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ARTICLE TYPE

A novel molecularly imprinted chitosan-acrylamide,graphene,ferrocene composite cryogel biosensor used to detect microalbumin

Amin Fatoni[a,b,c,1], Apon Numnuam[a,b,c], Proespichaya Kanatharana[a,b,c], Warakorn Limbut[a,b,d] and Panote Thavarungkul[a,b,e*]

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A novel highly sensitive and selective molecularly imprinted polymer (MIP) cryogel biosensor for determination of microalbuminin urine samples was fabricated. The MIP gel was prepared based on the graft copolymerization of acrylamide with N,N′-methylenebisacrylamide on chitosan using human serum albumin (HSA) as the template. The sub zero polymerization allowed the solvent to form ice crystals and left a macroporous cryogel structure when it was thawed. After removing the template, the specific imprinted surface on the cryogel pore walls was used to detect HSA via a redox mediator (ferrocene), entrapped in the cryogel, using differential pulse voltammetry (DPV). The electrochemical detection was improved by the presence of graphene that has been composited within the polymer. For determination of the albumin, the fabricated MIP cryogel biosensor showed a high sensitivity with a wide linear range of $1.0x10^{-4}$ to $1.0x10^{1}$ mgL^{-1} and a low limit of detection of $5.0x10^{-5}$ mgL^{-1} (S/N=3). The sensor also provided a very good reusability, *i.e.*, the sensitivity remained >90% after 9 cycles of binding-rewashing (18 analyses per cycle), while the sensitivity only decreased to 90% after 6 weeks of storage at room temperature. The biosensor also showed a good selectivity, both against bovine serum albumin (BSA) and some common possible interfering compounds normally present in urine (ascorbic acid, uric acid, urea, sodium, chloride, potassium and creatinine). The excellent performance of the biosensor was confirmed by analyzing the microalbumin in urine samples, and results were in good agreement with those obtained by the standard immunoturbidimetric method (P>0.05).

Introduction

Human albumin is a protein normally found in blood and when this protein leaks into the urine through damage to the kidneys it is known as microalbumin. Microalbumin plays an important role in the early detection of chronic kidney disease[1], endothelial dysfunction[2] and diabetic complications[3]. The importance of detecting microalbumin has attracted many research groups to develop various detection systems, including immunoturbidimetric[4], spectrophotometry[5], immunoresonancescattering[6, 7], radioimmunoassay[8], immunonephelometry[9], chemiluminescence immunoassay[10], fluorescence immunoassay[11], HPLC[12], electrophoresis[13] and by use of a biosensor[14]. Each of these methods has its own advantages and limitations, such as expensive instrumentation for HPLC and the high cost of using antibody based assays. On the other hand, the use of biological sensing elements has some advantages and has therefore promoted biosensors as suitable analytical devices in many fields. Biosensors, especially with electrochemical detectors have been widely studied due to their fast response, high sensitivity and low limit of detection, as well as their small size, and often low cost for construction[15, 16].

One of the areas of biosensor development is in the improvement of the biosensing elements. The expense and susceptibility to environment changes of many natural sensing elements have led research workers to develop mimicked counterparts. An interesting approach was to develop molecular imprinting methods[17]. A molecularly imprinted polymer (MIP) is a synthetic recognition element involving the creation of a recognition site in the polymer scaffold. Compare to biological recognition elements the MIPs offer benefits such as a specific recognition with a high binding affinity, higher physical robustness, less expense and the ease of mass fabrication[18]. These advantages have led to a study

[a]*Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[b]*Center of Excellent for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[c]*Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[d]*Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[e]*Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*

* Corresponding author email: panote.t@psu.ac.th, Tel: +6674288753
[1] On study leave from Department of Chemistry, Faculty of Science and Engineering, Jenderal Soedirman University, Purwokerto, Indonesia

of various MIP based sensors[19, 20].

In addition to the development of the sensing element, the support of the sensing part also plays an important role. Various porous structural supporting materials with large surface areas have been employed to improve the sensors performance such as a porous filter membrane[21], nanoporous aluminum oxide[22], porous silicon[23], a hydrogel[24] and a cryogel[25, 26]. The large surface area of a porous cryogel has been reported to increase the biosensor sensitivity and stability[25].In this work, the advantages of the MIP and the cryogel were used to fabricate a microalbumin biosensor. The cryogel was prepared by polymerization at a sub-zero temperature from monomers dissolved in water. When the ice crystal are formed and melted away when thawed, they leave a macroporous gel network[27]. The MIP cryogel was produced from a biocompatible material of chitosan grafted polyacrylamide (CS-g-PAM) using human serum albumin (HSA – the analyte) as a template together with entrapped ferrocene (Fc) as an electroactive element. The oxidation of Fc was recorded using differential pulse voltammetry (DPV) where the signal was related to the binding event of the MIP and HSA. In addition, graphene (Grp), with its excellent electrochemical performance that has been widely applied to increase the electron transfer in sensor and biosensor development[29-30], was also employed. The combination of Grp, entrapped in the pores wall, and the large surface area of the cryogel helped to enhance the biosensor performance.

