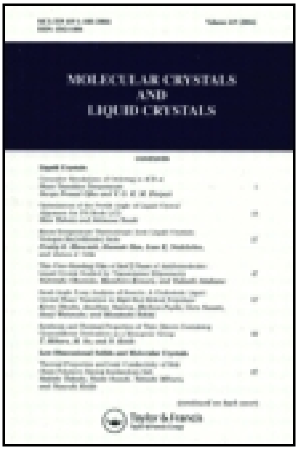

Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Electrochemical Characterization of Fluid Vesicles in Natural Waters

Vera žutić [a] , Tinka Pleše [a] , Jadranka Tomaić [a] & Tarzan Legović [a]

[a] Center for Marine Research, "Rudjer Bošković" Institute, Zagreb, Yugoslavia

To cite this article: Vera žutić , Tinka Pleše , Jadranka Tomaić & Tarzan Legović (1984): Electrochemical Characterization of Fluid Vesicles in Natural Waters, Molecular Crystals and Liquid Crystals, 113:1, 131-145

To link to this article: http://dx.doi.org/10.1080/00268948408071678

Mol. Cryst. Liq. Cryst., 1984, Vol. 113, pp. 131-145
0026-8941/84/1134-0131/$20.00/0

Printed in the United States of America

ELECTROCHEMICAL CHARACTERIZATION OF FLUID VESICLES IN NATURAL WATERS

VERA ŽUTIĆ, TINKA PLEŠE, JADRANKA TOMAIĆ and
TARZAN LEGOVIĆ

Center for Marine Research, "Rudjer Bošković"
Institute, Zagreb, Yugoslavia

Abstract The electrochemical method of polarographic maximum based on the interfacial instability of a positively charged interface dropping mercury electrode/seawater, allows a direct characterization of single events of coalescence and transformation of aggregates of fluid surfactants into adsorbed monolayers at the interface (relaxation times 10 - 500 ms, aggregation numbers $> 10^9$).

Highly surface active aggregates, resembling those of unsaturated lipids, were detected in the sea surface microlayer samples and in the mixing layers in the estuaries, although the unsaturated lipids represent only a minor fraction (<10%) of the aquatic organic matter. Products of flocculation of dissolved organic matter during estuarine mixing resemble closely fluid vesicles released by marine microflagellate, such as *Dunaliella tertiolecta*, that was studied in a greater detail.

The analysis of complex electrochemical responses in natural heterodispersion is developed using artificial dispersions of oleate and methyl oleate in seawater, as models.

INTRODUCTION

In natural waters hydrophobic compounds commonly exist in a micellar or colloid accomodated state rather than as true molecular solutes[1]. It is well known that the degree of aggregation influences transport properties and interactions of hydrophobic organic matter with surfaces and solutes in marine environment. However, fluidity of aggregates that affects dramatically the rate of interactions at interfaces[2], has not been adequately taken into account[3].

A possibility to elucidate size, distribution and reactivity of surface active aggregates in diluted heterodispersions (>100 µg/l) from polarographic response[4-6] is most interesting for the characterization of a highly reactive and important component of natural aquatic system[7], which is not amenable to analysis by the standard oceanographic methods.

EXPERIMENTAL

Measurements are performed in a 100 ml all glass cell open to air (so that measured solutions are equilibrated with atmospheric oxygen) and termostated at 20°C. The working electrode is a fast dropping mercury electrode (the capillary used in this work had the flow rate 5.2 mg/s and drop time 2.2 s) and an Ag/AgCl electrode is used as reference. Pt counter electrode completed the three-electrode configuration.

10^{-3} M Hg(II) (0.5 ml of 0.2 molar $HgCl_2$ solution) is added to a 100 ml sample just prior to measurement. If not specifically stated natural samples and dispersions were not filtered.

PAR 174 Polarographic Analyser in connection with a 7004 B Hewlett-Packard recorder and Gould digital storage oscilloscope OS 4000 has been used for registration of i-t curves at constant potential -0.3 V, where the mercury electrode is positively charged.

Registration of the i-t curves is usually completed within 10 minutes after insertion of the dropping mercury electrode into the test solution. Reproducibility of independent measurements is ± 2% at the concentration level of 1 mg/l (of dissolved surfactants).

