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International Journal of Pharmaceutics 287 (2004) 113–121
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

# A study of liposome formation using a solution (isoperibol) calorimeter

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Received 12 May 2004; received in revised form 31 August 2004; accepted 5 September 2004

**Abstract**

A solution (isoperibol) calorimeter has been employed to study the process of formation of phospholipid vesicles from natural and synthetic phospholipid films. Phospholipid films were hydrated in the solution calorimeter at temperatures exceeding the main phospholipid phase transition temperature, with continuous agitation to ensure conversion of the hydrating bilayers into multilamellar liposomes. It was seen that retention of chloroform in phospholipid films altered the apparent enthalpy change of vesicle formation to a far greater extent than would be expected from the contribution of the enthalpy of solution of chloroform; this indicates that chloroform alters the hydration process of the lipid. The overall measured enthalpy change for the formation of egg phosphatidylcholine vesicles was exothermic, whilst that for dimyristoylphosphatidylcholine was endothermic. This difference, it is suggested, results from the influence of the hydrocarbon chains mostly on the hydration process and also on the process of vesicle formation.


*Keywords:* Enthalpy; Liposome; Phosphatidylcholine; Phospholipid; Solution calorimetry; Thermal analysis

## 1. Introduction

Despite the increasing applications of phospholipid vesicles in the pharmaceutical, biotechnological and biological arenas, there is still a lack of fundamental knowledge on the thermodynamics of vesicle formation. Previous studies on the formation of phospholipid vesicles have focused mainly on the development of preparation procedures, geometrical aspects (Lasic, 1988; Thompson, 1990) and the conformational behaviour of the bilayer (Lipowsky, 1991). However, calorimetric data are helpful for understanding the hydration of lipids and the formation and stability of phospholipid vesicles.

The energy associated with formation of phospholipid vesicles is difficult to investigate due to the com-

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doi:10.1016/j.ijpharm.2004.09.004

plexity of the physicochemical parameters associated with the organization of phospholipids in water and organic solvents. Due to their lyotropic mesomorphism, phospholipids do not pass from the crystal to a true solution when interacting with water. Depending on the water content and temperature, different hydrated phases form until, at infinite dilution, thermodynamically stable structures are formed. The chemical structure of the phospholipid determines the swelling behaviour on hydration. Pure phosphatidylcholines (PCs) swell by sorbing up to approximately 40% water content, and above this limit a two-phase system comprising a fully hydrated lamellar phase and water is present (Chapman et al., 1967). The swelling of PC bilayers is initiated and facilitated above the main phospholipid phase transition temperature ($T_m$). Essentially, all neutral and isoelectric phospholipids exhibit a limiting swelling behaviour in water similar to pure PC. The thermodynamically stable structure of neutral and isoelectric phospholipids in excess water at temperatures above the $T_m$ is predicted to be the fully hydrated multilamellar vesicle (MLV) (Hauser, 1993).

Calorimetric data on the hydration of phospholipid bilayers have been obtained by use of isothermal microcalorimetry of water sorption (Markova et al., 2000), humidity titration calorimetry (Binder et al., 1999), differential scanning calorimetry (DSC) (Ulrich et al., 1994; Bach and Miller, 1998) and differential thermal analysis (DTA) (Lundberg et al., 1978; Bach and Miller, 1998). However, the thermodynamic instability of the intermediate (hydrated) states makes it difficult to obtain reproducible calorimetric data on the overall formation process. To date, no systematic study on the thermodynamics of phospholipid vesicle formation has been carried out, though solution calorimetry has provided some experimental information on the formation of dimyristoylphosphatidylcholine (DMPC) vesicles (Craig et al., 1990).

Solution calorimetry is a technique which measures the heat change over time resulting from the mixing of a solid and a liquid, or two liquids, in a temperature controlled environment. Although termed "solution calorimetry" the technique is employed in this study to investigate the enthalpy changes associated with hydration of a phospholipids film and its dispersion to form a liposome suspension. The commercial solution calorimeter that was used is in fact an isoperibol calorimeter, which functions by following a slow temperature drift towards the temperature of a water bath. Although the term solution calorimeter is frequently used, as the samples studied here do not go into solution the term isoperibol calorimeter will be used throughout this paper. The principal advantage of isoperibol calorimetry for studies of phospholipid systems lies in the direct measurement of the overall energy change associated with the formation of vesicles from phospholipid films. For the purposes of this work, the enthalpies of formation of MLVs from a natural and synthetic PC were studied in order to shed light on the mechanism of liposome formation.

