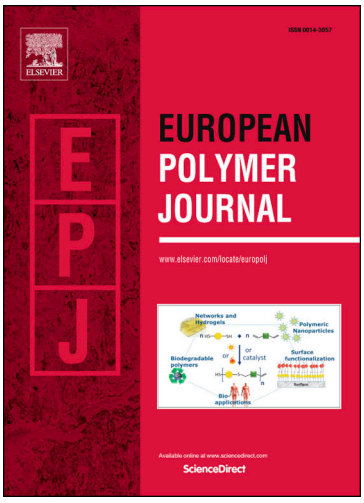

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Enantiomeric resolution of ephedrine racemic mixture using molecularly imprinted carboxylic acid functionalized resin

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Abstract

In the current work, we prepared an enantio-selective imprinted resin adsorbent ((+)-EMIP) with remarkable affinity for (+)-ephedrine ((+)-Eph) enantiomer. The phenolic amide derived from 4-hydroxybenzoic acid (HBA) and (+)-Eph ((+)-Eph-HBA) was first synthesized via N,N′-diisopropylcarbodiimide (DIC) activation and then copolymerized with resorcinol and formalin. The template (+)-Eph was then expelled from the resin by alkaline degradation of the amide linkage and the finally obtained (+)-EMIP resin particles exhibited a considerable selectivity toward the (+)-Eph with a capacity reached 220±1 mg/g. Also, the selectivity studies indicated a higher affinity toward the imprinted (+)-Eph enantiomer as a result of the formation of configuration-matching receptor sites that were able to fit the targeted enantiomer better than its mirror-image. Moreover, the prepared resin was successfully employed in the chiral resolution of (±)-Eph racemate using batch technique with (+)-Eph 87.1% enantiomeric excess in the loading supernatant solution and (-)-Eph 44.6% excess in the recovery eluant solution.

Keywords:
Molecular-imprinting
Ephedrine
Carboxylic acid resin
Chiral resolution

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1. Introduction

All biological systems are depending on proteins and proteins are mainly constructed from chiral amino acids, thus chirality has an essential role in controlling the physiological and pharmacological processes. Although the enantiomeric pairs behave similarly in achiral media, they behave differently in chiral media, like biological systems. If an enantiomer gives a certain pharmacological action, its mirror image enantiomer can display higher, lower or even opposite response [1, 2].
For these reasons, the pharmacological effect of the enantiomers of any innovated asymmetric drugs has to be individually investigated and quantified before commercializing as a legitimate medicine in the market. Moreover, it is necessary to examine the physiological and toxic effects of the individual enantiomers and compare it to their racemates, which necessities the development of enantiomeric resolution techniques in the fields of drug researches and pharmaceutical industry [3-7].

Various techniques were effectively utilized to obtain and detect pure enantiomers such as diastereomeric crystallization [8, 9], chiral catalysis [10], enzyme-based chiral separation [11-13], liquid, and supercritical fluid chromatography [14-17]. Among these methods, chromatographic methods such as liquid chromatography (LC) and electrochromatography (CEC) had attracted much interest as efficient and economic strategies in separation and purification of enantiomeric species since the 1980s [18]. In these techniques, the chiral separation could be effectively achieved by passing the racemates through appropriate chiral stationary phases (CSP), which are usually composed of cellulose or amylose derivatives, crown ethers, enzymes, and antibiotics [19].

Since the 1970s, molecularly imprinted polymers (MIPs) have attracted the interest of many researchers as promising, effective, and low-cost materials, which are extensively employed in various pharmaceutical

and biomedical applications including the chiral resolution of various racemic mixtures [20-22]. CSP derived from MIPs could be classified as a type of smart polymers containing stereo-selective cavities within its construction that fit well with the configuration of a certain enantiomer and hence can selectively recognize and bind with this enantiomer in its corresponding racemic mixture [23, 24]. The high mechanical and thermal stability besides the tolerance with various types of solvents and acidic or basic conditions make these imprinted polymers are competitive and challenging compared to other traditional CSP materials [25, 26].

The central nervous stimulant ephedrine is considered one of the commonly used drugs, which medically prescribed for some medical conditions including blood pressure disorders, asthma, narcolepsy and nasal congestion [27]. There are two enantiomers for ephedrine which are (1*S*, 2*R*)-(+)-ephedrine ((+)-Eph) and (1*R*, 2*S*)-(−)-ephedrine ((-)-Eph). These enantiomers have different pharmacological responses with different metabolic rates, (+)-Eph is 80% more active than (-)-Eph. As a result of the employment of ephedrine in the illegal manufacturing of amphetamine and methamphetamine, the identification and quantification of each enantiomer is an important process during the pharmaceutical formulation of the medical prescriptions and in the forensic applications [27, 28].

Due to the presence of both –NH- and –OH functional groups in the ephedrine molecule, it is able to interact with carboxylic acid-functionalized monomers, which make it a suitable template molecule in various molecular imprinting studies [29-34]. Here in this work, a polymerizable amide derived from (+)-Eph and *p*-hydroxybenzoic acid was synthesized and copolymerized with formaldehyde and resorcinol under acidic conditions. The resulted phenolic resinous material was then treated with NaOH to hydrolyze the amide bonds that link the (+)-Eph,

followed by HCl to remove the hydrolyzed (+)-Eph out of the polymer network and finally obtain (+)-Eph imprinted resin ((+)-EMIP). Instrumental and spectroscopic techniques were utilized in the investigation of the prepared materials and the selectivity of the obtained resin toward the imprinted (+)-Eph was investigated. In addition, the chiral separation of the (±)-Eph was attempted using a separation column filled with the prepared (+)-EMIP resin.

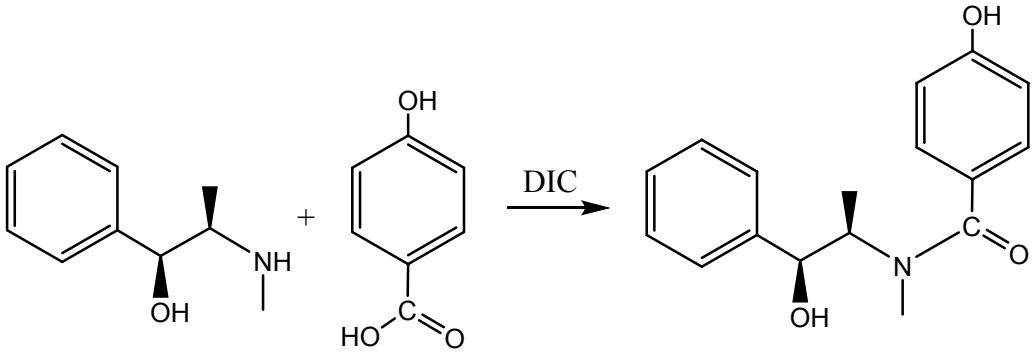
2. Materials and methods

2.1. Materials

(1R,2S)-(-)-Ephedrine ((-)-Eph) (98%) and (1S,2R)-(+)-ephedrine ((+)-Eph) (98%), 4-hydroxybenzoic acid (HBA) (99%), N,N′-diisopropylcarbodiimide (DIC) (99%), resorcinol (99%) and formaldehyde solution (37% (v/v)) were provided from Sigma-Aldrich (USA). All other solvents and reagents were obtained from different suppliers and used without any treatments.

2.2. Synthesis of (+)-ephedrine-4-hydroxybenzoic amide ((+)-Eph-HBA)

The synthesis of the amide derivative was performed in accordance with modified procedures by Fattahi et al [35] and as shown in Scheme 1. 3 g HBA was stirred with 20 mL ethyl alcohol/water (1:1) until completely dissolved and 2.74 g DIC was mixed and stirring was continued for 1 h at 30 ^{o}C. The template (+)-Eph was then added to the activated HBA and stirring was continued for 12 h at the same temperature. The obtained solid (+)-Eph-HBA amide derivative was then separated and washed with the same utilized solvent to extract the by-product and the unreacted materials.



