Research Article

DISSOLUTION CHARACTERISTIC OF CHLORAMPHENICOL PALMITATE-LIPOSOMAL PREPARATION *

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Abstract

Solid dispersions of chloramphenicol palmitate and dipalmitoyl-phosphatidylcholine (lecithin) have been produced both as coprecipitate and physical mixtures. The dissolution behavior of both forms were compared with pure chloramphenicol palmitate at different weight ratios of chloramphenicol palmitate-lecithin (liposomal system); as well as various pH. The dissolution characteristic of physical mixtures for different weight ratios of chloramphenicol palmitate-lecithin was similar to the pure drug. Whereas, the coprecipitates produced a 2.8 fold greater initial dissolution rate (IDR) and a 2.4 fold greater drug release concentration after 60 min at a chloramphenicol palmitate-lecithin weight ratio of 19:1. However, lecithin content enhancement to 9:1, 4:1 and 1.5:1 compositions, resulted in a further increase of 6%, 21%, and 24%, respectively in the initial dissolution rate. Increasing the lecithin content shows only a slight increase (8.5%) on drug release after 60 min when, the chloramphenicol palmitate lecithin weight ratio was 1.5:1. However, other weight ratios did not show any effect on the improvement of drug release after 60 min. The effect of pH of the medium on dissolution was slight, but varied with composition of the system.

In conclusion, liposome encapsulation of chloramphenicol palmitate has a significant effect on dissolution improvement of this drug.

Introduction

Liposomes (phospholipid vesicles) are lyotropic liquid crystals predominantly of amphiphilic bilayers which are formed when naturally occuring phospholipids such as lecithin are equilibrated with excess water or aqueous salt solution (1-2). Dimension of liposomes vary from 25 nm to 4 um (3). Since liposomes can encapsulate drugs, proteins and enzymes, the system can be administered intravenously, orally, intramusculary and topically in order to decrease toxicity, to increase specificity of uptake of drugs and in some cases, to control release (2). Liposomal preparations, when used by various routes showed to enhance the bioavailability of the entrapped drug (4). Liposomes might be included among the ranks of classic pharmaceutical vehicles designed to simply dissolve or solubilize drugs (1). The superiority of liposomal encapsulation of ophthalmic drugs as a drug delivery system in ocular therapy was compared with suspension dosage form by Singh & Mezei (5). The use of liposomal system to increase the dissolution of low water soluble drugs are well recognized (6). The dissolution improvement of griseofulvin from liposomal form has been reported (6). As chloramphenicol palmitate is practically insoluble in water (7). The purpose of this paper is to evaluate the dissolution behavior of chloramphenicol palmitate liposomal system.

Experimental Section

Materials-Pure chloramphenicol palmitate (obtained from Lepetite Co., Italy), pretreated by dissolving in chloroform, then removing the solvent by evaporating over a water bath. Drying of crystals was performed under vacuum over desiccants. The dried crystals passed through an 80-mesh sieve prior to test. Dipalmitoylphosphatidylcholine (purchased from Sigma Co., Germany), claimed to be 98% pure, was used as received. All solvents and chemicals were analytical reagent grade (Merck Co., Germany).

Formulation of Liposomes and Physical Mixtures

The techniques used in this study to prepare solid dispersions of chloramphenicol palmitate-lecithin were previously described in detail (5). Briefly, the lecithin and chloramphenicol palmitate were dissolved in organic solvents (chloroform + methanol) in a conical flask. The solvents were evaporated under vacuum on a rotary evaporator until a thin, smooth and dry film of lipids was deposited on the walls of the flask. The flask was immersed in a water bath at 60°C for 15 min. Methanol solution was added to the flask which was mechanically

shaken until the film was dissolved. To separate the liposomes from the unencapsulated chloramphenicol palmitate crystals, the dispersion was filtered through a membrane filter having a pore size of 0.22 micrometer. Liposomal fraction was diluted with methanol and centrifuged at 5000 rpm for 25 min. The supernatant layer was removed and detected for unencapsulated drugs. Physical mixtures were prepared by triturating appropriate quantities of drug and lipid using a mortar and pestle, then transferring to a vacuum desicator until ready for use.

