Amperometric Adhesion Signals of Liposomes, Cells and Droplets

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Individual soft microparticles (liposomes, living cells and organic droplets) in aqueous media are characterized by their adhesion signals using amperometry at the dropping mercury electrode. We confirmed that the general mechanism established for adhesion of hydrocarbon droplets and cells is valid as well for liposome adhesion within a wide range of surface charge densities. Incidents and shape of adhesion signals in liposome suspensions reflect liposome polydispersity, surface charge density and properties of phospholipid head group. Major distinction in adhesion behavior of liposomes when compared to organic droplets was identified as: (i) different values of critical interfacial tensions of adhesion at the positively and the negatively charged electrode, and (ii) appearance of signals revealing the specific interactions of phospholipid polar head groups with the electrode.

Key words:
Amperometric adhesion signals, DOPC liposomes, dropping mercury electrode, microparticles, PS liposomes

Introduction

The aim of this study is to develop electrochemical adhesion imaging for a direct characterization of organic microparticles in their aqueous environments such as seawater and biological fluids. The significance of adhesion phenomena in single particle/electrode interaction became apparent since the discovery of adhesion signals of vesicles in seawater samples at the mercury electrode.[1,2] Mercury-aqueous electrolyte interface is the simplest electrified interface where by varying the applied potential by 2 V (from 0 to –1.9 V vs. an Ag/AgCl reference electrode), surface charge density changes from +22 to –26 μC cm^{-2} and interfacial energy varies in the range from 241.7 to 426.7 mJ m^{-2}.[3] The shape of amperometric adhesion signals at mercury drop electrodes captures the primary structure of a microparticle in its aqueous environment. In previous work from this laboratory the adhesion signals of dispersed droplets of organic liquids,[4–6] living algal cells,[7–9] bacterial cells[10,11] and liposomes[12] were studied and compared with adhesion signals of organic microparticles in marine environments.

Liposomes present the classical model in studying the physical mechanism of cell adhesion.[13] Liposomes are also the most widely used drug carriers but they still need further development studies in order to elucidate the mechanisms of their actions in the body. In that respect, the factors that affect the physicochemical properties of liposomes (size, composition, surface charge) and the drug entrapment efficiency have been widely investigated.[14,15]

Interaction of liposomes from aqueous suspension with mercury electrode and characterization of formed layers have been extensively studied by electrochemical and optical techniques.[16–18] Insight into the mechanism of the potential-induced transformations of lipid-coated mercury drop was obtained using *in situ* fluorescence microscopy.[19] While capacitance measurements reveal properties of the already formed monolayers at the mercury interface,[17,20,21] time-resolved adhesion signal traces transformation kinetics of a single liposome to a film of a finite surface area.[22,23] Adhesion signals of liposomes were also studied by Scholz and coworkers with the general aim to extract kinetic parameters of adhesion and characterize effects of a pore-forming polypeptide.[24–27]

Electrochemical method

Adhesion and spreading of organic droplets at the charged mercury/water interface causes double-layer charge displacement from the inner Helmholtz plane, and the transient flow of compensating current can be recorded as an electrical adhesion signal. Signals are current transients defined

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by the maximum signal amplitude (I_{max}), signal duration (τ) and displaced charge (q_D).[4,7] Initial steep rise portion of adhesion signal corresponds to the attachment and deformation of a particle, while slower decay of current corresponds to the spreading of a particle to monomolecular film. Displaced double-layer charge, q_D, is obtained by integrating the area under the adhesion signal.

$$q_D = \int_{t_1}^{t_1+\tau} I\mathrm{d}t \qquad (1)$$

If a complete charge displacement takes place, as in the case of droplets of nonpolar organic liquids, the area of the contact interface, A_c, is determined from the amount of displaced charge,

$$A_c = \frac{q_D}{\sigma_{12}} \qquad (2)$$

where σ_{12} is the surface charge density of the mercury/aqueous electrolyte.

