Thermochimica Acta, 204 (1992) 145–155
Elsevier Science Publishers B.V., Amsterdam

Study of state transitions within multiple W/O/W emulsions using calorimetry (DSC) [1]

L. Potier [a], S. Raynal [b], M. Seiller [b], J-L. Grossiord [c] and D. Clausse [a]

[a] *Laboratoire de Thermodynamique et Physicochimie de Procédés Industriels, Université de Technologie de Compiègne, BP 649, 60206 Compiègne Cedex (France)*
[b] *Laboratoire de Pharmacie Galénique et de Biopharmacie, URA CNRS 1218, UFR des Sciences Pharmaceutiques, 5, rue J-B. Clément, 92296 Châtenay-Malabry Cedex (France)*
[c] *Laboratoire de Physique Pharmaceutique, URA CNRS 1218, UFR des Sciences Pharmaceutiques, 5, rue J-B. Clément, 92296 Châtenay-Malabry Cedex (France)*

(Received 20 January 1992)

Abstract

We report results obtained by differential scanning calorimetry of multiple water–oil–water emulsions. Samples were submitted to cooling (-2°C min^{-1}) between $+20$ and -60°C. DSC curves showed liquid/solid transitions of both dispersed and continuous aqueous phases at subzero temperatures due to undercooling phenomena. The mean freezing temperatures found were around -15°C for the continuous aqueous phase and around -44°C for the dispersed phase. Oil did not crystallize over the temperature range studied.

Furthermore, during the cooling of a multiple emulsion sample we observed a transfer of water from the internal to the external frozen aqueous phase. This transfer was attributed to the difference of vapor pressures between ice and undercooled water at the same temperature. The influence of urea, solubilized in the dispersed aqueous phase, on the observed phenomena was also studied. An evolution of the mean freezing temperature of the dispersed water was observed, showing a water transfer from the outer to the inner aqueous phase. Additional viscosity measurements performed at room temperature confirmed the occurrence of a mass transfer within the diluted multiple emulsion.

INTRODUCTION

In 1925 Seifriz [1] described a multiple emulsion formed by phase inversion of a simple emulsion. A water in oil in water (W/O/W) multiple

---

Correspondence to: D. Clausse, Laboratoire de Thermodynamique et Physicochimie de Procédés Industriels, Université de Technologie de Compiègne, BP 649, 60206 Compiègne Cedex, France.

[1] This paper was presented during the 22nd annual meeting of the French Association of Calorimetry and Thermal Analysis (AFCAT) at Paris-XI–Châtenay-Malabry (27–29 May 1991).

emulsion is composed of oil globules, containing one or several aqueous compartments, dispersed in an aqueous suspending fluid. This means that two aqueous phases, called the dispersed and the continuous phase, are separated by a thin oil layer. Such a feature enables the encapsulation of hydrophilic molecules [2,3]. Hydrophobic particles may also be encapsulated in an oil in water in oil (O/W/O) multiple emulsion.

Multiple emulsions are considered either as drug capsules or as solvent reservoirs. Applications have been developed to prolong drug delivery [4–6] or to improve the efficiency of overdose treatments [6,7]. Results in the cosmetics field have also been reported [8]. However, only a few publications deal with fundamental aspects of multiple emulsions [9–11].

The aim of this article is, first, to describe the state transitions within a multiple emulsion during cooling by calorimetric measurements. Our final purpose is to study mass transfer between both aqueous phases using differential scanning calorimetry (DSC). Mass transfer leads to an evolution of both aqueous phase concentrations, and then to a variation of the inner and the outer aqueous phase crystallization temperatures. As far as we know there are no published data on the subject.

MATERIALS AND METHODS

Sample formulation

We used a two step process to obtain a multiple emulsion (Fig. 1). [12–14]. First a simple emulsion, referred to as the primary W/O-A emulsion, of the following composition (w/w) was prepared: 4.0% of

