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A Novel Poly(ionic liquid) Interface-Free Two-Dimensional Monolithic Material for the Separation of Multiple Types of Glycoproteins

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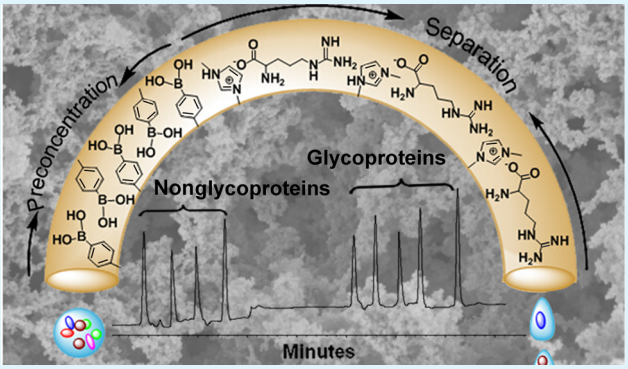
Supporting Information

ABSTRACT: Currently, many types of affinity materials have been developed for the enrichment of glycoproteins potentially considered to be clinical biomarkers; however, they can not effectively distinguish between different glycoproteins and thus lack the functionality that may be the key to the diagnosis of specific diseases. In the present work, a novel interface-free 2D monolithic material has been developed for the separation of multiple types of glycoproteins, in which boronate-functionalized graphene acts as preconcentration segment and poly(guanidinium ionic liquid) acts as separation segment. The resultant 2D material was characterized by X-ray photoelectron spectroscopy, elemental analysis, and electroosmotic flow analysis to demonstrate successful modification at each step. The performance of this 2D material was evaluated by capillary electrochromatography and allowed the successful online concentration and separation of five standard glycoproteins. The high separation efficiency can be largely attributed to the good orthogonality of boronate-functionalized graphene monolith and poly(guanidinium ionic liquid) monolith.



KEYWORDS: *monolithic material, two-dimensional, guanidinium ionic liquid, glycoprotein, preconcentration and separation*

INTRODUCTION

Glycosylation, one of the most significant post-translational modifications of proteins, plays a vital role in many biological processes.[1] Aberrant glycosylation has been implicated in numerous diseases, and numerous studies have revealed that many clinical biomarkers are glycoproteins.[2,3] Because of their relatively low abundance, many types of affinity materials have been developed for the enrichment and detection of glycoproteins.[4−11] However, these technologies usually perform the simple function of glycoprotein capture or release. They can not effectively distinguish between different glycoproteins; thus, they lack the functionality that may be the key to the diagnosis of specific diseases. Therefore, the development of a new material that can capture and separate multiple types of glycoproteins would dramatically increase the use of glycoproteins in diagnostics and prognostics.

Multidimensional separation, a flourishing research area, allows for the analysis of complex mixtures; considering different structures or physicochemical properties of analytes, the multidimensional separation systems are usually designed on the basis of the combination of multiple separation modes, including hydrophilic interaction, reversed-phase, size-exclusion, ion-exchange, and so on.[12−14] Nowadays, multiple types of interfaces between the two dimensions have been developed, such as leak-free connection, etched porous interface, electronically controlled switching valve, high-pressure two-position valve, and so on.[13−16] Although these systems are flexible and allow the combination of chromatographic and electrophoretic separation techniques, the successful preparation of these meticulous interfaces often relies on the skill of the chemists.[17,18] Besides, the presence of dead volume usually results in low column efficiency, resolution, and even sensitivity.[18] By comparison, a interface-free multidimensional separation system, as a technically simple approach omitting any dead volumes and sample dilution problems, attracts much more attention as a system in which online sample concentration and separation can be sequentially implemented in the first and second dimensions, respectively.[17] Monolithic materials, commonly possessing high permeability, loading capacity, and separation efficiency, have been extensively studied and widely applied in separation science.[19,20] Wang et al. reported the first study about biphasic monolithic capillary column comprising ethylene glycol methacrylate phosphate-based strong-cation-exchange monolith and lauryl methacrylate-based reversed-phase monolith.[21] Chen et al. synthesized a sulfo/vinyl biphasic silica hybrid monolithic column. The

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applicability of this system was demonstrated using standard amines, single peptide, and diluted BSA hydrolysate, indicating that the system has a capacity for preconcentration of low abundance peptides.[22] To date, research on glycoprotein separation by interface-free 2D monolithic material has not been reported yet. In our previous work, a novel graphene-doped poly[ionic liquid ($ViOcIm^+Cl^-$)] boronate-affinity monolithic material was prepared.[23] The electrochromatographic evaluation showed that the affinity material exhibited satisfying specific recognition toward glycoproteins even in acidic media, exhibiting a wider pH application range. The incorporation of graphene greatly improved the large specific surface area (133.64 m^2/g) of the monolithic material, which gave rise to higher binding capacity for glycoproteins compared to that of other boronic acid-functionalized monolithic materials.[5,11,24−27] Unfortunately, like other traditional enrichment materials, it could not distinguish between different glycoproteins.

