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Computational modeling and molecular imprinting for the development of acrylic polymers with high affinity for bile salts

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Abstract

This work has focused on the rational development of polymers capable of acting as traps of bile salts. Computational modeling was combined with molecular imprinting technology to obtain networks with high affinity for cholate salts in aqueous medium. The screening of a virtual library of 18 monomers, which are commonly used for imprinted networks, identified N-(3-aminopropyl) methacrylate hydrochloride (APMA·HCl), N,N-diethylamino ethyl methacrylate (DEAEM) and ethyleneglycol methacrylate phosphate (EGMP) as suitable functional monomers with medium-to-high affinity for cholic acid. The polymers were prepared with a fix cholic acid:functional monomer mole ratio of 1:4, but with various cross-linking densities. Compared to polymers prepared without functional monomer, both imprinted and non-imprinted microparticles showed a high capability to remove sodium cholate from aqueous medium. High affinity APMA-based particles even resembled the performance of commercially available cholesterol-lowering granules. The imprinting effect was evident in most of the networks prepared, showing that computational modeling and molecular imprinting can act synergistically to improve the performance of certain polymers. Nevertheless, both the imprinted and non-imprinted networks prepared with the best monomer (APMA·HCl) identified by the modeling demonstrated such high affinity for the template that the imprinting effect was less important. The fitting of adsorption isotherms to the Freundlich model indicated that, in general, imprinting increases the population of high affinity binding sites, except when the affinity of the functional monomer for the target molecule is already very high. The cross-linking density was confirmed as a key parameter that determines the accessibility of sodium cholate to the binding points. Materials prepared with 9% mol APMA and 91% mol cross-linker showed enough affinity to achieve binding levels of up to 0.4 mmol/g (i.e., 170 mg/g) under flow (1 ml/min) of 0.2 mM sodium cholate solution.

Keywords: Computational modeling; cholic acid; Freundlich isotherm; molecularly imprinted polymer (MIP); trap systems.

1. Introduction

Hypercholesterolemia represents a serious health problem in wealthy economies and its incidence is rising in developing countries and poor communities owing to shifts in the alimentary habits [1]. Such a global concern on hypercholesterolemia makes cheap and patient-friendly therapeutic approaches particularly attractive. Diet control and prescription of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors are common efficient strategies, but the first one does require willpower and the second one is not exempt of collateral effects [2,3]. The use of traps that can capture bile acids involved in the emulsification of fatty acids at the intestine is a particularly useful alternative or coadjutant in a broad range of therapies for patients [4,5]. Bile acids consist of a curved steroidal skeleton with a hydrophilic α face, which includes a carboxylic acid group, and a hydrophobic β face, which provides amphiphilic character and self-associative behavior [6]. The commercially available anionic resins (colestipol and cholestyramine, among others) exchange their chloride anions with anionic bile acids in the gastrointestinal tract, resulting in insoluble complexes that are eliminated in the feces [7]. This leads to lower adsorption of fats and to the conversion of plasma cholesterol to bile acid in order to normalize bile acid levels, which results in a decrease of cholesterol levels [3].

The search on novel polymeric materials capable of acting as selective and efficient traps is mostly based on the optimization of hydrophobic and ionic interactions [8-11]. The results obtained with natural and synthetic polymers indicate that a bile acid sequestrant should meet the following features [12-15]: i) to posses cationic groups that enable a fast interaction with bile salts; ii) to contain hydrophobic groups to enhance the stability of the complexes; and iii) to swell to a certain extent to make the network accessible to the bile salt. Molecular imprinting technology has been tested as tool to optimize performances of selective traps [16-18]. This technology consists in adding the target molecule to a monomers solution for enabling the arrangement of the monomers around the target analyte according to their interaction capability. Such an arrangement is fixed during polymerization. After extraction of the template molecules, the resultant polymeric networks exhibit pockets with size and shape specific for the template and with the most favorable chemical groups for the reuptake once in contact again with the

template molecules [19-21]. Both non-covalent and covalent imprinting have been applied for creating bile acids traps using vinyl or acrylic monomers [16-18]. Although the imprinting effect in aqueous environment is harder to achieve than in organic media, imprinted networks have shown a greater uptake of sodium cholate, both *in vitro* and *in vivo*, than the non imprinted networks [16-18].

Typically, the selection of the nature and the relative proportion of the monomers used for creating polymeric traps is based on literature data, previous experience of researchers, and the results of trial and error assays. Such a procedure involves hard and time consuming experimental work and remarkable costs for materials. Recent approaches to the rational design of functional polymeric networks have shown that *in silico* screening of suitable monomers for each specific target molecules can significantly shorten the process and improve the success rate [22]. Despite molecular imprinting technology has been routinely used for more than 20 years, implementation with computational modeling is still relatively novel [23]. The aim of this work was to apply computational modeling for the screening of monomers with affinity for cholic acid and use some of the selected monomers for synthesizing cholate-imprinted and non-imprinted networks. Adsorption isotherms were analyzed to test the predictive value of the computational modeling results. For the networks with better performance, the cross-linking density was tuned in order to elucidate the incidence of the mesh size in the capture of sodium cholate from an aqueous environment. The experiments were carried out both at equilibrium state to obtain relevant parameters of the adsorption process and in dynamic mode (i.e., under a certain flow of cholate solution) to simulate physiological conditions. The results were compared with those obtained using commercially available colestipol and conclusions about the incidence of computational design and molecular imprinting on the structure of the binding sites were extracted.