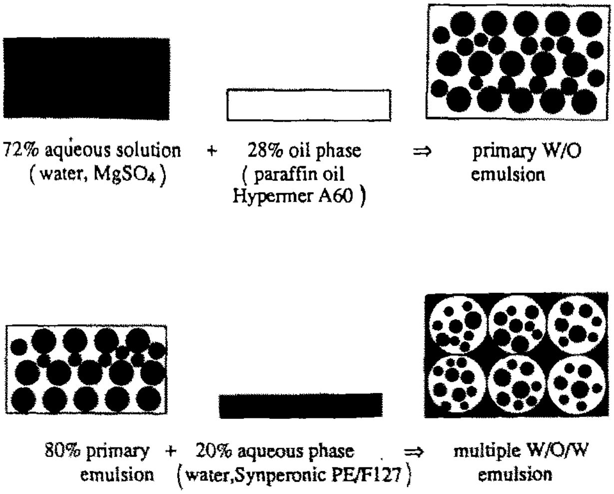

Fig. 1. Formulation process of multiple W/O/W emulsion.

hydrophobic surfactant (ICI Hypermer A60), 24.0% of paraffin oil, and 72.0% of aqueous solution (distilled water + 0.5% $MgSO_4$)

The aqueous and oil phases were heated at 85°C separately and mixed immediately after with a homogenizer (Rayneri) under high stirring. The second step of this process consisted in re-emulsifying the primary emulsion into water, the proportions (w/w) being 80.0% of primary W/O-A emulsion, 0.8% of hydrophilic surfactant (ICI Synperonic PE/F127), and 19.2% of distilled water.

The primary emulsion was added dropwise and slowly at room temperature to the aqueous external phase, containing the hydrophilic surfactant, under slow stirring. A multiple emulsion, the structure of which was checked by optical microscopy, was obtained after about 1 h. The multiple emulsion prepared as described, and referred to as multiple W/O/W-A emulsion, was white and macroscopically homogeneous.

We used urea in order to increase the osmotic pressure gradient between the two aqueous phases. An aqueous solution containing 10% (w/w) of urea was used to prepare a primary emulsion referred to as the W/O-B emulsion. Following the formulation process, the multiple emulsion obtained (referred to as W/O/W-B emulsion) was white, macroscopically homogeneous and highly viscous. We checked that both multiple emulsions did not undergo significant alterations during the period of analysis (i.e. at least one week).

The theoretical composition (w/w) of these multiple emulsions is given in Table 1 assuming that there is no mass loss and no mass transfer and no solubilization of the hydrophobic surfactant in the inner aqueous phase.

Analysis methods

Calorimetric analysis was carried out on a DSC 111 (Setaram). Samples were submitted to cooling between +20 and −60°C. The scanning rate was

TABLE 1

Composition of multiple emulsions

	W/O/W-A	W/O/W-B
Continuous aqueous phase		
Hydrophilic surfactant (ICI Synperonic PE/F127)	0.8	0.8
Distilled water	19.2	19.2
Oil phase		
Hydrophobic surfactant (ICI Hypermer A60)	3.2	3.2
Paraffin oil	19.2	19.2
Dispersed aqueous phase		
$MgSO_4$	0.3	0.3
Urea	–	5.8
Distilled water	57.3	51.5

-2°C min^{-1}. The experiments were made on about 180 mg of multiple emulsion.

Viscosity measurements were made on a cone–plate viscosimeter (Brookfield). Samples were submitted to a constant shear rate (26.9 s^{-1}) at constant temperature (25°C). We observed the shear stress evolution, and the apparent viscosity variation was recorded versus time.

Calorimetric and viscosimetric experiments were made on freshly prepared samples.

RESULTS AND DISCUSSION

As explained in the introduction, a multiple W/O/W emulsion is composed of dispersed water droplets and a bulk water continuous phase. Before testing the multiple emulsion itself, we studied separately the solidification of, first, pure water and then of the primary W/O emulsions. These preliminary tests were fundamental and will be used as references to explain state transitions in the multiple emulsion.

Pure water sample

Figure 2a shows the DSC curve obtained by cooling down a pure water sample. The freezing temperature T_c is defined as the intersection of the tangent at the beginning of the curve with the baseline [15,16].

Crystallization is a random phenomenon. For this reason several experiments should be made in order to determine a mean freezing temperature (T_c*). Table 2 gives the values of the freezing temperatures of four pure water samples.

