DOI 10.1007/s11094-019-02002-2
Pharmaceutical Chemistry Journal, Vol. 53, No. 4, July, 2019 (Russian Original Vol. 53, No. 4, April, 2019)

MEDICINAL PLANTS

SORPTION PROPERTIES OF SUNFLOWER HUSK MELANINS

N. V. Gracheva[1,*] and V. F. Zheltobryukhov[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 53, No. 4, pp. 34 – 38, April, 2019.

Original article submitted October 17, 2017.

The sorption properties of sunflower husk melanins were studied according to pharmacopoeial monograph GPM.1.2.3.0021.15 using marker compounds methylene blue, methyl orange, gelatin, and iodine. The sorption capacities of the studied samples were found to be 190.9 ± 4.2 mg/g for methylene blue; 302.1 ± 1.8, methyl orange; 114.7 ± 2.8, gelatin; and 38.4 ± 2.4%, I_2. The sorption capacities of the samples were greater than those of the sorbents Polifepan and Polysorb and comparable with that of activated charcoal for medium-molecular-mass toxicants. The melanins showed high affinity for anionic substances. The protein-binding capacities of the melanins were inferior only to that of Polysorb and significantly greater than those of Polifepan and activated charcoal. The sorption capacity for I_2 matched that of Polifepan and was >1.5 times less than that of activated charcoal. The results showed that enterosorbents based on melanins could be developed.

Keywords: melanins, sunflower husks, sorption properties.

Efferent therapeutics used in practice cover a broad spectrum of sorbents with different compositions, structures, materials, and forms [1]. However, new sorbents with expanded applications and increased enterosorption efficiency are actively sought. Development of sorbents based on natural substances, including those of plant origin, is a promising area [2 – 5].

Melanins are natural high-molecular-mass condensed polymers that exhibit sorption properties [6 – 9]. Highly stable paramagnetic centers, polyconjugated bonds, and various functional groups (carboxyl, aldehyde, carbonyl, etc.) are responsible for these properties [10 – 13] and determine the complexation capability and ion-exchange capacity of melanins. However, their widespread use is prevented, on one hand, by the limited natural feedstocks and, on the other, by the economics of biotechnological cultivation of melanin producers.

Sunflower husks are a side product of vegetable oil manufacturing, amount to 1 – 2 million tons per year in Russia alone, and have not until recently been considered as raw material for producing melanins.

[1] Volgograd State Technical University, Volgograd, 400005 Russia.
[*] e-mail: gracheva.tasha@yandex.ru

Sunflower husks were shown to be a promising raw-material source for producing melanins that met requirements for availability, cost, and renewability [12, 14].

The goal of the present research was to study the sorption properties of melanins isolated from sunflower husks and to determine the potential for developing enterosorbents based on them.

EXPERIMENTAL PART

Melanins isolated from sunflower husks (waste from oil extraction manufacturing, OOO Dobryi Spas, Novoanninskii, Volgograd Oblast) and the reagents distilled H_2O produced by distillation of tap water (pH = 6.8), $CHCl_3$ (chemically pure, TU 2631-066-44493179-2001), EtOAc (chemically pure, GOST 22300–76), BuOH (chemically pure, TU 2632-021-44493179–98), EtOH for chromatography (chemically pure, TU 6-09-1710–77), *i*-PrOH (chemically pure, TU 2632-181-44493179-2014), *n*-hexane (chemically pure, TU 2631-158-44493179-2013), petroleum ether (chemically pure, TU 2631-074-44493179-2001), conc. HCl (high purity, GOST 14261–77), NaOH (analytically pure), $KMnO_4$ (analytically pure), $FeCl_3$ (chemically pure), H_2O_2 (high purity, TU 20.13.63-207-44493179-2016), methylene blue (analyti-

cally pure), methyl orange (analytically pure), iodine titration standard (0.1 N) (TU 2642-581-00205087-2007), NaS_2O_3 titration standard (0.1 N) (TU 2642-581-00205087-2007), edible gelatin (powder), soluble starch (GOST 10163–76), KI (analytically pure, GOST 4232–74), $CuSO_4 \cdot 5H_2O$ (analytically pure, GOST 4165–78), and K-Na tartrate tetrahydrate (analytically pure, GOST 5845–79) were used in the work.

Solutions of the required concentrations were prepared by dissolving accurately weighed reagents in distilled H_2O or diluting a stock solution with distilled H_2O to the required concentration. Biuret reagent was prepared according to the procedure in GOST R 53030 – 2008.

Melanin isolation and identification. Melanins were isolated by the published method [12] except that reprecipitated and dried melanins were extracted exhaustively and sequentially with $CHCl_3$, EtOAc, and BuOH. Melanins were extracted from the raw material using NaOH solution (0.25 M) with a raw-material–extractant mass ratio of 1:5 and 1:3 for extractions 1 and 2, respectively.

