International Journal of Pharmaceutics, 51 (1989) 39–46
Elsevier

IJP 01712

Comparison of solute partitioning and efflux in liposomes formed by a conventional and an aerosolised method

S.J. Farr [1], I.W. Kellaway [1] and B. Carman-Meakin [2]

[1] *Welsh School of Pharmacy, University of Wales College of Cardiff, Cardiff (U.K.) and* [2] *3M Health Care, Loughborough (U.K.)*

(Received 4 July 1988)
(Modified version received 27 August 1988)
(Accepted 30 August 1988)

Key words: Liposome; Partitioning; Efflux; Salbutamol; Hydrocortisone 21-octanoate; Aerosol; Pressure pack

Summary

The liposomal partitioning and efflux of two solutes, salbutamol and hydrocortisone 21-octanoate, were studied in multilamellar vesicles (MLVs) produced either in a conventional manner or following actuation of a chlorofluorocarbon based pressure pack formulation in a multistage liquid impinger (MLI). Partitioning of salbutamol in MLVs following hydration of egg phosphatidylcholine (EPC) films was independent of the addition of cholesterol but proportionately increased when dicetylphosphate (DCP) was included up to a maximum value when the salbutamol : DCP molar ratio was equal to 1. Increased entrapment was not accompanied by any electrostatically induced increase in vesicle size suggesting that the improved entrapment was a function of the formation of a lipophilic, membrane associated complex between salbutamol and DCP. Salbutamol partitioning in liposomes derived from aerosols emitted from drug/lipid pressure pack formulations in the MLI was comparable to conventional systems and was independent of the stage of the MLI on which the liposomes were generated (i.e. originating from different size aerosol fractions). Similar conclusions were drawn from studies using hydrocortisone 21-octanoate as a model hydrophobic compound. In addition, equivalent release kinetics of the steroid ester from conventional and aerosolised liposome preparations was observed which suggests MLVs produced from phospholipid aerosols in the MLI possess similar structural characteristics to conventionally prepared liposomes.

Introduction

Control of drug release within the respiratory tract is a subject of increasing interest, the relevance of which has been addressed recently (Byron, 1986; Gonda, 1988). Several approaches have been proposed: the formation of poorly soluble drug (Gonda et al., 1985) or drug/excipient coprecipitate particles (Hickey and Byron, 1986) and the entrapment of drugs within vesicular carriers (liposomes) (Woolfrey et al., 1988; Taylor et al., 1988). In the first two cases, drug availability in the lung is a function of the particles' aqueous solubility, whilst in the latter, availability is dependent on drug efflux from liposomes.

An earlier paper (Farr et al., 1987) has highlighted the concept of forming liposomes within the respiratory tract following deposition of inhaled microfine phospholipid aerosols derived from solution-type pressure pack formulations. This depends upon the phenomenon of sponta-

Correspondence: S.J. Farr, Welsh School of Pharmacy, University of Wales, College of Cardiff, PO Box 13, Cardiff CF1 3XF, U.K.

0378-5173/89/$03.50 © 1989 Elsevier Science Publishers B.V. (Biomedical Division)

neous, entropic formation of liposomes within a water-rich environment and was evaluated in-vitro through use of a multi-stage liquid impinger (MLI).

By employing the same apparatus this further study aimed to assess drug partitioning and efflux in liposomes formed following deposition of aerosols emitted from pressure packs formulated to contain homogeneous phospholipid/drug mixes. Such data are routinely determined for liposomes prepared by more conventional means prior to in-vivo evaluation (Alpar et al., 1981; Arrowsmith et al., 1983) and can be considered equally as appropriate for the aerosol systems designed to create a liposomal controlled release system in the lung.

This paper examines the relationship between partitioning of two drug compounds in liposomes prepared either by a conventional method (i.e. by hydration of a thin-lipid film) or by the aerosolised method, and evaluates the potential of the former method as a means of primary screening of drug compounds that may be usefully formulated into the pressure pack system. Salbutamol was chosen as an example of a hydrophilic compound expected to partition within the aqueous bilayers of liposomes. In addition, its relatively short duration of action (4–6 h) following inhalation might be extended usefully by liposomal encapsulation. Hydrocortisone 21-octanoate, a model drug shown to partition favourably into phospholipid bilayers (Arrowsmith et al., 1983), was selected as an example of a hydrophobic species.

