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3D cryogel composites as adsorbent for isolation of protein and small molecules

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ABSTRACT

A green and promising sample pretreatment method was successfully established, which efficiently isolated proteins and small molecules in human serum. This method was achieved based on the multifunctional polymer, cryogel, as a solid phase extraction (SPE) monolith easily equipped in a syringe. The cryogel (pDC/GO-DE) was composed of diallyldimethyl ammonium chloride (DC) and 2-hydroxyethyl methacrylate (HE), which was further modified with graphene oxide (GO) and N-diethylethanamine hydrobromide (DE). Various proteins, including bovine serum albumin (BSA), lysozyme (Lys), γ-globulins, immunoglobulin G (IgG), transferrin, small molecules (ribavirin, adenosine, ofloxacin, estriol, rutin, amoxicillin, ibuprofen, 1-methyl-3-phenyl-propylamine, and benzylamine) and their mixtures were successively studied as model analytes to evaluate the new material and demonstrate the isolation mechanism, which was mainly dependent on mixed-mode ion-exchange and the hybrid hydrophobicity-hydrophilicity property of pDC/GO-DE cryogel. Moreover, the three-dimensional macroporous structure contributed to the underlying size-selective isolation. When 10 times diluted human serum was used as the sample, more than 95% of proteins were adsorbed within 10 min under physiological conditions, and the interference matrix in serum was also efficiently reduced. After recycling three times, the extraction ratio of proteins in human serum was still higher than 90%. When four small molecules (camptothecin, ribavirin, 1-methyl-3-phenylpropylamine and ofloxacin) were added to blank human serum, their recoveries were within 65.6–81.8%, and were comparable to those obtained by protein precipitation method (63.7–83.2%).

1. Introduction

In recent years, scientific and public attention has been focused on human health [1], diet safety [2], and environmental pollution [3]. It is necessary to develop valid methods to assess the target analytes in these fields in order to provide accurate and reliable information. However, during the development of analytical methods, negative effects frequently occur; for example, the use or generation of large amounts of hazardous chemical solvents resulting in harm not only to the natural environment but to laboratory personnel. Therefore, 12 Green Analytical Chemistry (GAC) principles were proposed and have been increasingly implemented [4–6].

Sample pretreatment is a critical and essential step in chemical analysis to ensure precise and accurate analytical results, especially in the analysis of biological samples with complex matrices containing salts, small organic molecules, abundant proteins and lipids [7,8]. Protein precipitation is the most widely used sample pretreatment method for isolation of proteins before instrumental analysis for the determination of a drug, a metabolite or a biomarker in complex biological samples [9,10]. This method uses the harmful organic solvents acetonitrile or methanol, and can even cause a series of issues such as instrument contamination, chromatographic column damage and signal interference if proteins are incompletely precipitated [11]. Furthermore, the denatured proteins are difficult to purify for other usage. Therefore, in response to these problems and under the terms of the GAC principles, various strategies such as solid phase microextraction (SPME) [12–14] have been developed. These new methods show a trend toward miniaturization and/or possible automation, environment-friendly and higher extraction efficiency [15–17]. For example, Guibin Jiang [18] reported a size-selective enrichment method for hazardous compounds on an ordered mesoporous carbon to avoid protein precipitation. Rafael Brüschweiler [19] used nanoparticle-assisted silica to remove proteins in human serum for metabolomics studies by NMR. However, it should be noted that these functional materials were prepared using a large amount of organic solvents. The substitution or reduction of these harmful solvents and simplification of the synthesis

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process would be beneficial.

Monolithic cryogels are attractive hydrogels, and are prepared by copolymerization of monomers and a crosslinker in water below freezing point. Following cryogelation, ice crystals thaw at room temperature and the cryogel is formed. Water acts as both a solvent and a type of porogen [20]. These monolithic cryogels exhibit several superior properties such as a continuous three-dimensional (3D) polymer network, large and interconnected pores (10–100 μm), high porosity and toughness, good permeability and hydrophilicity, and varied shapes [21,22]. Nevertheless, they also have some vital shortcomings that restrict their application, e.g. few functional monomers (usually, allyl glycidyl ether) crosslinkers (usually, N,N'-methylenebisacrylamide) with high activity, good water solubility, and low specific surface resulting in low binding capacities. To address these difficulties, some strategies have been successfully developed such as the synthesis of new functional monomers [23] or crosslinkers [24], cryogels with functional particles embedded during cryogelation [25,26] or immobilization of these functional particles on the pore surface of cryogels [27], functionalization of cryogels by chemical grafting [28], changing water to another solvent (e.g. cyclohexane) to dissolve hydrophobic functional monomers [29], and changing the polymerization irradiation system [30]. However, most of these improved fabrication approaches unavoidably use organic solvent and have complicated or tedious operations. Therefore, it is a requirement and a challenge to fabricate cryogels using a green method.

Cryogels have diverse applications ranging from immobilization of biomolecules, chromatographic separation or capturing biomolecules to environmental separation [31–34]. To our knowledge, the isolation of proteins and small molecules and the simultaneous reduction of matrix interferences in serum samples have not yet been reported.

