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DRUG RELEASE RATE METHOD FOR A LIPOSOME PREPARATION

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ABSTRACT

An in vitro method was developed to determine the rate of drug release from a liposome formulation. Liposome formulation containing metaproterenol sulfate was evaluated for release of the drug over a 24-hour period in an end-over-end tumbler device. Drug release appeared to be a function of all the operating parameters, such as rotational speed of the end-over-end tumbler, temperature of suspension, and the geometry of sample container that is placed in the end-over-end tumbler device.

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INTRODUCTION

Liposomes are microscopic vesicles consisting of membrane-like lipid bi-layers surrounding aqueous media. The aqueous media entrapped in lipid vesicles may contain many types of drugs. Variations in lipids composition can yield liposomes of varied physico-chemical characters, such as drug trapping ability, surface charges, and permeability characteristics.

Several articles have bene published about liposomes as drug carriers and controlled drug delivery systems (1-4). However, at the present time, very little attention was given to the development of an in vitro drug release method for liposome formulations. Several liposome drug delivery systems are in clinical studies and may prove useful as drug carries of prolonged activity.

In order for a liposome formulation to enter market as a long-acting drug delivery system, the manufacturer must quality control the drug delivery system routinely. Thus, a simple and reproducible in vitro drug release procedure for liposome formulations is necessary.

In this study, the authors investigated the effectiveness of agitation, temperature, and container configuration on drug release rate of a liposome formulation in an end-over-end tumbler device. Metaproterenol sulfate served as a model drug in this study.

MATERIALS

Egg phosphatidylcholine, 95% (Asahi Chemical Industry Company, Ltd., Japan), egg phosphatidylglycerol, 95% (Avanti Polar Lipids, Inc.,

Birmingham, AL), cholesterol (Croda, Inc., Mill Hall, PA), metaproterenol sulfate, USP (Vinchem, Inc., Chatham, NJ) were purchased and tested for purity before use. dl-Alpha-tocopherol, disodium phosphate dibasic, heptahydrate, and edetate disodium were obtained from Sigma Chemicals (St. Louis, MO). Sodium phosphate monobasic monohydrate, USP, was supplied by Mallinckroft Chemical Company.

METHODS

Preparation of Liposomes

Egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG), cholesterol, and dl-alpha-tocopherol were dissolved in chloroform. Subsequently, the solvent was removed by rotary evaporation. The resultant dry phospholipid mixture was slowly hydrated with an aqueous solution of metaproterenol sulfate (125 mg/ml, pH 7.0). The liposome suspension was sized to 0.2 micron mean diameter by a membrane filtration technique (5) and then concentrated to a paste consistency by ultrafiltration using a 100 K polysulfone membrane (Sartocon II, Sartorius, Hayward, CA). The resultant liposome paste is a mixture of encapsulated and unencapsulated forms of drug. This is called "liposome concentrate".

Release Rate Assay

The liposome concentrate diluted 1:10 with saline and aliquots were dispensed into vials and placed into the chambers of an end-over-end tumbler device (Van-Kel Industries, Edison, NJ). These samples were then rotated at a pre-selected rpm for various lengths of time. At various time

intervals (0 - 24 hours), triplicate vials were collected. Each suspension was centrifuged at 100,000 rpm for 30 minutes to pellet the liposome in an ultracentrifuge (Model TL100, Beckman Instruments, Inc., Palo Alto, CA). The supernatant was collected and assayed for drug concentration. The pellet was treated with 1% Triton X-100 to render a clear solution and then assayed for drug concentration. Triplicate samples were analyzed in an IBM Model 9533 HPLC system coupled to an IBM Model 9000 computer. A Whatman C-18 ODS analytical column, 10 micron particle size, was used. The flow rate was 1 ml/min. The mobile phase consisted of 70% phosphate buffer (Ph = 7.0) and 30% methanol. The UV detector's wave length was set at 278 nm to detect metaproterenol sulfate. Drug concentration was calculated from a standard curve. The drug concentration in a given sample was determined by comparing the values against the standard curve.

RESULTS AND DISCUSSION

Several parameters of release rate were examined in an attempt to optimize an in vitro drug release rate method. Effects of container geometry, rotation speed (rpm) of end-over-end tumbler device, temperature of sample, volume of sample, and concentration of liposome in the experiment on drug release were investigated.

