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# DEVELOPMENT AND VALIDATION OF A DRUG RELEASE RATE METHOD FOR A WATER SOLUBLE DRUG IN A LIPOSOME PREPARATION

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## ABSTRACT

An in vitro method was developed to determine the rate of drug release from a liposome preparation. Three batches of liposomes containing orciprenaline sulphate were evaluated for release of drug over a 24 hour period in an end-over-end tumbler device. Intra and inter day precision studies indicate good reproducibility for this test method. This method is easy to use in drug development and quality control laboratories to evaluate drug release from liposome formulations.

## INTRODUCTION

Numerous papers have been published concerning liposomes as drug carriers and controlled delivery systems (1-4). Several drug delivery systems are in clinical studies and may prove to be useful as drug carriers of prolonged activity. Clinical trials of liposomes containing anticancer drugs (5) antifungal drugs (6), or immunomodulators (7,8) have put the liposome preparations in the path for marketability. Until recently, relatively few reports appeared in the scientific literature on the pharmaceutical aspects of liposomes. In a recent review article, it has been correctly pointed out that the pharmaceutical concerns of liposomes were seldom reported in the published literature (9). For liposomes to be acceptable as marketable parenteral dosage form, the dosage form preparation must be sterile and free of pyrogens, have good shelf-life or physical stability, the process used for

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liposome preparation must be reproducible, and the drug release method to evaluate the liposomes must be validated. Development and evaluation of drug release rate from a sustained release dosage form is essential and is likely to be mandated for any liposome preparation which gains admission to USP. However, at the present time, very little attention appears to have been given to the development of methods to monitor *in-vitro* drug release method for liposome formulations although in 1991, a report was published on drug release testing method and the influence of geometry of container on drug release rate, which is of interest to pharmaceutical scientists (10).

In order for a liposome formulation to enter the market place as a long acting drug delivery system, effective quality control procedures to monitor the product integrity as a function of time must be developed. Thus a simple and reproducible *in-vitro* drug release procedure for liposome formulations is necessary.

The present study was undertaken to develop, evaluate, and validate a drug release procedure for a water soluble drug (model drug: orciprenaline sulphate) in liposomes. The concentration of drug in both the supernatant (free drug) and the pellet (liposome encapsulated) was determined at various time intervals and under a variety of conditions. Intra-run and inter run precision studies were performed to verify the reproducibility of the method. Three batches of drug-liposome preparations were evaluated to investigate batch-to-batch variability.

## EXPERIMENTAL

**Materials:** Egg phosphatidylcholine (Avanti Polar Lipids, Inc., Birmingham, AL), egg phosphatidylglycerol (Avanti Polar Lipids, Inc., Birmingham, AL), cholesterol (Croda, Inc., Mill Hall, PA), and orciprenaline sulphate, USP (Vinchem, Inc., Chatham, NJ), were obtained and used as received. dl-Alpha-tocopherol, disodium phosphate dibasic heptahydrate, and edetate disodium were obtained from Sigma Chemicals (St. Louis, MO). Sodium phosphate monobasic monohydrate,USP and chloroform were supplied by Mallinckrodt Chemical Company. The reference standard for orciprenaline sulphate was obtained from the United States Pharmacopeial Convention,Inc.(Rockville, MD).

**Preparation of Liposomes with a Water Soluble Drug:** The model drug orciprenaline sulphate was encapsulated into lipid bilayers using six different unit operations. They are described as i) roto-evaporation; ii) drug solution for hydration; iii) hydration of lipids; iv) mixing; v) sizing; and vi) concentration by ultrafiltration. A brief description of the encapsulation procedure is given below: 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, thin layer of phospholipid mixture was slowly hydrated with an aqueous solution of orciprenaline sulphate (100 mg/ml, pH7.0). The resultant liposome dispersion was sized to 0.2 micron diameter by a membrane filtration technique (11) and then concentrated to a paste

consistency by ultrafiltration technique using a 100 K polysulfone membrane. The resultant liposome paste is a mixture of encapsulated and unencapsulated forms of drug. The liposome paste is also referred to in this text as "liposome concentrate"

**Equipment:** Orciprenaline sulphate content of various samples was assayed by an HPLC system. The chromatography system (IBM Instruments, Danbury, CT) consisted of an IBM model LC/9560 and a variable wavelength UV detector (LC/9563) equipped with an autosampler (LC/9505). The output of the detector signal fed to an IBM 9000 computer system to generate the chromatogram.

