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K^+ and Ca^{2+} modified Na-X zeolites as possible bile acids sequestrant

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Abstract Modified zeolite X, previously exchanged with K^+ and Ca^{2+} cations were characterized by XRD, FT-IR, chemical analysis and BET techniques. Different masses of these solids were mixed with an ox bile solution at different reaction times. The supernatants obtained by centrifugations were submitted to bile acid and phospholipids quantitative determinations. The amount of bile acids adsorbed was 65, 28 and 77% and for phospholipids was 45, 67 and 98% for KX, NaX and CaX respectively. As expected, as the amount of mass used increases more bile acids and phospholipids are adsorbed due to a larger surface of the solid being available for adsorption. On the other hand, 120 min of reaction time were sufficient for the adsorption of both components. The solids, after incubations with bile solutions were treated with abundant distilled water and dried at room temperature. The FT-IR analysis of these solids did not detect any bile and on the zeolite surface with suggested that the bile acid adsorption on the exchanged zeolites is moderated by weak and non covalent interactions.

1 Introduction

Cholesterol is an important component in cell membranes, and is used by the body in making sex hormones, adrenal hormones, and vitamin D. Chemically, cholesterol is a complex alcohol of a type known as sterols, a waxy, fatlike compound that is found throughout the body. However, when cholesterol level in the blood is elevated (>200 mg/dL), it could produce a sickness called hypercholesterolemia. A close relationship exists among levels of blood cholesterol in the body, those of other fats or lipids, and the development of atherosclerosis. Cholesterol is notorious for its role in clogging arteries and thus contributing to heart disease and stroke. In this disorder, plaques containing cholesterol are deposited on the walls of arteries, particularly those of small and medium size, reducing their inside diameter and the flow of blood. Clotting of blood, such as may occur in the coronary arteries to cause a heart attack, is most likely to develop at places where arterial walls are roughened by such plaques [1].

Persons who are born with a predisposition to have abnormally high levels of cholesterol can reduce their risk of heart attack by lowering their blood cholesterol. This is done by following a diet low in cholesterol and saturated fats, getting sufficient exercise, and using drugs that inhibit cholesterol production.

Cholestyramine is a strongly basic anion exchange resin in the chloride form; chemically it binds to bile salts in the gastrointestinal tract exchanging chloride ions by bile acids and thereby preventing the body from producing cholesterol. After the exchange the bile salts are excreted in the faecal matter and the body synthesizes bile acids from cholesterol, producing a significant decrease of cholesterol in the blood. This resin is quite effective in lowering cholesterol; however, the sandy sensation it produces in the

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mouth and the tendency to induce haemorrhoids formation increase the search for more adequate and less traumatic preparations.

Zeolites are an alternative to these resins they are economic and easily modifiable to fulfil the requirements for adsorption of bile acids. The zeolite structure consists of a perfect ensemble of SiO_4 and AlO_4^- tetrahedrons tightly bound to form a crystalline open net with pores of molecular diameter. Due to its low toxicity, zeolites are effective in different type of pathologies such as stomach acidity [2], bacterial focus [3] and diabetes [4]. They have also been used as hypocholesterolemia agents [5–8], with good results shown by Ca^{2+} exchanged clinoptilolite zeolites [7]. However, the adsorption of cholesterol or bile acids on these types of solids is still not clear. Rao et al. [9] and Berezein et al. [10] adsorbed and separated mixtures of different sterols such as campesterol, β-sitosterol or cholesterol using Y zeolite.

It is important to take into account the complexity of bile matrix and that adsorption of bile salts is not possible based only in the size of the molecular kinetic diameter and zeolites pores diameter. Inside the bile matrix, bile acids already are forming very complex mixed macro micelles with other bile components.

