

Polarography of Marine Particles: A Model Study*

Nadica Ivošević and Vera Žutić

*Ruđer Bošković Institute, Center for Marine Research, P.O.B. 1016,
10001 Zagreb, Croatia*

Received March 14, 1996; accepted July 18, 1996

A simple polarographic method for direct measurement of surface active constituents of seawater was used to investigate the effect of variable levels of dissolved surfactants on the detection of attachment signals of surface active particles at the artificial interface.

A mixture of dextran solutions and dispersion of insoluble squalene droplets simulates electrochemical signals in natural samples and allows prediction of the behaviour of natural organic particles.

For the same distribution of particles in the aqueous dispersion, attachment signals frequency decreases exponentially with an increasing bulk concentration of dissolved molecules. Attachment rate of particles at the dropping mercury electrode (DME) depends on the extent of surface coverage with adsorbed molecules (θ). At full coverage of the electrode, no particle attachment signals can be detected.

The actual distribution of surface active particles can be determined by direct measurement of attachment signals when $\theta < 0.5$, which is the case of the majority of seawater samples. For $\theta \geq 0.5$, dilution with organic free electrolyte and/or sampling within the initial part of the DME life-time should be used.

INTRODUCTION

In one of his seminal papers,^{1,2} Branica with coworkers proposed a simple polarographic method for direct measurement of the surfactant activity of seawater samples. The method is based on suppression of the polarographic maximum of dissolved oxygen^{3,4} by adsorption of organic constitu-

* Dedicated to Marko Branica on the occasion of his 65th birthday.

ents of seawater at the DME/seawater interface. The surfactant activity of seawater was expressed as the equivalent amount of nonionic synthetic surfactant, Triton-X-100 ($\bar{M} = 600$). Dilution of seawater to ionic strength 0.1 M amplifies the signal.⁵⁻⁸ The procedure has later been modified to include addition of Hg(II) to seawater samples prior to the measurement in order to enhance the polarographic maximum current without dilution.^{9,10}

Hunter and Liss,¹¹ have empirically established a relationship between the surfactant activity and the dissolved organic carbon (DOC) of seawater:

$$\text{surfactant activity (mg/L)} = 1.3 \text{ DOC (mg/L).}$$

Thus, the electrochemical approach seemed to offer an alternative to measurement of dissolved organic matter as DOC, which itself has recently become a rather controversial issue.¹²

Upon more extensive application to various locations in the Adriatic and Mediterranean seas,^{9,13-16} it was observed that polarograms in samples of productive and polluted coastal sea and sea-surface microlayer were occasionally strongly perturbed by irregular oscillations. This revealed a new interfacial phenomenon – attachment signals of surface active particles.^{14,17-19}

In this way, surface active particles have been identified as a new class of organic constituents of seawater. Their size was in the μm range (0.4–10 μm), maximum concentration was estimated to reach up to $5 \times 10^5/\text{mL}$ in productive surface waters and in the sea surface microlayer.¹⁶ They are fluid or flexible, since they behave like liquid droplets. They are highly reactive^{14,20} and gradually disappear with the aging of samples. Measurements at the freshwater/seawater interface revealed a continuous transformation of biogenic dissolved organic matter into surface active particles.^{14,19,21} Surface active particles seem to be ubiquitous, since they were also detected in fresh seawater samples taken at different depths in the Pacific.²² There is now expanding evidence on the continuum in the size distribution of organic matter in the sea²³⁻²⁸ and a need to quantify its spatial and temporal heterogeneity.²⁹⁻³⁰ The electrochemical approach, though a priori lacking molecular specificity, might sense the organic matter field at a microscale.

As adsorption of dissolved organic molecules at the mercury/seawater interface results in gradual and highly reproducible decrease of the polarographic maximum, the collision and the attachment of fluid or flexible organic particles to the interface results in distinct signals in polarograms, *i.e.* in sharp perturbations of variable amplitudes and frequencies.¹⁹ For a more detailed analysis of attachment signals, a chronoamperometric mode (current-time curves at constant potential in the polarographic maximum region) was more appropriate. Each perturbation was found to correspond to the attachment and spreading of a single surface active particle, while a decrease of the current at the end of perturbation reflects the contact area after the attachment.

