Original Article

Preparation of Monolithic Chromatographic Column for Propranolol Hydrochloride Determination

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Abstract

Pharmacuticalcal compounds can be separated, identified, and quantified using high-pressure liquid chromatography (HPLC) in analytical chemistry. A monolithic column of glycidyl methacrylate-ethylene dimethcrylate-co-acryl amide was prepared by in-situ co-polymerization inside; a borosilicate tube as a mould (60mm in length) with 1.5mm and 3.0 mm (i.d. and o.d.) for LC separation. Monomers (glycidyl methacrylate and acrylamide) and a cross-linker (ethylene dimethacrylate) were dissolved in a porogenic solvent of 1-propanol, and hexanol is a suitable solvent for monomers and cross-linker. Nevertheless, it is a poor solvent for the prepared monolith. The polymer was formed after 3 min using UV- polymerization at 365 nm. Glycidyl methacrylate's epoxy groups were changed to diol groups by pumping 0.2 M HCl through the column. FT-IR, BET and FE-SEM were utilized to investigate and prove the monolith formation. The monolith was used to determine propranolol hydrochloride in medications.

Keywords: Monolith, HPLC, propranolol hydrochloride, glycidyl methacrylate

1. Introduction

Drug discovery and development labs have implemented various novel technologies to boost sample throughput over the past decade. [1][2]. Separation and identification of specific samples in a mixture can be achieved via chromatography, a technique that relies on the assumption that the components in the mixture have distinct propensities to adsorb onto a surface or dissolve in a solven[3]. Liquid chromatography is a generic term for any chromatographic technique with a liquid mobile phase; samples can be separated when the optimal conditions are applied. Therefore, the sample will react differently to the two phases.[4].

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.04.226

High-pressure liquid chromatography (HPLC) has been one of the crucial techniques in the pharmaceutical industry, biochemistry and analytical chemistry [5][6]. It is one of the most widely used procedures for purification, chemical separations, identification, clinical and pharmaceutical applications [7][8].

Monolith is a Greek word consisting of mono, and lithos, meaning one and stone, respectively. At the same time, geologically, It is definite as a single enormous rock with holes of various sizes and forms [9]. The polymerization mixture must continuously contain a vinylic monomer and across linker with at least two double bonds and inert solvents called porogen[10]. The porogenic solvents should dissolve monomers and cross-linker. In contrast, it does not dissolve the polymeric chains [10-14]

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How to cite this article Alsultani S N, Alkarimi A A, Aljazaery A I, Preparation of Monolithic Chromatographic Column for Propranolol Hydrochloride Determination, J Pharm Negative Results 2022;13(4):1629-1636

Monolithic materials contain macropores, or through pores (> 50 nm), mesopores (2-50 nm), and micropores (< 2 nm). The advantages of this porous structure are that macropores control the column permeability by decrease the back pressure of the column when high flow rates are applied because they allow the solvent to pass through the monolithic column faster than the packed column [15][16]. The mesopores are responsible for increasing the monolith's surface area and load capacity; this pore structure influences quick extraction with a high flow rate and moderate backpressure. In comparison, micropores play an essential role in the sorption capacity of small solutes on the surface area[17].

Propranolol hydrochloride is a non-selective beta-blocker or inhibitor, inhibiting beta-1 and beta-2 receptors, decreasing heart rate, myocardial contractility, blood pressure, and oxygen required for the heart muscle[18]. This work demonstrates that it is possible to quickly determine propranolol hydrochlorid in aqueous solutions in different medications.

Materials And Methods

Materials

Glycidyl methacrylate GMA (97%), acrylamide, ethylene dimethacrylate (EDMA), 3-(trimethoxysilyl) propyl methacrylate, 2,2-dimethoxy-2-phenyl acetophenone DAP, acetonitrile, and acetone were supplied from Sigma-Aldrich. While 1-Propanol, Hexanole from Merck-Schuchardt. Hydrochloric acid, sodium hydroxide (Fluka). In addition, propranolol hydrochloride was purchased from (Samarra pharmaceutical company. Iraq). Ethanol, 2-propanol, 2-Butanol, formic acid, chloroform (Gulf Cooperation Council).