Since X zeolites have a Si/Al = 1 ratio which will allow a high ionic exchange together with its hydrophilic properties comparable to that of bile polar components in aqueous solution, it emerges as a promising hypocholesterolemia agents to be used as bile acids sequestrant. The objective of the present work is to test K^+ and Ca^{2+} modified Na-X zeolites in order to determine their capacity as possible bile acids sequestrant and to shed some light on how these solids work.

2 Experimental

2.1 Cationic exchange of Na-X zeolites

Samples of Na-X zeolites from Grace-Davison were exchanged with 0.01 M KCl or $Ca(NO_3)_2$ solutions, with a 3 mL salt solution/g zeolite. The slurry was kept in a reflux for 3 h, centrifuged and the solid was again refluxed twice. After the last reflux, the slurry was centrifuged again and the solid was washed with distilled water and dried at 80 °C for 18 h in a convection oven.

2.2 Characterization of Na–X exchanged zeolites

The exchange zeolites were characterized by techniques such as X-ray diffraction (XRD), Fourier transform infrared (FT-IR), chemical analysis and BET specific surface area.

XRD experiments were conducted using a Phillips 3442 advanced diffractometer with a CoKα (1.54060 Å) radiation for crystalline phase detection between 4° and 80° (2θ). JCPDS-ICDD standard spectra software was used to determine the phases. The surface areas were measured by a single-point BET procedure using nitrogen-argon adsorption at –196 °C with an N_2/Ar ratio of 30/70 on a Beckman Coulter SA 3100. Inductively Coupled Plasma Emission Spectroscopy (ICP) using a Perkin Elmer ICP/5500 instrument to analyze the chemical compositions of the original and exchanged zeolites. The presence of functional groups and impurities in the solids was determined by FT-IR. The spectra were recorded in a Perkin-Elmer 283 spectrometer in the range 2,000–200 cm^{-1}.

2.3 Zeolite–bile acids interactions

To measure zeolite–bile acid interactions, the modified zeolites were contacted with solutions of ox bile at a concentration that mimic the total bile acid concentration within the range found in human bile, according to data reported by Carballo et al. [7]. Analyses were performed to determine the concentration of bile acids (colic acid) and phospholipids. Two types of experiments were performed. In the first experiment, the influence of zeolite mass was determined using: 50, 100, 250, 500 and 1,000 mg of zeolite mixed with 5 mL of bile solution. The slurry was stirred for 5 h at 25 °C and centrifuged at 3,500 rpm for 10 min. The supernatant was analyzed to determine the concentration of bile acids and phospholipids. A similar portion of bile, without the zeolite was used as control.

In the second experiment a fix mass of the exchanged zeolite (500 mg) was used and mixed with 5 mL of the bile solution with the same characteristics as those specify for the first experiment. Samples were stirred at different times: 15, 60 and 120 min at 25 °C. After centrifugation the supernatant and solid were treated and analyzed as in the first experiment.

3 Result and discussion

3.1 Characterization of the solids

Similar XRD patterns were observed for K^+ and Ca^{2+} exchanged solids characteristic of faujasite-type zeolites. Figure 1 shows the K^+ exchanged patterns where the most prominent peaks appear at inter planar distances: 14.5; 3.81; 3.34 and 2.89. No other zeolite phases or oxides were

