PHYSICOCHEMICAL STUDIES
OF SYSTEMS AND PROCESSES

Regularities in Adsorption of Anthocyans by Natural Clays

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Abstract—Regularities of adsorption by clay of some commercial sorts of anthocyans extracted from plant materials are investigated. The interrelation between sorption ability and structure of sorbates is revealed. Introduction of organic solvent is shown providing a possibility of targeted changing sorption properties of system and sorption purification of anthocyans.

Anthocyans are organic substances with the basic structure in the form of cation; the anthocyans are valuable colorants for the food and medical industry, whose important property is a wide spectrum of biological activity. However, during their extraction from plant material simultaneously are extracted in a significant amount some associated substances, whose nature depends on the source material and the method of extraction. Therefore purification of anthocyanate-containing extracts is vital problem.

In this communication is considered concentration and purification of anthocyans with natural clays. About the high sorption activity of some commercial accessible clays has been reported earlier [1–3]. It was established that the sorption activity of clays depends on the structure of anthocyans, mainly not of the aglycone part, but in essence on the structure of carbohydrate moiety. Use of talc, another laminar inorganic mineral, for concentration and purification of the grape anthocyans was described [4], whereas in the foreign literature was described application for these purposes of substantially more expensive synthetic sorbents on the basis of styrene–divinylbenzene copolymers (SDVB) [5–7]. In [8] was described investigation of 11 sorbents for the concentration of anthocyan from the Hungarian red wine, therewith the octadecylsilane sorbents showed the highest sorption capacity; the silica gels modified by aminopropyl groups was inferior to them, while not modified silica

gel and alumogel were proved to be ineffective for the investigated process.

There is little information on the application of clays in the technological stages with the participation of anthocyans. It is known the use of bentonite for treating wines for the purpose of increase their stability on the storage [9], and in [10] was investigated adsorption of anthocyans on the clay with the subsequent desorption as a method of purification of industrial extracts from the red radish. It is curious also the method of purification disclosed in the patent [11], which includes transformation of anthocyans into the not colored hydrosulfite complexes. These complexes are sorbed in the dynamic regime on macroporous nonionogenic styrene, divinylbenzene sorbents, with the subsequent reextraction of anthocyans with acidified alcohol.

Purpose of this work is development of the technology for the concentration purification of anthocyans with the use of clay materials.

EXPERIMENTAL

The anthocyans were extracted from the plant material by infusion in 0.1 M solution of hydrochloric acid. Extract was separated from the source material by filtration through a paper filter. The adsorption of anthocyans was determined by the static method: to a measured volume of extract was added the necessary quantity of clay and

the mixture was maintained with the periodic agitation during 30–40 min (experimentally determined time of reaching equilibrium). Solution was separated from the clay by decantation after centrifugation during 10 min at 5000 rpm. In the work were used the commercially accessible sorts of the clays of two producers, "Poly-Synthes-M" (PSM) and "Stimul-Color-Cosmetics" (SCC), without any chemical transformations. For the additional preparation of extracts (if necessary) and at the spectrophotometric determination of anthocyans [12] the solutions were diluted with 0.1 M solution of hydrochloric acid. At the quantitative spectrophotometric determinations of anthocyans in water mixtures were introduced the experimentally obtained corrections to the extinction coefficients (in this case is changed the position of the adsorption maximum). The qualitative composition of anthocyanic complexes was investigated by the method of reverse phase HPLC [13, 14]. The most important characteristics of the clays used were established earlier by the method of the low-temperature nitrogen adsorption [15].

The analysis of adsorption of the extracts of natural materials and the comparison of obtained data using the
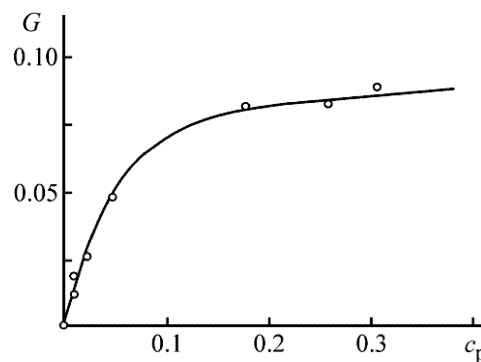

