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Bovine Serum Albumin Pre-concentration Using Glycidyl Methacrylate-co-ethylene Glycol Dimethacrylate-co- (2-diethylamino) ethyl Methacrylate Monolith

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Abstract

Abstract. Solid Phase Extraction (SPE) is one of the important as tool in the process of Pre-concentration of some biological compounds. such as proteins based on a solid support phase that interacts with the material to be Pre-concentration and then displaces that material with a suitable solvent in the lowest volume of the solvent. monolithic sorbent was prepared using a homogeneous mixture of two monomers Glycidyl methacrylate (GMA) and (2- diethylamino) ethyl methacrylate (2DEAMA) . In the presence of a cross-linker substance Ethelyn glycol dimethacrylate (EDMA) using a suitable solvent (porogenic solvent) consisting of 1-propanol and 2-butanol and a Initiator (2,2-dimethoxy-2-phenyl acetophenone) to the polymerization proces (photoelectric) . This monolithic was prepared inside a borosilicate tube. Evaluation of monolith performance was carried out by extraction of Bovine serum albumin standard protein and the extraction recovery was in the range (89 %) .

Keywords: (2- diethylamino) ethyl methacrylate; solid-phase sorbent; glycidyl methacrylate; extraction

1. Introduction

Solid-phase extraction (SPE) is the most widely used method and alternative for the extraction, clean-up, concentration of analytes materials as well as the recoveries achieved and fractionation of organic compounds from several samples[1].

At present, there are many types of solid phase extraction adsorbents that have been used in accordance with the analytical objective. Examples of these adsorbents are (1) silica-based polymers (2) co-polymers or cross-linked polymers dependent on the properties of the adsorbent, which can be hydrophobic, hydrophilic or exchange Ionic, (3) mixed mode copolymers [2]. Where extraction is usually used for isolation, pre-concentration, modification of sample properties prior to separation, and identification of compounds by means of techniques based on Chromatography and others [3]. One of the main advantages of solid-phase extraction is that the analytes sorbed onto the SPE column, cartridge,disc which may It she prevented from decomposing and thus be stored for a certain duration without any change in its identity or concentration[1,4] . The principle Of SPE solid phase extraction is based on the sample spread between the liquid mobile phase and the solid phase. As the materials to be analytes move from the liquid phase to the active sites of the solid phase or the adsorbent material [5]. Extraction occurs when the material to be analytes has a greater affinity for the solid phase than the sample mixture so that the material to be analytes is retained and concentrated on the solid phase (the adsorption step). Then all the materials to be analytes that have been detained and concentrated on the solid phase are recovered by eluting them with suitable solvents [6], Where the nature of the solid phase must be known (hydrophobic, hydrophilic, ion exchange) can drive the retention mechanism of the extraction process[7].

Monolithic materials are widely utilized in the separation of both biological and chemical molecules,

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for example, proteins, peptides, nucleic acids, and synthetic polymers[8]. These monolithic materials are prepared using a homogeneous or heterogeneous mixture within a continuous single rod with a porous structure made of micropores, macropores and mesopores [9-11]. The macropores (>50 nm) work as flow-through channels because they allow to the solvent to pass throw through the monolithic materials, whereas mesopores (2-50 nm)for increasing the surface area to provide efficient interaction with molecular analytes. Micropores (< 2 nm) It has a large capacity to absorb solutes in the mobile phase[12-14]. therefore, monolithic can be used with high flow rates with moderate back pressures[15]. This work aims to prepare a solid-phase sorbent based on a monolithic polymer that was investigated using in situ polymerization inside borosilicate tube for pre-concentration of protein.