Release rate experiments were followed in an end-over-end tumbler device (Van-Kel Industries, Edison, NJ). Samples were subjected to centrifugation in an ultracentrifuge (Model TL100, Beckman Instruments, Inc., Palo Alto, CA).

**Drug 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. These samples were then rotated at a pre-selected speed (rpm) for various lengths of time. At various time intervals (0 - 24 hours) , triplicate vials were collected. Each suspension was subjected to centrifugation at 100,000 rotation speed (rpm) for 30 minutes to pellet the liposome in an ultracentrifuge. The supernatant was collected and assayed for drug concentration. The pellet was treated with 1% Triton X-100 (a surfactant) to obtain a clear solution and then assayed for drug concentration. Samples were analyzed on an HPLC system. 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 orciprenaline sulphate. A typical chromatogram of orciprenaline sulphate lysed from liposomes is shown in Fig. 1. Drug concentrations were calculated from a standard curve.

## RESULTS

The free and total drug concentrations of orciprenaline sulphate were determined by a HPLC method. Since liposome preparations have drug on both the inside and outside of the vesicles, it is necessary to lyse them with a suitable agent to determine the total drug content in sample preparation. In preliminary studies, four different surfactants, viz sodium dodecyl sulfate, Tween-20, Triton X-100, and deoxycholate were screened to select a detergent which does not interfere with the analysis but can lyse the liposome vesicles completely. Each detergent was used at a concentration of 1% for 10 minutes at room temperature to lyse the liposome vesicles. Detergent treated liposomes yielded a clear solution which is a result of complete dissolution of liposomes in reaction mixture. There was no difference in their ability in recovering drug from liposome vesicles. Any one of these detergents could be selected to use in further studies. Triton X-100 was used in this study.

Standard curve: Drug concentrations were estimated from a standard curve generated using USP Reference Standard metaproterenol sulfate (orciprenaline

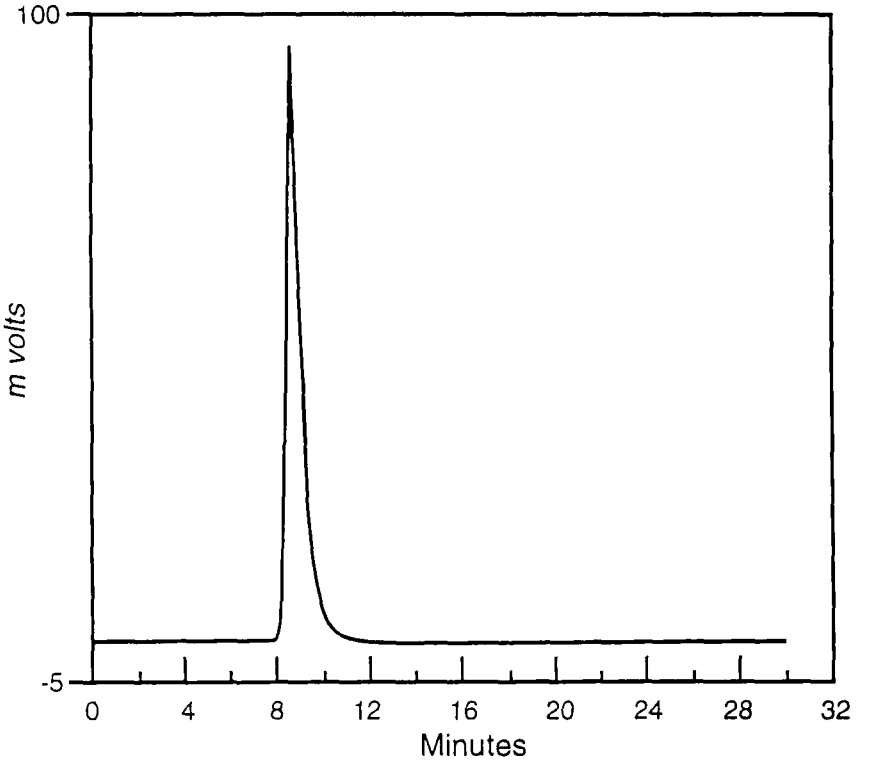


Fig. 1. A typical chromatogram of orciprenaline sulphate recovered from a liposome preparation.