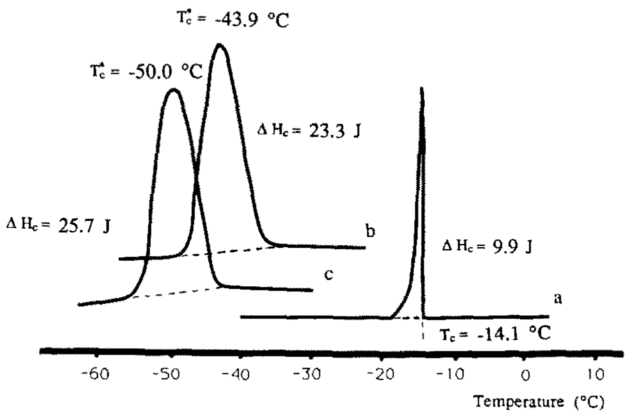

Fig. 2. Crystallization of (curve a) 88.9 mg of pure water, (curve b) 147.1 mg of primary W/O-A emulsion and (curve c) 161.0 mg of primary W/O-B emulsion.

TABLE 2

Freezing temperatures

Sample mass (mg)	T_c (°C)
35.1	−15.3
79.6	−17.8
88.9	−14.1
70.5	−12.3

According to these results, the mean freezing temperature T_c^* can be expected at around −15°C.

Primary W / O emulsions

It was checked that paraffin oil does not crystallize over the temperature range (from +20 to −60°C) studied.

Primary W / O-A emulsion

In this emulsion, the mean diameter of dispersed water droplets is about 1 μm. By cooling down a W/O emulsion sample, several thousand water droplets are submitted to the same condition. On the DSC curve (Fig. 2b) we observe a freezing temperatures distribution. The Gaussian shape of the curve is caused by two antagonistic phenomena: the increasing nucleation rate and the decreasing number of water droplets remaining liquid [17]. As has been shown [18], the top of the exothermic signal corresponds to the crystallization of 50% of water droplets and represents the mean freezing temperature (T_c^*) of the dispersed phase, $T_c^* = -43.9$°C. This temperature is significantly lower than that observed in dispersed pure water, namely around −40°C [18]. The presence of $MgSO_4$ leads to a decrease of T_c^*; however, its low concentration can not explain the observed decrease of temperature. It could be possible that a significant amount of the hydrophobic surfactant, solubilized in the inner aqueous phase, also induces a decrease of the mean freezing temperature.

Primary W / O-B emulsion

Figure 2c is an example of the DSC curve obtained with a primary emulsion containing 10% (w/w) of urea solubilized in water. The mean freezing temperature is found at $T_c^* = -50.0$°C. This result shows that the presence of urea induces a decrease of the mean freezing temperature of the dispersed water, as can be expected from previous work [15] on the influence of a solute on the undercooling of dispersed aqueous solutions.

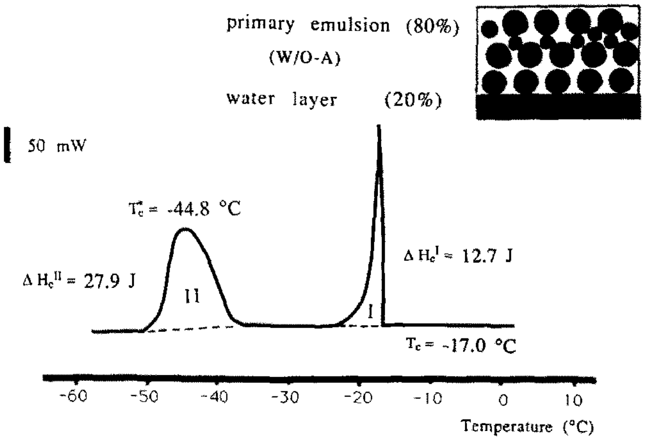

Fig. 3. Cooling of a sample containing bulk water (46 mg) as lower layer and primary W/O-A emulsion (196 mg) as upper layer.

Multiple W / O / W emulsions

Preliminary test

The following test will be used as reference to analyze the behavior of a multiple emulsion sample during the cooling. This test consists in filling an analysis cell with a drop of pure water and a drop of primary W/O-A emulsion. This experiment is interesting in as far as the whole sample composition can be the same as the multiple emulsion composition. However, the two structures are totally different.

The DSC curve obtained (Fig. 3) shows two exothermic signals which are clearly distinct. The first signal (I) corresponds to the crystallization of the lower layer of water and the second (II) corresponds to the crystallization of the dispersed water droplets in the primary W/O-A emulsion. Since the freezing temperature found for the dispersed phase of the W/O-A emulsion ($T_c^* = -44.8$°C) is not very far from the one observed on the W/O-A emulsion alone ($T_c^* = -43.9$°C), it can be concluded that there is no significant interaction between the bulk water layer and the W/O-A emulsion set above it. The difference between the freezing temperatures of the lower water layer and of a pure water sample (Fig. 2, curve a) must be attributed to the randomness of the crystallization events, as was explained in the section above headed Pure water sample.

