Article

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Synthesis of Molecularly Imprinted Polymer for Removal of Effective Impurity (Benzhydrol) from Diphenhydramine Hydrochloride Drug

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The present study describes the synthesis and preliminary testing of molecularly imprinted polymers (MIPs) as scavenger resins for removal of the genotoxic impurities (GTI) benzhydrol from active pharmaceutical ingredients (API). A new molecularly imprinted polymer was synthesized using benzhydrol (template molecule), methacrylic acid (functional monomer), ethylene glycol dimethacrylate (cross-linker), 2,2′-azobisisobutironitril (intiator) and chloroform (porogenic solvent). To compare the performance of this polymer, a control polymer or non-imprinted polymer (NIP) was prepared under the same conditions without the use of template molecule. The synthesized polymers were characterized by FT-IR spectroscopy. Selectivity of the molecularly imprinted polymer for absorption benzhydrol impurities through adsorption experiments reviews and the results were compared with the adsorption of impurities by NIP. Various parameters were optimized such as time, pH, type of eluent for elution of impurities from polymer, concentration of sample and saturation of polymer. The proposed method was applied for removal of benzhydrol from Diphenhydramine hydrochloride syrup and passing it through the MIPs led to the quantitative removal of benzhydrol.

Keywords: Molecularly imprinted polymer; Benzhydrol; Diphenhydramine; Solid phase extraction.

INTRODUCTION

Diphenhydramine hydrochloride (abbreviated DPH, sometimes DHM) is a first-generation antihistamine possessing anticholinergic, antitussive, antiemetic, and sedative properties which is mainly used to treat allergies.[1] This drug has many impurities, which in this project was working on benzhydrol. Benzhydrol or Diphenylmethanol is one of the effective impurities in the drug diphenhydramine hydrochloride.[2]

In pharmaceutical manufacture, it is used as a fundamental component in antihistamines, antihypertensive agents and antiallergenic agents. Benzhydrol is used as an intermediate of pharmaceuticals (including antihistamines), agrochemicals, and other organic compounds. Benzhydryl is a skeleton for Histamine H1 antagonist which an ethylamine group is attached to a diphenylmethane structure. It is also used as a fixative in perfumery and as a terminating group in polymerizations. It is carcinogen and Oral (rat) LD50 is 5000 mg/kg.[3]

The need for efficient methods for sample preconcentration and clean up in medical, food and environmental analyses is constantly increasing. The advantages of SPE over liquid-liquid extraction (LLE) are that it is faster and more reproducible, cleaner extracts are obtained, emulsion formation is not an issue, solvent consumption is minimized and smaller sample sizes are required. Moreover, SPE can be easily incorporated into automated analytical procedures.[4-5]

Polymeric network materials capable of recognizing target molecules by molecular imprinting technique are available. This is a process where functional and cross-linking monomers are copolymerized in the presence of a target analyte (the imprint molecule), which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric network. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the analyte. In this way, a molecular memory is introduced into the polymer, which is now capable of selectively rebinding the analyte.[6-9]

In this context, it is not surprising that much of the current research in the molecular imprinting field is focused on SPE, as here the advantages of MIPs, especially their low price and their stability in different environments, come into play, whereas some of the limitations are less important than other separation techniques. MIPs are not only more selective than common sample treatment methods using C18 or ion exchange materials, but are at the same time more stable than (also very selective) immunoextraction

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matrices. Since MIPs are compatible with organic solvents, MIP-SPE can be applied directly after a solvent pre-extraction step. On the other hand, the low resolution factors are not an issue since SPE works in the adsorption-desorption mode. Thus, SPE seems to be one of the most promising application niches for MIPs today and at the same time the application that is closest to commercialization. This is also reflected in the comparatively large number of reports dealing with real samples. The need for separation of specific compounds from complex mixtures, industrial or biological, has led to an increase in the synthesis and use of molecularly imprinted polymers (MIPs), which in fact act as biomimetic materials. MIP-SPE has been used to extract the target analyte from blood plasma and serum,[10-11] urine,[12-14] bile, liver extract,[15] chewing gum, environmental water and sediment,[16-18] plant tissue,[19-20] etc.[21-22] In the present work, benzhydrol-molecularly imprinted polymer was synthesized using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as cross-linking agent.

This study was performed to ascertain the optimum conditions for maximum recovery of benzhydrol from drug diphenhydramine hydrochloride using a molecularly imprinted polymer as solid phase extraction adsorbent and the MISPE-eluate fractions were analyzed by spectrophotometry. Different experimental conditions, e.g. adsorption time, the type of eluting solvent, the effect of pH and concentration of sample have been investigated.

