

Propranolol HCl Imprinted Polymeric Microspheres: Development, Characterization and Dissolution

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Summary

Specificity to target molecule, high selectivity and sensitivity to release and targeting were achieved using molecularly imprinted polymer (MIP) systems. In this study, propranolol HCl (PHCL) imprinted methacryloylamidohistidine-Co/PHCL (MAH-Co/PHCL) microspheres were prepared by suspension polymerization. Differences in pH, concentration and time are very important parameters in determining the adsorption capacity of the imprinted microspheres. In the adsorption studies, propranolol HCl was desorbed first from imprinted microspheres using 40% methanolic potassium hydroxide solution. Following the determination of appropriate pH and concentration values for adsorption of PHCL onto imprinted microspheres, time to reach adsorption equilibrium and maximum adsorption values were calculated. In vitro dissolution of imprinted microspheres was compared to that of pure drug and its commercial tablet. In the in vitro dissolution studies, flow-through-cell method defined in USP XXIV was used with UV spectrophotometric quantification method. Comparing the dissolution test results, it was found that PHCL imprinted microspheres released the active agent in a prolonged pattern.

Key Words: MIP system, propranolol hydrochlorid, dissolution

Received□ :□10.07.2009

Revised□ :□10.08.2009

Accepted□ :□15.09.2009

Propranolol HCl Bellekli Polimerik Mikroküreler: Geliştirme, Karakterizasyon ve Çözünme

Özet

Moleküler bellekli polimer (MIP) sistemler ile hedef moleküle spesifiklik, salım ve hedeflemede yüksek derecede seçicilik ve duyarlılık konularında başarı elde edilmektedir. Bu çalışmada, propranolol HCl (PHCL) baskılı metakriloamidohistidin-Co/PHCL (MAH-Co/PHCL) mikroküreleri, süspansiyon polimerizasyonu yöntemi ile hazırlanmıştır. pH, derişim ve zaman deęişimi, bellekli mikrokürelerin adsorpsiyon kapasitesinin belirlenmesinde çok önemlidir. Adsorpsiyon çalışmalarında, öncelikle % 40'lık metanollü potasyum hidroksit çözeltisi ile bellekli mikrokürelerden PHCL desorbe edilmiştir. Bellekli mikrokürelere PHCL'in adsorpsiyonu için uygun pH ve derişim deęerleri belirlendikten sonra, adsorpsiyon dengesine ulaşma süresi ve maksimum adsorpsiyon deęeri hesaplanmıştır. Bellekli mikrokürelerin in vitro çözünmesi, saf etkin madde ve piyasa tableti ile karşılaştırılmıştır. İn vitro çözünme deneylerinde, USP XXIV'de tanımlanan sürekli akış yöntemi ile birlikte UV-spektrofotometrik miktar tayini yöntemi kullanılmıştır. Yapılan çözünme karşılaştırmalarında, piyasa tabletine oranla PHCL bellekli mikrokürelerin salımının çok daha kontrollü olduęu görülmüştür.

Anahtar Kelimeler: MIP sistem, hidroklorür, çözünme

INTRODUCTION

Molecular imprinting is a method of introducing a specific molecular arrangement into a uniform polymeric matrix. It requires the mixture of a monomer and a template molecule with an appropriate solvent using a crosslinking agent in a porogenic environment to obtain a synthetic specific molecular recognition(1).

In the molecular imprinting process, a template molecule

is allowed to form reversible interactions (covalent/non-covalent) with suitable functionalized monomers in the presence of a cross-linking monomer. As a result of polymerization, a record of the template molecule in shape and chemical properties is obtained in the macroporous polymer. Removal of the template by competitive extraction procedures yields a matrix structure that can re-adsorb the template molecule. This novel structure exhibits antibody-

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like and enzyme-like activities comparable to those observed in biological recognition systems (2).

Extensive attention is on the pharmaceutical application of this technology due to the high affinity and selectivity similar to natural receptors, superiority of stability (physical and chemical) over biomolecules, long storage endurance (several years), recovery yield over 99%, simplicity of preparation, and repeated use with no loss in memory effect.

The potential pharmaceutical applications of molecularly imprinted polymer (MIP) drug delivery systems include prolonged drug delivery, chiral resolution of racemic mixtures, separation and quantification of drug and bioactive materials, trap of toxic materials in the biological fluids, as a diagnostic sensor, screening new materials with potential pharmacological effects, and detection of materials in drug abuse (3,4).