2. Experimental

2.1. Materials

Each chemical materials are used in this research highly purity from: 3- (trimethoxysilyl) propylmethacrylate (γ-MAPS), glycidyl methacrylate, ethylene glycol dimethacrylate, (2- diethylamino) ethyl methacrylate, protein, 2,2-dimethoxy-2- phenyl acetophenone, acetonitrile, sodium phosphate dibasic, Acetone, sodium hydroxide, hydrochloric acid 1-propanol, 2-butanol, water. Stainless steel union 1/8" to 1/16" adapter (Kinesis, Cambs, UK), polyetheretherketone (PEEK) tubing (Thames Restek Ltd., Saunderton, UK), borosilicate tube (1.5 mm I.D and 3.0 mm O.D) (Smith Scientific, Kent, UK) , microtight adapter (Kinesis, Cambs, UK), glass syringe (SGE, Kinesis, Cambs, UK) were used for the preparation of the SPE sorbent.

2.2. Instrumentation

The instruments used were FT-IR 380 spectra (Bruker), UV light lamp (Iraq),UV-Vis spectrophotometer double-beam (UV-1700, Japan) syringe pump (Bioanalytical System Inc., USA), stirrer VWR (West Chester, PA, USA).

2.3. Procedure

2.3.1. Fabrication of the monolithic materials Silanization step [16].

The inner surface of the tube (borosilicate) was washed with acetone and water. The borosilicate tube was then activated by washing the inner surface of the tube by pumping in a NaOH solution (0.2 M) at a speed of 5.0 µl min-1 for 1 hour using a syringe pump. After that, HCL solution was used to wash the tube at (0.2 M) at 5.0 µL min-1 for 1 hour using a syringe pump, and then wash with water and ethanol to convert the inner tube surface into (Si-OH) groups. in final was silanized the tube using 3-(trimethoxysilyl) propylmethacrylate (γ-MAPS) 20% in ethanol at pH of 5.0 through pumping at 5.0 µL/min for 1 hour. The tube were dried with nitrogen gas and left for 12 hour; then, the tube will be ready for in-situ polymerization.

2.3.2. In-situ polymerization of the monolith [17].

The monolith was prepared inside a borosilicate tube from a mixture consisting of two monomers of glycidyl methacrylate and 2-(diethylamino) ethyl methacrylate with the ratio of 80:20. The crosslinker used was ethylene glycol dimethacrylate , while 2,2-dimethoxy-2-phenyl acetophenone was used as an initiator (0.1%).The binary porogenic solvent consisted of 2-butanol and 1-propanol was used with the ratio of 40:60, respectively, to dissolve monomers, crosslinker, and initiator. an ultrawave sonicator was used to sonicate the mixture for 10 min. and then was removing the oxygen from the mixture by purging with nitrogen gas for 5 min. The borosilicate tube were filled with the polymerization mixture using a syringe then closed from both sides and exposed to UV light lamp for UV polymerization at 365 nm for anticipated irradiation time (4 min for borosilicate tube). The preparation method was based on Ueki et al [17], with some modifications.

2.3.3. Solid-phase extraction for pre-concentration

The prepared monolithic sorbent was used for pre-concentration of protein. However, the performance of the monolithic sorbent was evaluated using bovine serum albumin of standard. It was prepared by dissolving bovine serum albumin in a solvent consisting of (8 mL of water and 2 mL of acetonitrile) with a weight of 0.006g at room temperature. The next step was loading the protein sample by pumping 10 mL of protein, and then the solid phase sorbent was washed with 2mL of water. The final step was eluting the protein using 2mL acetonitrile.

3. Results and Discussion

3.1. Pre-Concentration of Protein Using monolithic Sorbent that was Fabricated inside the Borosilicate Tube

the monolithic column (GMA-co-EDMA-co-2DEAMA) can be used for the pre-concentration of the proteins Which have high pH value according to the pI of the protein. because the organic monoliths do not suffer from residual silanol interaction problems Therefore, it can be used in a wide pH range[18]. pre-concentration method was used from the methods published elsewhere [19]. The process consisted of several different steps. Initially, conditioning of the sorbent was performed by pumping 2 mL water using syringe pump and discard it, Than and was loading the sample by pumping 10 mL of protein. The next step, the sorbent was washed using 2 mL of water, and Finally was eluting the protein using 2mL of CAN. The flow rate was 10 μL min–1 for all the steps except the protein loading step which was 5 μL min–1 to allow protein to interact with the sorbent. the FT-IR spectra have been studied Using FT-IR technology to characterize the formation of the monolith. From the FT-IR spectrum, it can be see that several main prominent peaks could be used to confirm the formation of the monolithic sorbent in Table (1).

