ELSEVIER

Biochimica et Biophysica Acta 1193 (1994) 301–306

BBA Biochimica et Biophysica Acta

# An assessment of the biochemical applications of the non-ionic surfactant Hecameg

M. Begoña Ruiz, Adelina Prado, Félix M. Goñi, Alicia Alonso *

*Department of Biochemistry, University of the Basque Country, P.O. Box 644, 48080 Bilbao, Spain*

Received 21 February 1994

**Abstract**

A number of properties and effects of the novel non-ionic detergent Hecameg (6-*O*-(*N*-heptylcarbamoyl)-methyl-α-D-glucopyranoside) have been examined in view of its possible biochemical applications. In particular, its critical micellar concentration has been measured, and its effects on pure lipid membranes, soluble and membrane-bound enzymes have been recorded. Hecameg has some advantageous and some less advantageous properties; its relatively high critical micellar concentration (16.5 mM), almost insensitive to pH or ionic strength changes, makes it suitable for reconstitution procedures in which detergent must be removed by dialysis. It is also an effective lipid-solubilizing agent, producing leakage of vesicle contents at detergent concentrations well below the solubilizing range. Among the drawbacks, the presence of an amide group in the molecule may interfere with the protein amide group in spectroscopic measurements. It also appears to be less gentle than other nonionic surfactants towards certain enzyme activities.

*Key words:* Detergent; Surfactant; Membrane solubilization; Sarcoplasmic reticulum; Hecameg

## 1. Introduction

More than two decades of extensive use of detergents in biological research [1–3] have convinced the workers in this field that the 'universal detergent' does not exist, and that a variety of surfactants has to be tried whenever a new system is studied [4]. Novel detergents are thus always a welcome addition to the biochemist's armoury. Hecameg (6-*O*-(*N*-heptylcarbamoyl)-methyl-α-D-glycopyranoside) is a newly synthesized detergent [5] whose properties appear to make it suitable for membrane research.

Previous studies from our group have dealt with the characterization of surfactant effects on model and cell membranes [6–10] as well as on purified membrane proteins [11,12]. Our work has included well-tried [7,9,11,12] and also novel [8,10] surfactants. The present paper is devoted to examining the properties of Hecameg from the point of view of its putative application to biomembrane studies: the critical micellar concentration, very important for eliminating the detergent in reconstitution studies, the parameters for Hecameg solubilization of pure lipid bilayers, and the effects of this surfactant on selected soluble and membrane enzymes. Our results are compared to manufacturers'data [5], as well as to previously published studies of detergent effects on soluble enzymes [13] and on sarcoplasmic reticulum ATPase [14].

## 2. Materials and methods

Hecameg was purchased from Vegatec (Villejuif, France) and used without further purification. Egg

Abbreviations: SUV, small unilamellar vesicles; LUV, large unilamellar vesicles; MLV, multilamellar vesicles; $R_e$, effective ratio, molar ratio of detergent/lipid in the mixed aggregates (vesicles or micelles); $R_e^{SAT}$, $R_e$ value at which the vesicles are saturated with the detergent; $R_e^{SOL}$, $R_e$ value at which the solubilization of the lipid is complete; $R_e^{REL}$, $R_e$ value at which release of vesicle aqueous contents starts; $D_{on}$ and $D_{100}$, total detergent concentrations producing the onset and completion of solubilization, respectively; $D_w$, detergent concentration in the aqueous phase; $K$, distribution coefficient of Hecameg between vesicles and aqueous medium; ANTS, 1,3,6-trisulphonate-8-aminonaphthalene; DPX, *N,N'-p*-xylenebis (pyridinium bromide); PC, phosphatidylcholine.

* Corresponding author. Fax: +34 4 4648500.


*SSDI* 0005-2736(94)00109-3

phosphatidylcholine was grade I from Lipid Products (South Nutfield, England). Merocyanine 540 was supplied by Serva (Heidelberg, Germany); fluorescein 548 was from Exciton (Dayton, OH); 1,3,6-trisulphonate-8-aminonaphthalene (ANTS) and *N,N'-p*-xylenebis (pyridinium bromide) were purchased from Molecular Probes (Eugene, OR). Sarcoplasmic reticulum from rabbit muscle was purified as described by Nakamura et al. [15]. Soluble enzymes and the corresponding substrates were from Sigma (St. Louis, MO).

