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ON THE POSSIBILITY OF USING CARBON ENTEROSORBENTS TO NORMALIZE CHOLESTEROL METABOLISM

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The creation of effective drugs for the prevention and treatment of atherosclerosis is one of the urgent interdisciplinary tasks for modern chemistry and pharmacology. Given the role of hypercholesterolemia in the development of this disease, it is necessary to remove excess amounts of cholesterol from the body. As an alternative to means of lowering total cholesterol and low-density lipoprotein (LDL) cholesterol, the possibility of using carbon enterosorbents for efferent therapy is considered.

Aim. The purpose of the study was to evaluate the sorption capacity of the adsorbents developed by the authors in terms of the possibility of cholesterol adsorption.

Methods. Using the spectrophotometric method, the sorption of cholesterol on samples of adsorbents obtained by chemical activation of waste from the processing of lignocellulosic raw materials — dogwood and coffee residue has been studied.

Results. A comparison of sorption isotherms with the isotherm obtained on the industrial adsorbent SORBEX has been performed. It was shown that the adsorption capacity of carbon adsorbents is primarily determined by their porous structure. The highest sorption values (7.3 mg/g) have been revealed by the sorption material obtained by chemical activation of cornel seed, an intermediate position (6.3 mg/g) is occupied by the adsorbent obtained from the coffee residue. Industrial carbon SORBEX has the lowest sorption values (5.3 mg/g).

Conclusions. Calculations by Langmuir's and Freundlich's models testify about the accordance of the experimental data to Langmuir's model. The use of the obtained activated carbons may be one of the effective alternative ways to lower blood cholesterol.

Key words: cholesterol; atherosclerosis; low-density lipoprotein (LDL); enterosorbents; metabolism.

An increased concentration of cholesterol in the blood (i.e., hypercholesterolemia) is widely recognized as a risk factor for coronary artery disease. Cholesterol-LDL — one of the classes of lipoproteins, particles circulating in the blood, transporting cholesterol from the liver to other organs and tissues, a risk factor for the development of atherosclerosis and coronary heart disease — "bad cholesterol". This fraction of cholesterol refers to the so-called "bad cholesterol" because it contains a large amount of cholesterol. Deposited in the vessels, in the form of plaques, cholesterol, together with other metabolites, can lead to the development of atherosclerosis and the progression of coronary heart disease. Reducing plasma levels of total and low-density lipoprotein (LDL) cholesterol by diet, drugs or lifestyle modification is thus of principal importance in treating and preventing cardiovascular disease. In humans, blood cholesterol is derived from two sources. It is either absorbed from food by the intestine, or it is synthesized from precursor molecules in the liver [1].

Hepatic cholesterol synthesis can be pharmacologically regulated with statins by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase, the enzyme responsible for cholesterol synthesis. Statins are very potent lipid-lowering agents and they may significantly reduce significantly reduce coronary morbidity and mortality [2].

However many patients do not reach currently defined treatment goals and there is considerable interest in finding additional ways to reduce plasma and LDL cholesterol levels. This has led to the development of a new family of drugs that inhibits intestinal cholesterol absorption [3, 4]. Ezetimibe is a 2-azetidinone compound that reduces cholesterol absorption by inhibiting the protein responsible for cholesterol transport into enterocytes. As monotherapy, ezetimibe decreases LDL-C levels by 15–20 percent [2]; in combination with statins it reduces LDL-C by an additional 20–25% [3]. Increasing use of these therapeutic agents has refocussed interest on enterosorbents that have the potential to reduce intestinal cholesterol absorption. For this purpose, sorbents based on cellulose granules and modified cellulose [5, 6], alumina obtained by the sol-gel method [7], polyacrylate [8], a mixture of activated carbon and microcellulose [9], lignocellulose based activated carbons [10–13], are used for selective adsorption of LDL in order to prevent atherosclerosis. However, the elaboration of enterosorbents with high capacity for cholesterol is still topical. The oral intake of activated carbon may be helpful in therapy of atherosclerosis and diseases associated with an increased level of cholesterol and lipids.

The objective of the study was to evaluate the sorption capacity of the agricultural waste-based enterosorbents developed by the authors in terms of the possibility of cholesterol elimination.

Materials and Methods

Cholesterol was purchased from Sigma Chemical Co. (USA). Procaine hydrochloride solution was purchased from AO "Chemfarm" (Kazakhstan). Sodium nitrite was purchased from Co. "Stirol" (Ukraine). Hydrochloric acid ch. p. was purchased from Co. "Chemlaborreactive" (Ukraine).

Samples of adsorbents were prepared by chemical activation of waste from the processing of lignocellulosic raw materials — dogwood and coffee residue.

Spectrophotometric measurement of cholesterol for the purpose of sorption from alcoholic solution was determined according to the methodology of guidance [14].