## 2. Materials and methods

### 2.1. Materials

Dimyristoyl-L-α-phosphatidylcholine (DMPC) (approximately 99%) was purchased from Sigma Chemical Co., St. Louis, USA. Egg phosphatidylcholine (PC) was a gift from Lipoid (Lipoid GmbH, Ludwigshafen, Germany). Lipoid E PC (98.0% phosphatidylcholine; 0.1% phosphatidylethanolamine; 0.2% lysophosphatidylcholine; 1.0% sphingomyelin) was used without further purification. Egg PC was also supplied as Ovothin 200 (97.3% phosphatidylcholine; 0.0% phosphatidylethanolamine; 0.9% lysophosphatidylcholine; 1.9% sphingomyelin; Lucas Mayer, Hamburg, Germany), which was chromatographically purified (Lea et al., 1955) and fractions selected having $R_f$ equivalent to Lipoid E PC. Chloroform and potassium chloride (AnalaR grade) were supplied by BDH Laboratory Supplies, Poole, UK, phosphotungstic acid (analytical reagent grade) by Hopkin and Williams, Essex, UK, and phosphorous(V) oxide (99 %) by Avocado Research Chemicals Ltd., Lancaster, UK. All reagents were used as received. Deionised water was further purified by passing through an elgastat option 3 water purification unit (ELGA, High Wycombe, England).

### 2.2. Isoperibol calorimetry

All experiments were carried out in a Thermometric 2225 precision solution calorimeter. The sample was held in a sealed 1.1 ml crushable glass ampoule (Thermometric AB, Järfälla, Sweden) and placed in

the reaction vessel containing water as a dispersant. The reaction vessel was mounted in one channel of the Thermometric 2277 thermal activity monitor (TAM) (Thermometric AB, Järfälla, Sweden), which was used as a precise water bath only. After thermal equilibrium was reached (the instrument software permits an option to check that the system is reasonably stable and that an experiment can begin), an electrical calibration took place and then the ampoule was broken within the calorimeter vessel and the sample and the dispersant mixed. The contribution of the heat of ampoule breaking was investigated by breaking empty, sealed ampoules in the reaction vessel of the isoperibol calorimeter filled with 100 ml deionised water at 25 or 37 °C. The average heat of ampoule breaking was $8.3 \pm 1.6$ mJ ($n = 3 \pm$ S.D.), which was negligible in relation to the enthalpies measured in the experiments.

The reliability of the equipment was determined by measuring the change in enthalpy of solution of a standard, potassium chloride (KCl), in deionised water at 25 °C. The agreement between the experimental ($\Delta H = 235.8 \pm 0.87$ J/g, $n = 3 \pm$ S.D.) and the literature ($\Delta H = 235.8 \pm 0.23$ J/g (NIST: SRM 1655)) values indicated good isoperibol calorimeter performance.

### 2.3. Formation of phospholipid films and vesicles

The change in enthalpy on formation of egg PC or DMPC vesicles was measured by mixing phospholipid films with water in the calorimeter. Films were deposited on the inner surface of isoperibol calorimeter ampoules from solutions in chloroform. The required amount of phospholipid was directly weighed into the ampoule. Chloroform (0.5 ml) was added into the ampoule, and the ampoule gently agitated to ensure complete dissolution. Organic solvent was removed under a constant nitrogen flux (7.5 L/min) for 2 h. The drying process was completed by placing the ampoules under vacuum in a desiccator over phosphorous pentoxide for a further 17 h. Each film weight was equal to the initial amount of phospholipid deposited in the empty ampoule, indicating complete removal of organic solvent. The film-containing ampoules were closed with a silicone stopper, sealed twice with beeswax and immediately used in the experiment. The ampoules were first temperature-equilibrated and then an electrical calibration was performed prior to ampoule breaking into 100 ml deionised water under continuous agitation at 600 rpm for 1 h. To ensure formation of liposomes the experimental temperature was higher than the main phase transition temperature ($T_m$) of the phospholipid under study: 25 °C or 37 °C for egg PC and DMPC, respectively. After readings returned to the baseline, a further electrical calibration was performed. Preliminary experiments showed that under the same experimental conditions, the heat flow value approached that of the baseline after about 5 min for DMPC and 30 min for egg PC. To permit comparisons between data sets, all reactions were recorded for 1 h following ampoule breakage. The overall experimental duration exceeded 1 h, since electrical calibrations were performed before and after ampoule breakage to calculate the calibration constant.