The critical micellar concentration (cmc) of Hecameg was estimated as a shift of about 20 nm in $\lambda_{max}$ of the absorption spectrum of merocyanine 540, as described by Kaschny and Goñi [16]. In these measurements the final concentration of merocyanine was fixed at 4 $\mu$M.

Multilamellar liposomes (MLV) from egg PC were prepared in a 10 mM Hepes, 150 mM NaCl, pH 7.0 buffer. SUV were prepared by sonication [17] and LUV by extrusion through 0.1 $\mu$m Nuclepore filters [18]. Lipid P was measured according to Böttcher et al. [19]. Liposome suspensions were mixed with the same volumes of the appropriate detergent solutions (in the same buffer). The samples were left to equilibrate for 24 h at room temperature, and solubilization was assessed as a decrease in light scattering [20]. For each lipid concentration, 0% and 100% solubilization were established as the scattering of the liposomal suspension in the absence of detergent and in the presence of 5% Triton X-100, respectively. Total detergent concentrations producing the onset and the completion of solubilization ($D_{on}$ and $D_{100}$, respectively) were determined graphically as shown in a previous paper [20].

Surfactant-induced release of liposomal contents was measured with the ANTS/DPX method [21] as described by Nieva et al. [22]. Briefly, ANTS is a water-soluble fluorescent molecule, that may form a complex with the quencher DPX; when both are trapped together inside a liposome fluorescence is very low, but when they leak out dilution produces the breakdown of the complex, and fluorescence increases considerably. Liposomes were formed in a solution containing 12.5 mM ANTS, 45 mM DPX, 10 mM Hepes, 80 mM NaCl (pH 7.0). The vesicles were freed from non-encapsulated probes by means of a Sephadex G-75 column, eluted with a 10 mM Hepes, 150 mM NaCl, pH 7.0 buffer. Isotonicity of inner and outer solutions was checked with an Osmomat 030 (Gonotec, Berlin) osmometer.

Four soluble enzymes, previously tested with a variety of detergents [13], were also assayed with different concentrations of Hecameg: pig liver esterase (EC 3.1.1.1), *Helix pomatia* sulphatase (EC 3.1.6.1), wheat germ lipase type I (EC 3.1.1.3) and *Escherichia coli* alkaline phosphatase (EC 3.1.3.1). The enzymes were incubated with the appropriate detergent concentrations for 30 min at 25°C. Then enzyme activities were assayed spectrofluorometrically as described by Womack et al. [13].

Sarcoplasmic reticulum solubilization was assessed as a decrease in turbidity ($A_{540}$) as detailed previously [14]. $Ca^{2+}$-dependent ATPase activity was assayed at 37°C with an ATP-regenerating system, also as described in [14].

UV-Visible absorption spectra and ATPase assays were performed in a Kontron Uvikon 860 spectrophotometer. Fourier-transform infrared spectra were recorded in a Nicolet 520 spectrometer, fitted with a low-temperature, high-sensitivity, mercury-cadmium telluride detector; samples in water solution were deposited in a Harrick cell (Harrick, Ossining, NY) with 6 $\mu$m spacers and $CaF_2$ windows. Light scattering measurements were carried out in a Shimadzu RF-540 spectrofluorometer, with both emission and excitation monochromators at 500 nm. Fluorescence was also measured in a Shimadzu RF-540 spectrofluorometer.