Multiple W / O / W-A emulsion

Figure 4 represents the DSC curve obtained during the cooling of this emulsion. We can distinguish three exothermic signals.

Signal I corresponds to the crystallization of the continuous phase at $T_c = -12.5$°C. Several experiments should be made if the most probable

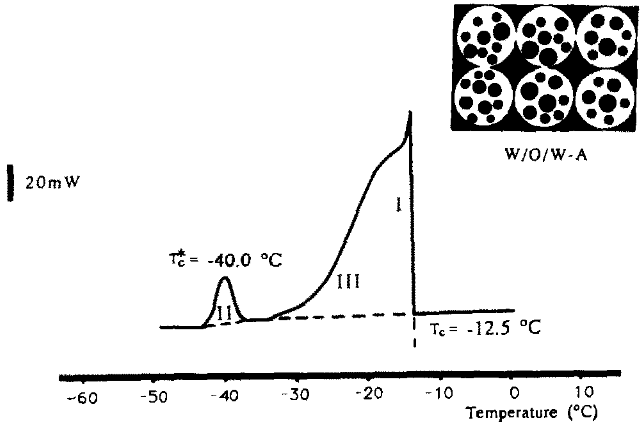

Fig. 4. Cooling of a sample of multiple W/O/W-A emulsion.

freezing temperature of the continuous phase is required, as was explained in the section above headed Pure water sample.

Signal II corresponds to the crystallization of the dispersed aqueous phase. The most probable freezing temperature (T_c*), given by the top of the signal, is −40.0°C. Comparing with the preliminary test, we observe an increase of 4.8°C in the mean freezing temperature of the dispersed phase, but this increase is not yet explained. Nevertheless, we can assume that this increase could correspond either to a significant increase of the droplet radius or to a dilution of the dispersed phase. More experiments are needed to give a reliable explanation.

Signal III reveals an unexpected phenomenon occurring during the cooling of the multiple emulsion, in as far as it was not observed during the preliminary test. We explain it by a transfer of water from the internal to the external aqueous phase due to the difference between the vapor pressure of ice and the vapor pressure of undercooled water at the same temperature. Table 3 gives the variation of the vapor pressure of ice and undercooled water versus temperature [19].

To confirm this hypothesis, isothermal experiments were carried out.

TABLE 3

Vapor pressure values

Temperature (°C)	Vapor pressure (mmHg)	
	Ice	Undercooled water
−10	1.950	2.149
−12	1.632	1.834
−14	1.361	1.560
−15	1.241	1.436

Three samples (primary W/O-A emulsion, bulk water plus primary W/O-A emulsion and multiple W/O/W-A emulsion) were stored during 1 h at −10°C. We noticed that water droplets of the primary W/O-A emulsion sample did not crystallize during the experiment. Concerning the second sample (water layer plus primary W/O-A emulsion) we observed the crystallization of bulk water without dispersed water droplets freezing. However, the crystallization of the continuous phase of the multiple W/O/W-A emulsion occurred rapidly, followed by a very slow (45 min) exothermic phenomenon. After 1 h this sample had been cooled from −10 to −60°C. There was no longer an exothermic signal around −40°C, which means that dispersed water droplets had disappeared as the result of complete water transfer from the inner to the outer phase. The hypothesis of freezing of the dispersed water during storage at −10°C has to be rejected, because no freezing was observed for the primary W/O-A emulsion in the same conditions. Also, the water mass transfer observed during cooling (signal III) is observed at fixed temperature, the only requirement being that the continuous phase is frozen.

In conclusion, when a sample contains both macroscopic and microscopic water volumes their crystallization occurs at very distinct temperatures. However, the special structure of a multiple emulsion (a very thin intermediate oil layer) leads to a transfer of water from the internal to the external aqueous phase caused by a vapor pressure gradient due to the freezing of the external phase.

Multiple W/O/W-B emulsion

The presence of urea creates an osmotic pressure gradient between the inner and outer phases. Because of this osmotic pressure gradient we expect a transfer of water from the external to the internal aqueous phase and a transfer of solutes ($MgSO_4$ and urea) in the reverse sense [20,21]. Those transfers are under study and will be reported in more details in a future paper. In this paragraph and the following, only preliminary results are reported.