Dissolution Studies-The USP dissolution apparatus II (paddle system, Erweka model DT6), was used. Rotation speed was maintained at 250 rpm in 900 ml of dissolution medium, which was either HCl-KCl buffer at pH 2.0 with polyethylene glycol (PEG) 400, or phosphate buffer at pH 5.0 with PEG 400. Temperature of all dissolution studies were kept at 37°C. Drug content was determined by ultraviolet absorption at 271 nm. The presence of lecithin in the dissolution medium did not interfere with the analysis of the drug. All tests were performed in duplicate and the results averaged, although at no time was a significant difference found between duplicates.

Stability Studies-Aliquots of chloramphenicol palmitate coprecipitates containing 40% lecithin were suspended in PEG 400 and stored at -20°C for 48 h. Frozen samples were kept at an ambient temperature (23-25°C) for 24 h. After thawing, the liposomes were washed in the sample liquid as that in which they were frozen. Replicate determinations of percentage chloramphenicol palmitate lost following a freeze/thaw cycle were made on three batches of liposomes.

Scanning Electron Microscopy (SEM)

Photomicrographs of chloramphenicol palmitate coprecipitate crystals were taken with a scanning electron microscope.

Results and Discussion

Dissolution Studies-Figure 1 depicts the dissolution profile of coprecipitates and physical mixtures of chloramphenicol palmitate-dipalmitoylphosphatidylcholine at pH 2.0 and 37°C. Apparently, the initial dissolution rate (IDR)(computed over the first 5 min of dissolution) and the amount of chloramphenicol palmitate dissolved after 60 min from coprecipitates exceeded those of pure chloramphenicol palmitate or the corresponding physical mixtures. Table I shows a comparison of chloramphenicol release from coprecipitates, physical mixtures (at different weight ratios of chloramphenicol palmitate to lecithin) and pure substances, at different times, pH 2.0 and 37°C. The results show that

Table I. A comparison of chloramphenicol palmitate release from coprecipitates, physical mixtures and pure substances, versus time at pH 2.0 and 37°C and different weight ratios of chloramphenicol palmitate to dipalmitoylphosphatidylcholin.

Time (min)	Conc. (mg/L) Coprecipitates				Conc. (mg/L) Pure	Conc. (mg/L) Physical mixtures			
	19:1	9:1	4:1	1.5:1	1:0	19:1	9:1	4:1	1.5:1
5	5.87 (0.08)	6.22 (0.03)	7.12 (0.03)	7.25 (0.05)	2.10 (0.10)	2.08 (0.08)	2.08 (0.08)	2.13 (0.03)	2.13 (0.03)
10	9.78 (0.03)	9.85 (0.00)	10.12 (0.03)	12.62 (0.10)	3.25 (0.05)	3.13 (0.12)	3.13 (0.03)	3.23 (0.03)	3.20 (0.00)
20	15.08 (0.08)	15.13 (0.06)	16.28 (0.08)	17.50 (0.08)	6.68 (0.03)	6.58 (0.10)	6.55 (0.05)	6.72 (0.04)	6.68 (0.03)
30	17.57 (0.06)	18.27 (0.03)	21.50 (0.00)	22.10 (0.09)	7.68 (0.03)	7.62 (0.03)	7.63 (0.03)	7.67 (0.07)	7.65 (0.03)

release of chloramphenicol palmitate from 1.5:1 coprecipitate in the first 30 min was greater than the 19:1, 9:1 and 4:1 composition. The IDR and concentrations of chloramphenicol palmitate released after 60 min at pH 2.0 and 37°C from physical mixtures and coprecipitates are compared in Table II. The IDR of chloramphenicol palmitate coprecipitate exhibits an increase of 3.5 fold for a sample containing 40% (1.5:1 weight ratio) of lecithin, and an increase of 2.8 fold for coprecipitate containing 5% (19:1 weight ratio) of lecithin. In contrast, the IDR of chloramphenicol palmitate from physical mixtures did not change in proportion to higher concentrations of lecithin. The amount of drug released after 60 min, based on the results of Table II indicates, an increase of 2.6 fold for coprecipitate containing 40% (1.5:1 weight ratio) of lecithin and 2.4 fold for sample containing 5% lecithin (19:1 weight ratio). Whereas, an increased fraction of lecithin in the physical mixture

