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Development of molecularly imprinted poly(methacrylic acid)/silica for clean-up and selective extraction of cholesterol in milk prior to analysis by HPLC-UV

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In the present paper the assessment of a novel molecularly imprinted polymer – poly (methacrylic acid)/silica, for clean-up and selective extraction of cholesterol in milk samples is described. The relative selectivity coefficients (*k*) values for cholesterol/5-α-cholestane and cholesterol/7-dehydrocholesterol systems were found to be 5.08 and 6.08, respectively, thus attesting the selectivity of MIP for cholesterol under competitive adsorption with structurally analogue steroid compounds. The milk analysis was initially based on saponification followed by liquid-liquid extraction with n-hexane. Then, the protocol of molecularly imprinted solid phase extraction (MISPE) was carried out by loading the milk hexanic extract through 200 mg of MIP or NIP (non imprinted polymer) packed into SPE cartridges at a flow rate of 0.6 mL min^{-1}. The washing step was performed by using n-hexane followed by further elution with ethanol and HPLC-UV analysis at 208 nm. From breakthrough curve the maximum adsorption capacity of MIP towards cholesterol was found to be 29.51 mg g^{-1}. The precision of MISPE protocol was assessed as intra and inter days yielding RSD (relative standard deviations) lower than 4.10%. Cleaner HPLC chromatograms were obtained for milk samples submitted to the MISPE protocol in comparison to the solid phase extraction using NIP or modified octadecyl silica (C_{18}). Recoveries varying from 96.6 up to 102.2 % for milk samples spiked with cholesterol were achieved, thus assuring the accuracy of the proposed method.

Introduction

Cholesterol (CHO) is one of the steroids that exerts the most concern in the medical science due to its essential low concentration in the human body playing an important role in the biologic functions.[1] On the other hand, it is very well known that high CHO intake raises plasma cholesterol levels and can be associated with high risk of cardiovascular disease (CVD), especially in adults with type 2 diabetes[2,3], although the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recognize that this observation is contradictory and cannot be considered as an absolute parameter.[4] Nevertheless, it is a currently consensus that to decrease the incidence of CVD, the diet should be low in total fat, cholesterol and saturated fatty acids. Anyway, as the blood cholesterol is originated from diet and endogenous synthesis, it is advisable to keep the intake as low as possible as, usually lower than 300 mg/day for adults.[4] Therefore, for the determination of cholesterol content in food, such as milk samples, is very important to select a diet with low consumption of this compound. Gas chromatography (GC) and high performance liquid chromatography (HPLC)[5] are the most used techniques for the cholesterol determination in food samples, but due to the very complexity of matrix, previous sample pre-treatments methods based on liquid-liquid (LLE) or solid phase extractions (SPE) for sample clean-up are highly recommendable prior to chromatographic analysis.[6,7] Although LLE is still very used for total cholesterol extraction from food samples after direct saponification method, its present drawbacks including time-consuming, large amount of toxic organic solvents and poorly selectivity to remove interfering species from sample.[7,8] In this sense, the use of molecularly imprinted polymers (MIPs) has been successfully employed to overcome some disadvantages of LLE and even regarding SPE methods when using non selective adsorbents such as modified octadecyl silica (C_{18}).[9,10] MIPs are synthetic polymers, in which the analyte binds preferentially to a receptor with high selectivity in the presence of structurally analogues compounds. The molecular imprinting technology is nowadays a well known area of research and is being increasingly used for separation processes, binding assays, sensing and many other applications.[11,12] Different approaches can be employed for MIPs preparation exploiting radical polymerization using organic monomer, such as bulk, emulsion, precipitation, suspension and two-step methods.[13] In addition, sol-gel processing using organically modified silane precursors (ORMOSILS) and grafting process to the surface of inorganic materials can also be cited.[14,15] More recently, molecular imprinted polymers have been synthesized by combination of random free-radical polymerization followed by sol–gel process to prepare hybrid materials.[16] The polymerization reaction in homogenous medium between the organic and inorganic precursors makes possible to obtain hybrid materials with better textural and morphological properties providing higher specific surface area and low swelling effect in organic solvent with regard to conventional organic imprinted polymers as well as better chemical stability under a wide pH range

