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Investigation of a solvent-cast organogel to form a liquid-gel microinterface array for electrochemical detection of lysozyme☆

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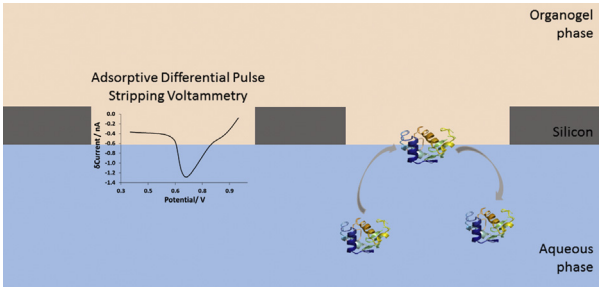
HIGHLIGHTS

- Protein detection by adsorption at aqueous-organogel microinterface array.
- Organogel prepared by solvent casting.
- Combined adsorption with differential pulse voltammetric detection.

GRAPHICAL ABSTRACT



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ABSTRACT

Ion transfer at aqueous-organogel interfaces enables the non-redox detection of ions and ionisable species by voltammetry. In this study, a non-thermal method for preparation of an organogel was employed and used for the detection of hen-egg-white-lysozyme (HEWL) via adsorptive stripping voltammetry at an array of aqueous-organogel microinterfaces. Tetrahydrofuran solvent casting was employed to prepare the organogel mixture, hence removing the need for heating of the solution to be gelled, as used in previous studies. Cyclic voltammetry of HEWL at the microinterface array revealed a broad adsorption process on the forward scan, at positive applied potentials, followed by a desorption peak at ca. 0.68 V, indicating the detection of HEWL in this region. Application of an adsorption step, where a constant optimized potential of 0.95 V was applied, followed by voltammetric detection provided for a linear response range of 0.02–0.84 μM and a detection limit of 0.030 μM for 300 s adsorption. The detection limit was further improved by utilizing differential pulse stripping voltammetry, resulting in detection limits of 0.017 μM, 0.014 μM, and 0.010 μM for adsorptive pre-concentration times of 60, 120 and 300 s, respectively, in unstirred solutions. These results are an improvement over other methods for the detection of HEWL at aqueous–organic interfaces and offers a basis for the label-free detection of protein.

1. Introduction

Biomolecules, such as proteins, play a vital role in maintaining the functionalities of every activity within living species. Hence, understanding and detecting protein behaviour can be beneficial for a number of biomedical applications [1]. One of the commonly studied model proteins is lysozyme, a protein found

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in mammals that is responsible for the cleavage of an acetal group located in the polysaccharide walls of bacteria [2]. Composed mainly of 129 amino acids residues held together by cysteine disulphide bonds [3], it is usually available as hen-egg-white-lysozyme (HEWL) since it comprises 3.5% of egg white protein [4]. Its molecular weight is ca. 14,600 g mol^{-1} [2] and isoelectric point is 11.35, making it positively charged at physiological pH [5]. Aside from being a model protein analyte, the investigation of lysozyme was propelled by its use as an indicator for several diseases [6–8].

For the past 45 years, the study of electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) has been rapidly increasing [9–11]. One of the main themes of research in this area in recent years has been the electrochemistry of proteins, since protein detection at the ITIES offers advantages for bioanalytical applications such as label-free detection, due to charge transfer processes at the ITIES, and amenability to miniaturization [12,13]. Amongst several biomolecules of interest at the ITIES or μITIES, dopamine [14], heparin [15], and cytochrome C [16] have been investigated in addition to HEWL. Scanlon et al. examined the electrochemical behaviour of HEWL at the ITIES and showed its adsorption at both all-liquid ITIES and gellified μITIES. The proposed mechanism for its detection included adsorption of the cationic protein at the interface and the protein-facilitated transfer of the organic electrolyte anion across the interface, resulting in a protein-anion complex [17,18]. This proposed complexation between the lysozyme and the organic electrolyte anion (hydrophobic) was demonstrated by Hartvig et al. [19] using an online mass spectrometry method to reveal this complexation. Subsequent mass spectrometry studies revealed partial unfolding of lysozyme following its electroadsorption at the aqueous-organogel interface [20]. Similar detection mechanisms were suggested for other biomacromolecules, including insulin [21] and haemoglobin [22,23].

