Journal of Thermal Analysis and Calorimetry, Vol. 92 (2008) 2, 643–648

ON THE STABILITY OF EXTREMELY DILUTED AQUEOUS SOLUTIONS AT HIGH IONIC STRENGTH
A calorimetric study at 298 K

V. Elia[*], *E. Napoli and M. Niccoli*

Department of Chemistry, University 'Federico II' of Naples, Complesso Universitario di Monte S. Angelo, Via Cintia
80126 Naples, Italy

An extensive study has been carried out on extremely diluted aqueous solutions (EDS). These solutions revealed a really intriguing physico-chemical behaviour, characterized by multiple independent variables. Because of their behaviour, EDS can be described as far-from-equilibrium systems, capable of self-organization as a consequence of little perturbations.

In this paper we investigate the stability of the calorimetric behaviour of EDS with a high ionic force, due to the presence of the sodium chloride electrolyte. We measured the excess heats of mixing of EDS with basic solutions, both with and without a high concentration of NaCl, and compared the results. In particular, we explored these concentrations: 0.5 and 1 M mol kg^{-1}). The analysis of the experimental results shows that the calorimetric response of the EDS is stable when they are in a concentrated solution of NaCl. That is of great relevance for the eventual pharmacological action of these solutions, since it involves the interaction with fluids of complex chemical composition and high concentration.

Keywords: *age of the EDS, calorimetry, dissipative structures, EDS, extremely diluted solutions, high ionic strength*

Introduction

In previous papers [1–11], EDS have been the object of several kinds of measurements, among which the calorimetric and conductivity measures stood out as the most useful in describing them. The EDS are peculiar because of their preparation technique: by the term extremely diluted solutions (EDS), we mean solutions in which a substance has been diluted through a special iterative process of successive dilutions and succussions. The latter are violent vertical shakings in between each dilution step. Through the iterated dilutions, the concentration of the solute becomes negligible in a few steps. It is therefore reasonable to expect the physico-chemical properties of such dilutions to be identical to those of the solvent used. In practice, however, the EDS exhibit different physico-chemical proprieties compared to their solvent. EDS behave as complex systems, influenced by peculiar aspects of both their preparation technique and storage conditions [1–11].

We measured the heats of mixing of basic solutions with the EDS and their electrical conductivity, comparing with the analogous heats of mixing and electrical conductivity of the solvent. Some relevant exothermic excess heats of mixing were found, as well as higher electrical conductivities than those of the untreated solvent [3, 4]. These physico-chemical results let us suppose that a supermolecular organization is present in the EDS [10, 11]. We evidenced the influence of several parameters on the calorimetric and conductometric behaviour of the solutions under study: nature of solute; degree of dilution; succussion procedure; volume of the samples and their age [3, 9, 11].

These and other studies [10–22], as a whole, back up the concept of water as a complex system, led to self-organization by perturbations of small entity (dilution, succussions, electromagnetic fields, etc.), with variations of the physico-chemical properties that can be either permanent, temporary or oscillating.

In this work, in order to test the stability of high ionic force EDS, we carried out calorimetric measures of mixing with sodium hydroxide solutions, in presence of sodium chloride at high concentration. The aim of this experimental work is that of gaining insight on the physico-chemical behaviour of EDS in concentrated solutions of salts (NaCl). The effect of the administration of the EDS to biological systems, of various nature and complexity, strictly depends on the stability of the physico-chemical behaviour of these solutions when mixed with fluids of complex composition and high concentration.

Experimental

Materials

The solutes were Carlo Erba, Sigma or Fluka products, of the highest purity commercially available. The ex-

---

[*] Author for correspondence: vittorio.elia@unina.it

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

tremely diluted solutions were prepared using as solvent a solution of sodium bicarbonate, at low and known concentration ($5\cdot10^{-5}$ mol L^{-1}). The laboratory glass containers were treated with $H_2SO_4/K_2Cr_2O_7$. The sodium chloride solutions (by Sigma) were prepared by mass, employing the previously prepared solute, desiccated for some hours at 130°C. As solvent we used: twice distilled water (to determine the heat of dilution); sodium bicarbonate solutions (to determine the chemical contribution to subtract from the heat of mixing, in order to obtain the excess heat); the EDS themselves; and finally solutions of NaOH 0.01 M (to measure the heat of mixing).