However, there is huge potential in drug delivery for technologies that can bring about intelligent drug release or can target a therapeutic pay-load to a particular site of action. Intelligent drug release refers to the release, in a predictable way, of a therapeutic agent in response to specific stimuli such as the presence of another molecule, whilst drug targeting is best exemplified by the "magic bullet" approach, where a drug conjugated to a targeting vector, such as an antibody or a peptide, interacts with specific sites of interactions. A good example of this might be a cell surface epitope. In both of these areas molecular imprinting has very real potential (5).

One further potential advantage of imprinted polymers as drug delivery devices is that, in the case where a racemic mixture of a drug is used, they can selectively release the more effective enantiomer (6). This novel approach of enantiomer-selective administration can perhaps increase the therapeutic window of racemic drugs, which are usually considerably cheaper to produce than the pure enantiomer. The incorporation of MIPs within delivery systems is of potential value in the administration of chiral drugs, where significant pharmacological activities are associated with only one enantiomer. A number of *in vitro* studies have demonstrated the potential use of MIPs in the enantioselective-controlled delivery of chiral drugs (i.e.,

β -blockers and non-steroidal anti-inflammatory drugs) by both the oral and transdermal routes (7).

For example, a current study demonstrated the potential of MIP composite membranes based on cellulose in controlling the release of S-propranolol into the skin. The degree of stereoselectivity demonstrated would result in considerably higher therapeutic advantage when considering the differential pharmacological activities of the two enantiomers of propranolol (8).

Since biological recognition mainly occurs in aqueous systems, and many biologically important compounds are not very soluble in non-polar organic solvent, it is quite important to make MIPs capable of recognition in aqueous media⁹. For this purpose, combining the features of β -cyclodextrin (β -CD) and the molecular imprinting method, the molecularly imprinted poly(β -CD) was prepared and was successfully used to separate some biomolecules, such as steroids and cholesterol in aqueous solution⁽¹⁰⁾. Molecularly imprinted CDs (MI-CDs) have three-dimensional structures that bind the template strongly and selectively. MI-CDs are referred to as artificial antibodies and will prove to be useful materials in several fields. However, pharmaceutical application of MI-CDs is difficult because MI-CDs ground with mortar and pestle are irregular in size and shape. In order to extend the application of MI-CDs, researchers attempted to prepare microspheres of MI-CD (MSs-MI-CD) (11).

The aim of this study was to achieve the prolonged release of propranolol HCL (PHCL), the beta-adrenoreceptor antagonist, with rather short elimination half-life. For this purpose, MIP systems were chosen for their high selectivity, high stability, practical preparation and low production costs.

MATERIALS and METHODS

Apparatus

Equipment used in the study: Differential Scanning Calorimeter (DSC) (Shimadzu-DSC 60, Japan); elemental analysis equipment (Elemental Analyzer EA 1108, Italy); Fourier Transferring Infrared Spectrophotometer (FTIR) (Perkin Elmer, UK); mechanical stirrer (Heidolph RZR 2051, Germany); Ultraturrax (Janke&Kunkel IKA LaborTechnik, Germany); particle size analyzer (Malvern

Mastersizer Hydro 2000-S, UK); pH-meter (Profi-Lab WTW 597, Germany); flow-through-cell dissolution apparatus (Sotax-CE70, Switzerland); Scanning Electron Microscope (SEM) (CamScan S4, UK); UV Spectrophotometer (Shimadzu-160 A, Japan); and X-Ray Diffraction (XRD) equipment (Rikagu, Japan).

Materials

PHCL was purchased from Amphar b.v., Holland. Commercial tablet (Dideral[®]) was supplied by Sanofi Doğu İlaç A.Ş., Turkey.

Methods

Physicochemical properties of PHCL

Physicochemical properties of PHCL were specified, including UV, IR spectrophotometric and thin layer chromatographic (TLC), DSC, XRD and elemental analyses, to follow any possible changes that may appear after the imprinting process.

Stability of PHCL

Physical and chemical stabilities of PHCL in media used in the study were investigated using different methods. FTIR, DSC, XRD and TLC methods were used to determine the stability of PHCL under imprinting, desorption and adsorption conditions. The results obtained were compared with those of the pure PHCL and the non-imprinted polymer (NIP).

Preparation of PHCL imprinted microspheres

Following the synthesis of 2- methacryloylamidohistidine monomer, 2- methacryloylamidohistidine Cobalt (II) complex [MAH-Co(II)] was synthesized (12).

