Macromolecular sensing at the liquid-liquid interface

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Abstract: We report here the electrochemical sensing of macromolecules, such as polyLysine dendrimers, at the polarised liquid | liquid interface. Electrochemistry at the liquid | liquid interface is a powerful analytical technique which allows the detection of non-redox active molecules via ion transfer reactions at a polarised water – oil interface. We demonstrate here that different parameters of the polyLysine dendrimers (charge number, molecular weight) have a strong influence on the sensitivity and limit of detection of these macromolecules. This work will help to the development of sensors based on charge transfer at the liquid | liquid interface.

1. Introduction

Electrochemistry at the liquid | liquid interface allows the electrochemical investigation of non-redox active ions, based on their transfer between two immiscible phases. The interface between two immiscible electrolyte solutions (ITIES) is polarised by the means of two reference electrodes and ion transfer can be potentially driven [1]. In recent years, significant advances were made to improve the sensing capabilities of electrochemistry at the ITIES [2]. A wide variety of ions have been detected at the ITIES: K^+, dopamine, peptides, proteins, DNA, dendrimers… Miniaturisation of the liquid | liquid interface has improved the sensitivity and the limits of detection [3]. This improvement comes from increased mass transfer rates at interfaces which have dimensions smaller than the diffusion layer thickness and is analogous to the phenomena observed at micro- and nanoelectrodes [4]. Proteins are biological macromolecules of primary importance as they are the molecular expression of the genetic information. Their nature and function are governed by their complex structure. The presence or absence of a protein, its concentration or alteration of its structure can be indicative of a disease state. Label-free electrochemical detection of proteins have thus attracted a lot of interest in recent years [5]. The electrochemical behaviour of proteins at the liquid | liquid interface is a particularly complex process, involving adsorption of the protein at the interface and the assisted transfer of the anion of the organic electrolyte to the aqueous phase. This process at the liquid | liquid interface has been thoroughly investigated for insulin [6], haemoglobin [2] and lysozyme [7-8]. Electroanalysis at the ITIES can also be indicative of the structure of a protein as changes in the electrochemical

behaviour have been investigated in the case of chemical denaturation [9] or enzymatic proteolysis [10]. Dendrimers are a class of synthetic monodisperse and highly branched three-dimensional macromolecules. They are made up of a central core, branched units and terminal groups. By increasing the number of branched units, the dendrimer generation increases. The electrochemical behaviour of poly(propylenimine) (DAB-AM-*n*) and poly(aminoamine) (PAMAM) dendrimer families at the liquid | liquid interface was investigated in different experimental conditions [11].

We report here the electrochemistry of poly-Lysine dendrimers from generation 2 (G2) up to generation 5 (G5) at the liquid | liquid interface. We have investigated the evolution of the electrochemical signal as a function of the poly-Lysine dendrimer concentration and how the structural characteristics of the dendrimer impact on the analytical sensitivities and the limits of detection. We have compared these values with those reported in the literature for protein detection at the ITIES.

2. Materials and methods

2.1 Reagents

The poly-Lysine dendrimers of generation G2, G3, G4 and G5 were purchased from Colcom (Montpellier, France). The core of the dendrimer molecules consists of a linear peptide of 8 lysine groups. For G2, 8 polypeptide units are grafted on the core. The dendrimers characteristics provided by the supplier are gathered in Table 1. All the other chemicals were purchased from Sigma-Aldrich Ltd Ireland. All chemicals were used as received.

Table 1:Dendrimer characteristics

Generation	G2	G3	G4	G5
Molecular weight (g mol^{-1})	8600	22000	65300	172300
Diameter at pH 7 (nm)	4.5	7	11	16
Number of lysine groups	48	123	365	963

2.2 Electrochemical experiments

The experiments were done in a custom made glass cell as shown in Figure 1. Two Ag|AgCl wires serve as reference electrodes (one in the aqueous phase and one in the organic phase) to monitor the interfacial potential difference and the current is measured by two counter Pt mesh electrodes (one in each phase). The organic phase electrolyte was 1,2-dichloroethane (1,2-DCE) containing 10 mM bis-(triphenylphosphoranylidene) ammonium

tetrakis(4-chlorophenyl)-borate ((BTPPA$^+$ TPBCl$^-$) and the aqueous phase was 10 mM HCl. The geometric area of the interface was 1.12 cm^2.