In preliminary experiments, egg PC films (purified Ovothin 200) with varying amounts of residual chloroform were formed. Solutions of egg PC in chloroform were produced in glass-crushing ampoules and the organic solvent removed under a constant nitrogen flux (7.5 L/min). Interrupting the evaporation process at selected time intervals allowed production of egg PC films containing differing amounts of chloroform (5–30 mg). In each case, the film weight was determined by weight difference between the ampoule plus film and the weight of the empty ampoule using a five figures balance. If the film weight did not correspond to the initial amount of phospholipid deposited in the empty ampoule, then the excess weight was chloroform. These films were mixed with 100 ml water at 25 °C in the isoperibol calorimeter under continuous stirring for 1 h, and the calorimetric signal was recorded. All films contained 120 mg egg PC. The amount of chloroform in the film was determined by weight difference. Experimental values of the heat of solution of chloroform in water were also determined.

### 2.4. Transmission electron microscopy (TEM) analysis

Samples were collected from the reaction vessel after isoperibol calorimetry analyses and were examined immediately using TEM. The samples were deposited on copper grids (400 mesh), negatively stained with phosphotungstic acid (1%, w/v, aqueous solution) and viewed using a Philips CM 120 BioTWIN microscope (Phillips, UK).

### 2.5. Pre-hydration of egg PC phospholipid films

In some experiments, egg PC films were hydrated prior to analysis in the isoperibol calorimeter. Using a micro-syringe (Hamilton, UK), 27.5 or 160.5 μl deionised water at ambient temperature was added to 120 mg egg PC films. The sample-containing ampoule was hand-rotated to ensure homogeneous distribution of the water over the surface of the film. The ampoule was sealed with beeswax and loaded into the calorimeter vessel at 25 °C. Temperature equilibration and calibration were necessary before dispersing the film in the calorimeter vessel. Therefore, a total of approximately 2 h passed between pre-hydration of the film and mixing of the film with the water in the calorimeter vessel, the time being kept as short as possible to minimise the opportunity for oxidation.

### 2.6. Data analysis

The heat evolved during the experimental reaction was calculated by multiplying a calibration constant obtained in an electrical calibration experiment (the input from the heater divided by the temperature change), by the experimentally measured temperature change.

## 3. Results and discussion

### 3.1. Residual solvent

When films containing 120 mg purified Ovothin 200 and no residual solvent were mixed with water at 25 °C the average enthalpy of reaction was −6.7 (±0.9) J/g ($n = 5 \pm$ S.D.). However, the presence of 4–20% chloroform (w/w) in the film affected the enthalpy of reaction as shown in Fig. 1. Values greater than 20% chloroform were not investigated, as investigators would not attempt to form liposomes from films having such a high proportion of residual solvent. Each symbol, except those at 0 and 100 wt.% chloroform, represents the result of a single experiment, since the final residual solvent content of films could not be accurately controlled using the evaporation protocol employed. Data at 0 and 100 wt.% chloroform are the mean of five experiments ± S.D.