Ionic liquids, a class of organic salts with melting points at or below 100 °C, have attracted tremendous interests in the past decade because of their beneficial characteristics including good solubility, low volatility, high thermal stability, recyclability, nonflammability, conductivity, and especially excellent mechanical properties and biocompatibility.[28−33] The versatile properties of ionic liquids are closely connected to their exchangeability and modifiability of the cation and anion parts. "Task-specific" ionic liquids can be obtained by incorporating various functional groups, such as amino, alkyl, hydroxyl, vinyl, and carboxyl groups.[34] As a result, their ability to interact with other molecules through hydrophobic/hydrophilic interactions, hydrogen bonds, ion exchange, $\pi-\pi$ stacking, and electrostatic interactions imparts beneficial characteristics to ionic liquids. In recent years, ionic liquids have been widely used as mobile/stationary phases in gas chromatography (GC), capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC) to improve separation efficiency.[35−37] It has been reported that poly(ionic liquid) monolithic materials can improve the separation efficiency of proteins because of the biocompatibility of ionic liquids. Besides, the limited content of organic solvent in mobile phase is very beneficial to analysis of protein containing samples because the high content of organic solvent in loading buffer may give rise to protein precipitation or dennaturation.[30,38] Functional ionic liquids are ionic liquids with functional cations or functional anions. Guanidinium ionic liquids, as functional ionic liquids, have not only common performance of ionic liquids but also unique molecular recognition function of guanidinium, such as its ability to form up to five hydrogen bonds with nucleic acids, to recognize phosphate anions through ion pairing and hydrogen bonding, and to interact with aromatic amino acids existing on the surface of proteins by cation$\cdots\pi$ interactions.[39,40] Owing to the strong biological activity and unique molecular recognition function of guanidinium, guanidinium ionic liquids exhibit unique potential in protein purification.[41,42]

In this study, a novel boronate-functionalized graphene-coupled poly(guanidinium ionic liquid) interface-free 2D monolithic material has been developed for the separation of multiple types of glycoproteins. Boronate-functionalized graphene, as a preconcentration segment, is located at the inlet of capillary column, which was synthesized according to ref 23. Close to the preconcentration segment, poly(guanidinium ionic liquid) material acts as separation segment that was synthesized by modifying guanidinium ionic liquid onto the poly(N,N-methylenebis(acrylamide) (MBA)-*co*-methacrylic acid (MAA)) monolithic material via the reaction between the $-NH_2$ of the ionic liquid and the $-COOH$ of MAA. The applicability of this system was demonstrated by preconcentrating and separating standard glycoproteins in capillary electrochromatography (CEC).

EXPERIMENTAL SECTION

Instrumentation. Scanning electron microscopy (SEM) micrographs of the monolithic material were obtained on a SU-1510 SEM (Hitachi, Japan). The elemental (C, H, O, and N) contents of the resultant monolithic material were determined on Vario MACRO cube (ELEMENTAR, German) by using a TCD detector. The X-ray photoelectron spectroscopy (XPS) analysis experiment was carried out on an XPS PHI1500VersProbe (ULVAC-PHI, Japan). Adsorption/desorption analysis were carried out using a nitrogen surface area analyzer (TriStar 3000, Micromeritics, Georgia, USA). The separation performance of the resultant interface-free 2D monolithic material was evaluated on a P/ACE MDQ CE system (Beckman-Coulter, California, USA) equipped with a UV detector.

