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Original Article

Pantoprazole oral liquid liposomal formulation: Design and Characterization

Masoud Ali Karami[a,b], Ali Saeidi[b], Eskandar Moghimipour[a*]

[a]*Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran,* [b]*Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*.

Abstract

Pantoprazole (PPZ) is one of the proton pump inhibitors which is used to prevent the secretion of excess gastric acid in both adults and children. However, it is difficult to use in patients under 5 years of age or elderly patients due to the lack of appropriate liquid formulation. In this study, a liquid liposomal formulation of pantoprazole with improved physicochemical stability has been introduced to facilitate the drug administration in above mentioned patients. Lecithin and cholesterol in different ratios were used to prepare liposomes by the thin-layer hydration method. Optimization studies were performed to find the best formulation. Liposomes were examined for particle size, entrapment efficiency, and stability at ambient temperature. The morphology of liposomes was also investigated by scanning electron microscopy and light microscopy. The release rate of the drug from the liposomal formulation in the aqueous phase was monitored for 24 hours. The results showed more than 60% loading of pantoprazole into the liposomes. The particle size of liposomes decreased with increasing levels of lecithin. Analysis of variance showed a significant difference in drug release within 24 hours. The effects of time, temperature, and light on the apparent instability of the drug were restrained by liposomal formulation and the entrapped drug was more stable compared to the pantoprazole solution. The results of the present study demonstrated that the formulation of pantoprazole in liposomes could provide a new perspective for the treatment of gastric reflux.

Keywords: Liposomes, Pantoprazole, Gastric reflux.

1. Introduction

The effect of excess acid in gastric and duodenal ulcers and gastric reflux disease has long been known. Over the past 30 years, various drugs have been developed to control the secretion of acid to treat the above-mentioned diseases [1]. In the past, the most effective treatment for acid-related diseases was surgery. However, in the early twentieth century, the development of acetylcholine and histamine antagonists suppressed antacid uses as well as providing effective treatment without surgery for the first time [2, 3]. There are

Corresponding Author: Dr. Eskandar Moghimipour, Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. E-mails: moghimipour@yahoo.com

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currently several proton pump inhibitors in the clinic, including omeprazole, pantoprazole, lansoprazole, and rabeprazole. To protect the acid-sensitive drugs from rapid degradation in the gastric lumen, they have been formulated with acid-resistant intestinal coating. Pantoprazole (PPZ) is a substituted benzimidazole with a long-acting selective proton pump inhibitor. The partition coefficient of the drug base is about 3.6 which indicates the lipophilic nature of it, however, the sodium salt of the drug is freely soluble in water and practically insoluble in n-hexane. The drug is used to treat gastrointestinal diseases caused by high acid secretion such as duodenal, esophageal, and gastric ulcers [4, 5]. The drug specifically inhibits the K+ATPase H+/pump which is responsible for secreting stomach acid in parietal cells.

The stability of drugs in aqueous environments is very important for the preparation of oral formulations including suspensions. Since the prescription of the existing formulations of PPZ is associated with problems and difficulty for children under 5 years of age or elderly patients, there is a need to introduce liquid oral formulations such as suspension or solution. Furthermore, preparing a formulation of insoluble or low-soluble pharmaceutical compounds in water is a challenge in the pharmaceutical industry [6, 7]. The use of complexes such as cyclodextrins or the addition of safe organic solvents to liquid solvents to increase the low saturation solubility of drugs have been proposed, but in practice, these methods have not been successful especially when the solubility of the drug is low in both aqueous and organic media [8]. Recently, a decrease in the particle size of a drug carrier in nanometer size has shown the potential to increase the dissolution of these drugs [9].

Today, nanoparticles have been a major part of the research. The use of such emerging technology has opened up new approaches for drug delivery and treatment with nanocarriers. Liposomes are nanocarrier vesicles composed of two layers of phospholipids. Phospholipids have both hydrophilic heads and hydrophobic tails surrounding the aqueous phase. The most important feature of liposomes is that they allow the administration of either hydrophilic or hydrophobic drugs [10, 11]. The most important characteristics that have led to the widespread use of liposomes in drug delivery include high biocompatibility, lack of toxicity, structural similarity to cell membranes, ability to increase drug stability, high efficiency to entrap drugs, reducing drug side effects, ability to retard drug release, and finally improve drug distribution and biological half-life [12-14]. This study aimed to prepare PPZ in liposomal formulation to protect the drug from environmental destructive factors and to provide a stable liquid dosage form for patients not able to use solid dosage forms.

2. Materials and Methods

2.1. Materials

Pantoprazole was provided by the Iranian Drug Distribution Company. Lecithin and cholesterol were obtained from Sigma, Germany. Chloroform and ethanol solvents were prepared from Merck, Germany. Diethyl ether and methanol were obtained from

Somchon, Korea. Triton-x100 was purchased from Kala Zist, Iran.