The data in Fig. 1 indicate either that there is a linear effect of chloroform load up to a certain limiting value, after which there are no further changes in enthalpy change as concentration is increased, or more probably that there is a negative deviation from linearity, in other words the presence of small quantities of chloroform (4–20%, w/w) markedly changed the enthalpy of liposome formation from egg PC films. Approximately 1 ml of chloroform dissolves in 200 ml of water (Merck Index), so there is no prospect that the limit of chloroform aqueous solubility is the cause of the deviation from linearity in Fig. 1. Consequently, it must be concluded that the chloroform alters the process itself. It is most probable that the hydration process is facilitated by the presence of chloroform in the phospholipid, hence giving a net favourable change in enthalpy. It is also clear that the smallest amount of chloroform that we tested has a substantial impact on the formation, clearly greater than expected simply from the enthalpy of solution of the chloroform in water. Residual chloroform present in phospholipids films is likely to cause variability in the formation, behaviour and stability of liposomes.

A further consequence of the measured effect of chloroform was the realisation that consistency of data would only be achieved by ensuring complete removal of the organic solvent from phospholipid films (as it is almost impossible to standardise any concentration other than 0%). Complete removal of the solvent was arduous due to the small opening of the ampoule (∼3 mm) and required nitrogen flushing and vacuum evaporation for a total of 19 h in order to return to a mass equivalent to the added mass of phospholipids.

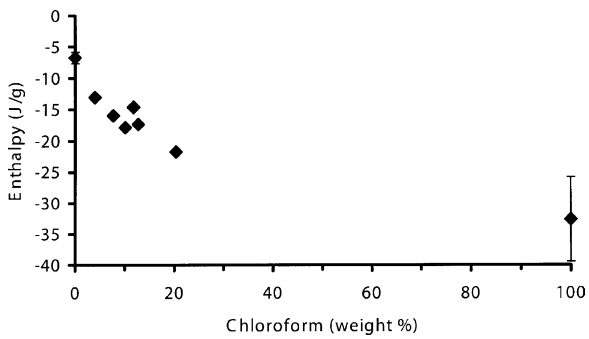


Fig. 1. Enthalpies of reaction of egg PC films containing various amounts of chloroform in water at 25 °C.

### 3.2. Enthalpy of formation of egg phosphatidylcholine vesicles

Stirring is required to facilitate equilibration of the temperature of the liquid inside the reaction vessel, and contributes to the mixing of the sample and dispersant. However, stirring will also affect the equilibration temperature of the reaction liquid. Consequently, in a preliminary experiment the stirrer was operated at 300 or 600 rpm for 24 h to investigate the effect of the stirrer. There was no difference between the observed changes in enthalpy of reaction, indicating that heat arising from stirring was adjusted for, using the information obtained from electrical calibration, which was carried out for each experiment. As the higher stirring rate was likely to be advantageous in dispersing hydrated phospholipids, all work was carried out at 600 rpm.

The reaction between an egg PC film and water at 25 °C in the isoperibol calorimeter with 1 h of continuous agitation resulted in formation of multilamellar phospholipid vesicles (Fig. 2A) and produced an overall exothermic response in all cases, independent of the source of PC (Fig. 3, Table 1). The heat of reaction measured in the solution calorimeter is the sum of the heats of all reactions taking place during the process under investigation. The formation of phospholipid vesicles by the film hydration method comprises many stages: hydration of the phospholipid, growth of myelin figures, detachment of the growing blisters, and self-closure of the phospholipid bilayer into vesicles (Lasic, 1988). Each of these processes can be affected by the sample history and the experimental conditions, so the film preparation, storage and the hydration processes were strictly controlled and reproduced for each experiment, minimizing experimental variability.

The interaction of egg PC with water has been plotted as heat flow versus time in Fig. 3 where the deviation from the baseline indicates the initiation of the reaction on breaking the ampoule. An endothermic (downward) peak followed by an exothermic (upward) peak was a common feature for all egg PC samples. At any given time, the heat flow recorded corresponds to the sum of the reactions taking place, for instance a neg-