Chemicals and Materials. 1-Vinyl-3-octylimidazolium chloride ($ViOcIm^+Cl^-$) and 1-aminopropyl-3-methylimidazolium bromide ($ApMeIm^+Br^-$) were purchased from Lanzhou Institute of Chemical Physics (Lanzhou, China). Thiol graphene (TG, 500 m^2/g) was purchased from Nanjing Jcnano Tech Co., Ltd. (Nanjing, China). (The structure information for the thiol graphene sheets is shown in the Supporting Information.) γ-Methacryloxypropyltrimethoxysilane (γ-MAPS), ethylene dimethacrylate (EDMA), N,N′-dicyclohexylcarbodiimide (DCC) and N,N-dimethylformamide (DMF) were obtained from Sigma (St. Louis, MO, USA). 4-Vinylphenylboronic acid (VPBA) was purchased from Alfa Aesar (Ward Hill, MA, USA). MBA and MAA were purchased from Bio Basic Inc. (Shanghai, China) and Tokyo Chemical Industry (Shanghai, China), respectively. The free radical initiator 2, 2-azobis(isobutyronitrile) (AIBN, 99%) was obtained from Tianjin Chemical Reagent Factory (Tianjin, China) and recrystallized from ethanol before use. 1,4-Butanediol (BDO) was purchased from Kangkede Technology Co., Ltd. (Tianjin, China). Bovine serum albumin (BSA), bovine hemoglobin (BHb), myoglobin (MB), cytochrome c (Cyt c), lysozyme (Lyz), horseradish peroxidase (HRP), ovalbumin (OVA), transferrin (TF), ribonudease A (RNase A), and alpha fetoprotein (AFP) were purchased from Sangon BioTech Co., Ltd. (Shanghai, China). Fused-silica capillary (100 μm i.d., 375 μm o.d.) was purchased from the Yongnian Optic Fiber Plant (Hebei, China). Healthy human serum sample was purchased from Shanghai Fankei Biological Technology CO., Ltd. (Shanghai, China). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Kangkede Technology Co., Ltd. (Tianjin, China). All of the other chemicals were of analytical grade or higher.

Preparation of the Boronate-Functionalized Graphene-Coupled Poly(guanidinium ionic liquid) Interface-Free 2D Monolithic Material. To covalently anchor the polymer to the capillary wall, the fused-silica capillary was pretreated sequentially by 1.0 mol/L NaOH for 12 h, H_2O for 30 min, 1.0 mol/L HCl for 12 h, H_2O for 30 min, and MeOH for 30 min to activate silanol groups. Then, the inner wall of the pretreated capillary was further vinylized with a solution of 50% (v/v) γ-MAPS in MeOH at 40 °C for 12 h.

Preparation of the boronate-functionalized graphene-coupled poly(guanidinium ionic liquid) interface-free 2D monolithic material involved four procedures: (1) The preconcentration segment was prepared in a single step via Michael addition reaction and radical polymerization. Details are as follows: TG was dissolved in a mixed solvent of DMF (1.0 g) and BDO (0.4 g) under ultrasonication for 2 h to form a homogeneous dispersion (0.3 mg mL^{-1}). Then, $ViOcIm^+Cl^-$ (0.15 g), VPBA (0.025 g), EDMA (0.12 g), and AIBN (0.005 g) were added and sonicated to obtain a homogeneous solution. The mixture was manually injected into the vinylized capillary from the inlet with the effective length of 14 cm by a syringe. After both ends were sealed with silicone rubbers, the capillary was incubated in 70 °C water bath for 12 h. The resultant monolithic material was washed with DMF and

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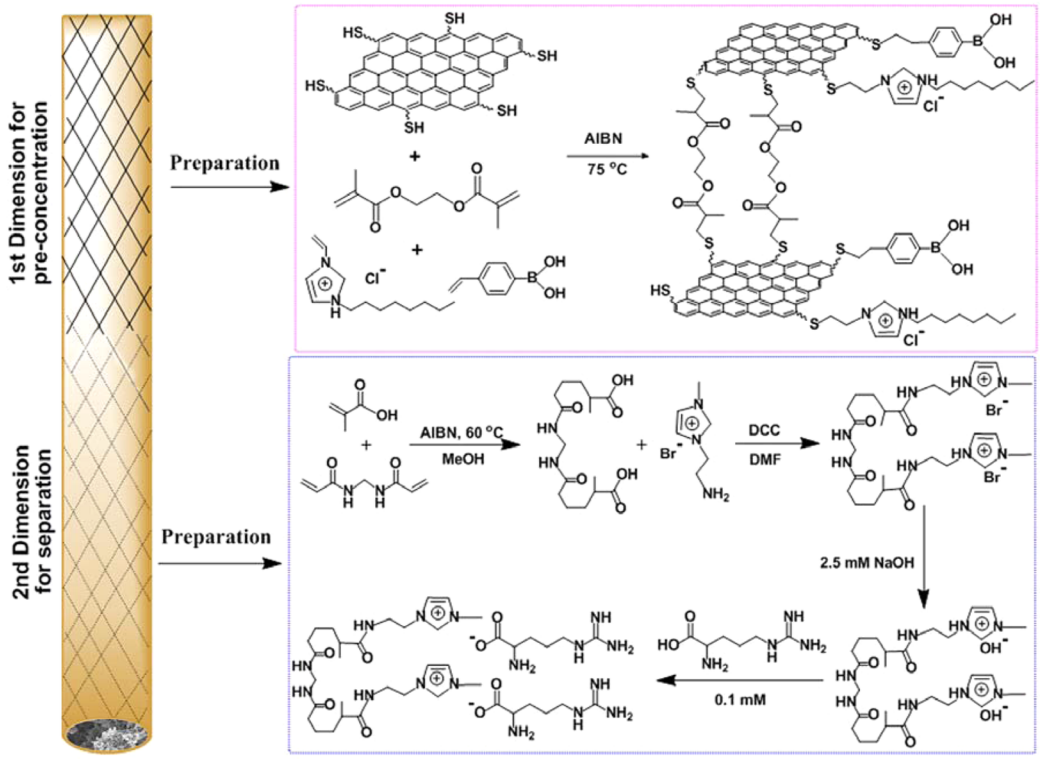