Materials and Methods

Chromatographically pure egg phosphatidylcholine (EPC) was prepared by a column separation technique (Martin et al., 1978) from a 90% pure source (BDH Chemicals, UK). Dicetylphosphate (DCP) and cholesterol (CHOL; 99%, porcine) were obtained from BDH Chemicals, U.K. and used without further purification. Salbutamol was a gift from 3M Health Care, U.K. Di-[1-^{14}C]palmitoyl L-α-phosphatidylcholine ([^{14}C]DPPC), (spec. act. 3–4.4 GBq/mmol), and [1,2,6,7-^{3}H]hydrocortisone (spec. act. 3.07×10^3 GBq/mmol) were purchased from Amersham International, U.K. Hydrocortisone was obtained from Sigma Chemical Co., U.K. The tritiated and non-tritiated steroids were esterified with octanoyl chloride (Sigma Chemical Co., U.K.) according to the method of Arrowsmith et al. (1983). Reaction yield was 49% for [^{3}H] hydrocortisone 21-octanoate and 73% for the non-tritiated ester. The chlorofluorocarbon propellants trichlorofluoromethane (P11) and dichlorodifluoromethane (P12) were purchased from ICI (Mond Division) U.K. Other chemicals were of at least analytical quality and obtained from BDH Chemicals, U.K.

Liposomes formed by the conventional method

Multilamellar vesicles containing salbutamol were prepared by hydrating at 37 °C with gentle agitation mixed films of lipid (300 mg) and salbutamol (30 mg) with 0.01 M phosphate-buffered saline, pH 7.4 (PBS) in 50 ml Quickfit round-bottomed flasks. The films which contained EPC, EPC : DCP (90 : 10, 80 : 20, 70 : 30 mol) or EPC : CHOL (50 : 50 mol%) and salbutamol were obtained by rotary evaporation of ethanolic solutions under vacuum at 40 °C.

Liposomes containing hydrocortisone octanoate were prepared similarly by hydrating mixed films of EPC (25 mg) and steroid ester (0.1 mg spiked with 30.7 kBq of [^{3}H]ester) with 50 ml normal saline at 37 °C.

Liposomes formed by the aerosolised method

Solution phase pressure packs (10 ml volume) were prepared containing lipid/salbutamol or lipid/steroid ester mixes in a chlorofluorocarbon blend by a previously reported method (Farr et al., 1987). The actual constituents of each formulation are listed in Table 1.

Between 20 and 40 actuations (50 μl) of each primed, inverted pack were delivered on an airstream at 60 litres/min to an MLI fitted with a glass "throat" and containing 10 ml of either PBS (for salbutamol-containing aerosols) or normal saline (for hydrocortisone ester-containing aerosols) on each stage. Following aerosol deposition, the contents of each stage were removed and transferred to sealed bottles before equilibration at 37 °C.

TABLE 1

Formulae used in the preparation of drug/lipid homogeneous pressure packs.

Component	Formulation code		
	S1	S2	H1
Salbutamol (g)	0.040	0.040	–
Hydrocortisone octanoate (mg) [a]	–	–	0.025
EPC (g) [b]	0.244	0.196	0.135
DCP (g)	–	0.060	–
Ethanol (g)	1.830	1.020	–
P11 (g)	1.920	2.290	3.075
P12 (g)	8.200	9.160	10.295
Density of liquid blend (g/ml)	1.221	1.277	1.353

[a] Containing 154 kBq [^{3}H]ester.
[b] Containing 37 kBq [^{14}C]DPPC.

Aerosol characterisation

Aerosol characterisation was undertaken by relating the fractional deposition of the aerosol constituents to the effective cut-off diameter of each stage. Estimates of aerosol deposited in the adaptor, throat and terminal filter were made following quantitative washing of the respective components with absolute ethanol.