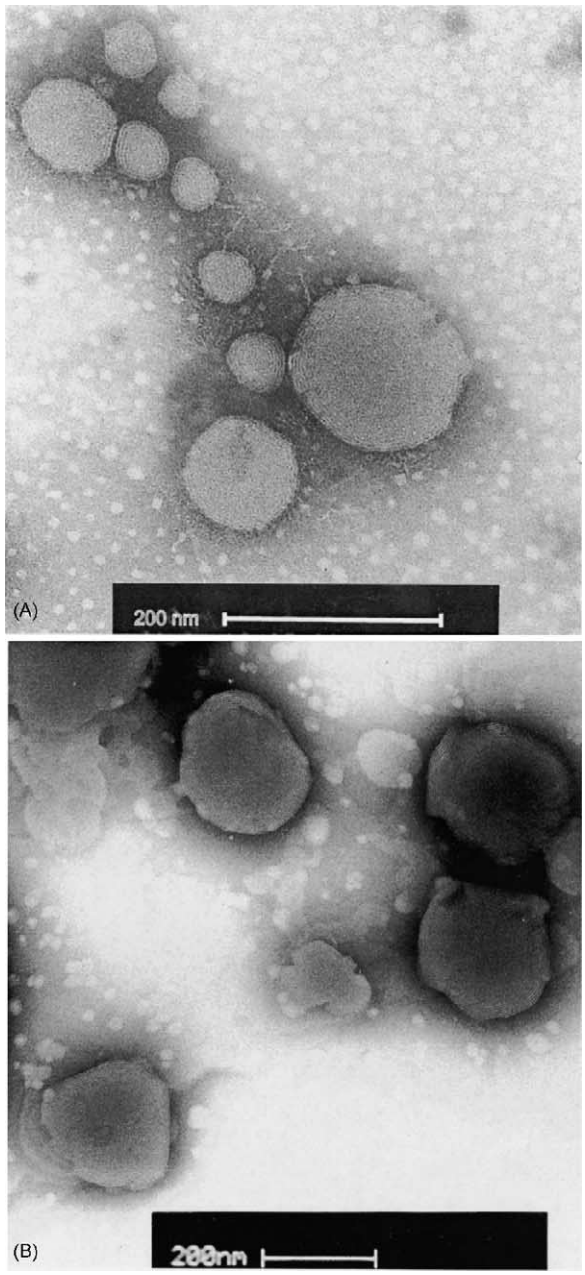


Fig. 2. Transmission electron micrographs of dispersions within the calorimeter formed from (A) egg PC film and (B) DMPC film.

Table 1
Enthalpies of reaction for egg PC in water at 25 °C ($n = 5 \pm$ S.D.)

| Phospholipid source | Weight of film (mg) | $\Delta H_m$ (kJ/mol) | $\Delta H$ (J/g) |
|---|---|---|---|
| Lipoid E PC | 122.9 (±1.4) | −5.1 (±0.7) | −6.7 (±0.9) |
| Purified Ovothin 200 | 119.2 (±1.1) | −5.2 (±0.7) | −6.8 (±0.9) |

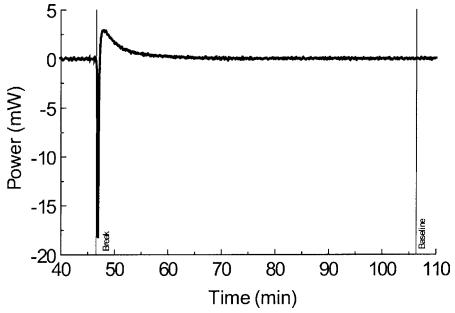


Fig. 3. Typical power–time curve for the reaction of an egg PC film (120 mg) in water at 25 °C.

ative heat flow value indicates that the exothermic reactions at that point are greater in magnitude than the endothermic ones. For egg PC the process of formation of liposomes comprised a net disruption of intermolecular interactions (endothermic, e.g. disruption of the pre-existing headgroup-headgroup bonds) and a net formation of interactions (exothermic, e.g. hydration). The overall reaction depends on the magnitude of these processes.

In order to obtain the largest possible signal in the solution calorimeter, it was desirable to use a substantial mass of phospholipid in the film. The film thickness could possibly influence the formation of vesicles, so this was investigated using films containing either 120 or 160 mg egg PC (purified Ovothin 200). The average molar enthalpy for the thicker films ($n = 3 \pm$ S.D.) was −5.5 (±0.7) kJ/mol, which is in good agreement with the values reported for films containing 120 mg egg PC (Table 1), indicating that (a) all the material contributed to the calorimetric response and (b) the calculated enthalpies are meaningful; and (c) film thickness is not critical, over the range tested.