Figure 1. Scheme of the boronate-functionalized graphene-coupled poly(guanidinium ionic liquid) interface-free 2D monolithic material preparation.

MeOH to remove any unreacted monomers and porogen, and then dried with nitrogen for further use. (2) The separation segment was synthesized by a prepolymerization solution that was composed of MeOH (1.2 mL), MBA (0.1 g), MAA (50 μL), and AIBN (0.004 g). After sonicating, the homogeneous prepolymerization solution was manually introduced into the capillary from the outlet (the end closest to the detection window). Both ends of the capillary were sealed with rubber, and the separation segment with 18 cm length was incubated in a water bath at 60 °C for 12 h. The resultant interface-free 2D monolithic column was then flushed with MeOH to remove any unreacted monomers and porogen. (3) Preparation of the ApMeIm$^+$Br$^-$ modified monolithic material was as follows. ApMeIm$^+$Br$^-$ (0.036 g) and DCC (0.038 g) were dissolved in 2 mL of DMF; the mixture was manually pumped through the monolithic column at room temperature for 12 h to modify the separation segment. (4) To obtain guanidinium-grafted monolithic material, anion exchange was carried out in the column (according to the procedures described in the literature)[43] by pumping 2.5 mM NaOH solution and 0.1 mM arginine solution at 5 μL min^{-1} for 4 and 24 h, respectively. The total length of the 2D monolith was 42 cm with the effective length of 32 cm. Figure 1 shows the preparation procedure of the current interface-free 2D monolithic material.

For comparison, single preconcentration monolith, single separation monolith, and ApMeIm$^+$Br$^-$-grafted 2D monolith were also prepared in the same way and evaluated by the standard protein mixtures.

Typical Separation in the Interface-Free 2D Monolithic Material. Prior to operation, the monoliths were preconditioned by rinsing with running buffer for at least 30 min and then equilibrated at a low voltage (−5 kV) in CEC until a stable current was obtained. Typical two-step separation was conducted as follows: (1) After injecting the standard protein mixtures, glycoproteins were captured on the first dimension under the high pH condition. (2) Through electrolyte switch, glycoproteins were released from the first dimension and then moved into the second dimension for separation under the low pH condition. Separation electrolytes for preconcentration and separation were 30 mM phosphate (pH 5.0) and 30 mM phosphate (pH 3.0), respectively. Electrolyte switch was set at 30 min after injection when nonglycoproteins were eluted completely.

RESULTS AND DISCUSSION

Preparation of the Boronate-Functionalized Graphene-Coupled Poly(guanidinium ionic liquid) Interface-Free 2D Monolithic Material. The current interface-free 2D monolith is composed of two segments. For the separation segment, MBA, usually used to prepare protein adsorption materials,[44,45] was selected as the cross-linker. The guanidinium group (pK_a 13.6) can stabilize two parallel hydrogen bonds over a wide pH range. In nature, enzymes often bind anionic substrates by using the guanidinium-containing side chain of arginine in their active sites.[46] To modify the guanidinium group onto the separation segment, MAA was selected as the functional monomer, which allowed modification of the guanidinium group onto the separation segment via the reaction of between $-NH_2$ of ionic liquid and $-COOH$ of MAA.

Considering the hydrophilicity of MAA and MBA, MeOH, and H_2O were selected as solvents to investigate the effects on the morphology of the resultant monolith. As shown in Figure S3, when H_2O was used as solvent, the capillary column was almost empty. This might be attributed to the low solubility of MBA in pure H_2O. In contrast, the MeOH system produced a uniform pore structure easily attached to the inner wall of the capillary. Furthermore, the amount of MAA and MBA was investigated from 0.03 to 0.08 g and from 0.06 to 0.12g, respectively. (Details are shown in Table 1.) The results showed that an increase in the amount of MAA or MBA led to a reduction in the permeability of the monolith, whereas lower content of MAA resulted in a reduction in the electroosmotic

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Table 1. Effects of Synthesis Parameters on Separation Monolith in Segment B

monolith	H_2O (g)	MeOH (g)	MAA (g)	MBA (g)	morphology	back pressure (MPa)[a]
1	1.2	0	0.05	0.03	inhomogeneous	
2	0.6	0.6	0.05	0.06	inhomogeneous	
3	0	1.2	0.05	0.1	homogeneous	2.5
4	0	1.2	0.08	0.1	homogeneous	5.8
5	0	1.2	0.03	0.1	homogeneous	3.1
6	0	1.2	0.05	0.12	homogeneous	6.4

[a]Flushed with methanol. Flow rate, 2 μL/min; column length, 18 cm.

flow (EOF) for CEC. Thus, 0.05 g of MAA and 0.1 g of MBA were appropriate.