Adhesion equilibrium and spreading in the three-phase system mercury (1), water (2) and organic liquid (3) is defined according to the modified Young-Dupré equation. The total Gibbs energy of interaction between a droplet and the aqueous mercury interface is

$$-\Delta G = A\ (\gamma_{12} - \gamma_{13} - \gamma_{23}) \qquad (3)$$

γ_{12}, γ_{13} and γ_{23} are the interfacial energies at mercury/water, mercury/organic liquid and water/organic liquid interfaces, respectively. The expression in parentheses is the spreading coefficient, S_{132}, at the three-phase boundary.[28] When $S_{132} > 0$ attachment and spreading are spontaneous processes. For $S_{132} < 0$ the spreading process will not proceed spontaneously. The reason for that is stronger interaction of mercury with electrolyte and water molecules than the interaction with the liposomes. The critical interfacial tension of adhesion, $(\gamma_{12})_c$ is defined by $S_{132} = 0$ as $(\gamma_{12})_c = \gamma_{13} + \gamma_{23}$.

The experimental values of the critical potentials for adhesion of hexadecane droplets at the positively, E_c^+ and the negatively, E_c^- charged interface and the corresponding critical interfacial tensions $(\gamma_{12})_c$ are indicated on the electrocapillary curve (Fig. 1a). The determined $(\gamma_{12})_c$ is in a good agreement with calculated values[5,6] according to Young-Dupré and Good-Girifalco-Fowkes relationships.[31,32] The amplitude and duration of signals depends on droplet size as well as on the polarity and the surface charge density of the mercury electrode (Fig. 1b). At the potential of zero charge of the electrode (E_{pzc}), adhesion signals of hexadecane droplets cannot be detected as there is no electrode double-layer charge to be displaced.

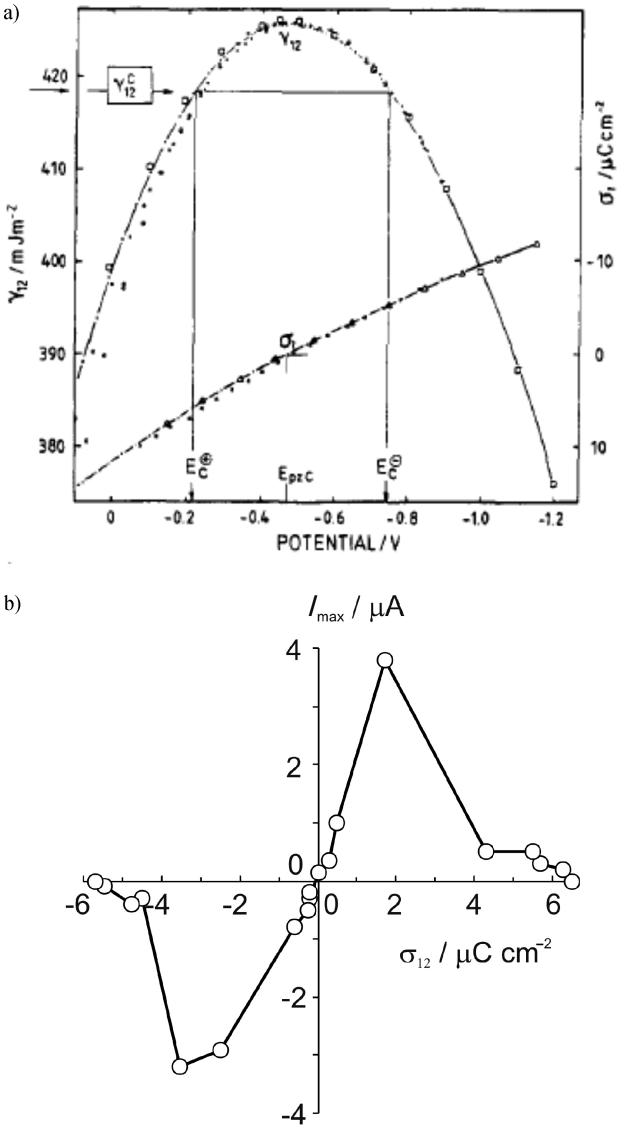

Fig. 1 – *(a) Electrocapillary curve and charge-potential curve at the mercury/0.1 M NaF interface.[29,30] An illustration is added on the determination of critical potentials of adhesion at positively charged (E_c^+) and negatively charged (E_c^-) mercury electrode (Fig. 2 in reference 5, reprinted with permission, copyright 1993, American Chemical Society) (b) Effect of charge density at the mercury electrode on the maximum signal amplitudes recorded in dispersion of 180 mg L^{-1} hexadecane in 0.1 M NaF.*

