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Application of lactobionic acid and nonionic surfactants as solubilizing agents for parenteral formulation of clarithromycin

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ABSTRACT

Purpose*:** The purpose of this paper is to enhance the solubility of clarithromycin (CLR) using nonionic surfactants and some type of acids for preparation of the new formulations. ***Methods*:** Myrj 52 and chremophor (2.5 and 5% w/v) were used in two concentrations. To investigate solubility, the formulations were shaken for 48 hours at room temperature. For stability test, lyophilized samples were maintained in refrigerator at 4° C, and in oven at 40° C. Drug analysis was performed by reverse phase high performance liquid chromatography (HPLC) with ultraviolet detection**.** ***Results*:** Solubility tests indicated that lactobionic acid was the most effective to increase clarithromycin solubility and chremophor showed higher enhancing effect than myrj 52 on CLR solubility. The stability tests results also confirmed that shelf-lives of all formulations have been the equivalent to 24 months**.** ***Conclusion: On the whole, formulations described in this article may be very suitable for industrial-scale manufacturing and clinical application.

Introduction

Clarithromycin is a relatively new, second-generation, semi synthetic macrolide antibiotic with a methoxy group (OCH3) attached to the C6 position of erythromycin. This derivatization makes clarithromycin more acid stable than erythromycin [1,2]. Clarithromycin has broad-spectrum activity against gram-positive and gram-negative aerobes, atypical bacterial pathogens of the respiratory tract, mycobacterium species, Legionella and helicobacter pylori [3]. Many dosage forms of clarithromycin, such as immediate and extended release tablets, capsules, dry suspensions, liposome-encapsulated, microspheres, cyclodextrin-compressed and IV formulation have been developed due to its wide antibacterial spectrum [2,4-9]. Nevertheless considering adverse effects of oral dosage forms such as gastrointestinal disorders and low bioavailability which doesn't exceed 55%, and the existence of some situations such as unconscious patients for whom the only usable dosage form is parenteral form and also the absence of generic intravenous dosage form in Iran, there is considerable interest in the development of a parenteral formulation of clarithromycin. However, the main problem related with clarithromycin intravenous form is its practical insolubility in water. Clarithromycin has the dimethylamino moiety as the only ionizable group (pK8.8) and hence its aqueous solubility at pH 7.0 is only about 500 μg/ml [2,10]. Using binary systems with a hydrophilic carrier by mixing, melting or solvent methods emulsion formulations for its IV use have been studied. However, leaving residual solvent, thermal degradation of clarithromycin during thermal sterilization and complication of these processes has limited the application of these methods [2,10]. Therefore it is necessary to develop a new formulation to improve its solubility for intravenous administration of clarithromycin [11]. We hypothesized that surfactants and gluconic acids can enhance clarithromycin solubility [10]. Based on this hypothesis myrj52 and chremophor (in different proportions) and lactobionic acid were investigated and results were compared and the best formulations were reported.

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Materials and methods

Materials

Clarithromycin (Elder Nerul, Pawane and Patalg, India), Lactobionic acid (Sigma – Aldrich, Co. St. Louis , MO , USA), Chremophor (BASF Florham Park, New Jersey), Myrj 52 (Atlas San Diego, USA), Anhydride citric acid(Merck Dermstadt, Germany), Sulfuric acid (Merck Dermstadt, Germany), Stearic acid (Merck Dermstadt, Germany), Boric acid (Merck Dermstadt, Germany), Hydrochloric acid (Merck Dermstadt, Germany), Phosphoric acid (Merck Dermstadt, Germany), Salicylic acid (Merck Dermstadt, Germany), NaOH (Merck Dermstadt, Germany), Sodium dihydrogen phosphate (Merck Dermstadt, Germany), Acetonitril (Merck Dermstadt, Germany), Tartaric acid (Seelze- Hannover Riedel _ Dehaen AG , Germany).

Formulation

To increase clarithromycin solubility in water to prepare an injectable solution, above mentioned acids were used. Results indicated that lactobionic acid is the only acid which can improve drug solubility. Nonionic surfactants, myrj 52 and chremophor also were used for solubilization. To produce 5 formulations, different concentrations of two surfactants were selected, added to appropriate amount of double distilled water and were sonicated (Julabo USR 3 Labortechnik, Germany) until complete dissolution. Then 500 mg of clarithromycin was added up and agitation was continued for 15 min. Acid solution was then added and after 5 minutes of stirring, pH was measured (Metrohm, Switzerland). Acid addition was continued until a clear solution was achieved. However to increase stability the pH was kept above 4.8. pH of solutions were adjusted using phosphoric acid 85% and NaOH 1N in the range of 5 to 6.5. Then the solutions were filtered through the 0.22µ membrane filter. To prepare samples for stability tests, 20 vials of each formulation were prepared. The samples were dried and lyophilized for 48 hours in the freezer (- 20°C), and then were maintained in refrigerator at 4°C.