In model systems with organic droplets or living cells, which were not accompanied by any significant level of dissolved surfactants, the mean frequency of the attachment signals was found to be a linear function of particle concentration.¹⁴ However, in natural seawater, the organic surface active particles are present together with variable amounts of dissolved surface active molecules.

In this paper, we shall use a model system to investigate the effect of increasing levels of dissolved surfactants upon detection of attachment signals of surface active particles. This is particularly relevant to bloom conditions, marine snow and laboratory cultures of marine microorganisms. We shall return to the original measurement procedure¹ (dilution of seawater to the ionic strength of 0.1), without addition of Hg(II), which is more appropriate for natural systems if we wish to keep the microorganisms alive.³¹

EXPERIMENTAL

Preparation of Dispersions

All solutions and dispersions were prepared in a 0.1 M NaCl aqueous solution containing 5×10^{-3} M NaHCO₃ to maintain the pH at 8.4. Aqueous stock dispersion of squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) was prepared by shaking (for 1 hour) 50 μ L of squalene in 250 mL of 0.1 M NaCl solution. The level of polydispersity was characterized by Coulter-Counter measurements (an example in Figure 1). The size distribution of organic droplets was fairly reproducible for independent preparations and it remained virtually unchanged over a period of 20 min, which was sufficient to run the electrochemical experiment. Only freshly prepared dispersions were used. Addition of dextran ($M = 70,000$, Serva) solution did not influence the size distribution of squalene droplets in the dispersion.

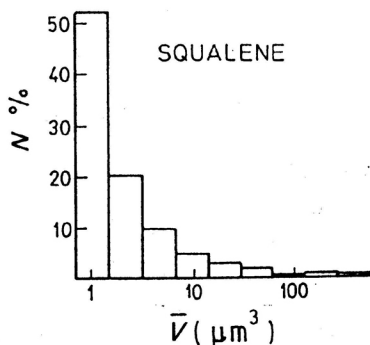


Figure 1. Polydispersity level of squalene dispersion ($c = 3.6 \mu\text{L/L}$) in aqueous solution of 0.1 M NaCl, as measured by Coulter-Counter, 70- μm orifice counting tube.

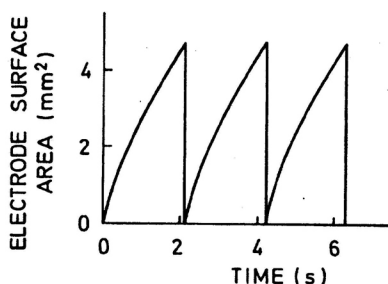


Figure 2. Periodic change of surface area of the DME (flow rate 6.0 mg/s, drop lifetime 2.08 s).

Commercial chemicals of highest purity were used throughout. Water was Millipore-MilliQ and precautions were made to avoid organic contamination.

Electrochemical Measurement

The DME had a flow rate of 6.1 mg/s, drop time of 2.08 s (at -0.4 V) and maximum surface area of 4.7 mm^2 (Figure 2). All potentials are referred to an Ag/AgCl (0.1 N NaCl) reference electrode which was separated from the measured dispersion by a ceramic frit. Its potential was + 2 mV *vs.* calomel electrode (1 N KCl).

The electrochemical measurements were performed with a PAR 174A Polarographic Analyser. The polarograms were recorded using a 70045 Hewlett-Packard *x-y* recorder. The current-time curves at a constant potential were recorded with a time resolution of 50–200 μs per point and stored using a Nicolet 3091 digital oscilloscope connected to a PC computer.

All measurements were performed in 25 mL volumes of dispersions saturated with air and thermostated at 20 ± 1 °C. The oxygen reduction polarograms were recorded at a scan rate of 10 mV/s, and the current-time curves at constant potential of -400 mV.

