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Comparison of Dialysis and Dispersion Methods for In Vitro Release Determination of Drugs from Multilamellar Liposomes

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ABSTRACT

The aim of these studies was to compare dialysis and dispersion methods for determining in vitro release of propranolol, metoprolol, pindolol, and atenolol from multilamellar liposomes. Multilamellar vesicles (MLV) were prepared using hydrogenated soy-lecithin phospholipon 90H (Ph 90H) as the primary lipid. The same volume of pH 7.4 phosphate buffered saline was used as a receptor medium for both methods. Samples were withdrawn, and drug concentration was determined using HPLC. All drug-containing liposomes exhibited an initial burst release followed by a slower rate of release. The rate and extent of drug release from MLV was dependent on the physicochemical properties of the drug. For all drugs investigated, the rate of release was higher for the dispersion method as compared with the dialysis method.

INTRODUCTION

Liposomes have been used as biocompatible and biodegradable dosage forms for small molecules and biologically active peptides and proteins for targeting as well as prolonging release of active pharmaceutical ingredients (APIs). Formulation development of systemically active compounds necessitates the establishment of an appropriate in vitro release method as an indirect method of determining drug availability for quality control purposes, to assess formulation factors, and to substantiate product label claim (*1*, *2*). Furthermore, correlations between in vitro and in vivo drug release are often sought, not only to define biorelevant in vitro release models, but to reduce the development time of an optimized formulation.

The aim of the present study was to evaluate the in vitro release from drug-loaded multilamellar liposomes for comparison between a dialysis and a dispersion method. Four model drugs with different physicochemical properties were included in the investigation (Table 1).

Table 1. Lipophilicities and Ionization Constants for the β-Adrenoceptor Antagonists Investigated (ref 7).

Drug	pK_a	Log *P*
Propranolol	9.45	1.49
Metoprolol	9.70	0.20
Pindolol	8.80	0.08
Atenolol	9.55	–0.11

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MATERIALS AND METHODS

Materials

Propranolol hydrochloride, metoprolol tartrate, pindolol, and atenolol were purchased from Sigma-Aldrich GmbH, Germany. Phospholipon 90H® was purchased from Phospholipid GmbH, Germany. Sodium dihydrogen phosphate monohydrate and Triton® X-100 were obtained from Merck KGaA, Germany. Phosphate buffered saline was purchased from Biochrom AG, Germany. Acetonitrile HPLC grade was purchased from Fisher Scientific, Germany.

Methods

Preparation of MLV

MLV were prepared by the freeze-and-thaw method (*3*). Phospholipon 90H (100 mg) was added to 2 mL of drug solution (19 mg/mL) in phosphate buffered saline (PBS). The dispersion was rotated at 10 rpm (Neolab-Reagenzglas-Mischer, Neolab GmbH, Germany) for 30 min and then vortexed intermittently for 30 min (2 min vortexing, 3 min interval in a water bath at 68 °C, the temperature above the phase transition temperature of Ph 90H, followed by six freeze-thaw cycles). The freeze-thaw cycles involved the immersion of the MLV in liquid nitrogen for 3 min followed by 6 min of thawing at 68 °C. The freezing process was used to aid the encapsulation of the drug inside the liposomal vesicular structure, whereas the thawing process was used to break multilamellar vesicles and to promote the mixing of the enclosed contents with the release medium (*4*). Thus the repeated freezing and thawing processes enhanced the encapsulation efficiency of the drug. After the freeze-thaw treatment, the liposomal dispersion was left for an hour to allow for the liposomes to be formed and to further encapsulate the drug. The liposomes were washed twice in 10 mL PBS with the aid of centrifugation at 10,000 × *g*

for 30 min. The MLV that formed were resuspended in PBS for all further investigations.

Determination of Encapsulation Efficiency (%)

Fifty microliters of the multilamellar liposomes was taken before and after washing and centrifugation, and the drug content was determined by HPLC. The encapsulation efficiency (%*EE*) was determined by the following equation:

$$\%EE = \frac{AD_a}{AD_b} \quad 100$$

where AD_a and AD_b are the amounts of drug in liposome after and before washing, respectively.