2. Experimental

2.1. Materials

Cholic acid, sodium cholate, ethyleneglycol dimethacrylate (EGDMA), 1,1′-azobis(isobutyronitrile) (AIBN), ethyleneglycol methacrylate phosphate (EGMP), and 2-(diethylamino)ethyl methacrylate (DEAEM) were from Sigma-Aldrich (Spain). N-(3-aminopropyl)-methacrylamide hydrochloride (APMA·HCl) was from Polysciences

(Germany). 2-Hydroxyethyl methacrylate (HEMA) was from Merck (Germany). Dimethylsulfoxide (DMSO) was puriss. grade (≥ 99.5%) from Fluka (Spain) and used as received in bottles containing molecular sieves ($H_2O \leq 0.01\%$). All other chemicals were analytical or HPLC grade and used without further purification.

2.2. Molecular modeling

The computational design used for preparing the networks has been described elsewhere [23-26]. Briefly, the workstation used to simulate monomer–template interactions was a Silicon Graphics Octane running IRIX 6.5 operating system, configured with two 195MHz reduced instruction set processors, 1GB memory and a 20GB fixed drive. The system was used to execute the software package SYBYL 7.0™ (Tripos Inc., St. Louis, Missouri USA). The structure of cholic acid was drawn and its energy minimized using the dielectric constant value of DMSO to get stable conformations. A virtual library containing 18 of the most commonly used monomers in molecular imprinting was used and the energy of these monomers minimized as the template 'in DMSO'. The LEAPFROG™ algorithm (30000 iterations) was applied to screen the library of functional monomers for their possible interactions with the template.

2.3 Synthesis of the networks

Each functional monomer EGMP, DEAEM or APMA·HCl (9% mol) was mixed with the cross-linker EGDMA (91% mol). The monomers solutions (3 ml) were diluted with the same volume of DMSO (Table 1) and 30 mg (1.2% mol) AIBN were added. In the case of the imprinted networks, 150 mg cholic acid (2.5% mol) were incorporated too. The reaction mixtures were purged with nitrogen and then left to polymerize at 70 °C for 24 h. The bulk polymers were ground in methanol using a manual mortar and then wet-sieved through 125 and 38 μm meshes (Endecotts, UK). The particles retained on the 38 μm mesh underwent Soxhlet extraction with methanol (500 ml, 24 h) and then were dried under vacuum. Networks containing APMA·HCl were also prepared with lower degrees of cross-linking, replacing a certain volume of EGDMA by HEMA, as indicated in Table 2.

2.4. SEM images

Particles of each polymer network were analyzed by scanning electron microscopy (LEO-435VP SEM, Leo Electron Microscopy, UK). Samples were mounted on double-sided tape on aluminum stubs and sputter-coated with gold/palladium, and micrographs were taken at various magnifications.

2.5. Degree of swelling

50 mg dried particles were immersed in 1 ml water and mechanically shaken for two hours. Then, the wet particles were gently filtered, weighed and placed in an oven at 50ºC for two days. The degree of swelling was calculated using the equation:

$$Q = \frac{(Ww - Wd)}{Wd} \cdot 100 \qquad (1)$$

where *Ww* and *Wd* are the weights of the particles after swelling and once dried, respectively. The experiments were carried out in duplicate.

2.6. Adsorption isotherms

Equilibrium batch binding experiments were performed, in triplicate, with 1.5 mg of polymers that were loaded in ultracentrifuge columns (Pierce Centrifuge Columns 0.8 ml, ThermoScientific, Rockford IL USA) and mixed with 500 µL of sodium cholate solution (0.1-0.5 mM in water, pH values ranging from 6.40 to 6.73). The columns were placed into Eppendorf tubes and mechanically shaken at room temperature for 12 h and then centrifuged for 2 min at 600 rpm. The concentration of sodium cholate remaining in the medium was spectrophotometrically quantified as previously reported [27]. Briefly, 3 ml of sulfuric acid 96% w/w were poured into test tubes containing 1 ml of sodium cholate solution inside a water/ice bath. After mixing, the test tubes were heated to 70ºC for 30 min and then the absorbance was measured at 389 nm (Agilent 8354, Germany).