Fig. 1. Isotherm of adsorption of the anthocyan of Aronia fruits on the kaolin (PSM) (23°[S]). (G) the value of Gibbs adsorption (mmol g^{-1}), (c_p) equilibrium concentration of anthocyan in the solution (mol l^{-1}).

adsorption isotherms is sufficiently complicated. The basic problem at obtaining the isotherms of adsorption is connected with the impossibility to exclude influence on the kinetics of the sorption processes of other extractive substances (sugar, polysaccharides, proteins, etc.) those by their mass exceed several times the mass of the anthocyans to be extracted. The well reproducible results

Table 1. Adsorption of anthocyan on two stamps of the clays of SK production

Extract of objects	Anthocyan concentration[a] $c \times 10^5$, mol l^{-1}		Sorbed amount, μmol g^{-1}
	initial	equilibrium	
Clay white cosmetic			
Aroniya (fruits)	9.94	3.81	17.9
Carcade (lobes)	9.94	4.23	16.3
Rosa (lobes)	9.91	4.44	15.8
Nightshade (fruits)	10.03	4.77	15.3
Black currant (fruits)	9.91	5.26	13.4
Cherry (fruits)	10.0	6.79	9.3
Clay blue cosmetic			
Aroniya (fruits)	9.88	5.38	12.7
Carcade (lobes)	9.94	6.88	8.7
Rosa (lobes)	9.94	7.06	8.2
Nightshade (fruits)	9.97	7.42	7.2
Black currant (fruits)	9.94	7.75	6.2
Cherry (fruits)	9.94	8.35	4.5

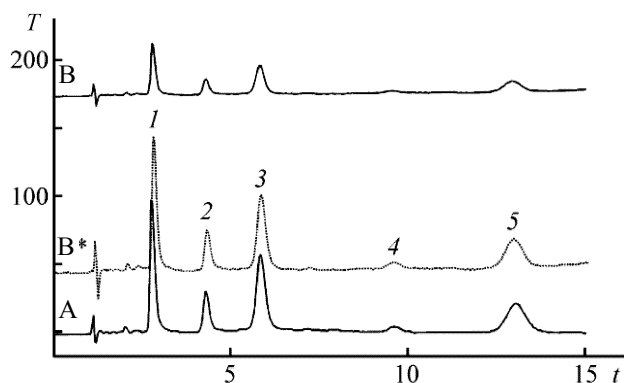[a] Recalculated on cyanidin-3-glucoside.

Fig. 2. Separation of the anthocyans of the extract of grape. (*T*) peak intensity (mV), (*t*) retention time (min); the same for Fig. 3. Conditions: column 150 × 4 mm Diasfer-110-C18, 5 m; the mobile phase 10 vol % of HCOOH, 10 vol % of CH_3CN in water, 1 ml min^{-1}, detector 505 nm; the same for Fig. 3. (A) initial extract, (B) and (B*) equilibrium extract after adsorption with the kaolin (PSM) with scaling in the case B*. Anthocyans are 3-glucosides: (*1*) of delphinidin, (*2*) of cyanidin, (*3*) of petunidin, (*4*) of peonidin, (*5*) of malvidin.
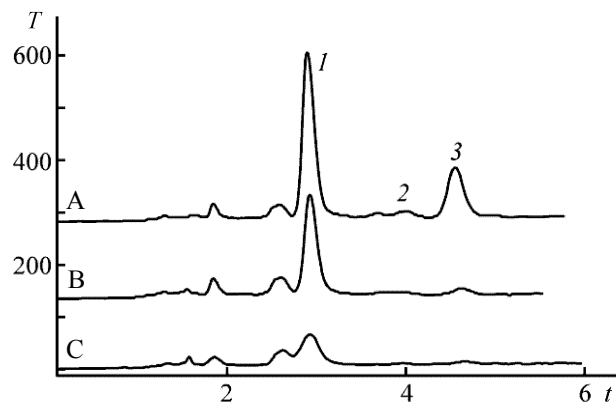

Fig. 3. Separation of the cherry anthocyans. (A) initial extract; (B), (C) the equilibrium extracts after adsorption by the kaolin (PSM). Anthocyans are 3-glycosides of cyanidin: (*1*) 2'-glyucosylrutinoside (**V**), (*2*) glucoside, (*3*) rutinoside.