RESULTS AND DISCUSSION

Squalene droplets were selected as model particles since they yield well pronounced attachment signals in a broad potential range at positively as well as at negatively charged mercury electrodes.³² Attachment signals for the majority of natural particles were also found to appear in a broad range of potentials, although particular dependence on their type was also observed.³³ Dispersion of droplets in the size range $\geq 1 \mu\text{m}$ of about $10^6/\text{mL}$ was obtained for dispersions of 20 μL of squalene per litre of aqueous electrolyte solution. Dextran was chosen as a model for dissolved organic matter since it is adsorbed in a broad range of potentials with suppression of the

polarographic maximum of oxygen comparable to natural seawater samples. Aqueous solution of 0.1 M NaCl with addition of 5×10^{-3} M NaHCO_3 to maintain the natural pH of seawater was used throughout. At this ionic strength, the amplitude of polarographic maximum is higher than at ionic strengths of seawater and the attachment signals are better pronounced.³⁴

Figure 3A shows a polarogram of oxygen reduction in the presence of 10 mg/L dextran. The polarogram is perfectly regular, and highly reproducible. Its form indicates adsorption of dissolved organic molecules in the potential range from -0.125 to -1.8 V. Such polarograms are typical of seawater samples with the concentration of surface active particles $N < 5 \times 10^3/\text{mL}$. A polarogram of oxygen reduction in the mixture of 10 mg/L dextran and dispersion of 20 $\mu\text{L/L}$ squalene is shown in Figure 3B. Irregular perturbations are

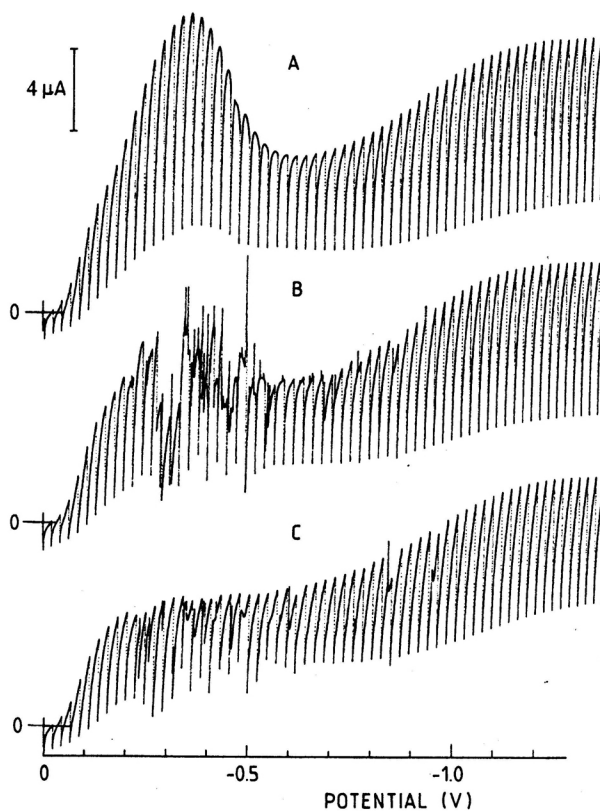


Figure 3. Polarograms (actual recordings) of oxygen reduction in 0.1 M NaCl + 5×10^{-3} M NaHCO_3 solution, in the presence of a) 10 mg/L dextran, b) 10 mg/L dextran + 20 $\mu\text{L/L}$ squalene, c) 60 mg/L dextran + 20 $\mu\text{L/L}$ squalene.

caused by attachment signals of squalene droplets. They appeared only in the potential range from -0.15 to -0.99 V. By increasing the concentration of dextran (Figure 3C), polarographic maximum is almost completely suppressed and the intensity of perturbations is significantly reduced, both in terms of amplitude and frequency, although the particle concentration is the same as in Figure 3B.

To get a better insight into this behaviour, current-time curves were recorded on consecutive drops at various constant potentials with high time resolution. The effects of dextran molecules adsorption and squalene droplets attachment are most pronounced at -400 mV.

Current-time curves ($I-t$ curves) for oxygen reduction in solutions containing 10 and 60 mg/L dextran are shown in Figure 4. For solutions with no dextran added, the shape of the $I-t$ curves reflects zero coverage of the electrode surface ($\theta = 0$). The oxygen reduction current has a maximum value at $\theta = 0$, and it decreases with increasing dextran concentration. In the presence of 10 mg/L dextran (curve 1), suppression of oxygen reduction current gradually increases towards the end of the drop life of the DME in a consistently reproducible way. At dextran concentration of 60 mg/L, the current is further suppressed and at 1.3 seconds, t_c , it reaches the diffusion limited value, i_d , which corresponds to the full coverage of the electrode sur-