With the hydrocortisone ester/phospholipid aerosols, 1 ml aliquots of the liquid from each stage or the ethanolic washings were incorporated into 10 ml volumes of Cocktail T and counted for 10 min using an instrument (RakBeta, LKB Wallac, UK) providing automated subtraction of background and conversion of cpm to dpm. Dpm for ^{14}C and ^{3}H were related to the weight of lipid and steroid ester in each sample, respectively. With the salbutamol/lipid aerosols, lipid levels were similarly measured and the amount of salbutamol was determined by reverse phase ion-pair HPLC (Kleinberg et al., 1980). 20 μl (determined by loop volume) of the ethanolic washings was injected directly onto the column (Spherisorb ODS) whilst liquid from the various stages of the MLI was diluted 50% with absolute ethanol prior to injection. The amount of salbutamol was calculated with reference to a calibration curve of peak height versus concentration.

Assessment of drug partitioning and efflux rates

Time profiles for salbutamol partitioning between liposomes and PBS at 37°C were followed in conventional systems by withdrawing serial aliquots (3 ml) from the shaking suspensions over 24 h. The liposomal phase of each sample was separated from the continuous aqueous medium by ultrafiltration through a PM10 membrane in a 10 ml ultrafiltration cell (Amicon, U.K.) under a nitrogen pressure of 155 kPa. The ultrafilter was shown not to adsorb salbutamol and possessed a suitably small pore size (10,000 mol. wt. cut-off) to prevent liposome permeation into the matrix. 1 ml fractions of the filtrates were diluted 10-fold with 0.1 M HCl and assayed spectrophotometrically at 276 nm. Assessment of the partitioning of salbutamol in liposomes formed on stages 3 and 4 was carried out by HPLC assay of ultrafiltered samples to determine free drug concentration and of samples diluted with ethanol (to disrupt liposomes) to determine the total drug concentration.

The partitioning of hydrocortisone 21-octanoate between EPC and 0.9% w/v saline was determined for the conventional systems and for systems generated on stages 3 and 4 of the MLI following equilibration (48 h; Arrowsmith et al., 1983) at 37°C by scintillation counting of duplicate aliquots of the liposome suspension and of the supernatant obtained by ultracentrifugation of a 3 ml sample at 195,000 *g* for 1 h. Prior to steroid efflux, the conventional liposome preparations were diluted with 0.9% w/v saline to produce a phase ratio factor of 2000. The suspensions were re-equilibrated resulting in two thirds of the steroid liposome associated. Efflux of the steroid ester was monitored by periodic centrifugation at 195,000 *g* for 1 h following a 1 in 10 dilution of the equilibrated suspensions with 0.9% w/v saline.

Calculations

For a sustained release liposomal system, the amount of drug residing with the lipid phase is the important parameter in the determination of its efficacy. Therefore for salbutamol, the entrapment of the drug was expressed in terms of vesicle composition, thus mg of drug per 100 mg of lipid (mg/mg%).

With hydrocortisone 21-octanoate, entrapment was expressed as a partition coefficient in order to compare the data with those previously reported (Shaw et al., 1976; Arrowsmith et al., 1983). Hence,

$$K = \frac{^3\text{H dpm in lipid phase}}{^3\text{H dpm in aqueous phase}} \times F \qquad (1)$$

where K is the partition coefficient and F is the phase ratio factor (aqueous/lipid) included to compensate for the difference in volume between the aqueous and lipid phases.

^{3}H dpm in the aqueous phase was calculated from the radioactivity in the supernatant and ^{3}H dpm in the lipid phase was obtained from the difference in activity between the suspension and supernatant.

The release of entrapped steroid ester was expressed indirectly by determination of % retained (latency) at various time intervals. Thus,

$$L_t = \frac{e_0 - (f_t - f_0)}{e_0} \qquad (2)$$

where L_t is the latency at time t; e_0 is ^{3}H dpm entrapped at equilibrium; f_0 is ^{3}H dpm free at equilibrium and f_t is ^{3}H dpm free at time t.