## 3. Results and discussion

### 3.1. The critical micellar concentration of Hecameg

The cmc of Hecameg in double distilled water was measured at 25°C by the merocyanine 540 method, as described above. $\lambda_{max}$ of the dye absorption spectrum is plotted as a function of surfactant concentration in Fig. 1A. A sharp increase in $\lambda_{max}$ occurs at the cmc of 16.5 mM in this case. The same value is found when the assay is performed at 37°C. The cmc of Hecameg is also insensitive to pH at least in the 2–9 range (merocyanine becomes colourless at pH $\geq$ 9.5). Our value is in agreement with those given by Plusquellec et al. [5], between 14.5 and 19.5 mM after the various methods. It is a relatively high cmc value [23], ensuring good detergent removal by dialysis, since detergent monomers, but not micelles, pass through the usual

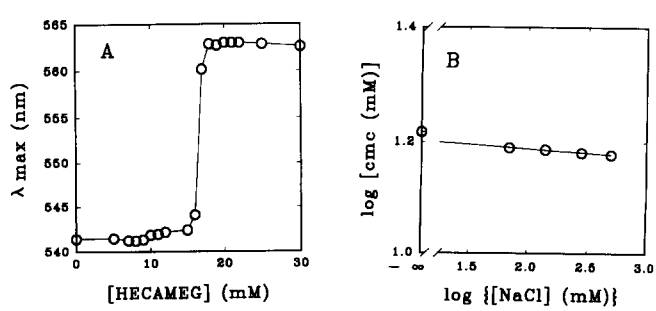


Fig. 1. The critical micellar concentration of Hecameg determined by the merocyanine 540 method. (A) The shift in $\lambda_{max}$ of the absorption spectrum of merocyanine 540 at the cmc of the surfactant. Solvent: distilled water. (B) The variation of the cmc of Hecameg with ionic strength. The equation of the straight line is: log(cmc) = 1.23–0.019 log([NaCl]).

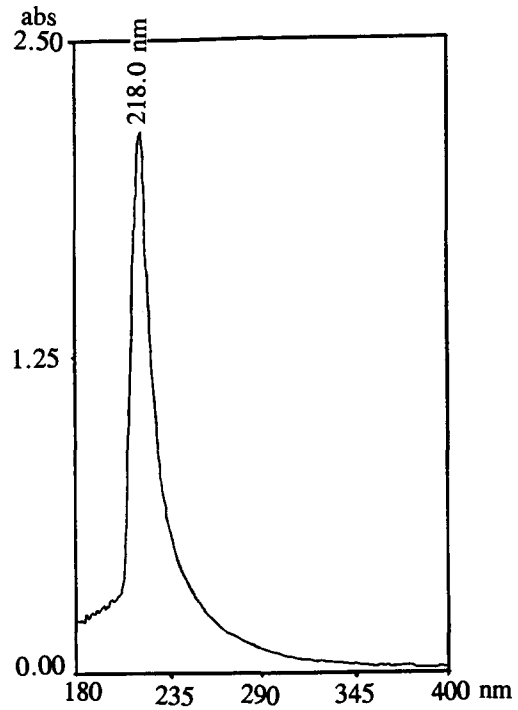


Fig. 2. The UV-Vis spectrum of Hecameg. The spectrum was recorded at 25°C. Hecameg was 100 mM in a 10 mM Hepes, 150 mM NaCl (pH 7.0) buffer; the reference cuvette contained the buffer only. The optical path was 1 cm.

dialysis membranes. The cmc value decreases very slightly with ionic strength: when NaCl concentration increases up to 500 mM, the cmc of Hecameg is 15.1 mM (Fig. 1B); this behaviour is the expected one for a non-ionic surfactant [24].

### 3.2. Absorption spectra

The UV-Vis absorption spectrum of Hecameg is shown in Fig. 2. A single maximum of absorbance is detected, at 218 nm. This portion of the ultraviolet spectrum is not frequently used in biochemical studies, thus the absorption band of Hecameg should not constitute a significant inconvenience.