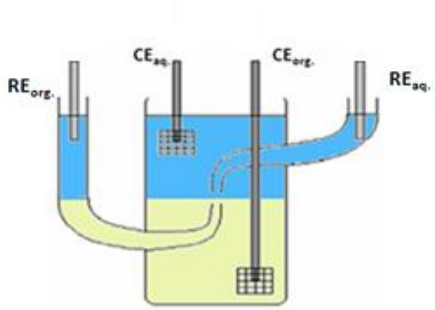

Figure 1: Four electrode electrochemical cell for liquid | liquid experiments. $RE_{org.}$: Reference electrode for the organic phase, $RE_{aq.}$: Reference electrode for the aqueous phase, $CE_{org.}$: Counter electrode for the organic phase, $CE_{aq.}$: Counter electrode for the aqueous phase.

The potential window is limited by the transfer of the background electrolyte salts present in both solutions. As a convention, positive currents are the result of cations transferring from the aqueous to the organic phase or from anions transferring from the organic to the aqueous phase. Similarly, anions transferring from the aqueous to the organic phase and cations transferring from the organic to the aqueous phase result in a negative current. The electrochemical cell used was as described in scheme 1 where x represents the concentration of poly-Lysine dendrimer and N the dendrimer generation.

Scheme 1: Electrochemical cell used

Ag	**AgCl**	**10 mM BTPPA$^+$ Cl$^-$ in 10 mM LiCl**	**10 mM BTPPA$^+$ TPBCl$^-$ in 1,2 dichloroethane**	**10 mM HCl + x μM GN**	**AgCl**	**Ag**

3. Results and discussion

The electrochemical behaviour of polyLysine dendrimers of four generations (G2 to G5) have been investigated at the polarised liquid | liquid interface by cyclic voltammetry. Figure 2 shows the cyclic voltammograms (CV) for increasing concentrations of G2 in the aqueous phase. When the potential window is scanned in the positive direction, a peak is observed at + 0.85 V and on the reverse scan, a peak is observed at + 0.7 V. In our previous work on proteins [2, 6, 8], this pair of peak was attributed to the assisted transfer of TPBCl$^-$, the organic electrolyte anion, to the aqueous phase and to the adsorption onto the aqueous side of the interface, of the protein – TPBCl$^-$ complex. A similar behaviour is observed for the dendrimers. At + 0.45 V, the peak observed is due to the transfer of tetraethylammonium (TEA$^+$) from the aqueous phase to the organic phase. TEA$^+$ is a model ion commonly used in electrochemistry at the liquid | liquid interface. Its transfer is inhibited by the presence of dendrimer at the interface. Indeed, the peak current corresponding to the transfer of TEA$^+$ is

decreasing as the dendrimer concentration increases. This was observed for all dendrimer generations. This observation confirms the adsorption occurring at the interface.