Materials and Methods

Materials

Chitosan (CS) from shrimp shells (low viscosity, < 200 mPa.s, 1 % in acetic acid at 20 °C), acrylamide (AM), N,N′-Methylene bisacrylamide (BAM), ferrocene (F408 – 98%), human serum albumin (HSA), bovine serum albumin (BSA), L-ascorbic acid, creatinine and uric acid were all from Sigma-Aldrich (Steinheim, Germany). Ammonium peroxodisulfate (APS) and urea were from Merck (Darmstadt, Germany). Sodium dihydrogen *o*-phosphate and disodium hydrogen *o*-phosphate were from Ajax Finechem (Sydney, Australia). Graphene nanoplatelets (4-5 layers, thickness of 8 nm, surface area 600-750 m^2 g^{-1}, particle diameter < 2 micron) were from Cheap Tubes Inc (Brattleboro, USA).

Preparation of a protein-imprinted cryogel modified electrode

A protein-imprinted cryogel was prepared using CS-g-PAM. The grafting was prepared according to previously described work[31] with some modification to make an MIP cryogel. In brief, CS (50 mg) was dissolved into a 1.0 % acetic acid solution (5.0 mL), mixed with AM (135 mg), BAM (15 mg) and adjusted to pH 4.6 by adding sodium acetate. An appropriate amount of Grp and Fc (see optimization study) were then added and mixed thoroughly followed by an appropriate amount of the HSA (see optimization study) and sonicated for 5 min to obtain the CS-AM-Grp-Fc HSA solution.

The electrode was made from a gold rod (3.0 mm diameter, 99.99% purity) that was polished using alumina slurries (5.0, 1.0, and 0.3 µm, respectively) and electrochemically etched in 0.5 M sulfuric acid for 25 cycles (0.1 – 1.5 volt, scan rate 100 mVs^{-1}). To modify the electrode (Fig. 1) a 200 µL of 20% APS solution was added to the CS-AM-Grp-Fc-HSA solution to initiate the polymerization. Then 5.0 µL was dropped onto the electrode and immediately stored at -20 °C overnight. The modified electrode was then thawed at 4°C for one hour and rinsed with deionized water. A non cryogel modified electrode was also prepared in the same way without the cryogelation process (freezing and thawing). The HSA template was removed from the modified electrode by soaking in a 10% (v/v) acetic acid solution containing 10% (w/v) sodium dodecylsulfate (SDS) for 2 h providing an MIP modified electrode with Fc and Grp entrapped in the polymer matrix. A non MIP (NIP) cryogel modified electrode was also prepared with the same procedure in the absence of the HSA template.

Characterization of the MIP Cryogel modified electrode

The morphology of the MIP cryogel was examined with a JSM-5200 LV scanning electron microscope (SEM) (JEOL, Japan). The pore size of the cryogel was also estimated by measuring the pores as seen in the SEM images. The binding of the MIP cryogel to the analyte was electrochemically studied using a three-electrode system, *i.e.*, a fabricated working electrode, a Ag/AgCl reference electrode and a Pt counter electrode, connected to an eDAQ potentiostat (Model EA161, EDAQ, New South Wales, Australia) controlled by EChem v2.1.12 software. The MIP-cryogel modified electrode response was the oxidation peak of the electroactive Fc entrapped in the cryogel and detected by differential pulse voltammetry (DPV). The DPV was operated from 0.0 to 0.4 V, against the Ag/AgCl reference electrode, with a scan rate of 50 mV s^{-1}, a step width of 100 ms, a step potential of 5.0 mV, the pulse width and pulse amplitude were 60 mV. The DPV was performed in a 10 mL batch system with 50.0 mM sodium phosphate buffer contained 50.0 mM of KCl as the medium.

Optimization study

The optimization study was performed for the MIP cryogel fabrication (amount of Grp and HSA) and the operational conditions (buffer concentration and pH). A series of HSA concentrations ($1.0x10^{-4}$ to $1.0x10^{1}$ mg L^{-1}) was tested and the DPV response (3 replicates per concentration) versus the logarithm of the analyte concentration of each optimization condition was plotted to get the sensitivity (slope of the calibration plot), where the highest sensitivity indicated the optimal conditions.

The Grp configuration on the fabricated biosensor was studied in each of two ways. One was to add Grp as the first layer followed by the layer of the CS-g-PAM, Fc and HSA composite (Grp/CS-AM-Fc-HSA). The other was to add only one layer of the composite of all the components *i.e.*, CS-g-PAM, Grp, Fc and HSA (CS-AM-Grp-Fc-HSA). The better configuration was further tested for the Grp concentration between 0 and 10.0 mg mL^{-1}.

