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# Synthesis of imprinted beads by aqueous suspension polymerisation for chiral recognition of antihistamines

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ABSTRACT

A novel non-stabilised aqueous suspension polymerisation methodology for the preparation of spherical molecularly imprinted polymers is described with chlorpheniramine (CP), *d*-chlorpheniramine (*d*-CP), brompheniramine (BP) and *d*-brompheniramine (*d*-BP) as the templates, respectively. Using this rapid and simple technique, controlled polymer beads in the low micron range with narrow size distributions were generated by photo-polymerisation. The use of agitation speed as a method of controlling bead size distribution was demonstrated. Enantioselective properties of the imprinted beads were examined and the polymers prepared using *d*-chlorpheniramine and *d*-brompheniramine were capable of discriminating between the enantiomers of the template. Cross-selectivity studies were performed by batch rebinding with the influence of template size and functional group orientation of analytes on the recognition properties of the imprinted polymers investigated. Physical characteristics of all polymers were studied by nitrogen sorption porosimetry, particle size analysis and scanning electron microscopy (SEM) in order to gain an insight into the role of such properties on retention behaviour.



## 1. Introduction

Molecular imprinting is a technique whereby selective recognition sites can be created in highly cross-linked synthetic polymers for target analytes (templates) [1]. The three-dimensional binding pockets, produced in the polymer network in the presence of a template, consist of cavities complimentary to the shape, size and functionality of the imprint molecule. The science community have paid considerable attention to the application of MIPs in several areas of analytical chemistry, including sensor technologies [2], chiral chromatography [3] and solid phase extraction [4].

MIPs are prepared in various formats by different polymerisation techniques. Strategies for generating imprinted beads have been developed to improve on the characteristics of the bulk monolith formations. Spherical particles have been deemed to be superior HPLC packing materials over irregular shaped particles in terms of efficiency and mass-transfer properties [5]. MIPs with adjustable morphologies such as those prepared by suspension polymerisation in water [6,7], liquid perfluorocarbon [8,9] and multi-step swelling and polymerisation [10,11] have been developed. One of the drawbacks of these methodologies is that the incorporation of surfactants or stabilisers is required at the pre-polymerisation stage, which may interfere with the creation of specific binding sites. Another significant disadvantage is the cost associated with the purchase/synthesis of specialised surfactants. As a result, to date these approaches have not been widely used in formation of imprinted polymers.

The work presented here details the synthesis of molecularly imprinted polymer beads by an aqueous suspension polymerisation methodology with chlorpheniramine (CP), brompheniramine (BP) and their enantiomers as template molecules (Fig. 1). CP and BP are antihistamine ($H_1$-receptor antagonist) drugs often used as ingredients in 'over-the counter' treatments for the common cold and allergic conditions [12]. Both CP and BP bear chiral centres and exhibit enantioselectivity in their pharmacological responses [13]. The antihistaminic drugs are marketed as a racemic mixture. Antihistamine activity is mainly associated with the *d*-isomer (*d*-CP) [14], while the *l*-isomer is mainly responsible for the sedative side effects of this drug [15]. The greater need to distinguish between isomers in pharmaceutical formulations is being driven by regulatory authorities such as the FDA and, therefore, the relevance of this research and the result data obtained is demonstrated.

The approach presented here involves the formation of micro-droplets of the pre-polymerisation solution by vigorous agitation in water, which serves as the continuous phase, followed by photo-induced free-radical polymerisation. This was based on a modified suspension polymerisation synthetic strategy initially published by Kempe and Kempe [16]. Chloroform was chosen as the pre-polymerisation solvent, forming a stable suspension following mechanical dispersion. Imprinted polymer beads with narrow size distributions were generated. The main advantages of this polymerisation strategy is that it is fast and simple, is cheap due to

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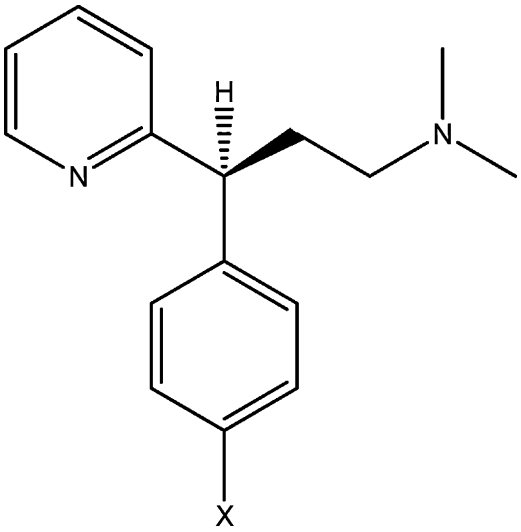


**Fig. 1.** The chemical structures of *d*-chlorpheniramine (X = Cl); *d*-brompheniramine (X = Br); pheniramine (X = H).

the absence of stabilisers, is relatively environmentally benign due to the use of an aqueous continuous phase and produces beads in chromatographically useful sizes. Additionally the chromatographic media produced demonstrated chiral recognition. The influence of column temperature on the chromatographic performance of these chiral phases was also examined.

Physical characteristics of these imprinted materials were studied by SEM, particle size analysis and nitrogen sorption porosimetry. Factors influencing bead size were also investigated. This characterisation facilitated a study of the impact of this novel synthetic strategy on material morphology which is important in an evaluation of MIP performance [17].