The first-order rate constants of efflux (k) were calculated from least squares linear regression analysis of plots of ln latency versus time through equation (3):

$$L_t = L_0 \cdot e^{-kt} \qquad (3)$$

Results and Discussion

The degree of liposomal association of salbutamol was initially high after hydration of the conventional lipid film although a rapid decline to equilibrium occurred at approximately 3 h (Fig. 1). Further values of entrapment at 6 and 24 h were similar and so in all further studies liposome suspensions were shaken for 6 h to ensure that the entrapment values obtained were equal to those at equilibrium. The low entrapment efficiency is of the order expected for a hydrophilic species fully ionised at pH 7.4 (pKa of the basic moiety of salbutamol is 10.3, Newton and Kluza, 1978). The insignificant effect of cholesterol on salbutamol partitioning at equilibrium suggests that salbutamol resides in the aqueous channels of liposomes and remains unassociated with the lipid bilayers. Incorporation of the negatively charged lipid, DCP, into egg PC liposomes resulted in an increased uptake of salbutamol with an insignificant effect on vesicle size (Table 2). The inclusion of a charged lipid into lecithin bilayers increases liposome size through electrostatic separation of the bilayers (Bangham et al., 1967; Johnson, 1973) and is a method by which uptake of drugs associated with the entrapped aqueous volume may be improved (Alpar et al., 1981). The absence of a size increase in this case indicates that the charge was counteracted and suggests that the improved

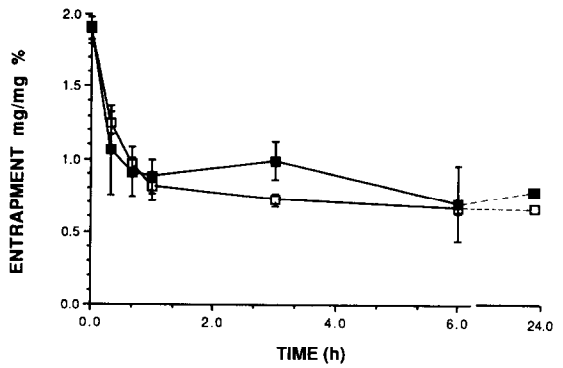

Fig. 1. Entrapment of salbutamol in MLVs formed using a conventional lipid hydration method. Symbols refer to lipid compositions of EPC:CHOL, 1:1 (■) and EPC (□). Each point is a mean of 3 determinations with S.D. bars.

TABLE 2

Entrapment of salbutamol and mean size of liposomes as a function of EPC/DCP molar ratio (n = 3, ± S.D.)

Lipid composition (mol%)		Entrapment (mg/mg %)	Mean size (μm)
EPC	DCP		
100	0	0.66 ± 0.04	1.26 ± 0.04
90	10	1.38 ± 0.02	1.38 ± 0.03
80	20	2.29 ± 0.14	1.05 ± 0.04
70	30	2.77 ± 0.21	1.11 ± 0.02

entrapment of salbutamol is due to the formation of a lipophilic ion-pair between the positive centre of salbutamol and the negative moiety of DCP. The increased salbutamol entrapment in the presence of DCP reflects the relative hydrophobicity of the complex in EPC/DCP liposome systems. For ion-pair formation, a 50:50 molar ratio of salbutamol and DCP would confer greatest hydrophobicity to salbutamol. In these experiments, this was equivalent to a lipid composition of 30 mol% DCP and 70 mol% EPC which resulted in the greatest liposomal association of salbutamol. For systems with a salbutamol: DCP molar ratio > 1, it is probable that a portion of the salbutamol resides unbound in the aqueous channels with the remainder anchored to a molar equivalent of DCP incorporated into the bilayers.

In the development of pressurised systems capable of forming drug loaded liposomes in situ following aerosol deposition, a micronised dispersion of drug in a phospholipid/chlorofluorocarbon solution is considered inappropriate owing to the following reasons

(1) Relatively high phospholipid concentrations (1–2% w/w) may encourage partial solubilisation of the suspended drug resulting in a tendency for crystal growth to occur on storage.

(2) It has been demonstrated (Malton et al., 1982) that suspended and solubilised components of a pressure pack can display different aerosol size distributions following actuation.