In Vitro Release

Dialysis Bag Method

A volume of 0.5 mL of liposomal preparation was put in a dialysis bag (3.8 cm in length). Dialysis tubing consisted of regenerated cellulose, a material chemically and physically treated to increase its resistance (MWCO 12,000–14,000 Da, 25-Å pore diameter, SERVA Electrophoresis GmbH, Heidelberg, Germany). Both ends were tied. The dialysis bag was suspended in 25 mL PBS at pH 7.4 and maintained at 37 ± 0.5 °C. The dispersion was rotated at 200 rpm in a shaker (GFL 3032 Shaker, LABOTEC, Germany). At predetermined time intervals of 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 69, 120, 168, and 336 h, 1-mL aliquots were sampled and replaced with 1 mL fresh pH 7.4 PBS, which was maintained at 37 ± 0.5 °C. Drug concentrations were quantified using HPLC, and all experiments were conducted in triplicate.

Dispersion Method

An aliquot of 0.5 mL of liposomal preparation was dispersed in a screw-capped glass vial (30 mL) containing 25 mL of PBS at pH 7.4 by shaking at 200 rpm in an incubator (GFL 3032 Shaker, LABOTEC, Germany) maintained at 37 ± 0.5 °C. At predetermined time intervals (0.5, 1, 2, 4, 6, 12, 24, 48, 72, 69, 120, 168, and 336 h), 0.25 mL of the dispersion was withdrawn and replaced with 0.25 mL of fresh PBS. The aliquot was centrifuged (Eppendorf centrifuge, Eppendorf, Germany) at 14,000 × *g* for 30 min, and the supernatant was analysed using HPLC. All experiments were conducted in triplicate.

High Performance Liquid Chromatography Method

The HPLC system (Merck-Hitachi) was equipped with an HPLC System Manager Chromatography Data Station Software® Model D-7000, a D-7000 interface, a programmable L-7250 autosampler, a pump model L-7100, and a UV variable wavelength detector model L-7420 (Merck Hitachi, Germany). The chromatographic separation was performed on a LiChrospher® 100 RP-18e column (250 × 4 mm, 5 µm). The conditions used for the analysis of each drug are listed in Table 2. Linear calibration curves were obtained in the concentration range 25–150 µg/mL for all model drugs. The correlation coefficients were always >0.999. Accuracy and precision were <5% (CV).

Table 2. Summary of HPLC Conditions for β-Adrenoceptor Antagonists from Dissolution Media.

Drug	Mobile Phase Composition (%)		UV detection wavelength (nm)	Flow rate (mL/min)
	Phosphate buffer*	Acetonitrile		
Propranolol	50	50	294	1.20
Metoprolol	50	50	224	1.00
Pindolol	70	30	264	1.00
Atenolol	70	30	224	0.80

*Phosphate buffer is composed of 0.067 M $NaH_2PO_4 \cdot H_2O$ with 0.2 % triethylamine.

Statistical Analysis

From each liposomal preparation, three samples were taken to study the in vitro release behavior. The data presented are expressed as mean ± standard deviation. The f_1 and f_2 statistics were used to compare the two methods.

RESULTS AND DISCUSSION

Figure 1 exhibits the encapsulation efficiency of the selected β-blockers. EE was dependent on the type of drug and—with the exception of pindolol—followed the lipophilicity of the drug in the following order: propranolol > metoprolol > atenolol > pindolol.