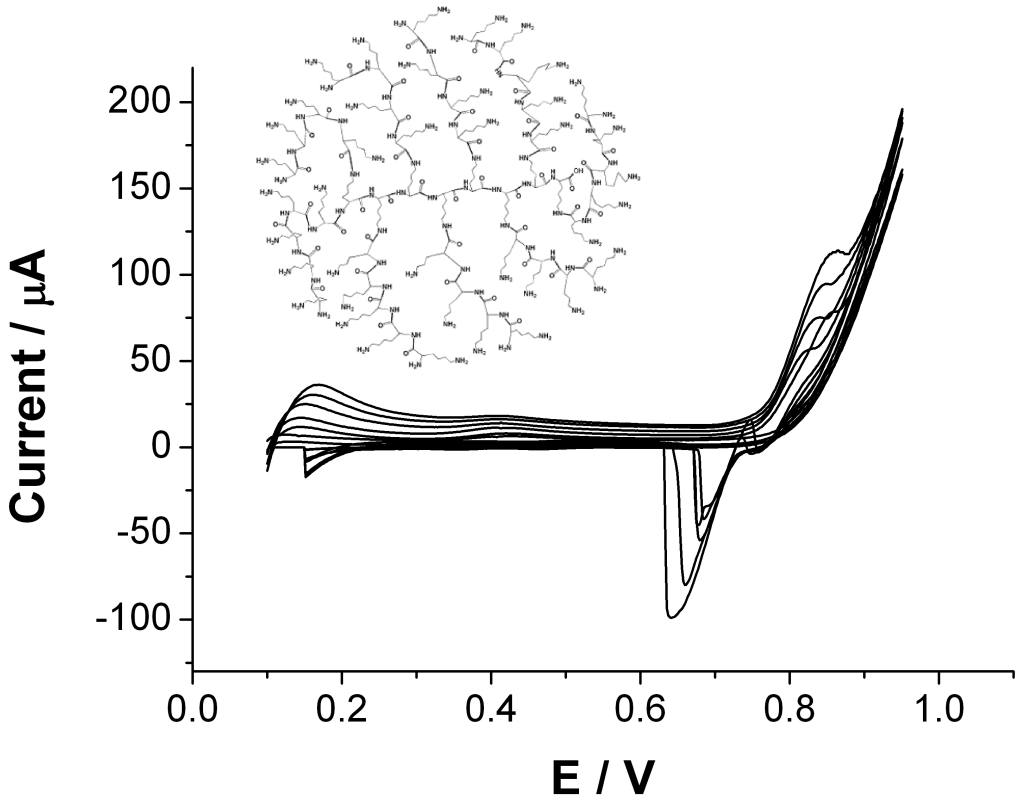

Figure 2: CV of increasing concentration of G2 (0, 11.1, 22, 32.8, 43.4 and 53.9 μM). The concentration of TEA^+ was 146 μM and the scan rate was 5 mV s^{-1}. Inset: Chemical structure of the polyLysine G2 dendrimer.

The peak current increased linearly with the concentration of dendrimers in the aqueous phase, which was observed for each of the dendrimer generation investigated. Indeed, calibration curves of the reverse peak current for the dendrimers G2 to G5 were built. The sensitivity (defined as the slope of the calibration curve i / C, where i is the current value and C the dendrimer concentration) increased with the dendrimer generation (Figure 3). The charge of the dendrimer, z, increased with the generation. Under the present conditions, z is considered equivalent to the number of lysine groups in the macromolecule, as were are at pH 2 and fully protonation of the end lysine groups are expected. Sensitivity values reported in the literature for proteins are much higher than we measured for dendrimers . The large difference indicated that not only charge has an influence on the sensitivity. Indeed, proteins have a tertiary structure with hydrophobic pockets, which play a role in complex formation between the organic electrolyte anions and the proteins adsorbed at the interface [7]. The dendrimers studied are made of lysine, which are strongly hydrophilic.

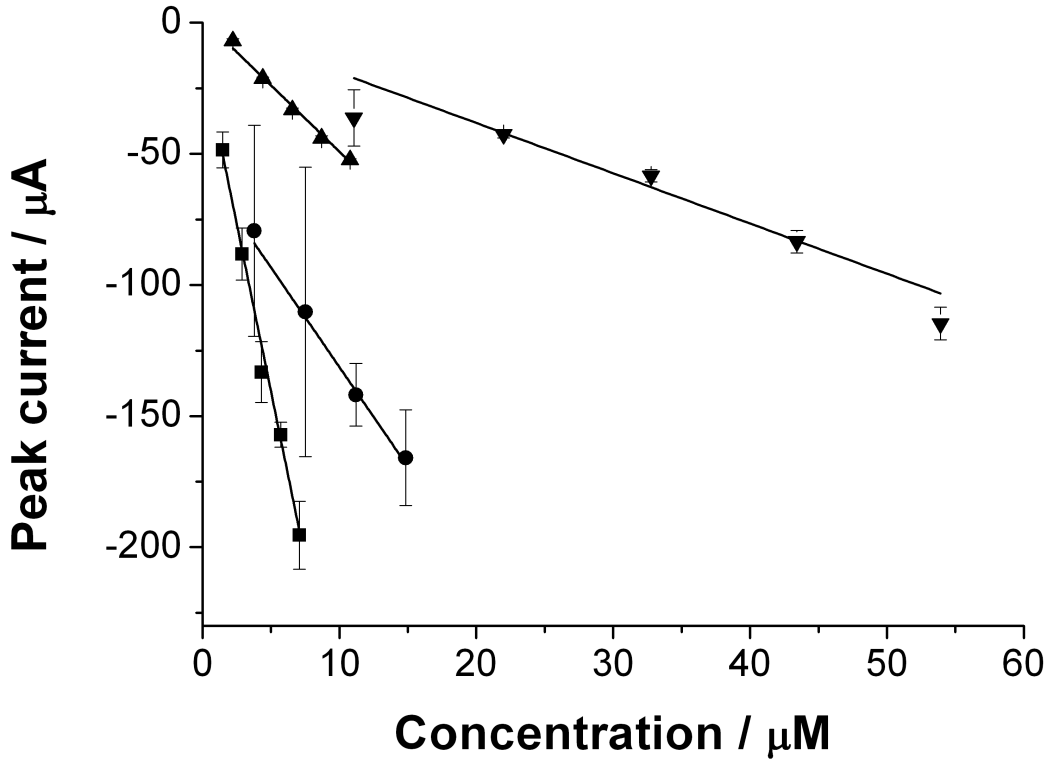

Figure 3: Calibration curves for G2 (▼), G3 (▲), G4 (●) and G5 (■) polyLysine dendrimers.