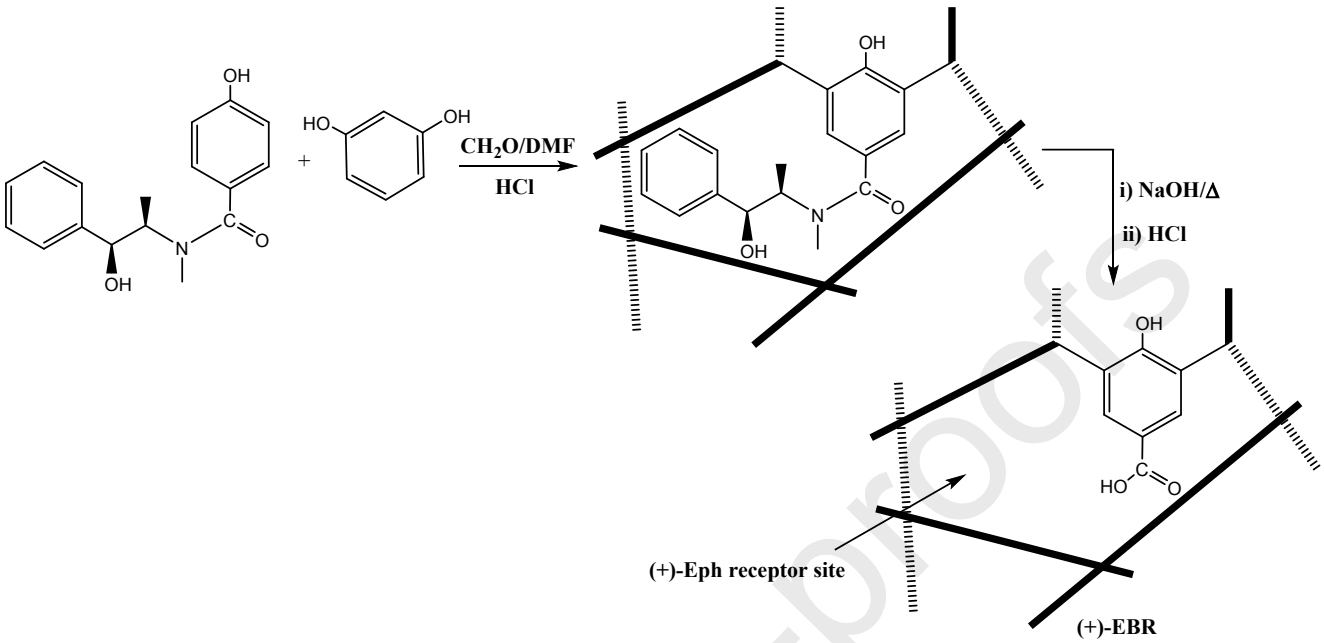
(+)-Ephedrine 4-Hydroxybenzoic acid (+)-Eph-HBA

Scheme 1. Synthesis of (+)-ephedrine-4-hydroxybenzoic amide ((+)-Eph-HBA).

2.3. Synthesis of imprinted (+)-EMIP resin

5 g of the synthesized amide (+)-Eph-HBA and 5 g resorcinol were placed in a reaction flask containing 30 mL DMF and magnetic stirring started until the solid materials had completely dissolved. A previously prepared mixture of 20 mL formalin and 6 mL glacial acetic acid was poured to the flask and the stirring continued for 10 min before gradual addition of 3 mL HCl solution (5 M). The solid polymeric material has appeared within 5 min before raising the temperature to 80 ^{o}C under continuous stirring for 1 h. The solid resin was then extracted and rinsed with distilled water followed by ethanol and DMF to extract the by-product and unreacted substances. The obtained solid polymer was then dried and weighed (10.7 g), then crushed using a porcelain mortar and pestle and sieved to around 200 μm particle sizes. The (+)-Eph templates were extracted from the cross-linked polymer by heating the resin particles with 100 mL sodium hydroxide solution (2 M) for 4h under magnetic stirring. The particles were then filtered and immersed in 100 mL, 0.5 M HCL solution and shaking for 2 h. The (+)-Eph free resin was filtered and rinsed multiple times with distilled water for neutralization and then dried at 40 ^{o}C. Scheme 2 demonstrated the imprinting process. For comparison

purposes, non-imprinted resin particles (NIP) were prepared under the same condition in the presence of HBA instead of the amide (+)-Eph-HBA.



Scheme 2. Imprinting of (+)-ephedrine.

2.4. Characterization

The elemental analysis of the amide derivative (+)-Eph-HBA was performed using a Perkin–Elmer 240 C instrument (2400 CHNS/O Series II System (100V), USA). The functional groups within the amide (+)-Eph-HBA, (+)-EMIP, and NIP were investigated by an attenuated total reflectance (*ATR*) supported Perkin–Elmer Fourier-Transform Infrared (FT-IR) spectrometer (USA).

ICP-MS Portfolio mass spectrometer instrument (Thermo Fisher Scientific Co, USA) was utilized to record the mass spectrum of the amide derivative (+)-Eph-HBA. Also, the NMR spectra were obtained by dissolving the (+)-Eph-HBA in DMSO using an Oxford NMR spectrometer (Model Unity Inova 500 MHz, USA).

Scanning electron microscope (SEM) (FEI Quanta-200, FEI Company, Netherlands) was employed to examine the surface morphology of (+)-EMIP, and NIP particles after coating the resin particles with Au at 15 mA for 50 s and at 20 kV. The measurements of the surface area of both

(+)-EMIP, and NIP particles were performed by nitrogen adsorption-desorption isotherms on an ASAP 2010 Micromeritics instrument (Model 2010, USA). The elemental analysis of the (+)-EMIP particle surfaces was recorded before and after expelling the (+)-Eph template molecules using Energy-dispersive X-ray spectroscopy (EDX, HITACHI S-4800, Japan).

The degree of the carboxylic acid functionalization was determined using the back titration technique. A definite amount of the resin particles was equilibrated with a 100 mL of a standard sodium hydroxide solution over a shaker for 4 h at 30 ^{o}C. The mixture was then allowed to stand and 10 mL of the supernatant solution was taken to determine the residual NaOH concentration by titration against a standard HCl solution. The carboxylic acid functionalization (mmol/g) was calculated using Eq. 1.

$$\text{-COOH functionalization (mmol/g)} = \frac{(V_1\text{-}V_2)\,M}{W} \times 1000 \qquad (1)$$

Where V_1 (mL) is the HCl volume equivalent to the 100 mL initial NaOH solution; V_2 (mL) is the HCl volume equivalent to the 100 mL NaOH solution after shaking with the resin particles; M (mol/L) is the molar concentration of HCl solution and W (g) is the weight of the resin particles.

2.5. Uptake of ephedrine enantiomeric species

Ethyl alcohol/water (1:1) mixture was used in all enantio-selective adsorption experiments of ephedrine enantiomers.

2.5.1. Effect of pH

0.05 g of the selected (+)-EMIP or NIP resin was equilibrated with (+)-Eph solution (50 mL, 200 mg/L) for 3 h at 30 ^{o}C and 200 rpm. The pH changed from 1 to 12, the acidic pHs were obtained by 0.01 M HCl solution while the alkaline pHs were obtained by 0.01 M NaOH solution. The remaining contents of (+)-Eph was determined in the supernatant solution

by Perkin-Elmer Bio UV-visible (LAMBDA XLS, USA) Spectrometer at λ_{max} 240 nm and the extracted Eph amounts had been determined by Eq. 2.

$$q_e = \frac{(C_i - C_e)V}{W} \qquad (2)$$

where q_e (mg/g) is the extracted Eph amount; C_i (mg/L) is the initial Eph concentration; C_e(mg/L) is the equilibrium concentration of Eph after adsorption; V (L) is the solution volume and W(g) is the mass of the adsorbent.