## 2. Experimental

### 2.1. Reagents

Analytical grade chemicals were employed for this study. Methacrylic acid (MAA, 99%) and trimethylolpropane trimethacrylate (TRIM) were purchased from Sigma–Aldrich (Dublin, Ireland). AIBN was purchased from Acros Chimica (Geel, Belgium). Potassium dihydrogen phosphate ($KH_2PO_4$) (98–100.5%), dipotassium hydrogen phosphate ($K_2HPO_4{\cdot}3H_2O$) (>99%) and orthophosphoric acid (<85%) employed as buffer reagents were obtained from Merck. Chlorpheniramine (CP), *d*-chlorpheniramine (*d*-CP), brompheniramine (BP) and *d*-brompheniramine (*d*-BP) were a kind donation from Schering-Plough (Avondale, Rathdrum, Co. Wicklow, Ireland). Antihistamines employed in this study were used in their free base form. Chloroform, methanol and acetonitrile were HPLC grade and obtained from Lennox (Dublin, Ireland). All organic solvents were used without further purification and to maintain the absence of moisture, chloroform, methanol and acetonitrile were stored over molecular sieves (3 Å, 3.2 mm).

### 2.2. Synthesis of spherical beads by suspension polymerisation in an aqueous media

The instrumentation used for the synthesis of spherical beads was adapted from a method employed for the preparation of MIPs by suspension polymerisation using mineral oil [16]. Molecularly imprinted polymers for chlorpheniramine ($CP_{MIP}$), *d*-chlorpheniramine (*d*-$CP_{MIP}$), brompheniramine ($BP_{MIP}$) and *d*-brompheniramine (*d*-$BP_{MIP}$) were prepared by dissolving 2 mmol of template, 3 mmol MAA, 10 mmol TRIM and 0.7 mmol AIBN in chloroform (8 ml). Each pre-polymerisation solution was purged with a stream of nitrogen gas for 3 min, followed by suspension of 3 ml of this solution in 57 ml of water in a 100 ml graduated cylinder. The pre-polymerisation mixtures were dispersed at 9500 rpm for 30 s with a DI 25 basic dispersing device, equipped with an S25-N18G dispersing tool (IKA® Werke, Gmbh & Co., Germany). To study the influence of agitation speed on bead size distribution, imprinted polymers were also prepared using dispersion speeds of 13,500 and 24,000 rpm. The sealed mixtures were placed in a Rayonet photochemical mini-reactor (Model RMR-600 (Branford, CT, USA) and polymerised at 350 nm in a 4 °C cold-room for 6 h. Once the polymerisation was complete, the polymer beads were isolated and washed with 50 ml of a methanol–acetic acid solution (90:10, v/v). Successive washings with methanol were performed to remove the template and any unreacted monomers. Template and monomer removal was monitored by UV–vis spectroscopy.

### 2.3. Physical characterisation of imprinted polymers

#### 2.3.1. SEM and particle size distribution analyses

A Hitachi S-2460N scanning electron microscope (SEM) was utilised to obtain images of the beads. Particle size distribution measurements were conducted on a Mastersizer 2000 (Malvern) equipped with a Hydro 2000S wet sample dispersion unit, with methanol as a dispersant. Prior to analyses, approximately 100 mg quantities of polymer beads were suspended in 10 ml methanol and placed in an ultrasonic bath for 20 min. Results were reported as *d*(0.1), *d*(0.5) and *d*(0.9). *d*(0.1) and *d*(0.9) corresponded to the size of particles below which 10% and 90% of particles lie, respectively and *d*(0.5) was the median of the particle size distribution.

#### 2.3.2. Nitrogen sorption analyses

Nitrogen sorption porosimetry studies were carried out using a Gemini VI nitrogen sorption porosimeter (Micromeritics). A quantity of 180 mg of dry polymer was weighed out into a sample tube and degassed using a constant flow of nitrogen for 2 h at 120 °C prior to analysis. The exact mass of polymer present in the tube was determined prior to the commencement of analysis. Nitrogen desorption analysis followed adsorption on to the polymer samples in order to generate a 109 point sorption isotherm in the relative pressure ($P/P_0$) range 0.01–0.99. Specific surface areas ($m^2/g$) for samples were obtained (using the BET method) from adsorption isotherms in the $P/P_0$ range from 0.05 to 0.3 using a six point plot. In addition, pore analyses were carried out using the Barrett, Joyner and Halden (BJH) method.

### 2.4. Equilibrium batch rebinding studies

Equilibrium binding studies were conducted to evaluate the binding properties of the polymers. Triplicate 50.0 mg quantities of both MIP and NIP polymers were accurately weighed out in 10 ml volumetric flasks. Each polymer (MIP and NIP) was loaded with 5 ml of 0.2 mM template solution in chloroform. Samples were placed on an orbital shaker for 16 h at room temperature. Following equilibration, the supernatants were filtered and the quantity of analytes rebound was determined by HPLC using a chiral column (Chiralpak AD-H (5 μm particle size, 250 mm × 4.6 mm i.d.). The mobile phase consisted of n-hexane:propan-2-ol:diethylamine (90:10:0.1, v/v), with a flow rate of 0.75 ml/min. Detection was carried out at 254 nm with a sample injection volume of 5 μl. Imprinting factors (IFs) were calculated as the ratios of the amount of analyte rebound to the MIP to that of the NIP (IF = MIP/NIP).