Solution phase pressure packs of salbutamol (4 mg/ml) and EPC (2% w/w) required the addition of 15% w/w ethanol as cosolvent in a 19/81, P11/P12 blend (formulation S1). The measured internal pressure was 536 kPa at 25 °C. The inclusion of 30 mol% DCP (expressed as a function of the lipid concentration) allowed a reduction of ethanol included in the blend to 8% w/w in 20/80, P11/P12 yielding the same internal pressure (formulation S2). Depositon profiles of emitted aerosol droplets for S1 and S2 using the MLI are represented as histograms in Fig. 2. In each case, no significant difference occurred between the deposition of EPC and salbutamol confirming each pack was a true homogeneous system. The respective recoveries of aerosolised phospholipid and salbutamol (expressed as percentages of valve delivery) were 89.6 ± 5.9% and 92.2 ± 6.5% for S1 and 88.6 ± 5.6% and 89.0 ± 6.7 for S2. In all cases, the majority of the aerosol cloud was lost before entry into the MLI. Kirk (1972) and Bell et al. (1973) reported that the formulation of isoprenaline as a solution-type pressure pack using ethanol as cosolvent dramatically reduced the therapeutic fraction of the emitted aerosols compared to suspension products. In this study, the inclusion of $\leq$ 15% w/w ethanol had no significant effect on the respirable component compared to formulations containing 2% w/w EPC as the sole constituent (Farr et al., 1987). This indicates that the poor MLI penetration efficiencies were primarily a function of retarded propellant evaporation due to the presence of EPC rather than ethanol.

Equilibrium partitioning data at 37 °C for salbutamol in liposomes formed on stages 3 and 4 were determined for deposited aerosols generated

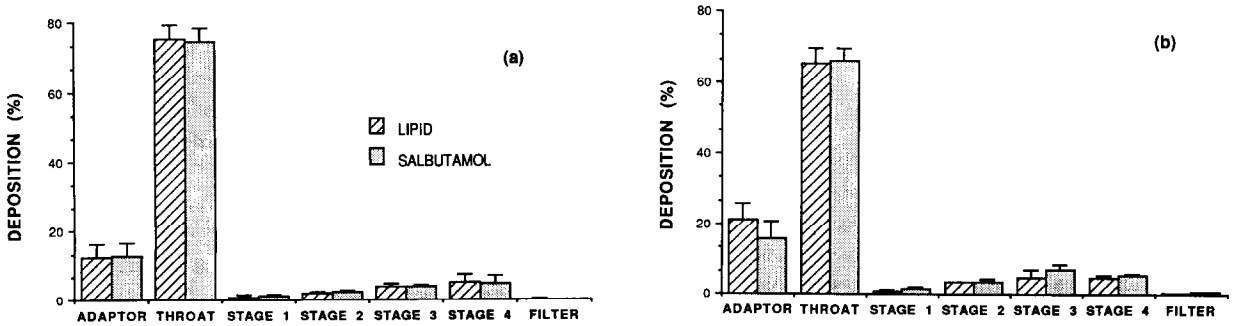

Fig. 2. Histograms demonstrating the mean relative retention ($n = 3$; S.D. bars) of aerosol droplets in the MLI following 40 actuations of pressure pack formulations S1 (a) and S2 (b).

TABLE 3

Equilibrium partitioning ($n = 3$, $\pm$ S.D.) of salbutamol in EPC:DCP (70:30 mol%) liposomes generated in the MLI by the aerosol method

MLI stage no.	Size fraction of original aerosol (μm)	Entrapment (mg/mg%)	Phase ratio ($\times 10^{-3}$)
3	3.59–5.51	2.67 ± 0.97	12.20 ± 6.13
4	1.25–3.59	2.65 ± 1.10	10.60 ± 2.49

from formulations S1 and S2. Negligible entrapment was measured with samples derived from S1 as only minuscule quantities of lipid and drug were detected in aliquots of liquid taken from the later stages of the MLI. The resultant phase ratio factors (aqueous/lipid) were disproportionately high and prohibited the accurate determination of the liposomally entrapped concentration of drug. For S2, where the lipid component contained 30 mol% DCP, partitioning of salbutamol into liposomes was observed (Table 3). It is likely that the high variability between samples was due to the low levels of salbutamol present in addition to the large aqueous/lipid phase ratios. Another consequence was that drug efflux experiments could not be undertaken as further dilution of equilibrated samples resulted in salbutamol concentrations below the assay sensitivity.

Studies with liposomes (MLVs) prepared by conventional methods have illustrated that hydrophobic drugs are incorporated into liposomes to a higher degree than hydrophilic moieties (Juliano and Stamp, 1978). The degree of liposomal association of steroidal esters can be increased by extending the 21-acyl chain length to yield partition coefficients greatly in favour of the lipid phase (Shaw et al., 1976; Arrowsmith et al., 1983). Such compounds would assist greatly in the accurate detection of entrapment and efflux in suspensions composed of very low phospholipid concentration formed by the aerosolised method. Moreover, the use of radiolabelled drug would necessitate the use of only trace levels precluding problems in the formulation of solution phase pressure packs.