in comparison to the inorganic polymers.[17,18] Despite the obvious outstanding features, at the best of our knowledge, no report on the development of molecularly imprinted hybrid materials based on the use of organic and inorganic monomers, were carried out for cholesterol extraction. A survey of literature demonstrates that few attempts have been made for the preparation of molecularly imprinted polymers selective to the cholesterol extraction. Polymerization with template-immobilized silica gel[19], cross linked poly(methacrylic acid–*co*-ethylene glycol dimethacrylate) by means of bulk method[20], seed suspension polymerization[21] and cholesterol-imprinted poly(2-hydroxyethyl methacrylate-*N*-methacryloyl-(l)-tyrosine methyl ester) particles synthesized by bulk polymerization[22] are examples of approaches and polymers for cholesterol extraction. According to the aforementioned, in this work, we have synthesized a novel molecularly imprinted poly(methacrylic acid)/silica hybrid material for clean-up and selective extraction of cholesterol in milk samples prior to analysis by HPLC-UV. The imprinted hybrid material was synthesized with methacrylic acid (MAA) as organic monomer, cholesterol (CHO) as specific template, tetraethoxysilane (TEOS) as inorganic monomer, 3 - propyl methacrylate trimethoxysilane (KH 570) as coupling agent and 2,2´-azobis-isobutyronitrile (AIBN) as initiator, involving reactions of coupling free radical addition and sol-gel processing. The feasibility and selectivity of molecularly imprinted poly(methacrylic acid)/silica hybrid material has been compared to the corresponding blank polymer (NIP) and commercial adsorbent material (modified octadecyl silica-C_{18}).

Experimental

Chemicals

All reagents used in the experiments were of analytical grade Cholesterol (Sigma-Aldrich, 95%), Methanol (Sigma-Aldrich, HPLC grade ≥ 99.9%), Chloroform (Sigma-Aldrich, ≥ 99.8%), hexane (Sigma-Aldrich, ≥ 95%), Methacrylic acid (Acros Organics, 99.5%), Tetraethoxysilane (Sigma-Aldrich, 98%), 3 - propyl methacrylate trimethoxysilane (Sigma, 95%) Dimethylsulfoxide (DMSO) (Fmaia, 99.5%), 2,2 '-azoisobutilnitrila (Sigma-Aldrich, 98%), Hydrochloric acid (Panreac, 37%), Acetic acid (JTBaker, 99.9%), Isopropanol (Sigma-Aldrich, 99.9%), Ethanol (JTBaker, 99.7%), Toluene (Vetec, 99.5%), Acetonitrile (Sigma-Aldrich, 99.0%), 5-α-cholestane (5-α-CHO) (Sigma-Aldrich, 95%) and 7-dehydrocholesterol (7-DHCHO) (Sigma-Aldrich, ≥98% (HPLC)). The water used to prepare the mobile phase was ultrapure Milli Q (Millipore) and filtered through a 0.45 µm nylon membrane daily. The water and solvents used in mobile phase were also degassed using an ultrasonic Bath model USC 1400 (Marconi®, Piracicaba, Brazil).