The suspension polymerization method is much less time-consuming and simpler to carry out with a small sacrifice in the size distribution of the microspheres. Therefore, this technique was used to prepare PHCL-imprinted microspheres. MAH-Co(II) (1.0 mmol) and PHCL (1.0 mmol) were dissolved in a small bottle containing 3 mL of ethanol and shaken for 20 min. The dispersion medium was prepared by dissolving 0.2 g polyvinylalcohol (PVA) in 60 mL distilled water. 1 mmol solution of MAH-Co(II)-PHCL monomer complex was added to the mixture of 8 mL ethylene glycol dimethacrylate (EGDMA) and 12 mL toluene/acetonitrile and dissolved in 0.09 g

azobisisobutyronitrile (AIBN) monomer mixture. This solution was then added to the dispersion medium and kept at the polymerization reactor with a thermostatic water bath and magnetic stirrer (constant stirring rate of 600 rpm). The reactor was degassed using nitrogen and closed. The polymerization process was performed with a reactor temperature of 75°C for 6 h followed by 90°C for 3 h. The monomer residue was washed with water-ethanol mixture and dried at 70°C under vacuum for 48 h. NIPs were prepared with the same method using MAH-Co(II) and EGDMA.

Desorption and adsorption studies

For the desorption of PHCL, the imprinted polymer was put in 200 mL 40% methanolic potassium hydroxide solution and stirred at 60°C and 750 rpm for 4 days with a magnetic stirrer. Following filtration, quantification of PHCL was performed at 235 nm using UV spectrophotometer in the filtrate. The recovered polymer by this filtration was washed with distilled water 4 times, dried at a vacuum oven at 60°C and stored for further analyses. Desorption was calculated using the equation below:

$$\text{Desorption (\%)} = \frac{\text{PHCL released into the desorption medium}}{\text{PHCL adsorbed}} - 100$$

For the adsorption of PHCL onto the imprinted microspheres, the effects of medium pH and PHCL concentration on the adsorption capacity and time to reach adsorption equilibrium were investigated.

Effect of medium pH on adsorption

Adsorption studies were performed in 6 different pH media (pH 3, 4, 5, 6, 7 and 8) to investigate the pH value at which PHCL adsorbs onto the imprinted microsphere (n=3 only for pH 6). Propranolol concentration was kept constant in all studies.

Effect of PHCL concentration on adsorption

Following the determination of the pH value at which adsorption is maximum (pH 6), the effect of PHCL concentration was tested at 9 different concentrations: 25, 50, 100, 200, 500, 1000, 1500, 2000 and 3000 ppm (n=3 only for 1500 ppm). Solvation medium was distilled water. Determination of the time to reach adsorption equilibrium

Time to reach adsorption equilibrium was determined at pH 6 medium using 100 ppm PHCL and samples at 1, 3, 6, 10, 15, 25, 30, 40, 50 and 60 minutes (n=3). Magnetic stirring at 750 rpm and room temperature were used.

Characterization of MIP systems

Surface characteristics and the shapes of PHCL imprinted microspheres and microspheres following desorption and adsorption were determined using SEM after distribution on carbon band and coating with gold. Particle sizes and distributions of PHCL imprinted microspheres were determined using a laser diffraction apparatus.

Elemental analyses of PHCL imprinted microspheres and the microspheres following desorption and adsorption were performed using 1-2 mg samples at 1020°C. FTIR spectra of pure PHCL, NIP and PHCL imprinted microspheres, and the microspheres following desorption and adsorption were taken at a range of 4000-200 cm⁻¹ using potassium bromide disks. XRD analyses of pure PHCL and PHCL imprinted microspheres were performed at a range of 5-40°C, using 40 kV voltage, 30 mA current and 2°.min⁻¹ scanning rate. DSC was used for the thermal analyses of pure PHCL and PHCL imprinted microspheres. Samples were weighed accurately on aluminium and closed under pressure. Thermal analyses were performed using aluminium reference, 10°C.min⁻¹ temperature increase and under 50 mL.min⁻¹ nitrogen gas flow. Heating was applied at the temperature range of 40°C-400°C.

In vitro dissolution studies

PHCL release from microspheres after desorption and adsorption procedures were tested in in vitro conditions using flow-through-cell method defined in USP 31, 200813. Medium of distilled water, flow rate of 8 mL.min⁻¹, temperature of 37°C and powder cells were used for the dissolution studies. Samples collected automatically at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 and 210 min were evaluated using UV spectrophotometer. Six cells were used for each test.

In vitro dissolution test results of the microsphere prepared were compared to those of pure PHCL and a commercially available tablet using the same test conditions.