The adsorption isotherms were characterized using the Freundlich model:

$$LogB = m \cdot \log F + \log a \qquad (2)$$

where *B* (µmol per gram of polymer) and *F* (mol/l) are the concentrations of bound and free sodium cholate, respectively, and *a* and *m* are fitting constants that yield a measure of physical binding parameters [28]. The preexponential factor *a* is a measure of the capacity (number of binding sites) and average affinity of the network. The constant *m* is a heterogeneity index; *m* values close to 1 indicate that all binding sites are identical

from an energetic point of view, while *m* values near 0 indicate heterogeneous binding points [29].

The affinity distribution of the binding sites (i.e., the plot of the number of sites, N, that have association constant, K) was estimated using the following equation [28]:

$$N(K) = 2.303 \cdot a \cdot m \cdot (1 - m^2) e^{-2.303 \cdot m \cdot \log K} \qquad (3)$$

within the limits $K_{min} = 1/F_{max}$ and $K_{max} = 1/F_{min}$.

2.7. Binding of sodium cholate from aqueous medium

20 mg of each polymer were packed in 1 ml filtration tubes (Supelco, Bellefonte PA USA), which were placed in a VisiPrep 12-vial vacuum manifold (Supelco, Bellefonte PA USA) connected to a vacuum pump. The cartridges containing the polymers were washed with 2 ml of water, 2 ml of 0.1% NaOH in 50:50 ethanol:water solution, 2 ml of 0.1 M formic acid, and 2 ml of methanol. Then, 1 ml aliquots of 0.2 mM sodium cholate solutions in phosphate buffer pH 7.4 were successively passed through the polymers up to a total volume of 30 or 50 ml. The flow rate of the solution through the cartridge, with the vacuum pump connected since the beginning of the experiment, was 1 ml/min. Each extracted portion was collected and the concentration of unbound sodium cholate was spectrophotometrically determined as explained in section 2.6. The experiments were carried out in triplicate.

3. Results and discussion

3.1. Design of the polymer networks

In silico screening of the most suitable monomers for preparing a certain functional polymer is gaining attention owing to the considerable number of monomers that can be tested in short time and without consumption of materials [25,26]. Our strategy comprised the development of a library of monomers, the selection of monomers with affinity for the target molecule, the synthesis of polymers using these monomers, and their testing in re-binding experiments. A virtual library of 18 commonly used functional monomers that are capable of interacting with cholic acid through electrostatic, hydrophobic, van der Waals forces or dipole-dipole interactions was screened against the template. The interaction energies obtained by docking template and monomer structures, minimized using the dielectric constant value of DMSO, are

reported in Table 3. DMSO was selected for the modeling as it was the porogen chosen for the preparation of MIPs by radical polymerization. In fact it has been previously shown that MIPs prepared in polar organic solvents (DMF and DMSO) also possess good affinity for the template in aqueous solutions [30-32]. Strong interactions such as electrostatic, present between monomers and template at the polymerization stage in a polar solvent, have high chances to take place also in aqueous solution during the rebinding. Normally, when MIPs need to work in aqueous solutions, the modeling is also repeated minimizing molecular structures using the dielectric constant of water. Nevertheless, since the molecules of solvents are not physically included during the screening process, the energy values for monomers-template complexes, when structures are minimized either in water or in polar organic solvents, are usually very similar.

The results in Table 3 show that allylamine, in its charged (protonated) form, demonstrated the strongest energy of interaction with sodium cholate. However this monomer was not considered for the MIP preparation, since, as shown previously [33], allylamine is prevalently neutral in a wide range of pH values and practically never protonated. APMA·HCl and EGMP were also among the monomers with the highest affinity for cholic acid (Table 3). Aminoethyl methacrylate both in HCl salt and free base showed strong interactions with the template and was initially considered for the preparation of MIPs. However it was subsequently discarded because of its limited solubility in DMSO and other polar solvents. DEAEM, which is the most basic monomer among those tested [33], showed a binding energy lower than APMA and EGMP but slightly greater than the cross-linker agent EGDMA. Most of the monomers that appear in Table 3 between EGMP and DEAEM are either very weak bases, which are known to be neutral, or possess anionic acid groups, which interacted with cholic acid through the same positions as EGMP and were not therefore considered for further experiments. According to the modeling, EGMP and DEAEM can interact with cholic acid through hydrogen bonds, while APMA·HCl can also electrostatically interact with the carboxylic groups (Figure 1). Since hydrogen bonds cannot be established in aqueous solutions, among the three monomers APMA HCl was from the beginning the most promising for production of polymeric networks with high affinity for cholic acid.

Nevertheless also EGMP and DEAEM were selected as functional monomers and used for the production of MIPs specific for the target molecule.