Apparatus and chromatographic analytical conditions

The chromatographic determinations were performed on a liquid chromatograph model LC-20AT, Shimadzu, operating isocratically. CLC-ODS column (250 mm x 4.6 mm id, 5 mm in particle size) and a guard column Phenomenex (4.0 mm x 3.0 mmm i.d., 5 µm in particle size) were used. The peak purity was determined on a photodiode-array detector (PDA) and monitored at λmax 208 nm. The flow rate of the mobile phase consisted of methanol (MeOH) and water (9:1, v / v) was 1.0 mL min^{-1} and the injection volume was 20 µL. The temperature of chromatographic separation (25˚C) was controlled by using a column oven. All samples submitted to the MISPE procedure were evaporated to dryness at 40˚C. The residues were dissolved in the mobile phase. The morphology of the polymers was evaluated by scanning electron microscopy (SEM), using a microscope JEOL JSM-6360 LV equipped with dispersive energy microscopy. The polymers were coated with a thin layer of gold, using a Bal-Tec MED 020 equipment, in order to minimize charging under the incident electron beam. A manifold system (Bio-Rad) with a capacity for 12 cartridges, coupled to a vacuum pump (Marconi MA 2057) was used in the MISPE procedure. Average pore sizes and volumes of the polymers were estimated by the Barrett-Joyner-Halenda (BJH) method based on nitrogen adsorption experiments using a Quantachrome Nova 1200e automatic instrument coupled to an automatic gas analyzer (all – Quantachrome). Specific surface areas were determined from adsorption isotherms according to the Brunauer-Emmett-Teller (BET) method.

Preparation of MIP

The synthesis procedure was based on our previous publication with some modification.[17] For this task, 0.320 g of cholesterol (as template) were dissolved in 7.0 mL of $CHCl_3$:DMSO (3:1, v/v), and mixed with 5.0 mL of methacrylic acid (54.5 mmol). The mixture was stirred for 1 h at room temperature, and then 1.0 mL of 3 - propyl trimethoxysilane methacrylate (KH 570, 4.21 mmol) and 0.20 g of 2,2'-azoisobutyronitrile (AIBN, 1.22 mmol) were added following sonication for 2 min. A chain structure pre-polymer was formed by copolymerization of MAA and KH 570 in the presence of AIBN and the template molecule. After the mixture was stirred and heated in an oil bath at 60.0˚C for 2 h. Tetraethoxysilane (2.0 mL) was dissolved in 7.0 mL of $CHCl_3$:DMSO and 2 mL of 3% HCl were added under continuous flow of nitrogen gas for 10 min. The reaction was allowed to proceed under stirring at 60.0˚C for 24 h in the oil bath. When the pre-polymer mixed with the TEOS hydrolysis solution, a poly(methacrylic acid)/silica hybrid material was prepared (Figure 1). The obtained material was removed from the flask, dried at 60.0˚C for 24 h, crushed, grounded and sieved to obtain regularly sized particles between 106 and 63 µm. A similar procedure without template was used to prepare the non imprinted polymers (NIP) as control material. For both syntheses, to ensure the complete removal of the excess of reagents and template, the materials were Soxhlet extracted with a mixture of methanol/acetic acid (9:1, v/v) for 48 h and finally they were dried at 60.0˚C for 24 h.

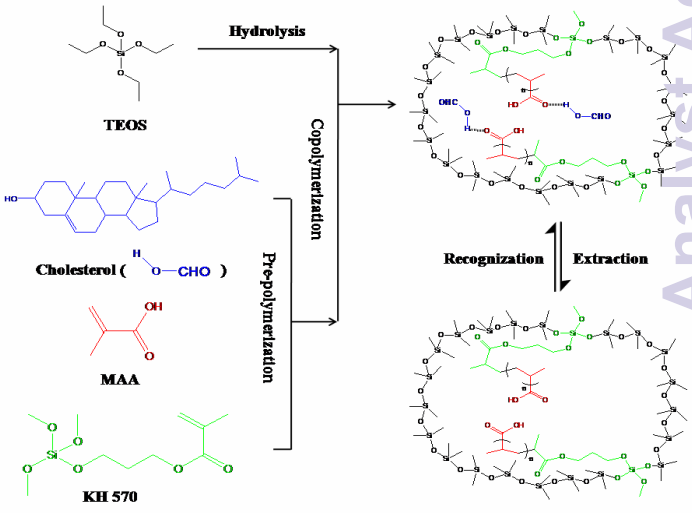


Figure 1. Synthesis of poly(methacrylic acid)/silica hybrid material and recognition mechanism.