Figure 5 shows the results obtained by DSC during the cooling of a sample of the multiple W/O/W-B emulsion containing a 10% (w/w) urea solution as the dispersed aqueous phase. Two exothermic signals are observed. The freezing temperature (T_c) of the continuous phase (signal I) is −16.9°C. The most probable freezing temperature (T_c^*) of the dispersed phase (signal II) is −48.0°C. Compared to the result observed with the W/O-B emulsion, $T_c^* = -50.0$°C, an increase of 2°C is observed. This increase might be explained by a dilution of the inner aqueous solution due to the osmotic pressure gradient. But this increase of the mean freezing temperature is rather small. To complete this study, we have performed additional experiments with a view to increasing the kinetic transfer of water.

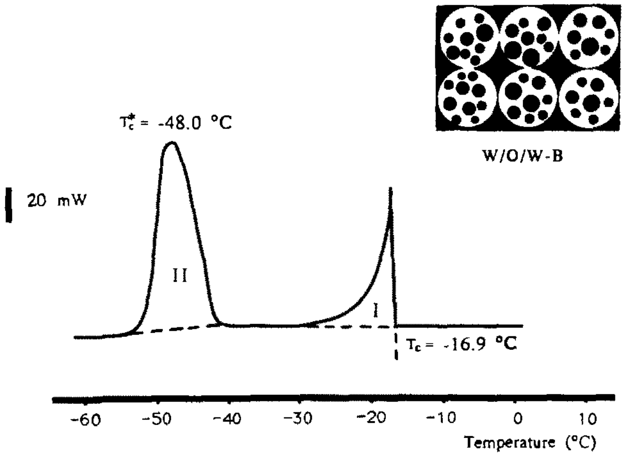

Fig. 5. Cooling of a sample of multiple W/O/W-B emulsion.

Diluted multiple W / O / W-B emulsion

Previous works [20,21] have shown that the dilution of the multiple emulsion increases the mass transfer. We diluted the multiple W/O/W-B emulsion, first with pure water and then with an aqueous solution of urea (10% w/w). The term "mother multiple emulsion" refers to the multiple emulsion prepared following the formulation process described in the section above headed Sample formulation, in contrast to the term "diluted multiple emulsion". Samples diluted as follows, 1 g of mother emulsion plus 2 g of aqueous solution, were studied by calorimetry and viscosity analysis. The dilutions conserve the osmotic pressure gradient existing in the mother W/O/W-B emulsion by adding 2 g of pure water, or suppress almost completely this osmotic pressure gradient by adding 2 g of a 10% (w/w) urea solution.

Results obtained with samples diluted by a 10% urea solution show no variation of water droplet crystallization ($T_c^* = -47.8$°C), which means no variation of the droplet concentration and no transfer of water between the two aqueous phases. Diluting with pure water leads to an increase of water droplet freezing temperature from −48.0 to −43.6°C. Such an evolution shows a decrease of the solute concentration of the dispersed aqueous solution and then a water transfer from the external to the internal phase. More details will be published in a further article.

Furthermore, viscosity analysis confirmed this phenomenon. When dilution was made with a 10% urea solution we did not observe any significant apparent viscosity variation (less than 5 mPa s) versus time. However, when we used pure water, we observed two steps in the apparent viscosity variation: first an increase from 8 to 26 mPa s, followed by a decrease (Fig. 6). This may be explained by, first, the oil globules swelling as a result of

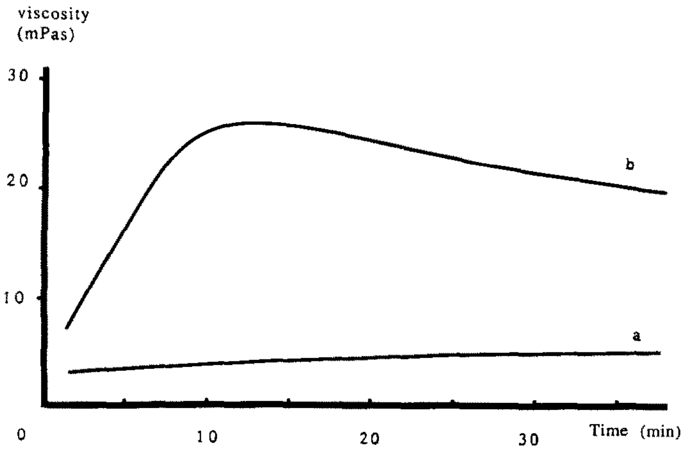

Fig. 6. Apparent viscosity variation versus time of diluted multiple W/O/W-B emulsion samples: (curve a) suppressing the osmotic pressure gradient and (curve b) conserving the osmotic pressure gradient.

the water transfer from the outer to the inner aqueous phase, and then by rupture of the oil film due either to oil vesicles bursting or to shearing effects [20,21].