### 2.5. Chromatographic evaluation of polymers

HPLC columns were packed using a dual piston pump system ('Pack in a Box' Column Packing System – Lab Alliance/Scientific Systems Inc., PA, USA). The MIPs used were prepared at 9500 rpm employing *d*-CP and *d*-BP as template molecules and were abbreviated to *d*-$CP_{MIP(9500\,rpm)}$ and *d*-$BP_{MIP(9500\,rpm)}$, respectively. Beads were packed into 50 mm stainless steel columns (i.d. 4.6 mm) with methanol as the packing solvent and a constant pump pressure of 7000 psi. A HP Agilent liquid chromatograph (model 1050) with a UV spectrophotometer detector, employing ChemStation software was utilised in this study. The flow rate was maintained at 0.075 ml/min (unless otherwise stated), with an injection volume of 5 μl and detection wavelength set to 254 nm at ambient temperature. The mobile phase consisted of acetonitrile and a buffer composed of 50 mM potassium dihydrogen phosphate ($KH_2PO_4$) and 50 mM dipotassium hydrogen phosphate ($K_2HPO_4{\cdot}3H_2O$) (70:30, v/v). A phosphate buffer was selected to study responses over a pH range from 3 to 9 in order to optimise the enantioseparation of the analytes. The pH was adjusted with orthophosphoric acid or sodium hydroxide (5%). All analytes were prepared at a concentration of 0.2 mM standard solution in mobile phase. The pH values were 'apparent' because a mixture of phosphate buffer and acetonitrile was used as the mobile phase. Prior to analysis, the mobile phase was degassed and filtered using 0.45 μm filter membranes. The column was then connected to the HPLC and equilibrated with mobile phase until a stable baseline was obtained. The retention factor ($k$) was calculated from the equation, $k = (t - t_0)/t_0$ where $t$ and $t_0$ were retention times of retained and unretained solutes, respectively. In this study, $t_0$ was estimated based on the retention time of the solvent peak. The *d*-isomers were used as standards. An enantioseparation factor was determined from the relationship, $\alpha = k_d/k_l$, where $k_d$ and $k_l$ were the retention factors of the eluted enantiomers, respectively. The resolution ($R_s$) was calculated from the equation, $R_s = (t_2 - t_1)/1.7 \times 0.5(w_{0.5,1} + w_{0.5,2})$, where $t_1$ and $t_2$ were the retention times of the first and second eluted enantiomers, respectively, and $w_{0.5,1}$ and $w_{0.5,2}$ were the peak widths at half the peak height of the first and second eluted enantiomers, respectively.

## 3. Results & discussion

Chlorpheniramine and brompheniramine molecularly imprinted polymers were prepared in bead formats, by a non-stabilised suspension polymerisation methodology. As water was used as the continuous phase, this methodology for producing MIP beads was found to be cost-effective and environmentally friendly. MIP beads with controllable narrow size distributions (2–10 μm) were generated with enantioselective properties demonstrated chromatographically, by employing MIPs as HPLC chiral stationary phases. A range of physical characteristics of these novel polymer materials was examined in order to gain a better understanding of the nature of the imprinted media produced utilising this suspension polymerisation approach.

### 3.1. Physical characterisation of imprinted polymers

#### 3.1.1. SEM and particle size distribution analyses

SEM images of polymers were obtained to ensure that the suspension polymerisation methodology utilised in this study generated polymers in bead formats. SEM images of *d*-CP imprinted polymers prepared using different agitation speeds at the pre-polymerisation stage are illustrated in Fig. 2. While spherical beads were formed, appreciable differences in bead sizes were observed between the beads prepared at different speeds. To gain a better insight into bead size and distribution, particle size analysis was performed and size distribution profiles were generated. Fig. 3 is a representative example of bead size distribution for the *d*-CP imprinted polymers prepared using different agitation speeds. Beads prepared at high agitation speeds were found to have a narrower distribution and smaller median bead size ($d(0.5)$ 2.34 μm at 24,000 rpm compared to $d(0.5)$ 5.15 μm at 9500 rpm).