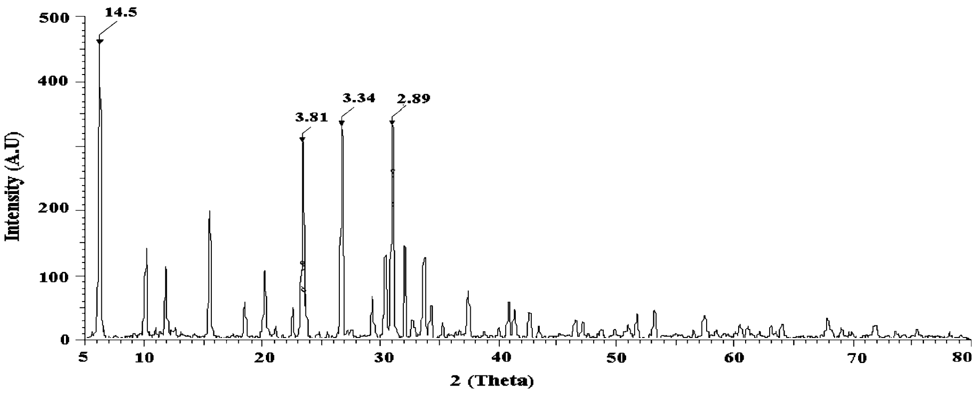

Fig. 1 XRD patterns of K^+ exchanged Na-X zeolites

detected, corroborating that the crystallinity of the zeolite was preserved.

Figure 2 shows the FT-IR spectrum of K-X zeolite. The band at 3,457 cm^{-1} corresponds to OH^- groups associated to water molecules occluded in the zeolite. The band at 1,644 cm^{-1} is assign to occluded water molecules. Other observed bands are assigned to Si–O–Al vibrations inside the zeolite structure. The intensity of bands at 753 and 559 cm^{-1} correspond to the finger prints of X-zeolite. It is observed that bands observed in the original Na-X zeolite remain unchanged after K^+ and Ca^{2+} exchanged process.

Surface area of the solids was determined once the absence of structural changes in the exchange process was corroborated. Since bile solutions could form mix complex micelles, it is possible that their molecular diameter be larger than that of the zeolite pores; for that reason, both total and external surface areas were measure and are reported in Table 1. As observed, there is a decrease in surface area with K^+ and Ca^{2+} exchange being more pronounce on Ca-X zeolite. The lower surface area observed for the Ca-X zeolite could be due to the tendency of Ca^{2+} cation to hydrolyze producing a 1^+ net charge instead of a 2^+, as previously observed by Xie et al. [11].

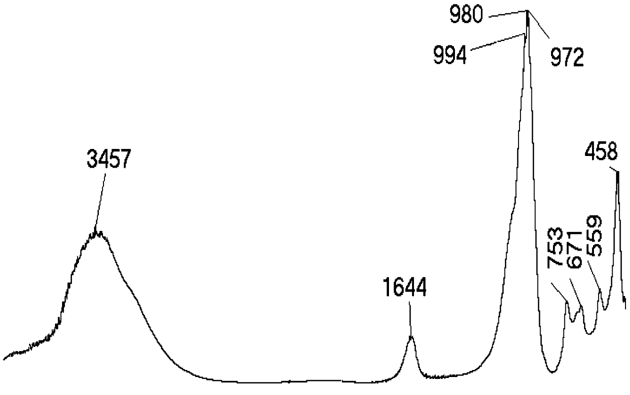

Fig. 2 FT-IR spectra of K^+ exchanged Na-X zeolites

Table 1 Specific surface areas of modified zeolites X

Zeolites	Total specific SA (m^2/g)	External SA (m^2/g)
NaX	760	74
KX	707	69
CaX	687	53

No modification of the Si/Al ratio was shown in the chemical analysis. As seen in Table 2, sodium cations are only partially replaced by K^+ and Ca^{2+}, this is to be expected since some Na^+ are located in very small inaccessible places inside the zeolite. Higher degree of exchange was achieved with Ca^{2+} cation. For all solids the observed structural formulae are consistent with those previously reported [12].

3.2 Interactions zeolite–bile acids

3.2.1 Influence of zeolite mass

The quantitative determination of bile acids and phospholipids in the ox bile after contact with the zeolites showed an important reduction in the initial amount measure in the supernatants as a function of zeolite mass. The amount of bile acids adsorbed was 65, 28 and 77% for NaX, KX and CaX respectively. As expected, as the amount of mass used increases more bile acids are adsorbed due to a larger surface of the solid being available for adsorption. Thousand milligram was the higher amount of solid used, higher masses were not tested since technically a person will not be able to ingest higher amounts in one doses.