### 3.3. Thermal response of hydrated egg phosphatidylcholine films

Films containing 120 mg egg PC (Lipoid E PC) were hydrated with 27.5 or 160.5 μl water prior to isoperibol calorimetry investigations (equivalent to 9.8 and 57.1 mol water/mol phospholipid, respectively). A small exothermic peak was observed when previously hydrated films were added to excess water at 25 °C and stirred for 1 h in the isoperibol calorimeter vessel. The average heat of reaction ($n = 3 \pm$ S.D.) for films containing 120 mg egg PC and 27.5 μl added water or 120 mg egg PC and 160.5 μl added water was $-0.1 \pm 0.0$ J in both cases. Since the heat of ampoule breaking was less than 10 mJ, the heat of reaction obtained for hydrated films was significant. The average molar enthalpy of three experiments was −0.6 kJ/mol (Table 2).

Table 2
Enthalpies of reaction for egg PC films containing different amounts of added water, mixed with water at 25 °C

| Sample | $\Delta H_m$ (kJ/mol) (±S.D.) | $n$ |
|---|---|---|
| Anhydrous | −5.1 (±0.7) | 5 |
| Partly hydrated | −0.6 (±0.2) | 3 |
| Fully hydrated | −0.6 (±0.1) | 3 |

The amount of water that can associate with neutral phospholipids is limited, and any additional water remains in the lipid dispersion as bulk water (McIntosh and Magid, 1993). X-ray diffraction studies have indicated that when egg PC was directly mixed with bulk water, the maximum number of water molecules per lipid molecule (nw) was less than or equal to 27 mol water/mol phospholipid (Klose et al., 1988). Klose et al. (1988) concluded that this represented the real swelling capacity of egg PC. Other studies have reported that the maximum number of water molecules a phosphatidylcholine molecule can "accommodate" before an excess phase forms ranges from 20 to 34 water molecules per lipid molecule (Offringa et al., 1987; McIntosh and Magid, 1993). The differences in the reported values probably result from difficulties in determining the precise water content at which an excess water phase forms. It has also been argued that the formation of defects in and between the multilayers caused by the preparation method influences the maximum water content determined (Klose et al., 1988). The films in this investigation were partly (9.8 mol water/mol phospholipid) and fully hydrated (57.1 mol water/mol phospholipid) prior to calorimetric analysis.

Lipid hydration comprises two stages (McIntosh and Simon, 1986). Firstly, the water locates in the head group region of the bilayers. In the second stage the water occupies the space between opposing bilayers. X-ray studies (Pearson and Pascher, 1979) have shown that fully hydrated egg PC contains less than 10 water molecules per lipid molecule in the head group region of the bilayers, whereas the remaining molecules of hydration water (outer waters of hydration) are located

in the water space between adjacent bilayers. In these investigations, when partly hydrated films (9.8 mol water/mol phospholipid) were added to the bulk water in the isoperibol calorimeter the head group regions of the bilayers were already hydrated and the additional water would be expected to hydrate the space between bilayers. However, no difference was observed in the thermal response of partly and fully hydrated films indicating that the hydration energy of the outer water molecules ($nw > 10$) was not detected by isoperibol calorimetry. The response observed in the calorimeter would correspond to the transformation of the hydrated intermediates into their equilibrium state. The disappearance of the endothermic peak, associated with disruption of intermolecular interactions, indicated that before hydration could take place it was necessary to disrupt the molecular arrangement of the phospholipid in the film.

The results obtained for anhydrous, partly hydrated and fully hydrated egg PC films are summarised in Table 2. It may be suggested that the difference in the enthalpy change between anhydrous and previously hydrated phospholipids, corresponded to the enthalpy of hydration (−4.5 kJ/mol). The observation that previously hydrated films still gave a measurable response indicated that the heat evolved during the reaction in the isoperibol calorimeter was associated with formation of vesicles and not only with hydration.