Procedures

The EDS studied were prepared according to the procedure mentioned in the Introduction section. For example, to prepare a *centesimal dilution* of 2,4-dichlorophenoxyacetic acid (2,4-D), 1 g of 2,4-D was added to 99 g of solvent (dilution step). The solution was then subject to the process of 'dynamization' (succussion step), consisting in a violent shaking by means of a mechanical apparatus (DYNA HV 1 by Debofar N.V.S.A. Belgium). The final solution is called '1 centesimal hahnemannian' or 1 CH in short. So in the preceding example, 2,4-D 1 CH was obtained. In order to prepare the 2 centesimal hahnemannian (2,4-D 2 CH), 1 g of the 1 CH solution was added to 99 g of solvent, and the resulting solution was 'dynamized'. The dilution and succussion steps were iterated until the desired degree of dilution was met. Potentized water, W, is obtained using the same procedure described above, starting with pure solvent without active principle.

Methods

Calorimetry

The EDS were studied through mixing-flow microcalorimetry. The heats of mixing were monitored using a Thermal Activity Monitor (TAM) model 2227, by Thermometric (Sweden), equipped with a flow mixing vessel. A P3 peristaltic pump from Pharmacia sends the solutions into the calorimeter through Teflon tubes. The experimental apparatus is detailed in previous papers [1–3]. The value of the mixing heat, Q_{mix}, was obtained using the following formula:

$$Q_{\mathrm{mix}}(m_x^i m_y^i \to m_x^f m_y^f) = (\mathrm{d}Q/\mathrm{d}t)/P_w$$

where $\mathrm{d}Q/\mathrm{d}t$ is the heat flux (W), P_w is the total mass flow-rate of the solvent (kg s^{-1}) and m_x^i, m_y^i and m_x^f, m_y^f are the initial and final molalities. Q_{mix} is given in J kg^{-1} of solvent in the final solution.

The experimental procedure we used can be outlined as such:

- In the first step, we determined the excess heat of the EDS by using a 'probe' solution. The latter was a NaOH 0.01 M solution in twice distilled water. The excess heat of mixing, $-Q_{\mathrm{mix}}^{E}$, is defined as the heat of mixing of the probe solution with the EDS, $-Q_{\mathrm{mix}}$, minus the heat of mixing of the same probe solution with the solvent (sodium bicarbonate solutions), having the same contents of chemical impurities as the EDS [15].
- In the second step, we measured the excess heat of the EDS again, but this time at a high ionic force, at two different concentrations of sodium chloride: NaCl 0.5 and 1 M. The probe solution was a NaOH 0.01 M solution in aqueous NaCl solutions at 0.5 and 1 M; The excess heats of mixing, $-Q_{\mathrm{mix}/H_2O}^{E}$ (J kg^{-1}) in NaCl is defined as the heat of mixing of the probe solution NaOH 0.01 M (in NaCl 0.5 or 1 M) with the EDS (in NaCl 0.5 or 1 M).

Analytical determination of impurities

Care was taken to account for impurities, released by the glass containers that can affect the calorimetric results. The glass may release sodium oxides that, in contact with the atmospheric CO_2, get converted to sodium bicarbonate. Therefore we determined the sodium concentration, through atomic absorption spectrometry carried out with a Varian Spectra A instrument. Before measuring the samples with the instrument, a calibration curve was obtained using standard solutions. To prepare the standard solutions, NaCl was dissolved in water and diluted to 1 L to obtain 1000 μg mL^{-1} Na. The working conditions were: lamp current: 5 mA; fuel: acetylene; support: air; flame stoichiometry: oxidizing. The wavelength used depends on the concentration range of sodium. For our samples it was 589.6 nm. Sodium is partially ionised in the air-acetylene flame. To suppress ionisation, some potassium chloride solution was added to produce a final concentration of 2000 μg mL^{-1} in all solutions, including the blank [23]. Impurities were in the ppm range.

The glass may also release silica, giving rise to silicic acid. The silicic acid concentration was determined by measuring the UV absorption of complexes with ammonium molybdate.

