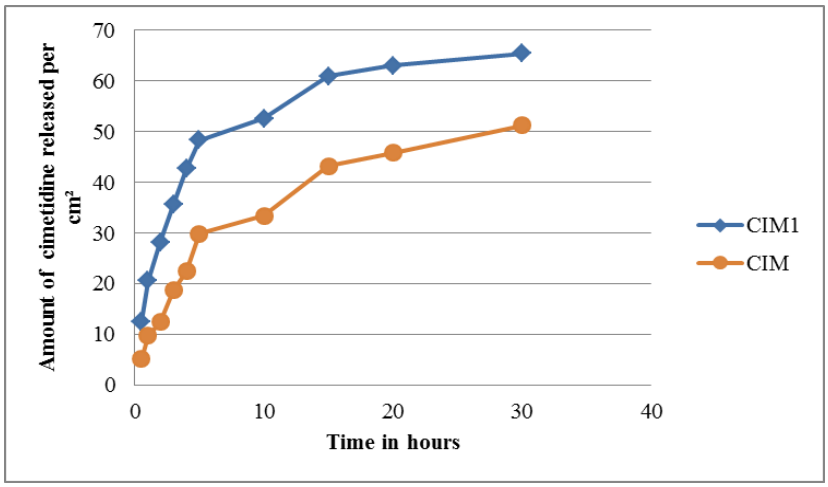

Fig. 2: *Ex vivo* permeability studies.

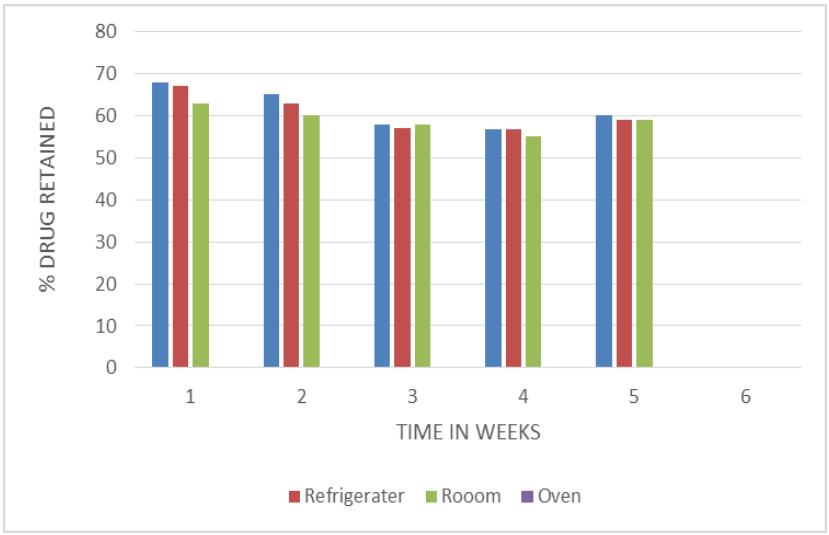

Fig. 3: percentage of cimetidine retained different temperatures.

DISCUSSION

Niosomes formed using Span 40 has higher encapsulation efficiency. It is also noted that cholesterol beyond a certain level disrupts the regular bilayer structure. In in vitro drug-release profile, there was the initial fast release of the drug due to the presence of the un-entrapped drug, but later on drug release was slowed down due to proniosomal derived niosomal entrapped drug. CIM2 was found to sustain the drug release due to high cholesterol content, this may be due to the fact that cholesterol is known to provide rigidity to the membrane, and also abolishes the gel to liquid phase transition of niosomes and thus prevent the leakage of drug from the vesicle.[15] The values of in vitro drug-release profile that fit best into the Higuchi model shows that the batches follow super class II transport mechanism of drug release from proniosomes-derived niosome is by diffusion. The ex vivo data of the release of cimetidine from proniosomal formulations have shown increased percent release and flux when compared to the same dose of marketed preparation of cimetidine. Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the stomach. Several factors including adsorption and fusion of niosomes onto the surface would facilitate drug permeation. All these factors would help to achieve a sustained release of cimetidine into the bloodstream.

CONCLUSION

It can be concluded that the encapsulation efficiency and percentage drug release are functions of surfactant and cholesterol concentration when compared to the marketed formulation of cimetidine proniosomal system prolong the drug release rate up to a considerable period. This is supported by the ex vivo data of the release of cimetidine from the proniosomal formulation. Proniosome vesicles are quite stable at room temperature, but more at refrigerated conditions. Thus, the method of formulation of proniosomes is more simple, stable and scalable than conventional niosomes and can provide prolonged delivery of cimetidine.

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