MATERIALS

Artificial seawater, used as the electrolyte, was of the following composition: 0.6 M NaCl, 10^{-3} KBr and 5×10^{-3} M $NaHCO_3$. The Adriatic seawater had salinity 38 ‰ and pH = = 8.2. Stock dispersions of fatty acids and esters were prepared by shaking a given amount (5 - 100 mg) in the artificial seawater, or 5×10^{-3} M $NaHCO_3$ solution for four hours. Freshly prepared dispersions were measured to avoid any significant transformation, such as oxidation of double bonds. Surfactants of the highest commercial purity were used without any further purification. A special care was taken with respect to purity of water, glassware and the atmosphere in the laboratory.

Marine phytoplankton cell suspensions were prepared as previously described[5]. Microflagellate *Dunaliella tertiolecta* and diatom *Pheodactylum tricornutum* were axenic.

Stock suspensions of hydrous δ-Al_2O_3 were prepared from the Degussa monodispersed product with BET surface area 110 m^2/g.

METHOD

Stationary and non-stationary convective streamings might be generated by gradients of surface tension $d\gamma/dE$ (where γ is surface tension and E potential at the liquid-liquid interface dropping mercury electrode-aqueous solution). Microscopic perturbations at the interface are amplified to macroscopic instabilities (Maragoni instability[9-10]) demonstrated by a measurable increase in current (polarographic maxima of the first kind) due to the coupling of the perturbations in a cyclic chain: surface tension → surface motion → bulk motion → diffusional mass transport → surface electrochemical potential → surface tension. Under controlled experimental conditions (rate of mercury flow, concentration of reducible solute and electrolyte, absence of trace surfactants) the effect is highly reproducible[5,11].

Soluble surfactants adsorb at the mercury/seawater interface and decrease the surface tension gradient $d\gamma/dE$ slowing down the convective streaming (Fig. 1), which provides a basis for their determination[5,11] (curves 1 and 2 in Fig. 2).

However, heterodispersions of fluid surfactants, such as unsaturated fatty acids and their esters, produce irregular current oscillations of polarographic maximum of Hg(II) in seawater due to random collisions of aggregates of variable size with the interface mercury electrode/solution (Fig. 3)[6].

The perturbations in the current-time curves (Fig. 3) of variable amplitude and frequency indicate a stochastic process, which can be interpreted as random collisions of surface active aggregates of variable size with the interface mercury electrode/aqueous solution. Thus, each pertur-

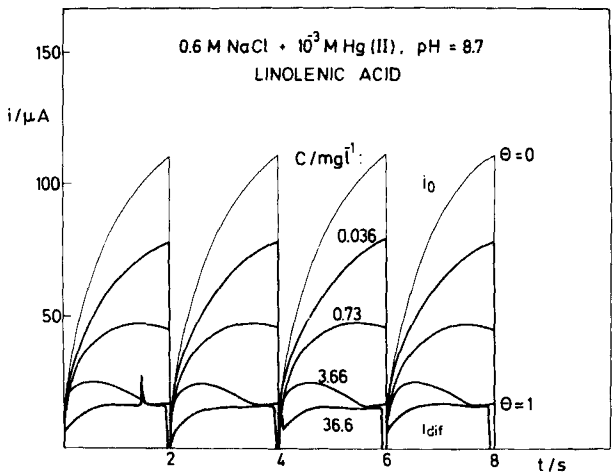

FIGURE 1. Current-time curves at the dropping mercury electrode for the reduction of 10^{-3}M Hg(II) in artificial seawater (i_0) and in presence of linolenic acid (0.036, 0.73, 3.66 and 36.6 mg l^{-1}), 20°C, E = -300 mV.

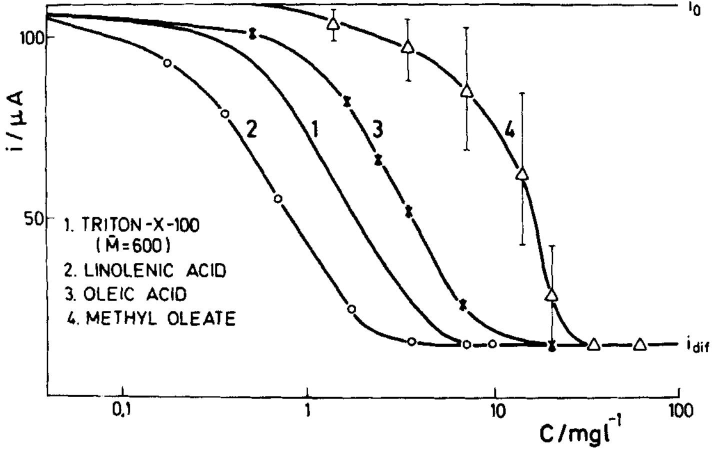

FIGURE 2. Dependence of current (at the end of the drop life, t = 2 s) on the concentration of (1) nonionic detergent Triton-X-100, (2) linolenic acid, (3) oleic acid and (4) methyl oleate.