sulphate) dissolved at 1.0, 0.5, and 0.1 mg/ml in 0.01 N HCl. Ten microliter injections of the standards were made into the HPLC. Triplicate samples were analyzed at each concentration. The peak area counts were calculated and the mean of each concentration plotted against known concentration. The plot is linear with a correlation coefficient (r) of 1.000 for three points. Fig. 2 illustrates the reproducibility of the USP Reference orciprenaline sulphate sulfate Standard at 10 separate determinations. It is quite clear the method is reproducible.

The kinetics of orciprenaline sulphate release from liposomes: Several parameters of release were examined to optimize the appropriate analytical configuration. Fig. 3 illustrates the kinetics of drug release at three different rotation rates. This figure shows the effect of rotation speed (rpm) on drug release over 24 hours. These results indicate that not only the rate of release increase with increasing rotation rates but also the total amount of drug released over 24 hours also increases with increasing rotation speed.

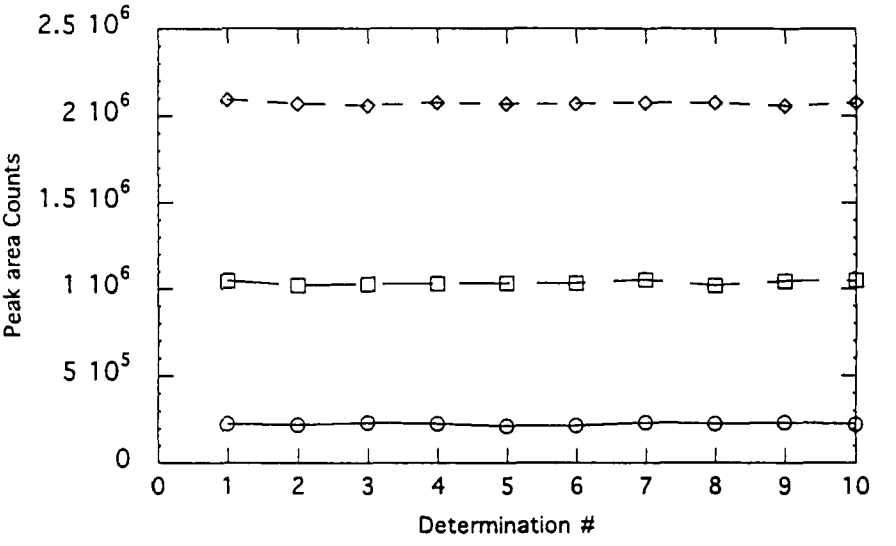


Fig. 2. Reproducibility of USP reference orciprenaline sulphate standard assayed by HPLC technique. Key: (circle) 0.1 mg/mL orciprenaline; (square) 0.5 mg/mL orciprenaline; and (diamond)1 mg/mL orciprenaline.