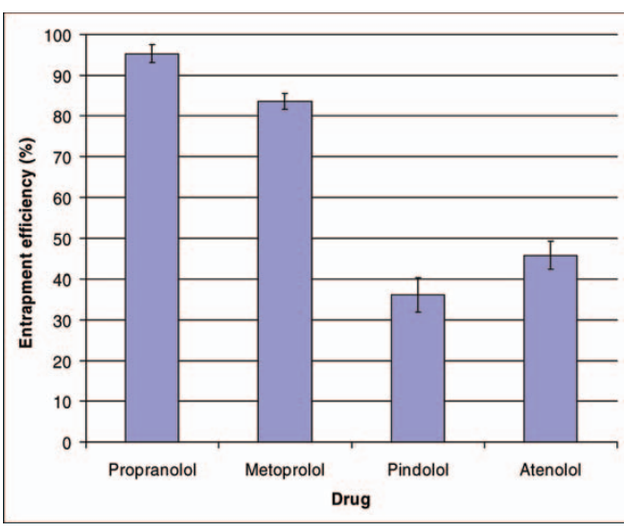

Figure 1. Entrapment efficiencies for β-adrenoceptor antagonists in multilamellar liposomes.

Figure 2 depicts the in vitro release profiles of the model drugs from the multilamellar liposomes using either the dispersion or dialysis dissolution methods. Regarding the results for liposomes containing propranolol hydrochloride, 32% and 23% were released after 4 h using the dispersion and the dialysis methods, respectively. The burst-release phase was followed by a plateau over 336 h, the duration of the experiment. This long duration of the release experiment may be impractical for use in a QC setting; yet future studies may focus on stress conditions that aid in minimizing the duration of such investigations. The release of propranolol was incomplete, probably due to an interaction between propranolol and the phospholipid. Similar results were previously obtained by Ahn et al. (*5*), who reported that interactions between propranolol and the phospholipid may slow down the release of the drug from proliposomes or hamper its complete dissolution. The release was not hampered by insufficient sink conditions, because propranolol concentrations in the dissolution fluid did not exceed 20% of its saturation solubility. Nor was this result due to insufficient loading of the liposomes, since the reported data measured the fraction of drug released as a function of the encapsulated drug material.

In contrast to propranolol, the release of metoprolol was complete using both dissolution methods. In the dispersion method, it took 24 h to reach complete release, whereas it was considerably slower for the dialysis method. The deviations in the release kinetics between the two methods may be explained mainly by the differences in shear stress exerted on the liposomal preparations. This point has been made by Saarinen-Savolainen et al. (6), who stated that drug release from the dialysis bag is strongly affected by the stirring inside the bag as well as the dialysis membrane permeability.

In our experiments, limited permeability of the dialysis membrane is most likely not occurring, since control experiments on the release of drug from an aqueous solution within the dialysis bag resulted in a complete release within 30 minutes (data not shown).

The release rate of atenolol from liposomal formulations was the highest of the four drugs studied. This may be because the log *P* of atenolol is lower than for the other drugs investigated (Table 1). Likewise, as has been observed for the other drugs studied, the release rate of atenolol and pindolol was more rapid for the dispersion method than for the dialysis method.

Based on the lipophilicity parameters alone, this result was unexpected, since pindolol shows similar lipophilicity when compared with metoprolol. Yet, in studies on transdermal permeation, the skin flux reported for pindolol was much higher than expected based on its lipophilicity, which was attributed to the unique nature of the indole group (heterocyclic ring) as an aromatic substituent (*7*).

In general, the release rates of the incorporated drugs were consistently higher when using the dispersion method as compared with the dialysis method for in vitro release testing. This might be due to (1) differences in the hydrodynamics of the system when comparing the liposomal formulation within the dialysis bag to the formulation dispersed within a flask. These differences relate to variations in the shear-effect exerted upon the formulation; (2) the density of the liposomal vesicles in