can be obtained for the fruits richest in the anthocyans, where the content of the associated extractive substances in the concentrated extract is relatively low, for example, extracts of Aronia pistils (Fig. 1). For this reason in the present work for the comparison of sorbents and extracts of different anthocyane complexes is used the following procedure: the extracts with equal concentrations of anthocyans were prepared by dilution of extracts to identical optical density; the identical weighted samples of clays were added to the aliquot portions of the prepared extracts, the mixtures were maintained

with the periodic stirring at room temperature during approximately 40 min; extracts were separated from the sorbents by centrifugation (10 min, speed 5000 rpm) for the subsequent spectrophotometric analysis. For two different models of the clays of production SCC the obtained results are represented in Table 1.

The sorption properties of kaolin 1.5–2 times exceeded the properties of blue clay. This is consistent with the ratio of their specific surface areas: 74.1 and 37.3 m^2 g^{-1} respectively (the adsorbent characteristics were derived from the isotherms of nitrogen adsorption measured at 73.30 K on an automatic adsorbometer ASAP 2010N of the firm "Micromeritics", USA [15]). It is significant that the silica gel (KSK-2) which possesses 4 times larger specific surface area (266.6 m^2 g^{-1} [15]) is found to be much weaker sorbent in comparison with the investigated clays under the similar conditions. Hence, the mechanisms of anthocyan adsorption on the clay and on the silica gel are principally different. Let us note that we tested also the sorption properties of talc (TMK-28) under the analogous conditions: in this case was obtained the sorption activity (per unit of mass), that was approximately 20 times inferior to the samples of the kaolin SCC and PSM.

Investigation of the dependence of sorption activity of anthocyans on their structure was carried out for six different extracts, the species composition of anthocyan in which was monitored by the method of HPLC. The extract of Aronia (Black chokeberry) fruits contained in essence cyanidin-3-galactoside (**I**) with the small additive of cyanidin-3-arabinoside. The extract of the lobes of dark red rose used in this work proved to be also sufficiently simple: by chromatography was revealed practically only one component, cyanidin-3,5-diglyucoside (**II**). The extract of carcadè (grassy tea, Ginger Red "Greenfield") contained two 3-diglycoside derivatives, delphinidin and cyanidin (sambubiosides) (**III**). In essence diglycoside derivatives (cyanidin and delphinidin 3-rutinosides) predominated in the extract of fruits of black currant: the content of 3-glucosides with the same aglycones was approximately 4 times less. The basic components of the extract of cherry fruits containing triglucoside residue in *3* position of cyanidin [(2'-glyucoszylrutinozid) (**IV**), more than 70%]. Finally, the basic components of the extract of nightshade garden fruits were the product of rutinoside radical acylation in position *3* by *para*-coumaric acid (petunidin-3-rutinosid-5-glucoside) (**V**).

The data obtained unambiguously confirm decrease of adsorption with an increase in the size (and in the

Table 2. Values of molar volume of different anthocyan complexes

Compound	Carbohydrate substituents in structure (VI) in the position				Molar volume, cm^3
	R$_1$	R$_2$	3'	5'	
Pelargonidine-3-hexoside	C$_6$	–	H	H	251.3 ± 5.0
Delphinidine-3-hexoside	C$_6$	–	OH	OH	244.9 ± 5.0
Pentunidine-3-hexoside	C$_6$	–	OH	OCH$_3$	269.8 ± 5.0
Cyanidine-3-hexoside (**I**)	C$_6$	–	OH	H	248.1 ± 5.0
Cyanidine-3,5-dihexoside (**II**)	C$_6$	C$_6$	OH	H	332.3 ± 5.0
Cyanidine-3-dihexoside (**III**)	C$_6$+C$_6$	–	OH	H	335.6 ± 5.0
Цианидин-3-тригексозид (**IV**)	C$_6$+C$_6$+C$_6^a$	–	OH	H	418.7 ± 5.0
Pentunidine-3-(*p*-coumaroyl-rutinoside)-5-glucoside (**V**)	C$_6$+C$_6$+*p*-Cu	C$_6$	OH	OCH$_3$	515.7 ± 5.0