Results and discussion

Table 1 contains the results of the study on the EDS, performed with mixing-flow microcalorimetry and electrical conductivity. Table 1 reports: active principle; degree of dilution; excess conductivity χ^{E}

Table 1 Excess conductivity (χ^E/µS cm^{-1}) and excess heats of mixing of the EDS with NaOH 0.01 M, in water ($-Q^E_{mix/H_2O}$/J kg^{-1}), NaCl 0.5 M ($-Q^E_{mix}$ (0.5 M)/J kg^{-1})) and 1 M ($-Q^E_{mix}$(1 M)/J kg^{-1}))

System	χ^E	$-Q^E_{mix/H_2O}$	$-Q^E_{mix}$ (0.5 M)	$-Q^E_{mix}$ (1 M)
H_2O	1.2±0.2	1.05±0.05[a]	0.93±0.05[b]	1.00±0.04[c]
W 12 CH	–	0.40	–	0.62
W 30 CH	2.05	0.09	–	0.11
W 30 CH	7.23	0.74	–	0.84
W 3 CH	1.56	0.51	0.55	0.37
W 12 CH	1.13	0.09	0.17	–0.01
W 9 CH	1.80	0.54	0.62	0.49
W 3 CH	1.56	0.52	0.55	0.37
W 12 CH	1.13	0.09	0.17	–0.01
W 3 CH	2.98	0.49	0.59	0.37
W 6 CH	2.36	0.45	–	–0.03
W 30 CH	1.23	0.32	–	–0.08
W 30 CH	2.64	0.10	–	0.25
W 30 CH	1.04	0.74	0.83	0.28
W 30 CH	0.62	0.15	–	0.22
W 7 CH	6.56	2.56	–	1.86
W 9 CH	3.67	0.65	0.75	0.15
W 5 CH	2.58	1.07	1.18	1.09
W 7 CH	2.09	1.14	–	0.85
W 30 CH	3.05	1.28	–	0.81
W 12 CH	5.05	1.16	1.65	1.08
W 12 CH	0.70	0.05	0.15	0.05
W 5 CK	0.80	0.15	–	0.59
W 12 CK	2.10	0.26	–	0.34
W 12 CK	1.51	0.05	0.18	0.16
W 12 CK	2.20	0.37	0.42	0.53
W 30 CK	1.60	0.21	–	0.16
W 12 CH	–	0.30	0.68	0.59
W 30 CH	–	–	–	0.33
W 12 CH	–	0.68	0.42	0.86
2,4–D 3 CH	–	0.51	0.69	0.22
2,4–D 7 CH	8.08	0.98	0.88	1.03
2,4–D 6 CH	3.22	2.34	2.36	1.77
2,4–D 9 CH	1.52	1.58	1.92	1.28
2,4–D 12 CH	7.47	4.45	4.31	4.11
2,4–D 6 CH	1.27	2.68	2.73	2.60
2,4–D 9 CH	3.84	0.86	0.72	0.67
2,4–D 12 CH	3.31	0.65	0.65	0.40

[a] heat of mixing (J kg^{-1}) of an aqueous solution of NaOH 0.01 M. Mean and standard deviation are calculated using 60 experimental measures of dilution with bidistilled water

[b, c] heat of mixing (J kg^{-1}) of NaOH 0.01 M, prepared with an aqueous solution of NaCl as solvent, with NaCl 0.5 and 1 M solutions. Mean and standard deviation calculated using 12 experimental dilution measures with aqueous solution of NaCl 0.5 and 1 M

(μS cm^{-1}); excess heats of mixing in water, $-Q^{E}_{mix/H_2O}$ (J kg^{-1}); excess heats of mixing in NaCl 0.5 M, $-Q^{E}_{mix/NaCl-0.5}$ (J kg^{-1}); excess heats of mixing in NaCl 1 M, $-Q^{E}_{mix/NaCl-1}$ (J kg^{-1}). To obtain the excess heats of mixing (in water and in NaCl) we subtracted the contribute coming from impurities, whose concentration was analytically determined.

As previously stated, the aim of this study was to verify whether the introduction of a perturbation, such as NaCl at high concentration, would influence the physico-chemical behaviour of EDS. The choice of the NaCl concentrations, 0.5 and 1 M, was meant as a preliminary test based on the potentiometric experiments performed on EDS in concentrated NaCl.

The outcome of these new experiments reveals that the presence of the electrolyte does not influence the amount of excess heat in a relevant way. Figure 1 is a graph of the excess heat, $-Q^{E}_{mix/NaCl}$, as a function of the NaCl concentration. It can be noted that the heat is almost unaltered by the increase of ionic strength. The same trend can be found in Fig. 2, showing the excess heat of the EDS in water, $-Q^{E}_{mix/H_2O}$ (J kg^{-1}), compared to that obtained in NaCl, $-Q^{E}_{mix/NaCl}$ (J kg^{-1}). This figure also contains a line with slope equal to one that, as can be seen, fits the experimental data well.