Table 1. The FT-IR data for the main peaks of the monomers and the co-polymer before and after double bond reaction

Monomers/Polymers	C=O (cm^{-1})	C=C (cm^{-1})	Epoxy group (cm^{-1})
GMA [22]	1716.48	1637.10	907.60
2DEAMA [22]	1717.69	1637.40	
EDMA [22]	1717.69	1636.85	
GMA-co-EDMA-co 2DEAMA polymer	1724.19	Disappeared	907.10

In the procedure, the effect of various parameters on the formation of the prepared monolith was investigated

3.2. Effect of irradiation time

The irradiation time was investigated for monolithic column that illustrated in Table (2) to obtain the effective irradiation time that can be formed the monolith properly inside the borosilicate tube to prepare monolithic column that has high surface area and reasonable pore size. therefore the irradiation time was in the range 0.5-6 min.

It can be conclusion from Table 2 the irradiation time is play important role in changes the monomers mixture to the solid polymer, When the irradiation time increases it will form a dense monolith due to formation of a micro porous structure either When using a low irradiation time, this may lead to the formation of less polymerized material inside the borosilicate tube and It will affect the fabricated monolith performance, While when using appropriate irradiation time that gives surface area and reasonable back pressure.

Table 2. The effect of irradiation time on monolith formation

Irradiation time (min)	**Result**
0.5	The monolith did not form
1	The monolith started forming
2	The monolith formed with very low back pressure
3	The monolith formed with low back pressure
4	The monolith formed with suitable back pressure
5	The monolith formed with high back pressure
6	The monolith formed with very high back pressure

3.3. Effect of porogenic solvents

The porogenic solvent play important role in the monolith formation, therefore, using different solvents alongside with 1-propanol that listed in Table (3) was investigated to form the monolith column that has high surface area and reasonable pore size.

Table 3. the types of porogenic solvents and effect in the monolith formation

	Porogenic solvents		Result
1	1-propanol	Methanol	The monolith did not form
2		Ethanol	The monolith started forming
4		2-butanol	The monolith formed with suitable back pressure
5		Hexanol	The monolith formed with high back pressure

3.4. The Morphological Properties of the monolith GMA-co-EDMA-co-2DEAMA

The morphological properties for the monolith GMA-co-EDMA-co-2DEAMA have been investigated using a SEM and BET analyzer. The results showed that the solid phase sorbent has an average surface area of 83.49 m^3 /g . This surface area could provide sufficient interactions between protein and the functional groups on the solid phase (sorbent)

surface. the average pore size was 26.88 nm, due to the micro and mesopores which allows the sample molecules to move through the solid phase (sorbent) with a suitable backpressure. The morphology of the monolith GMA-co-EDMA-co-2DEAMA is shown in Fig. 1.

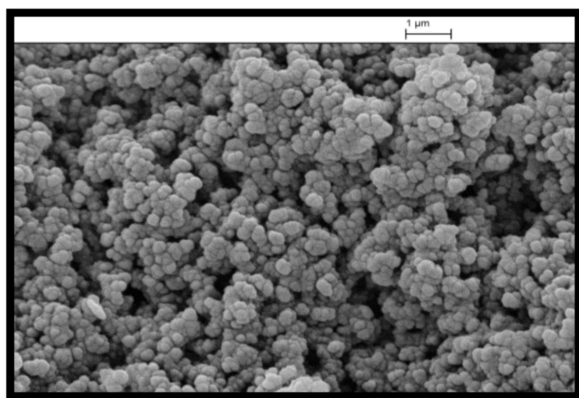

Fig 1. SEM images of the monolith GMA-co-EDMA-co-2DEAMA

3.5. Determination of extraction recovery

Determination of extraction recovery was for pre-concentration the protein by UV-Spectrophotometer system to study the peak area for protein before and after pre-concentration to calculate the extraction recovery (ER). can be calculated using the following equation [20]:

Extraction recovery (%) = (I eluent / I total) x 100

Where (I eluent) is the amount eluted form the sorbent, and (I total) is the amount of protein introduced to the sorbent.