Instruments

UV-Visible spectrophotometer model (UV-1700 double-beam Shimadzu, Japan). Digital analytical balance model (Denver Instrument, Germany TP-214). Magnetic motor with electric heater model (VWR West Chester, PA, USA). Ultrasonic bath (India). Homemade irradiation device (220V-50HZ). A syringe pump (Bioanalytical System Inc., USA). HPLC pump with isocratic elution system model (Kd Scientific, Holliston, MAU.S.A), FT-IR 380 spectra (Bruker) and (Shimadzu), FE-SEM (TESCAN, Model: Mira3, Czech Republic), BET technique model (BEL, BELSORP MINI II, Japan).

Fabrication of the monolithic materials Silanization step[19]

The inner surface of the borosilicate tub was purged using ethanol and water, then activated the internal surface of the tube by pumping NaOH solution (0.2 M 5.0µl min-1) for 60 min using a syringe pump. Afterwards, HCL solution (0.2 M 5.0 µL.min-1) was used to wash the tube for 1 hr, then washed with water and ethanol. The final step was silanized the tube using 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS) 20% in ethanol at a pH of 5.0 through pumping at (5.0µL.min-1) for (one hour). The tube is dried with nitrogen gas, which will be ready for in-situ polymerization.

In-situ polymerization

The monolith was prepared inside a borosilicate tube from a mixture consisting of two monomers of 650 µL (GMA) with 0.282g (A.Am) and 50µL (EDMA) as cross-linker. (DAP) with one weight per cent of the monomers used as an initiator. Porogenic solvent (750 µL1-propanol + 900 µL hexanol) was used to desolve monomers, cross-linker, and washed with ethanol and water to eliminate un-reacted components. The preparation method was based on Ueki et al[20] with some modifications, and the epoxy groups in glycidyl methacrylate were opened to form diol groups using hydrochloric acid (0.2M)as a catalyst[21][22].

The effect of irradiation time

The effect of irradiation time was investigated on monolith formation; the irradiation time ranged from 1-5 minutes.

The morphological characteristics of the monolithic column

The morphological properties were tested using a scanning electron microscope (SEM) and a Brunauer-Emmett-Teller model (BET).

GMA – co- EDMA - co – A.AM for propranolol hydrochloride determination

and initiator. The polymerization mixture was mixed well for (10 min) using a magnetic stirrer. Ultra wave sonicator was used to sonicate the mixture for (10 min). Then oxygen was removed from the mixture by purging with nitrogen gas for (5 min).

After that, the borosilicate tube was filled with the polymerization mixture using a glass syringe, then closed from both sides by a rubber stopper and exposed to a UV light lamp for UV polymerization at (365 nm) for 3 min. After monolith formation, the column was

The prepared monolith was used to determine propranolol hydrochloride; it was tested using several standard solutions of propranolol hydrochloride (0.1-20) ppm at room temperature. Then the test solution of propranolol hydrochloride supplied by the different companies was tested using the HPLC technique.

Preparation of Propranolol hydrochloride solutions

The standard solution of propranolol hydrochloride was

prepared with a concentration of 30 ppm, and several concentrations were prepared from the standard solution.

Results And Discussions

Preparing the tube's inner surface

Preparing the inner surface of the tube is the most crucial stage of monolith production in a borosilicate tube, as it requires the reaction of 3-trimethoxysilyl propyl methacrylate with silanol groups (Si-OH) on the inner wall of the tube. This technique has the benefit of securing the monolith to the inner wall of the tube and preventing the polymer from escaping at high pumping speeds. Besides that, it helps prevent the shrinkage effect during the polymerization process[23].