[a] C$_6$ is cyclic form of hexose, Cu is the residue of para- coumaruc acid.

number) of carbohydrate substituents. Therefore the greatest difference is revealed at the adsorption of relatively simple 3-glycosides compound **I** in comparison with the adsorption of anthocyan **IV** with more bulky 3-triglycosides. This situation is correct for the both investigated clays. For the diglycosides, in spite of two diverse variants of their formation (compounds **II** and **III**), there are no difference in the adsorption practically for each clay. Clearly fall out from the examined sequence the data on the adsorption of the anthocyan of black currant, that, probably, is a feature of properties of the relatively the more hydrophobic ramnosyl fragment of rutinoside radical. Clays as sorbents are especially differed from octadecylsilane sorbents by ever lower sorption of the acylated anthocyans than the not acylated ones, while under the conditions of reverse-phase HPLC the acylated derivatives show substantially larger retention time in comparison with the not acylated. It is necessary take in mind that the adsorption of different forms of anthocyans, strictly speaking, was investigated under the not equivalent conditions (for example, in respect of the concentration and form of the associated extractive substances). But conclusions about the different sorption of anthocyans were confirmed by the HPLC study which allows measuring the ratio of anthocyans in one and same extract before and after adsorption. For

example, the independence of adsorption from the form of aglycone is confirmed by the invariability of the ratio of quantities of different anthocyans (determined from the areas of peaks on the chromatograms) for the anthocyans of grapes, which include 3-glucosides of delphinidin, peonidin, malvidin, cyanidin and penidin (Fig. 2).

In Fig. 2 are depicted two chromatograms: of the initial extract and of the equilibrium solution after

Table 3. Adsorption of anthocyans by the sorbent "kaolin" of SCC production

Sample	Equilibrium concentration of anthocyan,^{a} mmol l^{-1}	Adsorbed A, μmol g^{-1}	S^*_{sp}, m^2 g^{-1}
Aronia	0.144	60.4	20.1
Carcade	0.231	51.9	20.5
Rose	0.114	51.9	21.0
Nightshade	0.182	45.5	24.7

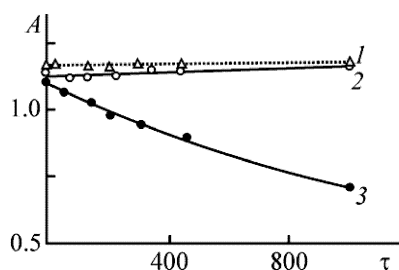[a] Recalculated on cyanidin-3-glucoside.

Fig. 4. Stability upon storage of anthocyans of the extract of bilberry in water solutions. The volume fraction of organic solvent 40%. (*A*) the fraction of stable substance(rel. units), (τ) time (min). (*1*) CH₃CN, (*2*) C₂H₅OH, (*3*) CH₃COCH₃.

adsorption on kaolin (PSM) at the 2.5 times decrease in the total concentration of anthocyans. The chromatograms for convenience in the visual comparison of the peaks are recorded at different dilution of samples. The calculation of the peaks parameters showed that fractions of the peaks areas of individual anthocyans did not change after adsorption in the range of determination error (±1.5%). Another result is obtained for adsorption of the cherry fruits anthocyans (Fig. 3). It is obvious that cyanidin-3-rutinosid disappears faster from the extract in comparison with the anthocyan of more complex structure (**IV**). In the case of the pith black, the plant, whose fruits relate to the richest sources of anthocyans, the extract contains two different 3-glycosides: cyanidin-3-glucoside (monohexoside) and 3-sambubioside (pentozylhexoside). The fraction of sambubioside in the equilibrium solution increased from 42 to 62% at the five-fold decrease of the total concentration of anthocyans after adsorption on kaolin.