The length of preconcentration and separation segments was optimized. For the preconcentration monolith, a short preconcentration monolith resulted in poor enrichment effect for glycoproteins, whereas a too-long preconcentration monolith led to high back pressure. A 14 cm length of preconcentration monolith was selected for further experiments. The effect of the length of separation monolith on the separation performance was also investigated. As shown in Figure 2, with the length of separation monolith increasing

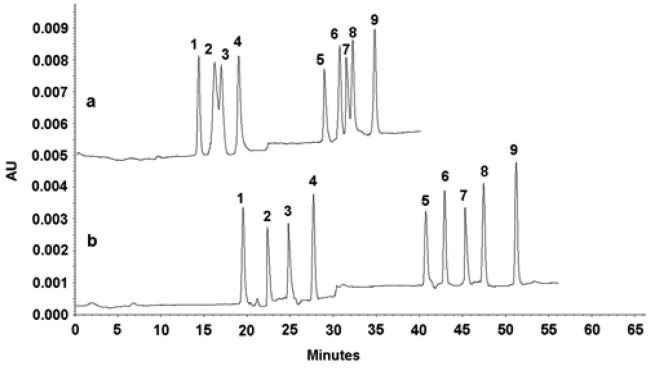

Figure 2. Effect of the length of separation monolith on the separation performance: (a) 10 cm and (b) 18 cm. CEC conditions: mobile phase, pH 5.0, 30 mM Na_2HPO_4–H_3PO_4 buffer was adopted for 0–30 min then switched to 30 mM phosphate (pH 3.0) at 30 min; applied voltage, −8 kV; injection, 5 psi for 25 s; and detection wavelength, 210 nm. Analytes: 1, BSA; 2, Mb; 3, Cyt c; 4, Lyz; 5, OVA; 6, TF; 7, AFP; 8, HRP; and 9, RNase A.

from 10 to 18 cm, the resolution of AFP and HRP improved from 1.33 to 1.75. Thus, an 18 cm length of separation segment was selected.

Characterization of the Boronate-Functionalized Graphene-Coupled Poly(guanidinium ionic liquid) Interface-Free 2D Monolithic Material. For the preconcentration segment, boronate-functionalized graphene had been characterized systematically in our previous work.[23] The TEM image (Figure S4a) confirmed the presence of TG sheets in the monolithic matrix. A typical S 2p XPS spectrum (Figure S4b) was shown at 162 eV, and the S% (atm %) was determined to be 0.12%, which indirectly reflected the content of thiol graphene sheets in the monolith. In this study, the 2D monolithic material was synthesized by a two-step process. To avoid the creation of blockage between the two segments, a partial thermal polymerization method was employed as a previous report described.[22] Figure 3 shows the morphologies