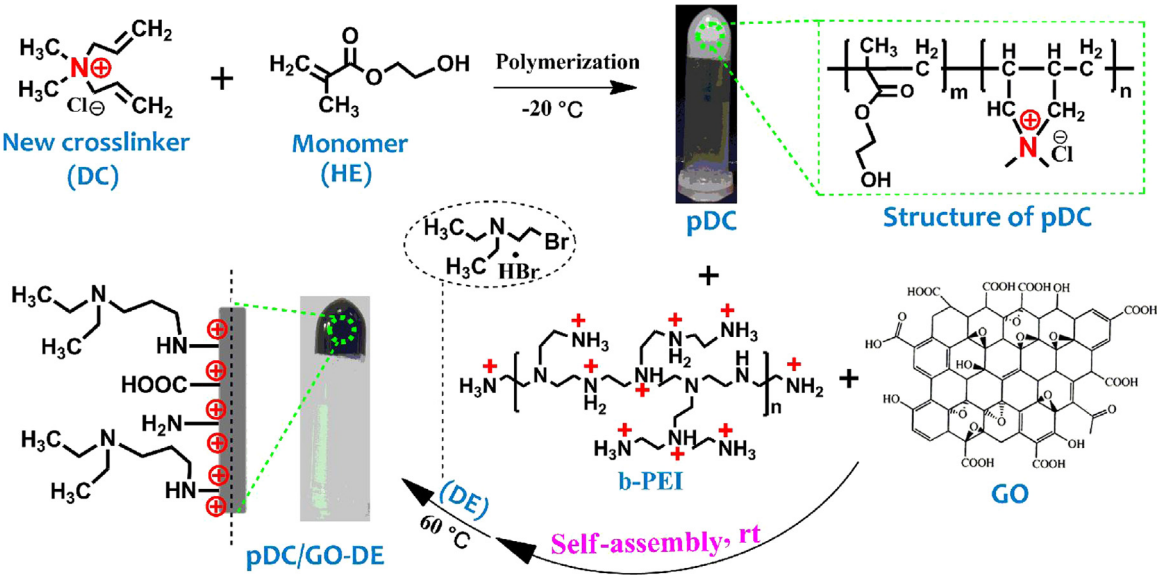
In this study, a simple strategy for the construction of a multifunctional cryogel (pDC/GO-DE) was developed (Scheme 1). In order to realize large-scale batch preparation of monolithic cryogels with small volume, 2.0 mL centrifuge tubes were chosen as the mold. The water-soluble diallyldimethyl ammonium chloride (DC) containing the quaternary ammonium group was chosen for the first time as the new crosslinker to prepare the positively charged 3D ionic cryogel (pDC). Subsequently, negatively charged amphiphilic nanosheets of graphene oxide (GO) and positively charged branched polyethyleneimine (b-PEI) were self-assembled in the pores of pDC cryogel to adjust their hydrophilicity, which provided the ion-exchange function and hybrid hydrophobicity-hydrophilicity property. The pDC/GO cryogel was further functionalized with N-diethylethanamine hydrobromide (DE) to improve the adsorption performance. Water was the only solvent used and the cryogels were prepared using mild reaction conditions. The multifunctional cryogel (pDC/GO-DE) was used as a SPE monolithic adsorbent and equipped in a syringe to establish a green sample pretreatment method, which efficiently isolated proteins and small molecules, and reduced other interference matrices in human serum sample.

2. Results and discussion

2.1. Characterization

The SEM images (Fig. 1**(a)**) show that the basic pDC cryogel had a continuous 3D polymer network and an interconnected macropore (ranging from 10 to 100 μm). In the pDC/GO-DE cryogel (Fig. 1**(b)-(c)**), 2D nanosheets of GO appeared on the macropore surface, and the macropore structure was maintained. Digital photos of the monolithic cryogel are shown in Supplemental information (Fig. S2). After self-assembly, the color changed from white to black, and the internal section of the pDC/GO-DE cryogel was also black due to the good electrostatic interaction. The specific surface area of the cryogel is listed in Table S3. The specific area of pDC/GO-DE (S_{BET} or S_{BJH}) was approximately two times or eight times higher than those of the basic pDC cryogel. The characteristics of GO prepared using Hummers' method are shown in Supplemental information (Fig. S3), and indicated that GO was a single layer, a single crystal form and had good hydrophilicity, which was helpful for GO self-assembly on cryogel composites.

Fourier-transform infrared spectroscopy (FTIR) and element analysis further confirmed that the cryogel was successfully fabricated. The FTIR spectra of GO, pDC and pDC/GO are shown in Fig. S4(A). The adsorption bands at 3434 cm^{-1} (-OH stretching vibrations), 2943 cm^{-1} and 2886 cm^{-1} (methyl vibration), 1729 cm^{-1} (the carbonyl group), 1451 cm^{-1} (C-H asymmetrical deformation vibration due to N-CH_3) and 1026 cm^{-1} (quaternary ammonium group) showed that the novel pDC cryogel containing quaternary ammonium was successfully prepared. In addition, both the original GO and the pDC/GO-DE cryogel showed the representative C=C (aromatic) stretching vibration at 1661 cm^{-1}, which demonstrated the successful self-assembly of GO on the basic cryogel. As pDC/GO and pDC/GO-DE contained similar substituted amino groups, it was difficult to use FTIR analysis to accurately illustrate the successful fabrication of pDC/GO-DE cryogel with b-PEI and DE.



Scheme 1. Schematic illustration for preparation of the multifunctional cryogel composites (pDC/GO-DE).