The process of preparing the inner surface of the tube includes several steps. In each of these steps, the solutions necessary for the conditioning process are pumped into the borosilicate tube using a syringe at a flow rate of 5 µL.Min-1 for one hour. The first step was to wash the inner wall of the tube with acetone to eliminate any organic matter, then rinse with distilled water to eliminate any acetone residue. Then, sodium hydroxide solution was used at a concentration of (0.2M) to decompose the siloxane groups and increase the density of the silanol groups, then it was washed with distilled water to remove any remaining base solution[24]. In addition, a (0.2 M) concentration of hydrochloric acid was used to eliminate the residual alkali metal ions. After that, the borosilicate tube was rinsed with distilled water to remove any remaining hydrochloric acid and then washed with ethanol to remove the distilled water. The 3-trimethoxysilyl propyl methacrylate was injected into the borosilicate tube and left to react for 1 hour. The tube was dried using nitrogen gas. Trimethoxysilane groups are linked to silanol groups on the tube's surface; the attached methacrylate groups will contribute to the polymerization reaction, binding the monolith to the inner walls of the glass tube[25]. The silanization process is shown in Figure (3-1).

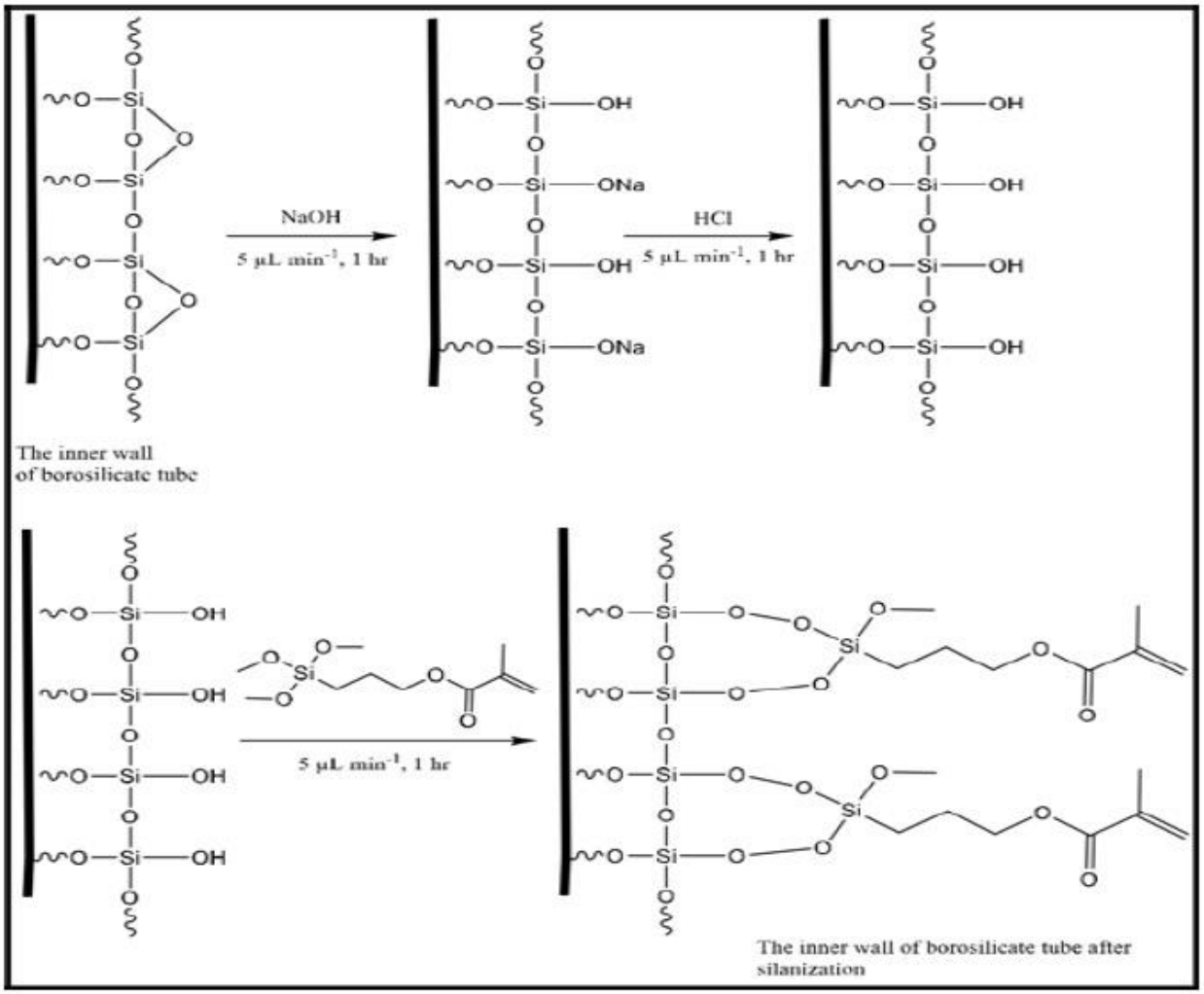


Figure (3-1) : Silanization process

Polymerization process

The polymerization process involved radical polymerization of two monomers, glycidyl methacrylate and acryl amide in precence of cross-linker. Glycidyl methacrylate was selected since it contains two functional groups, the methacrylate double bond that contributes to the photopolymerization reaction and the epoxide groups that can be used in numerous chemical reactions as post-polymerization modification reactions to yield distinct functional groups that can provide a numerous separation mechanism[26][27]. The cross-linker, porous solvents, and a reaction initiator have a vital role in the polymerization reaction and the final morphology [28]. Ethylene glycol dimethacrylate (EDMA) is a typical cross-linking agent used to make solid large-pore monolithic polymers[29]. 