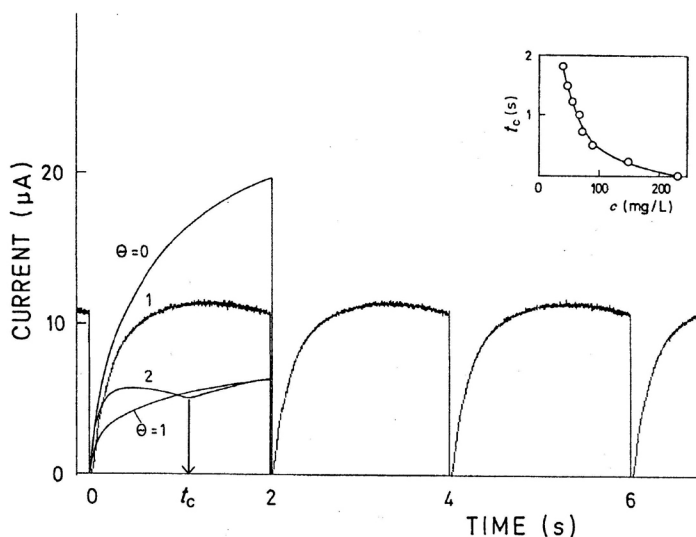


Figure 4. $I-t$ curves for oxygen reduction at -400 mV for three consecutive mercury drops for solutions of 0.1 M NaCl + 5×10^{-3} M NaHCO₃ + various concentrations of dextran (mg/L): 10 (curve 1); 60 (curve 2); 300 ($\theta = 1$, full surface coverage); no dextran added ($\theta = 0$, free surface). The insert shows dependence of time, t_c , when $\theta = 1$, on dextran concentration.

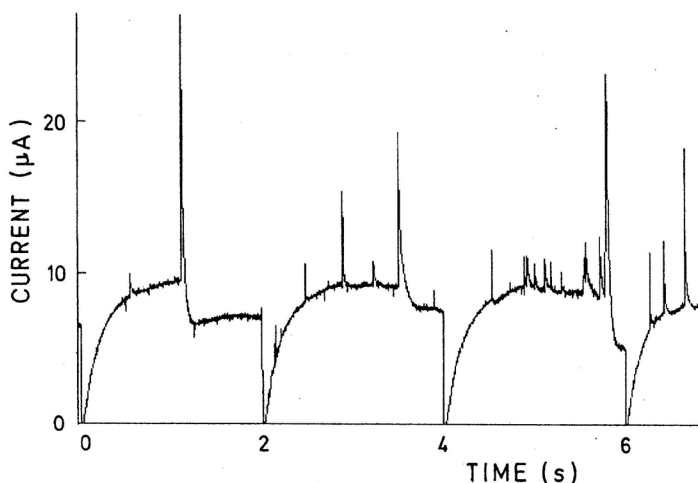


Figure 5. $I-t$ curves for oxygen reduction in the dextran solution of 10 mg/L containing 20 $\mu\text{L/L}$ squalene dispersion.

face with dextran molecules $\theta \sim 1$. In dextran solution of 300 mg/L, the current is totally suppressed to i_d during the whole drop life-time ($t_c = 0$).

Randomly selected $I-t$ curves of solutions containing 10 mg/L dextran and squalene droplets are shown in Figure 5. Irregular perturbations of variable amplitude and frequency indicate a stochastic process corresponding to random collisions, with and subsequent attachment of droplets of variable sizes to the mercury interface. For a heterodispersion system, each $I-t$ curve is unique and reflects the distribution of particles and molecules around the electrochemical probe at a given time interval. $I-t$ curves contain a number of spikes of variable amplitude and frequency of appearance. The higher spikes are followed by a decrease of the base-line current which corresponds to a measurable increase in the surface coverage, $\Delta\theta$, due to the spreading of the particle at the electrode. It has been proved earlier^{13,14,20} that with this experimental set-up, the amplitudes of attachment signals for small droplets, $\leq 0.4 \mu\text{m}$, are within the instrumental noise level.

When a series of $I-t$ curves on 50 subsequent mercury drops were analyzed, the mean number of perturbations, $\bar{N}_p = 5.2$, was obtained. It is important to note that perturbations appeared over the whole drop life-time.