Both imprinted (MIP) and non-imprinted (NIP) networks were prepared in order to test also the effect of the molecular imprinting on the affinity of the networks for sodium cholate. Cholic acid was used as template molecule since the sodium salt is not soluble in the monomers solution. The functional monomer:template molar ratio was set at 4:1, which is a common molar ratio in non-covalent imprinting for ensuring the saturation of the binding points of template [34,35]. Non-imprinted networks were prepared in the absence of template. After polymerization, the polymers were mechanically crashed and wet-sieved in methanol, and the portion of particles retained between 38 and 125 μm meshes was used for the following experiments. The particles were subjected to Soxhlet extraction with methanol to ensure the complete removal of both fines and unreacted monomers and template molecules. SEM micrographs of APMA·HCl-based networks are shown in Figure 2. The high DMSO proportion used during synthesis led to porous networks. Similar morphologies were observed for the other polymer networks.

3.2. Sodium cholate binding isotherms

Binding isotherms clearly showed the incidence of the functional monomer on the affinity of the polymers for sodium cholate (Figure 3). Despite the high binding energies identified for EGMP by the computer modeling, the networks prepared with this monomer showed a binding isotherm similar to that of networks prepared without functional monomer. This finding was not, however, surprising since it can be easily explained by the inability of the monomer to form hydrogen bonds in aqueous medium and also by the electrostatic repulsion between the anionic groups of the functional monomer and cholate molecules. Such electrostatic repulsion was not highlighted by the modeling because cholic acid was screened against the monomers in its neutral form. Oppositely, DEAEM and APMA·HCl significantly enhanced the binding capability of the polymer networks. The isotherm obtained for APMA·HCl-based networks showed a high binding affinity with remarkably low concentrations at equilibrium, which means that most sodium cholate was absorbed by the network. This isotherm, which resembles that described for colestipol, suggests that the APMA·HCl-based networks could

efficiently retain bile salts in the gastrointestinal tract [7]. As predicted by the modeling, the protonated amine group of APMA enables ionic interactions with the carboxylic acid group of cholate, while hydrophobic interactions between the apolar face of cholate and the cross-linking points can help to stabilize the adsorption.

The binding isotherms were fitted to the Freundlich model (Statgraphics Plus 5.1, Statistical Graphics Corp.) in order to gain insight into the heterogeneity of the binding sites (which is a common phenomenon in imprinted networks) from an energy point of view [36]. Freundlich isotherm measures the heterogeneity of binding site as affinity distributions (AD) and heterogeneity index (*m*) [29]. The values in Table 4 show that control networks have *m* values above 1, which indicates that the networks are homogeneous; i.e., there are not specific binding points (Table 4). The other polymers have *m* values between 0 and 1, confirming the existence of binding sites of varying affinity and selectivity, probably due to differences in chemical groups distribution and in depth and shape of the binding pockets. APMA·HCl-based networks show the lowest *m* values. This means that in these polymers there are both high and low affinity domains; the first ones may correspond to the sites in which APMA·HCl is located. No incidence of the cross-linking degree on the binding isotherms was observed in the range evaluated (data not shown). EGMP and DEAEM MIPs showed *m* values lower than the corresponding non-imprinted networks. This suggests that the presence of template during the polymerization contributes to the heterogeneity of the material, increasing the affinity of the binding sites by causing an adequate spatial arrangement of the monomers. The networks with APMA·HCl showed such a high affinity for the template that the imprinting did not seem to improve the binding sites. Such a high affinity is also responsible for the relatively worse fitting to the Freundlich model compared to the other networks.

Affinity distributions of the binding sites of each polymer are shown in Figure 4. The exponential decay (linear log-log plot) is characteristic of the isotherm region far from saturation [29]. The subsaturation region, in which the high affinity binding sites are preferentially filled, is the most interesting for a wide range of applications of non-covalently imprinted networks. This is because the difference between imprinted and

non-imprinted networks is particularly evident at low loadings. In addition it is very difficult to reach saturation in most non-covalently imprinted polymers because of the heterogeneity of the material. As expected from the *m* values, the affinity distributions shown in Figure 4 indicate that the networks have association constants ranging from 3100 to 160,000 l/mol, with predominance, in all polymers, of sites with low affinity. When compared with the networks prepared with the other functional monomers, EGMP polymers showed the least amount of binding points with also the lowest affinity. The imprinting notably enhanced both the number of binding sites and their affinity. On the other hand, the APMA·HCl networks possessed many more binding sites of high association constant, but the contribution of the imprinting was not evident. These results confirm that APMA·HCl itself was able to create high affinity binding sites and that the arrangement during synthesis did not lead to a relevant improvement in the association constant. Networks prepared with DEAEM showed an intermediate behavior and the imprinting contributed to increase the number of high affinity binding sites. On the bases of these results EGMP polymers were abandoned and further testing was continued using only APMA HCl and DEAEM networks.