Similar to the salbutamol/lipid formulations, the pattern of deposition of lipid and hydrocortisone ester in the MLI were indicative of a homogeneous system. Less EPC was included in this case which explains the higher respirable fractions of each component ($20.9 \pm 2.8\%$ for EPC and $20.2 \pm 2.7\%$ for the steroid ester). Table 4 compares the EPC/saline partition coefficient for conventional and aerosolised systems. There was no significant difference between the values (which also concur with previously reported data, Arrowsmith et al., 1983) suggesting that liposomes produced from phospholipid aerosols possess similar structural conformations as those prepared by more conventional means. The similar solute partitioning in liposomes formed on stages 3 and 4 of the impinger further suggest this is independent of the aerosol size fraction from which the liposomes originated.

Additional evidence of similar structural characteristics arose from comparison of steroid efflux data (Fig. 3) following a 1 in 10 dilution of liposome suspensions formed on stage 4 of the MLI and conventionally prepared liposomes. In both systems an initial period of rapid release, also observed with other compounds (Layton et al., 1979; Alpar et al., 1981; Arrowsmith et al., 1983), preceded a first-order efflux process. Half-lives of efflux 2 h post dilution were similar: 48.3 h and 50.2 h for the aerosolised and conventional systems, respectively.

The overall conclusion arising from these studies is that partitioning and efflux studies of drugs in liposomes prepared by conventional means can serve as a useful screening process for prospective drug candidates intended for incorporation into the aerosol generated systems. Potential problems with respect to the use of hydrophilic drugs were highlighted. Formulation of pressure packs con-

TABLE 4

Comparison of the partitioning (mean $\pm$ S.D., 37 °C) of hydrocortisone 21-octanoate in EPC liposomes formed by the conventional or aerosolised method

System	Partition coefficient (EPC/saline) $\times 10^{-3}$	Phase ratio (Aqueous/Lipid)
Conventional	5.50 ± 1.04 ($n = 4$)	300
Aerosolised		
stage 3	4.50 ± 1.66 ($n = 3$)	4919 ± 558
stage 4	5.68 ± 0.95 ($n = 3$)	2793 ± 479

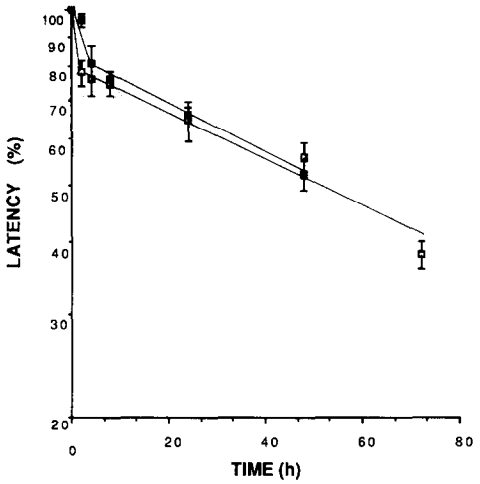

Fig. 3. Release of hydrocortisone octanoate from EPC liposomes formed by conventional (□) and aerosolised (■) methods. Each point is a mean of 3 determinations with S.D. bars.

taining such drugs will necessitate the inclusion of low volatility cosolvents which will diminish the aerosol fraction available for inhalation. Dependent on the drug's physicochemical character, liposomal entrapment is likely to be low and therefore will require the delivery of a relatively high lipid dose. The presence of high lipid concentrations within the pressure pack formulation will further complicate the production of a respirable aerosol.

Results with the hydrophobic model compound, however, suggest lipophilic drugs (eg. amphotericin B) might usefully be employed in such systems. Rendering a hydrophilic drug more lipophilic through either ionic complexation with a lipid of opposite charge incorporated in the liposome bilayer, or by the chemical synthesis of a lipophilic prodrug may improve the applicability of this type of compound. The potential toxicity of charged lipids in-vivo (Adams et al., 1977) would, in reality, invalidate the former approach, whilst with prodrugs of negligible intrinsic pharmacological activity, drug availability is likely to be a complex function of liposomal efflux as well as prodrug to drug reaction kinetics.