### 3.4. Enthalpy of formation of DMPC vesicles

When films containing 25 and 120 mg DMPC were measured in the isoperibol calorimeter (water at 37 °C) the reaction was endothermic and MLVs were formed (Fig. 2B). As with egg PC, the mass of sample did not alter the measured molar enthalpy change for 25 and 120 mg DMPC films, which were 31.1 (±3.8) and 35.7 (±3.7) kJ/mol, respectively. These values are in good agreement with a previous isoperibol calorimetry study for 10 mg DMPC in water at 37 °C, where a value of 35.4 kJ/mol was reported (Taylor et al., 1990), indicating that all the sample was involved in the process that was measured.

The power–time curves for DMPC showed a single endothermic peak (Fig. 4), indicating that in the formation of DMPC vesicles either the magnitude of the endothermic reactions was greater than that of the exothermic reactions or that exothermic reactions did not take place. The absence of exothermic processes is unlikely because the formation of vesicles in the calorimeter required hydration of the phospholipid film. The uptake of the first three to four water molecules by a DMPC film was previously measured as an exothermic process by sorption microcalorimetry, whereas the remaining water was incorporated endothermically (Markova et al., 2000). The most likely interaction sites for the first few water molecules were believed to be the oxygen atoms of the choline-phosphate groups. The remaining water molecules probably interacted with the quaternary ammonium group and the ether-like oxygen atoms. Although in the sorption microcalorimeter the phospholipid film adsorbed water from the vapour phase (Markova et al., 2000) and in the isoperibol calorimeter the film was directly mixed with bulk water, the study by Markova et al. (2000) illustrates the complexity of the water sorption by phospholipid systems and shows that incorporation of water involves exothermic and endothermic reactions.

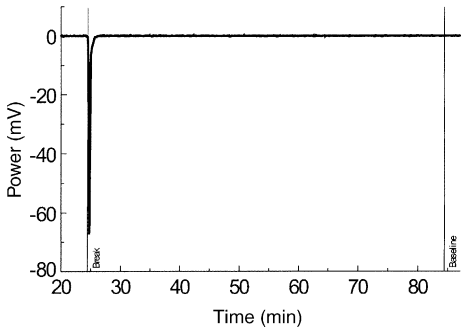


Fig. 4. Typical power–time curve for the reaction of a DMPC film (25 mg) in water at 37 °C.

### 3.5. Effect of phospholipid structure on the energetics of liposome formation

The experimental conditions, temperature of reaction above the $T_m$ of the phospholipid and continuous agitation, were chosen to favour vesicle formation. However, to allow a better comparison between the two phospholipids, egg PC vesicles were also formed at 37 °C.

The response for the formation of egg PC vesicles was exothermic at both 25 and 37 °C. The transformation of an egg PC film into vesicles resulted in an overall exothermic response, whereas the formation of

DMPC vesicles appeared as an endothermic process. It is clear that the film hydration/disruption and the vesicle formation are very different for these two materials, with one being enthalpically favoured and the other not. As values for free energy change have not been determined, it is not possible to calculate values for entropy change for the systems studied. The process of vesicle formation will be expected to be greatly affected by entropy.

Phospholipid hydration is the process producing the biggest effect on the overall enthalpy of vesicle formation. Therefore the differences observed in the enthalpies of formation of egg PC and DMPC vesicles may be related to differences in the enthalpies of hydration. The energetics of phospholipid hydration have been related to the number of water molecules taken up by the phospholipid (McIntosh and Magid, 1993). The amount of water sorbed from the vapour phase is associated with the number of unsaturations, length and physical state of the hydrocarbon chains and with the methylation and the electrical charge of the head group (Jendrasiak and Smith, 2000). While egg PC and DMPC have the same zwitterionic polar head group, they differ in the length and structure of the hydrocarbon chain. The $C_{14}$ hydrocarbon chains of DMPC are fully saturated (Silvius, 1993). In egg PC, the predominant chain lengths in the 1 and 2 positions are 16 and 18 carbon atoms, respectively. The fatty acids attached in positions 1 and 2 of egg PC are usually different from each other; that in position 2 is unsaturated, while that in position 1 is usually saturated (Huang, 2001). Comparison of the water adsorbed by unsaturated and saturated phospholipids showed that the composition of the hydrocarbon chains affected the amount of water adsorbed (Jendrasiak and Smith, 2000); with the number of water molecules adsorbed increasing as the number of double bonds increased.