With increasing the dextran concentration to 60 mg/L, a significant overall decrease of the current is observed (Figure 6). In the two first $I-t$ curves maximum suppression is achieved at $t_c = 1.3 \text{ s}$, which is the same as in dextran solution without squalene (curve 2 in Figure 4). In the third curve, the full coverage of the electrode is achieved already at $t_c = 0.7 \text{ s}$ due to attachment of a large squalene droplet. Attachment signals appear only in the be-

ginning of the drop life-time, at times $t < t_c$ and with a comparable frequency to that in Figure 5. At times $t > t_c$, when full coverage of the electrode is achieved, not a single attachment signal could be detected in a large number of $I-t$ curves (> 50) recorded.

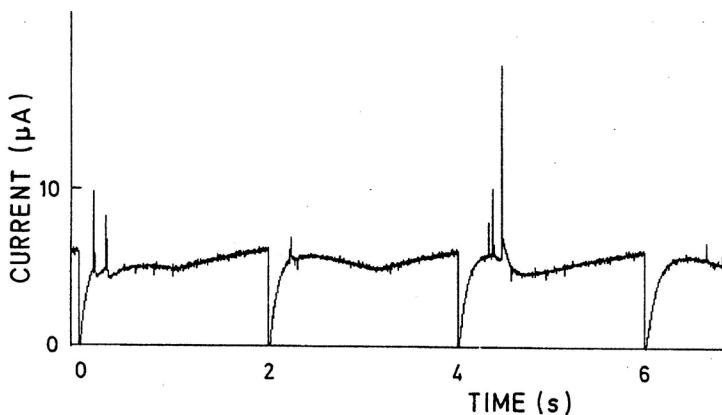


Figure 6. $I-t$ curves for oxygen reduction in the dextran solution of 60 mg/L with 20 $\mu\text{L/L}$ squalene dispersion.

There are two alternative interpretations for the disappearance of attachment signals for an electrode surface fully covered with adsorbed dextran molecules:

(1) squalene droplets have no affinity for the dextran covered mercury surface because its interfacial tension drops below the critical value for wetting by squalene,³³

(2) squalene droplets attach to the dextran covered mercury electrode, but there is no measurable electrical response, since there is no net displacement of double layer charge or net increase of the surface coverage.

Microscopic observations at a mercury pool electrode³⁵ support this second scenario.

Dependence of the attachment signal frequency on dextran concentration is given in Figure 7. The range of perturbation frequencies, N_p , is determined from 50 subsequent $I-t$ curves. The mean frequency, \bar{N}_p exponentially decreases with increasing the dextran concentration and drops to zero for a solution of 300 mg/L dextran. At this dextran concentration $t_c = 0$, meaning that full coverage by dextran molecules is maintained during the whole drop life-time. In Figure 8A, the mean perturbation frequency is plotted as a function of the average surface coverage of the mercury drop electrode by dextran molecules, as evaluated directly from the polarogram. An interesting linear

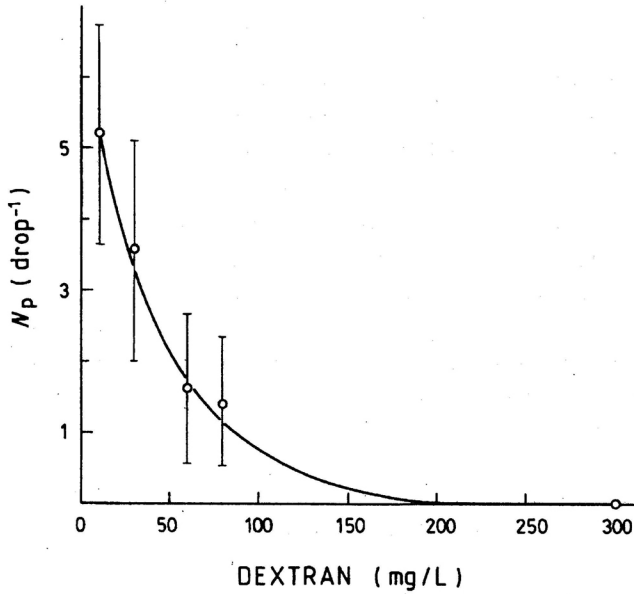


Figure 7. Dependence of the perturbation frequency, N_p for solutions containing $20 \mu\text{L/L}$ squalene dispersion on concentration of dissolved dextran.

relationship is obtained when the mean perturbation frequency (\bar{N}_p) is plotted against the duration of the mercury electrode drop life-time before the electrode is fully covered with adsorbed dextran molecules, t_c (Figure 4).