Experimental

Liposome suspensions

DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), PS (1,2-diacyl-*sn*-glycero-3-phospho-L-serine from bovine brain), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), Egg-PC (L-α-phosphatidylcholine, type XI-E from fresh egg yolk), cholesterol from porcine liver and dicetylphosphate were purchased from Sigma Chemical Co (St. Louis, MO, USA) and used as received. Multilamellar DOPC, PS and DPPC liposomes were prepared by dissolving 10

mg of lipid in 2 mL $CHCl_3$. After rotary evaporation of the solvent, the remaining lipid film was dried in vacuum for an hour and then dispersed by gentle hand shaking in 1 mL of phosphate-buffered saline (PBS), 0.15 M, pH 7.47. The liposome solution was left overnight at 4 °C to swell and stabilize. Multilamellar liposomes of Egg-PC/cholesterol/dicetylphosphate in molar ratio of 7:5:1 was prepared as described previously.[33,34] The suspension submitted for electrochemical measurements was characterized by Coulter-counter to determine liposome concentration and the size distribution using a 100 μm-diameter sampling orifice tube. The normal-size distribution was fairly reproducible, remaining unchanged over time of the electrochemical experiment. Liposome suspension of $2 \cdot 10^8$ particles L^{-1} contained predominant size fraction in the range from 3.2 μm to 16.0 μm.

Cell suspensions

We used laboratory culture of unicellular marine algae *Dunaliella tertiolecta* Butcher (Chlorophyceae). *D. tertiolecta* cell suspension in 0.1 M aqueous electrolyte solution represents a well-studied model system suitable for electrochemical detection due to the cell-size (6–12 μm) and membrane properties. Details of cell-growth and separation procedures have been described earlier.[7,9]

Dispersions of organic droplets

The aqueous dispersions of hexadecane (99 % GC, Aldrich) and methyl oleate (≥ 99 %) were prepared by shaking 50 μL of organic liquids in 250 mL of 0.1 M aqueous electrolyte solution, pH 8.2 at 300 rpm for an hour.

Electrochemical measurements

Dropping mercury electrode (DME) had a drop-life of 2.0 seconds, flow-rate of 6.0 mg s^{-1} and a maximum surface area of 4.57 mm^2. All potentials are referred to an Ag/AgCl (0.1 M NaCl) reference electrode, which was separated from the measured dispersion by a ceramic frit. Electrochemical measurements were performed using a PAR 174A Polarographic Analyzer interfaced to a PC. Analog signals data acquisition was performed with DAQ card-AI-16-XE-50 (National Instruments) input device and the data were analyzed using the application developed in LabView 6.1 software. The current time (*I-t*) curves over 50 mercury drop lives were recorded at the constant potentials, with the time-resolution of 100 μs. The experiments in liposome suspensions were performed in deaerated PBS at 20 °C and those with hexadecane droplets and *D. tertiolecta* cells in 0.1 M NaCl at 25 °C under purging with nitrogen. A separate experiment in suspension of DPPC liposomes was performed at 45 °C.