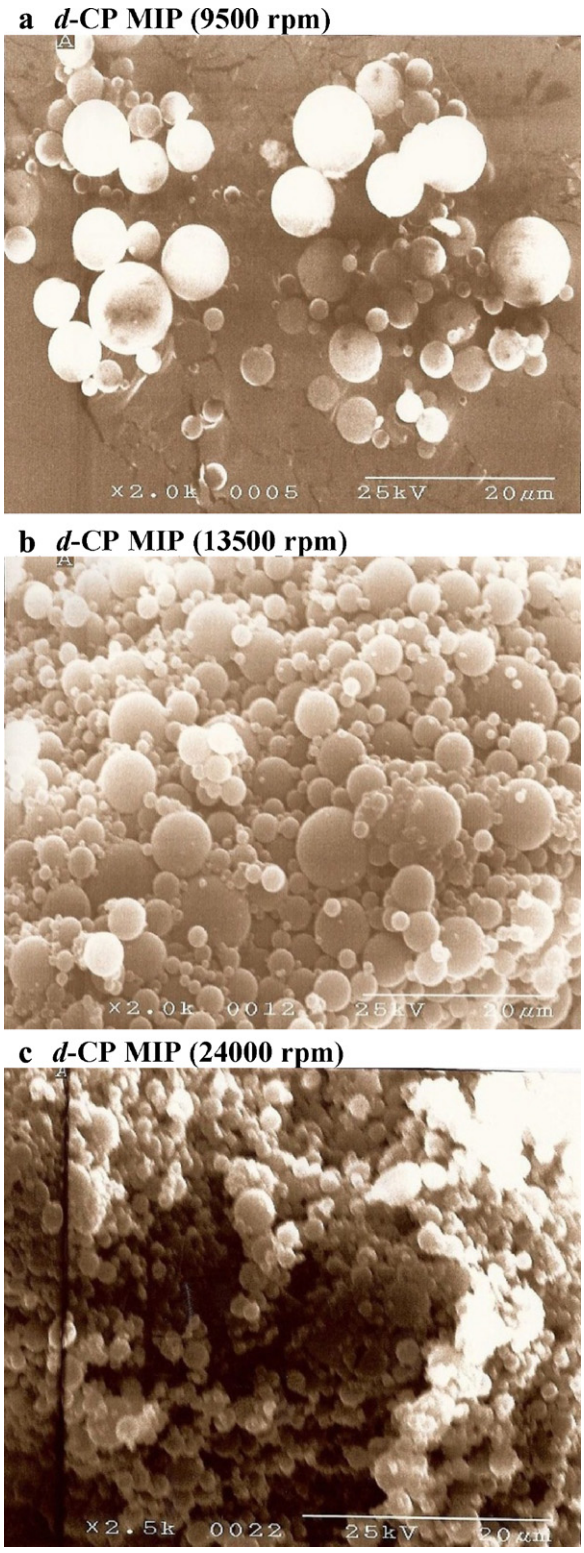


**Fig. 2.** SEM images of *d*-CP imprinted polymers prepared using different agitation speeds at the pre-polymerisation stage.

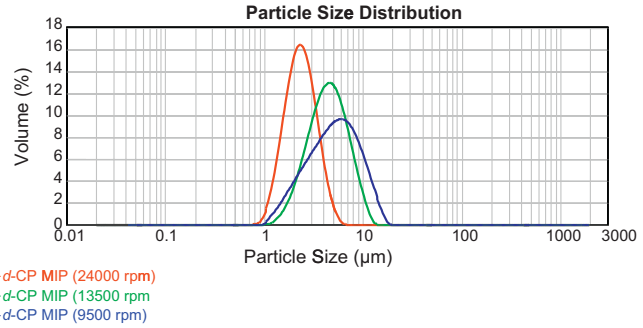


**Fig. 3.** The particle size distribution obtained for *d*-CP MIPs prepared at 9500, 13,500 and 24,000 rpm.

**Table 1**
Dv10, Dv50 and Dv90 values obtained for *d*-CP suspension MIPs prepared at 9500, 13,500 and 24,000 rpm. Data are based on average values from triplicate analyses.

| *d*-CP MIP polymer | Dv10 (μm) | Dv50 (μm) | Dv90 (μm) |
|---|---|---|---|
| 9500 rpm | 2.16 ± 0.001 | 5.15 ± 0.008 | 10.45 ± 0.043 |
| 13,500 rpm | 2.42 ± 0.002 | 4.45 ± 0.004 | 7.78 ± 0.008 |
| 24,000 rpm | 1.48 ± 0.001 | 2.34 ± 0.003 | 3.71 ± 0.003 |

The change in agitation speed at the pre-polymerisation stage is clearly reflected in the variations in average particle size values observed. A summary of the imprinted bead size distributions at each agitation speed is given in Table 1. The bead sizes produced are appropriate for use as new uPLC phases.

### 3.1.2. Nitrogen sorption analyses

Sorption isotherms of the imprinted polymers were generated from the plots of the quantity of gas sorbed against relative pressure. All polymers, irrespective of the agitation speed, displayed the same general shape of Type II isotherms (as classified by the IUPAC [18]), a characteristic of non-porous or macroporous materials.

Sorption isotherms allowed the calculation of the BET surface area of the polymers, using a six-point plot in the relative pressure region ($P/P_0$) of 0.05 to 0.3, with correlation coefficients of greater than 0.999. The surface area of the MIPs was found to be dependent on the size of the polymer beads, with the MIP prepared at the lowest speed (9500 rpm) showing the lowest surface area. Values varied from 1.68 (±0.01) $m^2/g$ for polymers prepared at 9500 rpm to 4.55 (±0.04) $m^2/g$ for 24,000 rpm. Table 2 is a summary of the sorption porosimetry result data. The agitation rate, therefore, provides control over the pore structure of the final beads. From BET surface area analyses, polymers appear to be non-porous as they demonstrated very low surface areas in the dry state. Low specific surface area points to a recognition mechanism mainly associated with adsorption on the external surface of the materials and also to their ability to withstand the higher pressures associated with use.

The specific pore volumes and average pore diameters were calculated according to the BJH method (Table 2). The cumulative volume of pores and pore diameter for the MIP polymers prepared at 9500 rpm and 13,500 rpm was approximately half (or less) of the corresponding MIP prepared at 24,000 rpm. This suggests that overall, the polymers prepared at the lower agitation speeds were less porous than the polymers prepared at the higher speed.

Porosity differences result from the phase separation of the solvent and the growing polymer during polymerisation. Depending on synthesis parameters, polymers with low solubility in the porogen should separate early [19]. Sellergren and Shea reported preparation of monolith polymers with chloroform as the solvent [17]. The ground polymers were found to be non-porous and had very low surface areas (3.5 $m^2/g$) with essentially no internal pore volume (0.007 ml/g) and an average pore size of 9.1 nm. Our results agree with these observations.

### 3.2. Rebinding studies using chiral HPLC analysis

Successful generation of MIP beads by suspension polymerisation in an aqueous medium was achieved for CP, *d*-CP, BP and *d*-BP imprinted polymers. Equilibrium rebinding of the analytes was determined in chloroform and analysed using chiral HPLC with a Chiralpak AD-H column. Additionally, binding characteristics of the structural analogues of the template were assessed in a cross-selectivity study. Fig. 4 represents the equilibrium binding results for the aqueous suspension imprinted polymers (i.e. $CP_{MIP(24,000)}$, $d\text{-}CP_{MIP(24,000)}$, $BP_{MIP(24,000)}$ and $d\text{-}BP_{MIP(24,000)}$) reloaded with CP, *d*-CP, BP and *d*-BP standard solutions, respectively. As observed from Fig. 4, superior rebinding of the analytes was achieved by the MIPs in comparison to the NIPs. This affinity is attributed to the imprinting effect produced during polymerisation in the presence of the template, which led to the formation of affinity binding cavities in the imprinted polymer.