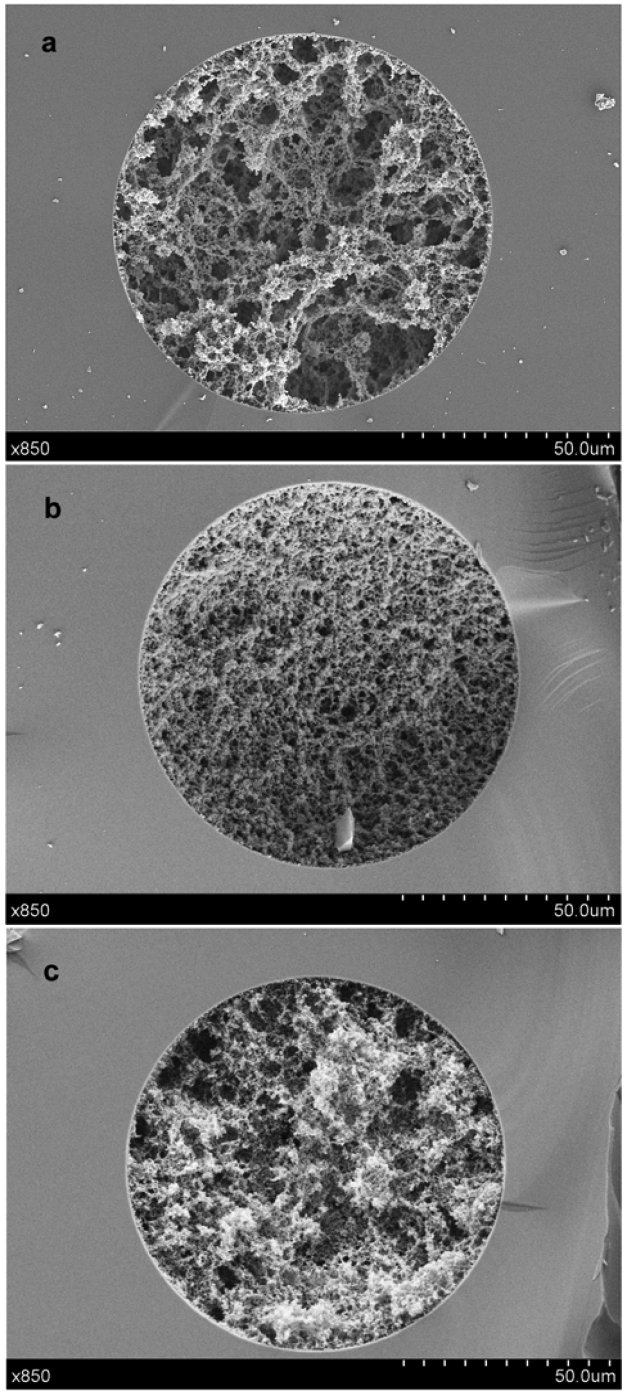

Figure 3. SEM images of the 2D monolithic material at 850× magnification of the (a) preconcentration monolith, (b) separation monolith, and (c) border of the two segments.

of the preconcentration monolithic matrix, separation monolithic matrix, and the border of the two segments, respectively. The results showed that uniform pore structure attached well to the inner wall of the capillary, and no blocking appeared at the border of the two segments. For demonstrating the successful graft of $ApMeIm^+Br^-$ onto the separation monolithic matrix, EOF analysis in the pH range of 2.0–11.0 was employed (Figure 4). The monolith prior to grafting $ApMeIm^+Br^-$ produced positive EOF (from anode to cathode) generated by the negatively charged −COOH of MAA; after grafting of $ApMeIm^+Br^-$, the EOF was reversed because of the positive

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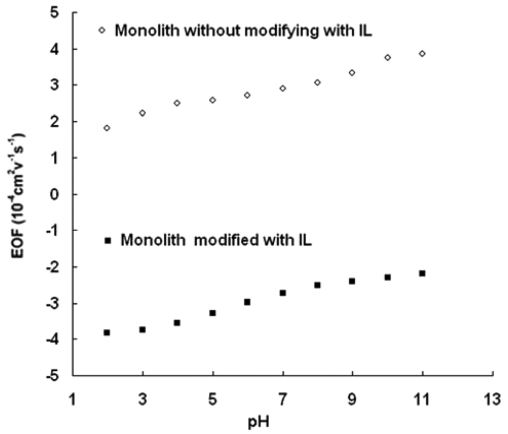

Figure 4. Influence of mobile-phase pH on the EOF of (a) poly(MBA-*co*-MAA) monolith and (b) ApMeIm$^+$Br$^-$-grafted poly(MBA-*co*-MAA) monolith. CEC conditions: 30 mM phosphate buffer (H_3PO_4–Na_2HPO_4 for pH 2.0–9.0, Na_2HPO_4–NaOH for pH 9.0–12.0) containing 30% (v/v) ACN at various pH (2.0–12.0); separation voltage, −10 kV; injection, 0.5 psi for 5 s; detection wavelength, 214 nm; and EOF marker, thiourea.

charges of the imidazolium ring. The alternation of the EOF direction could serve as a support of successful modification of ApMeIm$^+$Br$^-$. XPS analysis was employed (Figure S5), and a typical Br 3d XPS spectrum was shown at 67.93 eV. The Br% (atm %) was determined to be 1.47%, whereas after the anion-exchange procedure, the characteristic spectrum was absent. Furthermore, the C%, H%, N%, and O% (w/w) of these monolithic materials were determined by elemental analysis. From Table 2, it can be found that the N content in

Table 2. C%, H%, N%, and O% (w/w) of the Monoliths

monolith[a]	C	H	N	O
1	54.95	6.63	12.05	26.35
2	49.34	6.51	15.69	12.06
3	53.60	7.60	22.47	15.46

[a]Monoliths 1–3 are poly(MBA-*co*-MAA) monolith, ApMeIm$^+$Br$^-$-modified monolith, and guanidinium-grafted monolith, respectively.

guanidinium ionic liquid grafted monolithic material was enhanced from 12.05 to 22.47% compared with ApMeIm$^+$Br$^-$-grafted monolithic material. These results demonstrated the formation of poly(guanidinium ionic liquid) monolithic material. The specific surface area and the average pore size of the resultant material were measured by the Brunauer–Emmett–Teller method. As shown in Figure 5, the specific surface area and the average pore size were 40.24 m^2/g and 17.18 nm, respectively.