Results and discussion

Adhesion signals of droplets and DOPC liposomes of comparable signal amplitudes displayed similarity at higher surface charge densities of the electrode (Fig. 2a). Differences in signal shape become pronounced only around the potential of the zero charge of the mercury electrode (E_{pzc}). The effect of electrode potential on adhesion behavior of multilamellar DOPC liposomes will be examined in terms of signal amplitude and signal shape. Characteristic adhesion signals of DOPC liposomes captured in the time frame of 100 ms are presented in Fig. 3. The adhesion signals appeared randomly due to a spatial heterogeneity in liposome suspension and the stochastic nature of liposome encounter with the electrode. At a given potential, signal amplitude reflects the size of adhered liposome, while signal frequency reflects liposome concentration in the suspension. At potentials of –100 mV and –300 mV, where the electrode is positively charged, the sign of displacement current is the same as that for reduction and opposite to the charging current of the free mercury surface. At the negatively charged electrode (–800 mV and –1200 mV) the signals appeared in the opposite direction.[12] Signal shape changes from drawn-out at the potential of –100 mV and –1200 mV to sharp and narrow signals at the potentials of –300 mV and –800 mV. Signal duration depends on particular spreading coefficient, S_{132}, i.e the duration becomes shorter by increasing S_{132}. Estimates of S_{132} in PBS was obtained using electrocapillary data. At potential of –100 mV, where S_{132} value is 14 mJ m^{-2} sig-

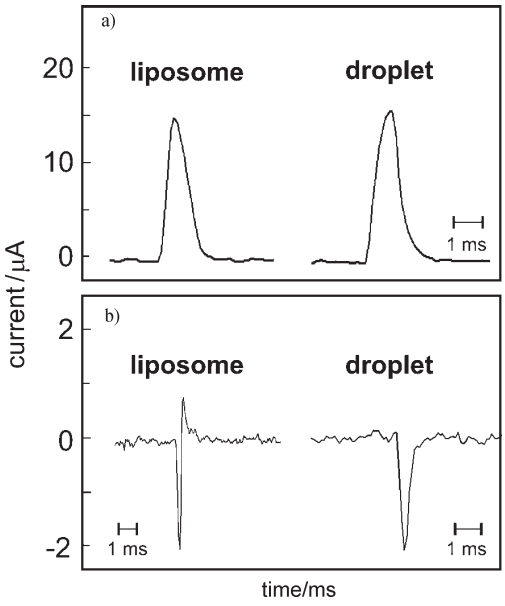

Fig. 2 – *Comparison of adhesion signals of DOPC liposomes and methyl oleate droplets having the same amplitude: (a) at –400 mV ($\sigma_{12} > 0$); (b) at –600 mV ($\sigma_{12} < 0$)*

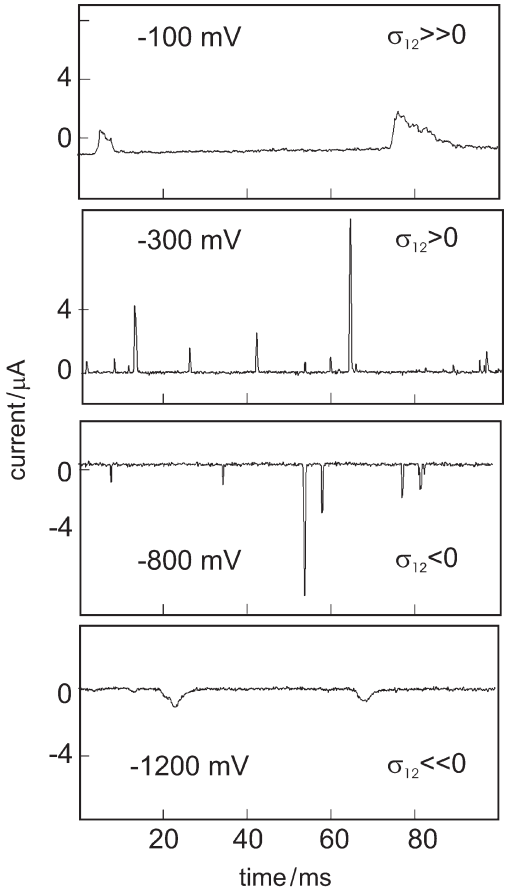

Fig. 3 – *Effect of potential on appearance of adhesion signals of DOPC liposomes at the mercury/PBS interface*