RESULTS and DISCUSSION

Physicochemical properties of PHCL

Since UV-spectrophotometry was used for the calculation of PHCL quantity in desorption and adsorption medium and in vitro dissolution studies, this method was validated. Following the UV spectrophotometric studies, wavelengths for the maximum absorbances were determined to be at 289 nm in distilled water and pH 6 medium and at 235 nm in the 40% methanolic potassium hydroxide solution. The I_{max} value determined for distilled water was in accordance with the USP XXIV. Linearity was obtained with the same correlation coefficients (r=0.999) in three media (n ≥ 3).

In the validation studies of the UV spectrophotometric method, repeatability and reproducibility-RSD (relative standard deviation) values were both found to be less than 2% which are appropriate values (14). Accuracy, which shows the percentage of recovery of a known amount of active agent, was determined to be 101.63% and 100.86% for the two concentrations tested which is in the range of 98-102% reported in the literature (14). With respect to sensitivity values of the analytical method, limit of detection (LOD) for PHCL was found to be 2.74 µg.mL⁻¹ while the limit of quantification (LOQ) was 8.31 µg.mL⁻¹.

Results of the elemental analysis of PHCL revealed 5.2 N, 66.6 C and 5.1 H. R_f value of PHCL in TLC studies was determined to be 0.26 (n=3).

Stability

Results of the stability tests of imprinted microspheres are given under the title of 'characterization studies'.

Testing the stability in the analytical validation procedure comprises testing the effects of carrier and work conditions on the analyte. TLC method was selected for the stability tests. During the TLC studies, the system parameters given in the pharmacopeia were used for the identification of PHCL and to follow its stability in the working conditions and media (15). Silicagel 60 G was the coating material and concentrated ammonium-methanol (1:99) was the mobile phase in this system.

R_f value of PHCL in the imprinted polymer was found to be 0.26 while there was no spot for the non-printed polymer in the TLC analyses. This shows that there is no interaction

between the carrier and the active agent. TLC analysis under the desorption conditions of pure PHCL showed Rf value of 0.23. The similar color and size of the spot and the Rf value obtained showed that PHCL remained stable even under severe conditions. Rf value under adsorption conditions at pH 6 was 0.29; it was 0.22 in the dissolution medium and conditions.

In a previous study on the stability of PHCL, aqueous suspensions of 1 mg.mL⁻¹ concentration obtained from PHCL tablets were kept at 25°C and 2°C for a period of 4 months (16). After testing the concentration of PHCL, pH value, microbiological character and redispersibility, PHCL was determined to be stable in aqueous medium. Stability tests under storage conditions for the imprinted microspheres prepared, the solid particulate system, were not performed based on the results of this study on aqueous suspension.

PHCL imprinted microspheres

Molecular imprinting can be approached in two ways: the self-assembly and the preorganized approaches. These two approaches differ with respect to the interaction mechanism in prepolymerization. The self-assembly molecular imprinting approach involves host-guest complexes produced from weak intermolecular interactions (e.g. ionic or hydrophobic interactions, hydrogen bonding and metal coordinations) between the template and the monomer (17). Self-assembled complexes are established in the liquid phase and are then sterically fixed by polymerization with a high degree of crosslinking. The preorganized molecular imprinting approach involves formation of strong, reversible, covalent arrangements of the monomers with the template molecules before polymerization. Thus, the template molecules need to be "derivatized" with the monomers before the actual imprinting is performed (17). Removal of the template from the network obtained affords complementary binding sites that can selectively rebind the same template.

Synthesizing macroporous MIPs or creating surface imprinting using metal-ligand monomers may be used to imprint large molecules as peptides and proteins to develop delivery systems with high loading capacity and controlled release (12). Therefore, PHCL was imprinted in a monomer system MAH-Co(II).

Different imprinting methods to obtain polymer beads with various size ranges are normal suspension polymerization, suspension polymerization in perfluorocarbon, seed polymerization, graft polymerization, polymerization of reactive surfactants, and precipitation polymerization (18,19). Suspension polymerization technique was used in this study, and microspheres with good PHCL recognition were obtained. In the suspension polymerization technique, the monomer is dispersed as liquid spheres in a medium containing a stabilizing agent (usually buffered aqueous phase). The polymerization starter is solubilized in the monomer phase. Dispersion is maintained by mechanical agitation. Following the application of appropriate temperature and stirring parameters, each of the liquid monomer spheres polymerizes independently.

Of the imprinting strategies used, it has become evident that the use of noncovalent interactions between the template molecule and the functional monomers is the most versatile. The noncovalent method was also involved in this study. These kinds of interactions resemble the recognition pattern observed in nature. Binding to the surface of the polymer and also to the internal volume could be achieved. In this course, the bioadhesion and volume characteristics of the carrier were affected. Following polymerization, the extraction of excess monomer and the template molecule is a complementary procedure for the functions of the remaining polymer-template molecule (20).