RESULTS AND DISCUSSION

The aim of this work was to evaluate the feasibility of using an imprinted polymer as scavenger resins for removal of the genotoxic impurities (GTI) benzhydrol from active pharmaceutical ingredients (API).

FT-IR spectra

Synthesized molecularly imprinted and control polymers were subjected to characterization by FT-IR. Both polymers have similar IR spectra indicating the similarity in the backbone structure.

In the IR spectra, the absorptions due to carboxyl OH stretch (ca. 3500 cm^{-1}), carbonyl group stretch (ca. 1730 cm^{-1}), C—O stretch (ca. 1260 cm^{-1}) and C—H vibrations (ca. 756, ca. 1390, ca. 1460, and ca. 2956 cm^{-1}) were observed. In addition to backbone similarity concluding, two important results were also acquired from spectra that are:

(1) The absorbances pertaining to benzhydrol, stretching of aromatic C=C (ca. 1493.92, and ca. 1597.70 cm^{-1}), stretching of aromatic C—H sp2 (ca. 3027.21, and ca. 3059.97 cm^{-1}), stretching of alcohol OH (ca. 3379.80 cm^{-1}), C—O stretch of alcohols (ca. 1077.13 cm^{-1}) are not observed in the MIP spectrum. This difference proves that imprint molecule (benzhydrol) has been sufficiently leached from MIP in the soxhlet extraction step.

(2) This feature is clear that absorbances attributed to the C—H stretch of methylene group (ca. 2954.48 cm^{-1}), carbonyl group stretch (ca. 1721.63 cm^{-1}), C—O stretch (ca. 1250.20 cm^{-1}) and C—H bend of CH_2 (ca. 1451.32 cm^{-1}) for the molecularly imprinted polymer are relatively stronger than for non-imprinted polymer. From this comparison, it was found that presence of imprint molecule (benzhydrol) causes incorporation of ethylene glycol dimethacrylate in the preparation of polymer to be increased.

Optimization of adsorption conditions of impurities on polymer

Effect of time on adsorption of impurities

Six portions of standard or sample solutions (100 mL) containing benzhydrol (0.1 mmol) were transferred into 250 mL beakers. Then exactly 1 g of MIP adsorbent were added to each beaker, and the mixtures were shaken vigorously for 30, 60, 90, 120, 150 and 180 min to facilitate adsorption of the benzhydrol onto the imprinted polymer particles. After the solutions were centrifuged, the amount of unadsorbed benzhydrol in the filtrate solutions was directly determined by spectrophotometry. Figure 1 shows that an equilibration time of about 150 min was required for 91% sorption. The amount of benzhydrol bound to the polymer was calculated by subtracting the amount of unadsorbed substrate from the initial amount of template.

Effect of sample pH on adsorption of impurities

The effect of varying pH values on impurities uptake was investigated using the batch procedure. Six portions of

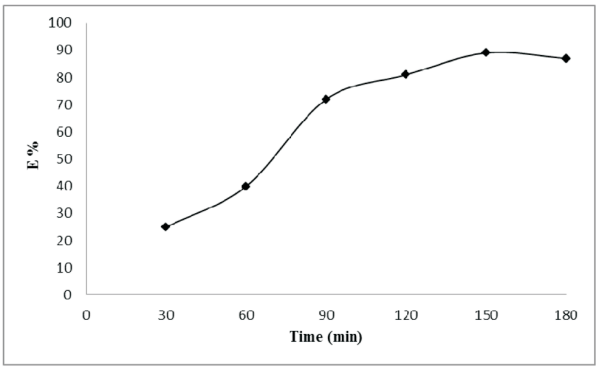

Fig. 1. Influence of adsorbing time on the Extraction of impurities.

standard or sample solutions (100 mL) containing benzhydrol (0.1 mmol) were transferred into 250 mL beakers, and the pH value was adjusted in range 3-8 with 0.01 mol.L^{-1}, HCl or NaOH. Then exactly 1 g of MIP adsorbent was added to each beaker, and the mixtures were shaken vigorously for 150 min to facilitate adsorption of the benzhydrol onto the imprinted polymer particles. According to the results shown in Figure 2 was found the adsorption quantity of impurities increases with the pH value increases and polymer does maximum adsorption at pH = 5. So pH 5.0 is chosen for this experiment and after pH 5.0 the adsorption capacity of the polymer decreases. The decrease of adsorption of bezhydrol at the pH values other than 5 may be due to formation of ionic compounds, which those are more soluble in water.