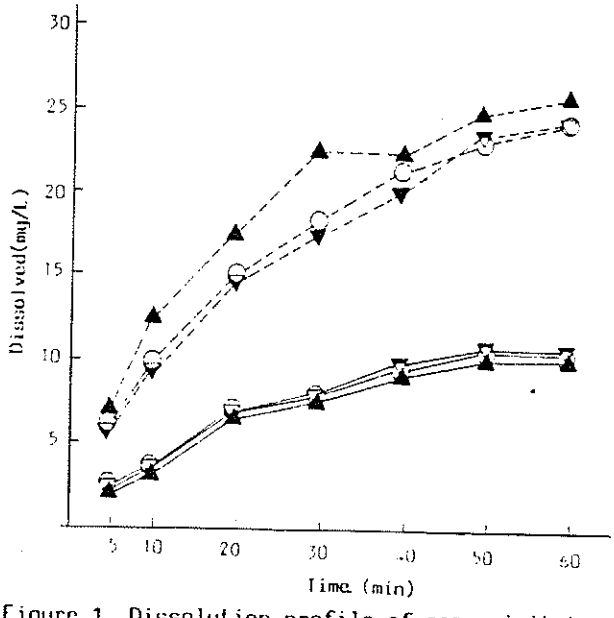

Figure 1. Dissolution profile of coprecipitates (---); and physical mixtures (——) of chloramphenicol palmitate-dipalmitoylphosphatidylcholin at pH 2.0 and 37°C. Symbols: ○ 19:1; ▼ 9:1; ▲ 1.5:1 composition of drug to lecithin.

Table II. Dissolution of chloramphenicol palmitate-dipalmitoylphosphatidylcholin composition at pH 2.0 and 37°C .

Composition (Weight ratio)	Initial Dissolution Rate (mg/L/min)		Amount released after 60 min (mg/L)	
	Coprecipitate	Physical mixture	Coprecipitate	Physical mixture
1:0	0.42	0.42	10.20	10.20
19:1	1.17	0.43	24.10	10.23
9:1	1.24	0.44	24.18	10.20
4:1	1.42	0.41	24.08	10.17
1.5:1	1.45	0.42	26.13	10.17

compositions did not effect the dissolution behavior of chloramphenicol palmitate Dissolution of chloramphenicol palmitate from coprecipitates, physical mixtures and pure drug at pH 2.0 and 37°C is exhibited in Table III. The amount of chloramphenicol palmitate dissolved from coprecipitates is more than twice that released from physical mixtures at corresponding times. Figure 2 shows the dissolution profile of pure and coprecipitates of chloramphenicol palmitate-dipalmitoylphosphatidylcholine at pH 5.0 and 37°C. It is clear, that the IDR and amount of chloramphenicol palmitate released after 60 min from coprecipitates surpassed those of pure chloramphenicol palmitate. The IDR and the amount of drug dissolved after 60 min at pH 2.0 and pH 5.0 are compared in Table IV. The IDR of chloramphenicol palmitate coprecipitates (as seen in Table IV), are slightly decreased at pH 5.0. However, the effect of pH on dissolution behavior of this drug is negligible after 60 min. Figure 3 shows scanning electron micrographs (SEMs) of chloramphenicol palmitate coprecipitate crystal containing 40% phospholipid (1.5:1 weight ratio). The enhanced dissolu-

Table III. Comparison of percent dissolved from chloramphenicol palmitate-dipalmitoylphosphatidylcholin coprecipitates, physical mixtures and pure chloramphenicol palmitate at pH 2.0 and 37°C.

Composition (Weight ratio)	Coprecipitates				Physical mixtures			
	5 min	20 min	40 min	60 min	5 min	20 min	40 min	60 min
1:0	4.2	13.4	18.3	20.4	4.2	13.4	18.3	20.4
19:1	12.4	31.8	41.9	50.7	4.5	14.1	19.3	21.5
9:1	13.8	33.6	46.9	53.7	4.8	14.9	20.3	22.7
4:1	17.8	40.7	59.7	62.7	6.8	16.4	22.9	25.6
1.5:1	24.2	58.3	74.8	87.1	6.1	21.9	30.3	33.9

Table IV. Effect of pH on the dissolution of coprecipitates of chloramphenicol palmitate and dipalmitoylphosphatidylcholin.