In order to verify whether such stability is kept with time, we carried out a series of calorimetric measures, for some selected EDS in NaCl, over a period of 257 days, as reported in Table 2 and Fig. 3. The thermal excess of the EDS in NaCl follows the same trend as that of the EDS with no electrolyte: an increase with time. This behaviour is further proof of the extraordinary (and unexpected) stability of the EDS at high salt concentration.

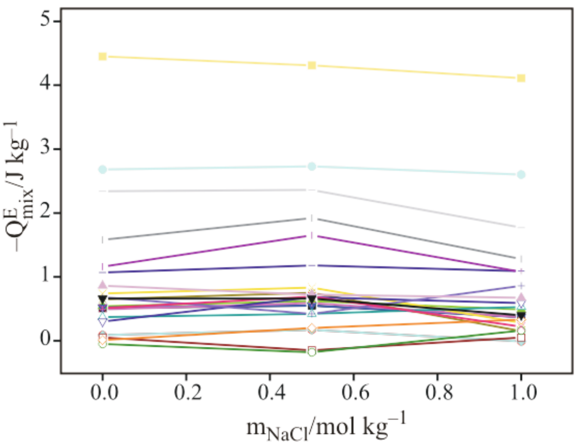

Fig. 1 Excess heat as a function of the NaCl concentration in the EDS

A large number of thermodynamic and transport measurements were conducted on EDS [1–11]. The body of calorimetric and conductometric measures suggests that the EDS are complex systems in a

Table 2 Excess heats of mixing of the EDS with NaOH 0.01 M, in water ($-Q^{E}_{mix/H_2O}$), NaCl 0.5 M ($-Q^{E}_{mix}$ (0.5 M)) and 1 M ($-Q^{E}_{mix}$ (1 M)), as a function of the age of samples

System	$-Q^{E}_{mix/H_2O}$ (0 day)[1]	$-Q^{E}_{mix/H_2O}$ (257 days)[2]	$-Q^{E}_{mix}$ (0.5 M) (0 day)[1]	$-Q^{E}_{mix}$ (0.5 M) (257 days)[2]	$-Q^{E}_{mix}$ (1 M) (0 day)[1]	$-Q^{E}_{mix}$ (1 M) (257 days)[2]
H_2O	1.05±0.05[a]	1.05±0.05				
NaCl			0.93±0.05[b]	0.83±0.05	1.00±0.04[c]	1.00±0.04
W 12 CH	2.76	3.4	3.09	3.73	3.09	3.85
W 30 CH	2.33	3.21	2.68	3.5	2.7	3.73
W 12 CH	2.46	2	2.63	2.34	2.71	2.62
W 6 CH	2.55	3.18	2.76	3.8	2.84	3.96
W 12 CH	3.02	3.4	3.21	3.74	3.4	3.8
W 12 CH	2.76	4.39	3.12	4.7	3.16	5.06
W 3 CH	4.42	4.87	4.7	5.5	5.19	5.67
W 9 CH	3.75	3.95	4.34	4.6	5.15	4.86
W 12 CH	4.01	4.16	4.56	4.76	4.73	4.87
W 30 CH	4.28	4.46	4.33	4.56	4.36	4.61
W 30 CH	4.66	4.32	4.73	5.42	5.12	5.6

[1] Excess heats of mixing determined at 537 days of age for the EDS (*t*=0 day)

[2] Excess heats of mixing determined at 794 days of age for the EDS (*t*=257 days). The samples at high ionic strength were stored in NaCl (0.5 or 1 M) from *t*=0, to the calorimetric measure at 794 days (*t*=257)

[a] heat of mixing (J kg^{-1}) of an aqueous solution of NaOH 0.01 M. Mean and standard deviation are calculated using 60 experimental measures of dilution with twice distilled water

[b, c] heat of mixing (J kg^{-1}) of NaOH 0.01 M, prepared with an aqueous solution of NaCl as solvent, with NaCl 0.5 and 1 M solutions. Mean and standard deviation calculated using 12 experimental dilution measures with aqueous solution of NaCl 0.5 and 1 M