Selectivity studies

The selectivity of MIP particles for cholesterol was estimated from competitive adsorption performed in batch procedure by using 5-α-cholestane and 7-dehydrocholesterol as structurally analogue compounds. From previous experimental results (data not shown), the best cholesterol adsorption onto MIP was achieved under chloroform medium. Thus, the batch procedure employed for assessing the selectivity of MIP was performed in chloroform. 50 mg of polymers (MIP or NIP) and 10.0 mL of binaries solutions containing 1000 mg L^{-1} of cholesterol and 200 mg L^{-1} of 5-α-cholestane and in another experiment 1000 mg L^{-1} of cholesterol and 1000 mg L^{-1} of 7-dehydrocholesterol were added in polyethylene flasks. The mixture was stirred in a horizontal shaker at 150 rpm for 12 min for the MIP and NIP. The polymers suspensions were filtered through a 0.45 µm nylon (GVS, New York, USA) filter and the liquid phase was dried at 60˚C. After this step, the residue was dissolved into mobile phase and the CHO was quantified by HPLC-UV. The experimental chromatographic condition to quantify cholesterol and 5-α-cholestane was accomplished by using a mobile phase MeOH:H_2O (8:2, v/v) at a flow rate of 0.5 mL min^{-1} and wavelength of at 208 nm. For the experiments involving the competitive adsorption of cholesterol and 7-dehydrocholesterol, the mobile phase was comprised of MeOH using a flow rate of 1.5 mL min^{-1} and the wavelength was set at 208 nm.
After the stirring procedure and knowing the concentrations of cholesterol, 5-α-cholestane and 7-dehydrocholesterol in the supernatant by HPLC-UV, the distribution (K_d), selectivity (k) and the relative selectivity (k') coefficients according to Equations (1-4) were determined as follow:

$$K_d = \frac{(C_i - C_{eq})}{C_{eq}} \frac{V}{M}, \quad (1)$$

where V is the solution volume (mL), and M is the polymer (MIP or NIP) mass (mg);

$$k = \frac{K_d(cholesterol)}{K_d(5-\alpha-choles\tan e)} \quad (2)$$

$$k = \frac{K_d(cholesterol)}{K_d(7\text{-}dehydrocholesterol)} \quad (3)$$

$$k' = \frac{k_{MIP}}{k_{NIP}} \quad (4)$$

Extraction of total cholesterol from the cow milk

Whole UHT milk samples were acquired from a local supermarket in Londrina city (Brazil). 5.0 L of the milk from the same brand were mixed and 10.0 g of the milk were accurately weighed and placed in a 50.0 mL flask. Then, 8.0 mL of 50% KOH solution and 12.0 mL of ethanol were added to the sample and thoroughly mixed. Subsequently the flask was placed in a water bath under stirring until complete solubilization of sample for 10 min at 40˚C. In the next step, 10.0 mL of water and 3x10.0 mL of n-hexane were added to the unsaponified supernatant. The mixture was separated in a separatory funnel.[23] The hexanic extract was then submitted to the MISPE procedure. The extractions were performed in triplicate.

MISPE procedure

200 mg of the MIP and NIP were packed into SPE cartridges and capped with fritted polyethylene disks at the top and bottom. The SPE cartridges were coupled to a manifold. After conditioning the solid phase with 5.0 mL of n-hexane, 5.0 mL of the milk hexanic extract were percolated through the cartridges at a flow rate of 0.6 mL min^{-1}. Then the cartridges were washed with 5.0 mL of n-hexane. The CHO was eluted with 5.0 mL of ethanol. The eluate was then collected, dried at 40˚C and the residues were redissolved in the mobile phase and analyzed by HPLC-UV at 208 nm.