Cholesterol solution was prepared by dissolving 0.05 g in ethanol and the volume was made up in 500 mL volumetric flask. Solutions of further dilute concentrations were prepared from this working standard solution.

Precisely weight of 0.0818 g of procaine hydrochloride was dissolved with less amount of distilled water, then 3 mL of $NaNO_2$ 0.1 M and 3 mL of HCl 1 M were added to the beaker. The solution was allowed to stand for 5 min at 5–10 °C, the solution was transferred into 50 mL volumetric flask and the volume was made up to mark with distilled water where temperature at 5–10 °C was kept.

1 mL of cholesterol solution (100 μg/mL) was added into 10 mL volumetric flask. 2 mL of the diazotized procaine hydrochloride solution and 2 mL of 2M NaOH were added to the volumetric flask. The solution was mixed thoroughly, the volume was made up to mark with distilled water and the solution allowed to stand for 5 min. Adsorbance of a colored product is proposed to measure at 428 nm in case of cholesterol against reagent blank.

Spectrophotometric measurements were carried out using the ultraviolet spectrophotometer Shimadzu UV-2500 with 1.00 cm glass cells.

Results and Discussions

Table 1 includes the characteristics of agro-based adsorbents, obtained with H_3PO_4 activation of dogwood stone (DS-AC) and coffee residue (CR-AC), and, for comparison, commercial sorbent SORBEX. It is seen that a rather high content of mesopores is typical for the considered sorbents.

In [14], the wavelength for measuring cholesterol was chosen mistakenly (for our opinion). To determine the optical density of the colored product, a blurred region of the spectrum (not having a peak) in the range of 360–430 nm was chosen.

In present work the solution was mixed thoroughly, the volume was made up to mark with distilled water and the solution allowed to stand for 24 hours to acquire permanent coloration. The wavelength of 318 nm has been chosen to determine cholesterol in a colored product. Obtained adsorption spectra of cholesterol are presented in Fig. 1. As it seen, the spectra are arranged in proportion to the concentration of the prepared solutions, the adsorption maxima are close, and the spectral shapes are similar. The adsorption maximum corresponds to the wavelength 318 nm.

The calibration curve obtained at different concentrations of cholesterol is presented in Fig. 2. It may be seen that solutions with different cholesterol content fit well along this straight line.

Table 1. **Characteristics of agro-based sorbents, obtained with H_3PO_4 activation of dogwood stone and coffee residue, and commercial sorbent SORBEX**

Characteristics	DS-AC	CR-AC	SORBEX
Granulometric composition, mm	0.5–1.0	0.25–1.0	0.25
Ash content,%	4.6	6	3.7
Yield,%	46	48	–
Bulk density, g/cm^3	0.35	0.36	0.40
S_{BET} specific surface area, m^2/g	2 169	1 480	1 228
S_{me} specific surface area, m^2/g	573	431	84
Total pore volum, VΣ, cm^3/g	1.0	0.84	0.58
Mesopore volume, V_{me} cm^3/g	0.67	0.32	0.12
Average pore radius, Å	10.6	9.8	9.5
MB sorption capacity	145	120	72

Note: DS-AC — dogwood stone activated carbon; CR-AC — coffee residue activated carbon; SORBEX — commercial activated carbon.

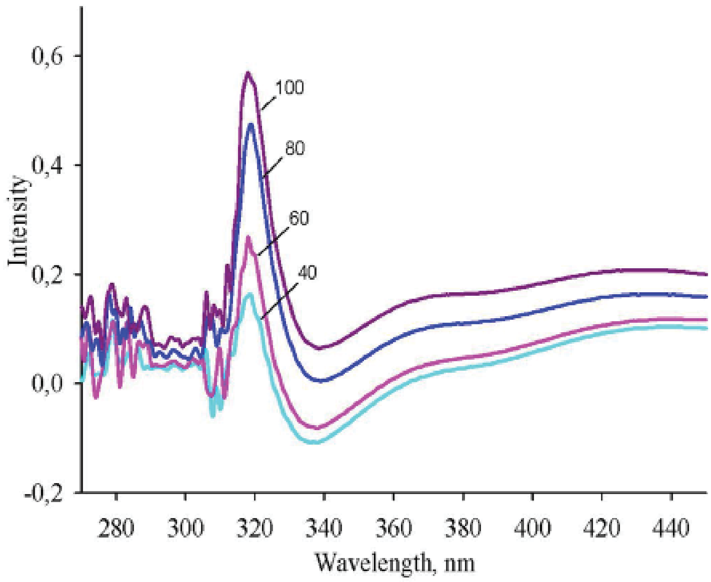

Fig. 1. **Adsorption spectra of cholesterol at different concentrations (mg/L)**

Photometric methods for determining cholesterol are based almost exclusively on the use of chemical reactions. Among the classical colorimetric methods, the Lieberman-Burkhard method is of the greatest importance, which is based on the measurement of the intensity of the greenish-blue color, which appears as a result of the treatment of cholesterol with a mixture of rather aggressive reagents — sulfuric acid and acetic anhydride. Toxic reagents such as acetic acid and chloroform are also used as solvents. In addition, the rate of color of the complex and its stability are strongly dependent on temperature, which significantly complicates the determination procedure. That is why, in this work, the method, described in [14], was chosen for the spectrophotometric determination of cholesterol on the obtained samples.