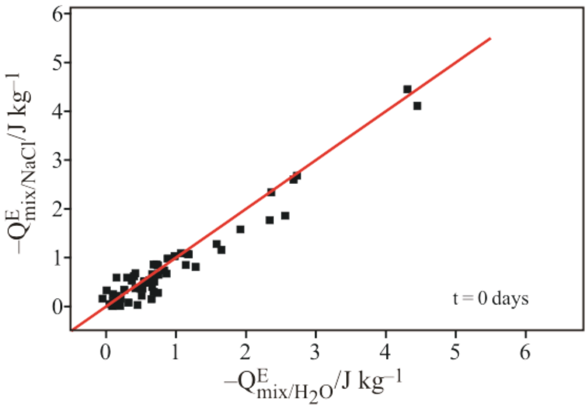

Fig. 2 Excess heat of the EDS in presence of NaCl, as a function of the excess heat determined for the same EDS in water. The measure was carried out at the time of preparation of the EDS in NaCl (*t*=0 day)

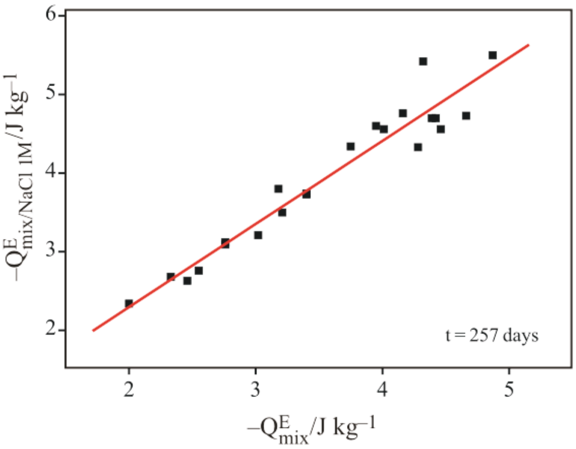

Fig. 3 Excess heat of the EDS in presence of NaCl, as a function of the excess heat determined for the same EDS in water. The measure was carried out 257 days after the preparation of the EDS in NaCl (*t*=257 days)

far-from equilibrium condition [7, 9–11, 24–26]. Fortunately, despite an extremely varied physico-chemical picture, some behaviours are reproducible on a qualitative level: both the excess heat of mixing, $-Q^E_{mix}$ (J kg^{-1}), and the excess conductivity, χ^E (μS cm^{-1}), grow as the samples get older; the excess electrical conductivity was found to be correlated to the excess heat of mixing with NaOH 0.01 M [3–4]. The correlation between excess heat and excess conductivity is linear, with a slope growing with the samples age [5–6].

In order to explain the observed behaviour, we were forced to focus our attention on the solvent and, in particular, on possible physico-chemical changes induced by the preparation procedure. Such an experimental picture is compatible with a model for the solvent that involves molecular aggregates and provides a mechanism for their formation [7, 9–11]. A first hypothesis is that the EDS system, because of the peculiar preparation protocol, is led to the attainment of a minimum of energy, an equilibrium condition. The observed time scale is so long as to be incompatible with such a scenario, though. A possible alternative hypothesis is that the succussion procedure triggers a macro oscillation that allows for the formation of an ordered structure from the molecular chaos of the liquid. Such an ordered structure would be far from the equilibrium, and would keep far from it by dissipating the energy available from the external environment. The extremely slow temporal evolution of the EDS that is further influenced by the volume of the samples during their storage would be compatible with such a scenario [7, 9–11].

The hypothesis of a far-from-equilibrium system and of the formation of dissipative structures does not lead to a stable system but, on the contrary, to a system whose existence depends on the possibility to dissipate energy from the environment, and that is thus intrinsically unstable [24–25].

The studies performed in presence of high concentrations of NaCl show that such dissipative structures exhibit a truly remarkable stability of their calorimetric behaviour, and that their evolution is not influenced by the electrolyte.

The calorimetric behaviour of the EDS can be explained on the basis of two possible interaction mechanisms [6]:

- in mixing with the EDS, the Na^+ and OH^- ions would more efficiently hydrate than in water. The ions present in the probe solution could probably use the aggregates of water molecules found in the EDS solvent to build their hydration sphere, with a consequent increase of heat of mixing.
- The increase of pH when mixing with NaOH solutions would induce a reduction, or a disruption, of the water molecules aggregates. Such a reduction or disruption would account for part of the measured thermal excess. To explain the exothermic excess of mixing we need to invoke the effect of the destructured hydration sphere of the water molecules of hydrophilic solutes [27].