Cross-selectivity studies revealed marginal differences in analyte rebinding. Although both CP and BP have similar molecular structures and $pK_a$ values, in all cases the imprinting factors were found to be slightly higher for brompheniramine, which may have resulted from the differences in polarisability between the halogen groups of each analyte. The high IF values for the *d*-BP MIPs make these MIPs very useful as group-recognition material for CP, BP and their enantiomers.

Using chiral HPLC, it was possible to test for enantioselectivity in the equilibrium rebinding of CP and BP racemates from the ratio of the HPLC peak areas for the *d* and *l*-isomers. Reloading of CP racemate to both $d\text{-}CP_{MIP(24,000)}$ and $d\text{-}BP_{MIP(24,000)}$ MIPs showed evidence of a slight excess in the binding of the *d*-isomers for each substrate (data not included). However no enantioselectivity was observed in the rebinding of the BP racemate to either $d\text{-}CP_{MIP(24,000)}$ or $d\text{-}BP_{MIP(24,000)}$. Thus rebinding studies in the polymerisation solvent ($CHCl_3$) did not provide significant evidence of enantioselective imprinting.

### 3.3. Applicability of MIPs as tailor-made HPLC chiral stationary phases

In order to further investigate the chromatographic performance and chiral recognition properties of these MIP beads, $d\text{-}CP_{MIP(9500\,rpm)}$ was employed as a HPLC stationary phase. The structurally related antihistamine, pheniramine (PHEN) was included to test the selectivity of the stationary phase.

### 3.3.1. Optimisation of chiral separation in aqueous media

Different mobile phase pHs ranging between 3 and 9 were selected for evaluation of the $d\text{-}CP_{MIP(9500\,rpm)}$, since it is the pH

**Table 2**
Nitrogen sorption data for *d*-CP MIPs prepared at 9500, 13,500 and 24,000 rpm. Data are based on average values from duplicate analyses.

| *d*-CP MIP polymer | BET surface area ($m^2/g$) | Cumulative surface area of pores ($m^2/g$) | Cumulative volume of pores ($cm^3/g$) | Average pore diameter (nm) |
|---|---|---|---|---|
| 9500 rpm | 1.68 (±0.01) | 2.63 ± 0.11 | 0.005 ($\pm 3 \times 10^{-4}$) | 7.50 ± 0.10 |
| 13,500 rpm | 2.11 (±0.04) | 3.04 ± 0.10 | 0.01 ($\pm 1 \times 10^{-4}$) | 9.66 ± 0.45 |
| 24,000 rpm | 4.55 (±0.04) | 5.07 ± 0.20 | 0.02 ($\pm 3 \times 10^{-3}$) | 13.11 ± 0.11 |

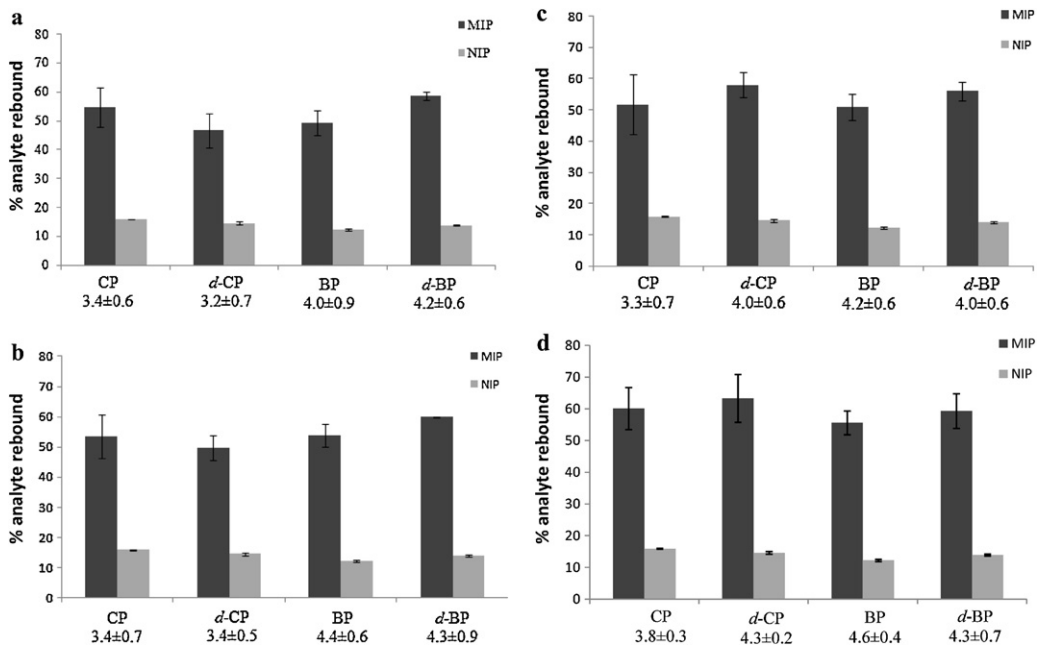


**Fig. 4.** Quantities of CP, *d*-CP, BP and *d*-BP rebound to: (a) CP MIP and NIP; (b) *d*-CP MIP and NIP; (c) BP MIP and NIP; (d) *d*-BP MIP and NIP. Data are based on average values from triplicate analysis. Imprinting factor data obtained from batch rebinding studies all performed on polymers produced at speeds of 24,000 rpm.