Using the optimal Grp condition, the template concentration (HSA) was studied in the range of 5.0 to 60.0 mg mL^{-1}.

For the operational conditions, the concentration of the sodium phosphate buffer (25, 50 and 100 mM) and pH (5.50 - 7.50) were

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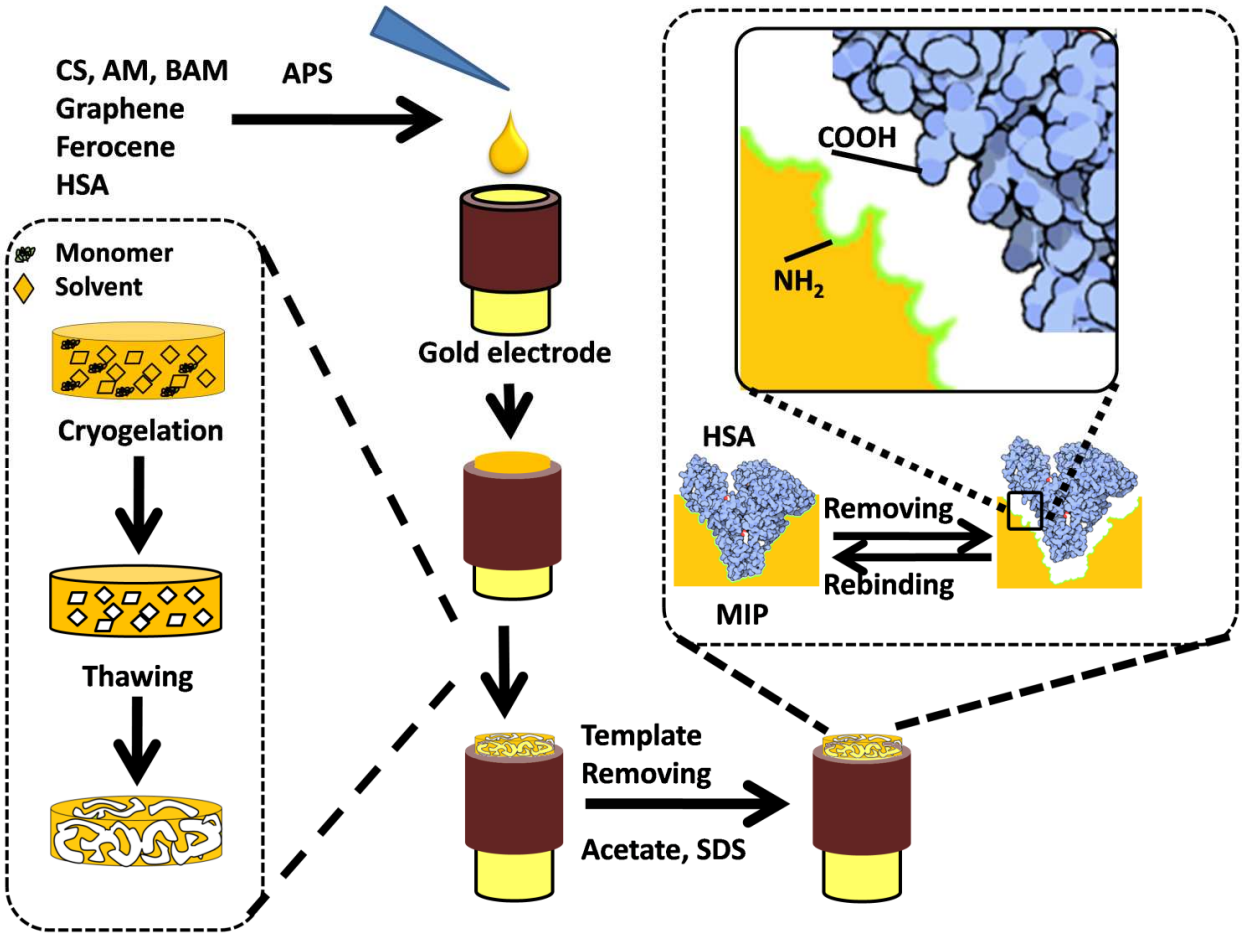

Fig. 1 The Chitosan grafted polyacrylamide MIP cryogel with graphene and entrapped ferrocene using a human serum albumin template electrode preparation. The –COOH groups of HSA molecules can form a hydrogen bond with –NH2 group of polymer. CS : chitosan; AM : acrylamide; BAM : N,N′-methylenebisacrylamide; APS: ammonium peroxodisulfate; HSA : human serum albumin; SDS : sodium dodecyl sulfate.

optimized with the electrode prepared with the optimal conditions of HSA and Grp.