It was observed that Ca-X zeolites are more active than Na-X and K-X indicating that the nature of the exchanged cation also places an influence in the adsorption process. This behaviour could be related to the zeolite acidity: the order of acidity is $Ca^{2+} > Na^+ > K^+$ [13]. The pH of the bile solution used was ~ 8; in this reacting medium, colic acid will be in the form of its conjugated base R–COO$^-$

Table 2 Chemical analyses and formulae of modified X zeolites

Zeolite	Chemical composition				
	Na (%)	K (%)	Ca (%)	Al (%)	Si (%)
NaX	10.3 ± 0.2			10.9 ± 0.5	16.9 ± 0.3
KX	10.4 ± 0.5	0.94 ± 0.01		10.5 ± 0.3	16.3 ± 0.6
CaX	9.87 ± 0.02		1.45 ± 0.01	10.7 ± 0.4	16.9 ± 0.5
Chemical formulae					
NaX	$Na_{77,11}(Al_{77,11}Si_{114,89}0_{384})$				
KX	$Na_{77,15}K_{4,95}(Al_{77,11}Si_{114,89}0_{384})$				
CaX	$Na_{77,11}Ca_{3.84}(Al_{76,19}Si_{115,81}0_{384})$				

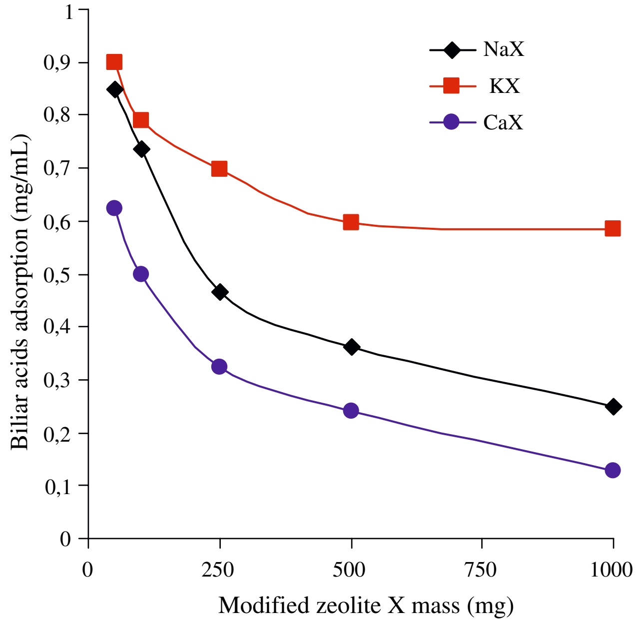

Fig. 3 Influence of modified zeolite X mass on bile acids adsorption

[14]. In this condition as the acidity of the cation increases the interaction with the zeolite increases and adsorption will be higher (Fig. 3).

For phospholipids, the amount adsorbed was 45, 67 and 98% for KX, NaX and CaX respectively. The behaviour was similar to that observed for adsorption of bile acids (Fig. 4).

Similarly to the observation for bile acids adsorption, in the case of phospholipids the cations also play an important roll: as the acidity of the cation increases more phospholipids are adsorbed. A probable adsorption mechanism could be that bile salts are previously deposited in the surface of the zeolites followed by the phospholipids in a hydrophobic interaction tail–tail of the two anphipatic compounds (bile acids and phospholipids). Such mechanism could be based in the high bile acids concentration compare to that of phospholipids, which hinders phospholipids competition for the external surface of the zeolite.