Adsorption capacity of impurities by MIP

Adsorption of impurities from sample solution was investigated in batch experiments. At this stage, the effect of sample concentration on the adsorption impurities was studied to obtain the best concentration for the sample solution. Solution with 0.4, 0.6, 0.8, 0.9, 1.0, 1.2 1.5 and 2.0 mmol L^{-1} concentrations of impurities were prepared and the pH value was adjusted to 5.0 with 0.01 mol.L^{-1}, HCl or NaOH. Then exactly 1 g of MIP adsorbent were added to each beaker, and the mixtures were shaken vigorously for 150 min to facilitate adsorption of the benzhydrol onto the imprinted polymer particles. In order to reach the Saturation the initial impurities concentrations were increased until the plateau values (adsorption capacity values) were obtained. The data were shown in Figure 3. The average maximum adsorption capacity was 98.3 μmol g^{-1} for three replicate measurements.

Comparison of MIP and NIP adsorption

Two solutions were prepared at a concentration of 10^{-3} mol L^{-1} of impurities and the pH value was adjusted to 5.0 with 0.01 mol.L^{-1}, HCl or NaOH. Then were added 1 g of MIP to a solution and to another 1 g NIP and the mixtures were shaken vigorously for 150 min to facilitate adsorption of the benzhydrol onto the imprinted polymer particles. Then Both filtrate and adsorption the filter solution was measured with a spectrophotometer. The results in Table 1 show that MIP performs better adsorption than the NIP and this confirms the accuracy of the molecular format.

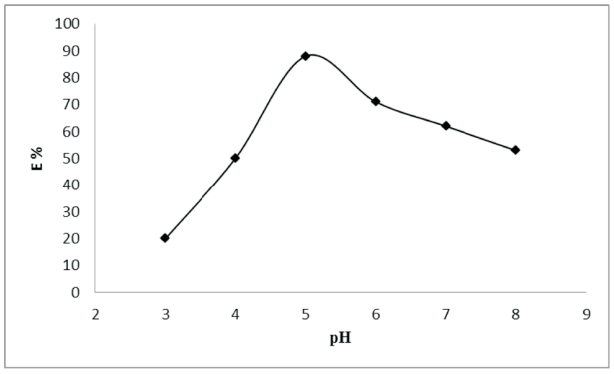

Fig. 2. Effect of pH of sample solution on impurities uptake.

Table 1. Comparison of MIP and NIP

Polymer Type	Initial concentration[a]	Final concentration[a]	K_d	Extraction (%)
MIP	1000	47	0.2	95.3
NIP	1000	962	3.9×10^{-4}	3.8

[a] μmol L^{-1}

Efficient eluent for removal of the retained benzhydrol

In order to choose a proper eluent for the retained benzhydrol, after the extraction of 0.1 mmol benzhydrol from 100 mL of aqueous sample solution, the benzhydrol was stripped with 5 mL of various concentrations of different organic and mineral acids. Benzhydrol was stripped with 5 mL of eluents. In order to choose the most efficient eluent, different organic solvents and various concentrations of different acids in organic solvents were tested. As it is shown in Table 2, acidic eluents are more effective for stripping of benzhydrol from polymer. The lipophilicity of prepared particles assists that by the protonation of MAA carboxylic groups in binding sites with the help of H^+ ions, acidic eluent interacts, via hydrogen bonds, with the poly-

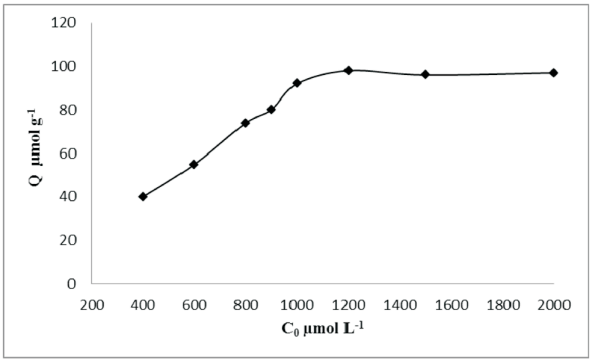

Fig. 3. The effect of benzhydrol initial concentration on the adsorption quantity of synthesized polymer. Other conditions: 1 g of synthesized polymer, pH 5.0, shaking time 150 min, temperature 25 °C.