range of interest. This is indicated by the p$K_a$ values of the antihistamines of 4.00, 3.59 and 4.20 at the pyridine ring and 9.20, 9.12 and 9.30 at the aliphatic amine group for CP and BP and PHEN, respectively [20–22], which has an influence on the retention of the analytes. The MIP stationary phase was able to discriminate between the enantiomers at pH 7 and a mobile phase composition of 70:30 (v/v) ACN:buffer. The retention factors ($k$) of both CP and BP varied over the pH range studied, reaching a maximum at pH 7 (Fig. 5). The pH influence on the retention effect was dramatic. At low pHs, CP and BP were protonated while the degree of ionisation of the carboxylic groups in the polymer decreased. Further increases in basicity of the mobile phase (pH 7) caused the carboxylic groups of the polymer to be deprotonated and a reduction of the protonation of the pyridyl and amino groups on CP and BP. The enantiomers of CP demonstrated the strongest retention and best separation on a *d*-$CP_{MIP(9500\,rpm)}$ column with a mobile phase composition of (70:30) ACN:buffer at pH 7. This composition and pH were selected for future studies investigating the enantioselectivity of the *d*-$CP_{MIP(9500\,rpm)}$ column.

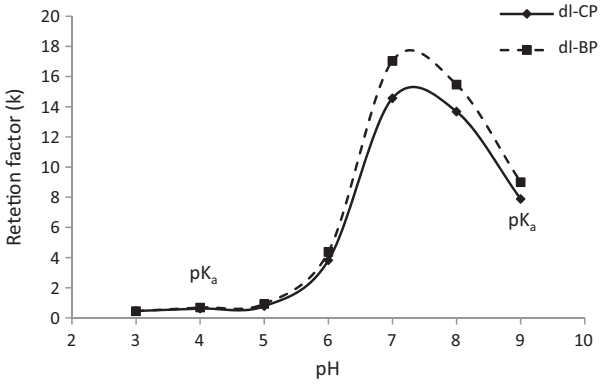


**Fig. 5.** Influence of mobile phase pH on retention factors ($k$) obtained on the *d*-$CP_{MIP(9500\,rpm)}$ packed column.

**Table 3**
Retention factors, enantioselectivity factors and resolution of CP and BP enantiomers on *d*-$CP_{MIP(9500\,rpm)}$ column. HPLC conditions: column temperature, ambient; mobile phase, ACN:buffer (70:30, v/v) at pH 7.

| Analyte | $k_l$ | $k_d$ | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| CP | 12.27 | 14.56 | 1.19 | 0.88 |
| BP | 14.98 | 17.04 | 1.14 | 0.60 |
| *d*-CP | | 14.8 | | |
| *d*-BP | | 17.3 | | |

*Note*: $k_l$ and $k_d$ are the retention factors of the eluted enantiomers respectively; $\alpha$, enantioseparation factor; $R_s$, resolution.

### 3.3.2. *Separation of antihistamines using a d-*$CP_{MIP(9500\,rpm)}$ *column*

The chromatographic behaviour and separation of CP, *d*-CP, BP and *d*-BP was investigated using the *d*-$CP_{MIP(9500\,rpm)}$ column, with a mobile phase at pH 7 and the results are illustrated in the chromatograms in Fig. 6. Enantioselectivity was achieved for CP and BP at pH 7 as shown in Fig. 6(a) and (c). While the retention of *d*- and *l*-BP in the *d*-$CP_{MIP(9500\,rpm)}$ column was marginally longer than *d*- and *l*-CP, higher enantioselectivity (1.19) and resolution (0.88) was obtained for *d*- and *l*-CP, as detailed in Table 3. At pH 7 both CP and BP were charged. The results obtained above suggest that ionic and hydrophobic interactions could be used to explain differences in retention between analytes but not between enantiomers. Enatioseparation is, therefore, seen as clear proof of the specific binding of the template on the *d*-$CP_{MIP(9500\,rpm)}$ column. The retention tendencies of both *d*-CP and *d*-BP (Table 3) are similar since they bear pyridyl and amino groups. The somewhat longer retention of *d*- and *l*-BP may be attributed to the more hydrophobic nature of BP (3.57 [11]) compared to CP (3.39 [11]). This difference in hydrophobicity was prominent when an aqueous-based solvent was used in the analysis, in comparison to the equilibrium rebinding studies which were performed in chloroform. However, even in chloroform some differences in rebinding were noted between the two antihistamines. It is also of interest that rebinding in the polymerisation solvent was less discriminating between the enantiomers of these analytes than the aqueous based solvent system used for the *d*-$CP_{MIP(9500)}$ HPLC application.