The most probable mechanism of adsorption on the clays of the substances existing in solution in the cation form (including anthocyan), is ion exchange [15]. For explaining the differences in the adsorption depending on the structure of substances it is possible to assume the different "screening" of ion-exchange centers in accordance with the molecular size. The program product

ChemSketch (ACD) allows calculation of the molar volumes of neutral molecules. In the case of anthocyans were used the results of calculation for the appropriate pseudobases (**VI**) (Table 2): The calculated molar volume of anthocyans grows in essence with a change in the number of carbohydrate fragments and little depends on the type of aglycone. In Table 3 are given calculations for sorbtion of anthocyans of four extracts. The high equilibrium concentrations of anthocyan in the solutions over the sorbents correspond to the high degree of filling of the clay surface. For further calculations we used the ratios between the molar volume V_m, the volume of one molecule V_1 and Avogadro number N_A:

$$V_m = N_A V_1.$$

For the surface area, occupied by one mole of the sorbate S_m and with one molecule S_1, we obtain: $S_m = N_A S_1$. At the unknown geometry of the sorption of anthocyans on the surface of sorbent in the first approximation we use relation $S_1 = V_1^{2/3}$. Finally, we obtain:

$$S_m = V_m^{2/3} N_A^{1/3}.$$

The product of the excessive adsorption of anthocyans and the surface area, occupied by one mole of sorbate on the studied sorbent must correspond to "effective (relative to anthocyans) specific surface area" of the sorbate:

$$S_{sp}^* = A\, S_m.$$

For three anthocyans of different structure the values of effective specific surface area proved to be sufficiently close (Table 3), somewhat greater value is obtained for the acylated anthocyan, but such anthocyans are differed from the not acylated by a number of properties, for example, by higher stability.

Table 4. Sequential adsorption- desorption operations on one and the same sample of clay with the identical Aronia extract

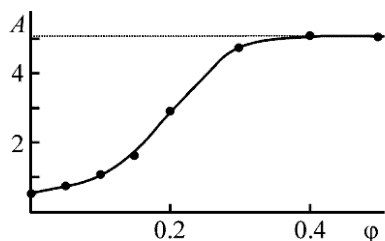| Operation	Number of operation				Totally
	1	2	3	4	
Adsorption, mmol g⁻¹	0.0499	0.0458	0.0418	0.0378	0.175
Desorption: mmol g⁻¹	0.0412	0.0453	0.0470	0.0374	0.171
	82.6	99.1	112.5	99.2	97.6

Fig. 5. Dependence of optical density φ (reduced) of equilibrium anthocyan extract on the volume fraction of ethanol A (mol l^{-1}). Dotted line is iitial solution: 1.7×10^{-4} mol l^{-1} (extract of rose).

The data obtained do not contradict the hypothesis about the ion-exchange mechanism of the adsorption of anthocyan taking into account the area occupied by molecule on the surface of sorbent. Effective specific surface area by the order of magnitude corresponds to the results found from the low-temperature adsorption of nitrogen (74.1 m^2 g^{-1}), but approximately one third as great. This nonconformity can be explained, first, by the obvious conditionality of the relation $S_1 = V_1^{2/3}$, since several times overestimation of the height of anthocyan monomolecular layer is completely probable. Second, anthocyans might miss the micropores of sorbent accessible for nitrogen.

In Table 3 there are no data on the adsorption of the anthocyan of cherry fruits, since in this case it is difficult to obtain the extracts with high initial concentration of anthocyans due to the relatively low content of anthocyans in the fruits of this plant and high content of the associated extractive substances. However, correlating the data of Tables 1 and 2, one can see that one ought not expect large deviations from the obtained regularity for sorption of cherries anthocyans.

Finally, the most important property of the anthocyans, clay system is the possibility of changing the adsorption by adding organic solvent. However, one must be careful at the selection of organic solvent. For example, it was found that adding of acetone (proposed as the most effective organic component for the extraction of anthocyan [16]) to the extract containing anthocyan leads to gradual destruction of the latter, which was not observed at the use of ethanol and acetonitrile (Fig. 4). The ethanol additives rapidly decrease adsorption of anthocyan: even at the 50% content of ethanol it is possible to carry out effective desorption of the anthocyans adsorbed on the clay (Fig. 4). Although acetonitrile somewhat exceeds ethanol by desorption activity, at the preparation of dyes for food and medical

industry is preferable to use ethanol. For the practically complete (more than 95%) desorption of anthocyan from the clays is sufficient two extractions by acidified (1% of conc. HCl) ethanol. However, it is better to use single desorption with repeated use of one and the same sample of clay for the sorption concentration (Table 4). Such a scheme makes it possible to obtain the more concentrated extracts with the practically quantitative yield of anthocyan (Fig. 5). Let us note that the purified extracts no longer form amorphous precipitates of the associated extractive substances, characteristic for initial aqueous solutions upon storage.