Adsorption capacities of PHCL imprinted microspheres prepared with different methods were compared in both organic and aqueous solvents (19). In the organic solvent (toluene + 0.5% acetic acid), adsorption percentages were 50, 40, 35, 15 and 10 for sedimentation, suspension, volume, emulsion and two-step swelling polymerization techniques, respectively. In the aqueous solvent (25 mM sodium citrate + 0.5% acetic acid + 2% ethanol, pH 4.6), adsorption capacities were 20%, 19%, 19%, 15% and lower for two-step swelling, suspension, volume, emulsion and sedimentation polymerization techniques, respectively. As can be followed, suspension polymerization method leads to a higher adsorption capacity than the other methods. Following the imprinting procedure in this study, the percentage of PHCL that could be adsorbed onto the recognition sites was determined to be 76.5% (n=3).

At the end of the studies for the selection of the appropriate pH value for adsorption (mg active agent/g polymer), adsorption was found to be maximum at pH 6 with a value of $45.84 \pm 0.35 \text{ mg.g}^{-1}$ (n=3). Results of these adsorption studies are shown in Figure 1.

Studies on the effect of PHCL concentration on adsorption revealed maximum adsorption of $213 \pm 7.34 \text{ mg.g}^{-1}$ (mg active agent/g polymer) with 1500 mg.L^{-1} PHLC (n=3), and the results are shown in Figure 2.

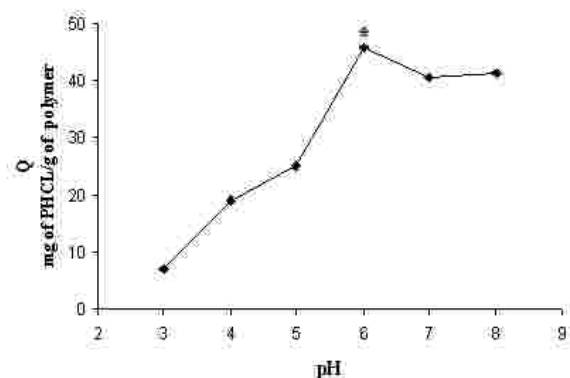


Figure 1 : Effect of medium pH on adsorption of PHCL onto the imprinted microspheres (*mean \pm SE, n=3).

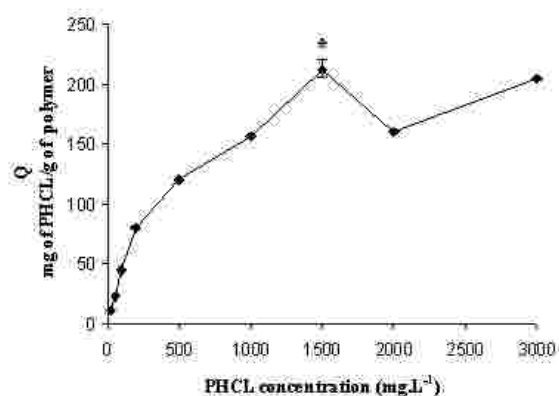


Figure 2 : Adsorption of different concentrations of PHCL onto imprinted microspheres in distilled water (*mean \pm SE, n=3).

Maximum adsorption was at the 30th minute with an adsorption value of $46.01 \pm 1.20 \text{ mg.g}^{-1}$ (mg active agent/g polymer) (n=3). Results of the adsorption equilibrium time determination studies are given in Figure 3. When the profile is examined, it can be observed that the values of adsorption at the first minutes are close to the maximum adsorption at 30 minutes. This shows that the equilibrium is reached rapidly.

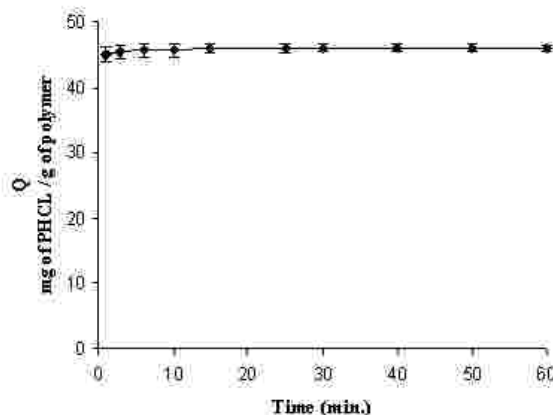


Figure 3 : Time to reach the adsorption equilibrium for PHCL (mean \pm SE, n=3).