2,2-dimethoxy-2-phenyl acetophenone was an initiator to form free radicals that attack the double bonds in the monomers to initiate the polymerization process.

Any alteration in the polymerization mixture ratio will affect the porosity qualities and chemical composition of the cross-linked material; for example, if the percentage of the bond is raised, the average pore size reductions due to the creation of microspheres with high cross-linking, which may be advantageous for obtaining a monolith with a large surface area. However, a monolith with a large surface area will have low permeability to solvents and raise backpressure. Therefore, the bond-to-monomer ratio must remain constant[10].

The polymerization process was carried out using ultraviolet rays to initiate the free radical polymerization process; this method has many advantages, such as controlling pore size, reducing preparation time, and avoiding high temperatures that lead to crystallization [30].

FT-IR analysis

The FT-IR spectrum gives a strong sign of polymer formation by detecting the main peaks in the monomers and the formed polymer. However, in the GMA monome, the main peak (1715.40cm-1) for (C = O), (1635.98cm-1) for (C = C), (906.70cm-1) for (epoxy ring), These peaks refer to the vibrational stretching of these functional groups[26]. On the other hand the FT-IR spectra for A.Am showed main peaks for (C = C) at (1605.83cm-1), (C = O) at (1665.02cm-1), and (NH2) at (3337.67cm-1) .

The FT-IR spectra of GMA-co-EDMA-co-A.Am showed clear peaks for (C = O), (NH2), and epoxy rings. Simultaneously, the (C = C) peaks in the monomers and cross-linker spectra disappeared, which is strong evidence of polymer formation. The "FT-IR" for the prepared monolith is shown in Fig. (3-2)