Preconcentration and Separation of Glycoproteins by the Interface-Free 2D Monolithic Material. The preconcentration of glycoproteins by the resultant monolithic material is based on the fact that reversible covalent ester bonds are formed between boronic acid functional groups and glycoproteins in the basic media and are then dissociated in the acidic media. In our previous work, the preconcentration material was systemically characterized and exhibited good recognition property to HRP and OVA.[23] Herein, its recognition property of AFP, TF, and RNase A was further investigated. As shown in Figure S6, the preconcentration monolithic material exhibited good retention for five

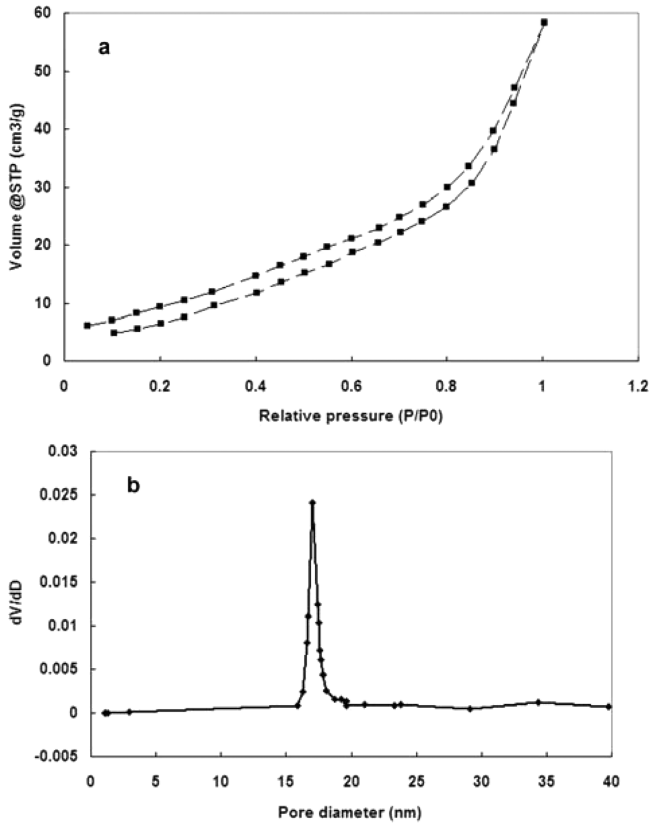

Figure 5. (a) N_2 adsorption–desorption isotherms and (b) corresponding pore size distributions of the poly(guanidinium ionic liquid) monolithic material.

glycoproteins, whereas the separation segment could not specifically recognize glycoproteins. Furthermore, it could be seen in Figure 6 that the peak height of all these proteins significantly increased with the injection time increasing from 15 to 55 s. These results indicated that the current 2D monolithic material exhibited excellent enrichment for glycoproteins. For the purpose of protein separation, the pH application range is a significant parameter. Herein, the binding capacity for glycoproteins was determined using frontal analysis with pH ranging from 5.0 to 9.0. It could be seen from Figure S7 that the binding capacity for the five glycoproteins was limited in acidic media. With the buffer pH increasing, the binding capacity increased obviously, which is in good agreement with the theoretical expectation that basic media favors the formation of covalent ester bonds between *cis*-diols and boronic acid functional groups. For disease markers TF and AFP, the binding capacity of the current preconcentration material could reach 10.16 and 10.47 mg/g at pH 9.0.

The separation performance of the developed 2D monolithic material was evaluated by CEC. For comparison, single separation monolith, single preconcentration monolith, and ApMeIm$^+$Br$^-$-grafted 2D monolith were also evaluated using the standard protein mixtures. As shown in Figure 7, neither the single preconcentration monolith nor the single separation monolith could baseline-separate these proteins. ApMeIm$^+$Br$^-$-grafted 2D monolith could isolate the glycoproteins from other nonglycoproteins, but the separation performance was not satisfactory. When the Br$^-$ was replaced by guanidinium, the separation efficiency of the monolithic material was greatly improved, and baseline separation of five glycoproteins and