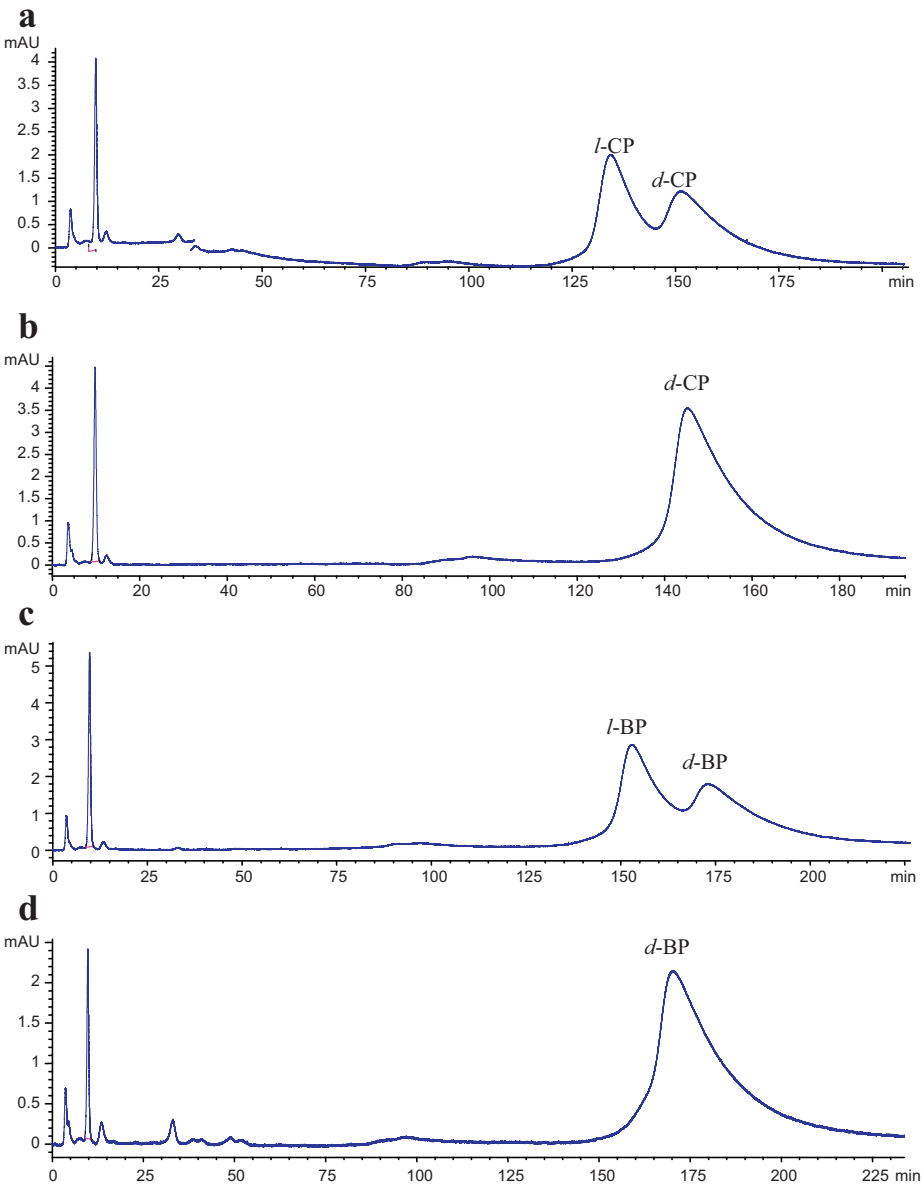


**Fig. 6.** Chromatograms of (a) CP, (b) *d*-CP, (c) BP and (d) *d*-BP isomers on the *d*-$CP_{MIP(9500\,rpm)}$ column.

### 3.4. Separation of PHEN, CP and BP enantiomers on *d*-$CP_{MIP(9500\,rpm)}$ column

The ability of the chiral stationary phase designed with the *d*-CP MIP to resolve other antihistamines was investigated. For this study the mobile phase composition was modified slightly (65:35 (v/v) ACN:phosphate buffer, instead of 70:30) in order to achieve partial separation of PHEN enantiomers, while the pH was maintained at 7. As illustrated in Fig. 7, PHEN eluted first and the peaks were overlapping for CP and BP. Table 4 presents the retention factors, enantioselectivity, and resolution of PHEN, CP and BP enantiomers on the *d*-$CP_{MIP(9500\,rpm)}$ column.

The highest retention factor, enantioselectivity and resolution values were observed for BP over the PHEN and CP enantiomers. The retention of these antihistamines can be related to hydrophobicity, as measured by Log $P_{o/w}$ (2.79, 3.39 and 3.57 for PHEN, CP and BP, respectively [11]) with the most hydrophobic BP retained the longest on the column.

Data for the CP enantiomers shown in Table 4 demonstrated lower resolution values in comparison to Table 3. In contrast the

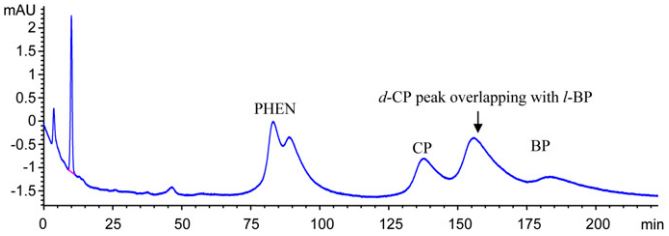


**Fig. 7.** Chromatogram of a mixture of PHEN, CP and BP racemates on a *d*-$CP_{MIP(9500\,rpm)}$ column.

**Table 4**
Retention factors, enantioselectivity and resolution of PHEN, CP and BP enantiomers on a *d*-$CP_{MIP(9500\,rpm)}$ column. HPLC conditions: column size, 50 mm × 4.6 i.d.; column temperature, ambient; mobile phase, ACN:buffer (65:35, v/v) at pH 7.

| Analyte | $k_l$ | $k_d$ | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| PHEN | 7.38 | 7.98 | 1.08 | 0.50 |
| CP | 12.72 | 14.66 | 1.15 | 0.64 |
| BP | 14.66 | 17.20 | 1.17 | 0.72 |

**Table 5**
Retention factors, enantioselectivity factors and resolution of CP and BP enantiomers on a *d*-$BP_{MIP(9500\,rpm)}$ column. HPLC conditions: column temperature, ambient; mobile phase, ACN:buffer (70:30, v/v) at pH 7; detection, 254 nm; flow rate, 0.1 ml/min.

| *d*-$BP_{MIP(9500\,rpm)}$ | | | | |
|---|---|---|---|---|
| Analyte | $k_l$ | $k_d$ | $\alpha$ | $R_s$ |
| CP | 10.30 | 12.30 | 1.19 | 0.68 |
| BP | 11.50 | 14.40 | 1.25 | 0.73 |
| *d*-CP | | 11.5 | | |
| *d*-BP | | 13.4 | | |

*Note*: $k_l$ and $k_d$ are the retention factors of the first and second eluted enantiomers respectively; $\alpha$, enantioseparation factor; $R_s$, resolution.