The study of possible interferences

The selectivity of the MIP cryogel biosensor to detect the albumin level in urine was tested against urea, sodium, chloride, potassium and creatinine that are generally found in the urine[32]. Sodium, chloride, potassium and creatinine were tested from 0.10 to 4.0 g L^{-1}, while urea was tested between 0.10 and 20 g L^{-1}. These selected concentrations covered the upper limit of each component found in normal urine, *i.e.* (in g L^{-1}) urea 9.3, chloride 1.87, sodium 1.17, potassium 0.75 and creatinine 0.67[32].Other electroactive interferences found in the blood such as ascorbic acid (0.10 to 10.0 mM) and uric acid (0.10 to 3.0 mM) were also tested. To prove the selectivity of the MIP cryogel for detecting albumin, bovine serum albumin (BSA), a protein with structure similar to HSA, was also tested in the range of $1.0x10^{-4}$ to $1.0x10^{1}$ mg L^{-1}.

Studies on stability

For the operational stability study, one modified electrode was used to detect a series of concentration of HSA ($1.0x10^{-4}$ to $1.0x10^{1}$ mg L^{-1}) after which the bound HSA was removed by dipping the modified electrode in a 1.0% (v/v) acetic acid solution containing 1.0% (w/v) sodium dodecylsulfate (SDS) for 15 min. This was called a cycle and the operational stability was determined by the number of cycles before the sensitivity decreased to less than 90%. For the storage stability, seven electrodes were fabricated and used to measure the HSA as in the operational stability test. Each electrode represented the storage for 1 to 7 weeks at room temperature. The sensitivities of the electrode to detect HSA for different certain storage periods were compared.

Urine samples analysis

Urine samples from Songklanagarind Hospital, Hat Yai, Thailand were tested. The slopes of a standard curve and a spiked curve (sample with a series of HSA concentration) were first compared and analyzed by Two-Way ANOVA to detect any matrix effect. The samples, with an appropriate dilution (according to the matrix effect test), were then analyzed with the biosensor and the result was statistically compared to those from the standard method of immunoturbidimetric (used by the hospital) using the Wilcoxon Signed-Rank test.

Results and discussion

Characterization of the MIP cryogel modified electrode

The scanning electron micrograph of the MIP cryogel showed an interconnected porous structure (Fig. 2a) while no pores were observed for the non cryogel form (Fig. 2b). The high magnification SEM image of MIP modified electrode showed a rough surface (Fig. 2c) while the NIP surface is smoother (Fig. 2d). The diameter of the pores of the cryogel of 5-20 µm, were much larger than the size of the HSA (13.6 x 2.7 x 2.7 nm^3 [33]), and this would allow for an easy transfer (rebind or remove) of the protein to and from the MIP of the cryogel. The cryogel porous structure not only facilitated protein transfer but also increased the surface area, thus increased the number of imprinting receptors that could improve the sensitivity of the fabricated biosensor. Besides the specific recognition site of the MIP, it has also been reported that the MIP cryogel had a larger hydrodynamic volume compared to the NIP cryogel, due to the larger surface area of the imprinted molecule on the cryogel surface[34, 35]. In this condition, the water molecules carrying the analyte can easily penetrate into the polymeric chain of the cryogel.

Electrochemical properties of the modified electrode

The detection of the HSA was based on the oxidation of the Fc entrapped in the cryogel matrix and analyzed using differential pulse voltammetry due to its high sensitivity[36]. The MIP-HSA binding is most likely based on the formation of hydrogen bonds between the NH_2 groups that are widely available on the chitosan and polyacrylamide MIP surface and the COOH groups on the HSA surface similar to a previous report of the interaction between a pyrrole based MIP and bovine serum albumin[37]. This MIP-HSA binding event was performed in a batch system with 1 min mixing after which the binding would take place.