Breakthrough Curve

The maximum adsorption capacity of MIP towards cholesterol under dynamic condition and breakthrough volume were determined from construction of a breakthrough curve. The assays were performed by percolating aliquots of 5.0 mL of 100.0 mg L^{-1} cholesterol dissolved in n-hexane through a SPE cartridge packed with 200 mg of MIP, previously activated with hexane, until saturation was reached. Each aliquot was collected and analyzed by HPLC-UV, and the amount of cholesterol adsorbed on MIP for each aliquot was determined according to equation (5):

$$Q_e(mgg^{-1}) = \frac{(C_o - C_f)V}{m} \quad (5)$$

where C_o is the initial cholesterol concentration (mg L^{-1}), C_f is the concentration of cholesterol in the column effluent (mg L^{-1}), V is the solution volume (in L) and m is the polymer mass (g).

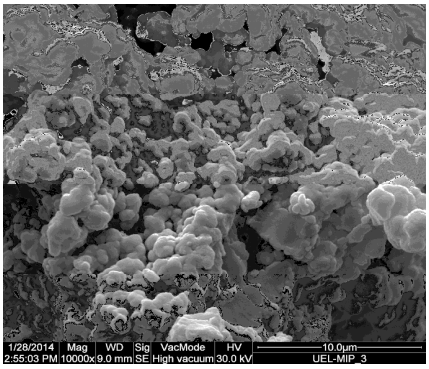
Validation and application of proposed method

The intra-day and inter-day precision (two consecutive working day) was assessed in terms of repeatability by analyzing (n=6) cholesterol concentrations of 75.0 mg L^{-1} and 150.0 mg L^{-1} and the relative standard deviations (RSD) were determined. The proposed method was applied for cholesterol determination in four different brands of whole UHT milk samples, whose accuracy was checked from addition and recovery test. The addition and recovery test was assessed by adding known amounts of cholesterol at two levels 35 and 70 mgL^{-1} in the milk samples.

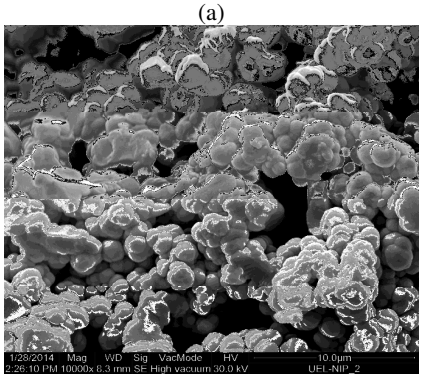
Results and discussion

Scanning electron microscopy (SEM) and textural analysis

The morphological characteristics of the MIP and NIP (Figure 2(a) and (b)) are exemplified by the scanning electron micrographs. As observed, the polymers show a morphological similarity containing particles with high degree of aggregation and a roughness surface. Such features are of paramount importance in the adsorption process. In spite of similar morphological characteristics of the polymers, great differences on the textural data were observed (Table 1). MIP presented a higher surface area as well mesoporos[24] in comparison to NIP, good conditions for solid-phase extraction with quick adsorption/desorption kinetics.



(a)



(b)

Figure 2. SEM images of (a) MIP and (b) NIP with magnification of 10000.

Table 1. Textural properties of the MIP and NIP.

Adsorbent	Pore volume ($cm^3\ g^{-1}$)	Specific surface area ($m^2\ g^{-1}$)	Pore size (nm)
MIP	0.22	42.44	5.47
NIP	0.01	2.23	1.65

Selectivity studies

As already mentioned, the imprinting effect created on MIP can be assessed under competitive adsorption with structurally analogue molecules to the template. The adsorption results are than compared to the corresponding non imprinted polymer. After

Table 2 and 3 show the retention parameters and selectivity of cholesterol on MIP and NIP under competitive adsorption from a binary mixture. As observed, the K_d values obtained for CHO adsorption on MIP were much higher when compared to the adsorption on NIP. On the other hand, very similar K_d values were observed for 5-α-CHO and 7-DHCHO adsorption on MIP and NIP. Thus, obviously the selectivity coefficients ratios (k) of MIP were much higher than those of NIP for CHO adsorption and the comparison of k for MIP with that for NIP shows that the relative selectivity coefficients (k') for MIP under competitive CHO/5-α-CHO and CHO/7-DHCHO adsorption were, respectively, 5.08 and 6.08 times greater than for NIP. From the results and taking into account the similarity among compounds we can confirm the imprinting effect create during polymer synthesis.

