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Research Article

ENHANCED SOLUBILIZATION OF AQUEOUS INSOLUBLE ANTI-HYPERTENSIVE DRUG

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ABSTRACT

Ramipril, an angiotensin converting enzyme inhibitor, has a relatively low bioavailability due to its poor aqueous insolubility. Microemulsion system composed of clove oil, tween 20 and water (5:30:65 v/v) was investigated as a potential drug delivery system to enhance the water solubility and stability of the drug through simple emulsion concept. Electrical conductivity was done to confirm whether the microemulsion system formed is oil-continuous or water-continuous. Extreme stress conditions were performed to delineate the microemulsions. Centrifugation test confirmed the physical stability of the microemulsion. Further, pH and viscosity measurements were carried out in characterizing the optimized formulation. The optically clear and low-viscous formulation with enhanced solubility and stability has a significant promise in increasing the effectiveness of very sparingly soluble drug.

Keywords: Ramipril, Clove oil, Drug delivery, Solubility, Stability

INTRODUCTION

Microemulsions are isotropic, optically clear and thermodynamically stable colloidal system, generally formed by mixing oil and surfactant dispersed in the water phase[1]. Microemulsion technique is the most preferred form of drug delivery system in pharmaceutical technology, due to the ease of manufacturing, long shelf life, improved solubilization capacity and good thermodynamic stability. They are potential in improving the bioavailability of poorly water-soluble drugs by enhancing their solubility, increasing the membrane permeability and protecting the encapsulated drug against enzyme degradation[2, 3].

In our present work, ramipril, an anti-hypertensive drug was chosen as the model drug due to its poor aqueous solubility. Ramipril [(2S, 3aS, 6aS)-1[(S)-N-[(S)-carboxy-3-phenylpropyl] alanyloctahydrocyclopenta[b] pyrrole-2-carboxylic acid, 1-ethyl ester], is found effective in patients suffering from hypertension and congestive heart failure. It works by inhibiting angiotensin-converting enzyme, thereby preventing the conversion of angiotensin I to angiotensin II. By this mechanism, the angiotensin II production is lowered and breakdown of bradykinin is decreased. Thus, arterial muscles are relaxed and enlarged, causing the heart to pump blood easily[4, 5]. Ramipril is a highly lipophilic drug (log p octanol/water, 3.32), with a relatively low bioavailability of 28–30%. Because of its water insolubility, it poses dissolution related problems during drug absorption. This could be solved by enhancing the solubilization capacity of the drug, which, in turn, would increase the dissolution rate[6].

In our study, we attempted to design a stable and aqueous soluble system for ramipril, to improve the oral bioavailability and to delineate dissolution related problems *in-vivo*. The drug was checked for highest solubility in different oil system to develop a suitable oral drug delivery system for ramipril. Clove oil/tween 20/water was investigated as a potential drug delivery system for the model drug. Stress tests were carried out to optimize the best microemulsion formulation under varying extreme conditions. Conductivity tests, pH and viscosity measurements were used to characterize the optimized formulation.

MATERIALS AND METHODS

Materials

Ramipril was procured from Morepen Laboratories Private Limited, India. Tween 20, castor oil, almond oil, and clove oil were obtained from Hi Media, India. For all experiments, double distilled water was used. All other reagents used were of analytical reagent grade.

Solubility study

Excess amount of drug was dissolved in 2 ml of various oils (such as castor oil, almond oil, and clove oil). The mixture was then vortexed and kept in an orbital shaker for 72 h and maintained at a temperature of 25 ± 1.0 °C to reach equilibrium. The equilibrated samples were then centrifuged at 3000 rpm for 15 min, in order to measure the concentration of drug in the supernatant. The double beam UV-visible spectrophotometer (UV-Vis Systronic-2201) was used in measuring the concentration at 210 nm.

Preparation of microemulsions

Microemulsions were prepared by gently mixing oil and surfactant together before adding the required volume of water. All the preparations were done at room temperature until a clear dispersion was formed. To check the drug delivery potential, drug was first dissolved in the oil, followed by addition of surfactant and water.

Characterization of the selected microemulsion formulations

Centrifugation

The selected microemulsion formulations were centrifuged at 10,000 rpm for 30 min to ensure stability.

Conductivity measurements

The conductivity measurements (Conductivity meter, Elco CM 180) help in determining whether the microemulsion system formed is oil-continuous or water-continuous. The solubilization of water phase in the selected oily mixture was monitored quantitatively by measuring the electrical conductivity (σ). The conductivity (σ) of the formulated samples was measured using a conductivity meter.

Stress tests

These tests were done to optimize the best microemulsion formulation under extreme conditions. Stress were carried out at 4 °C and 45 °C for 48 h each for a period of six cycles, followed by 25 °C and 21 °C for 48 h for about three cycles. The samples were checked for coalescence, cracking or phase separation.

pH measurements

The pH values of the optimized formulation were measured by immersing the electrode directly into the dispersion using a calibrated pH meter (model HI 8417, Hanna Instruments Inc., Woonsocket, USA), at 25 ± 1 °C.

Viscosity determination

The viscosity of the optimized formulation was determined as such without dilution using Brookfield Viscometer (LVF model)-UL-Adapter with spindle set, Spindle # 2 at 25 ± 1 °C.

RESULTS AND DISCUSSION

Solubility study

Oil system plays a major role in maintaining the drug stable in its lipophilic environment[7]. Thus, different oil system was chosen for

our work and their solubility shown in Table 1. Ramipril showed the highest solubility in clove oil (71.1 ± 1.33 mg/ml) as compared to other oils. Thus, clove oil was selected as the oil phase for the development of oral microemulsion formulation.