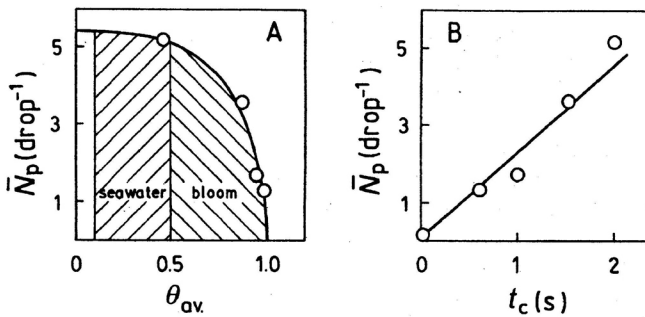


Figure 8A. Relationship between the mean perturbation frequency (N_p) and the surface coverage of the mercury electrode by dextran. The average surface coverage (θ_{av}) during the drop life-time was determined from the suppression of the polarographic maxima at -400 mV .

Figure 8B. The effect of t_c on the mean perturbation frequency, N_p for $20 \mu\text{L/L}$ squalene dispersion in solutions with increasing dextran concentrations.

CONCLUSIONS

The electrochemical response of a mixture of dextran solutions and dispersion of insoluble squalene droplets simulates electrochemical signals for natural samples, allowing predictions on the behaviour of surface active particles.

The following methodological implications of the experimental findings:

1. higher levels of dissolved organic constituents mask the electrochemical response of surface active particles by decreasing the number of attachment signals;

2. electrochemical counting of surface active particles cannot be applied directly to samples with increased levels of dissolved organic matter, such as samples of marine snow or samples from phytoplankton bloom areas (Figure 8A);

3. direct measurements should be made only during the initial part of the DME life-time, when $t < t_c$ (Figure 8B). Dilution of samples with organic-free electrolyte is an alternative;

should be considered in the electrochemical analysis of organic particles in natural samples.

Acknowledgments. – The authors are indebted to Milica Petek, one of the reviewers for improvements of the language and style of the manuscript. This research was sponsored by the Ministry of Science and Technology of Croatia and the UNEP/IOC project Link between eutrophication and interfacial processes.

REFERENCES

1. T. Zvonarić, V. Žutić, and M. Branica, *Thalas. Jugosl.* **9** (1973) 65–73.
2. H. W. Nurnberg and P. Valenta, *Polarography and Voltammetry in Marine Chemistry*, in: E. D. Goldberg (Ed.), *The Nature of Sea-water*, Dahlem Konferenzen, Berlin, 1975, pp. 87–136.
3. B. Gosman and J. Heyrovský, *Trans. Electrochem. Soc.* **59** (1931) 249.
4. R. G. Barradas and F. M. Kimmerle, *J. Electroanal. Chem.* **11** (1966) 163–170.
5. V. G. Levich, *Physicochemical Hydrodynamics*, Prentice-Hall, Englewood Cliffs, London, 1962, pp. 413–429.
6. A. N. Frumkin, *J. Electroanal. Chem.* **107** (1960) 461–470.
7. T. S. Sørensen, *Dynamics and Instability of Fluid Interface*, Lecture notes on physics, Vol. 276, Springer Verlag, Berlin, 1978.
8. R. Aogaki, K. Kitazawa, K. Fukai and T. Mukaibo, *Electrochim. Acta*, **23** (1978) 867.
9. T. Zvonarić, M. Sc. Thesis, *Electrochemical Determination of Surface Active Substances in Seawater*, University of Zagreb, 1975. (in Croatian)
10. V. Žutić, B. Čosović, and Z. Kozarac, *J. Electroanal. Chem.* **78** (1977) 269–283.
11. K. A. Hunter and P.S. Liss, *Wat. Res.* **15** (1980) 203.