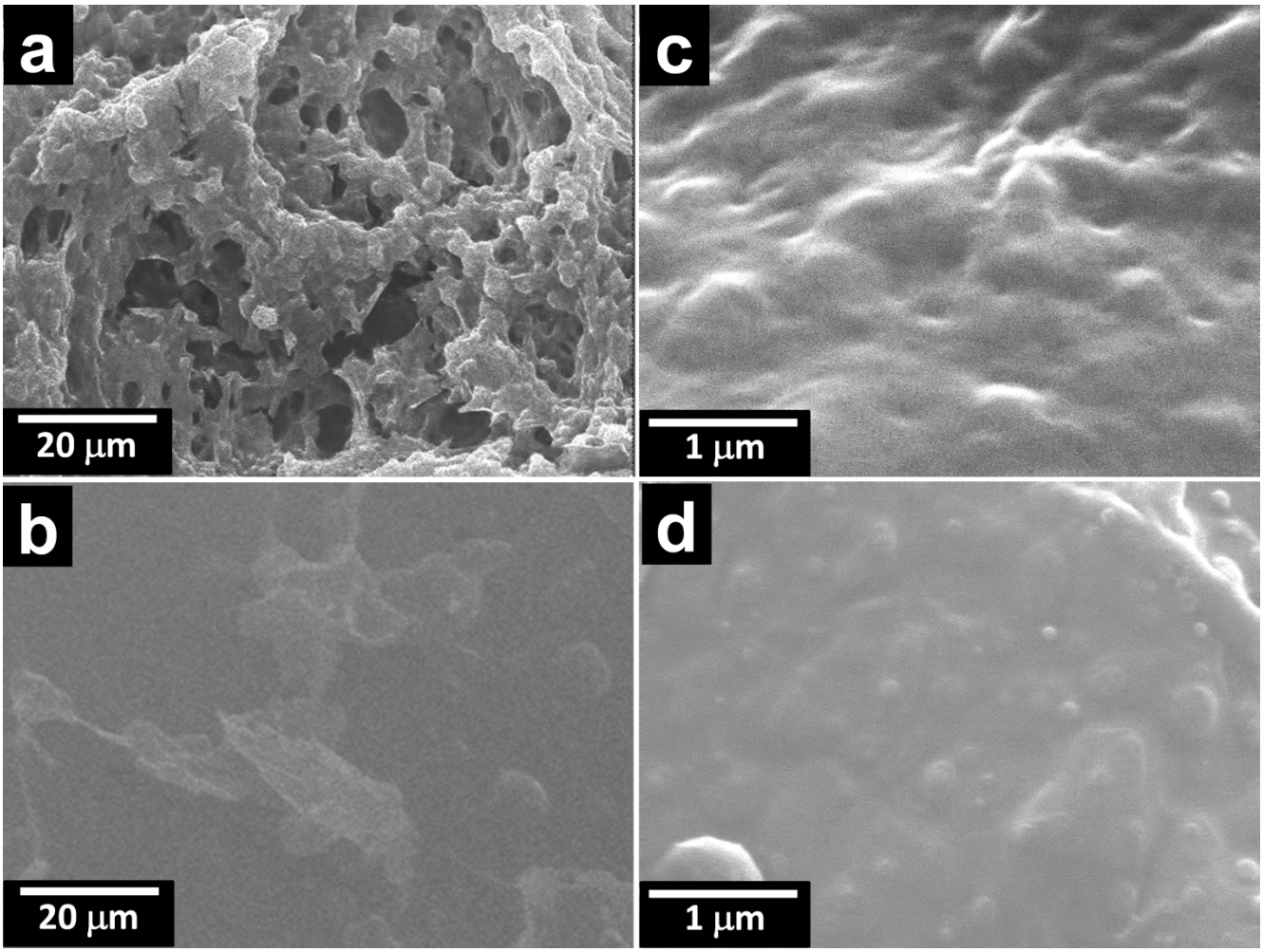

Fig. 2 SEM micrographs showing a porous structure ofCS-AM-Grp-Fc-HSA MIP cryogel at x2000 (a), an MIP non cryogel at x2000 (b). the high magnification of MIP surface at x20000 (c) and NIP surface at x20000 (d)

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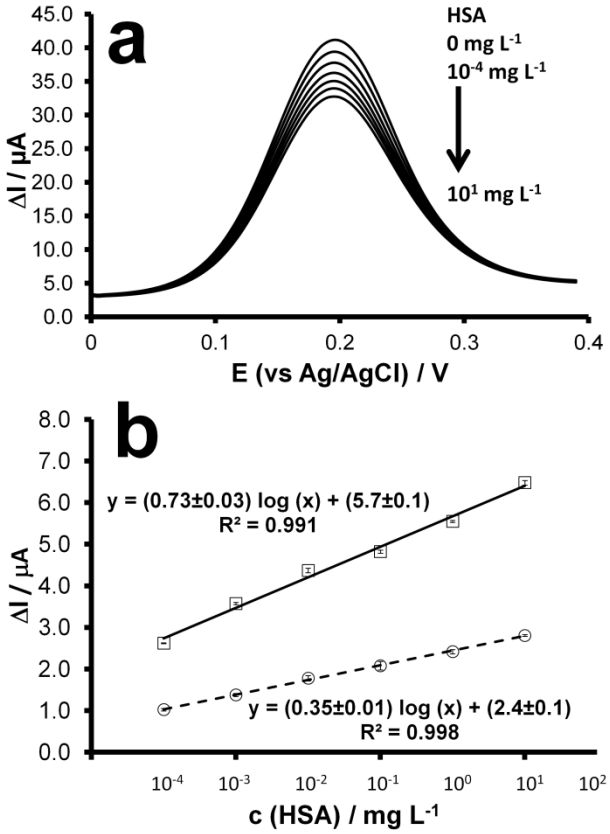

Fig.3 (a) An example of the decrease of the oxidation peak with the addition of the analyte. (b) Responses of the MIP cryogel modified electrode (solid line) shows a better sensitivity than the MIP non cryogel (dashed line).