The research has been ethically approved by the University Research Ethics Committee in Ahvaz Jundishapur University of Medical Sciences with Approval ID: IR.AJUMS.REC.1398.861, available at http://ethics.research.ac.ir/IR.AJUMS.REC.1398.861.

2.2. Preparation of Liposomes

The thin-layer hydration method was used to prepare liposomes. At first, cholesterol and lecithin were dissolved in different ratios in a mixture of chloroform and dimethyl ether with a molar ratio of 1:1. The resulting solution was transferred to a round bottom flask. Using a rotary evaporation device equipped with a water bath and adjusted at 45°C, while rotating at a speed of 150 rpm, the organic solvent evaporated completely. The lipid film was then hydrated by adding deionized water containing PPZ sodium using a rotary device. The temperature was adjusted at 45°C, rotation speed of 120 rpm for 30 minutes without applying a vacuum. The liposomal formulation was also homogenized for 5 minutes. The liposomes were then isolated using centrifugation for 30 minutes at 12000 rpm and finally, the sedimented liposomal formulation was freeze-dried for 48 h [15].

2.3. Morphology and Particle Size Determination

The morphology of optimal liposomal formulation was evaluated by Scanning Electron Microscope (SEM, MIRA3TESCAN-XMU, Czech Republic). Liposomal samples were diluted with distilled water, then a drop of the diluted solution was transferred on an aluminum foil. After drying, the samples were inspected. The particle size of liposomes was also determined using a particle size analyzer (Qudix, Scatteroscope I system, Korea) after diluting the samples with distilled water. Each sample was studied three times.

2.4. Determination of Entrapment Efficiency (EE %)

The percentage of encapsulation (EE%) was determined by both direct and indirect methods. In the indirect method, after centrifugation at 12000 rpm for 30 minutes, the supernatant solution was rinsed three times, passed through the 0.22-micron syringe filter, and then the unenclosed PPZ was assayed by UV spectrophotometry. This procedure was repeated three times for each sample. The EE% was calculated according to the equation 1:

(1) $EE\% = (D_f - D_t) \times 100 / D_t$

Where D_t is the amount of PPZ initially added to the formulation and D_f is the amount of the free drug in the supernatant after centrifugation [16]. The EE% was also determined directly. For this purpose, liposomes were separated from the supernatant solution and then were broken down using Triton-x100, diluted with a specified.

2.5. Drug release profile

A static diffusion cell equipped with a cellulose membrane was used to evaluate the drug release. Phosphate buffer solution (PBS, pH 7.4) was used as the release medium. A certain amount of each of the formulations was placed in the donor part. At specific time intervals (30 minutes, 1, 2,

3, 4, 5, 6, 7, 8, and 24 hours), 2 ml of the solution in the receiver part was withdrawn and replaced with an equal volume of distilled water. The amount of drug in each sample was measured using UV spectroscopy. Finally, the cumulative percentage of drug released from the liposomes was plotted against time.

2.6. Stability studies

2.6.1. Aqueous stability of the drug

In this study, after preparation and storage at 25ºC for three months, three PPZ liposomal formulations were placed separately in the donor compartment of a diffusion cell, and the amount of the drug passed through the cellulose membrane was measured for up to 24 hours.

2.6.2. Photostability

Because light, in some cases, causes instability of the samples, the effects of light on the formulations were investigated. To evaluate the photostability of the drug-loaded liposomes, test samples were wrapped in aluminum foil, and then regarding their appearance and drug content, samples were compared with control unwrapped samples after 2 months.

2.6.3. Thermal stability

To evaluate the effect of temperature on the stability of the liposomal formulations, the samples were kept in glass containers at either 25°C or 4°C for two months. At the end of this period, the formulations were evaluated in terms of appearance.

2.7. Statistical Analysis

All the measurements were done in triplicate and data were expressed as mean±SD. One one-way ANOVA test was used for the comparison of the results and the statistical significance was indicated by $p<0.05$.

3. Results and Discussion

3.1. Encapsulation Efficiency

The values given in **Table 1** indicate the Cholesterol/Lecithin (C/L) ratio and average EE%. The results show that the percentage of drug loading increases with increasing lecithin. Also, analysis of variance for the percentage of drug loading showed a significant difference between formulations with different ratios (P-value <0.05).

Table 1: Entrapment Efficiency for liposomal formulations with different ratios of Cholesterol/Lecithin.