3.3. Removal of sodium cholate from aqueous medium

The capability of the polymers to act as traps of sodium cholate in phosphate buffer pH 7.4 was tested in a dynamic mode, i.e., under a relatively rapid flow of cholate solution through the polymer bed. This was done in the attempt to simulate the conditions in the gut. Under these experimental conditions, only the networks with a high affinity and capable of rapidly capturing the target molecules would be able to effectively retain sodium cholate. The amounts of sodium cholate retained and non-retained by the networks made with and without (controls) functional monomers are depicted in Figures 5-7. Both DEAEM and APMA·HCl polymers showed higher binding capacity than the control polymers. Between the two monomers, APMA·HCl provided the networks with the highest binding capacity, significantly diminishing the concentration of sodium cholate in the aqueous medium.

3.4. Effect of the degree of cross-linking

In the attempt to optimize the performance of the APMA·HCl networks, we evaluated the feasibility of decreasing the degree of cross-linking of the polymer network, by replacing part of EGDMA with a structurally related monofunctional monomer such as HEMA. Cholate molecule is relatively large, about 17 Å length and 5.3 Å width estimated using CS Chem3D Std® (CambridgeSoft Corp., MA). The mesh size of the network prepared with 91% mol EGDMA (8.7 Å distance between two adjacent cross-linking points) [37] is too low to enable sodium cholate to reach all the binding points even after swelling in water. Therefore, a set of polymers were prepared with decreasing content of EGDMA and fix proportion of functional monomer APMA·HCl (Table 2). The capability of the resulting MIPs and NIPs to trap sodium cholate in the dynamic mode was evaluated. As can be seen in Figure 8, the loading significantly improved (2-fold) when the EGDMA was reduced up to 31.7 mol% (F networks in Table 2); below this proportion the amount loaded leveled off or even decreased. The initial improvement can be clearly attributed to the easiness of the cholate molecules to diffuse through the polymer network as the mesh size increases. The APMA·HCl particles, in the range of cross-linking evaluated, had similar degrees of swelling in water (250-300%), but are significantly different in mesh size. Upon synthesis, the distance between adjacent cross-linking points was estimated from the number of molecules of cross-linker EGDMA per unit of volume (cm^3) of network [37]:

$$R_X = \frac{10}{\sqrt[3]{EGDMA \cdot N_A}} \qquad (4)$$

with N_A being the Avogadro´s number. For example, the distance between adjacent cross-linking points is expected to be 12 and 14.3 Å for F and G networks, respectively. These values are 37 and 64% larger than the distance of the APMA·HCl networks prepared with the highest degree of cross-linking.

The non-imprinted polymers prepared with APMA·HCl behaved as well as the imprinted ones due to the ability of the functional monomer itself to bind strongly sodium cholate creating high affinity binding sites (F MIP and NIP profiles were superimposable, Figure 8). This is quite advantageous from the point of view of potential pharmaceutical applications of the materials as traps of bile acids *in vivo*. In fact MIPs usually require prolonged washing steps to achieve total removal of template

and in most cases the risk of template leaching, once in contact with physiological fluids, is not completely eliminated. This would be a major issue and could compromise MIPs approval for clinical use. It would be much easier to get approval for a sorbent like APMA·HCl NIP, which has got affinity and binding capacity for sodium cholate high enough to be used in clinical applications and at the same time would not be affected by issues as template leaching.

The results of this work revealed that computational modeling is adequate for a fast identification of the most suitable monomers to create efficient traps for a certain substance. Once the functional monomer is chosen, a conventional polymer synthesis enables the synthesis of high affinity networks avoiding waste of time and resources. This rational approach can therefore be of general application for creating traps for a wide range of substances with foreseeable high performances.

4. Conclusions

Computational modeling is a useful tool for the screening monomers with affinity for a given target molecule, enabling a rational design of functional networks. Nevertheless, since the modeling is performed using some approximations such as a 'virtual' inclusion of solvent through the use of its dielectric constant during minimization, differences can occur between modeling and experimental results especially when polymerization and rebinding steps are done in different liquids. In this specific case the use of functional monomers containing amine groups notably enhanced the capability of the acrylic networks to uptake sodium cholate. Combination of the screened functional monomers with molecular imprinting technology remarkably improved the performance of networks made of monomers with affinity for the target, through an adequate arrangement of the monomers into pockets suitable to host the target molecules. However, molecular imprinting technology is of less relevance when the functional monomer itself, as predicted by the computer modeling, has a strong affinity for the template/analyte. In addition, in this work we showed that by tuning the degree of cross-linking of networks with high affinity functional monomers it is possible to enable optimization of the loading capability, making the materials useful as traps of undesirable biological molecules.

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Figure captions

Figure 1. Computational modeling of the interaction of cholic acid with EGMP, DEAEM, and APMA·HCl.

Figure 2. SEM micrographs of APMA·HCl-based networks.

Figure 3. Binding isotherms of sodium cholate in water by imprinted (MIP) and non-imprinted (NIP) networks prepared with EGMP, DEAEM, APMA·HCl or without functional monomers (control). The functional monomer and cross-linker proportions upon synthesis were 9% mol and 91% mol, respectively. For preparation of imprinted networks, 2.5% mol of template was added to the polymerization mixture.