2.5.2. Isotherm experiments

0.5 g of (+)-EMIP or NIP particles were placed in 50 mL of either (+)- or (-)-Eph solution with initial concentrations 25-300 mg/L at 30 ^{o}C and initial pH 7 before shaking the bottles at 200 rpm for 3 h. The Eph content in the supernatant solution was finally determined to calculate the adsorbed amounts by the utilized adsorbent particles.

2.5.3. Selectivity studies toward Eph enantiomers.

The selectivity toward Eph enantiomers was examined by preparing four batches two for (+)-EMIP and the remaining batches for NIP adsorbents. Each adsorbent particles were tested with both (+)-Eph and (-)-Eph enantiomers individually using initial concentration 50 mg/L at 30 ^{o}C and pH 7. After shaking the four batches for 3 h, the equilibrium concentration of Eph was measured and the selectivity coefficient $\beta_{(+)\text{-Eph}/(-)\text{-Eph}}$ was calculated using Eq. 3.

$$\beta_{(+)\text{-Eph}/(-)\text{-Eph}} = \frac{D_{(+)\text{-Eph}}}{D_{(-)\text{-Eph}}} \qquad (3)$$

where $D_{(+)\text{-Eph}}$ and $D_{(-)\text{-Eph}}$ are the distribution ratios of (+)-Eph and (-)-Eph, respectively, and were calculated by Eq. 4.

$$D = \frac{(C_i - C_f)}{C_f} \, x \, \frac{V}{W} \qquad (4)$$

The relative selectivity coefficient (β_r), which can evaluate the potential of the (+)-Eph imprinting strategy on creating selectivity toward the template enantiomer was calculated using Eq. 5.

$$\beta_r = \frac{\beta_{imprinted}}{\beta_{non\text{-}imprinted}} \qquad (5)$$

where $\beta_{imprinted}$ and $\beta_{non\text{-}imprinted}$ are the selectivity coefficients of (+)-EMIP and NIP resin particles, respectively.

2.5.4. Resolution of (±)-Eph racemate

The enantiomeric separation of (±)-Eph was attempted using the batch technique. Two 100 mL bottle batches each containing 5g of the solid phase adsorbent resins, which are (+)-EMIP or NI-PR. The working adsorbate solution was prepared by dissolving the (±)-Eph in the ethanol/water solvent mixture to reach a concentration of 30 g/L and pH 7. 50 mL of the prepared racemic solution was poured in each bottle and allowed to equilibrate over the shaker for 4 h at 30 ^{o}C then allowed settle down and the supernatant solution was filtered and transferred to a polarimeter (PerkinElmer Inc. - Model 341, USA) to determine the optical purity. The eluant solution was prepared from the same ethanol/water solvent mixture but adjusted at pH 1 and the above separated Eph-loaded resin particles were eluted by equilibrating with 50 mL of this eluant for 4 h over the shaker at 30 ^{o}C. The batches were then allowed to stand and the eluant solutions were filtered to examine the optical purity.

3. Results and discussion

3.1. Synthesis and characterization of (+)-EMIP

The synthesis of the (+)-Eph-HBA amide derivative is demonstrated in Scheme 1. The percentages of C, H, and N were experimentally obtained using elemental analysis and were found to be 71.6%, 6.7%, and 4.9%, respectively, which are in a high match with the suggested molecular formula in Scheme 1.

Also, (+)-Eph-HBA exhibited a mass spectral peak at *m/z* 286.1 and there were no observed (+)-Eph and HBA characteristic peaks at *m/z* 138 or 166.1, which confirm the complete formation of the amide bond and the successful synthesis of the (+)-Eph-HBA amide derivative. Furthermore, the synthesized (+)-Eph-HBA gives a negative acidity test, which indicates the conversion of the HBA carboxylic into amide groups during the reaction with the template (+)-Eph.

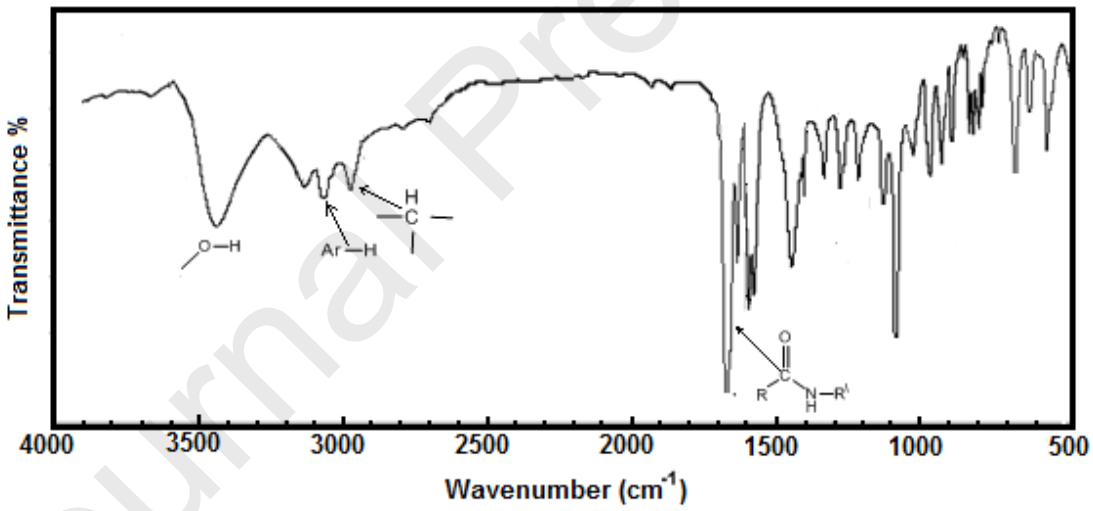


Fig. 1. FTIR spectrum of (+)-Eph-HBA amide.

The functional groups within (+)-Eph-HBA were also investigated using FTIR spectroscopy (Fig. 1), which demonstrates a characteristic broad -OH peak around 3455 cm^{-1} beside the aromatic and aliphatic C-H peaks around 3120 and 2940 cm^{-1}, respectively. The observed sharp peak

at 1675 cm^{-1} is related to the amide C=O groups and revealed the successful synthesis of the (+)-Eph-HBA amide derivative.