The oxidation peaks of the Fc decreased with time with the addition of the HSA, because the MIP-HSA binding blocked the electrontransfer of the Fc. The DPV responses reached a steady value at 10 min (5 min interval measurements from 0 to 20 min) where a complete binding occurred. Therefore, 10 min of binding was allowed before recording the response. The concentration of the HSA was quantified based on the decline of the peak current (Fig. 3a). In comparison to the MIP non cryogel modified electrode, the MIP cryogel electrode has a 2.1 time higher sensitivity (Fig. 3b). In contrast the NIP cryogel modified electrode showed only a very low response at a high concentration of HSA (about 0.5 µA for the NIP cryogel response compared to about 7 µA for the MIP cryogel with 10.0 mg L^{-1} HSA)

Optimization study

Graphene with its advantages, i.e., it has a high charge mobility (200,000 cm^2 V^{-1} s^{-1})[38], wide potential windows[28, 39], and can exhibit a well-defined redox peak[40, 41], was applied to increase the

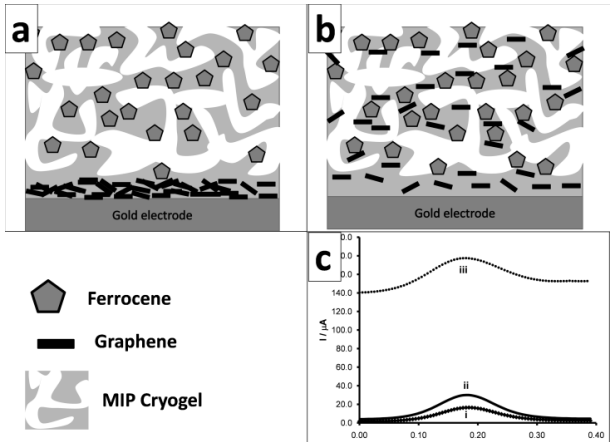

Fig. 4 The CS-AM-Fc MIP cryogel with the different configuration of Grp. (a) Grp on the first layer (Gr/CS-AM-Fc-HSA), (b) Grp as a composite (CS-AM-Grp-Fc-HSA). (c) the base line comparison of the modified electrode with the different Grp configurations, (i) without Grp, the oxidation peak is lower than CS-AM-Grp-Fc-HSA (ii), whereas the Gr/CS-AM-Fc-HSA(iii) showed a high background current.

electron transfer. A comparison of the two configurations showed that the Grp composite (CS-AM-Grp-Fc-HSA, Fig. 4b) had a better sensitivity (1.3 time higher) than when the Grp was added as the first layer on the electrode (Grp/CS-AM-Fc-HSA, Fig. 4a). This was because the Grp/CS-AM-Fc-HSA had a higher background current (Fig. 4c (iii)) that resulted in a lower peak height, hence, a lower sensitivity. When a composite without graphene was tested it showed a lower response than the one with graphene (Fig. 4c (i)-(ii)).

The Grp in the CS-AM-Grp-Fc-HSA composite was then optimized for its concentration and the sensitivities of the biosensor increased with the amount of Grp from 0 to 0.75 mg mL^{-1}. In this condition, the addition of Grp to the MIP cryogel composite increased the electron transfer, and resulted in a higher oxidation peak and finally improved the sensitivity. However, the sensitivity decreased when the concentration of Grp was more than 0.75 mg mL^{-1} due to the high background current that resulted in a low response, similar to that mentioned above. Therefore, 0.75 mg mL^{-1} of Grp was used for further studies.

The amount of the template was another important factor that affected the MIP efficiency[42]. Increasing the template from 5.0 to 30.0 mg mL^{-1} improved the sensitivity of the biosensor because the number of MIP holes increased. As a consequence the ability of the biosensor to capture the analyte was extended. However, too many templates (30.0 to 60.0 mg mL^{-1}) caused the sensitivity to decrease. This may be because when there are too many templates, they can become close to one another or exist as an aggregate. The intermolecular exchange of disulfide is one reason of albumin aggregation[43] and the possibility of aggregation will increase with the concentration. This resulting in MIP holes those was not ideal, so the effectiveness of the MIP to capture the

analyte was significantly reduced.

As for the effect of the HSA structure, the MIP–HSA interaction and the electrochemical detection were greatly affected by the buffer conditions and the concentration and pH of the sodium phosphate buffer were optimized. The optimal conditions, with the highest sensitivity, were obtained at 50 mM and at a pH of 6.50. The sensitivities increased from pH 5.50 to 6.50, then decreased at higher pH values. This may due to the HSA having a conformation change after being moved from a neutral to a basic condition[44], hence, the MIP-HSA binding was reduced.