The corresponding adsorption isotherms are shown in Fig. 3. The best sorption ability is possessed by a sample of dogwood stone activated carbon (the maximum value

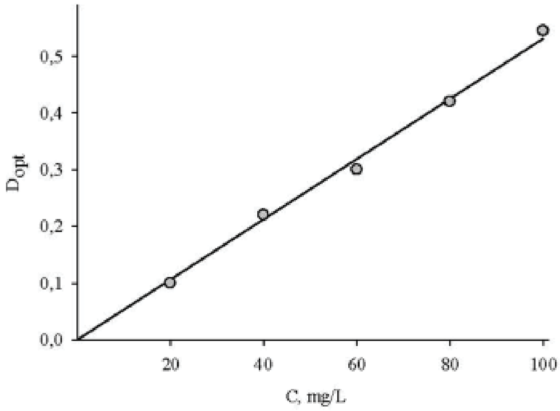

Fig. 2. **Calibration curve obtained at different concentrations of cholesterol**

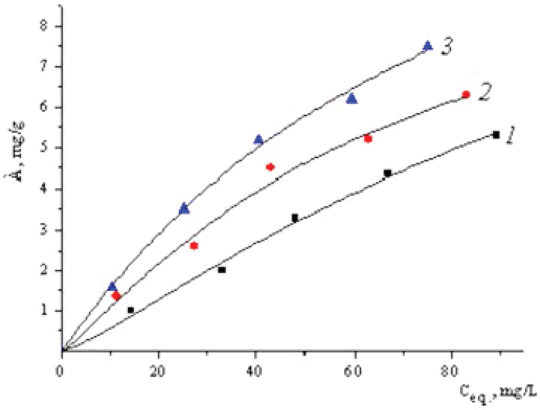

Fig. 3. **Cholesterol sorption isotherms by samples:** *1* — SORBEX; *2* — CR-AC; *3* — DS-AC

Table 2. **The parameters of the Langmuir and Freundlich isotherms calculated for isotherms of cholesterol sorption**

Samples	Langmuir isotherms			Freundlich isotherms		
	A_{∞}, mg/g	K_L, L/mg	r^2	K_F, mg/g	n	r^2
DS-AC	7.08	60.67	0.978	38.59	2.23	0.984
CR-AC	7.34	79.30	0.997	35.64	1.96	0.967
SORBEX	8.48	63.22	0.956	37.5	2.08	0.957

reaches 7.5 mg/g). An intermediate position is occupied by a sample of coffee residue activated carbon (the maximum value reaches 6.3 mg/g). SORBEX possesses the weakest adsorbing characteristics (the maximum value reaches 5.3 mg/g). It may be stated that the adsorption of cholesterol increases according to the growth of the specific surface area of mesopores.

With the help of the obtained isotherms, the parameters of the adsorption processes have been analyzed. Isotherms of adsorption were calculated by Langmuir and Freundlich equations:

$$a = a_0 \frac{KC_{eq}}{1 + KC_{eq}}, \quad (1)$$

where C_{eq} is equal concentration, mg/l; a_o — value of maximum adsorption, mg/g; K is a constant;

$$A_F = K_F \cdot C^{1/n}, \quad (2)$$

where A_F — adsorption value, mg/g; n — exponent index.

Table 2 shows the values of the maximum adsorption, Langmuir and Freundlich constants, as well as coefficients of correlation.

The characteristics of the correlation are quite high for all prepared samples that testified about the accordance of experimental data to Langmuir's model. It can be seen that the value of the maximum adsorption calculated using this equation also agrees well with the experimental data. Based on this, it is possible to make a guess about the potential mechanism of adsorption of cholesterol: adsorption is not carried on surfaces of the adsorbent, but on active centers, which are characterized by so-called free valency. Every active center is designed for interaction only with one molecule of the adsorbate; as a result, only one layer of adsorbed molecules may be installed on the surface. The process of adsorption is reversible and equally important — the adsorbed molecule is absorbed by the active center for a while, when it is desorbed; in this way, over time, a dynamic equilibrium will arise between the processes of adsorption and desorption.

Conclusions

A new method of spectrophotometric measurement of cholesterol in solutions has been mastered and improved. Using the spectrophotometric method, the isotherms of cholesterol adsorption on samples of adsorbents obtained by chemical activation of waste from the processing of lignocellulosic raw materials — dogwood and coffee residue have been studied. It was proved the advantages of chosen methodology of determining cholesterol.

