



# Application of isothermal titration calorimetry for detecting lipid membrane solubilization

H. Heerklotz <sup>a</sup>, G. Lantzsch <sup>a</sup>, H. Binder <sup>a</sup>, G. Klose <sup>a</sup>, A. Blume <sup>b</sup>

<sup>a</sup> Institut für Experimentelle Physik I, BIM, Universität Leipzig, Linnéstraße 5, D-04103 Leipzig, Germany

<sup>b</sup> Fachbereich Chemie, Universität Kaiserslautern, Erwin-Schrödinger-Straße, Postfach 3049, D-67653 Kaiserslautern, Germany

Received 25 October 1994; in final form 31 December 1994

---

## Abstract

Isothermal titration calorimetry is presented to be an excellent method of detecting lipid membrane solubilization upon the addition of detergents. As an example, the titration of the non-ionic detergent C<sub>12</sub>EO<sub>6</sub> to the lipid POPC is studied. The main contribution to the measured titration heat is the detergent's enthalpy difference upon transfer between micelles and membranes. The detergent added as micelles is transferred into membranes up to the saturating detergent per lipid mol/mol ratio,  $R_{\text{sat}} = 1.45 \pm 0.07$ . Beyond  $R_{\text{sat}}$  the membranes are solubilized to micelles. Subsequently, the titration heat changes its sign from endothermic to exothermic. After complete membrane dissolution at  $R_{\text{sol}} = 5.0 \pm 0.4$  the titration heat vanishes nearly completely.

---

## 1. Introduction

As a result of the rapid development of the technical equipment, isothermal titration calorimetry has become a standard method for the analysis of solute binding to biological and model membranes, [1–3]. Moreover, critical micelle concentrations and micellization enthalpies [4,5] as a function of temperature [6,7] have been determined by surfactant titration into water.

We found this method to be advantageous for detecting membrane solubilization by the addition of detergents, which, to our knowledge, has not yet been published.

Membrane solubilization is just the phase transition from lipid/detergent mixed membranes to mixed micelles driven by detergent addition. This transition is characterized by the three-stage model of Lichten-

berg and co-workers [8,9]. Accordingly, detergents are incorporated into the membranes up to a critical saturation detergent/lipid ratio  $R_{\text{sat}}$ . Beyond  $R_{\text{sat}}$  the membranes are destroyed and lipid saturated mixed micelles of a minimum detergent/lipid ratio  $R_{\text{sol}}$  coexist with detergent saturated membranes. When the total detergent content in the aggregates reaches  $R_{\text{sol}}$ , all membranes are dissolved leaving only mixed micelles, and thus solubilization is completed.

Compared to the traditional experimental approach to  $R_{\text{sat}}$  and  $R_{\text{sol}}$ , which are static and quasi-elastic light scattering, the application of isothermal titration calorimetry has several advantages. Because of the  $r^6$  dependence of scattered light, the first few micelles occurring at  $R_{\text{sat}}$  in addition to a nearly unchanged number of vesicles are scarcely detectable directly by light scattering techniques. How-

ever, employing extremely small vesicles ( $r \approx 30$  nm) a well-detectable growth of these vesicles to about 70 nm has been observed at  $R_{\text{sat}}$  [10,11].

Unfortunately, other investigations using slightly less stressed vesicles of 45–50 nm radius yielded no satisfactory indication of  $R_{\text{sat}}$  [12,13]. Altogether we find the extremely strong effect of  $R_{\text{sat}}$  on the titration heat to be clearly preferable with respect to the accuracy and evidence of the results as well as to the experimental effort.

## 2. Materials and methods

The non-ionic detergent hexa(ethylene oxide) dodecyl ether ( $C_{12}\text{EO}_6$ ) was purchased from Nikko Chemicals Ltd., Japan and the lipid palmitoyl oleoyl phosphatidylcholine (POPC) was from Avanti Polar Lipids, Birmingham AL. The substances were used without further purification.

The lipid vesicle suspensions were prepared by direct mixing of the lipid with water followed by

