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ORIGINAL ARTICLE

Molecular imprinting of β-cyclodextrin/cholesterol template into a silica polymer for cholesterol separation

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Abstract The molecular assembly formed by the inclusion complex of cholesterol in β-cyclodextrin (β-CD:chol) was used as a template for the molecular imprinting of a sol–gel polymer (MIP/β-CD:chol), produced with tetraethoxysilane (TEOS) as precursor. The MIP/β-CD:chol and pure silica matrix (PSM) were tested for the efficiency of cholesterol removal from solutions at different cholesterol concentrations (1–10 mg/mL). The adsorption tests were run at 25°C using 1% (w/v) solid/liquid suspensions during 24 h. The MIP/β-CD:chol data on cholesterol adsorption was fitted by the Langmüir isotherm model, giving a maximum adsorption capacity of 76.5 mg cholesterol/g-adsorbent. The PSM data did conform to the Langmüir model. The maximum cholesterol adsorption achieved with the PSM was higher, 251 mg/g, probably due to multilayer adsorption. The hydrophobic silica matrix, imprinted with the inclusion complex of β-CD and a target molecule, has the potential of being used as an adsorbent for other organic molecules.

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Introduction

Molecular imprinting is a process that utilizes the molecular recognition phenomena between a template molecule and the monomers employed in the polymerization of a rigid macroporous polymer, to create a unique network of microporous cavities within the polymer matrix. After removal of the template following the polymerization, the rigid macroporous polymer features a highly defined microporous environment, which retains a three-dimensional shape and thus, potential separative selectivity for chemical species related to the original template molecule [1–3]. Asanuma et al. [3] have devised a new molecular imprinting technique, in which several host molecules fit appropriated portions of a large target guest molecule. They have chosen cyclodextrins (CDs) as the functional hosts since they bind angstrom-sized guests through non-polar interactions.

Elevated cholesterol levels in blood are a major risk factor for developing coronary heart disease [4, 5]. A high dietary cholesterol intake can elevate its level in the blood considerably. Several methods such as extraction with solvents, supercritical fluid extraction, steam distillation and treatment with cyclodextrin or saponin have been adopted to reduce cholesterol in food [4]. Among these methods, removal of cholesterol complexed by β-CD has been widely implemented in the dairy industry at lower operation costs, compared to other methods. β-CD is a cyclic oligosaccharide with a

non-polar cavity in the center of the molecule, which has the capability of forming an inclusion complex with a great variety of compounds. The presence of these compounds inside the cavity of the CD molecule produces changes in the electronic environment of the CD atoms [6]. Our studies focus on creating hydrophobic solid matrices that can recognize and bind biologically significant molecules, such as cholesterol. These matrices are obtained by the sol–gel technique accompaigned by molecular imprinting of a target molecule in the presence (MIP/β-CD: target molecule) or absence β-CD (PSM, Pure Silica Matrix). The mutual conformation of β-CD and the hydrophobic matrices obtained by the sol–gel technique are successfully regulated by crosslinking them in presence of cholesterol.

Experimental

Materials

Tetraethoxysilane (TEOS), supplied by Across Organic (NJ, USA), was used without further purification as precursor for producing the molecular imprinted polymer. Cholesterol was supplied by Sigma Chemical Company Ltd-UK. Ethanol (minimum 99%), ammonia (minimum 28%), and hydrochloric acid (minimum 36%) were of analytical grade.

Methods

Synthesis of the imprinted polymers and immobilization of β-cyclodextrin

Tetraethoxysilane, β-CD and the target molecule (cholesterol) were dissolved in ethanol and the methodology previously established by Soares et al. [7] was adapted for preparing the molecular imprinted polymer (MIP/β-CD:chol). The system became opaque as the polymerization proceeded. After 2 h of stirring, a white precipitate was collected and washed with a large amount of hot water and acetone. As a control, non-imprinted β-CD: cholesterol polymer was prepared in the same manner as given above, except for the absence of β-CD and the target molecule, producing then the PSM.