Table 1: Solubility of Ramipril in various oils

Oils	Solubility (mg/ml)
Castor oil	0.027 ± 0.03
Almond oil	0.008 ± 0.01
Clove oil	71.1 ± 1.33

Mean ± S.D., n=3.

Table 2: Various formulations showing the Microemulsion Region

Formulations	Oil (v/v)	Surfactant (v/v)	Water (v/v)	Appearance
F1	5	5	90	Milky white
F2	5	10	85	Milky white
F3	5	15	80	Milky white
F4	5	20	75	Transparent
F5	5	25	70	Transparent
F6	5	30	65	Transparent
F7	5	35	60	Transparent
F8	5	40	55	Transparent
F9	5	45	50	Transparent

Preparation of microemulsions

Nine different compositions were prepared by gently mixing drug incorporated oil, surfactant and water as shown in Table 2. About 5 mg of drug equivalent to the prescribed dose was added for each formulation. At low surfactant levels, milky white formulations were seen. At higher surfactant levels, clear and transparent formulations were formed as seen from the table.

Characterization of microemulsions

Centrifugation

Formulations F1 to F3 clearly separated into two phases after centrifugation, which were milky white in appearance. Those formulations (F4 to F9) which were transparent were found stable even after centrifugation and hence were taken for further study.

Conductivity measurements

Conductivity measurement is the most widely used method to understand the structural changes in the microemulsion system based on percolation theory[8]. The electrical conductivity determines whether phase inversion has occurred in the microemulsion system i.e., from oil-continuous to water-continuous or vice versa[9]. The surfactant used in our system is a non-ionic amphiphile that exhibits electroconductive behavior. The electrical conductivity of water is very high compared to that of oil; hence, there was a gradual decrease in the conductivity from F4 to F9 formulation. As the water concentration decreased from F4 to F9, the electrical conductivity also gradually decreased. The electrical conductivity is expressed in terms of μS/cm and the water concentration as percentage. The electrical conductivity (σ) of oil and surfactant mixture, as a function of concentration of water is shown in Fig. 1.

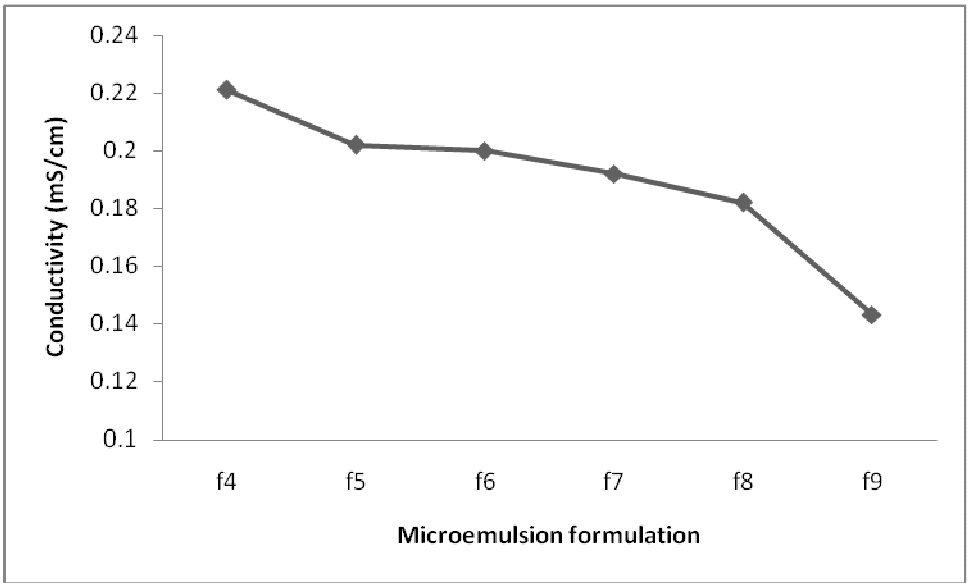

Fig. 1: Electrical conductivity measurements showing decrease in conductivity from F4 to F9 as a function of water phase volume fraction

Stress tests

Of the six formulations (F4 to F9), only two formulations (F6 and F8) passed through different stress conditions as shown in Table 3. This indicates that these formulations have good physical stability with no phase separation, creaming or flocculation. But F8 formulation has very high surfactant and hence rejected. Thus F6 formulation serves as the best formulation and is taken for further characterization. The results of stress tests conclude that the formulation is both physically and chemically stable[10, 11].

Table 3: Observations of stress tests

Formulations	Observations of stress tests		
	Centrifugation	(4 °C and 45 °C)	(−21 °C and 25 °C)
F4	✓	×	–
F5	✓	✓	×
F6	✓	✓	✓
F7	✓	✓	×
F8	✓	✓	✓
F9	✓	✓	×

pH measurement

The pH of the microemulsion formulations F6 and F8 was found to be 4.5 and 4.8 respectively. With increase in surfactant concentration from F6 to F8, the pH value also increased.

Viscosity

The viscosity of microemulsion formulations F6 and F8 were 50 cPs and 240 cPs respectively as determined by viscometer. The viscosity of the microemulsion increased with increasing concentration of the non-ionic surfactant, Tween 20. The increase in viscosity may be explained by trapping of water molecules into the cross-linking portions of surfactant as explained in previous literatures[12].

CONCLUSION

Ramipril microemulsion drug delivery system was prepared by simple hand mixing technique. The preparatory method of microemulsion did not use any external high-energy methods. The ease of manufacturing using simple oil system and bio-based non-toxic surfactant mixture emphasizes a special point. The clear, transparent and low-viscous formulation exhibited all the desirable properties of an ideal microemulsion with good stability and enhanced solubilization capacity. The optimized microemulsion would greatly improve the dissolution *in-vivo* also. However, further studies should be carried out using animal models followed by extensive clinical oriented research.

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