Table 2. Effect of type of eluent on extraction efficiency

Eluent	Recovery %
0.2 mol L^{-1} H_2SO_4	79.26
0.4 mol L^{-1} H_2SO_4	80.11
0.4 mol L^{-1} HCl	46.01
0.4 mol L^{-1} CH_3COOH	74.46
Methanol	56.89
Ethanol	51.89
0.2 mol L^{-1} KOH in methanol	5.76
0.2 mol L^{-1} HCl in methanol	92.11

mer to disrupt the interaction between benzhydrol and polymer. From the data given in Table 2, it is obvious that 5 mL of 0.2 mol L^{-1} HCl in methanol can strip the retained benzhydrol almost quantitatively. Thus, this eluting solvent was chosen for further studies.

Analysis of real samples

For evaluate the performance of synthesized MIP for absorption of impurity from the real sample, diphenhydramine syrup was chosen as a real sample and these were analyzed before and after of purification by high performance liquid chromatography. Thus, 12 mL of the syrup was diluted to 50 mL with water and its pH was adjusted to five. 20 mL form this solution was mixed with 0.5 gr of polymer for 150 min and its supernatant and solution of syrup without purification were injected to HPLC column separately. The condition for HPLC separation was mentioned in apparatus section.

The obtained results were shown in Figure 4. The peak corresponding to the benzhydrol appeared after 4 minutes, the results show that the intensity benzhydrol peak reduces apparently after purification. In addition, it demonstrates the selectivity of the polymer to the target molecule rather than diphenhydramine and these proves the performance of molecularly imprinted polymer as scavenger resins for removal of the genotoxic impurity.

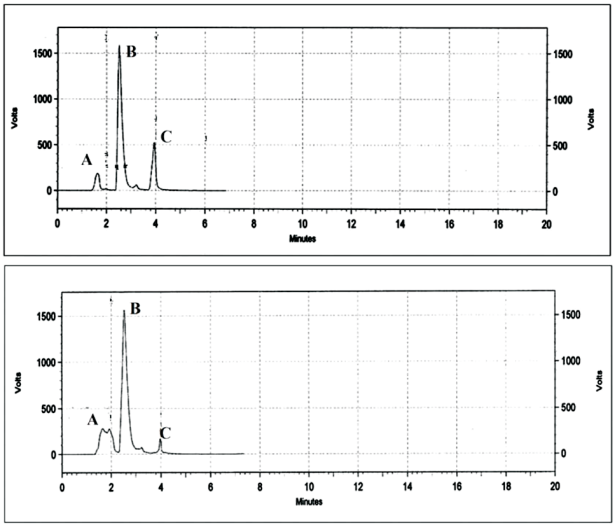

Fig. 4. Chromatogram of diphenhydramine syrup before and after of purification.

EXPERIMENTAL

Reagents: All materials that were required during the project include, Methacrylic acid (MAA), 2,2′-azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA), chloroform, methanol, hydrochloric acid, sodium hydroxide, sulfuric acid, ethanol, acetic acid, potassium hydroxide, potassium dihydrogen phosphate, phosphoric acid and acetonitrile were obtained from Merck. Diphenhydramine hydrochloride and benzhydrol were obtained from Sigma-Aldrich. All chemicals and reagents were of analytical grade and used without any further purification. A stock solution of benzhydrol was prepared by dissolving an appropriate amount of benzhydrol in ethanol and pH was adjusted with HCl or NaOH.

Apparatus: HPLC measurements were carried out on Kenauer D-14163 (Berlin, Germany). The high-performance liquid chromatography (HPLC) system was composed of a D-14163 pump (Knauer Co., Germany), a reversed C18 column (4.6 × 150 mmi.d.; Knauer, Germany), a D-14163-detector (Knauer, Germany) set at 220 nm, and a reversed C18 guard column (4.6 × 15 mm; Knauer, Germany). The mobile phase consisted of acetanilide: 0.1 mole L^{-1} of phosphate buffer, pH = 3 (35:65, v:v), the flow rate was 1.2 mL min^{-1} at 30 °C, and the injection volume was 20 µl. Quantification was carried out by the external standard method using a commercial standard sample of benzhydrol. Integration of the chromatograms was made with the Knauer EZChrom software package.

UV-VIS spectrophotometer (Varian-Cary100, Australia) was used for measuring impurities in Standard solutions after contact with polymer. Soxhlet extraction apparatus was used for removing the target molecule of the polymer network. A model 3510 Jenway pH-meter was used for the pH adjustments. The Fourier transform infrared (FTIR) spectra of template molecule (benzhydrol), non-imprinted and molecularly imprinted polymers were obtained using a 6700 Thermo Nicolet FTIR spectrometer which IR spectra were recorded in the range 400-4000 cm^{-1}.