12. J. I. Hedges, and C. Lee (Eds.), *Measurement of Dissolved Organic Carbon and Nitrogen in Natural Waters, Marine Chem.* **41** (1993) 1–290.
13. V. Žutić, B. Čosović, E. Marčenko, N. Bihari, and F. Kršinić, *Mar. Chem.* **10** (1981) 505–520.
14. V. Žutić, T. Pleše, J. Tomaić, and T. Legović, *Mol. Cryst. Liq. Cryst.* **113** (1984) 131–145.
15. B. Čosović, V. Žutić, V. Vojvodić, and T. Pleše, *Mar. Chem.* **17** (1985) 127–139.
16. J. C. Marty, V. Žutić, R. Precali, A. Saliot, B. Čosović, N. Smoldaka, and G. Cauwet, *Mar. Chem.* **25** (1988) 243–263.
17. J. Tomaić, V. Žutić, and T. Legović, *J. Electroanal. Chem.* **49** (1989) 49–57.
18. V. Svetličić, V. Žutić, and J. Tomaić, *Mar. Chem.* **32** (1991) 253–268.
19. V. Žutić, V. Svetličić, and J. Tomaić, *J. Pure and Appl. Chem.* **62** (1990) 2269–2276.
20. V. Žutić and J. Tomaić, *Mar. Chem.* **23** (1988) 5167.
21. V. Žutić and T. Legović, *Nature* **328** (1987) 612–614.
22. V. Žutić, unpublished results.
23. W. Stumm and J. J. Morgan, *Aquatic Chemistry*, John Wiley & Sons, New York, 1981.
24. J. Buffle and H. P. van Leeuwen (Eds.), *Environmental Particles*, vol. 1, Lewis Publishers, Chelsea, MI, 1992.
25. B. D. Johnson, K. Kranck, and D. K. Muschenheim, *Physicochemical Factors in Particle Aggregation*, in: R. S. Wotton, *The Biology of Particles in Aquatic Systems*, Lewis Publishers, London, 1994, pp.75–96.
26. I. Koike, S. Hara, K. Terauchi, and K. Kogure, *Nature* **345** (1990) 242.
27. M. L. Wells and E. D. Goldberg, *Nature* **353** (1991) 342–344.
28. M. L. Wells and E. D. Goldberg, *Limnol. Oceanography* **39**(2) (1994) 286–302.
29. F. Azam and B. C. Cho, *Bacterial Utilization of Organic Matter in the Sea: in Ecology of Microbial Communities*, SGM 41, Cambridge University Press, 1987, pp. 261–281.
30. F. Azam, D. C. Smith, G. F. Steward, and Å. Hagström, *Microb. Ecol.* **28** (1993) 167–179.
31. N. Ivošević, V. Žutić, V. Svetličić, and D. Fuks, *Ocean Sciences Meeting*, San Diego, 1996.
32. N. Ivošević, J. Tomaić, and V. Žutić, *Langmuir* **10** (1994) 2415–2418.
33. V. Žutić, J. Tomaić, J. Chevalet, S. Kovač, and V. Svetličić, *12th International Symposium on Chemistry of the Mediterranean*, Rovinj, 1992.
34. S. Kovač, M. Sc. Thesis, *Heterocoalescence of Organic Particles at Mercury Electrode in Aqueous Electrolyte Solution and Seawater*, University of Zagreb, 1993. (in Croatian).
35. N. Ivošević, unpublished results.

SAŽETAK**Polarografija morskih čestica: modelno istraživanje***Nadica Ivošević i Vera Žutić*

Polarografska metoda za izravno mjerenje površinske aktivnosti uzoraka morske vode primijenjena je za mjerenje utjecaja adsorpcije otopljenih organskih molekula na detekciju odziva površinski aktivnih čestica na međupovršini živina elektroda/morska voda. Smjesom otopine dekstrana i disperzije kapljica skvalena simulirani su odzivi u prirodnim uzorcima i predviđeno ponašanje prirodnih organskih čestica u moru.

Za jednaku raspodjelu čestica u vodenoj otopini elektrolita, učestalost signala prijanjanja eksponencijalno opada porastom koncentracije otopljenih molekula. Učestalost prijanjanja čestica na kapajućoj živinoj elektrodi (KŽE) ovisi o stupnju pokrivenosti živine površine adsorbiranim molekulama (Θ). Kod potpune pokrivenosti nestaju signali prijanjanja.

Stvarna volumna raspodjela površinski aktivnih čestica može se odrediti direktnim mjerenjem signala prijanjanja kada je $\Theta < 0.5$, što je slučaj u većine uzoraka morske vode. Za slučaj $\Theta \geq 0.5$, potrebno je razrjeđivanju uzorka otopinom elektrolita ili analiza odziva isključivo na početku vremena života KŽE.