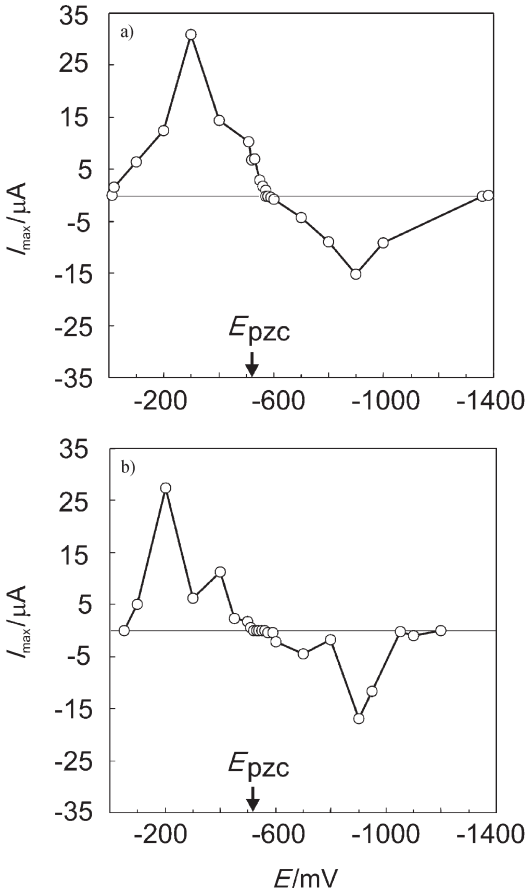

Fig. 4 – *Potential dependences of maximum signal amplitude of (a) DOPC liposomes and (b) PS liposomes in PBS*

nal durations are in the range 2.5–80 ms depending upon liposome size. Signal durations are in the range 0.5–10 ms at the potential of –300 mV where S_{132} value is 37 mJ m^{-2}. This behavior of decreasing signal duration with increasing S_{132} was identified as well in the case of hydrocarbon droplets independently on the polarity of the electrode charge[35] proving that interfacial energy governs the rate of spreading.

Adhesion behavior of DOPC liposomes differs from that of PS liposomes under the same experimental conditions. The adhesion signals of DOPC liposomes appeared in broader potential range; i.e. from –20 mV to –1360 mV, in comparison with adhesion range of PS liposomes that appeared in the potential range from –100 to –1100 mV. Fig. 4 shows potential dependence of maximum signal amplitude in liposome suspensions. Around the E_{pzc} adhesion signals of DOPC liposomes were detected having a sign of displacement current as that for reduction (Fig. 4a), while adhesion signals of PS liposomes ceased to appear in the vicinity of E_{pzc} (Fig. 4b).

Comparison of critical potentials of adhesion of liposomes at the positively and the negatively charged mercury interface with those of droplets and living cells is summarized in Table 1. Critical interfacial tensions of hexadecane droplets adhesion was found to be 419 mJ m^{-2} at positively and 420 mJ m^{-2} at negatively charged mercury electrode. This difference of 1 mJ m^{-2} could be ascribed to the effect of specific adsorption of chloride anions which takes place only at the positively charged mercury electrode. As shown in Fig. 1a, there is no difference between $(\gamma_{12})_c^+$ and $(\gamma_{12})_c^-$ of hexadecane droplets in 0.1 M NaF showing the importance of dispersion forces in hydrocarbon interaction with the mercury and water interface.[32] Critical interfacial tensions of *D. tertiolecta* adhesion equal to 394 mJ m^{-2} at positively and 374 mJ m^{-2} at negatively charged mercury electrode.[7] Our precision of determining $(\gamma_{12})_c$ from electrocapillary curve is 0.1 mJ m^{-2}. This higher value of $(\gamma_{12})_c^+$ corresponds to the contribution of electrostatic interactions since the cell exterior of *D. tertiolecta* bears negative charge at neutral pH.[9] Critical potentials of liposome adhesion are characteristic and sensitive to composition of nonpolar hydrocarbon chains and effect of specific polar groups (Table 1).