Fig. 3 shows the FT-IR spectra of Hecameg in aqueous solution and in KBr disk. In the former case the solvent absorption has been subtracted. The spectra show intense signals in the regions corresponding to methylene groups (2800–3000 $cm^{-1}$) and carbonyl groups (1600–1800 $cm^{-1}$), as well as at lower frequencies where the phosphate vibrations of phospholipids occur. The spectrum in KBr shows, in addition to the increased resolution inherent to the solid state, a shift of the band found in solution at 1616 $cm^{-1}$ (assigned to N-H vibrations) to 1681 $cm^{-1}$, indicating strong hydrogen bonding of this group in water. The main feature in these FT-IR spectra, from the point of view of Hecameg application, is the strong carbonyl absorption, that should interfere with the amide I band of any membrane protein solubilized with this surfactant. It should be noted that, once a protein is solubilized with detergent, some surfactant molecule(s) may remain irreversibly bound to the protein [25].

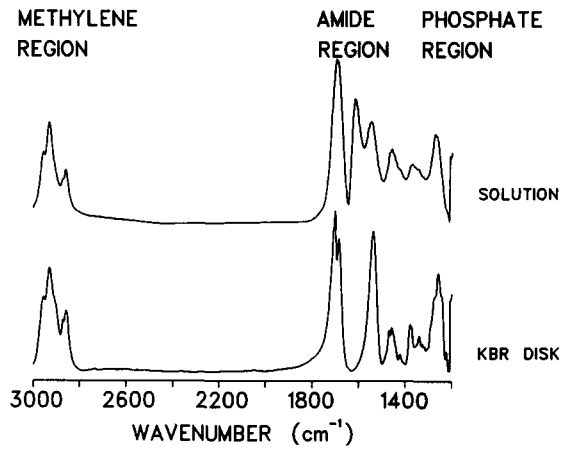


Fig. 3. The FT-IR spectrum of Hecameg. (A) 100 mM Hecameg in distilled water. (B) KBr disk. See text for details.

### 3.3. Solubilization of lipid bilayers

The solubilization patterns for the three liposomal preparations under study, MLV, LUV and SUV, at increasing concentrations of Hecameg, are shown in Fig. 4. The patterns are very similar to those found with other detergents, e.g., Triton X-100 [20], although, with Hecameg, solubilization occurs at higher detergent concentrations. From the plots in Fig. 4, the total detergent concentrations producing the onset and the completion of solubilization ($D_{on}$ and $D_{100}$, respectively), are determined graphically (see arrows 1 and 2) for each kind of liposome preparation.

When solubilization is assayed at different liposomal concentrations, and $D_{on}$ and $D_{100}$ are plotted as a function of phospholipid concentration, two straight lines are found (Fig. 5). According to Lichtenberg and

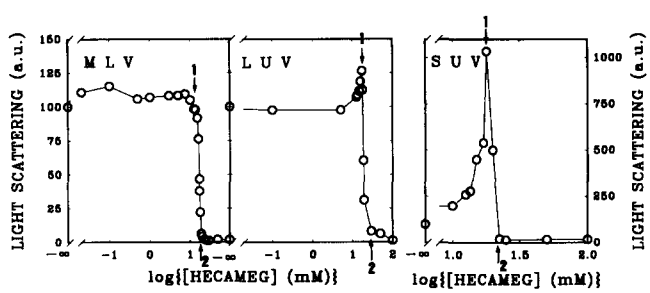


Fig. 4. Liposome solubilization by Hecameg. The change in light scattering of 2 mM egg PC liposomal suspensions as a function of Hecameg concentration, for three kinds of vesicle preparation. 100 = scattering in the absence of detergent. Arrows 1 and 2 correspond to $D_{on}$ and $D_{100}$ respectively (see text for details).

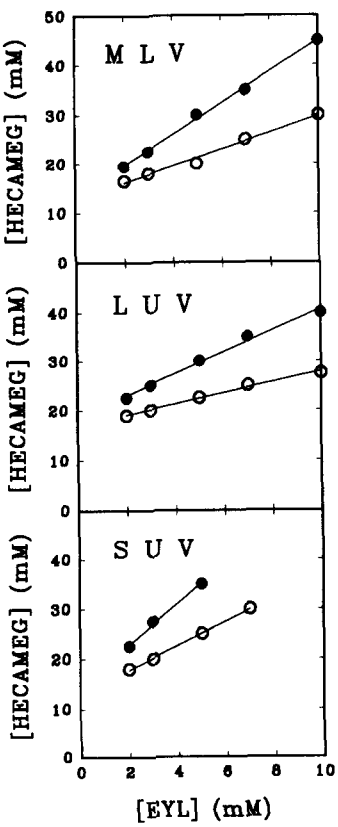


Fig. 5. Effective detergent/lipid mole ratios for bilayer solubilization by Hecameg. $D_{on}$ (○) and $D_{100}$ (●) are plotted against lipid concentration. The slopes of the resulting straight lines correspond to $R_e^{SAT}$ and $R_e^{SOL}$, respectively.