Table 2. Retention parameters and selectivity of cholesterol based on K_d, k and k' values for 5-α-CHO with respect to CHO.

Adsorbent	Initial Concentration ($mg\ L^{-1}$)		K_d ($mL\ g^{-1}$)		k	k'
	CHO	5-α-CHO	CHO	5-α-CHO		
MIP	1000	200	711.78	2.02	352.4	5.08
NIP			121.25	1.75	69.3	

Table 3. Retention parameters and selectivity of cholesterol based on K_d, k and k' values for 7-DHCHO with respect to CHO.

Adsorbent	Initial Concentration ($mg\ L^{-1}$)		K_d ($mL\ g^{-1}$)		k	k'
	CHO	7-DHCHO	CHO	7-DHCHO		
MIP	1000	1000	262.43	108.14	2.43	6.08
NIP			11.14	79.22	0.14	

Optimization of MISPE procedure

5.0 mL of milk hexanic extract were applied to MIP and NIP in order to find the optimized MISPE protocol. The solvent n-hexane was utilized in the loading step since it was used to extract the cholesterol from milk.[23] After sample loading, in which 88.3% of cholesterol was retained on MIP, the following solvents or mixtures n-hexane, toluene, n-hexane:toluene (1:9 v/v) and n-hexane:ethanol (1:4 v/v) were tested as washing solvents. 5.0 mL for each of washing solvent were used and 5.0 mL of ethanol was employed in elution step. All the fractions from washing step were collected and detected by HPLC-UV and each assay was carried out in duplicate. As observed in Figure 3, n-hexane was the best eluent to satisfactorily retain the loaded CHO on MIP. This nonpolar solvent was also capable to eliminate possible interfering molecules from sample and enhance the specific interactions between CHO and the binding sites of MIP. Thus, 5.0 mL of n-hexane was chosen for posterior experiments. As regards the use of elution solvent, chloroform, ethanol, acetonitrile:H_2O (9:1 v/v), hexane:ethanol (4:1 v/v), ethanol:acetic acid:chloroform (1:1:3 v/v/v) and methanol were investigated. From Figure 4 shows that CHO was eluted quantitatively from MIP by using 5.0 mL of ethanol, being this condition adopted as eluent in the MISPE procedure.

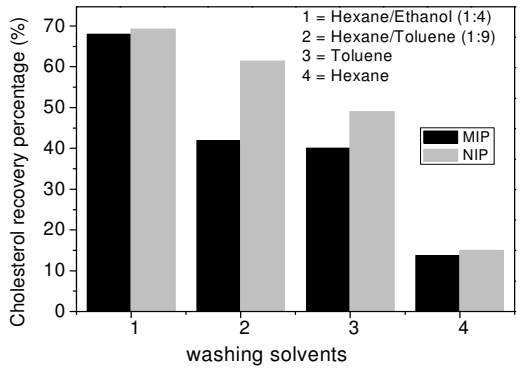


Figure 3. Cholesterol recovery percentage (%) on MIP cartridges using different washing solvents.