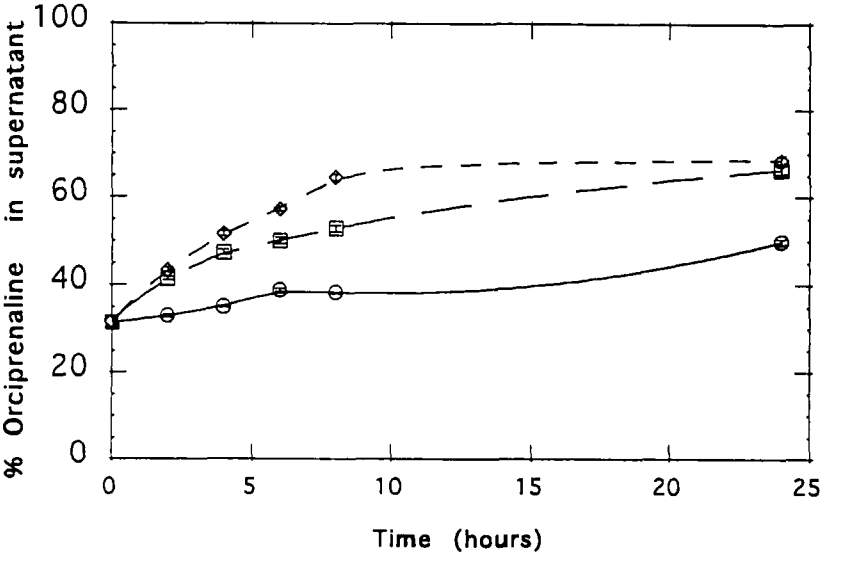


Fig. 3. Effect of different rotational speeds on release rate assay. Key: (open circle) - 20 RPM speed, (open square) - 50 RPM speed, and (open diamond) - 80 RPM speed.

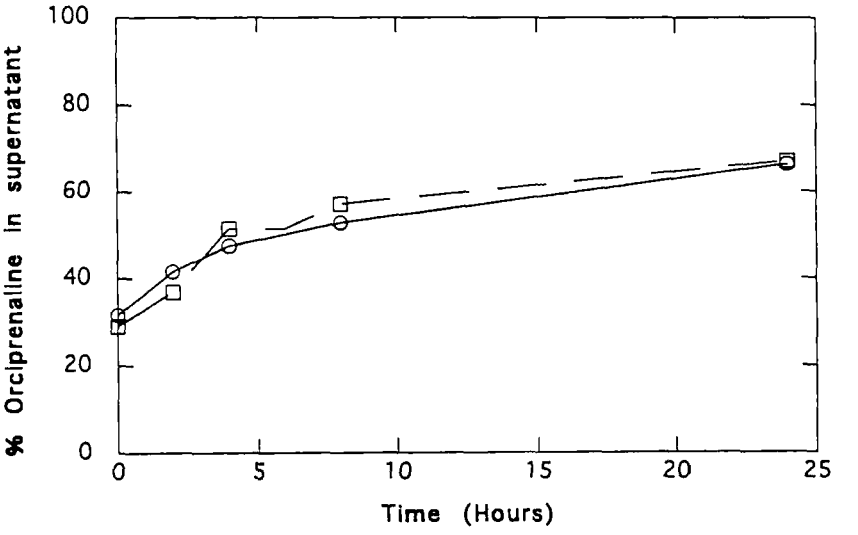


Fig. 4. Effect of temperature on release rate assay. Key: (open circle) - at room temperature, and (open square) - at 37°C.



Temperature Effect: The effect of temperature on release rate was investigated by maintaining samples at either room temperature or at 37° C . The results in Fig. 4 indicates that release rate is temperature-dependent, occurring more rapidly at an elevated temperature.

Volume Effect: The effect of volume was investigated by suspending a fixed weight of liposome paste in various volumes of assay suspension buffer. Table 1 summarizes this experiment. The results in Table 1 indicate similar rates of release for the three concentrations used. In the more concentrated formulation (2X) the reproducibility appears to decline as evidenced by the larger standard deviation (SD) values at several time points.

In another experiment designed to investigate the effects of liposome concentration on release rate, the volume of the suspension was maintained constant but the concentration of liposome was varied. The results of this experiment is shown in Table 2. The results in Table 2 are comparable to those observed in Table 1. The same drift in reproducibility as noted in Table 1 is again observed.

Precision Studies: Table 3 summarizes the day-to-day precision on one batch of liposomes. The coefficient of variation (% CV) for any day's determination ranged from 0.1 to 1.5% whereas the % CV for the mean of the four determinations ranged from 3.0 to 6.2%. Table 4 illustrates the day-to-day precision of a second batch of liposomes. Good precision is observed in these studies where the % CV of the means ranged from 2.3 to 6.6%.