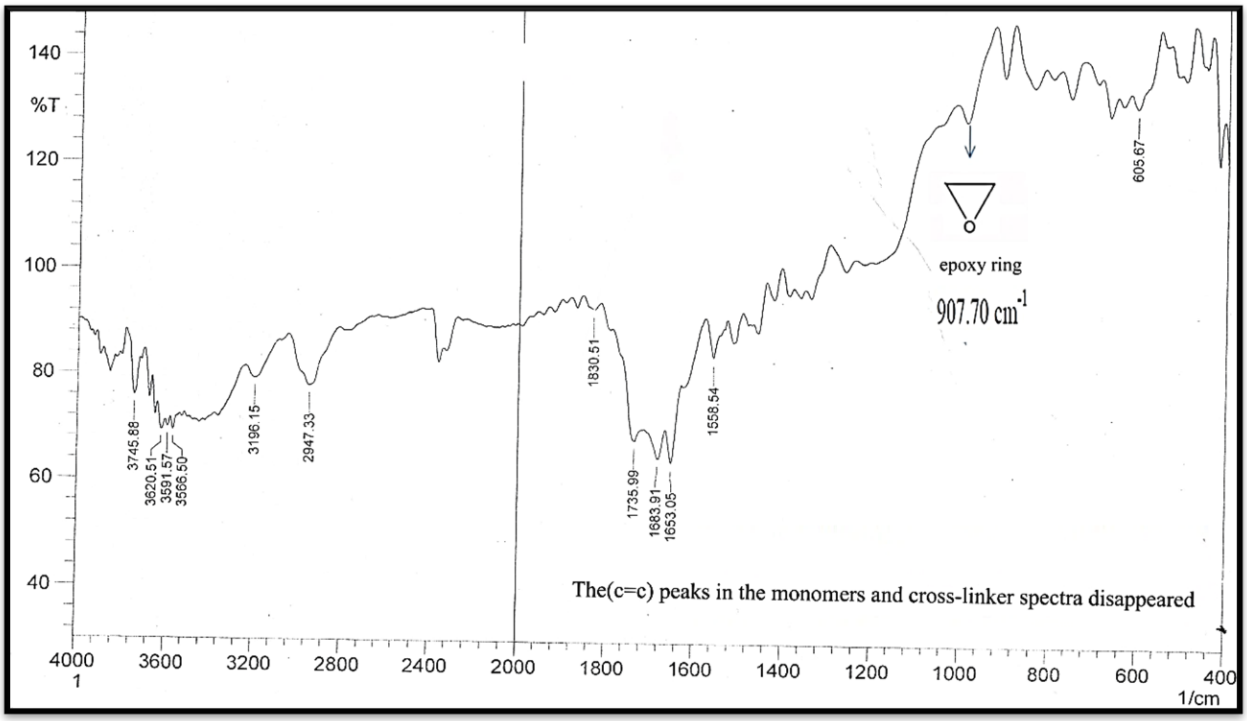


Figure (3-2) The FT-IR spectra of GMA-co-EDMA-co-A.Am

Diol groups formation

The epoxy rings were opened after polymer formation using a hydrolysis reaction by pumping hydrochloric acid (0.2 M) for three hours using a dual syringe at a flow rate of 20 µL.min-1. The epoxy group changed to diol groups, and the opening reaction and diol formed are demonstrated by FT-IR spectra that depicted in Fig (3-3).