It was established that cholesterol adsorption grows proportionally to the mesopore specific surface area of enterosorbents.

Calculations by Langmuir's and Freundlich's models testify about the accordance of the experimental data to Langmuir's model. The value of the maximum adsorption, calculated with the help of this equation, fit well with experimental data.

With great probability it is possible to survive, that application of the obtained active carbons may be one of the effective alternative ways of lowering the level of cholesterol in blood and other biological solutions.

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The authors declare that they have no conflict of interest.

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ПРО МОЖЛИВОСТЬ ВИКОРИСТАННЯ ВУГЛЕЦЕВИХ ЕНТЕРОСОРБЕНТІВ ДЛЯ НОРМАЛІЗАЦІЇ ХОЛЕСТЕРОЛОВОГО МЕТАБОЛІЗМУ

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Створення ефективних препаратів для профілактики та лікування атеросклерозу є одним із актуальних міждисциплінарних завдань сучасної хімії та фармакології. З огляду на роль гіперхолестеринемії у розвиткові цієї хвороби необхідно виводити надлишки холестеролу з організму. Як альтернативу засобам зниження загального холестеролу та холестеролу ліпопротеїдів низької щільності (ЛПНЩ) розглядається можливість використання ентеросорбентів для еферентної терапії.

Метою дослідження було оцінити сорбційну здатність адсорбентів, розроблених авторами, з погляду можливості адсорбції холестеролу.

Методи. За допомогою спектрофотометричного методу досліджено сорбцію холестеролу на зразках адсорбентів, отриманих хімічною активацією відходів пiл час перероблення лігноцелюлозної сировини — кизилової кісточки та залишків кави.

Результати. Проведено порівняння ізотерм сорбції з ізотермою, отриманою на промисловому адсорбенті SORBEX. Показано, що поглинальна здатність вуглецевих адсорбентів визначається насамперед їхньою поруватою структурою. Найвищі значення сорбції (7,3 мг/г) виявляє сорбційний матеріал, одержаний за допомогою хімічної активації кісточок кизилу, проміжне положення (6,3 мг/г) займає адсорбент, отриманий із кавових залишків. Промисловий вуглець SORBEX має найнижчі показники сорбції (5,3 мг/г).

Висновки. Розрахунки за моделями Ленгмюра та Фрейндліха свідчать про те, що експериментальні дані найбільш відповідають моделі Ленгмюра. Використання одержаного активованого вугілля може бути одним із ефективних альтернативних способів зниження холестеролу в крові.

Ключові слова: холестерол; атеросклероз; ліпопротеїди низької щільності (ЛПНЩ); ентеросорбенти; метаболізм.

О ВОЗМОЖНОСТИ ИСПОЛЬЗОВАНИЯ УГЛЕРОДНЫХ ЭНТЕРОСОРБЕНТОВ ДЛЯ НОРМАЛИЗАЦИИ ХОЛЕСТЕРОЛОВОГО МЕТАБОЛИЗМА

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Создание эффективных препаратов для профилактики и лечения атеросклероза является одной из актуальных междисциплинарных задач современной химии и фармакологии. Учитывая роль гиперхолестеринемии в развитии этой болезни, необходимо выводить излишки холестерола из организма. В качестве альтернативы средствам снижения общего холестерола и холестерола липопротеидов низкой плотности (ЛПНП) рассматривается возможность использования энтеросорбентов для эфферентной терапии.

Цель. Оценка сорбционной способности адсорбентов, разработанных авторами, с точки зрения возможности адсорбции холестерола.

Методы. С помощью спектрофотометрического метода исследованы сорбция холестерола на образцах адсорбентов, полученных химической активацией отходов при переработке лигноцеллюлозного сырья – кизиловой косточки и остатков кофе.

Результаты. Проведено сравнение изотерм сорбции с изотермой, полученной на промышленном адсорбенте SORBEX. Показано, что поглощающая способность углеродных адсорбентов определяется, прежде всего, их пористой структурой. Высокие значения сорбции (7,3 мг/г) обнаруживает сорбционный материал, полученный с помощью химической активации косточек кизила, промежуточное положение (6,3 мг/г) занимает адсорбент из кофейных остатков. Промышленный углерод SORBEX имеет самые низкие показатели сорбции (5,3 мг/г).

Выводы. Расчеты по моделям Ленгмюра и Фрейндлиха свидетельствуют о том, что экспериментальные данные наиболее соответствуют модели Ленгмюра. Использование полученного активированного угля может быть одним из эффективных альтернативных способов снижения холестерола в крови.

Ключевые слова: холестерол; атеросклероз; липопротеиды низкой плотности (ЛПНП); энтеросорбенты; метаболизм.