TABLE 1. Effect of Suspension Volume on Liposome Release Rate

| Time (Hours) | % Orciprenaline sulphate in supernate (+/- S.D.) 0.5 X | 1.0 X | 2.0 X |
|---|---|---|---|
| 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 Concentrate at Constant Volume on Release Rate

| Time (hours) | % Orciprenaline sulphate in supernate (+/- SD) 0.5X | 1.0X | 2.0X |
|---|---|---|---|
| 0 | 26.9 +/- 0.03 | 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 |

TABLE 3. Day-to-Day Precision of Release Rate Assay for Orciprenaline sulphate-Liposome (Batch 1).

| Time (hours) | % Orciprenaline sulphate in supernate (+/- SD) Day 1 | Day 2 | Day 3 | Mean +/- SD |
|---|---|---|---|---|
| 0 | 39.0 +/- 0.1 | 31.7 +/- 0.4 | 35.1 +/- 0.4 | 31.9 +/- 3.0 |
| 2 | 36.9 +/- 0.2 | 43.4 +/- 0.6 | 46.3 +/- 0.4 | 42.2 +/- 4.8 |
| 4 | 43.2 +/- 0.2 | 51.7 +/-0.6 | 55.3 +/- 0.6 | 50.1 +/- 6.2 |
| 6 | 51.4 +/- 0.3 | 57.3 +/-0.6 | 60.3 +/- 0.5 | 56.3 +/- 4.5 |
| 8 | 57.2 +/- 0.4 | 64.5 +/- 0.7 | 65.7 +/- 1.0 | 62.5 +/- 4.6 |
| 24 | 67.2 +/- 0.1 | 68.6 +/- 0.9 | 72.9 +/- 0.8 | 69.6 +/- 3.0 |
| % CV (range) | 0.1 - 0.7 | 1.0 - 1.4 | 0.9 - 1.5 | 3.0 - 6.2 |

TABLE 4. Day-to-day Precision of Release Rate Assay for Orciprenaline sulphate-Liposomes (Batch 2)

| Time (hours) | % Orciprenaline sulphate in supernate (+/- SD) Day 1 | Day 2 | Day 3 | Mean +/- SD |
|---|---|---|---|---|
| 0 | 35.3 +/- 0.02 | 34.5 +/- 0.03 | 36.5 +/- 0.03 | 35.4 +/- 1.0 |
| 2 | 48.4 +/- 0.04 | 49.9 +/- 0.04 | 53.3 +/- 0.03 | 50.5 +/- 2.5 |
| 4 | 53.1 +/- 0.01 | 59.2 +/- 0.06 | 60.0 +/- 0.03 | 57.4 +/- 3.8 |
| 6 | 66.4 +/- 0.01 | 63.4 +/- 0.02 | 64.6 +/- 0.03 | 64.8 +/- 1.5 |
| 8 | 67.7 +/- 0.01 | 69.9 +/- 0.02 | 66.2 +/- 0.01 | 67.9 +/- 1.9 |
| 24 | 73.8 +/- 0.01 | 70.5 +/- 0.03 | 70.5 +/- 0.22 | 71.6 +/- 1.9 |
| % CV (range) | 0.01 - 0.05 | 0.02 - 0.10 | 0.02 - 0.10 | 2.3 - 6.6 |

TABLE 5. Same Day Precision Study - Release Rate Assay.

| Time (hours) | % Orciprenaline sulphate (+/- SD) Run 1 | Run 2 | Run 3 | Mean +/- SD |
|---|---|---|---|---|
| 0 | 35.2 +/- 0.2 | 37.1 +/- 0.6 | 35.3 +/- 0.5 | 35.9 +/- 1.1 |
| 2 | 51.1 +/- 0.8 | 43.2 +/- 0.6 | 48.5 +/- 0.5 | 47.6 +/- 4.0 |
| 4 | 57.9 +/- 0.8 | 54.3 +/- 0.4 | 53.2 +/- 0.2 | 55.1 +/- 2.4 |
| 6 | 65.8 +/- 0.6 | 57.2 +/- 0.9 | 66.3 +/- 0.2 | 63.1 +/- 5.1 |
| 8 | 69.4 +/- 0.4 | 64.2 +/- 0.9 | 67.7 +/- 0.6 | 67.1 +/- 2.6 |
| 24 | 76.6 +/- 0.9 | 71.6 +/- 0.8 | 73.8 +/- 0.3 | 74.0 +/- 2.5 |
| % CV (Range) | 0.6 -1.4 | 0.7 - 1.6 | 0.3 - 1.4 | 1.1 - 5.1 |

Precision was also investigated by performing the assay three times on the same day. These results in Table 5 indicate CV ranges of 0.3 - 1.6% for individual runs and an overall CV for the mean of the three runs to be 1.1 - 5.1%.