bation corresponds to a single event of coalescence.

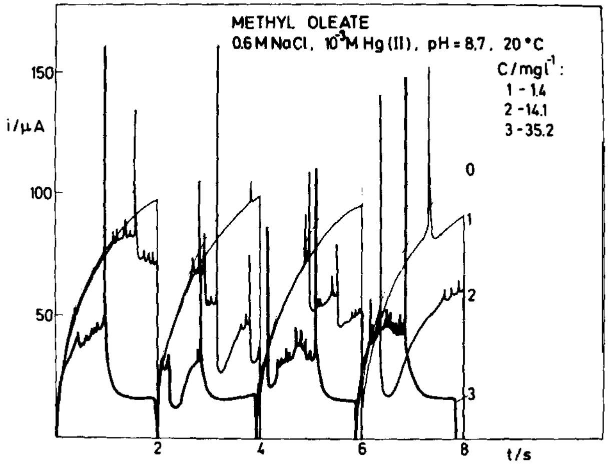

FIGURE 3. Current-time curves for reduction of 10^{-3} M Hg(II) in artificial seawater in presence of methyl oleate dispersions (1.4, 14.1 and 35.2 mg l^{-1}), 21°C, E = = -300 mV.

The characteristic form of perturbation (schematically presented in Fig. 4) is interpreted by the following sequence of events:

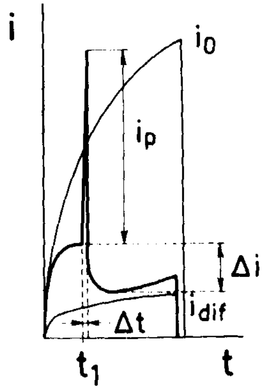

FIGURE 4. Schematic presentation of a single perturbation on the current--time curve.

(1) Before the arrival of an aggregate, current is controlled by convective diffusion driven by electrochemical and hydrodynamical instability at the interface (typical conditions of maxima of the first kind);

(2) An aggregate arrives at the interface. This has little effect on the current;

(3) The aggregate desintegrates, its material spreads over the surface and gives locally a patch of lower interfacial tension ($\gamma - \Delta\gamma$); time scale of spreading is much shorter than the drop life (2 s);

(4) Owing to the generated gradient $\Delta\gamma/\Delta x$, the patch rapidly spreads towards the regions of higher surface tension (Maragoni effect, driving force $(\Delta\gamma/\Delta C)(\Delta C/\Delta x)$[9] and this gives rise to a transient turbulent transport of matter from the bulk to the surface, superimposed on the initial flux. This is manifested as the peak current i_p. The time scale of the event is of the order 10^{-3} - 10^{-2} s. The peak height is a function of the size and fluidity of the aggregate[12] (Fig. 5);

(5) After this event, the surface concentration of surfactant is increased ($\Delta\Gamma$), and the current is lowered (Δi) with respect to the value before the onset of the perturbation.

An attempt can be made to assess the aggregation number m of an aggregate under the following simplifying assumptions:

1. aggregation number m of the aggregate is equal to the increase of the surface concentration, $\Delta\Gamma$ after the coalescence:

$$m_1 = \Delta\Gamma\, A_1\, N$$

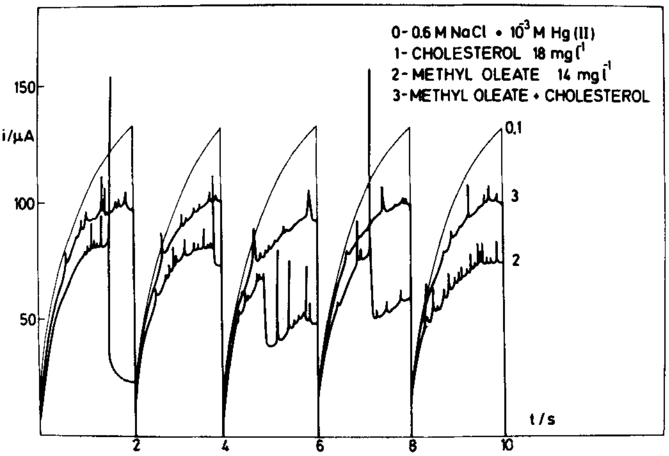

FIGURE 5. Effect of an equimolar addition of cholesterol (3) on the response in the methyl oleate dispersion, 14 mg l^{-1} (2), 30°C. Cholesterol alone (curve 1) does not affect current-time curve, i_o.

where Γ is the surface concentration of surfactant in mol/cm² and A_1 the surface area of the mercury drop at the time t_1, N is Avogadro number;

2. minimum surface coverage at which the polarographic maximum is totally supressed ($i = i_{dif}$) corresponds to a full monolayer;

3. drop of current after the peak, Δi, corresponds to an increase in the surface concentration $\Delta\Gamma$, due to the incorporation of the coalesced aggregate into the adsorbed layer:

$$\frac{\Delta i}{i_o - i} = \frac{\Delta\Gamma}{\Gamma_{max}}$$

The results obtained by this simplified calculation (measurable aggregation numbers are in the range $10^9 - 10^{10}$) are in good agreement with the experimental observations - effect of filtration and centrifugation (Fig. 6) and the particle size distribution as measured by Coulter counter technique (Fig. 7). This also indicates that the smallest aggregates, such as simple micelles cannot be distinguished from soluble surfactants because of a small amplitude of perturbation (in comparison to the noise level) and/or short time of their relaxation[13].

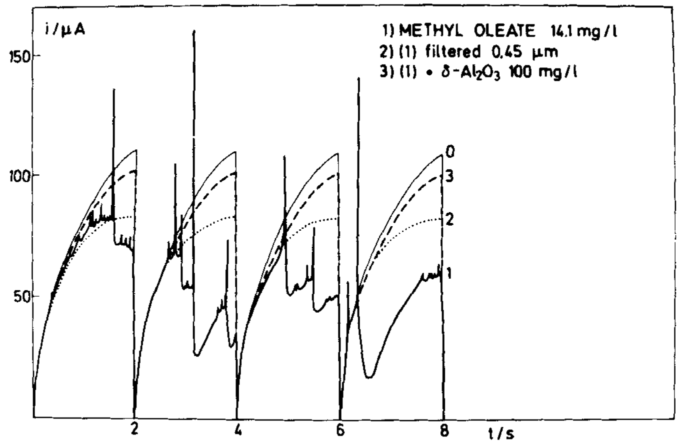

FIGURE 6. Current-time curves in presence of 14.1 mg l^{-1} methyl oleate before (1) and after filtration (2). (3) Current-time curves in dispersion 14.1 mg l^{-1} methyl oleate + 100 mg l^{-1} δ-Al$_2$O$_3$ after 2 hours shaking, 20°C.

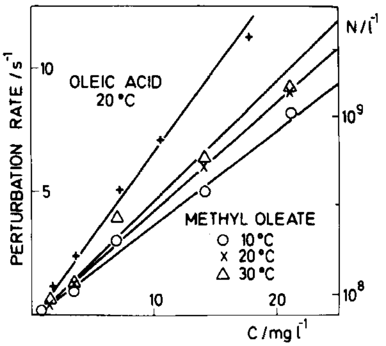

FIGURE 7. Dependence of number of perturbations on the current-time curves on the concentration of methyl oleate at different temperatures. Oleic acid at 20°C is presented for comparison. On the left hand side total particle number N`, as measured by Coulter counter (size fraction >1.3 μm) is indicated.

The relaxation time of the reorganization of an aggregate into the adsorbed layer at the electrode can be characterized more directly from the with of the current spike (Δt). The relaxation times observed for oleic acid (50-200 ms) correspond to the relaxation times of films at the air//seawater interface[14].