Composition (Weight ratio)	Initial Dissolution Rate (mg/L/min)		Amount released after 60 min(mg/L)	
	pH 2.0	pH 5.0	pH 2.0	pH 5.0
1:0	0.42	0.43	10.20	10.20
19:1	1.17	1.15	24.10	24.00
9:1	1.24	1.22	24.18	24.15
4:1	1.42	1.41	25.08	25.07
1.5:1	1.45	1.44	26.13	26.13

tion of low water soluble drug from a solid dispersion system containing lipid with significant surface activity (e.g. cholesterol stearate) has been reported by Kim & Jarowski. The ability of phospholipids to increase the wetting of hydrophobic drug particles were reported by Venkataram & Rogers (6). Simonelli et al. demonstrated that the rapid release from solid dispersion systems with the larger ratios of carrier to drug was increased (9). The present work demonstrated that high rates of dissolution of drug are observed from coprecipitates even with sample containing a low (5%) amount of lecithin. In addition, as the lipid content of coprecipitate was increased, (8-fold increase in amount of lecithin in the sample) the IDR of chloramphenicol palmitate increased by a further 24%, whereas the concentration of chloramphenicol palmitate released after 60 min at pH 2.0 increased by a further 8.5% (Table II). The improvement of chloramphenicol palmitate release from coprecipitate could be due to the formation of crystals of lower stability (6). It can also be related to the wetting and dispersing effect of phospholipid carrier in the formulation. These results are consistent with the reports of other investigators (6).

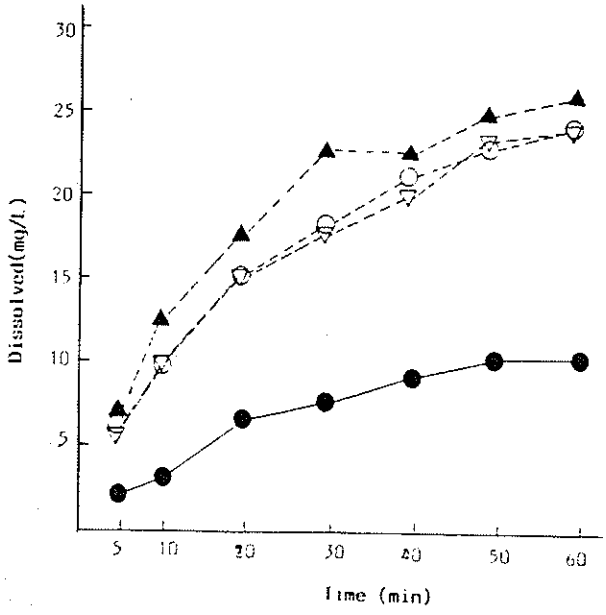

Figure 2. Dissolution profile of pure and coprecipitates of chloramphenicol palmitate-dipalmitoylphosphatidylcholin at pH 5.0 and 37°C. Symbols: ● pure; ○ 19:1; ▽ 9:1; ▲ 1.5:1 compositions of drug to lecithin.