Figure 4. Affinity distributions based on the fitting to the Freundlich model of the sodium cholate isotherms, obtained for imprinted (full symbols) and non-imprinted (open symbols) networks prepared with different functional monomers.

Figure 5. Sodium cholate retained by polymer particles (top graph) and remaining free in the medium (bottom graph) after flowing the solution through the polymer prepared with DEAEM.

Figure 6. Sodium cholate retained by polymer particles (top graph) and remaining free in the medium (bottom graph) after flowing the solution through the polymer prepared with APMA·HCl.

Figure 7. Sodium cholate retained by polymer particles (top graph) and remaining free in the medium (bottom graph) after flowing the solution through the polymer prepared without functional monomer.

Figure 8. Sodium cholate retained by polymer particles (top graph) and remaining free in the medium (bottom graph) after flowing the solution through the polymers prepared with APMA·HCl and different degrees of cross-linking and through commercially available colestipol granules. Profiles of MIP and NIP were superimposable for F and G networks.

Table 1. Composition (mg) of the polymer networks synthesized with or without (control) functional monomer, in the presence (MIP) or absence (NIP) of the template cholic acid.

Polymer network	**DEAEM**		**EGMP**		**APMA·HCl**		**Control**	
	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP
Functional monomer (mg)	272	272	262	262	262	262	0	0
Cholic acid (mg)	150	0	150	0	150	0	150	0
EGDMA (mg)	2578	2578	2539	2539	2737	2737	3000	3000
DMSO (ml)	3	3	3	3	3	3	3	3
AIBN (mg)	30	30	30	30	30	30	30	30

Table 2. Composition (% mol) of the polymer networks synthesized with APMA·HCl and various degrees of cross-linking.

Polymer network	Cholic acid	HEMA	APMA·HCl	EGDMA
APMA·HCl	0 (NIP)-2.5 (MIP)	0	9.0	91.0
A	0	6.2	9.9	83.9
B	0	12.1	9.7	78.2
C	0	23.3	9.3	67.4
D	0	33.5	8.9	57.5
E	0	43.1	8.6	48.3
F	0 (NIP)-2.5 (MIP)	60.0	8.3	31.7
G	0 (NIP)-2.5 (MIP)	92.3	9.2	21.5

Table 3. Binding energies, estimated using the LEAPFROG algorithm, of cholic acid with the monomers contained in the virtual library.

Monomer	**Binding energy (kcal mol^{-1})**
Allylamine protonated	-50.14
N-(3-aminopropyl) methacrylamide hydrochloride (APMA·HCl)	-48.43
Aminoethyl methacrylate·HCl	-47.87
Ethyleneglycol methacrylate phosphate (EGMP)	-41.75
N,N´- Methylenebis(acrylamide)	-39.73
Ethyleneglycol methacrylate phosphate deprotonated	-37.99
Acrylamido-2-methyl-1-propanesulfonic acid	-33.67
Aminoethyl methacrylate	-32.20
Itaconic acid	-31.42
Itaconic acid deprotonated	-30.01
1,3,5-Trihydroxylstyrene	-29.97
N,N-Diethylamino ethylmethacrylate protonated (DEAEM·HCl)	-29.82
Acrylamide	-27.39
N,N- Diethylamino ethylmethacrylate (DEAEM)	-27.14
Ethyleneglycol dimethacrylate (EGDMA)	-25.52
Methacrylic acid deprotonated	-25.16
N-(3-aminopropyl) methacrylamide (APMA)	-24.39
Allylamide	-23.69

Table 4. Fitting of the sodium cholate isotherms to the Freundlich model ($\alpha < 0.01$). Parameter *a* is related with the binding affinity and parameter *m* is the heterogeneity index. The standard errors are shown in parenthesis.

Polymer network	***m***	***Log a***	r^2	$F_{1,5\ d.f.}$
EGMP-NIP	0.811 (0.065)	4.824 (0.276)	0.969	153.50
EGMP-MIP	0.717 (0.042)	4.638 (0.186)	0.983	291.67
DEAEM-NIP	0.735 (0.038)	5.031 (0.169)	0.987	374.37
DEAEM-MIP	0.641 (0.042)	4.606 (0.189)	0.978	225.70
APMA·HCl-NIP*	0.306 (0.093)	3.577 (0.424)	0.781	10.70
APMA·HCl-MIP*	0.367 (0.104)	3.833 (0.555)	0.688	9.43
CONTROL-NIP	1.617 (0.138)	7.990 (0.530)	0.986	136.97
CONTROL-MIP	1.396 (0.084)	7.296 (0.329)	0.993	275.82

* The first two points of these isotherms were discarded for the fitting; $\alpha < 0.05$