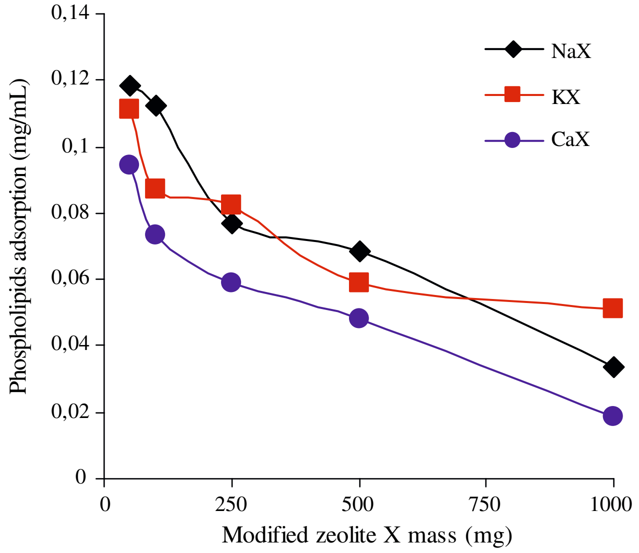

Fig. 4 Influence of modified zeolite X mass on phospholipids adsorption

It is also possible that adsorption of both molecules do not occur inside the zeolite, even that molecular diameter of both molecules allows their penetration inside the zeolite cavities [9, 10]. In solution, these molecules form mix macro micelles which exceed the size of the zeolite pores hindering their penetration inside the zeolite. The zeolites external surface area (Table 1) will play an important roll in the bile acids and phospholipids adsorption. Differences among external surface of the zeolites are not that important (74–53 m^2/g) so it is probably the cation acidity which plays a stronger influence in bile acid adsorption.

3.2.2 *Kinetics of adsorption*

As can be observed from Fig. 5, bile acids concentration decreases drastically within the first 120 min of reaction (NaX = 25%, KX = 38% and CaX = 44%), after which, change in concentration occurs slowly. This indicates that at the beginning of the reaction the active sites of the

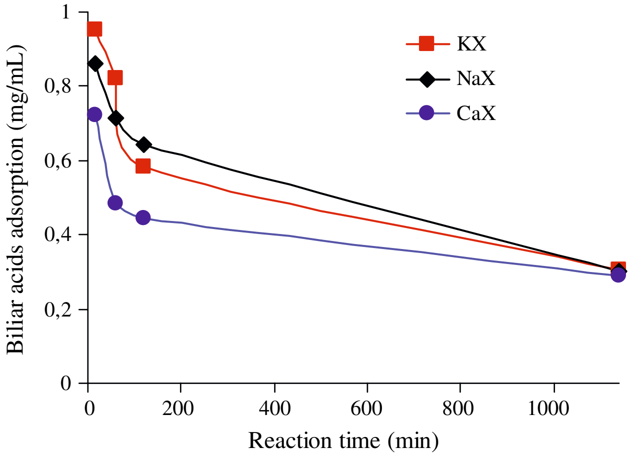

Fig. 5 Kinetics of bile acids adsorption on 500 mg of modified zeolite X

zeolites are easily occupied which is an important advantage since digestion takes place within 2–3 h. A similar behaviour was observed for phospholipids adsorption (Fig. 6).

Similarly, it is important to note the influence of the compensation cation of the zeolite: Ca–X showed the highest adsorption capacity compare to Na–X and K–X during the first 120 min of reaction; after that, the adsorption becomes independent of the compensation cation, attributed to reaching the adsorption–desorption equilibrium.

3.2.3 *Nature of adsorbed species*

Figure 7, shows the IR spectra for Ca–X zeolite before and after contacted with bile acid solutions at different reaction times. As can be observed, no significant changes were observed in the bands of the exchanged zeolites compare to the solids before reaction. This behaviour could be attributed to the formation of micelles between bile components and the zeolite. After exhaustive washing of the zeolites, micelles are broken and leave the solid which remains free of adsorbed molecules. A similar analysis performed to cholestyramine did not show the presence of bile acids on the surface of the solid (spectrum no shown).