Container configuration B and bromo-butyl stoppers were used in all the experiments unless indicated otherwise. Every point in figures is an average of three determinations carried out on three different lots.

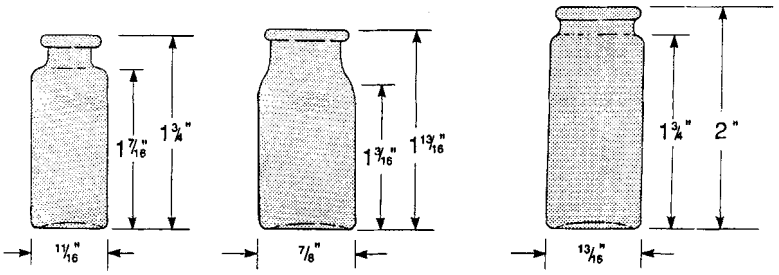

Figure 1. Geometric Configuration of Containers

The effect of container geometry on drug release rate was investigated on a known quantity of liposome suspension. Figure 1 shows the container configurations used in the study. The end-over-end tumbler device was operated at 80 rpm and 25°C. Results in Figure 2 indicate that not only does the rate of release increase with container geometries A and B, but also the total amount of drug released over 24 hours was complete. Drug release profiles obtained from containers A and B are practically identical. It is clearly seen that container C gives much slower release rates than containers A and B.

Three different rotational speeds (rpm) on drug release rate over 24 hours was studied. Figure 3 illustrates the kinetics of drug release at 25, 50, and 80 rpm. The results indicate that not only does the rate of release increase with increasing rotational speed, but also the total amount of drug released over 24 hours increases with increasing rotational speed.

The effect of temperature on release rate was studied by maintaining samples at either room temperature (22 - 25°C) or at 37°C. This study