Cholesterol adsorption isotherms

Isothermal adsorption studies were conducted at 25°C using 0.5 g of PSM or MIP/β-CD:chol and varying concentrations (1–10 g/L) of cholesterol in 50 mL of ethanol (95%). The absorption test medium was kept in constant agitation for 24 h and then, a sample of the liquid solution was taken for analysis. The extent of adsorption was calculated based on the difference between the cholesterol concentrations in the solution, before and after adsorption. The cholesterol concentration was determined by HPLC (Shimadzu LC9A, Japan) using a Hichrom C18 reverse phase column (Hichrom, Reading, UK) at 35°C. A mobile phase consisting of acetonitrile and isopropanol (70:30, v/v) was used. The concentration of cholesterol was obtained from a standard curve of the HLPC peak area as a function of the cholesterol concentration in acetonitrile solution.

Cholesterol removal

One percent (w/v) MIP/β-CD:chol or PSM was added to diluted cholesterol solutions (2.49 and 4.34 mg/mL) to carry out the cholesterol removal test. 1 mL of the cholesterol solution was pipetted out from the test medium at regular intervals and the cholesterol concentration was determined after adding 9.5 mL of acetonitrile. The percentage of removal was also calculated based on the difference between cholesterol concentrations in the cholesterol solution, before and after adsorption.

Results and discussion

Isothermal adsorption studies

The extent of cholesterol adsorption at 25°C and as a function of the initial cholesterol concentration is shown in Figs. 1 and 2, respectively for the PSM and

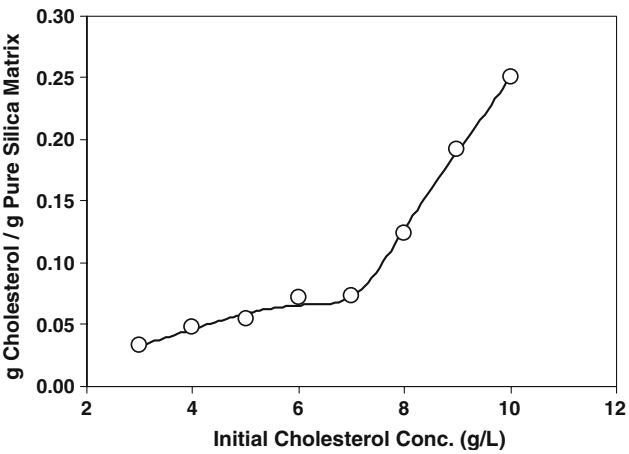

Fig. 1 Amount of cholesterol adsorbed on PSM as a function of the initial concentration at 25°C

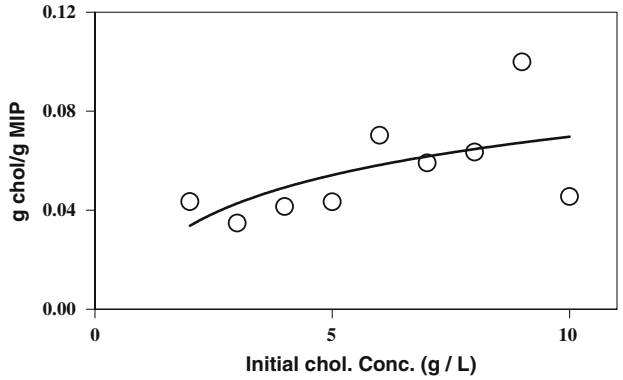

Fig. 2 Amount of cholesterol adsorbed on MIP/β-CD:chol as a function of the initial concentration at 25°C

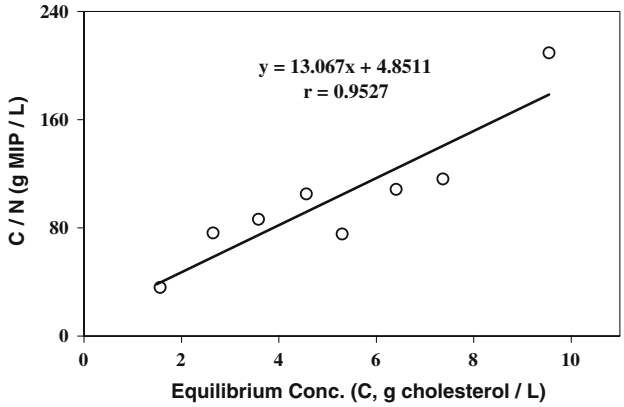

Fig. 3 Fitting of the Langmüir model, Eq. 1, to the adsorption of cholesterol into the molecular imprinted polymer (MIP/β-CD:chol)