Solubility study

CLA solubility samples were prepared in glass tubes at serial concentrations for all five solvent systems developed. For each system ten glass tubes with different concentration of drug were prepared. Then the samples were shaken for 48 hours at room temperature. The first tube in each series in which the fine un-dissolved drug particles were seen, was detected by visual inspection and finally the range of drug solubility were determined.

Drug analysis

A reverse phase high performance liquid chromatography (HPLC) (Knauer, Germany) analytical method with ultraviolet detection was employed for drug analysis. The mobile phase consisted of acetonitrile- sodium dihydrogen phosphate (2:3 v/v) containing phosphoric acid (3M) and NaOH (1N) to adjust the pH on 4.5. The flow-rate was 2 ml/min and the detection wavelength was set at 205nm. Column (Shimadzu C18:250mm×4.6mm, 4µm) temperature was maintained at 59°C using oven (Memmert, Germany) and the injection volume was 20µL.

Method validation

In this study a reverse phase HPLC method with ultraviolet detection was used. Standard solutions of 3.125 to 50 mg/ml were prepared in water, and linearity range and correlation coefficient were obtained. To evaluate the inter-assay precision and accuracy, samples were analyzed for 3 consecutive days, while intra-assay precision and accuracy were evaluated through analysis of samples at three different concentrations in the same run. Relative standard deviation (RSD) and the accuracy were calculated using the experimental concentration and the nominal concentration for each sample.

Stability tests

To investigate the short-term storage stability of clarithromycin in formulations and determine the shelf life, at least 6 vials of each formulation were kept for 6 month in undesirable conditions including constant relative humidity of 75% and temperature of 40° C which equals maintaining for 2 years in ambient conditions. Other lyophilized formulations were kept in -20° C. For stability study of newly prepared and ready to use injection solution of formulations, a sample of each formulation was tested over 120 h at 4° C.

For determination remained drug in formulations, monthly a vial from samples in oven and freezer was taken and was brought to volume 10ml with double distilled water. Samples were shaken and sonicated for 5 minutes and injected to HPLC column in triplicate. This test was also performed for ready to use solution within 5 days. Monthly a calibration curve was taken.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry is a rapid and exact way to examine drug and excipient compatibility and provides the most information about possible interactions. Samples (5 mg) were sealed in aluminum pans and heated at a rate of 20° C per minute from 30° C to 300° C (DSC-60, Shimadzu, Japan). Thermal behaviors of clarithromycin and exipients, the complex and the physical mixture of them were determined and compared.

Results

To examine the effect of different acids on CLR solubility, various acids such as lactobionic acid, anhydride citric acid, sulfuric acid, stearic acid, boric acid, hydrochloric acid, phosphoric acid, salicylic acid and tartaric acid were used. Lactobionic acid showed

the best result in improving drug solubility in water and forming a soluble dosage form suitable for injection. The results obtained for used surfactants, myrj 52 and chremophor were illustrated in Table 1.

Table1. CLR solubility in the presence of surfactants, chremophor and myrj 52 in two concentration levels.

Surfactant	Formulation code	Surfactant concentration (w/v)	Solubility (mg/ml)
-	1	-	65-70
Myrj 52	2	2.5	70-75
	3	5	75-80
Chremophor	4	2.5	75-80
	5	5	80-85

To determine shelf lives of various formulations that were kept under undesired conditions for 6 months, and also frozen samples, residual drug was determined monthly using HPLC method. Representative chromatograms of serial concentration of clarithromycin were shown in figure 1. The method was linear under the reported conditions between 3.125 and 50 mg/ml with a correlation coefficient of 0.9995. Calibration curve was taken monthly. Relative standard deviation (RSD) and recoveries were calculated from the theoretical and experimental concentrations in order to determine the precision and accuracy of the method. The with-in-day and day-to-day RSD values were determined to be 0 .096 and 0.103 respectively. The accuracy for with-in-day and day-to-day were determined as 107% and 108% respectively. The half-lives of various pharmaceutical formulations from accelerated tests were obtained from division of the respective area under curve in the chromatogram, on the area under the curve of that concentration in standard calibration curve taken on that month. The consequence results indicated that all formulations had shelf lives more than 24 months. Hence, it can be concluded that the formulated IV products had sufficient physicochemical stability for storage at room temperature for 24 months. Drug remained in various formulations kept in oven or freezer, was determined monthly and illustrated in Table 2. In order to check the stability of drug in ready to use form, the reconstituted product was kept for five days in refrigerator (4°C). For content analysis, HPLC method was also applied. The results indicated that for all formulations remained drug after five days were more than 90%. To screen drug and excipient compatibility, DSC was used and polymorph types and melting points of individual substances were determined. The melting points of lactobionic acid and myrj 52 were 61.75° C and51.78° C respectively. The clarithromycin endothermic peak temperature was 228.51° C, which had not been changed significantly in physical mixture of drug and excipients and also in formulations. An example of the thermograms of CLR, lactobionic acid and Formulation (F_2) is given in Figure 2.