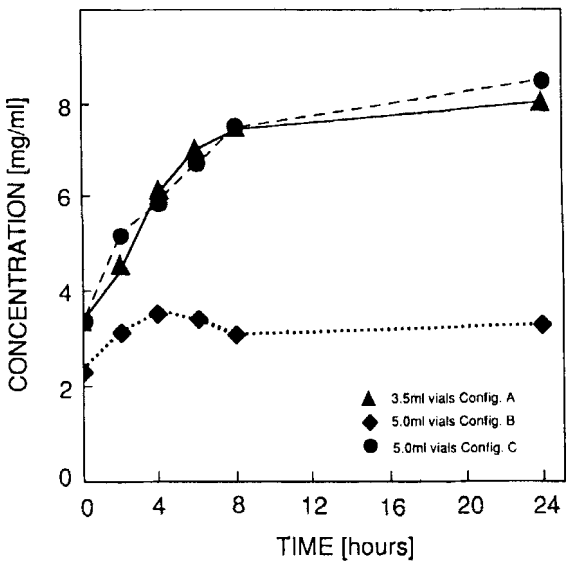

Figure 2. Effect of Container GeometryOn Drug Release Profile

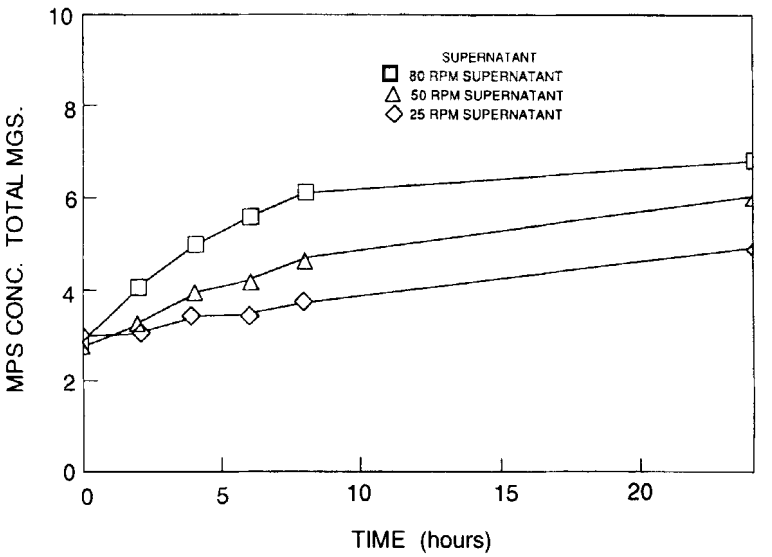

Figure 3. Effect Of Different Rotational Speeds On Release Rate Assay

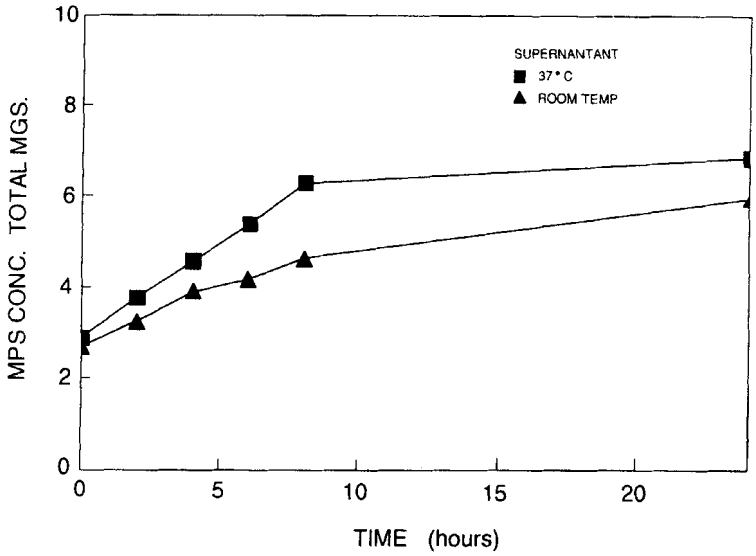

Figure 4. Effect Of Temperature On Release Rate Assy

was carried out at a rotational speed of 50 rpm. Figure 4 illustrates that the release rate is temperature-dependent, occurring more rapidly at an elevated temperature.

The effect of volume was studied by suspending a fixed weight of liposome paste in various volumes of assay suspension buffer. Table 1 summarizes results and it indicates similar rates of release for the three concentrations studied. However, it should be noted that in the more concentrated form (2X), the reproducibility appears to decline, which is evidenced by the larger standard deviation values on mean at several time points.

In a different experiment, effects of liposome concentration on release rate was studied by maintaining the volume of the suspensions but

Table 1

Effect of Suspension Volume on Liposome Release Rate

Time (hr)	% Drug in Supernate (± SD)* Release Rate Suspension Volume 0.5X	1.0X	2.0X
0	32.8 ± 0.2	32.7 ± 0.3	32.7 ± 0.7
2	49.6 ± 0.2	44.1 ± 0.2	44.4 ± 0.5
4	60.5 ± 0.4	56.1 ± 0.5	52.1 ± 0.7
6	63.1 ± 0.3	58.7 ± 0.6	45.7 ± 1.3
8	60.5 ± 0.3	64.2 ± 0.5	61.3 ± 1.4
24	68.6 ± 0.3	72.7 ± 0.4	70.6 ± 1.6

Table 2

Effect of Liposome Concentration at Constant Volume on Release Rate

Time (hr)	% Drug in Supernate (± SD)* Liposome Concentration 0.5X	1.0X	2.0X
0	26.9 ± 0.3	27.9 ± 0.2	32.1 ± 0.4
2	34.3 ± 0.2	41.5 ± 0.3	45.5 ± 0.6
4	51.1 ± 0.2	51.4 ± 0.4	58.9 ± 1.1
6	61.1 ± 0.5	59.4 ± 0.4	61.9 ± 0.9
8	65.2 ± 0.4	59.4 ± 0.5	65.5 ± 0.7
24	66.6 ± 0.1	68.9 ± 0.7	72.6 ± 1.2

the concentration of liposome was varied. Results of this experiment are summarized in Table 2. These findings are comparable to those obtained in Table 1.

CONCLUSION

This study was designed to develop and evaluate an in vitro method for rate of drug release from a liposome formulation. The purpose of this method was to determine the amount of free drug (model drug: metaproterenol sulfate) contained in the liposome suspension at zero time and then to evaluate rate of drug release in an end-over-end tumbler device over the studied time period. From this study, it was found that the release profile of drug from this liposome formulation can be changed by altering any of the operating parameters of the method. Particularly, the geometry of sample container and the rotation speed have more profound effect on drug release rate compared to the sample temperature. Although, this method may not correlate the in vivo kinetics, it can be a reproducible, in vitro, quality control tool.

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