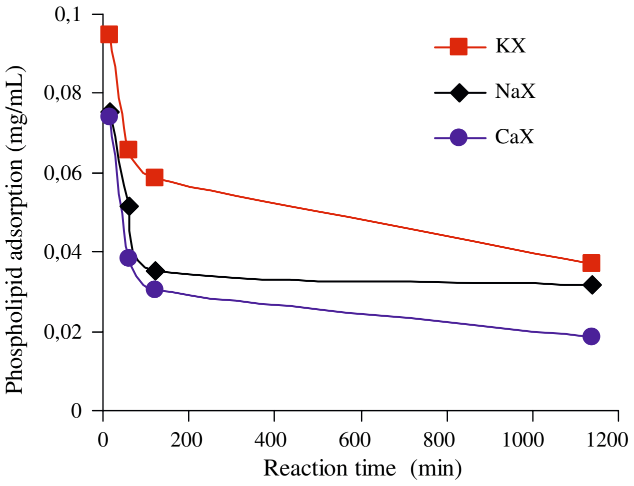

Fig. 6 Kinetics of phospholipids adsorption on 500 mg of modified zeolite X

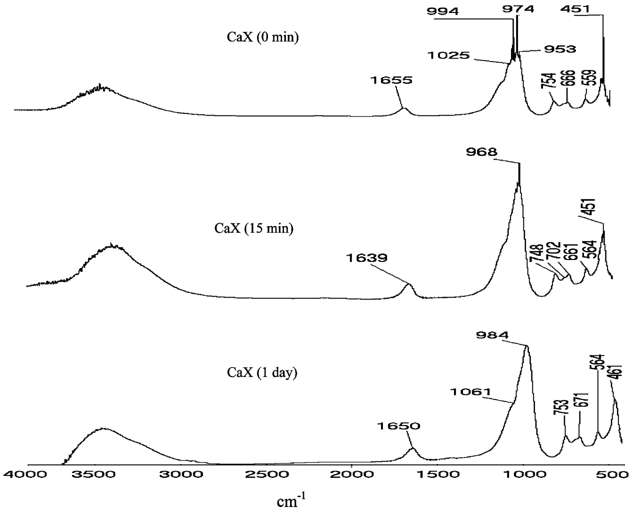

Fig. 7 IR spectra of CaX: before (0 min) and after (15 min and 1 day) contact with ox bile solutions

3.2.4 *Adsorption mechanism*

Different mechanisms could be invoked to explain adsorption of bullock bile components on the surface of zeolites. A possible one could involve cationic species exchange with zeolite cations. However, this mechanism is not probable because at pH = 8, bile acids are present as conjugated bases (R–COO$^-$) and no cationic species can be exchanged. Another mechanism could be associated with the hydrophilic character of zeolites [11] which induce electrostatic-type interactions between bases. This interaction produce a covering of the zeolite surface with bile salts; while its hydrophobic part is in contact with non-polar parts of phospholipids. A bile acids–phospholipids–zeolite interaction will favour formation of mix macro micelles. A primary interaction of phospholipids–zeolite followed by bile salts–zeolite interaction is not discarded; however, since bile acids concentration is higher than that of phospholipids, it is possible that this type of mechanism is secondary.

4 Conclusions

Modification of Na-X zeolites by Ca^{2+} and K^{+} was successfully achieved. It was observed that cation exchange did not affect the structural properties of the solids, only a decrease in surface area of CaX and K–X was observed due to its size/charge ratio.

The modified zeolites show to be effective sequestrants of bile acids and phospholipids, with maximum adsorption observed after 120 min on 500 mg of Ca–X zeolite.

IR spectra of solids after contacted with bile solutions did not show the presence of bile components, which indicate that after thorough washing; the adsorbed components leave the zeolite surface.

A mix micelle type mechanism is proposed in which adsorption of bile components (bile acids, phospholipids) on zeolite surface explain the sequestrant effect of modified zeolites.

Finally, it is important to emphasize that these solids represent an alternative to substitute exchange resins in decreasing high cholesterol risks. They are effective as sequestrant of bile acids, especially in its calcium form.

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