co-workers [3,26,27] these straight lines satisfy the equation:

$$D_T = R_e\{L + 1/[K(R_e + 1)]\}$$

where $K$ is the distribution coefficient of Hecameg between the vesicles and the aqueous medium. $D_T$ is the total detergent concentration, $R_e$ is the 'effective detergent/lipid ratio', defined as the detergent/lipid molar ratio in the mixed aggregates, vesicles or micelles [27], and $L$ is the lipid concentration. The equation means that the total detergent concentration, $D_T$, required for obtaining any effective ratio $R_e$, has a linear dependence on $L$. $R_e$ is given by the slope of the straight line defined by the equation, which should intercept the $x$-axis at $-1/[K(R_e + 1)]$. In addition, the intercept with the $y$-axis corresponds to the concentration of free detergent in water, $D_w$, which in turn represents the apparent critical micellar concentration of the surfactant in the presence of lipid. The corresponding values for the three liposomal preparations, derived from the straight lines in Fig. 5, are shown in Table 1.

$R_e^{SAT}$ and $R_e^{SOL}$ values, i.e., $R_e$ at which bilayer solubilization starts and reaches completion, respectively, indicate that bilayers become saturated with Hecameg at ≈ 1–2 detergent molecules per lipid molecule, while complete solubilization requires a detergent/lipid mole ratio of ≈ 3–4:1. In common with other detergents, (i) the whole solubilization process occurs within a narrow range of detergent concentrations (i.e., $R_e^{SAT}$ and $R_e^{SOL}$ are close values), (ii) $R_e^{SOL}$ are virtually the same for the different kinds of vesicles, and (iii) $R_e^{SOL}$ are very similar to those of other detergents, irrespective of the critical micellar concentrations, e.g., Triton X-100 (cmc = 0.24 mM) [20] or octyl glucoside (cmc = 25 mM) [28]. $R_e^{SOL}$ tends to be independent of cmc because $K$, the lipid/water distribution coefficient, does decrease with increasing cmc; compare the $K$ values in Table 1 (≈ 0.1–0.2) with those for Triton X-100 (≈ 1–2) [20]. Finally, note that $D_w$ values are near the measured cmc of Hecameg in water (16.5 mM, see above).

In summary, Hecameg is an efficient solubilizing agent towards phospholipid bilayers, and its relatively high cmc should ensure its rapid removal by dialysis.

### 3.4. Release of vesicle aqueous contents

Detergents are known to induce leakage of liposomal contents at concentrations below those producing solubilization [9,29,30]. This was checked for Hecameg with LUV and SUV at various concentrations. The peculiar structure of MLV makes a precise detection of the onset of leakage more complicated. As a representative example, the results with SUV are shown in Fig. 6. Values of $R_e^{REL}$, effective detergent/lipid mole ratio producing the onset of release of vesicle aqueous contents, are also given in Table 1 for LUV and SUV. As expected $D_{on}$ and $R_e$ values are significantly lower for leakage than for solubilization. Thus, also in this respect, the interaction of Hecameg with lipid bilayers follows the pattern of the previously studied detergents.