The power–time curves shown in Fig. 5 illustrate the differences in the response of 120 mg egg PC and DMPC. An endothermic peak at the beginning of the reaction was observed for both phospholipids. The reaction for egg PC (Fig. 5A) gave an endothermic peak followed by an exothermic peak (upward direction) while only a single, large endothermic peak was observed for DMPC (Fig. 5B). The overall reaction of a phospholipid film in water was exothermic for egg PC and endothermic for DMPC indicating that the magnitude of the reactions taking place depended on the

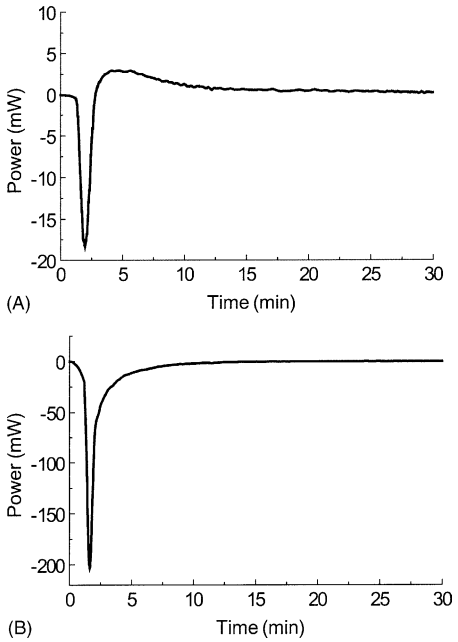


Fig. 5. Power–time curves for the reaction of (A) 120 mg egg PC and (B) 120 mg DMPC in water at 25 °C and 37 °C, respectively.

phospholipid composition. It was also noticeable that although both traces shown in Fig. 5 corresponded to films containing 120 mg phospholipid, the reaction for egg PC occurred over a longer time period. This indicates that the process of hydration and subsequent formation of vesicles was slower for egg PC than for DMPC. This may result from egg PC being a complex mix of phospholipids of differing chain lengths having varying degrees of saturation, whilst DMPC is a single synthetic compound.

## 4. Conclusions

The ability of isoperibol calorimetry to supply information on the process of formation of phospholipid vesicles from natural and synthetic phospholipid films has been demonstrated.

Formation of phospholipid vesicles from dry films requires hydration in the first stages, followed by rearrangement of the hydrated intermediates into MLVs (Lasic, 1988). Dry phospholipid films were hydrated in the isoperibol calorimeter at temperatures above the $T_m$ of the phospholipid with continuous agitation to ensure

the transformation of the hydrating bilayers into MLVs. The comparison between anhydrous and hydrated egg PC films indicated that the enthalpic response obtained in the isoperibol calorimeter for dry films corresponded to the sum of the different processes taking place.

The overall measured enthalpy of formation of egg PC vesicles was exothermic and that for DMPC vesicles was endothermic. The differences in the thermal response observed for egg PC and DMPC in excess water are likely to be related to the influence of the hydrocarbon chains both on the hydration process and on the evolution of the intermediate hydrated structures. The power–time measurements allowed estimation of the duration of the process under investigation. It was shown that DMPC vesicles formed more quickly than egg PC vesicles. This observation could be related to the formation of more intermediate structures in egg PC systems due to the higher mobility of the bonds in unsaturated molecules and a more favoured interaction with water. The applicability of isoperibol calorimetry to the study of phospholipids systems has been established. A detailed study in the future, using synthetic phospholipids, having variations in hydrocarbon chain length and degree of saturation, will permit a detailed and systematic understanding of the processes involved in the formation of vesicles from a hydrating film.

The different responses obtained for the phospholipid systems under investigation indicated that isoperibol calorimetry was capable of discriminating between compositions suggesting the potential application of the technique in qualitative identification of impurities and residual solvent.

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