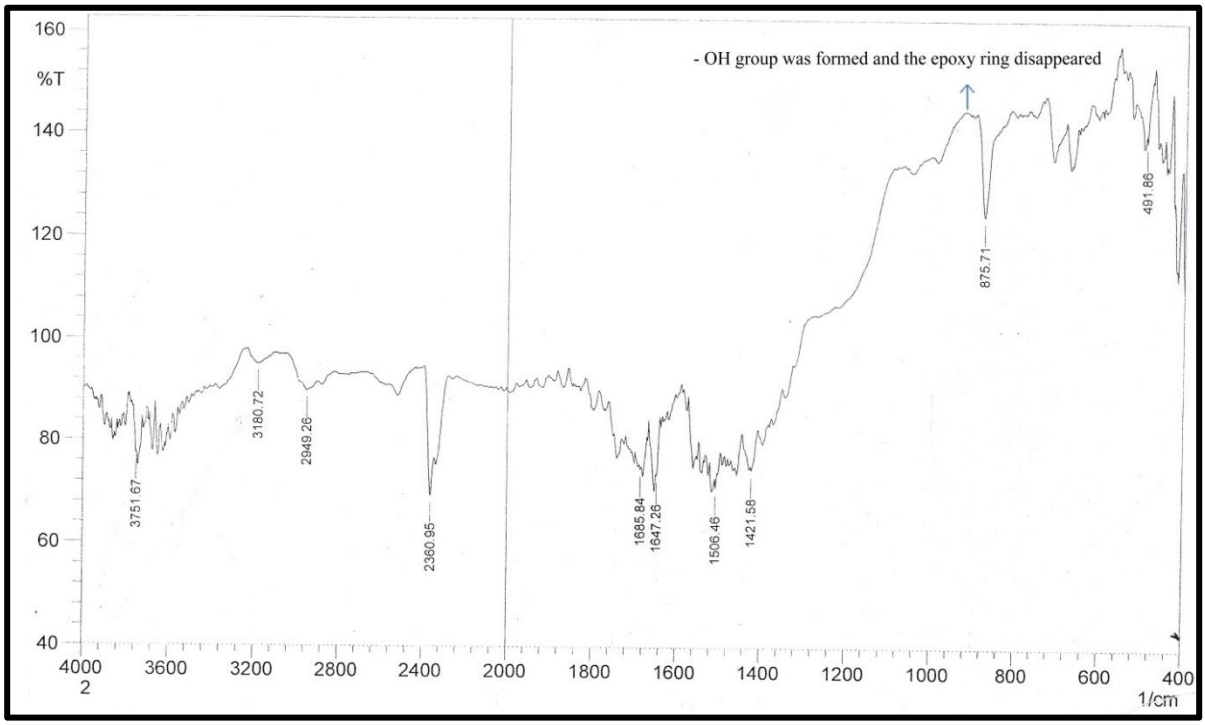


Figure (3-3) FT-IR spectra of the monolith after epoxy group opening

From figure (3-3), it can be seen that the epoxy group at 907 cm-1 disappeared. The peak of the OH group at (3751cm-1) appeared, indicating that the epoxy ring-opened and was not found in the FT-IR spectra of the monolith before the epoxy group opening.

Irradiation time effect

The irradiation period is one of the main factors that can determine the structure and morphology of the monolith, as it increases the polymeric series and the surface area while decreasing the pore size if the irradiation time is too long and vice versa. The irradiation time effect is illustrated in Table (3-1).

Table (3-1) :irradiation time effect on monolith formation.

Irradiation time min	*Result*
1	monolith did not form
2	monolith start forming
3	monolith formed with suitable back pressure
4	monolith formed but blocked
5	Blocked polymer

The Morphological Properties of the monolith GMA-co-EDMA -co-A.AM

The morphological properties of the monolith (GMA-co-EDMA-co-AAm) have been investigated using (SEM and BET) techniques. The SEM image demonstrated that the monolith's morphology reveals multiple cluster groups and macropores, as seen in Fig (3-4). It is possible to establish several requirements for the prepared column; firstly, monolithic media can be viewed as a vast channel network. Secondly, the mobile phase may readily flow through these pores, which is advantageous for minimizing back

pressure[31]. On the other hand, micropores and mesopores can enhance the monolith's surface area, enhancing its surface loading capacity [12].

Brunauer-Emmett-Teller (BET) analyses demonstrate the surface area was (14.461m2.g-1) and the pore size (5.26nm) of glycidyl methacrylate-co-ethylene dimethcrylate-co- acryl amide monolithic column.