RESULTS AND DISCUSSION

Natural heterodispersions, such as phytoplankton cultures (Cryptomonas sp., Dunaliella tertiolecta) containing phytoplankton cells and fragments, such as fluid vesicles[14-16] show a similar irregular pattern in current-time curves (Fig. 8) and analogous dependence on the electrode potential as oleic aggregates[6]. However, the individual perturbations are less pronounced - the current spike i_p is significantly lower, due to the difference in fluidity of the aggregates. Besides, methyl oleate and oleic acid aggregates seem to adsorb at mercury in two dymensional ordered structure, as manifested by desorption maxima of the third kind[6,17], while there is no indication of two dymensional order in adsorbed layers formed after coalescence of natural aggregates at mercury electrode.

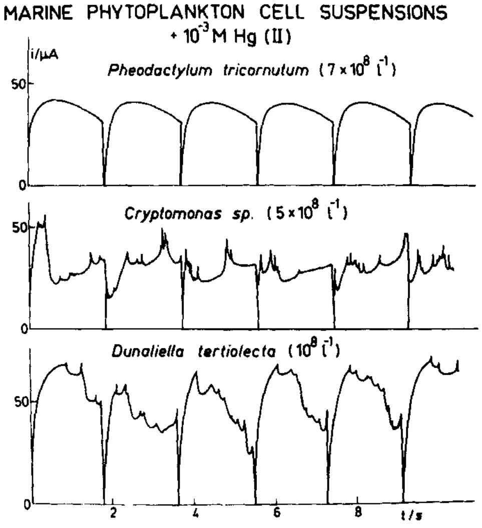

FIGURE 8. Current-time curves for the reduction of 10^{-3} M Hg(II) in the marine phytoplankton cell suspensions. Comparison of a typical response in diatom culture (Pheodactylum tricornutum) with two microflagellates (Dunaliella tertiolecta and Cryptomonas sp.) where the perturbations are clearly visible.

However, diatoms, such as *Pheodactylum tricornutum*, with rigid cell walls do not cause perturbations on the current-time curves (Fig. 8) but a smooth supression of convective current due to presence of soluble excretion products in the cell suspension[5].

The production of surface active vesicles seems to be inherent to flagellates[14,15] because of their characteristic cell membrane and the secretion of membrane structures with a high lipid content (<10%)[16]. However it is quite possible that the living cells themselves coalesce and partly reorganize into adsorbed layers in the time scale of the drop life (<2 s)[20,21]. The effect of hydrous alumina on *Dunaliella* cell suspension (Fig. 9) is similar to the experiment with

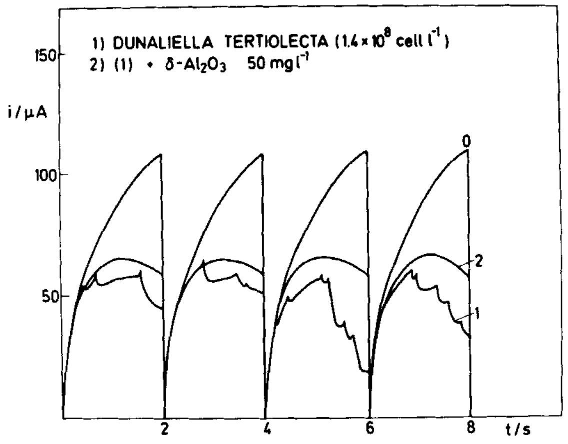

FIGURE 9. Effect of addition of δ-Al$_2$O$_3$ (50 mg l^{-1}) to the cell suspension of *Dunaliella tertiolecta* (1.4 x 10^8 cell l^{-1}), 20^{o}C, pH = 8.7.

dispersion of methyl oleate (Fig. 6) demonstrating clearly that natural as well as the artificial fluid aggregates are preferentially adsorbed at mineral interfaces, too.