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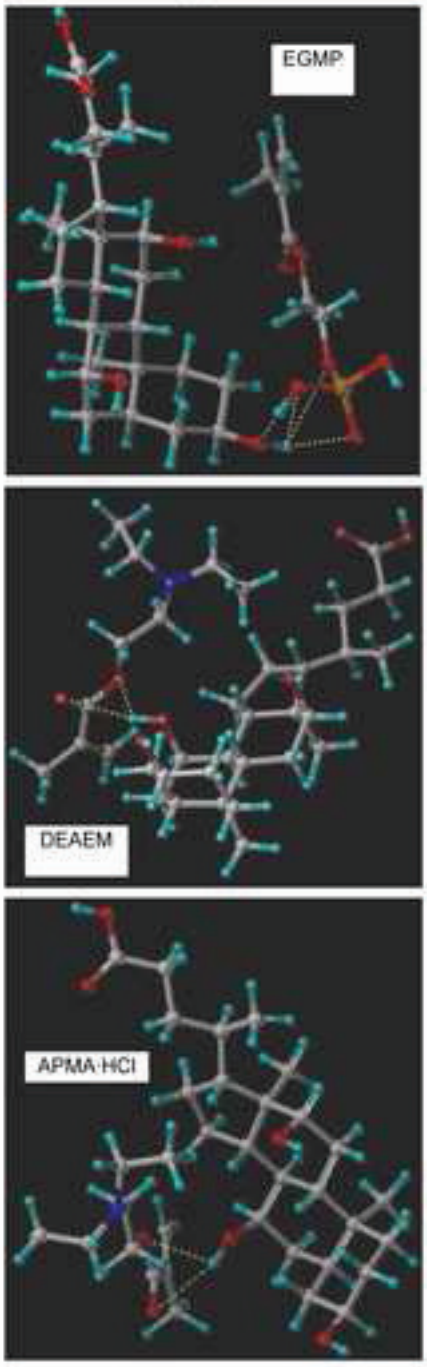

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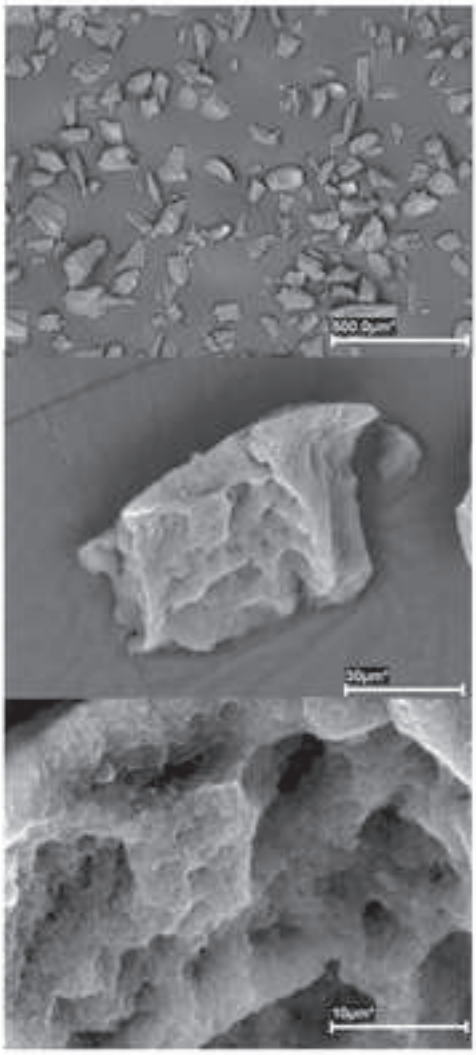

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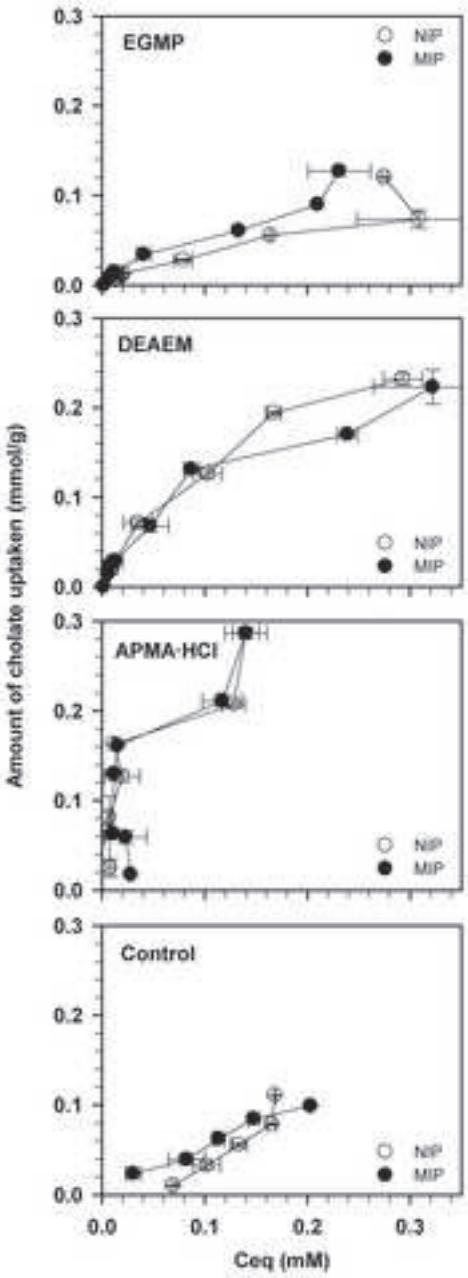