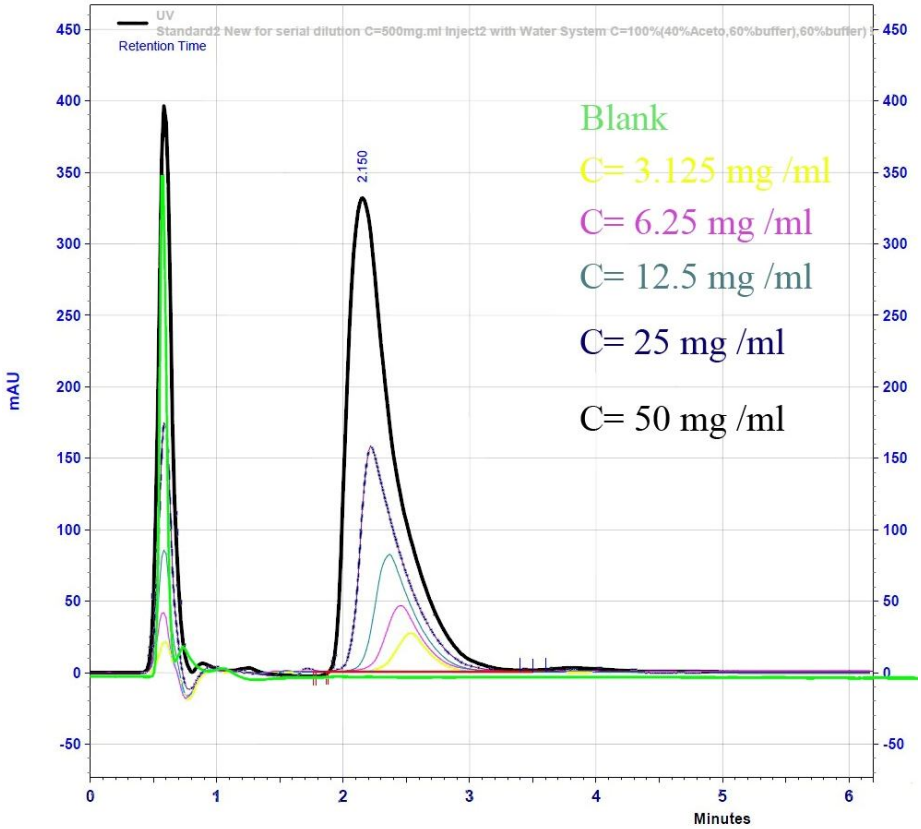

Figure1. HPLC chromatograms of different concentration of clarithromycin

Table 2. The percent drug remained in formulations kept in oven or freezer.

Formulation code	Month 4		Month 5		Month 6	
	Oven	Freezer	Oven	Freezer	Oven	Freezer
1	91.81	96.43	102.9	90.97	98.31	92.69
2	99.17	95.37	97.99	90.69	102.40	100.84
3	104.74	98.13	102.63	97.17	101.76	90.96
4	97.23	105.18	94.92	104.57	90.60	98.11
5	103.13	96.15	97.13	95.37	105.95	102.77