The reproducibility of each determination was evaluated by running triplicate determinations during each assay. The results in Table 6 summarize one such determination. CV values for the supernatant range from 0.8 to 2.1% whereas quantitative assay of drug in the pellet appears more variable (CV range =1.1 - 5.2%). The results suggest a heterogeneity of liposome-encapsulated drug.

TABLE 6. Accuracy of Each Determination of Release Rate

| | Orciprenaline sulphate contained in the fraction (mg) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time (hrs) | Sample1 | | Sample2 | | Sample3 | | Mean | | S+P | Release Rate (%) |
| | Supernate | Pellet | Supernate | Pellet | Supernate | Pellet | Supernate | Pellet | | |
| 0 | 3.65 | 5.71 | 3.80 | 6.29 | 3.74 | 5.81 | 3.73 +/- 0.08 | 5.94 +/- 0.31 | 9.67 | 38.6 |
| 2 | 4.60 | 5.26 | 4.68 | 5.13 | 4.74 | 5.08 | 4.68 +/- 0.07 | 5.16 +/- 0.09 | 9.84 | 47.6 |
| 4 | 5.35 | 4.46 | 5.33 | 4.46 | 5.55 | 4.37 | 5.41 +/ -0.12 | 4.43 +/- 0.05 | 9.84 | 55.0 |
| 6 | 6.06 | 4.18 | 6.09 | 4.48 | 6.03 | 4.38 | 6.06 +/- 0.03 | 4.35 +/- 0.15 | 10.41 | 58.2 |
| 8 | 6.49 | 4.36 | 6.40 | 4.27 | 6.40 | 4.37 | 6.43 +/- 0.05 | 4.33 +/- 0.06 | 10.76 | 59.8 |
| 24 | 8.62 | 3.27 | 8.72 | 3.40 | 8.38 | 3.18 | 8.58 +/- 0.17 | 3.28 +/- 0.11 | 11.86 | 72.3 |
| % CV (range) | | | | | | | 0.8 +/- 2.1 | 1.1 +/- 5.2 | | |

S+P = Total Orciprenaline sulphate in Supernate and Pellet (Mean = 10.40 +/- 0.83)

Release Rate = (Orciprenaline sulphate in Supernate / Total Orciprenaline sulphate) X 100%

TABLE 7. Release of Orciprenaline sulphate-Liposome: Batch to Batch Variation

| Time (hours) | % Orciprenaline sulphate in supernatant (+/- SD) Batch 1 | Batch 2 | Batch 3 |
|---|---|---|---|
| 0 | 34.5 +/- 0.1 | 35.1 +/- 0.4 | 34.8 +/- 0.4 |
| 2 | 52.8 +/- 0.2 | 46.3 +/- 0.5 | 45.5 +/- 0.4 |
| 4 | 56.9 +/- 0.2 | 55.3 +/- 0.6 | 51.5 +/- 0.5 |
| 6 | 61.7 +/- 0.1 | 60.3 +/- 0.6 | 61.2 +/- 0.6 |
| 8 | 61.6 +/- 0.1 | 65.7 +/- 0.8 | 59.5 +/- 0.7 |
| 24 | 65.3 +/- 0.2 | 72.9 +/- 0.8 | 64.1 +/- 0.6 |

Batch - to - Batch Variability: In order to examine the batch - to - batch variability, three batches of liposomes were made and evaluated for drug release. The results in Table 7 summarize the release rates observed for three batches of orciprenaline sulphate-liposomes. Based on the results reported in the Table 7 about 60% of the drug was found in the supernatant at 6 hours and no further drug release was observed beyond that time.