A limit of detection for the dendrimers G2 to G5 was determined experimentally. As the dendrimer generation increases, the limit of detection decreases to reach a minimum around 1 μM (Figure 4).

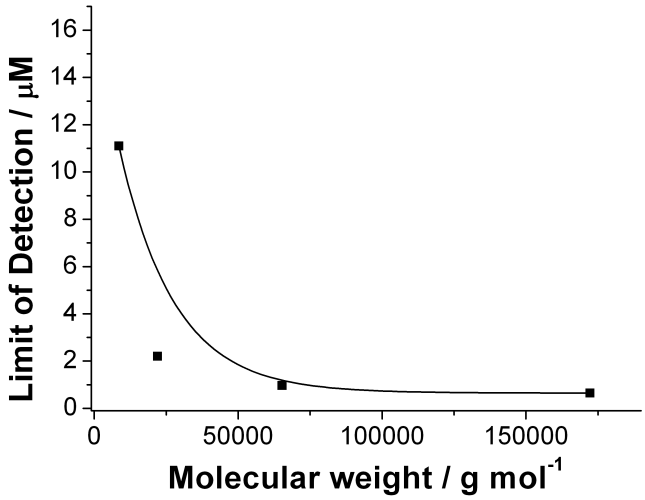

Figure 4: Limit of detection determined experimentally for polyLysine dendrimers G2 to G5 as a function of the dendrimer molecular weight.

The improved limit of detection for the dendrimers of higher generation is consistent with the increase of sensitivity with the dendrimer generation.

4. Conclusions

We report here the detection of polyLysine dendrimers via electrochemical detection at the liquid | liquid interface, A linear relationship is observed between the current measured and the dendrimer concentration. Structural characteristics of polyLysine dendrimers have a strong impact on the analytical performances at the liquid | liquid interface. Sensitivity increases with the charge of the dendrimers but not as fast as for proteins. Dendrimers cannot be used as a model macromolecules to predict the sensitivity and the limit of detection that could be achieved for a protein based on its overall charge. This is due to the tertiary structure of the protein which favours interactions with organic electrolyte anions.

5. References

[1] H.H. Girault, Electrochemistry at liquid-liquid interfaces, in: A.J. Bard, C.G. Zoski (Eds.) Electroanalytical Chemistry: A Series of Advances, vol. 23, CRC Press, Boca Raton, 2010, pp. 1-104.
[2] G. Herzog, V. Kam, D.W.M. Arrigan, Electrochim. Acta, 53 (2008) 7204-7209.
[3] M.D. Scanlon, D.W.M. Arrigan, Electroanalysis, 23 (2011) 1023-1028.
[4] X.J. Huang, A.M. O'Mahony, R.G. Compton, Small, 5 (2009) 776-788.
[5] G. Herzog, D.W.M. Arrigan, Analyst 132 (2007) 615-632.
[6] F. Kivlehan, Y.H. Lanyon, D.W.M. Arrigan, Langmuir, 24 (2008) 9876-9882.
[7] R.A. Hartvig, M.A. Mendez, M. van de Weert, L. Jorgensen, J. Ostergaard, H.H. Girault, H. Jensen, Anal. Chem., 82 (2010) 7699-7705.
[8] M.D. Scanlon, E. Jennings, D.W.M. Arrigan, Phys. Chem. Chem. Phys., 11 (2009) 2272-2280.
[9] G. Herzog, P. Eichelmann-Daly, D.W.M. Arrigan, Electrochemistry Communications, 12 (2010) 335-337.
[10] G. Herzog, A. Roger, D. Sheehan, D.W.M. Arrigan, Analytical Chemistry, 82 (2010) 258-264.
[11] A. Berduque, M.D. Scanlon, C.J. Collins, D.W.M. Arrigan, Langmuir, 23 (2007) 7356-7364.