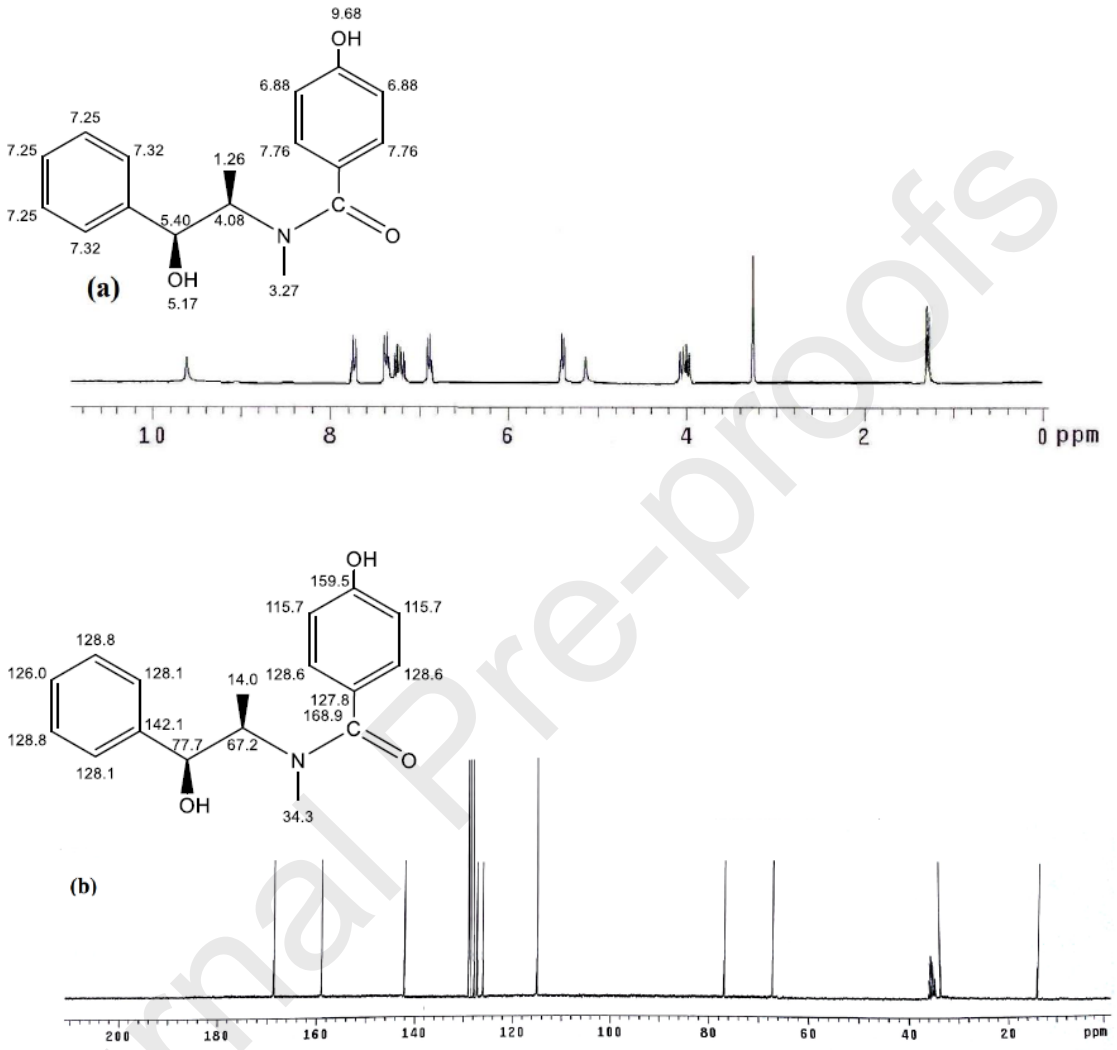


Fig. 2. (a) ^{1}H NMR spectra (b) ^{13}C NMR of (+)-Eph-HBA amide.

The (+)-Eph-HBA amide was investigated by ^{1}H NMR (Fig. 2a) and the spectrum presented doublet peaks at 6.88 and 7.67 ppm beside the phenolic –OH signal at 9.68 ppm that belonging to the 4-hydroxybenzoic moieties. The multiple peaks at 7.25 and 7.32 ppm are related to the aromatic protons of the (+)-Eph residue. Also, the doublet peak at 1.26 ppm, singlet peak at 3.27 ppm, multiple peaks at 4.08, singlet peak at 5.17 ppm, and doublet signal at 5.4 ppm are related to the C-CH_3, N-CH_3, C2*-

H, C1*-OH, and C1*-H, respectively. Fig. 2b shows the ^{13}C NMR spectrum of the (+)-Eph-HBA amide derivative and the (+)-Eph residue is indicated by the aromatic peaks at 126, 128.1, 128.8, and 142.1 ppm beside the C1* and C2* signals at 77.7 and 67.2 ppm, respectively. The 4-hydroxybenzoic residue is clarified by the aromatic peaks at 115.7, 127.8, 128.6, and 159.5 ppm beside the C=O signal at 168.9 ppm.

Also, using HyperChem (8.03) software, the chemical structure of the (+)-Eph-HBA amide derivative was investigated via PM3, (Polak Ribiere) RMS 0.01 kcal and the optimized conformation indicated the low steric hindrance around the ortho-positions of the –OH group, which can permit an easy condensation polymerization without any crowding as demonstrated in Fig. 3.

SEM images (Fig. 4) presents the surface appearance of both (+)-EMIP and NIP. It is clear that the appearance of (+)-EMIP is irregular and possesses a rough surface compared to the NIP surface morphology. This can be explained by the alternations that accompanying the alkaline amide bond degradation during the removal of the (+)-Eph enantiomer from the polymer matrix. Also, the irregular (+)-EMIP particles displayed a relatively higher surface area of 163.66 m^2/g compared to the NIP particles that displayed a surface area of 54.23 m^2/g, which could be in agreement with the SEM observations.

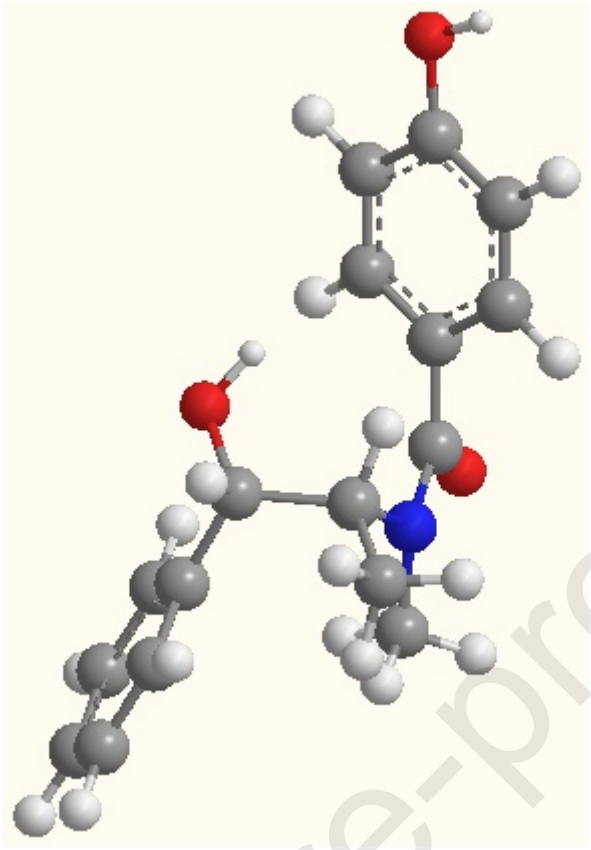


Fig. 3. Optimized molecular model of (+)-Eph-HBA.

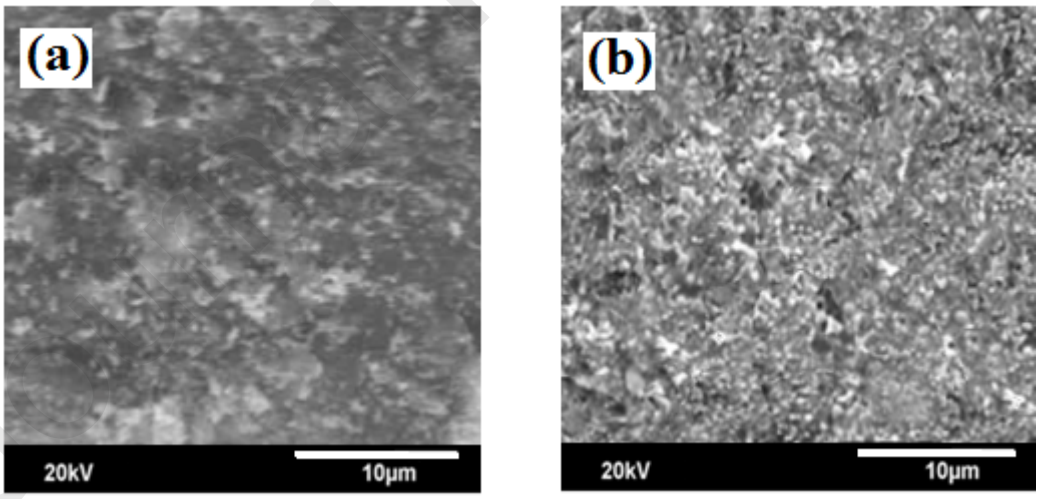


Fig. 4. SEM photos of (a) NIP (b) (+)-EMIP

The FTIR investigations of NIP particles along with (+)-Eph containing and free (+)-EMIP are collected in Fig. 5. The (+)-Eph containing (+)-EMIP resin demonstrated a spectrum close to that of the