The isolated melanins were identified as usual including comprehensive studies of their solubility in a variety of solvents, quinoid and phenol fragments in their structures, elemental composition, and spectral properties [13, 15].

Elemental analysis used a vario EL Cube automated analyzer (Netzsch EAS, Hamburg, Germany). Primary elemental analysis data were adjusted for the sample ash content of $(1.37 \pm 0.3)\%$ and hygroscopic moisture content of $(3.55 \pm 0.5)\%$. Oxygen was calculated from the difference of the ashless anhydrous mass and sum of C, H, N, and S (%). Table 1 presents the elemental analyses.

FT-IR spectroscopic studies of the melanins in KBr pellets used a Nicolet 6700 FT-IR spectrometer (USA) in the range $4000 – 400\ cm^{-1}$. Results were processed as before [16]. FT-IR spectrum, ν_{max}, cm^{-1}: 1013 (C–OH_{alc}), 1228 [C_{ar}–O–R(H)], 1538 (C_{ar}–C_{ar}), 1633 (C=C conjugated to C=O), 1709 (C=O), 2852 (–CH_2–), 2921 (–CH_2–), 3286 (–OH).

The presence of quinoids and phenols in the melanins was confirmed by qualitative reactions with H_2O_2, $KMnO_4$, and $FeCl_3$ [15]. Melanins (0.05%) in NaOH solution (0.1 N) were treated in the first instance with an equal volume of H_2O_2 (10%) and left for 24 h to react and, in the second, with an equal volume of $KMnO_4$ solution (0.1 N). In the third instance, melanins (0.01%) in basic solution were treated with $FeCl_3$ solution until its concentration reached 0.5 – 1 mg/mL.

Solubility was studied in organic solvents [EtOH, *i*-PrOH, hexane, petroleum ether, and EtOAc), H_2O, and NaOH solution (0.5%)] according to GPM.1.2.1.0005.15 Solubility.

Qualitative reactions (bleaching by 10% H_2O_2 solution and 0.1 N $KMnO_4$ solution) and solubility in organic solvents, H_2O, and NaOH solution (0.5%) were used to standardize the melanins because there are currently no satisfactory methods for standardizing them.

Study methods for melanin sorption capacity. The sorption capacity of the obtained melanins were determined using methods based on sorption of model marker compounds. The model compounds used in the work corresponded to pharmacopoeial monograph GPM.1.2.3.0021.15 Determination of adsorption capacity of enterosorbents" and included methylene blue, methyl orange, and gelatin. Also, the sorption capacity of the melanins for I_2 was also determined.

Cationic dye methylene blue is a marker mimicking medium-molecular-mass toxicants and characterizes the affinity of the sorbent for positively charged particles [17]. The sorption capacity for methylene blue gives an indication of mesopores in the structure that are responsible for the ability of the enterosorbent to absorb organic molecules.

Anionic dye methyl orange has a molecular mass close to that of methylene blue and is also a marker mimicking medium-molecular-mass toxicants but characterizes the affinity of the sorbent for negatively charged particles [17].

Gelatin is a marker for high-molecular-mass proteins of irregular structure and characterizes the protein-binding capacity of the sorbent that is responsible for absorptive detoxification of pathological protein agents such as microorganisms, their toxins, endogenous bioactive intestinal polypeptides, etc. [5]. The ability to absorb biological markers defined the possibility of using the materials as enterosorbents.

I_2 is a marker characterizing the sorbent microporosity [18]. Sorbents with a predominance of micropores are capable of adsorbing efficiently low-molecular-mass compounds.

Sorption capacity was determined using spectrophotometry and methylene blue according to the literature method [19]; methyl orange, the method of GOST 4453–74 for powdered wood bleaching activated charcoal; gelatin, the literature method [19]. Spectrophotometry used an SF-26 spectrophotometer (Russia). Sorption capacity of the obtained melanins for I_2 was determined according to the method given in GOST 6217–74.

Experiments were conducted in triplicate for each of the nine melanins isolated from raw materials taken at random. Results were processed using the parametric Student *t*-criterion and nonparametric Mann—Whitney *U*-criterion for error probability <5%.

RESULTS AND DISCUSSION

The elemental analyses of the melanins isolated from sunflower husks (Table 1) showed that the contents of major elements C, H, and O corresponded to the values for melanins [15, 20]. The N and S contents in the studied melanins were 2.31 and 0.31%, respectively. This could be related to incorporation of N-containing heterocyclic compounds and amino acids into the biopolymer and to the formation of melanin–protein complexes [15].

FT-IR spectroscopic studies found that the samples had a set of absorption bands characteristic of melanins.