Characterization of PHCL imprinted microspheres

The highly crosslinked network of MIP materials formed during the molecular imprinting process are part of a class of materials known as macroporous polymers. Due to the insoluble and intractable nature of the MIP systems, methods involving the solution states can not usually be used for characterization. Therefore, there are only a limited number of direct physical characterization methods for imprinted polymers (21,22).

Characterization of MIP systems may be achieved in three aspects: 1) chemical characterization, 2) morphological characterization, and 3) molecular recognition behavior characterization.

Surface morphology

Microscopic methods (light, SEM) are used to characterize MIPs morphologically. SEM in particular is often used to image (macropores). A picture of PHCL imprinted microspheres is given in Figure 4 which clearly illustrates its spherical form.



Figure 4 : SEM picture of PHCL imprinted microsphere.

Particle size distribution

The PHCL MIP system prepared using suspension polymerization technique ended with spherical polymeric particles. The size distribution depends on various parameters of polymerization conditions, principally the stirring rate. Polymeric particles with sizes greater than 50 μm can be produced by suspension polymerization (23). Freshly prepared microspheres were in agreement with the literature, with a mean particle size of 28.334 μm and homogeneous distribution.

Elemental analysis

Chemical characterization requires elemental micro-analysis and FTIR analyses. Elemental micro-analysis can be used in a routine manner to measure the percentage by mass of carbon, hydrogen, nitrogen, chloride, etc. within samples. Results obtained for the PHCL imprinted microsphere were 0.5% N, 63.9% C, 18.6% H; 0.5% N, 53.9% C, 6.9% H for microspheres after desorption and 0.5% N, 58.9% C, 6.4% H for microspheres after adsorption.

There was a decrease in the percentages of atoms following desorption of the imprinted microspheres and an increase in the atoms after adsorption when compared to the desorbed form. This provides a clue regarding the successful unloading and reloading of PHCL to the imprinted carrier. Unfortunately, the method is not sufficiently sensitive to enable the detection of trace quantities of template remaining in molecularly imprinted polymers (21).

FTIR analysis

FTIR spectroscopy can be applied in a similar fashion to elemental micro-analysis to extract quantitative information on the composition of the polymer.

Looking at the FTIR spectra (Figure 5), the band that is around 900 cm^{-1} in the PHCL imprinted microspheres was observed to disappear after desorption and reappear after adsorption. Following imprinting and adsorption, three significant peaks at 1729, 1261 and 1157 cm^{-1} (Figure 5C and 5E), which correspond to C=O and C-O-C stretching modes, respectively, support the successful occurrence of imprinting and adsorption. On the other hand, when Figure 5D was compared to 5E, intensities of the two bands in Figure 5C (carboxylic acid band at 3500 cm^{-1} and the band around 500 cm^{-1}) decreased after desorption while they

increased after adsorption. These differences display the formation of H bands between PHCL and the polymer. In summary, all the bands show the adsorption of PHCL onto/into the microspheres.

Characteristic peaks in the fingerprint region of PHCL are observed with difficulty in Figure 5C and 5E due to the low molar amount of PHCL relative to EGDMA in the polymer.

FTIR and solid state nuclear magnetic resonance (NMR) methods are useful for the measurement of functional group incorporation, especially for the quantification of the degree of polymerization and reactivity for each type of polymerizable group on the monomers. A measure for the degree of polymerization is assessed from the number of unreacted double bonds, which are quantified by integration of the area under the peak corresponding to the related wavelenghts (22).

There have been few attempts to probe the nature of the template-polymer interaction, not because it is considered unimportant, but because of the practical difficulties involved in characterizing intermolecular interactions within an amorphous, insoluble polymeric material. The formation of highly cross-linked porous polymers is difficult to characterize fully, even by solid-state NMR and FTIR spectroscopy (24).

X-ray diffraction analysis

XRD patterns of pure PHCL and PHCL imprinted microspheres are demonstrated in Figure 6. Characteristic peaks observed in the XRD patterns show high crystallinity of pure PHCL (Fig. 6A). Looking at the XRD patterns of the PHCL imprinted microspheres, all the principal peaks of PHCL were found to disappear (Fig. 6B). This indicated the formation of a new structure.