(+)-Eph-HBA amide derivative with the amidic carbonyl band at 1675 cm^{-1}. Upon the degradation of the amide bond via alkaline treatment and extraction of the (+)-Eph template, the spectrum of the (+)-EMIP resin became very close to that of the NIP with a characteristic carboxylic acid C=O peak at 1730 cm^{-1} and absence of any peak around 1675 cm^{-1}, which confirm the successful removal of the (+)-Eph molecules from the resin structure with the maintenance of the functional carboxylic acid groups intact within the polymeric matrix.

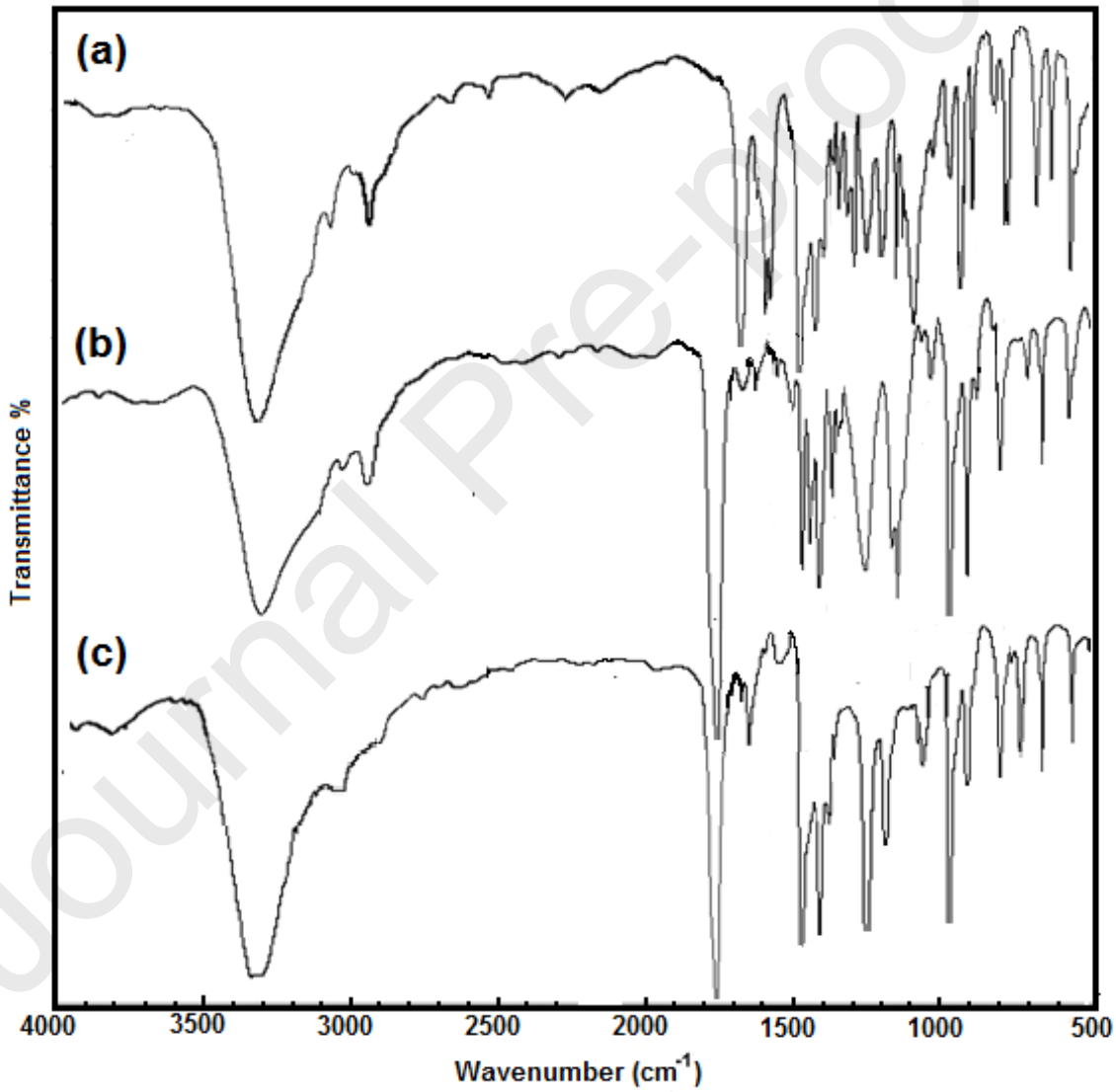


Fig. 5. FTIR spectra of (a) (+)-EMIP befor (+)-Eph leaching, (b) (+)-EMIP after (+)-Eph leaching, (c) NIP.