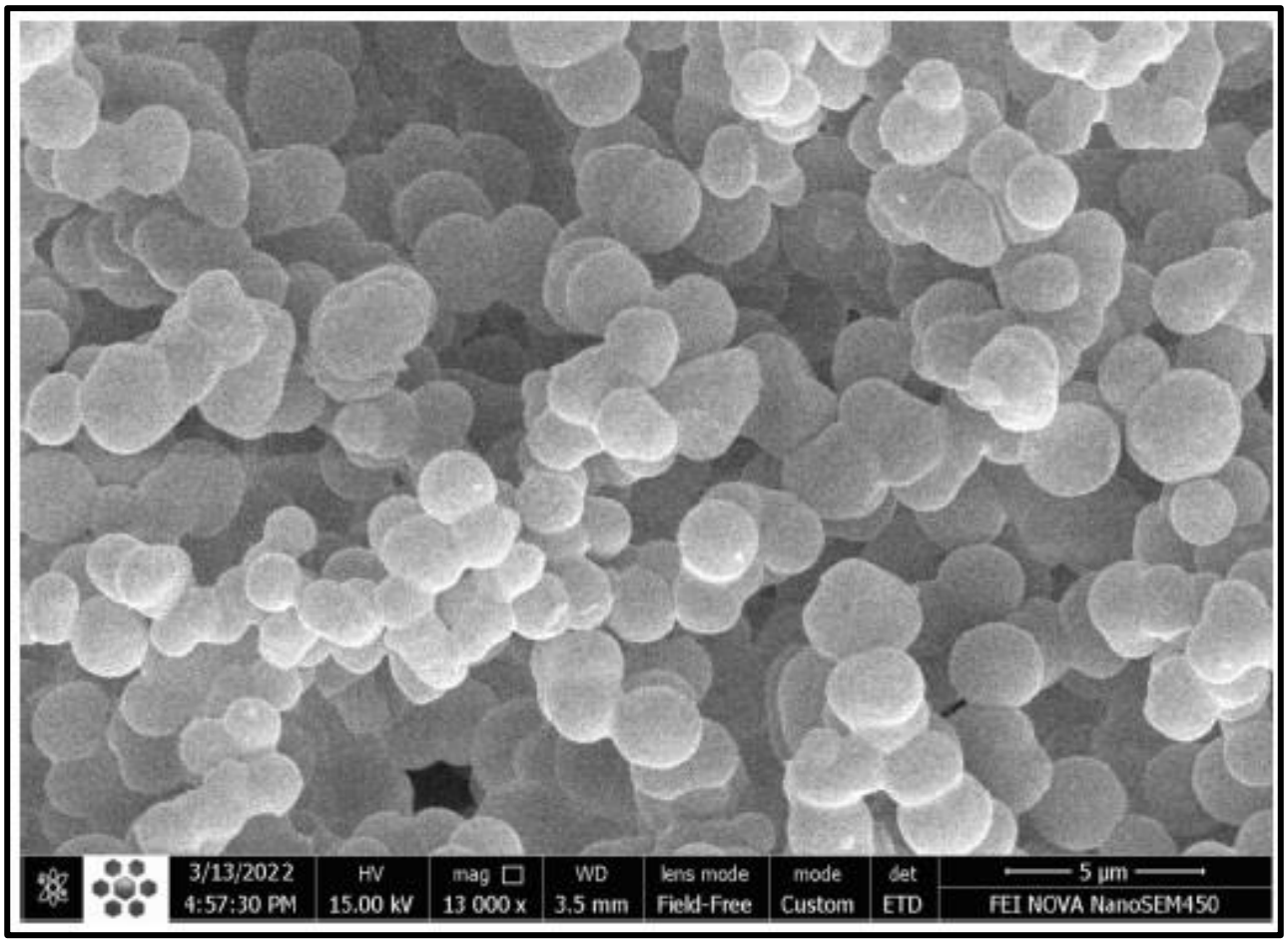
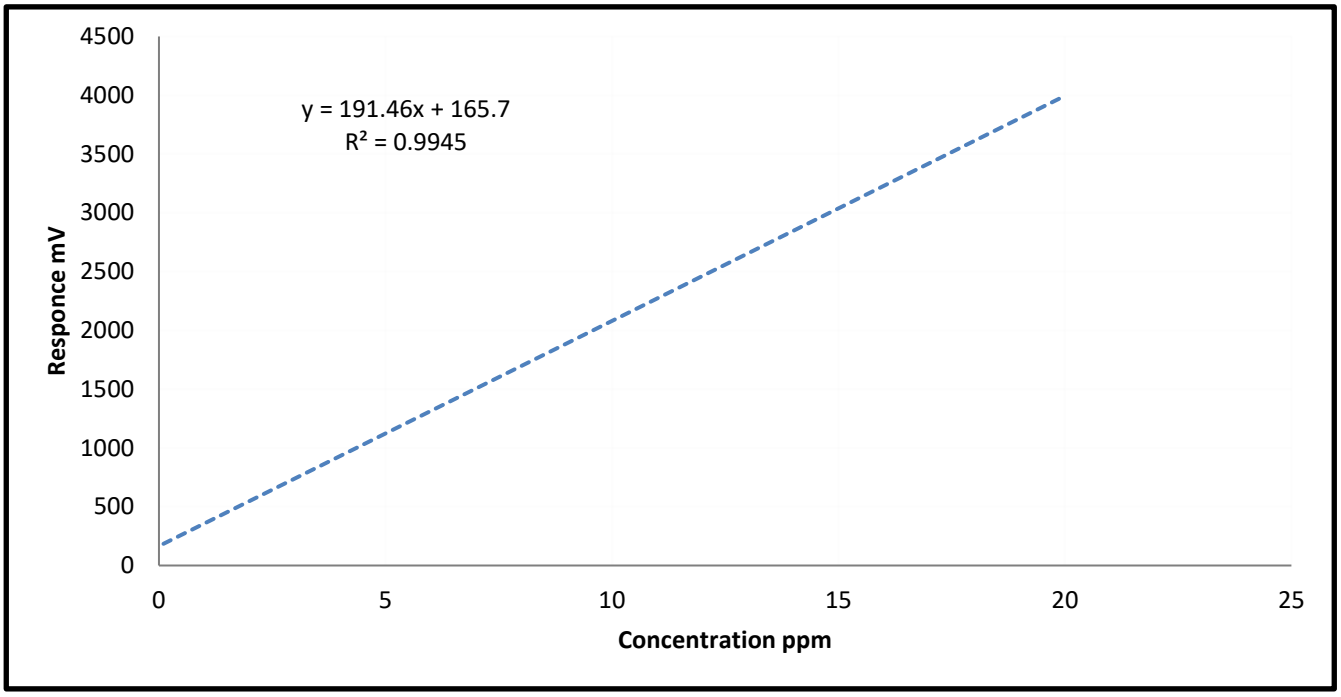