Thermal analysis

In this study, some evidence of complexation was obtained from the calorimetric studies by DSC. Thermograms of pure PHCL and PHCL imprinted microspheres are demonstrated in Figure 7. The PHCL thermal curve (Fig. 7A) is typical of crystalline anhydrous substances and is characterized by a sharp endothermic effect (peak temperature at 164°C) assigned to its melting. PHCL

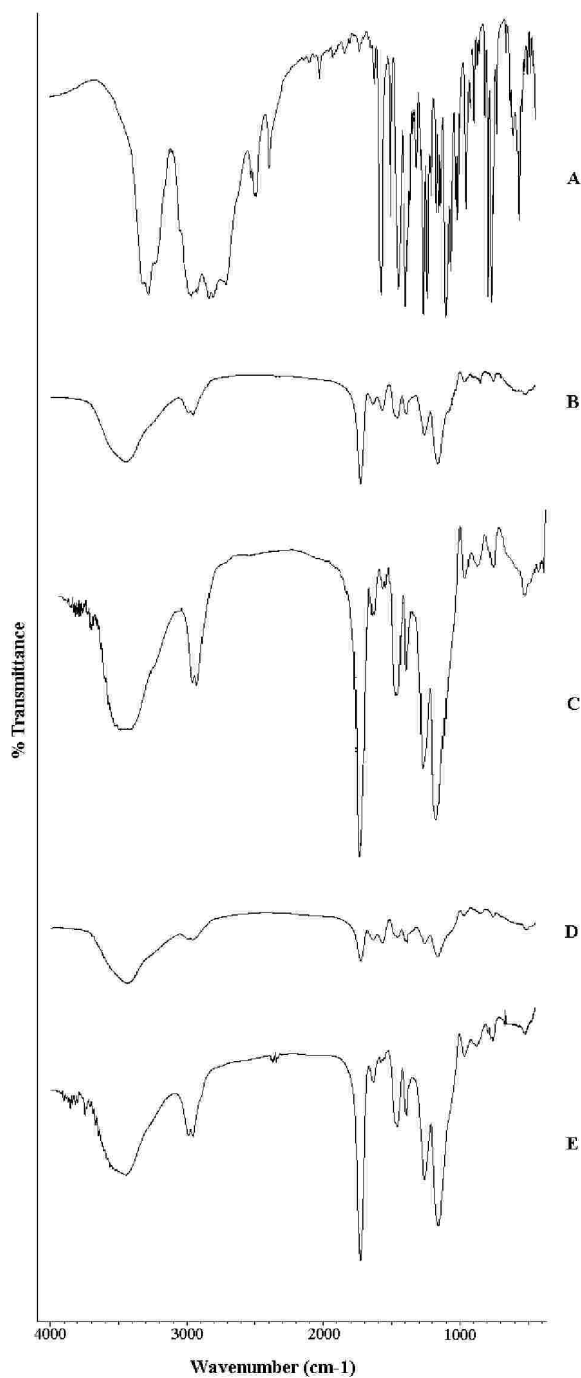


Figure 5: FTIR spectra of A: pure PHCL; B: non-imprinted polymer; C: PHCL imprinted microsphere; D: microsphere after desorption; E: microsphere after adsorption.

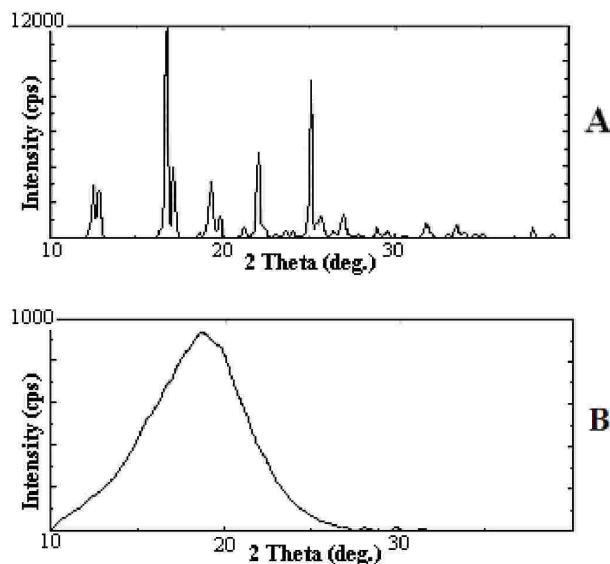


Figure 6: XRD patterns of pure PHCL and PHCL imprinted microsphere. A: pure PHCL; B: PHCL imprinted microsphere.

imprinted microspheres, in contrast, show a wide and strong endothermic effect in the range of 255-374°C (Fig. 7B) corresponding to a dehydration or fusion process of the polymer. In this Figure, peak temperature of PHCL is observed to change from 164°C to 145°C. This change may be related to the melting point shift when a guest molecule is embedded in a polymer cavity.

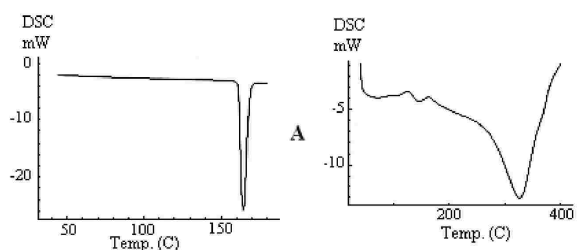


Figure 7: Thermograms of PHCL and its microsphere. A: pure PHCL; B: PHCL imprinted microsphere.