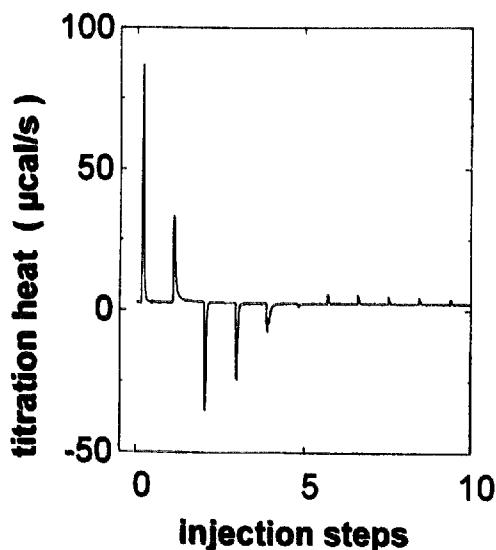


Fig. 1. Titration of a 100 mM micellar  $C_{12}\text{EO}_6$  solution to 1.3 ml of a 2 mM POPC vesicle suspension in 13 steps of 20  $\mu\text{l}$  each at 25°C. The plot shows the differential heat measured versus the number of detergent portions added step by step every 13th minute. By each of those portions, the molar detergent per lipid ratio  $R$  within the sample cell is increased by an amount of  $\Delta R = 0.8$ . Thus, the abscissa can be rescaled to  $R$  with a resolution of  $\Delta R$  (cf. Fig. 3). The three characteristic stages obtained correspond to the three stages of the Lichtenberg model.

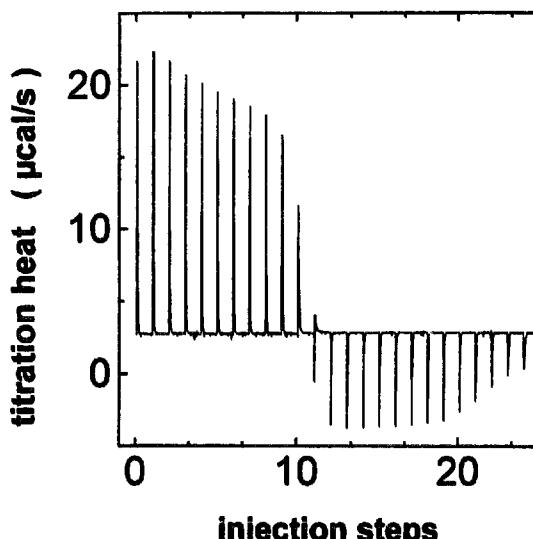


Fig. 2. Titration of a 33.3 mM micellar  $C_{12}\text{EO}_6$  solution to 1.3 ml of a 2 mM POPC vesicle suspension in 25 steps of 10  $\mu\text{l}$  each at 25°C. In a repetition of the experiment shown in Fig. 1 the detergent portions per step are diminished to  $\Delta R = 0.13$ .

rapid vortexing. We have found recently that this procedure yields almost unilamellar vesicles [14].

The experiments were carried out on a MicroCal™ Omega 5000 calorimeter at a temperature of 25°C. A 100 mM micellar solution of  $C_{12}\text{EO}_6$  was added to 1.3 ml of a 2 mM lipid vesicle suspension in the mixing cell in 12 steps of 20  $\mu\text{l}$  each (cf. Fig. 1). The measurement was repeated by adding 25 times 10  $\mu\text{l}$  of a 33.3 mM micellar  $C_{12}\text{EO}_6$  solution titrated to 2 mM POPC (cf. Fig. 2). This repetition was to enhance the resolution in the composition range up to  $R = 3.25$ .

For both experiments the interval between titrations was 13 minutes. The equilibration was observed to be fast compared to the time resolution of the apparatus, being about 30 s. The absence of additional long-term effects within the 10 min up to 6 h timescale was proved by fluorescence spectroscopy [15].

## 3. Results and discussion

The lipid concentration was chosen sufficiently high to ensure that almost all added detergent molecules are incorporated into the vesicles or mi-

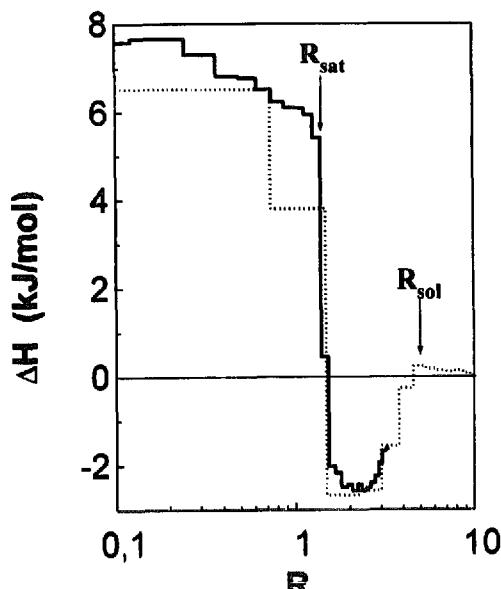


Fig. 3. Molar titration enthalpy differences  $\Delta H$  gained by integration of the heat pulses shown in Fig. 1 (···) and Fig. 2 (—) versus the detergent/lipid molar ratio in the sample cell,  $R$ .  $\Delta H$  is given per mole of detergent added. The steps correspond to the integral over one heat pulse each. The boundaries of the vesicle/micelle coexistence region,  $R_{\text{sat}} = 1.45 \pm 0.07$  and  $R_{\text{sol}} = 5.0 \pm 0.4$ , are indicated in consistence with light scattering results.