### 3.5. Effects on soluble enzymes

Womack et al. [13] carried out an extensive survey of the effects of detergents on soluble enzymes, and for

Table 1
Effective detergent/lipid molar ratios and related parameters in the solubilization of egg PC vesicles by Hecameg

| | Type of vesicles | | |
|---|---|---|---|
| | SUV | LUV | MLV |
| $R_e^{SAT}$ | 2.42 | 1.36 | 1.72 |
| $D_w$ (mM) | 13.0 | 15.9 | 12.6 |
| $K$ ($mM^{-1}$) | 0.19 | 0.08 | 0.13 |
| $R_e^{SOL}$ | 4.11 | 2.64 | 3.17 |
| $D_w$ (mM) | 14.6 | 17.0 | 13.3 |
| $R_e^{REL}$ | 0.73 | 0.68 | – |

Data are derived from the plots shown in Figs. 5 and 6B.

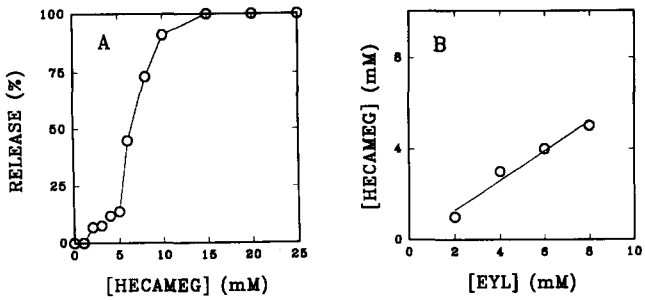


Fig. 6. Release of aqueous contents from small unilamellar vesicles. (A) Percent release from a 2 mM suspension of SUV, as a function of total Hecameg concentration. (B) Detergent concentrations producing the onset of leakage as a function of lipid concentration; the slope of this straight line gives the effective detergent/lipid molar ratio for the onset of release, $R_e^{REL}$.

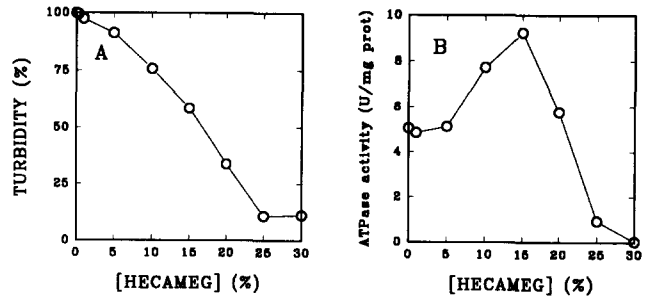


Fig. 8. Effects of Hecameg on the sarcoplasmic reticulum $Ca^{2+}$-ATPase. (A) Sarcoplasmic reticulum solubilization, assessed as a decrease in turbidity ($A_{540}$) of the membrane suspension with increasing Hecameg concentrations. (B) Specific activity of $Ca^{2+}$-ATPase in the presence of increasing concentrations of Hecameg. Average values of four independent measurements.

that purpose they selected four enzyme activities, as detailed in Materials and methods. Each enzyme was assayed after incubation with 0.3%, 1%, 5% and 10% detergent. We paralleled these studies with Hecameg, and the results are shown in Fig. 7. Sulphatase and phosphatase are unaffected by low surfactant concentrations, and activated above 5%. Plusquellec et al. [5] found an activation of the soluble enzyme lactoperoxidase above the cmc of Hecameg (16.5 mM $\approx$ 0.53%). Other commonly used detergents, e.g., CHAPS or octyl glucoside, have little effect on sulphatase or phosphatase [13]. Lipase activity displays variations of $\pm$50% the original value with increasing Hecameg concentrations.

The effect of Hecameg on esterase is clearly inhibitory, and already detectable at 0.3% (9 mM) surfactant. Womack et al. [13] found a similar esterase inhibition by Tween-80 or Triton X-100, while CHAPS or octyl glucoside did not affect this enzyme activity. Plusquellec et al. [5] also found an inhibitory effect of Hecameg on another soluble enzyme, $\beta$-lactamase. Those authors suggested that $\beta$-lactamase inhibition might be due to surfactant-substrate interaction; we have tested this point for esterase, but neither the substrate absorbance nor the quantum yield of the product is significantly modified by Hecameg concentrations up to 10%. In summary, Hecameg does not appear to have any particular advantage on other commercial surfactants with respect to its effect on soluble enzymes.