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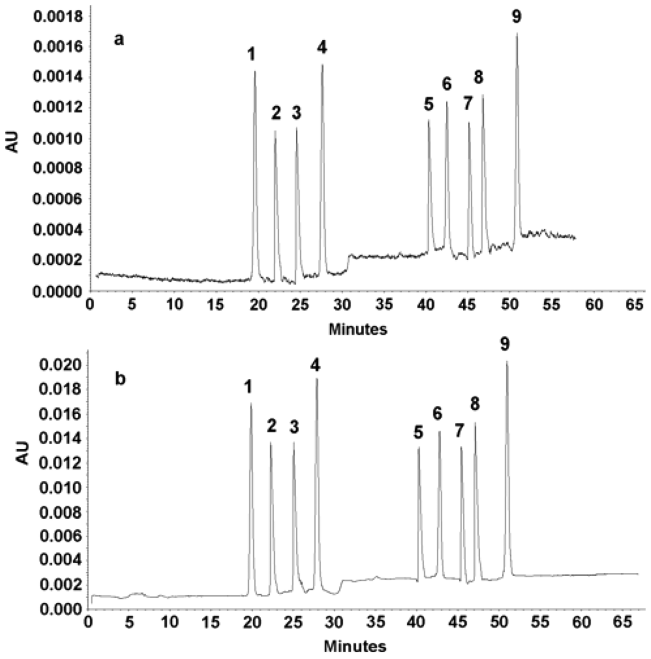

Figure 6. Effect of sample injection time on the separation efficiency of nine proteins. Injection at (a) 5 psi for 15 s and (b) 5 psi for 55 s. Other conditions and peak identification were the same as those in Figure 2.

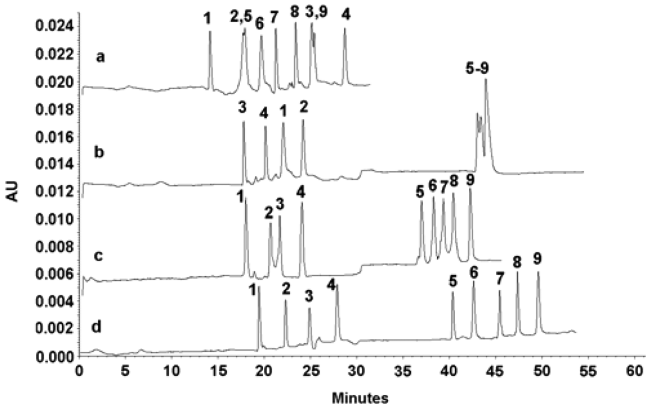

Figure 7. Separation of standard protein mixtures on (a) single separation monolith, (b) single preconcentration monolith, (c) ApMeIm$^+$Br$^-$-grafted 2D monolith and (d) guanidinium ionic liquid grafted 2D monolith. CEC conditions and peak identification were the same as those in Figure 2.

four nonglycoproteins was achieved. The high separation efficiency could be largely attributed to the strong biological activity and the unique molecular recognition function of guanidinium, the adjustability of the three nitrogen atoms, and the interaction between the guanidinium and aromatic amino acids (tryptophan, phenylalanine, and tyrosine) existing on the surface of proteins.[40,42]

The run-to-run and column-to-column repeatability of the 2D monolith were characterized by relative standard deviations (RSD) for the retention time of different proteins. The results were in the range of 0.92−1.85% (n = 5) and 3.5−6.9% (n = 5), respectively, demonstrating the good repeatability of the 2D monolith for the separation of glycoproteins.

CONCLUSIONS

In this study, we have successfully developed a novel boronate-functionalized graphene-coupled poly(guanidinium ionic liquid) interface-free 2D monolithic material. The developed 2D system allowed online glycoprotein concentration and separation to be sequentially implemented. Especially for disease markers TF and AFP, the binding capacity of the preconcentration segment could reach 10.16 and 10.47 mg/g at pH 9.0. Furthermore, the grafting of guanidinium onto the separation segment greatly improved the separation efficiency and allowed the baseline separation of multiple types of glycoproteins including OVA, HRP, TF, RNase A, and AFP. These results demonstrate the potential of the current 2D materials in clinical diagnosis. However, in this work, the major limitation of the proposed 2D system is lack of scalable automation fabrication technique. Furthermore, to take full advantage of the 2D system, more effort needs to be made to improve the sensitivity. In addition, the separation of other diol-containing small molecules, such as nucleosides and catechol derivatives, is also of great significance; thus, novel multidimensional separation systems will be developed by our further study.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b07668.

Preparation of the boronate-functionalized graphene monolithic material, X-ray photoelectron spectroscopy (XPS) analysis for demonstrating the successful graft of ionic liquid, comparison on the recognition performance between the preconcentration segment and separation segment, and binding capacity of boronate-functionalized graphene for glycoproteins. (PDF)

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Notes

The authors declare no competing financial interest.

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