## DISCUSSION

Several parameters of release rate were examined in an attempt to optimize an in-vitro drug release method. The purpose of such a method was to determine the amount of free drug (water soluble) contained in the liposome suspension at zero time and then to evaluate the rate of drug release in an end-over-end tumbler device over an interval of 24 hours. The assay was designed to monitor the drug content of the suspension at various times after tumbling in the device. Mass balance was to be considered by quantifying the amount of drug in the supernatant of the liposome suspension as well as by releasing (Triton X-100 treatment) any of the drug still contained within the liposome pellet at various time points. It was felt such a configuration although perhaps not representative of *in-vivo* kinetics might be useful as an *in-vitro* release test.

## CONCLUSION

The results of this study indicate the feasibility of such a release test to evaluate drug release from liposomes. Run-to- run and day-to-day precision studies indicate reliable and predictable coefficients of variations for this assay. The precision of the assay on any day is acceptable with CV values of less than 2%. Day-to-day variabilities indicate slightly higher CVs but the reason for this is mainly

attributed to the variations in sample handling, in ambient temperature. Whether this higher CV reflects influences of other components of the liposome suspension or of contaminants (varied levels of residual organic phase in different batches, effects of evaporation, effects of storage) remains unclear. The studies in this report investigating volume effects, concentration effects, and temperature effects suggest that this is a reliable assay procedure.

## REFERENCES

1. Laserman, L., Suzuki, H., and Machy, P., (1993) Comments on the application of liposome technology to specific cell targeting, In: Gregoriadis, G. (Ed) Liposome technology: entrapment of drugs and other materials, Vol III. CRC Press Inc., Boca Raton, FL., pp 139-152.

2. Juliano, R. L., and Layton, D., (1980) Liposomes as a drug delivery system, In Juliano, R. L. (Ed) Drug delivery systems. Oxford University Press, Oxford, UK, pp.189-236.

3. Martin, F.J., (1990) Pharmaceutical manufacturing of liposomes, In: Praveen Tyle (Ed) Specialized drug delivery systems: manufacturing and production technology. Marcel Dekker, Inc., New York, NY, pp 267-315.

4. Touitou E., Shaco-Ezra N., Dayan N., Jushynski M., Rafaeloff R., and Azoury R., (1992) Dyphylline liposomes for delivery to the skin, J. Pharm. Sci., 81: 131-134,

5. Treat, J., Greenspan, A.R., and Rahman, A., (1989) Liposome encapsulated doxorubicin preliminary results of phase I and phase II trials, In: Lopez-Berestein, G., and Fidler, I.J. (Eds) Liposomes in the therapy of infectious diseases and cancer. Alan R. Liss, New York, pp 353-360.

6. Lopez-Berestein, G. (1989) Treatment of systemic fungal infections with liposomal-amphotericin B, In: Lopez-Berestein, G., and Fidler, I.J. (Eds) Liposomes in the therapy of infectious diseases and cancer. Alan R. Liss, New York, pp 317-328.

7. Hanagan, J.R., Trunet, P., LeSher, D., Andrejcio, K., and Frost, H. (1989) Phase I development of CGP 19835A lipid (MTP-PE encapsulated in liposomes), In: Lopez-Berestein, G., and Fidler, I.J., (Eds) Liposomes in the therapy of infectious diseases and cancer. Alan R. Liss, New York, pp 305-316.

8. Creaven, P.J., Brenner, D.E., Cowens, J.W., Huben, R., Karakous, C., Han, T., Dadey, B., Andrejcio, K., and Cushman, M.K. (1989) Initial clinical trial of muramyl tripeptide derivative (MTP-PE) encapsulated in liposomes: an interim report, In: Lopez-Berestein, G., and Fidler, I.J., (Eds) Liposomes in the therapy of infectious diseases and cancer. Alan R. Liss, New York, pp 297-304.

9. Talsma, H., and Crommelin, D. J. A., (1992) Liposomes as drug delivery systems, Part I: preparation, Pharm. Tech., 16(10), pp 96-104.

10. Vemuri, S., Yu, C., Pushpala,S., and Roosdorp, N., (1991) Drug release rate method for a liposome preparation, Drug Dev. Ind. Pharm. 17(2): 183-192 .

11. Hunt, C.A., Papahadjopoulos, D. (1985) Methods for producing liposomes in selected size range, U.S. Patent 4,529,561, July 16.