C/L ratio	EE% (Indirect method)	EE% (Direct method)
1:4	72.16	73.07
1:3	63.83	66.32
1:2	59.83	60.79

In this study, three liposomal formulations were prepared to investigate the effect of lecithin and cholesterol with different ratios on the loading of PPZ. The results showed an increase in the loading percentage in liposomes as an outcome of an increase in the amount of lecithin. This finding is consistent with the results of previous studies that showed increasing the amount of phospholipids led to an increase in the drug-loading percentage in liposomes [17, 18]. Despite the previous reports regarding the low encapsulation efficiency of the thin hydration method [19], the prepared samples displayed a relatively high encapsulation. In comparison to a maximum EE% of about

30% for 5-fluorouracil using this method [20], our results indicated a much higher amount of 60 to 73 percent entrapment of PPZ, which may be mainly due to differences in drug molecular weights and/or polarities. In addition, it seems that due to the large number of liposomes, a lot of internal space is provided for trapping drugs and increases the drug loaded into liposomes [21, 22].

3.2. Particle size, particle size distribution, and morphology

The average particle size for all formulations was within 117 – 184 nm. Particle size distribution diagrams for different formulations of liposomes with C/L ratios of 1:4, 1:3, and 1:2 are shown in **Figure 1**.

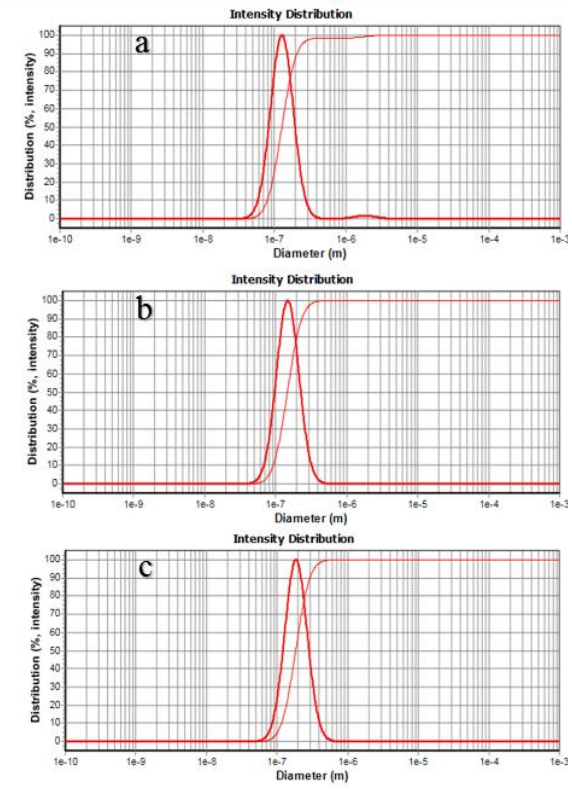

Figure 1. Liposomal particle size distribution with different ratios of C/L 1:2. **a.** C/L=1:4, **b.** C/L=1:3, and **c.** C/L=1:2.

The results of particle size variance analysis showed a p-value less than 0.05 which was significant. According to the results, as the amount of lecithin increased, the particle size of the liposomes decreased.

Previous studies have shown that nanoparticles with the size of 100 to 200 nm penetrate tumor cells through the EPR (enhanced permeability and retention) effects, and are therefore suitable for drug delivery [23]. In the present study, the particle size of all three types of liposomal formulations was in the suitable range, which has the above-mentioned benefits at the same time. It has also been observed that increasing the amount of lecithin reduces the particle size and the difference is statistically significant ($p<0.05$). Decreasing the particle size by increasing the number of phospholipids may be because large amounts of lecithin probably "altered the arrangement of the lipid layers, resulting in more liposomes."However, they are formed with smaller particle sizes. It can be inferred that by increasing the amount of phospholipid lecithin, liposomes may become denser with a smaller particle size.

SEM was also applied to confirm particle size and morphology. Images shown in **Figure 2** are two samples of SEM photographs with different scale bars. As it is clear, the particles have a suitable size distribution and spherical structure. Also, based on the images, the particles are homogeneous and have uniform morphology. The boundary of the particles is separated and there is no adhesion in the particles. A size

less than 200 nanometers can also be re-proven for particles.