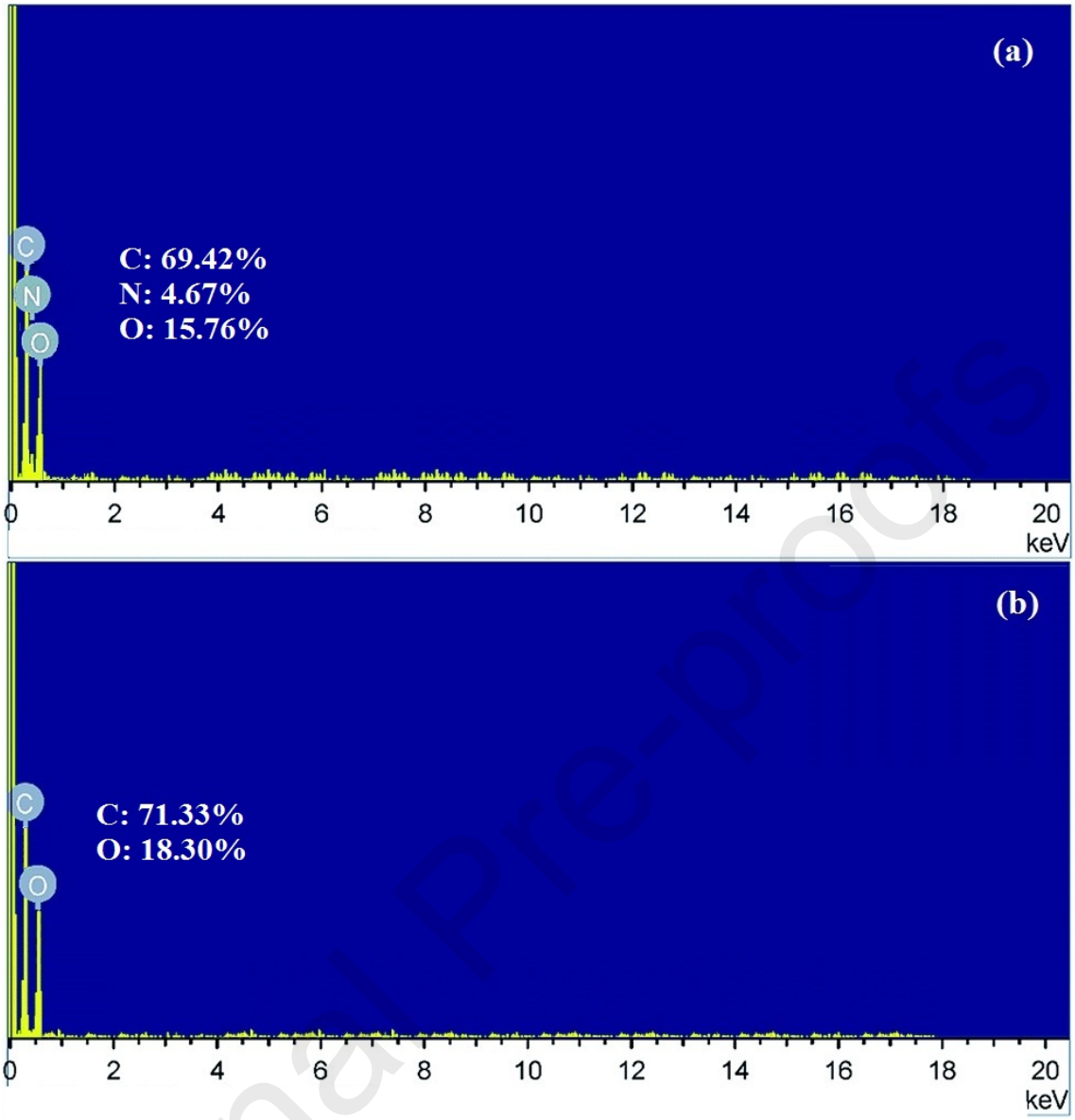


Fig. 6. EDX spectra of (+)-EBR (a) before (b) after (+)-Eph template removal.

Moreover, the complete degradation of the amide bond that links the (+)-Eph to the resin matrix and the consequent expelling from (+)-EMIP particles was confirmed by examining the (+)-EMIP particles before and after the alkaline treatment using EDX spectroscopy (Fig. 6). The (+)-Eph containing resin particles demonstrated a spectrum with 6.5% nitrogen content while the spectrum after the treatment and extraction of the (+)-Eph template molecules didn't show any indication for the presence of nitrogen within the structure of the (+)-EMIP resin, which can reveal the

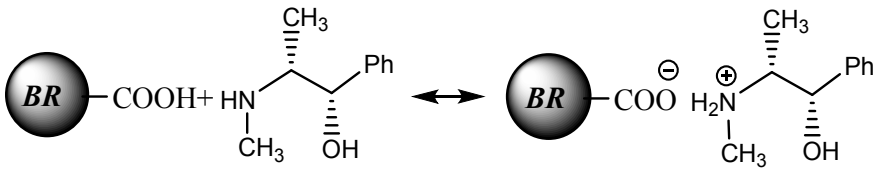
efficiency of the performed alkaline/acidic treatment in removing the (+)-Eph out of the resin structure.

Also, the carboxylic acid functionalization degrees of both (+)-EMIP and NIP that were determined using Eq. 1 were found to be around 1.55 and 1.58 mmol/g, respectively, suggesting the successful performance of the synthetic reactions with a considerably high efficiency.

3.2. Adsorption of Eph enantiomers

3.2.1. Effect of pH

The dependence of the (+)-Eph extraction using (+)-EMIP and NIP resins on the initial solution pH was investigated in batches with variable pH values (1-12). Fig. 7 demonstrated the pH profiles for both resin types. The maximum adsorption was obtained under the neutral pH 7 for both (+)-EMIP and NIP and around this optimum pH, the (+)-Eph uptake was dramatically reduced particularly under acidic conditions. By lowering the pH of the adsorption solution, the –OH rich adsorbent resin will be positively charged under the high H^+ ions concentration and the consequent protonation of these –OH groups. In the same time, the (+)-Eph adsorbate will also mainly exists as cationic species due to the protonation of both –OH and –NH- units, which will reduce the approach between the (+)-Eph enantiomer and the -COOH sites within both (+)-EMIP and NIP matrix. However, at pH 7, the absence of the excess H^+ ions will enhance the uptake of (+)-Eph enantiomer by the functional –COOH groups of the resin by the proton transfer mechanism as demonstrated below.



Under alkaline pH, the functional –COOH groups of the adsorbent will be ionized and mainly exist as –COO-, which will also decrease the

proton transfer opportunity with the (+)-Eph enantiomer and remarkably lower the enantiomer uptake.

Moreover, the pH profiles of both (+)-EMIP and NIP showed a high adsorption capacity with respect to (+)-EMIP resin under all studied pH, which could be related to the higher surface area along with the (+)-Eph imprinted sites provided by this resin.

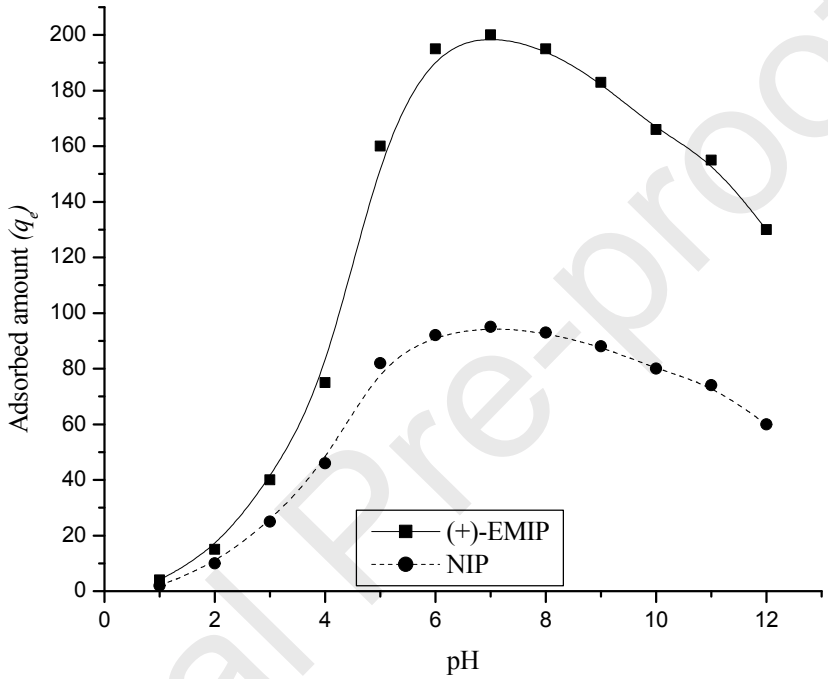


Fig. 7. Effect of pH on (+)-Eph extraction using (+)-EMIP and NIP (initial concentration 200 mg/L; adsorbent. 1 g/L; contact time 3 h; shaking rate 200 rpm, 30 ^{o}C).

3.2.2. Adsorption Isotherms

Fig. 8 presented the extent of (+)-Eph and (-)-Eph uptake on both (+)-EMIP and NIP resins against the equilibrium concentration of each enantiomer. It is clear from the isotherm pattern related to (+)-EMIP resin adsorbent that the adsorption of (+)-Eph is remarkably higher than that of (-)-Eph enantiomer suggesting a considerable stereo-selectivity toward the imprinted (+)-Eph enantiomer. On the other hand, the NIP adsorbent didn't

exhibit any differences regarding the isotherms of both (+)-Eph and (-)-Eph enantiomers and both enantiomeric species displayed almost the same adsorption trends with the same capacities, which indicate the absence of any stereo-selectivity toward any of the investigated enantiomers. These results revealed that the employed (+)-Eph-imprinting strategy was effective in creating receptor sites within the resin particle that fitted the shape and configuration of the targeted (+)-Eph enantiomer.

The Freundlich and Langmuir linear mathematical equations were utilized in treating the obtained adsorption data. According to the Langmuir model (Eq. 6), all adsorbed enantiomeric species are accommodated as a monolayer onto similar and equivalent adsorption sites [36].