Preparation of a benzhydrol-MIP and the corresponding non-imprinted polymer: An MIP and a non-imprinted polymer (NIP) should be prepared in parallel and with identical compositions (except that template is to be omitted from the NIP). The procedure for the polymer synthesis was as follows: To a three-

necked round-bottom flask were added template (benzhydrol: 0.5 mmol or 0.46 g), functional monomer (MAA: 10 mmol or 0.85 mL), cross-linker (EGDMA: 50 mmol or 9.45 mL) and initiator (AIBN: 1.25 mmol or 0.21 g) in chloroform (100 mL). The mixture was sparged with nitrogen for 10 min to remove dissolved oxygen, which can inhibit free radical polymerization. The polymerization was allowed to continue in a water bath at 60 °C for 18 h. After polymerization, a hard polymer monolith was obtained, which was crushed and ground into a fine powder with a mortar and pestle. Soxhlet extraction was performed to remove the template with 70/30 (V/V) methanol/acetic acid overnight. Then, the polymer was washed several times with pure methanol to remove the acetic acid and facilitate drying. The dried polymer is ready for testing. The method for preparation of NIP was exactly similar to procedure for the synthesis of MIP with an exception that benzhydrol (imprint molecule) was omitted in the preparation of NIP. Schematic illustration of the process of preparing the benzhydrol-MIP is shown in Figure 5.

Batch procedure: In 100 mL polyethylene bottles, previously cleaned with detergent, DDI water, dilute nitric acid and DDI water in sequence, added buffer solution and the benzhydrol solution, and immersed imprinted polymer with shaking at 25 °C. At pre-fixed time, an aliquot of the supernatant was separated and the benzhydrol were determined by UV-Vis spectrophotometer at 220 nm. The adsorbed benzhydrol was eluted with 0.2 M HCl in methanol and the desorbed benzhydrol was measured with UV-Vis spectrophotometer. The phase distribution ratio (Kd) and adsorption capacity (Q) were calculated using the following equations:

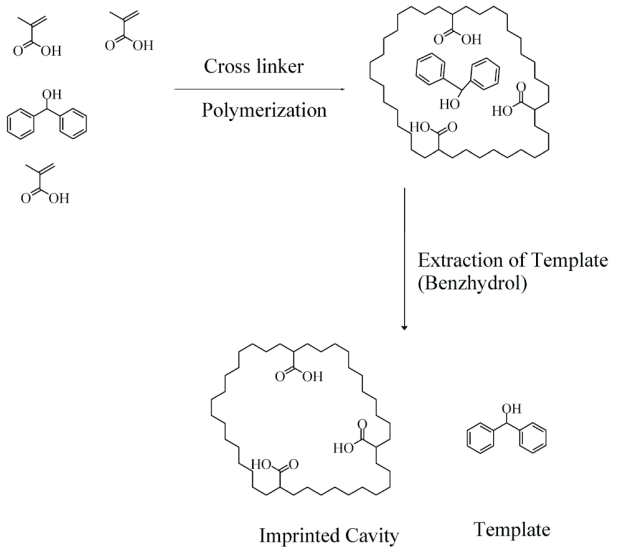

Fig. 5. Schematic illustration of the process of preparing the benzhydrol-MIP.

$$K_d = \frac{C_i - C_f}{C_f} \times \frac{V}{W} \quad (1)$$

$$Q = \frac{(C_i - C_f)V}{W} \quad (2)$$

where Q represents the adsorption capacity (μmol L^{-1}), C_i and C_f represent the initial and equilibrium concentration of metoprolol in the aqueous phase (μmol L^{-1}), W is the weight of the polymer (g) and V is the volume of the aqueous phase (L). The percent extraction, E, was calculated using the following equation:

$$E = \frac{C_i - C_f}{C_i} \times 100 \quad (3)$$

CONCLUSIONS

Novel molecularly imprinted polymers have been designed which can recognize benzhydrol as a pharmaceutical impurity. These MIPs have potentials to purify APIs as selective adsorbents due to high affinity binding sites. Rebinding tests indicates that high-affinity interactions are present between the binding sites of the hosts and the target impurities, which results in high efficiency purification and it is suitable for repeated use without considerable loss of adsorption capacity. The new MIPs developed answer to the industrial call for systems that provide a high binding between impurities and MIPs, as high recovery of the APIs is achieved.

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