BP enantiomers showed a higher resolution value (Table 4). The separation of PHEN, CP and BP racemates was performed under a different set of conditions i.e. the mobile phase composition was changed (with a overall change in polarity), therefore, this factor had an effect on the change in resolution values for both CP and BP enantiomers.

### 3.5. Separation of CP and BP on d-$BP_{MIP(9500\,rpm)}$ column

The chromatographic behaviour and separation of CP, and BP isomers were also studied on a *d*-$BP_{MIP(9500\,rpm)}$ column at a mobile phase pH of 7. Similar chromatograms were observed as in Fig. 6(a)–(d). The retention factors, enantioselectivity factors and resolution of CP and BP enantiomers and *d*-CP and *d*-BP isomers on the *d*-$BP_{MIP(9500\,rpm)}$ column are presented in Table 5. The retention factors, enantioselectivity factors and resolution were greater for BP enantiomers than CP on the *d*-$BP_{MIP(9500\,rpm)}$ column. A similar trend was observed in the retention factors for BP over CP to that already described with the *d*-$CP_{MIP(9500\,rpm)}$ column. However, an opposite trend was observed for the enantioselectivity factors and resolution, as they were greater for CP enantiomers than BP on the *d*-$CP_{MIP(9500\,rpm)}$ column, showing that the best resolution was achieved for the templates on their own imprinted polymers.

For all studies performed, separations of the CP and BP compounds on *d*-$CP_{MIP(9500\,rpm)}$ and *d*-$BP_{MIP(9500\,rpm)}$ columns were achieved in an aqueous mobile phase. Interactions were mainly attributed to the contribution of MAA functionality to the stationary phase and the tertiary amino group in the CP and its structural analogues, which plays a predominant role in the binding of MIPs in an aqueous environment. Again enantioselectivity in the *d*-$BP_{MIP(9500\,rpm)}$ is clear evidence of an imprinted site with chiral recognition.

### 3.6. Influence of column temperature on antihistamine separation

The effect of temperature on the *d*-$CP_{MIP(9500\,rpm)}$ column was also investigated. Fig. 8 presents the separation of a mixture of *d*-CP and *d*-BP on *d*-$CP_{MIP(9500\,rpm)}$ using a column temperature of ambient and 50 °C and Table 6 summarised the effect of

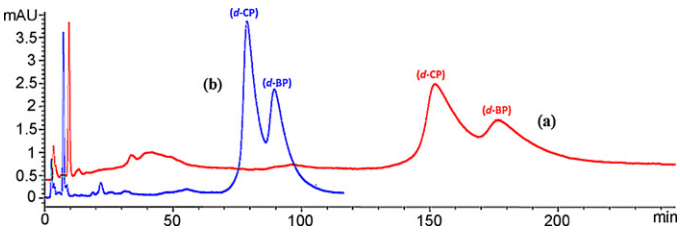


**Fig. 8.** Chromatograms of a mixture of *d*-CP and *d*-BP isomers on a *d*-$CP_{MIP(9500\,rpm)}$ using a column temperature of (a) 25 °C and (b) 50 °C.

**Table 6**
Effect of temperature on the retentivity, selectivity and resolution of a mixture of *d*-CP and *d*-BP isomers on *d*-$CP_{MIP(9500\,rpm)}$ using a column temperature of (a) ambient and (b) 50 °C. HPLC conditions: mobile phase, ACN:buffer (70:30, v/v) at pH 7; (a) flow rate, 0.075 ml/min; (b) flow rate, 0.1 ml/min.

| Column temp. (°C) | *d*-CP $k_d$ | *d*-BP $k_{d'}$ | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| (a) Ambient | 14.94 | 17.56 | 1.18 | 0.61 |
| (b) 50 | 9.56 | 11.18 | 1.17 | 0.86 |

temperature on the retention factors, selectivity factors and resolution of these analytes on the *d*-$CP_{MIP(9500\,rpm)}$ column. A reduction in retention times and sharper peaks were observed at the higher column temperature illustrating improved column performance. Because back pressures were also reduced, it was possible to increase the flow rate from 0.075 to 0.1 ml/min for the higher temperature study, which is a significant parameter in terms of improving the column performance. At the higher temperature and increased flow rate the selectivity remained unchanged while the resolution was increased. The improvement in resolution would be expected due to the sharper peaks produced and decreased tailing. Thus operating at 50 °C resulted in improved separation performance maintaining the selectivity with better resolution in a shorter analysis time.

## 4. Conclusions

This novel study demonstrated the controlled generation of spherical MIP beads with narrow size distribution, using a non-stabilised aqueous suspension polymerisation. This methodology is fast, straightforward, environmentally benign and reproducible, yielding imprinted polymer materials with molecular recognition properties. No surfactants or stabilisers were required to produce spherical beads in the low micron diameter range, which were isolated easily from the aqueous continuous phase. Two antihistamines, chlorpheniramine and brompheniramine, were employed as template molecules. Imprinted beads displayed enantioselective properties for their own templates and structural analogues.

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