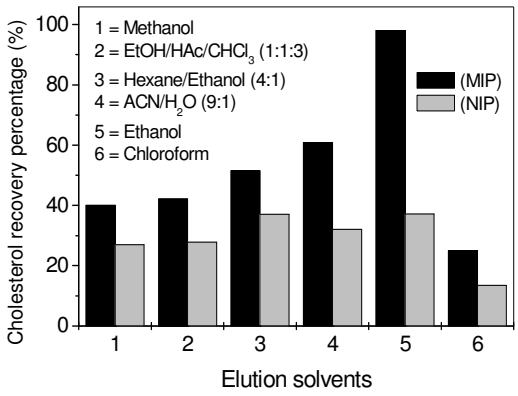


Figure 4. Cholesterol recovery percentage (%) on MIP cartridges using different elution solvents.

According to the results achieved from MISPE procedure, in which n-hexane and ethanol were chosen in the washing and elution steps, respectively, we can infer that the CHO interaction with binding sites of MIP takes place by means of hydrogen bond formation ascribed to the carboxyl group from methacrylic acid and hydroxyl group from CHO molecule. Figure 5 shows the chromatograms of milk hexanic extract not purified by SPE and those when the sample was applied to the clean-up produced on MIP and NIP. Figure 6 shows the chromatogram of milk hexanic applied to the clean-up produced on C_{18}. Clearly, a cleaner chromatogram can be observed for milk samples submitted to the MISPE procedure. The similarity of chromatograms for milk hexanic extract and the NIP or C_{18} procedure indicates, as expected, the very low efficient of NIP or C_{18} for sample clean-up.

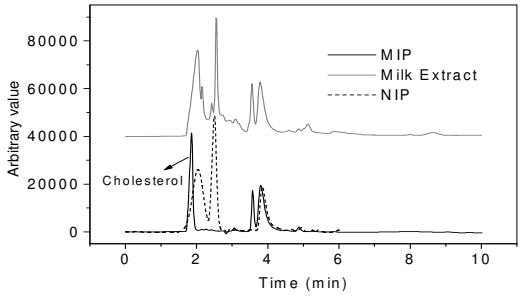


Figure 5. Chromatograms of milk hexanic extract after saponification and submitted to SPE procedure using MIP or NIP.

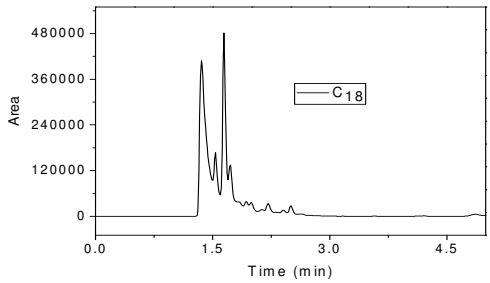


Figure 6. Chromatograms of milk hexanic extract after saponification and submitted to SPE procedure using C_{18}.

Breakthrough Curve

From breakthrough curve depicted in Figure 7, it was observed, under experimental previously mentioned, that the breakthrough volume was found to be 15.0 mL corresponding in mass to 7.49 mg g^{-1}. The column saturation was achieved by loading 140.0 mL of 100 mg L^{-1} cholesterol solution, reflecting in a maximum adsorption capacity (MAC) of 29.51 mg g^{-1}.

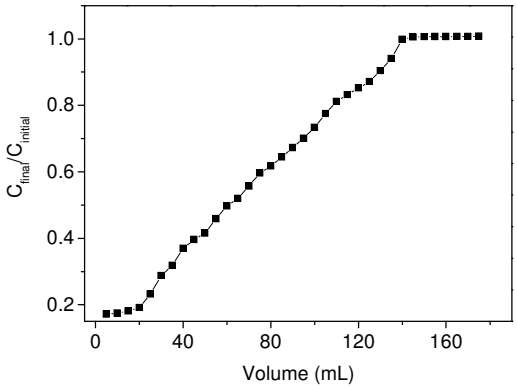


Figure 7. Breakthrough curve of MIP using 100 mg L^{-1} cholesterol at a flow rate 0.6 mL min^{-1}. C_{final} = cholesterol concentration in the column effluent and $C_{initial=}$ initial cholesterol concentration 100 mg L^{-1}

Validation and application of proposed method

Since the percentage of cholesterol adsorption on MIP in the sample loading (88.3%) as well as the loss of cholesterol (13%) in the washing step and the quantitative elution of cholesterol (100%) in the milk samples were in agreement with standard cholesterol solution prepared in hexanic medium submitted to the protocol of molecularly imprinted solid phase extraction (MISPE), the cholesterol quantification was carried out by using external analytical curve. Therefore, the use of standard addition method whereby the cholesterol standard is added directly to milk samples was not required in the proposed method.