celles. Taking into consideration the partition coefficient for  $C_{12}\text{EO}_6$  between POPC membranes and water [15], one obtains this detergent transfer to the water phase to be less than 3% of the added detergent in our case. In addition, the enthalpy of transfer of monomers from a micelle into water (demicellization) at 25°C is not essentially higher than the observed micelle to membrane transfer enthalpy [4,6,7]. Thus, at a lipid concentration of 2 mM set in the mixing cell, the results do not depend on the partition coefficient or binding constant [1]. Instead, the observed integrated heat  $\Delta H$  (i.e. enthalpy difference) includes mostly the micelle to bilayer transfer enthalpy difference for the added detergents and a contribution from micelle dilution (cf. Fig. 3). Altogether we find an endothermic enthalpy difference of about 7 kJ/mol, slightly decreasing with increasing detergent content of the mixed membranes. A quantitative analysis will be given elsewhere [13]. The situation changes rapidly at  $R_{\text{sat}}$ , which is found to be  $1.45 \pm 0.07$  (cf. Fig. 3). Now, the existing vesicles start to be dissolved to micelles, i.e. the included

detergents are transferred from the bilayer to the micellar states. Thus, at  $R_{\text{sat}}$  the detergent transfer direction changes and, subsequently, the sign of the integrated heat which becomes exothermic. We note that this is just the dominating but not the only contribution to the observed titration heat within the solubilization range [13].  $R_{\text{sat}}$  is clearly indicated by a change in the sign of the measured titration heat.

At the end of the bilayer micelle coexistence range ( $R_{\text{sol}} = 5.0$ ), we observe the titration heat to increase steeply towards about zero. Adding micelles to mixed micelles, no transfer heats occur and the other contributions to the titration heat seem to be low or to compensate each other to a great extent.

The observed values for  $R_{\text{sat}}$  and  $R_{\text{sol}}$  have been proved by static and by dynamic light scattering experiments; the results are quite consistent (cf. Ref. [13]).

We summarize that isothermal titration calorimetry is a suitable approach for the investigation of isothermal, i.e. composition controlled, phase transitions. Thus the phase boundaries of the bilayer micelle coexistence range (two-phase region) have been found to be indicated fairly well. Moreover, the typical titration heat values will give important information about the nature of the various aggregates [13].

### Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 294 and Vo 526/1-1).

### References

- [1] T. Wiseman, S. Williston, J.F. Brandts and L.N. Lin, *Anal. Biochem.* 179 (1989) 131.
- [2] E. Freire, O.L. Mayorga and M. Straume, *Anal. Chem.* 62 (1990) 950A.
- [3] R. Lehrmann and J. Seelig, *Biochim. Biophys. Acta* 1189 (1994) 89.
- [4] G. Olofsson, *J. Phys. Chem.* 89 (1985) 1473.
- [5] K.S. Birdi, *Colloid Polym. Sci.* 261 (1983) 45.
- [6] A. Blume, I. Tuchtenhagen and S. Paula, *Prog. Colloid Polymer Sci.* 93 (1993) 118.
- [7] A. Blume, S. Paula, W. Süs and I. Tuchtenhagen, in preparation.

- [8] D. Lichtenberg, R.J. Robson and E.A. Dennis, *Biochim. Biophys. Acta* 737 (1983) 285.
- [9] S. Almog, B.J. Litman, W. Wimley, J. Cohen, E.J. Wachtel, Y. Barenholz, A. Ben-Shaul and D. Lichtenberg, *Biochemistry* 29 (1990) 4582.
- [10] K. Edwards and M. Almgren, *J. Colloid Interface Sci.* 147 (1991) 1.
- [11] P. Schurtenberger, N. Mazer and W. Käntzig, *J. Phys. Chem.* 89 (1985) 1042.
- [12] T. Inoue, T. Yamahata and R. Shimozawa, *J. Colloid Interface Sci.* 149 (1992) 345.
- [13] H. Heerklotz, G. Lantzsch, H. Binder, G. Klose and A. Blume, in preparation.
- [14] G. Lantzsch, H. Binder and H. Heerklotz, *J. Fluorescence* 4 (1994) 339.
- [15] H. Heerklotz, H. Binder, G. Lantzsch and G. Klose, *Biochim. Biophys. Acta* 1196 (1994) 114.