Performance of the modified electrode

Linear dynamic range and limit of detection

Under the optimal conditions, the relationship between the decrease of the oxidation peak (ΔI) and the logarithm of the HSA concentration (mg L^{-1}) showed a linear correlation (Fig. 5) between $1.0x10^{-4}$ to $1.0x10^{1}$ mg L^{-1} (y = (1.36±0.06) log (x) + (6.3±0.1)) with a correlation coefficient (r) of 0.9916 and a limit of detection of 5.0×10^{-5} mg L^{-1}(S/N = 3).

Selectivity study

The selectivity test using the closely related BSA protein was performed under the optimal conditions using a series of concentration of BSA from $1.0x10^{-4}$ to $1.0x10^{1}$mg L^{-1}. Under the same concentration range the response of the MIP cryogel biosensor to BSA has a much lower relative sensitivity (0.16 times) compared to the HSA detection. Even at 10 mg L^{-1} of BSA the response was still much lower than the LOD of HSA.

Other possible interfering compounds such as urea, chloride, sodium, potassium and creatinine showed no redox peak that could affect the analysis. On the other hand, oxidation peaks were observed for the electroactive ascorbic acid and uric acid at relatively high concentrations, beginning at 3.0 mM for ascorbic acid and 1.0 mM for uric acid.

However, the potentials that these oxidation peaks occurred were not the same as the oxidation potential of Fc that was used to measure the MIP-analyte interference (Fig. 6). Thus, these electroactive compounds would not interfere with the HSA measurement.

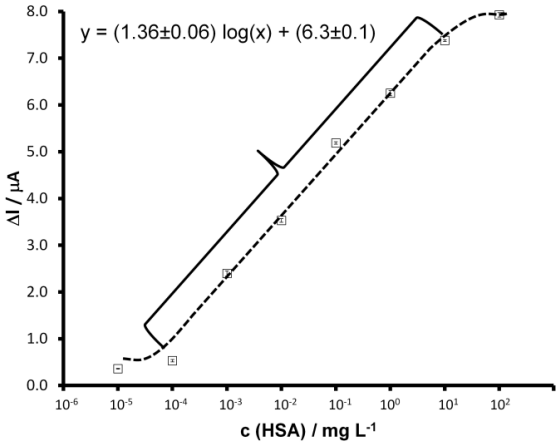

Fig. 5 The linear response of the CS-AM-Grp-Fc-HSA MIP cryogel to detect HSA. Inset, an example of the decrease of the oxidation peak with the addition of the analyte.

Stability study

For the operational stability test, the sensitivity after using a number of subsequent binding-washing cycles (18 injections per cycle) was compared to the first cycle. The relative sensitivities of the fabricated biosensor were greater than 90% after 9 binding-washing cycles (a total of 162 injections). The decrease in the biosensor sensitivity may due to the harsh conditions used for regeneration. Although the mild acid-detergent used to remove the template could disturb the stability of the HSA structure, the repeated process may also affect the MIP structure and affinity performance. The cavities of the MIP cryogel biosensor may also be blocked by small molecules during the adsorption process, and resulted in a lower sensitivity after certain binding-washing cycles[44].

In the case of the storage stability, results showed that the electrode could be stored at room temperature for up to 5 weeks, during which the biosensor sensitivity remained at more than 90% (RSD 6.1%). The sensitivity then decreased to 87.9% and 82.7% in the 6th and 7th week, respectively.

Urine samples analysis

The effect of the urine sample matrix showed that at a 10^{4}dilution, the sensitivities of the standard HSA and of samples spiked with HSA were not significantly different (*P*> 0.05). Thus, at this dilution factor any matrix effect can be ignored. The urine samples were then diluted with the running buffer at least 10^{4} times before being analyzed by the MIP cryogel biosensor. Table 1 shows the albumin level in the urine samples obtained by the biosensor and the immunoturbidimetric assay.

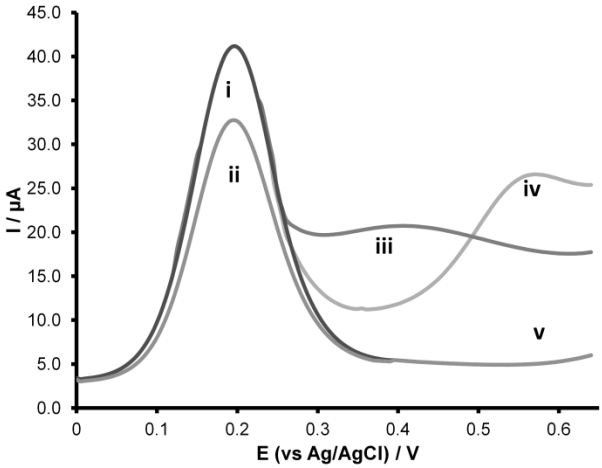

Fig. 6 The effect of possible interfering compounds on the DPV measurement of HSA detection by the CS-AM-Grp-Fc-HSA MIP cryogel biosensor, the peak of a blank signal (i), with the addition of $1.0x10^{1}$ HSA mg L^{-1} (ii), 3 mM of ascorbic acid (iii), 3 mM of uric acid (iv) while urea, creatinine, sodium, potassium, chloride provided similar peaks to the blank (v).