CONCLUSIONS

Differential scanning calorimetry performed on multiple W/O/W emulsions indicates freezing around −15°C and −44°C respectively for the continuous and for the dispersed aqueous phase. Furthermore there is a vapor pressure gradient between the outer frozen water and the inner undercooled water which leads to a water transfer from the dispersed to the continuous aqueous phase during the cooling of a multiple emulsion sample. The addition of solutes reduces the vapor pressure of the undercooled water and may be the way to limit and even to suppress the water transfer during cooling.

By using a 10% urea solution as dispersed phase, we created an osmotic pressure gradient in "a mother multiple emulsion". This gradient leads to mass transfers at room temperature between the two aqueous phases. It was shown that those transfers could be studied by calorimetry and viscosity analysis. Calorimetry and viscosimetry appear to be complementary methods to study mass transfer in a diluted multiple emulsion.

Calorimetric analysis is not the most appropriate method to study liberation of solutes in the external aqueous phase because crystallization is a random phenomenon. Even an evolution of 4.4°C in the freezing temperature of the external phase (Figs. 4 and 5) is not significant. Several experiments should be made in order to obtain the mean freezing temperature of the continuous phase and then to conclude that the liberation of solutes has occurred in the external phase by noticing a decrease of this mean temperature. Supplementary conductivity and dialysis studies are under investigation.

ACKNOWLEDGEMENT

This work was realized with FIRTECH financial support concerning the development of methods to study dispersed liquid–liquid systems.

REFERENCES

1 W. Seifriz, J. Phys. Chem., 29 (1925) 738.
2 W.J. Herbert, Lancet, 2 (1965) 771.
3 R.H. Engel, S.J. Riggi and N.J. Fahrenbach, Nature (London), 219 (1968) 856.
4 L.A. Elson, B.C.V. Mitchley, A.J. Collings and R. Schneider, Rev. Eur. Etud. Clin. Biol., 15 (1970) 87.
5 A.F. Brodin, D.R. Kavaliunas and S.G. Frank, Acta. Pharm. Suec., 15 (1978) 1.
6 D. Whitehill, Chem. Drug., 213 (1980) 130.
7 J.W. Frankenfeld, G.C. Fuller and C.T. Rhodes, Drug Dev. Commun., 2 (1976) 405.
8 C. Chiang, G.C. Fuller, J.W. Frankenfeld and C.T. Rhodes, J. Pharm. Sci., 67 (1978) 63.
9 S.S. Davis, Chem. Ind. (London), (1981) 683.
10 A.T. Florence and D. Whitehill, Int. J. Pharm., 11 (1982) 277.
11 S. Matsumoto, in M.J. Schick (Ed.), Nonionic Surfactants, Surfactant Science Series, No. 23, Marcel Dekker, New York and Basel, 1987, p. 549.
12 M. Seiller, C. Vaution, M. de Luca, Bull. Tech. - Gattefosse, 80 (1987) 27.
13 M. de Luca, C. Vaution, A. Rabarron and M. Seiller, STP Pharma, 4(8) (1988) 679.
14 M. de Luca, P. Rocha-Filho, J.L. Grossiord, A. Rabarron, C. Vaution and M. Seiller, Int. J. Cosmet. Sci., 13 (1991) 1.
15 D. Clausse, Encyclopedia of Emulsion Technology, Vol. 2, Marcel Dekker, 1987, p. 77.
16 J.-P. Dumas, D. Clausse and F. Broto, Thermochim. Acta, 13 (1975) 261.
17 F. Broto, D. Clausse, L. Babin and M. Mercier, J. Chim. Phys., 75 (1978) 10.
18 D. Clausse, Rev. Gen. Therm., 279 (1985) 263.
19 Handbook of Chemistry and Physics, CRC Press, Boca Raton, 62nd edn., 1982.
20 M. Tomita, Y. Abe and T. Kondo, J. Pharm. Sci., 71 (1982) 332.
21 S. Matsumoto, M. Kohda and S. Murata, J. Colloid Interface Sci., 62 (1977) 149.