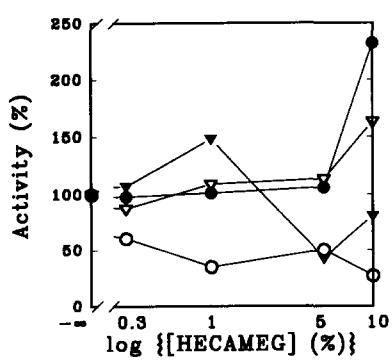


Fig. 7. Effects of Hecameg on the activity of soluble enzymes. (●) Sulphatase; (▽) phosphatase; (▼) lipase; (○) esterase. 100% = activity in the absence of surfactant. Average values of four independent measurements.

### 3.6. Effects on sarcoplasmic reticulum ATPase

Detergents are most frequently used in biochemistry in the solubilization of membrane-bound enzymes. Hecameg concentrations above its cmc (i.e., 30 mM) produced the solubilization and activation of bacterial succinate dehydrogenase and NADH oxidase [5]; similar effects have been found for Triton X-100 on various membrane-bound mitochondrial enzymes [6]. $Ca^{2+}$-ATPase from rabbit sarcoplasmic reticulum is a well-characterized enzyme, and its interaction with surfactants has been explored in detail [12,14,31]. In the present study, we have treated sarcoplasmic reticulum with Hecameg much in the same way as we did with sodium dodecylsulphate [12] or Triton X-100 [14] in past studies. Fig. 8 shows the surfactant-dependent decrease in membrane suspension turbidity (i.e., membrane solubilization) (Fig. 8A) and the change in specific activity of $Ca^{2+}$-ATPase in the presence of increasing surfactant concentrations (Fig. 8B). Unlike sodium dodecylsulphate or Triton X-100, Hecameg produces a gradual decrease in turbidity, suggesting that solubilization occurs over a wide range of surfactant concentrations. Most significantly, Hecameg concentrations producing full solubilization also destroy the enzyme activity, so that, with respect to $Ca^{2+}$-ATPase, Hecameg parts from the behaviour of non-ionic surfactants, e.g., Triton X-100, acting more like the negatively charged sodium dodecylsulphate.

## 4. Concluding remarks

In spite of considerable efforts in this field, the effects of a given detergent on a particular biological system are largely unpredictable, and a screening of detergents each time a new biological application is envisaged appears to be an unavoidable task. This said, it is only fair to add that, by examining the effects of an unknown detergent on a series of biological systems previously tested with a variety of surfactants, an overall evaluation of its usefulness may be performed. This is what has been carried out for Hecameg with the experimental observations reported above. Like any other detergent, Hecameg has positive and negative characteristics with respect to its biological applications. According to our results, Hecameg *advantages* are (i) its high critical micellar concentration, invariable under most experimental conditions, that should facilitate detergent removal by dialysis, and (ii) its ability to solubilize lipid bilayers, at concentrations well above those producing just vesicle leakage. Among Hecameg *disadvantages* we should note (i) the presence of an amide group in its molecule, leading to spectral interference in the case of protein-detergent mixtures, and (ii) its ability to inhibit or otherwise impair some enzyme activities that are not affected by other nonionic surfactants.

## Acknowledgements

The authors are indebted to Professor J.L.R. Arrondo for his help with the FT-IR spectra. This work was supported in part by funds from DGICYT (grant No. PB91–0441). M.B.R. is a pre-doctoral fellow of the Spanish Ministerio de Educación y Ciencia.