MIP/β-CD:chol. Figure 1 shows that in the case of PSM the adsorption data has two regions, suggesting that at cholesterol concentrations above 7 g/L, multilayer absorption might occur. For the MIP/β-CD:chol adsorption data, Fig. 2 shows more scatter than Fig. 1. From these figures, the maximum cholesterol adsorption capacity of the PSM and MIP/β-CD:chol matrices can be determined, within the experimental range of cholesterol solutions tested. The adsorption data was fitted to the Langmüir isotherm model, in the form given by the Eq. 1:

$$C/N = C/N_{\mathrm{m}} + 1/(N_{\mathrm{m}} \times K_{\mathrm{L}}), \quad (1)$$

where N (g/g) is the amount of cholesterol adsorbed by the solid matrices, at the final equilibrium cholesterol concentration, C (g/L), N_{m} is the maximum amount of cholesterol adsorbed at monolayer coverage (g/g), and K_{L} is the adsorption equilibrium constant (L/g).

Because of the form of data shown in Fig. 1, the PSM adsorption data was not amenable to fit to the Langmüir model, even using only the first region up to cholesterol concentration of 6 g/L.

The MIP/β-CD:chol adsorption data was reasonably well fitted by Eq. 1, giving the straight line shown in Fig. 3. From the slope and intercept of this line, Eq. 1 parameters were calculated, giving 0.0765 g/g for the maximum adsorption capacity (N_{m}) and 2.69 L/g for the adsorption equilibrium constant (K_{L}).

The Langmüir adsorption model is generally regarded as being the most sensitive model in describing sorption phenomena of the kind treated in this article, because of its assumptions, especially, that the surface adsorption sites are homogeneous with respect to the bonding energy for molecules such as cholesterol and that an adsorbed monolayer is formed [6]. Therefore, Langmüir model has been adopted by several researchers to describe the phenomenon of cholesterol adsorption [6]. However, Fig. 1 has shown that for the PSM and higher cholesterol concentrations, multilayer absorption might take place.

Theoretically, three mol of β-CD will form a complex with 1 mol of cholesterol [8], and the strategy developed by this research group consisted of assembling several host CDs in a polymer network, with a spatial distribution that allowed each of them to fit a designated portion of the target molecule. The result was that the assembly, as a whole, could recognize the guest molecule with high specificity, similarly to the method described by Alvarez-Lorenzo and Concheiro [2]. If each cholesterol molecule is bond to an assembly of three β-CD molecules, then the mass of cholesterol that could be absorbed by the solid matrix should be approximately equal to that of the β-CD (molar masses of cholesterol and β-CD are 386.7 and 1,135 g/mol, respectively). However, in this study, 0.17g of β-CD was immobilized on 1g of MIP/β-CD:chol beads, whereas the maximum capacity (N_{m}) of cholesterol adsorption was 0.0765 g/g, that is 2.2 smaller than the mass of bound β-CD. This could be the result of some β-CD molecules being occluded in the silica matrix. An opposite discrepancy was observed by Chiu et al. [6]. They explained the excess bound cholesterol by the chelation of additional molecules in the gaps between the silica polymer and the β-CD molecules, thereby causing an increased cholesterol adsorption. A comparison of the efficiencies of cholesterol adsorption using various adsorbents reported in the literature is listed in Table 1.

The cholesterol adsorption capacity of 0.0765 g/g achieved with the MIP/β-CD:chol in this study is lower than that obtained by using chol/β-CD or the PSM. However, it is thought that achieving a more open

Table 1 Comparison of the efficiency of cholesterol removal by different adsorbents

Adsorbents	Time (h)	Temperature (°C)	Cholesterol (mg/g sol)
Alumina/CO_2*	4	40	2.4
Terpolymers*	24	37	17
Ch/β-CD*	0.5	25	330
PSM**	24	25	251
MIP/β-CD:chol**	24	25	76.5

*Chiu et al. [6]; **This work

porous structure with the MIP/β-CD:chol matrix may lead to greater cholesterol adsorption capacity.

Conclusions

The molecular imprinting of a β-cyclodextrin/cholesterol complex assembly into a TEOS silica polymer was developed for the preparation of a solid adsorbent, which can be used for the separation of cholesterol from its solutions. It was demonstrated that a fair maximum cholesterol adsorption capacity could be achieved, 76.5 mg/g. The Langmüir equation could be used to describe the cholesterol adsorption phenomena by the imprinted polymer. The maximum cholesterol adsorption achieved with PSM was higher, 251 mg/g, probably due to multilayer adsorption. Studies are in progress to obtain a more porous matrix with the aim to improve cholesterol adsorption capacity.

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