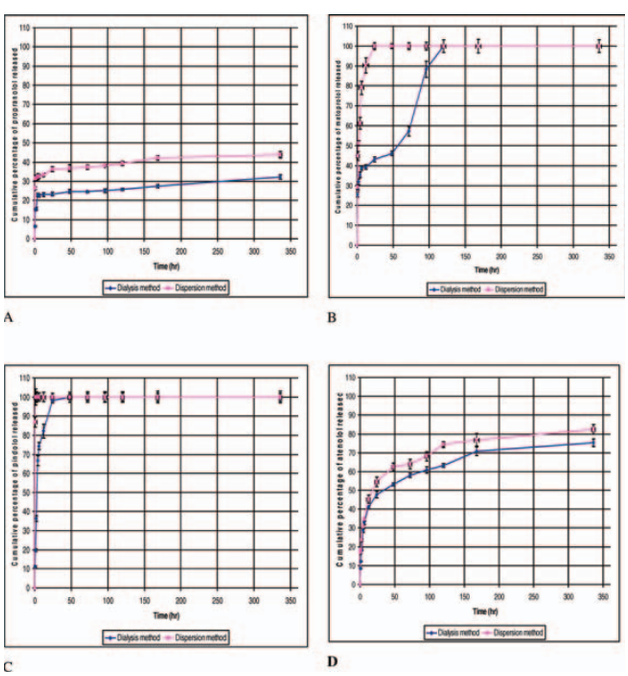

Figure 2. Release pattern of β-adrenoceptor antagonists from MLV using dialysis and dispersion methods. (A) Propranolol, (B) Metoprolol, (C) Atenolol, (D) Pindolol.

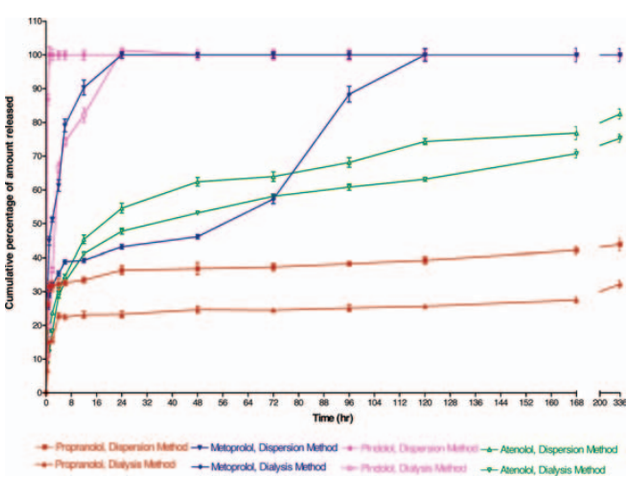

Figure 3. Comparative release pattern of selected β-adrenoceptor antagonists from MLV using the dialysis and dispersion methods.

Table 3. f_1 and f_2 Statistics for the Dissolution of the Model Drugs Using the Dispersion Method as Reference and the Dialysis Method as Test Model.

Drug	f_1	f_2
Propranolol	37.44	43.29
Metoprolol	30.45	24.46
Pindolol	23.19	21.27
Atenolol	12.52	58.18

their aqueous dissolution medium is higher in the dialysis method when compared with the dispersion method. Given that the probability of vesicle-to-vesicle contacts will increase with higher density, it may be postulated that the surface area of the vesicles available for contact with the dissolution medium should be higher in the case of the dispersion method. Consequently, under sink conditions, this mechanism should explain the higher release rate of the drug under the conditions of the dispersion method; (3) the membrane permeability of the dialysis membrane in general, which however has been determined not to be rate-limiting in the present case.

The release rate differences were evaluated using the f_1 and f_2 statistical measures. The results are given in Table 3. The difference in the release kinetics of the investigated model drugs was significant ($f_1 > 15$ and $f_2 < 50$) for propranolol, metoprolol, and pindolol, whereas it did not reach significance in the case of atenolol ($f_1 < 15$ and $f_2 > 50$).

CONCLUSION

The dialysis bag and the dispersion methods can be used to measure drug release from liposomal pharmaceutical preparations. Both methods gave similar shapes of in vitro release profiles at acceptable precision (<10%, CV). The dispersion method tended to show faster release rates from formulations than those observed using the dialysis bag method. The optimum method with respect to in vivo relevance in light of an IVIVC will need to be determined next.

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