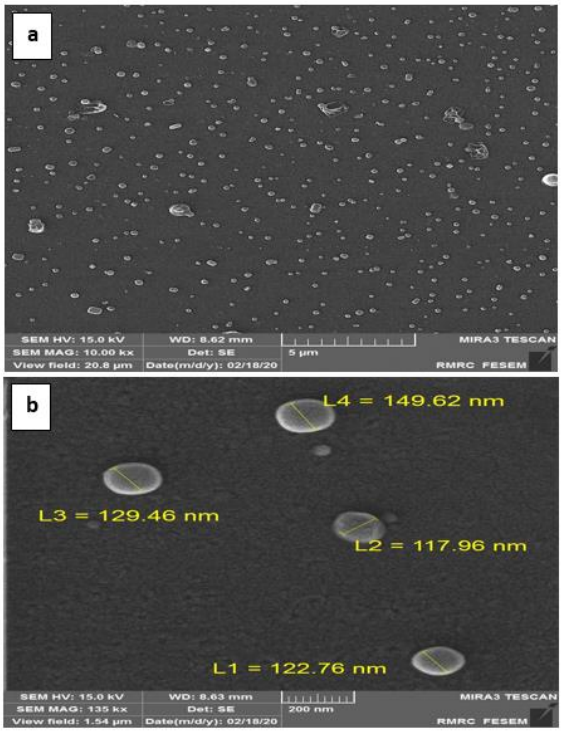

Figure 2. SEM images of pantoprazole of 1:4 liposomal formulation. **a**. SEM MAG: 10 kx, and **b**. SEM MAG: 135 kx.

3.3. Drug release profile

The patterns of the cumulative percentage of drug released from all formulations within 24 hours are shown in **Figure 3**.

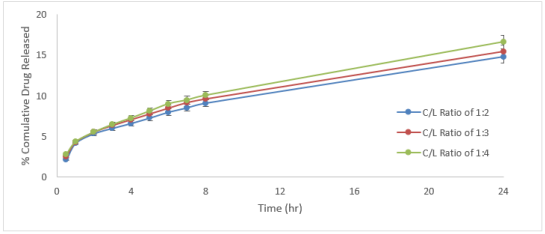

Figure 3. Cumulative percentage of drug released from liposomes.

Results obtained from analysis of variance, mean release rate in liposomes with different ratios of C/L showed a significant difference ($p<0.05$). In all formulations, a burst release within the first 2 hours was seen and then the rug was slowly released. Such a drug release profile can provide rapid and then prolonged pharmacological effects of the drug which is a desired condition in terms of drug delivery.

In this study, three liposomal formulations containing PPZ were placed separately in the donor phase, and the amount of drug passed through the cellulose membrane was measured for 24 hours. The results of the release rate of PPZ during this period indicated that all three formulations were unstable in water. On the other hand, the liposomal formulation with a Cholesterol/Lecithin ratio of 1:4, which had the highest drug load, showed more release than the other two formulations indicating the difference in concentration gradient in the donor and receptor phases. This makes the drug force out of the liposome and tends into the aqueous phase. According to previous studies showing the decomposition of liposomes in the stomach environment, the results obtained in this study indicated the instability of the drug in an aqueous environment [24]. Therefore, it is recommended that these formulations be coated with pH-sensitive polymers to prevent the release of the drug during storage and to prevent the destruction of the gastric medium.

3.4. Stability of liposomes

Measuring the amount of drug trapped in liposomal formulations after two months showed a decrease over time. The impact of

temperature on the stability of liposomal formulations was also studied by keeping the samples of formulations at 4°C or 25°C. The results demonstrated a significant decrease in the amount of drug in room samples and a relative decrease in refrigerated ones, indicating that keeping the formulations at 4°C delays the deterioration.

The results of the release rate of PPZ during this period indicated that all three formulations were unstable in water. On the other hand, the liposomal formulation with a cholesterol ratio of 1:4, which had the highest drug load, showed more release than the other two formulations, which indicates the difference in concentration gradient in donor and receptor phases. The drug tends to be released into the water phase. On the other hand, the stability of liposomal formulations of PPZ in water is one of the important factors in stability during period of storage. Following previous studies related to the instability of liposomes in the stomach environment, the results obtained in this study also indicated the release of the drug from the liposomes in aqueous environment, so it is recommended that these formulations be coated with pH-sensitive polymers to prevent the release of the drug and to prevent the destruction of liposomes in the gastric environment. The results showed that light significantly increased the rate of decomposition process and it is recommended that formulations be kept away from light. Further stability studies showed the instability of the formulations prepared at 25°C and relative stability at 4°C which indicates that the shelf life of the formulations may be prolonged by keeping them at 4°C. On the other hand, the stability of liposomal formulations of PPZ in water is one of the important factors in achieving this goal.

4. Conclusion

The stability of drugs in aqueous environments is very important for the preparation of oral formulations including suspensions. Since the prescription of the existing formulations of PPZ is associated with problems and difficulty for children under 5 years old or elderly patients, there is a need to introduce liquid oral formulations such as suspension or solution. On the other hand, in recent years, the use of nanotechnology in making pharmaceutical formulations of drugs associated with dissolution or absorption problems is increasing. Considering the results of this study, it can be concluded that pantoprazole-contained liposomal formulations can be used for the treatment of gastroesophageal reflux disease, especially in children under 5 years old or elderly patients, in a liquid dosage form.

Conflict of interest

The authors declare to have no conflict of interest.

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