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m} \quad (6)$$

where q_e (mg/g) is the extracted Eph at equilibrium, C_e (mg/g) is the equilibrium concentration of Eph in the adsorption solution, K_L (L/mg) is Langmuir equilibrium constant and q_m (mg/g) is the maximum adsorption capacity.

The Freundlich model (Eq. 7) validates the uptake of the adsorbate species as multilayer onto heterogeneous adsorbent sites [37].

$$ln\, q_e = lnK_F - \frac{1}{n} lnC_e \quad (7)$$

where *n* is the heterogeneous factor and K_F is the Freundlich constant.

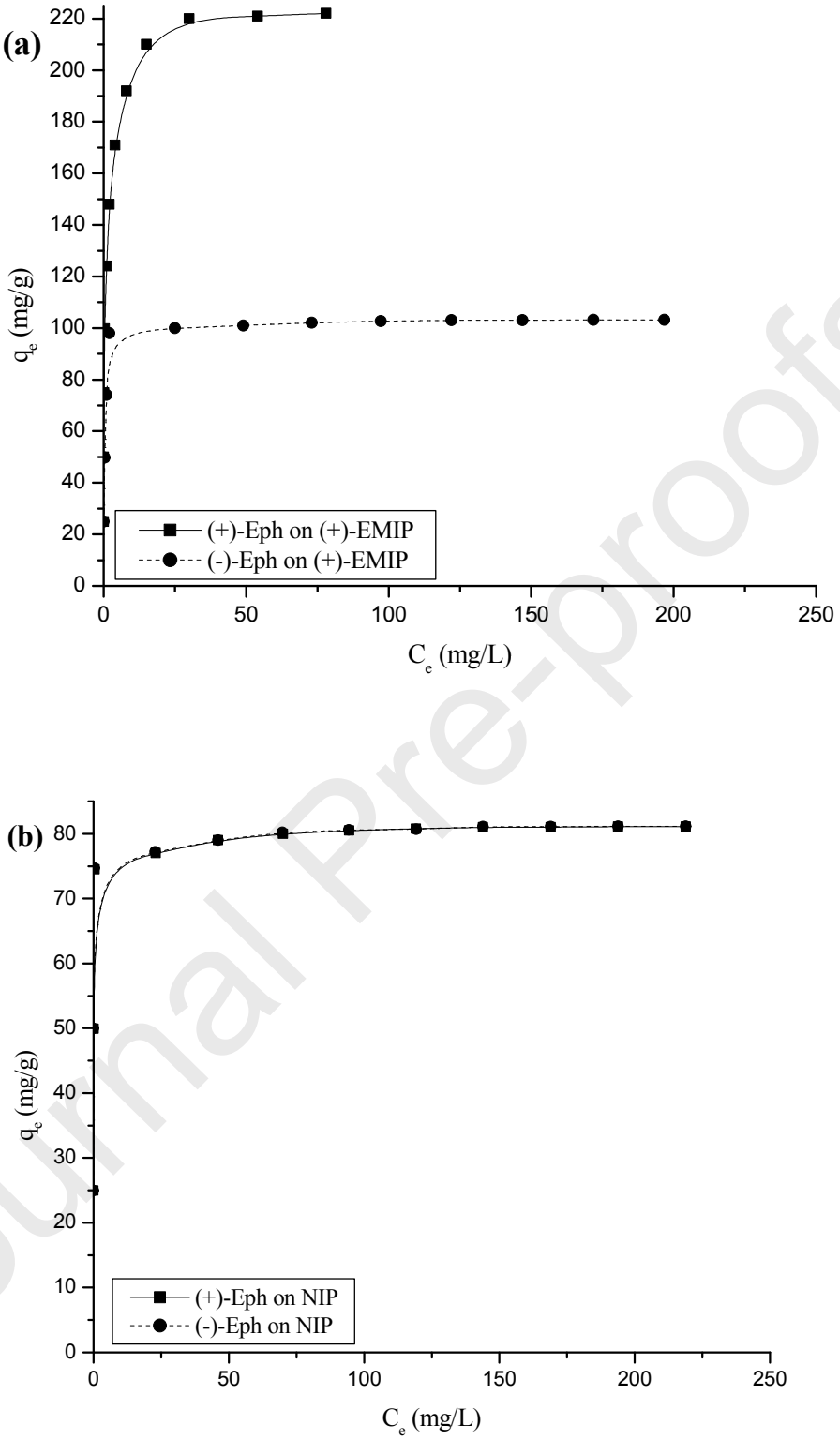


Fig. 8. Adsorption isotherms of (a) (+)- and (-)-Eph by (+)-EMIP. (b) (+)- and (-)-Eph by NIP (initial concentration 25-300 mg/L, (+)-EMIP or NIP 1 g/L, pH 7, stirring at 200 rpm, for 3 h 30 ◦C).

The analyzed data after fitting the results with the above-mentioned equations are summarized in Table 1 and the best fit was observed with Langmuir equations with the higher R^2 compared to the Freundlich model suggesting the monolayer adsorption of the Eph enantiomers onto the –COOH functionalized active sites. Moreover, the K_L value obtained for the adsorption isotherm of (+)-Eph onto (+)-EMIP resin was almost double that of related (-)-Eph on the same adsorbent, which implies a superior affinity of the imprinted resin toward the targeted (+)-Eph enantiomer due to the formation of –COOH functionalized receptor sites within the resin matrix that well-recognized the configuration the (+)-Eph molecules.

Table 1

Parameters for (+)- and (-)-Eph adsorption by (+)-EMIP and NIP according to different equilibrium models.

System	Langmuir isotherm constants		
	K_L(L g^{-1})	qm(mg g^{-1})	R^2
(+)-Eph on (+)-EMIP	$8.3x10^{-2}$	220±1	0.9998
(-)-Eph on (+)-EMIP	$4.7x10^{-2}$	105±1	0.9999
(+)- or (-)-Eph on NIP	$2.1x10^{-2}$	80±1	0.9998
System	Freundlich isotherm constants		
	K_F	n	R^2
(+)-Eph on (+)-EMIP	18.36	7.83	0.9675
(-)-Eph on (+)-EMIP	8.55	5.37	0.8896
(+)- or (-)-Eph on NIP	2.53	4.97	0.8136

3.2.3. Enantio-selectivity

Table 2 presents the calculated selectivity parameters after performing the uptake experiments using the adsorbents (+)-EMIP and NIP individually with either (+)-Eph or (-)-Eph within a single component working solution as described in the experimental section. The (+)-EMIP resin particles displayed a distribution ratio for (+)-Eph around 15 times higher than that related to (-)-Eph. Also, the other parameters including the

selectivity and relative selectivity coefficients demonstrated values above 14 with respect to the (+)-Eph enantiomer, which can give a clear evidence for the remarkable stereo-selectivity toward the imprinted (+)-Eph enantiomer as a result of the formation of chiral receptors within the polymer matrix that recognize and fit with the shape of (+)-Eph better than the (-)-Eph enantiomer. On the other hand, the NIP adsorbent particles give similar selectivity parameters values for both (+)-Eph and (-)-Eph, indicating no stereo-selectivity toward both enantiomers.

Table 2

Selective adsorption of (+)- and (-)-Eph from aqueous solution by (+)-EMIP and NIP (initial concentration 50 mg/L, adsorbent 1 g/L, shaking rate 200 rpm, solution pH 7.0, 30 ^{0}C).