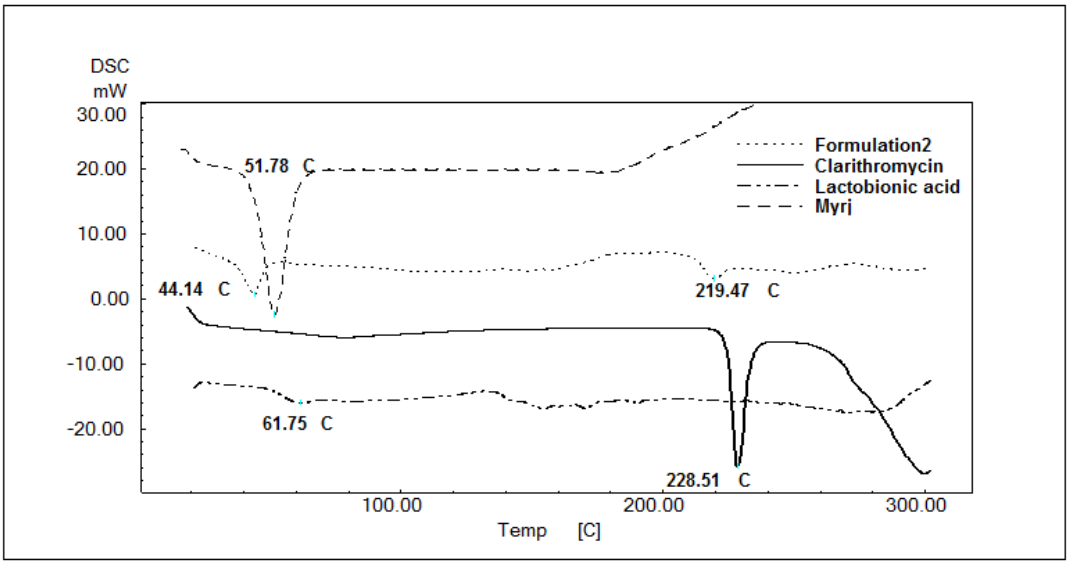

Figure 2. Thermograms of CLR, lactobionic acid and formulation (F_2) kept in the freezer - 20° C for 6 months.

Discussion

As stated before, clarithromycin is a drug that its solubility is very low (0.33 mg/ml) and it is practically insoluble in water[12]. Suitable form for intravenous injection of clarithromycin is a soluble form. Solubility tests for nine different acids with the aim of increasing the water solubility of clarithromycin were performed, and lactobionic acid found the most effective to increase clarithromycin solubility. In a similar study on erythromycin and clarithromycin, lactobionic acid was the best acid to increase their solubility [10], and in another study ascorbic acid 2-glucoside improved clarithromycin solubility [13]. Concerning the structural similarity of erythromycin with clarithromycin, and also the similarity of ascorbic acid 2-glucoside and lactobionic acid this result seems to be reasonable. On the other hand obtained results showed that drug solubility in formulations containing surfactants was higher than those without surfactants (65-70 mg/ml and 70-85 mg/ml respectively). Myrj 52 with concentrations of 2.5% and 5% w/v could increase CLR solubility 5 and 10 mg/ml respectively. Moreover chremophor showed higher enhancing effect than myrj 52 on CLR solubility (5mg/ml more increase in obtained solubility using the same concentrations). It has been suggested that formation of the stronger hydrogen bonds in chremophor than myrj is may be the reason of it's tough emulsification power [10]. Furthermore DSC technique was used to check the compatibility of drug and excipients. As illustrated in Figure 2, there is no significant difference in melting point peak of clarithromycin in formulations, and pure clarithromycin. This indicated that its polymorphism has not been changed by manufacturing process. From the sample analysis point of view, although previous studies have presented several HPLC methods with electrochemical, fluorescent, ultraviolet, and mass spectrometric detectors for CLA analysis [8,9,14-17], in the

present study we have developed a simple reverse-phase HPLC method with acceptable precision and accuracy. The results of this study also illustrated that (table 2) shelf-lives of all formulations have been the equivalent to 24 months. In previous studies CLR shelf life of 48 months was reported [18]. Hence, it can be concluded that the prepared formulations in this study have sufficient physicochemical stability for storage at -20° C for 24 months. There are situations such as kidney or liver failure or drug therapy in children, where administration of less than a vial dose is needed, Therefore the storage of prepared solution and its stability over the storage time is of importance, which was determined to be at least 120 hours in this investigation. The acceptance criterion was remaining at least 90% of initial dose in the formulations. In similar studies, the stability of the drug solution, kept at 2 to 8°C (refrigerator temperature) was reported to be 24 hours [19]. Although ready for injection drug solutions were stable until 5 days, however for reason of sterility consideration, it is recommended to limit the time elapsed between reconstituting and administration to 24 hours.

Conclusion

Based on obtained results using nonionic surfactants such as myrj52 and chremophor and gluconic acid derivatives like lactobionic acid can enhance clarithromycin solubility without any complication in manufacturing in industrial scale. The results obtained proved that clarithromycin had sufficient physicochemical stability in the prepared formulations. Therefore IV dosage form of clarithromycin can be a good candidate to be made in an industrial scale.

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Conflict of interest

There is no conflict of interest in this study.

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