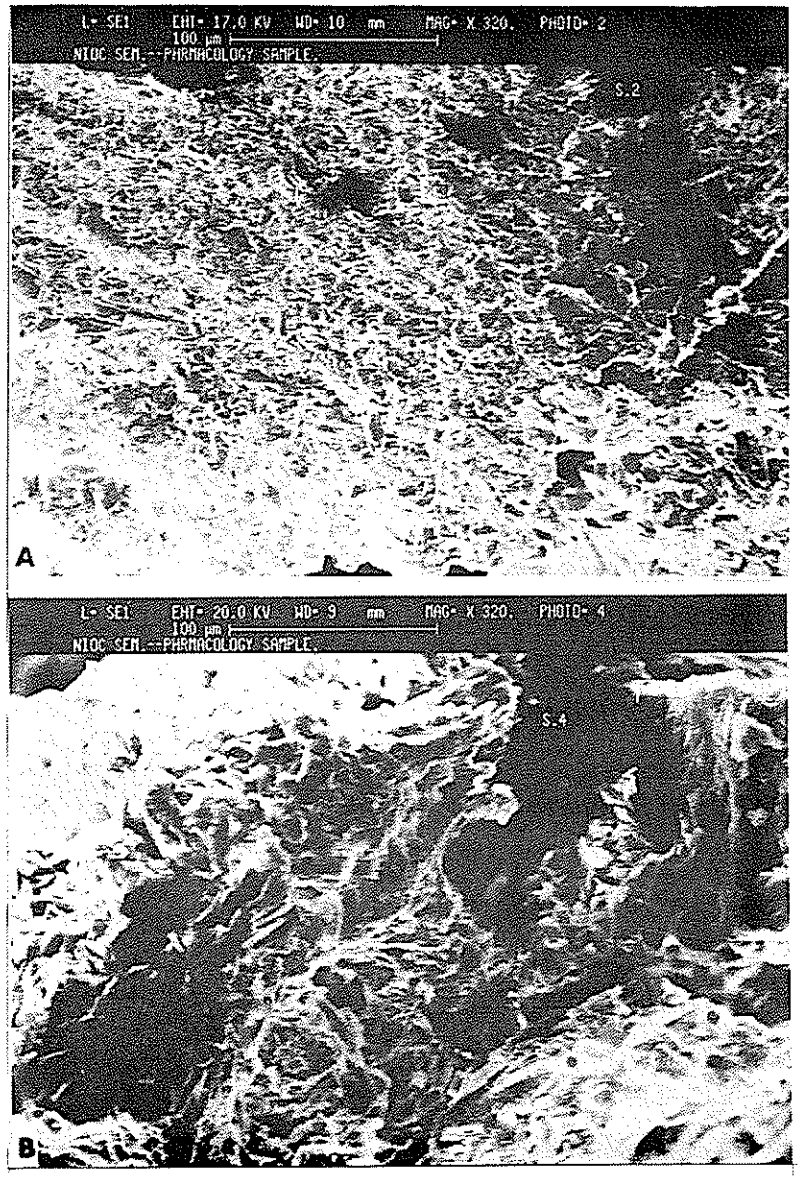

Figure 3. Photomicrographs of 1.5:1 chloramphenicol palmitate coprecipitate crystals dispersed in water at various time periods after preparation (320X magnification)
Key: (A) 1 min; (B) 3 min.

Stability Studies-A decrease of 0.7% of chloramphenicol palmitate from the coprecipitate containing 40% lecithin was observed, as the coprecipitate was stored under freeze/thaw conditions. The small leakage of chloramphenicol palmitate from liposomal form is due to a damage of membrane during the freeze/thaw cycle. It is widely recognized that freeze/thaw damage may result from two main sources; ice crystal formation and osmotic effects (10).

In conclusion, liposome encapsul-

ation of chloramphenicol palmitate has a significant effect on the dissolution improvement of this drug.

References

1. Ostro, M.J.*Liposomes;From Biophysics to Therapeutics;*Marcel Dekker : New York, *1987*,pp 2,339.
2. Florence, A.T.*Physicochemical principles of Pharmacy;*2nd edition; Macmillan Press:London, *1988;*pp 211-212.
3. Roseman, T.J.*Controlled release Delivery System;*Marcel Dekker : New York, *1983*,pp 27.
4. Singh, K.;Mezei, M.*Int.J.Pharm. 1984*,19, 263-269.
5. Singh, K.;Mezei, M.*Int.J.Pharm. 1983*,15, 339-344.
6. Venkataram, S.;Rogers, J.A.*J.Pharm. Sci. 1984*,73, 757-761.
7. *British Pharmacopoeia,BP88*,British Pharmacopoeia Commission,London. *1988;*Volume 1;pp 118-119.
8. Kim, K.H.;Jarowski, C.I.*J.Pharm. Sci. 1977*,66, 1536.
9. Simonelli, A.P.;Metha, S.C.;Higuchi, W.I.*J.Pharm.Sci, 1969*,58, 538.
10. Higgins, J.;Hodges, N.A.;Oliff, C.J.;Phillips, A.J.*J.Pharm.Pharmacol. 1986*,38, 259-263.

Acknowledgements

The authors wish to express their appreciation to Dr. Anvari of the National Iranian Oil Company (NIOC) Research Center, for his technical assistance in the preparation of SEMs and Dr. Kazem Mohammad from the School of Health, Tehran Medical Sciences University and Dr. T. Toliat from the School of Pharmacy, for their valuable help in the preparation of this manuscript.