Content of PHCL in microspheres

Following adsorption of PHCL onto the imprinted microsphere, 0.186 ± 0.01 mg of PHCL content was determined in 10 mg of microspheres ($n=3$).

In vitro dissolution studies

Cumulative percentages of release of pure PHCL (5 mg), PHCL from a microsphere after adsorption (containing 5 mg PHCL) and commercial tablet (containing 40 mg PHCL) are given in Figure 8. As a result of the solubility

studies, the best medium was found to be pH 6. However, the dissolution medium was distilled water since the solubility and the spectrophotometric behaviors of PHCL were very similar in both media.

In another study, R and S enantiomers of PHCL were imprinted using the two different polymers, methacrylic acid and N-acryloylalanine and the dissolution tests were

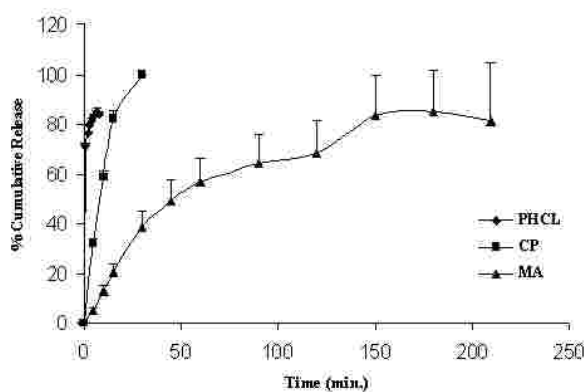


Figure 8: Release profiles of pure PHCL commercial preparation (CP) and microsphere after adsorption (MA) (mean \pm SE, n=6).

performed in pH 7.4 at 37°C using Apparatus II defined in USP XXIV (25). 80% of PHCL was released in 1 h and 100% was released in 4 h from the MIP system prepared with methacrylic acid. One hundred percent of PHCL was released in 3 h from the N- acryloylalanine based MIP system. In this study, a more prolonged release, 83% release in 3.5 h was obtained in distilled water at 37°C.

Solid polymers or hydrogels are frequently chosen as a material to allow controlled delivery of drugs. Drug release might be governed and controlled by diffusion, degradation of the matrix, or a combination of both. There is ongoing interest to identify additional tools to modify the release profile of a drug from a polymer matrix, and molecular imprinting has been suggested as one of those tools (26).

A recent study demonstrated that under physiological ocular volumetric flow rates the release kinetics is zero-order, or concentration-independent, for an extended time of at least 3.5 days. The imprinting process extends further control over the release kinetics independent of polymer swelling behavior, resulting in dramatic variations in diffusion coefficients (27).

Initial rapid release observed for the pure PHCL has disappeared with the imprinted microspheres. This is most probably due to the slow release from the recognition sites. Repetition of desorption and adsorption procedures may lead to a different release pattern, which has to be further tested.

Microspheres prepared are in hydrophobic character and thus relatively stable in the aqueous medium when compared to the hydrophilic carriers. Therefore, owing to the characteristic of the polymer, microspheres remained unchanged at the end of the dissolution tests. This indicates that the release is by a diffusion mechanism (28).

The 84.5% cumulative release of PHCL from the MIP system prepared and not complete release may be due to the flow rate of 8 mL.min⁻¹ of the flow-through system which may not supply the strict condition for the desorption of all the active agent(29). If we increased the time of the dissolution study, it would be possible to release the total amount of PHCL.

CONCLUSION

In this study, PHCL imprinted microspheres were prepared by suspension polymerization technique, using MAH-Co(II) as the functional monomer and EGDMA as the cross-linking agent. Desorption of PHCL from the MIP system was achieved by using 40% methanolic potassium hydroxide. Following the determination of the appropriate pH and concentration value for maximum adsorption, time to reach the maximum adsorption was found to be 30 min and the maximum adsorption value 213 mg.g⁻¹.

PHCL MIP system could be produced as a rate-attenuating selective excipient for a prolonged release drug delivery. Release profiles indicate a lower release for the MIP system compared to pure PHCL and the commercial preparation. As a result, new and interesting solutions to successful polymer network design and hence controlled release strategies will become available, like in this study. For the enhancement of the applicability of the MIP systems, predictive tools in the design of MIPs and assessment of the efficiency of the molecular imprinting processes need to be further studied.

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