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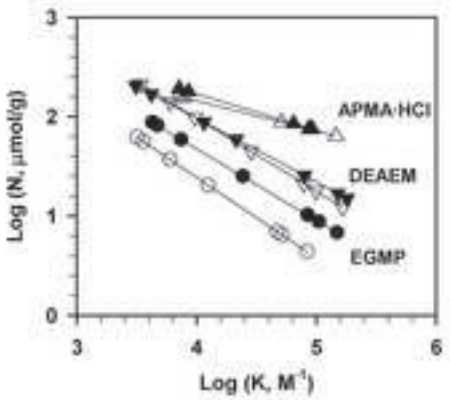

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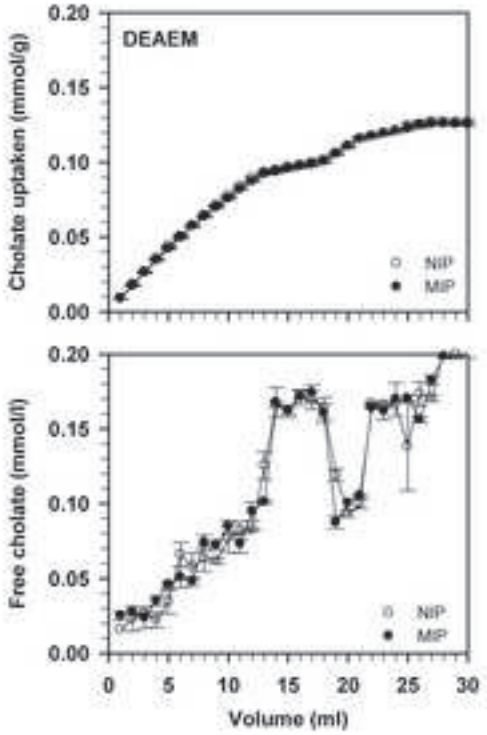

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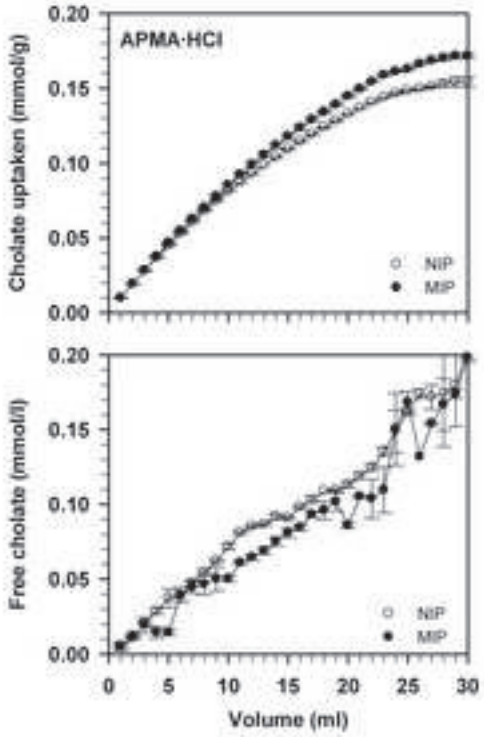

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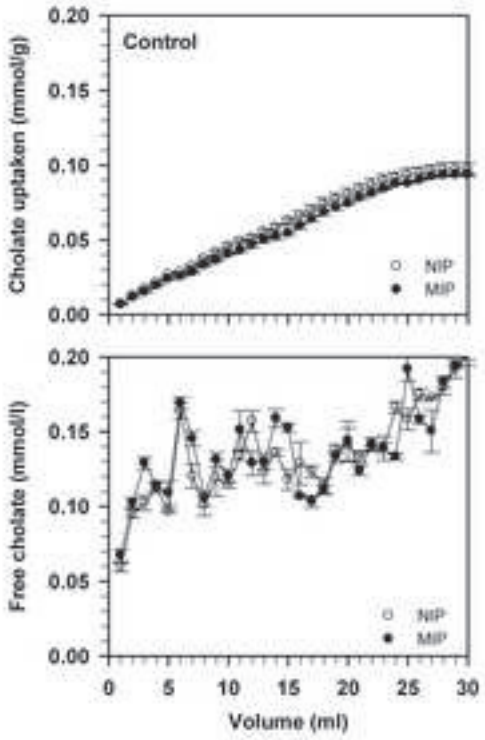

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