The calorimetric behaviour of high ionic force EDS can be used to prefer one model over the other. The small quantity of available bulk water in NaCl at high ionic strength leads to prefer the second model. The stable thermal excess of the EDS in concentrated NaCl hints towards the model in which alkaline solutions exhibit a disrupting potential on the supermolecular structure of water. That potential would probably be linked to the attitude, of basic solutions, to break the hydrogen bonds that are likely to aggregate the water molecules in the EDS.

Anyway work is in progress on the conductometric titration of the EDS, using basic or acid solutions as titrant.

References

1 V. Elia and M. Niccoli, Ann. N. Y. Acad. Sci., 879 (1999) 241.
2 V. Elia and M. Niccoli, J. Therm. Anal. Cal., 61 (2000) 527.
3 V. Elia and M. Niccoli, J. Therm. Anal. Cal., 75 (2004) 815.
4 V. Elia, E. Napoli, M. Niccoli, L. Nonatelli, A. Ramaglia and E. Ventimiglia, J. Therm. Anal. Cal., 78 (2004) 331.
5 V. Elia, M. Marchese, M. Montanino, E. Napoli, M. Niccoli, L. Nonatelli and A. Ramaglia, J. Solution Chem., 34 (2005) 947.
6 V. Elia, L. Elia, P. Cacace, E. Napoli, M. Niccoli and F. Savarese, J. Therm. Anal. Cal., 84 (2006) 317.
7 V. Elia, L. Elia, E. Napoli and M. Niccoli, Int. J. Ecodyn., 1 (2006) 1.
8 V. Elia, L. Elia, M. Marchese, M. Montanino, E. Napoli, M. Niccoli, L. Nonatelli and F. Savarese, J. Mol. Liq., 130 (2007) 15.
9 V. Elia, L. Elia, M. Montanino, E. Napoli, M. Niccoli and L. Nonatelli, J. Mol. Liq., 135 (2007) 158.
10 V. Elia, E. Napoli, M. Niccoli, N. Marchettini and E.Tiezzi, J. Solution Chem., (2007), accepted.
11 P. Belon, V. Elia, L. Elia, M. Montanino, E. Napoli and M. Niccoli, J. Therm. Anal. Cal., (2007), accepted.
12 V. I. Lobyshev, R. E. Shikhlinskaya and B. D. Ryzhikov, J. Mol. Liq., 82 (1999) 73.
13 V. I. Lobyshev, A. B. Solovey and N. A. Bulienkov, J. Mol. Liq., 106 (2003) 277.
14 l. Rey, Physica A, 323 (2003) 67.
15 S. Samal and K. E. Geckeler, Chem. Commun., (2001) 2224.
16 O. Mishima and H. E. Stanley, Nature, 392 (1998) 164.
17 G. Malescio, G. Franzese, A. Skibinsky, V. Buldyrev, V. Sergey and H. E. Stanley, Phys. Rev. E, 71 (2005) 061504/1.
18 H. J. Bakker, M. F. Kropman and A. W. Omta, J. Phys. Condens. Matter, 17 (2005) 3215.
19 G. W. Robinson, C. H. Cho and G. I. Gellene, J. Phys. Chem. B, 104 (2000) 7179.
20 S. Woutersen and H. J. Bakker, Nature, 402 (1999) 507.
21 S. Y. Lo, Mod. Phys. Lett. B, 10 (1996) 909.
22 S. Y. Lo, A. Lo, L. W. Chong, L. Tianzhang, L. H. Hua and X. Geng, Mod. Phys. Lett. B, 10 (1996) 921.
23 Varian Spectra Manual.
24 G. Nicolis, The New Physics, P. Davies, Ed., Cambrige University Press, New York 1989.
25 I. Prigogine, Nobel Lecture, 8 December, 1977.
26 A. Magnani, N. Marchettini, S. Ristori, C. Rossi, F. Rossi, M. Rustici, O. Spalla and E. Tiezzi, J. Am. Chem. Soc., 126 (2004) 11406.
27 G. Castronuovo, V. Elia and F. Velleca, Curr. Top. Solution Chem., 2 (1997) 125.

Received: October 15, 2007
Accepted: October 17, 2007

DOI: 10.1007/s10973-007-8840-y