CONCLUSIONS

1. Clays are effective sorbents for purification and concentration of natural pigments anthocyans. For absorbing the anthocyans the contact of clays with the aqueous extracts of the plant material acidified by hydrochloric acid (fruits or flower lobes) is necessary.

2. The sorption activity of clays increases with an increase in the specific surface area in going from the kaolin to the blue clay produced by "Stimul-Color-Cosmetics" firm.

3. It is assumed that adsorption of anthocyan is achieved by the ion-exchange mechanism, and difference in the adsorption of the different types of anthocyan is caused by a difference in the size of the anthocyan molecules.

4. For the desorption of anthocyan, to the used for desorption aqueous solution of hydrochloric acid should be added organic modifier (ethanol or acetonitrile).

REFERENCES

1. Deineka, V.I., Chulkov, A.N., Deineka, L.A., and Shamshurov, A.I., I*I Vseros. Nauch. Conf. s mezhdunar. uchastiem "Sorbenty kak faktor kachestva zhizni"* (All-Union Sci. Conf. with Int. Participation on Sorbents as Factor of Life Quality), Belgorod, 2006.

2. Chulkov, A.N. and Deineka, V.I., Abstracts of Papers, *Tret'ya mezhdunar. nauch.-prakt. konf. "Issledovanie razrabotka i primenenie* (3rd Int. Sci. Pract. Conf. on Invetigation, Development and Application of High Technologies in Industry), St. Petersburg: Politekhn. Univ., 2007, pp. 92–93.

3. Deineka, V.I., Khlebnikov, V.A., Chulkov, A.N. et al., *Khimiya Rast. Syr'ya*, 2007, no. 2, pp. 63–66.

4. Ptitsyn, A.V., Kaplun, A.P., Mukhtarov, E.I., Shvets, V.I.,

Biotekhnologiya, 2007, no. 2, pp. 13–20.

5. Scordino, M., Di, Mauro, A., Passerini, A., Maccarone, E., *J. Agr. Food Chem.*, 2005, vol. 53, pp. 651–658.

6. Di Mauro, A., Arena, E., Fallico, B. et al*., J. Agric. Food Chem.*, 2002, vol. 50, pp. 5968–5974.

7. Scordino, M., Di, Mauro, A., Passerini, A., and Maccarone, E*., J. Agric. Food Chem.*, 2004, vol. 52, pp. 1965–1972.

8. Csiktusnadi, Kiss, G.A., Forgacs, E., Cserhati, T. et al*., J. Chromatogr. A*, 2000, vol. 889, pp. 51–57.

9. Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, M. et al., *J. Wine Res.*, 2000, vol. 11, pp. 223–231.

10. Lopes, T.J., Quari, M.B., and Quari, M.G.N., *Braz. J. Food, Technol.*, 2006, vol. 9, pp. 49–56.

11. US Patent 4500556, Cl 426/540. *Anthocyanin Colorant from Grape Pomace.*

12. Deineka, V.I., Grigor'ev, A.M., Deineka, L.A. et al*., Rast. resursy*, 2005, no. 1, pp. 91–98.

13. Deineka, V.I. and Grigor'ev, A.M., *Zh. Fiz. Khim.*, 2004, vol. 78, no. 5, pp. 923–926.

14. Deineka, V.I and Grigor'ev, A.M*., Zh. Anal. Khim.*, 2004, vol. 59, no. 3, pp. 305–309.

15. Deineka, V.I., Lebedeva, O.E., Lazebnaya, M.A., and Anisimovich, I.P., *Sorbts. Khromatogr. Protsessy*, 2007, vol. 7, no. 3, pp. 473–480.

16. Garcia-Viguera, C., Zafrilla, P., and Tomás-Barberán, F.A*., Phytochem. Anal.*, 1998, vol. 9, pp. 274–277.