Table 1 Albumin level in urine samples obtained by the MIP cryogel biosensor compared to the standard method used by the hospital.

Sample	Biosensor (mg L^{-1})[a]	Immunoturbidimetric assay (mg L^{-1})
1	4.2±0.1	4.3
2	32±4	31.4
3	1440±20	1440.4
4	2170±40	2102.4
5	51±2	51.6
6	74±2	75.4

[a] mean ± SD, n = 3.

The recovery test has also been validated by spiking different concentrations of a HSA standard solution to a urine sample (1.0 to 10.0 mg L^{-1}) to obtain final concentrations after a 10^4 times.

In comparison to some other methods used to detect protein (Table 2), this fabricated biosensor showed a wider linear range (5 order of magnitude) with a lower limit of detection than most of other MIP based biosensors[45-51] and other microalbumin detection methods such as by HPLC, resonance scattering[6], chemiluminescence[10], fluorescence[11].

The biosensors with the better linear range and limit of detection were based on anti-HSA using capacitive[52] and electrochemical impedance spectroscopy[53] detectors. However, the fabricated MIP cryogel sensor provided a better stability with up to 162 analyses compared to 44 analyses for the anti-HSA using EIS. Furthermore, the antibody based biosensors required a low temperature storage, whereas the MIP biosensor, with no biological component, can be stored at room temperature.

Conclusion

This is the first report of an MIP cryogel based biosensor with an electrochemical detection for the determination of microalbumin in urine. The combination of MIP and cryogel on the developed biosensor provided an excellent performance, with a very wide linear range, a low limit of detection and high selectivity. The performance of the fabricated biosensor showed a good agreement (P> 0.05) with the urine sample microalbumin detection compared to the standard immunoturbidimetric method generally used by hospitals. In addition, this relative lower cost MIP cryogel biosensor, in comparison to the anti-HSA based biosensor, also provided a better stability with easier storage handling.

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Table 2 Comparison of the fabricated biosensor with other MIP based biosensor used to detect protein.

System	Analyte	Linear range / mg L^{-1}	LOD / mg L^{-1}	Detector	Selectivity against	Recycle	References
MIP based							
EMIP-coated CdTeQDs	BSA	33.5 – 670	6.7	Fluorescent	BHb	3 (93%)	46
dBSA -CdTe -QDs	Lys	0.20 – 122	0.10	Fluorescent	Cyt, mBSA, BSA	6 (83%)	47
3-APBA-BSA	BSA	20-100	20	SPR	Hb, Lys		48
PEDMAH-NPs	Lys	0.30 – 20.2	1.2×10^{-3}	SPR	BSA, cyt	5	49
PEDMAH-NPs	Lys	0.2–1500	1.2×10^{-3}	QCM	BSA		51
Si-NP/CdTe	BHb	1.3 – 135	0.6	Fluorescence	BSA, OB, Lys	6 (91%)	45
3-APBA – Au-Cr	Hb	50 – 5000	0.4	SPR	BSA, OB, Lys		50
Other sensing based							
Anti-HSA	HSA	0.03–0.96	0.02	Resonance scattering-Fluorescent	Interfered by other proteins		6
avidin–biotin, anti FITC	HSA	0.15–15	0.089	Chemiluminescent			10
cyanine 5	HSA	10 - 320	5.5	Fluorescent			11
HPLC	HSA	4 - 240	3.4	UV			12
Anti-HSA / AgNPs	HSA	6.6×10^{-11} – 6.6×10^{-3}	6.6×10^{-11}	Capacitive			52
Anti-HSA / porous gold	HSA	6.6×10^{-7} – 6.6×10^{-1}	6.6×10^{-7}	EIS	BSA, AFP	44 analyses	53
CS-AM-Grp-Fc	**HSA**	**1.0×10^{-4}– 1.0×10^{1}**	**5.0×10^{-5}**	**Amperometry**	**BSA, UA, AA, Crt, urea, K^+, Cl^-, Na^+**	**9 (>90%) (162 analyses)**	**This work**

EMIP: epitope molecularly imprinted polymer; QDs: quantum dots; BSA: bovine serum albumin; dBSA: denaturated BSA; 3-APBA: 3-aminophenylboronic acid; PEDMAH: poly(ethylene glycol dimethacrylate-Nmethacryloyl-l-histidinemethylester);NP: nanoparticle; OB: ovalbumin; lys: lysozyme ; BHb: bovine hemoglobin; Hb: hemoglobin; cyt: cytochrome; SPR: surface plasmon resonance; CZ: capillary zone, FITC: fluorescein-iso-thiocyanate; EIS: electrochemical impedance spectroscopy; UA: uric acid; AA: ascorbic acid; Crt: creatinine.

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