Table 1 – *Experimental values of critical potentials of adhesion at the positively and negatively charged mercury electrode*

Particles	Critical potentials of adhesion/mV	
	E_c^+	E_c^-
n-hexadecane droplets in NaCl	–300	–750
DOPC liposomes in PBS	–20	–1360
Egg-PC liposomes in PBS	–90	–1200
PS liposomes in PBS	–100	–1100
D. tertiolecta cells in NaCl	–80	–1220

Another specificity and differentiation between studied liposomes is manifested through the appearance of specific adhesion signals around E_{pzc} of mercury electrode. Fig. 5 illustrates differences in the adhesion signals between DOPC and PS liposomes: appearance of adhesion signals of DOPC liposomes while PS liposomes display only simple, unidirectional signals. The bidirectional adhesion signals were not detected in dispersions of organic droplets or in cell suspensions. Bidirectional adhesion signals of DOPC appeared in a narrow range of potential, from –570 mV to –700 mV and the ratios of negative to positive current maxima are found to depend on the potential. We have postulated earlier[26] that the bidirectional adhesion signals originate from specific interaction of positively charged choline groups of phospholipid polar heads when in immediate contact with the mercury electrode. Nelson and Leermakers[20] have assumed an effect of the lipid head group in the interaction of lipid monolayers with the mercury electrode, at potentials slightly negative to the E_{pzc}. Another indication of the lipid head group-mercury electrode interaction arises from studies of potential-dependent fluorescence of DOPC monolayers on mercury surface.[19] The bidirectional adhesion signals around E_{pzc} of mercury electrode were confirmed as well in the systems of the DPPC liposomes in PBS measured at 45 °C (DPPC gel to liquid transition is at 41 °C). In addition, appearance of bidirectional adhesion signals has been also observed in suspension of unilamellar DOPC liposomes.

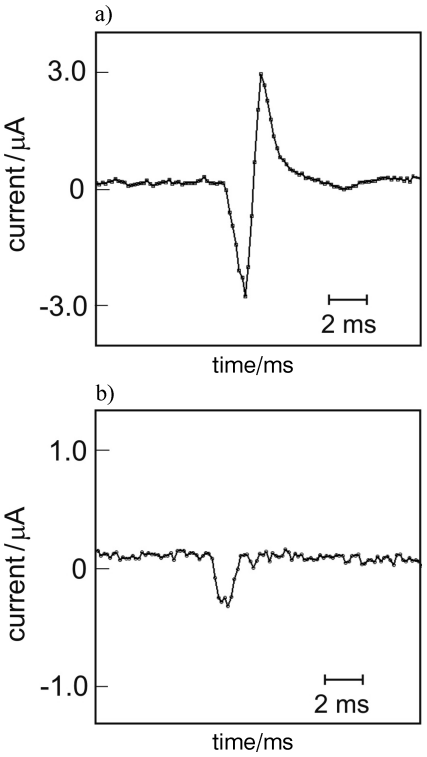

Fig. 5 – *Effect of the phospholipid head group on the type of adhesion signal of liposomes at potential of –580 mV in PBS. (a) bidirectional signal of DOPC liposome; (b) simple signal of PS liposome.*

Conclusion

Electrochemical adhesion imaging presents an approach for a direct characterization of organic particles in aqueous electrolyte solutions. During their attachment and spreading at the electrode, liposomes, cells and microdroplets displace the double-layer charge from the inner Helmholz plane of the electrode, and the transient flow of compensating current can be recorded as well-defined adhesion signal. The range of adhesion signals of liposomes are compared with those of organic droplets and living cells recorded under the same experimental conditions. We confirmed that general mechanism established for adhesion of hydrocarbon droplets and living cells is valid as well for the liposome adhesion within a wide range of surface charge densities. Major distinction with organic droplets was identified as: (i) difference in values of critical interfacial tensions of adhesion at the positively and the negatively charged electrode, and (ii) appearance of the signals around E_{pzc} revealing the specific interactions of phospholipid head groups at mercury electrode. Further studies of adhesion signals are in line with recent studies using STM[36] which are providing a molecular level information of liposome bilayers transformation at hydrophobic interfaces.

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