Figure (3-4): SEM image of the prepared monolith column GMA - co - EDMA - co – A.AM

Calibration curve for propranolol hydrochloride

The calibration curve of propranolol hydrochloride in the range of concentrations (0.1-20 ppm) obeyed Beer's law at the wavelength of (290 nm). The detection limit (LOD) was (0.005 ppm), the quantitative detection limit (LOQ) was (0.036), and Sandel's sensitivity was (5.223 µg.cm-2). The calibration curve is shown in Figure (3-5), and all the parameters are shown in Table (3-2)



Figure(3-5) Calibration curve for propranolol hydrochloride

Table (3-2): all the parameters obtained from the calibration curve of propranolol hydrochloride

Parameters	*Values*
Range	(0.1-20) ppm
slope	191.46
ɤmax	290nm
R2	0.9945
LOD	0.005
LOQ	0.036
Sandle Index µg.cm-2	5.223
ε (L.mol-1.cm-1)	56.633

Applications

The performance of the monolith column was evaluated by calculating the RSD% values for the determination process of propranolol hydrochloride using the prepared monolithic column connected to the HPLC system (Shimadzu 2010A). In these conditions, the mobile phase is (70:30) acetonitrile to water with a flow rate of 1 mL. min-1, the wavelength is 290 nm. The determination process of propranolol hydrochloride was carried out three times. The results can be seen in Table (3-3).

Table(3-3): the evaluation results for monolithic column

Pharmaceutical compounds	*Theoretical value mg*	*Experimental value mg*	*RSD% N =3*
Propanolol (accord)	10	9.62	1.02

Conclusion

The monolithic polymer was successfully prepared low-costly inside the borosilicate tube using UV polymerization. The ring-opening reaction of the epoxy group in the GMA monomer has given broad possibilities for forming and modifying different groups on the surface of the monolithic column. These groups can assist in determining various compounds such as pharmaceutical, biological and ionic compounds. Using the HPLC technique, the prepared

monolithic column was successfully used to determine the pharmaceutical compound (propranolol hydrochloride).

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