## References

[1] Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79.
[2] Lichtenberg, D., Robson, R.J. and Dennis, E.A. (1983) Biochim. Biophys. Acta 737, 285–304.
[3] Lichtenberg, D. (1994) in Handbook of Biological Membranes (M. Shinitzki, ed.), in press.
[4] Racker, E. (1986) Reconstitution of Transporters, Receptors and Pathological States, Academic Press, New York.
[5] Plusquellec, D., Chevalier, G., Talibart, R. and Wróblewski, H. (1989) Anal. Biochem. 179, 145–153.
[6] Gurtubay, J.I.G., Goñi, F.M., Gómez-Fernández, J.C., Otamendi, J.J. and Macarulla, J.M. (1980) J. Bioenerg. Biomembr. 12, 47–70.
[7] Goñi, F.M., Urbaneja, M.A., Arrondo, J.L.R., Alonso, A., Durrani, A.A. and Chapman, D. (1986) Eur. J. Biochem. 160, 659–665.
[8] Partearroyo, M.A., Aranda, F.J., Alonso, A., Gómez-Fernández, J.C. and Goñi, F.M. (1988) Arch. Biochem. Biophys. 262, 585–591.
[9] Ruiz, J., Goñi, F.M. and Alonso, A. (1988) Biochim. Biophys. Acta 937, 127–134.
[10] Partearroyo, M.A., Goñi, F.M. and Katime, I.A. (1989) J. Colloid Interface Sci. 132, 22–26.
[11] Valpuesta, J.M., Arrondo, J.L.R., Barbero, M.C., Pons, M. and Goñi, F.M. (1986) J. Biol. Chem. 261, 6578–6584.
[12] Prado, A., Muga, A., Castresana, J., Goñi, F.M. and Arrondo, J.L.R. (1990) FEBS Lett. 269, 324–327.
[13] Womack, M.D., Kendall, D.A. and MacDonald, R.C. (1983) Biochim. Biophys. Acta 733, 210–215.
[14] Prado, A., Arrondo, J.L.R., Villena, A., Goñi, F.M. and Macarulla, J.M. (1983) Biochim. Biophys. Acta 733, 163–171.
[15] Nakamura, H., Yilka, R.L., Boland, R. and Martonosi, A.N. (1976) J. Biol. Chem. 251, 5414–5423
[16] Kaschny, P. and Goñi, F.M. (1993) J. Colloid Interface Sci. 160, 24–30.
[17] Alonso, A., Villena, A. and Goñi, F.M. (1981) FEBS Lett. 123, 200–204.
[18] Mayer, L.D., Hope, M.J. and Cullis, P.R. (1986) Biochim. Biophys. Acta 858, 161–168.
[19] Böttcher, C.S.F., Van Gent, C.M. and Fries, C. (1961) Ann. Chim. Acta 24, 203–204.
[20] Partearroyo, M.A., Urbaneja, M.A. and Goñi, F.M. (1992) FEBS Lett. 302, 138–140.
[21] Ellens, H., Bentz, J. and Szoka, F.C. (1985) Biochemistry 24, 3099–3106.
[22] Nieva, J.L., Goñi, F.M. and Alonso, A. (1989) Biochemistry 28, 7364–7367.
[23] Helenius, A., McCaslin, D.R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749.
[24] Rosen, M.J. (1978) Surfactants and Interfacial Phenomena, Wiley, New York.
[25] Del Río, E., González-Mañas, J.M., Gurtubay, J.I.G. and Goñi, F.M. (1991) Arch. Biochem. Biophys. 291, 300–306.
[26] Lichtenberg, D. (1985) Biochim. Biophys. Acta 821, 470–478.
[27] Almog, S., Litman, B.J., Wimley, W., Cohen, J., Wachtel, E.J., Barenholz, Y., Ben-Shaul, A. and Lichtenberg, D. (1990) Biochemistry 29, 4582–4592.
[28] Levy, D., Gulik, A., Seigneuret, M. and Rigaud, J.L. (1990) Biochemistry 29, 9480–9488.
[29] Goñi, F.M., Urbaneja, M.A., Arrondo, J.L.R., Alonso, A., Durrani, A.A. and Chapman, D. (1986) Eur. J. Biochem. 160, 659–665.
[30] De la Maza, A., Sánchez Leal, J., Parra, J.L., García, M.T. and Ribosa, I. (1991) J. Am. Oil. Chem. Soc. 68, 315–319.
[31] Lund, S., Orlowski, S., De Foresta, B., Champeil, P., Le Maire, M. and Moller, J.V. (1989) J. Biol. Chem. 264, 4907–4915.