The Bovine serum albumin before and after preconcentration are shown in Fig. 2 and 3.It can be Note from the two figures that the peak intensity after the pre-concentration process increased, which indicates that monolith GMA-co-EDMA-co-2DEAMA sorbent could be used to pre-concentrate, and purify of Bovine serum albumin. The result of the extraction recovery for Bovine serum albumin standard protein was in the range (89 %) .

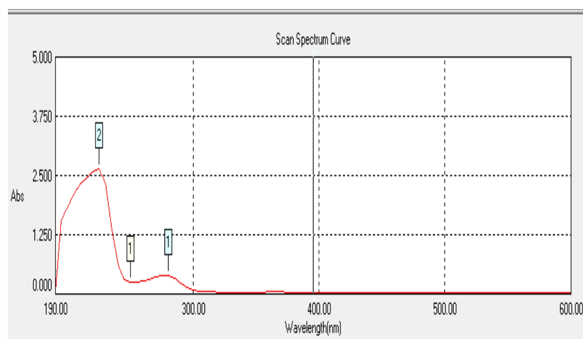

Fig 2. height Peak before to pre-concentration of bovine serum albumin using a UV system

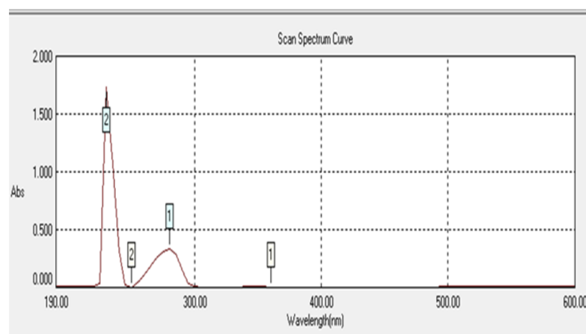

Fig 3. height Peak to after pre-concentration of bovine serum albumin using a UV system

3.6. Evaluation of Monolith Performance in Pre-concentration

The extraction performance for monolithic column was evaluated by calculating the% RSD values of the pre-concentration process for protein using two methods Run-to-Run and Batch-to-Batch method and was evaluated using long-term stability and the reproducibility, run-to-run reproducibility was evaluated through extracting is protein for three times utilizing the same borosilicate tube while Batch-to-Batch reproducibility was calculated by extracting bovine serum albumin for three times using a different borosilicate tubes As shown in the Table 4.

It can be conclusion from Table 4 that the extraction reproducibility using borosilicate tube was achieved because to the acceptable range of RSD% values, which could be because to the little volume of the protein that loaded to the solid phase (sorbent). Consequently, it will improve performance and reduce the extraction error. It was found that the monolithic sorbent (GMA-co-EDMA-co-2DEAMA) could be used for several times because to the stability of the sorbent with the time. Therefore, it could be used for 15 times inside the borosilicate tube.

Table 4. The RSD for the extraction recovery for the, run-to-run and for the batch-to-batch analysis [n = 3]

Method	BSA RSD% n=3
Run-to-Run	3.02
Batch-to-Batch	3.74

4. Conclusion

monolithic sorbent was successfully prepared inside borosilicate tube using UV polymerization method. The final product was used as a solid phase extraction sorbent for the pre-concentration of protein. The results showed the recovery value of bovine

serum albumin is(89 %) and detection limit 6.24×10^{-4} mM.

5. References

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