References

Adams, D.H., Joyce, G., Richardson, V.J., Ryman, B.E. and Wisniewski, H.M., Liposome toxicity in the mouse central nervous system. *J. Neurol. Sci.*, 31 (1977) 173–179.

Alpar, O.H., Bamford, J.B. and Walters, V., The in-vitro incorporation and release of hydroxycobalamin by liposomes. *Int. J. Pharm.*, 7 (1981) 349–351.

Arrowsmith, M., Hadgraft, J. and Kellaway, I.W., The in-vitro release of steroids from liposomes. *Int. J. Pharm.*, 14 (1983) 191–208.

Bangham, A.D., De Gier, J. and Greville, G.D., Osmotic properties and water permeability of phospholipid liquid crystals. *Chem. Phys. Lipids*, 1 (1967) 225–246.

Bell, J.H., Brown, K. and Glasby, J., Variation in delivery of isoprenaline from various pressurised inhalers. *J. Pharm. Pharmacol.*, 25 (1973) 32P–36P.

Byron, P.R., Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. *J. Pharm. Sci.*, 75 (1986) 433–438.

Farr, S.J., Kellaway, I.W. and Carman-Meakin, B., Assessing the potential of aerosol generated liposomes from pressurised pack formulations. *J. Contr. Rel.*, 5 (1987) 119–127.

Gonda, I., Drugs administered directly to the respiratory tract: modelling of the duration of effective drug levels. *J. Pharm. Sci.*, 77 (1988) 340–346.

Gonda, I., Khalik, A.F.A.E. and Britten, A.Z., Hexamethylmelamine aerosols prepared in an evaporation–condensation generator. *Int. J. Pharm.*, 27 (1985) 255–265.

Hickey, A.J. and Byron, P.R., Preparation, characterisation, and controlled release from coprecipitates of fluorescein and magnesium hydroxide. *J. Pharm. Sci.*, 75 (1986) 756–759.

Johnson, S.M., The effect of charge and cholesterol on the size and thickness of phospholipid vesicles. *Biochim. Biophys. Acta*, 307 (1973) 27–41.

Juliano, R.L. and Stamp, D., Interactions of drugs with lipid membranes. Characteristics of liposomes containing polar or nonpolar anti-tumour drugs. *Biochim. Biophys. Acta*, 586 (1978) 137–145.

Kirk, W.F., In-vitro method of comparing clouds produced from inhalation aerosols for efficiency in penetration of airways. *J. Pharm. Sci.*, 61 (1972) 262–264.

Kleinberg, M.L., Stauffer, G.L. and Latiolais, C.J., Stability of five liquid drug products after unit dose repackaging. *Am. J. Hosp. Pharm.*, 37 (1980) 680–682.

Layton, D., De Meyere, J., Collard, M.P. and Trouet, A., The accumulation by fibroblasts of liposomally encapsulated vinblastine. *Eur. J. Cancer*, 15 (1979) 1457–1483.

Malton, C.A., Hallworth, G.W. and Padfield, J.M., The association and particle size distribution of drug and surfactant discharged from a metered dose inhalation aerosol. *J. Pharm. Pharmacol.*, 34 (1982) 65P.

Martin, G.P., Kellaway, I.W. and Marriot, C., The solubilisation of progesterone by mixed bile salt–phospholipid sols. *Chem. Phys. Lipids*, 22 (1978) 227–238.

Newton, D.W. and Kluza, R.B. pK_a values of medicinal compounds in pharmacy practice. *Drug. Intell. Clin. Pharm.*, 12 (1978) 546–554.

Shaw, I.H., Knight, C.G. and Dingle, J.T., Liposomal retention of a modified anti-inflammatory steroid. *Biochem. J.* 158 (1976) 473–476.

Taylor, K.M.G., Taylor, G., Kellaway, I.W. and Stevens, J., The pharmacokinetics of sodium cromoglycate following inhalation of free and liposomal cromoglycate by human volunteers. *Proc. Br. Pharmacol. Soc.*, April, 1988, C26.

Woolfrey, S.G., Taylor, G., Kellaway, I.W. and Smith, A., Pulmonary absorption of liposome-encapsulated 6-carboxyfluorescein. *J. Contr. Rel.*, 5 (1988) 203–209.