A calibration curve was linear in the range of 0.0 (blank)-250 mg L^{-1} with correlation coefficient of r = 0.9972 was obtained by submitting the standard cholesterol solutions through protocol of MISPE. The limits of detection (1.21 mg L^{-1}) and quantification (3.75 mg L^{-1}) were calculated according to IUPAC recommendation[25] as 3std/s and 10std/s, respectively, where std is the standard deviation of ten blank measurements, and s is the slope of the calibration curve.

The precision of MISPE protocol was evaluated in terms of intra-day precision by analyzing (n=10) cholesterol concentrations of 75.0 mg L^{-1} and 150.0 mg L^{-1}, yielding relative standard deviations (RSD) of 1.70 and 1.91%, respectively. Moreover, inter-day precision (two consecutive working day) using six measurements were also evaluated for the same levels of cholesterol concentration. The RSD observed intra-day was varied from 4.10 up to 3.50%.

In order to check the applicability of the MISPE protocol for analysis of milk samples, different brands of milk were submitted to the developed method (Table 4). The labeled amount of cholesterol for brand B was 27 mg 200 mL^{-1} very similar to that determined by the proposed method (26.79 mg 200 mL^{-1}). After sample analysis, known amounts of cholesterol were added to milk samples and very good recoveries (96.6 to 102.2%) were obtained, thus attesting the accuracy of method. The data obtained also ensure that the MISPE

satisfactorily promotes sample clean-up and when associated to HPLC-UV it was very suitable for cholesterol determination in milk samples. It worth emphasize the high reusability of hybrid material because more than 100 cycles of extraction/washing/elution were carried out without loss of adsorption capacity. This value is higher than that one achieved for other polymers, in which the reusability is about 5 and 2 times for organic[22] and inorganic polymers prepared by sol-gel processing[26], respectively.

Table 4. Recovery of cholesterol in different milk samples using MISPE procedure.

Milk samples	Added cholesterol (mg L^{-1})	Determined cholesterol concentration (mg L^{-1})*	Recovery (%)
A	0	136.50 ± 0.52	-
	35	168.38 ± 1.38	98.2
	70	200.65 ± 0.61	97.2
B	0	133.95 ± 0.35	-
	35	172.66 ± 0.18	102.2
	70	207.42 ± 0.76	101.7
C	0	134.15 ± 0.53	-
	35	172.21 ± 0.12	101.8
	70	204.76 ± 1.24	100.3
D	0	137.20 ± 0.16	-
	35	166.39 ± 0.45	96.6
	70	202.64 ± 0.94	97.8

*The results are expressed as mean value ± S.D based on two replicates (n = 2) determinations.

Conclusions

The molecularly imprinted poly(methacrylic acid)/silica hybrid material synthesized offered several advantages for clean-up and selective extraction of cholesterol in milk samples. It can be pointed out the satisfactory morphological features of MIP in adsorption process providing high maximum adsorption capacity (29.51 mg g^{-1}) under dynamic conditions and high reusability of material because only one cartridge packed with MIP was employed throughout method developed, without losses of adsorptive efficiency.
Furthermore, the MIP also presented a high selectivity towards cholesterol in the presence of structurally analogue molecules.
Finally, the synthesis of hybrid material can be considered a facile and promising approach for enhancing the performance of molecularly imprinted polymers and expand it to another analytes.

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