Enantiomer	Distribution ratio (L/g)		Selectivity coefficient $\beta_{(+)\text{-}Eph/(-)\text{-}Eph}$		Relative selectivity coefficient β_r
	(+)-EMIP	NIP	(+)-EMIP	NIP	
(+)-Eph	453.38	35.21	-	-	11.18
(-)-Eph	40.54	35.21	11.18	1	

3.2.4. Chiral resolution

After equilibrating the (±)-Eph racemic mixture solutions with the (+)-EMIP and NIP as previously explained, the supernatant solutions were filtered and its optical purity was measured. Also, the adsorbed Eph enantiomers were eluted at pH 1 and the eluant solutions were also filtered to test their optical purity. The measured optical activities of both supernatant and eluant solutions along with the total Eph concentrations were employed to quantify the extent of the chiral separation via calculating the individual concentrations of both (+)-Eph and (-)-Eph using Eq. 8 and 9, respectively [38].

$$C_{(-)\text{-}Eph} = \frac{[ee_{(-)\text{-}Eph} + 0.5(100\text{-}ee_{(-)\text{-}Eph})]}{100} x\, C_t \quad (7)$$

$$C_{(+)\text{-}Eph} = C_t - C_{(-)\text{-}Eph} \quad (8)$$

where $C_{(-)\text{-}Eph}$ and $C_{(+)\text{-}Eph}$ are the (-)-Eph and (+)-Eph concentrations, respectively; C_t is the total Eph concentration in the supernatant solution, and $ee_{(-)\text{-}Eph}$ is the optical purity related to (-)-Eph in the supernatant solution.

The concentrations of both (-)-Eph and (+)-Eph enantiomers within the eluant recovery solution were also determined by applying the same above equations but by using the optical purity related to the excess (+)-Eph $(ee_{(+)\text{-}Eph})$.

The obtained data were tabulated in Table 3 and as expected, the obtained solutions from the NIP batch were racemic mixtures, which reveal the same tendency toward both (+)-Eph and (-)-Eph. On the other hand, the supernatant solution obtained from the (+)-EMIP batch was optically active with 87.1% (-)-Eph excess while the eluant was also optically active with 44.6% (+)-Eph excess, which confirms the uptake of a higher percentage of the (+)-Eph enantiomer by the (+)-EMIP resin particles. These results indicated the effective capability of the prepared (+)-EMIP resin in performing a successful chiral resolution of the (±)-Eph racemate.

Table 3

Enantiomeric resolution of (±)-ephedrine racemate using both (+)-EMIP and NIP

Adsorbent	Enantiomeric excess of the supernatant and eluant solutions (%)[a]		Total equilibrium concentration of ephedrine (g/L)		Individual enantiomeric concentration (g/L)			
	Supernatant	Eluant	Supernatant	Eluant	Supernatant		Eluant	
					(-)-Eph	*(+)-Eph*	*(-)-Eph*	*(+)-Eph*
(+)-EMIP	87.1 *((-)-Eph)*	44.6 *((+)-Eph)*	9.8	19.6	9.17	0.63	5.43	14.17
NIP	0	0	21.7	7.6	10.85	10.85	3.8	3.8

[a] Enantiomeric excess $(ee) = ([\alpha]_{obs} / [\alpha]_{max}) \times 100$, where $[\alpha]_{obs}$ is the rotation of the sample obtained from the batches and $[\alpha]_{max}$ is the maximum rotation of the pure (+)- and (-)-ephedrine.

4. Conclusion

An enantio-selective molecularly imprinted polymer based on carboxylic acid-functionalized resorcinol/formaldehyde resin was developed for specific interaction with (+)-ephedrine and effective enantiomeric resolution of (±)-ephedrine. The amide derived from N,N′-diisopropylcarbodiimide activated 4-hydroxybenzoic acid and (+)-ephedrine was synthesized and anchored with resorcinol/formaldehyde copolymerization followed by amide cleavage and releasing the (+)-ephedrine from the polymeric structure. The progress of the synthesis and polymerization were all confirmed using the spectral and instrumental methods and the morphology was observed using SEM. The selectivity and isotherm studies indicated a considerably higher affinity of the imprinted resin particles toward the (+)-ephedrine enantiomer and the enantiomeric resolution of (±)-ephedrine was effectively performed using the batch technique, which demonstrated 87.1% (-)-ephedrine excess in the loading racemate solution and 44.6% (+)-ephedrine excess in the recovery solution.

In general, the prepared (+)-ephedrine imprinted resin material showed high efficiency and successfully achieved a large part of the goals

of preparation, which can be explained due to the presence of the –COOH groups that can easily link to the (+)-ephedrine via proton transfer interaction as well as the utilized synthetic strategy, which effectively creates receptor binding sites that can recognize and fit with the (+)-ephedrine even in presence of its related enantiomer (-)-ephedrine.

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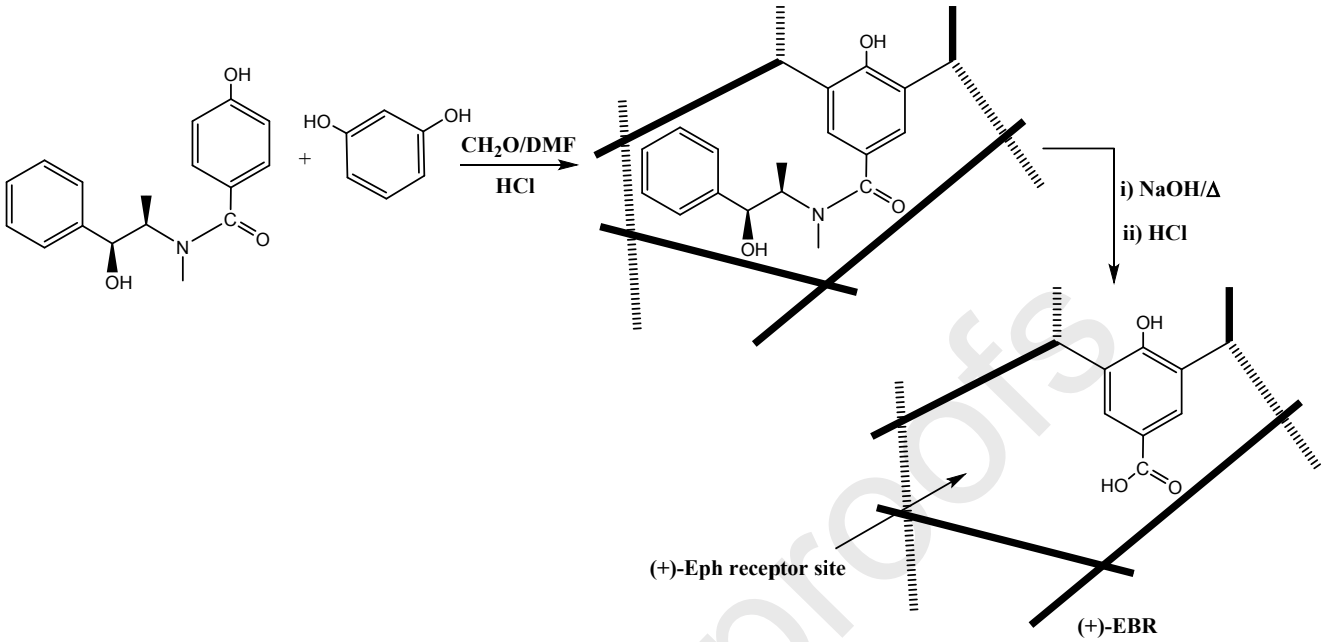
Graphical Abstract



Highlights

- Polymerizable (+)-ephedrine-4-hydroxybenzoic amide ((+)-Eph-HBA) was synthesized.
- The prepared (+)-Eph-HBA was implemented in polymerization with resorcinol and formalin.
- The (+)-ephedrine enantiomer were extracted from the polymer matrix.
- The polymeric resin was applied for chiral resolution of (±)-ephedrine racemate.

Conflict of interest

The authors declare that there is no any conflict of interest