In terms of detection limits for HEWL at the ITIES, reports have been within the low micromolar range, such as that based on background-subtracted cyclic voltammetry at a μITIES array that detected 0.5 μM [17]. However, lower detection limits are required for protein detection when applied in clinical diagnostics [24]. One common method that is utilized by researchers to address such concerns for low detection limits is in the form of preconcentration. In voltammetric analysis, this entails preconcentrating the analyte into or onto the electrochemical interface before application of the voltammetric analysis. This is referred to as stripping voltammetry and a recent review by Herzog and Beni [25] highlighted how this technique has been applied to μITIES arrays. In particular, its application to exploiting protein adsorption at the μITIES array has enabled lysozyme detection at 30 nM [26] as well as haemoglobin at ~40 nM [27] and insulin at 10 nM [28]. In all such methods, however, the organic phase was prepared using a high temperature process that involves pouring the hot gel mixture into the micro-interface-forming membrane and allowing it to cool [17,18,26–29]. However, alternative methods for organogel preparation, such as the solvent-casting methods widely used in potentiometric ion-selective electrode research [30,31], may offer a more convenient method for the preparation of the gelled organic phase.

The purpose of the work reported here was to examine whether the solvent-casting organogel preparation method was a viable approach for the development of a gelled μITIES array for protein detection. The combination of this method with adsorptive stripping voltammetry and differential pulse voltammetry will be described in the following sections, with a low detection limit of 10 nM achieved.

2. Materials and methods

2.1. Reagents

All reagents were obtained from Sigma–Aldrich Australia Ltd. and were used as received unless otherwise stated. The organic phase was prepared by dissolving bis(triphenylphosphoranylidene) tetrakis(4-chlorophenyl)borate ($BTPPA^+TPBCl^-$, 10 mM) in 1,6-dichlorohexane (1,6-DCH). A volume (2.5 ml) of this electrolyte solution was then gelled by the addition of 10% w/v low molecular weight poly(vinyl chloride) (PVC) [17,32]. In order to dissolve the PVC, a maximum equal volume (2.5 ml) of tetrahydrofuran (THF) was added dropwise to the mixture, with continuous stirring over a *ca.* 15–20 min time. The resulting solution was then set aside for 48 h to evaporate excess solvent before being used [30]. The gel mixture was used for a maximum of a further 24 h after this evaporation period. The organic electrolyte salt ($BTPPA^+TPBCl^-$) was prepared by metathesis of bis(triphenylphosphoranylidene) ammonium chloride ($BTPPA^+$ Cl^-) and potassium tetrakis(4-chlorophenyl)borate (K^+TPBCl^-) following the published procedure [33]. The HEWL stock solutions were prepared fresh in 10 mM HCl and then stored at 4 °C. Similarly, tetraethylammonium (TEA^+) chloride was dissolved in 10 mM HCl. All aqueous solutions used were made with MilliQ water from a USF Purelab plus UV, having 18.2 MΩ cm resistivity.

2.2. Apparatus

All electrochemical experiments were performed on an AUTOLAB PGSTAT302N electrochemical station (Metrohm, The Netherlands) through a NOVA 1.9 software interface. The μITIES array employed was defined by a micropore array silicon membrane, previously described [34,35]. The membrane employed in this study consisted of eight micropores in a hexagonal arrangement, each having a diameter of 22 μm and a pore centre-to-pore centre distance of 400 μm. These microporous silicon membranes were sealed onto the lower orifice of a glass cylinder using silicone rubber (acetic acid curing Selley's glass silicone). The solvent-cast organogel mixture was introduced into the silicon micropore arrays via the glass cylinder with the aid of a pre-warmed glass Pasteur pipette. The set-up was then set aside for at least 1 h before use. When ready, the organic reference solution (composition: 10 mM $BTPPA^+Cl^-$ in 10 mM LiCl) was then placed on top of the solvent-cast organic phase. The solvent-cast organogel/silicon membrane assembly was then immersed into the aqueous phase (10 mM HCl, HEWL in 10 mM HCl, and/or TEA^+ in 10 mM HCl) and voltammetric experiments were implemented. Scheme 1 summarizes the electrochemical cell employed. Each assembly of solvent-cast gel + silicon micropore array membrane was used for a maximum of 1 day of experiments.

2.3. Electrochemical measurements

A pair of Ag/AgCl electrodes were used for all measurements. The geometric area of the microinterface array was 3.04×10^{-5} cm^2. Cyclic voltammetry (CV) and adsorptive stripping voltammetry (AdSV) were carried out at a scan rate of 5 mV s^{-1}, unless noted otherwise. Optimal parameters for differential pulse voltammetry were found to be 75 mV as the modulation amplitude, 200 ms for the modulation time and 500 ms for the interval time, which resulted in a scan rate of 10 mV s^{-1}. Other parameters such as protein concentration, applied potential, and duration of the preconcentration step were varied accordingly. In order to compare all techniques utilized, all the calculated limits of detection were based on three times the standard deviation of the blank (n = 3)