Fluid surface active aggregates similar to those in phytoplankton cell suspensions, according to the type of perturbation in current-time curves, were detected in the mixing zone in estuaries[19] (Fig. 10). They seem to be generated by physiochemical processes - flocculation from dis-

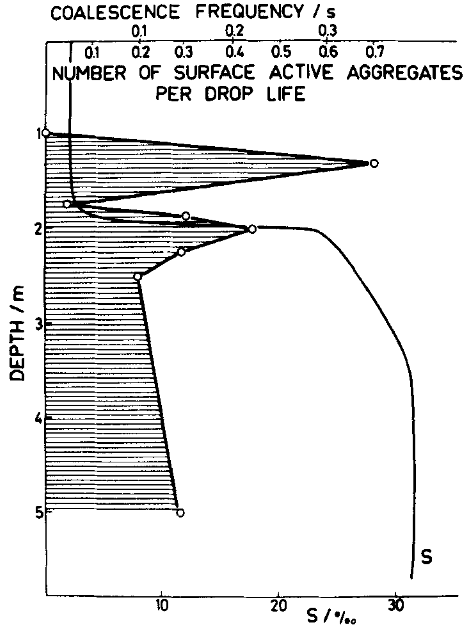

FIGURE 10. Relative distribution of surface active aggregates along the depth profile of a stratified estuary (river Aude, West Mediterranean, May 1983). The number of coalescence is defined as number of well defined perturbations on i-t curves at -300 mV.

solved riverine organic matter and smaller aggregates, and not by direct in situ primary production. High adsorbability of these aggregates at positively charged hydrophobic surface, as is mercury electrode, and a high rate of their reorganization into adsorbed layers at charged interfaces ($t < 500$ ms) has important implications in the transport and scavenging of hydrophobic organic matter and pollutants in estuaries. This highly reactive and instable fraction seems to be precursor of SEM visible organic coatings (persistent gel-like structures on mineral particles "voiles organiques") ubiquitous in estuarine zones[18].

ACKNOWLEDGEMENT

We wish to thank Dr. Damir Viličić from Biological Institute in Dubrovnik for preparation of phytoplankton cultures.

Financial support of Self-managing Community for Scientific Research of SR Croatia is gratefully acknowledged.

REFERENCES

1. C. Tanford, The hydrophobic effect; Formation of micelles and biological membranes, Wiley Interscience, New York, 200 p (1973).
2. D. Chapman (editor), Biological membranes. Physical facts and function, Academic Press, London, 438 p (1968).
3. E.K. Duursma and R. Dawson, Marine Organic Chemistry, Interactions and Chemistry of Organic Matter in Seawater, Elsevier, Amsterdam (1981).
4. J. Heyrovsky and J. Kuta, Principles of Polarography, Czechoslovak Acad. of Sciences, Praque (1965).
5. V. Žutić, B. Ćosović, E. Marčenko, N. Bihari and F. Kršinić, Mar. Chem. 10, 505 (1981).
6. T. Pleše and V. Žutić, J. Electroanal. Chem., in press.
7. W. Stumm and J. Morgan, Aquatic Chemistry, Wiley, New York (1981).

8. R. Aogaki, K. Kitazawa, K. Fukai and T. Mukaibo, Electrochim. Acta, 23, 867 (1978).
9. T.S. Sørensen, Dynamics and instability of fluid interfaces, Lecture notes on physics, Springer Verlag, Berlin, 276 (1978).
10. J. Reichenbach and H. Linde, J. Colloid. Interface Sci. 84, 433 (1981).
11. V. Žutić, J. Tomaić and T. Legović, submitted to J. Electroanal. Chem.
12. E. Lessner, M. Taubner and M. Kahlweit, J. Phys. Chem. 86, 3167 (1982); E. Lessner and I. Frahm, ibid. 3132.
13. Đ. Dragčević and V. Pravdić, Croat. Chem. Acta, 53, 1 (1980).
14. A. Aaranson, Limnol. Oceanogr., 16, 1 (1971).
15. D. Kahan, R. Oren, A. Aaranson and Z. Behrens, J. Protozool., 25, 30 (1978).
16. J.S. Patton and J.E. Burris, Mar. Biol., 76, 131 (1983); ibid. 137.
17. A.N. Frumkin, N.V. Fedorovich, B.B. Damaskin, E.V. Stenina and V.S. Krylov, J. Electroanal. Chem., 50, 103 (1974).
18. J.C. Alöisi, C. Millot, A. Monaco and H. Puc, C.R. Acad. Sci. Paris, 289, 879 (1979).
19. G. Bitton and K.C. Marshall (eds.), Adsorption of Microorganisms to Surfaces, Wiley and Sons, New York (1980).
20. Y. Avnimelech, W.B. Troeger and L.W. Reed, Science, 216, 63 (1982).
21. V. Žutić, A. Monaco and G. Cauwet, submitted to Geochim. Cosmochim. Acta.