A broad absorption band at 3250 – 3500 cm^{-1} was attributed to stretching vibrations of alcohol and phenol OH groups bound in inter- and intramolecular bonds. Absorption bands at 1140 – 1230 cm^{-1} were indicative of phenols in the melanins and were due to phenol C–O– and OH stretching and bending vibrations. Absorption bands at 1630 – 1644 cm^{-1} were assigned to quinone C=O vibrations. Absorption bands for aromatic C–C stretching vibrations at 1519 – 1538 cm^{-1} were consistent with aromatic moieties in the studied melanins. An absorption band at 1709 cm^{-1} showed that the structure contained carbonyls. Absorption bands at 2852 – 2853 and 2921 – 2922 cm^{-1} confirmed that the structure had $–CH_2–$ fragments. Polysaccharides were responsible for absorption bands at 1000 – 1030 cm^{-1}.

All obtained samples were insoluble in organic solvents and H_2O but soluble in basic solution, which was characteristic of melanins. The solutions were bleached by strong oxidants (H_2O_2 and $KMnO_4$). The solutions reacted with $FeCl_3$ to give a flocculent precipitate that dissolved with an excess of the reagent. The reactions were indicative of quinoid and phenolic components in the samples and confirmed that the obtained samples were melanins.

Table 2 presents the sorption capacity for marker compounds of the melanins and reference material.

The reference materials were industrially manufactured sorbents with different chemical compositions and structures. Structural differences of the industrially manufactured enterosorbents were responsible for their different properties and therapeutic effects and designated their indications. Thus, Polifepan is an enterosorbent of plant origin. Its main active ingredient is hydrolyzed lignin containing many functional groups that is a highly efficient sorbent for medium-molecular-mass toxins and metal ions [23, 24]. Polysorb is a nonselective inorganic polyfunctional enterosorbent based on highly disperse nonporous silica that is a highly efficient sorbent for high-molecular-mass toxic proteins [23, 24]. Activated charcoal is a porous carbon sorbent produced from C-containing material (wood, coal, coconut shells, etc.) via carbonization and activation. Its structure has all pore types. It exhibits high adsorption capacity for toxicants of medium and low molecular mass [24].

The experimental results showed that sunflower-husk melanins had high sorption capacity for methylene blue. This indicated that their structures were dominated by mesopores and organic molecules were absorbed efficiently. The sorption capacity parameter for melanins was more than twice those of Polifepan and Polysorb. This was related to structural features of these preparations, i.e., macroporous for Polifepan and nonporous for Polysorb. The sorption capacity for methylene blue was slightly lower for melanins than for activated charcoal because of the high specific surface area of activated charcoal. Differences in the sorption capacities for methylene blue of the studied melanins and the industrially manufactured sorbents were found to be statistically significant.

The sorption capacity of sunflower-husk melanins for methyl orange was also highly significant and was greater than the sorption capacity for methylene blue. This indicated that the melanins had high affinity for negatively charged organic molecules. The sorption capacity of the studied melanins for methyl orange was an order of magnitude greater than those of Polifepan and Polysorb and more than double that of activated charcoal. Differences in the sorption capacities for methyl orange of the studied melanins and industrially manufactured sorbents were also statistically significant.

The results could be explained as follows. Melanins are by nature redox active [15], i.e., contain groups capable of transferring electrons. Each electron transfer either forms (or destroys) positive charge or destroys (or forms) a positively charged ion. A unique property of melanins, i.e., a stable free-radical state, confirms this. Melanin pigment monomers can exist as phenoxyl or semiquinone radicals, depending on the conditions. These natural polymers react not only in the reduced phenolic hydroquinone form but also as a polyphenol–quinone system in which a semiquinone radical acts as the obligatory intermediate [13, 25]. Furthermore, the high sorption capacity of melanins for methyl orange could be due to the ability for complexation and chemisorption because of carboxyl, carbonyl, hydroxyl, and other functional groups in the structure.

Absorption of biological markers found that the melanins had high sorption capacity for gelatin and characterized their high affinity for high-molecular-mass compounds. The sorption capacity of the melanins was significantly greater than the corresponding value for Polifepan and activated charcoal but lower than that of Polysorb. This was due to the following factors. Gelatin is an amphoteric polyelectrolyte. Its isoelectric point was observed at pH 4.8 – 5.0 [26], i.e., it was negatively charged under the experimental conditions. The surface of hydrolyzed lignin (Polifepan) contains carboxyls and phenols so it is negatively charged. The same surface charges prevent them from interacting and reduce the

TABLE 1. Elemental Composition of Melanins Isolated from Sunflower Husks

Element	Content, mass%				
	C	H	N	O	S
Found	44.21 ± 1.05	5.98 ± 0.35	2.12 ± 0.05	…	0.28 ± 0.04
Calculated	49.11 ± 1.08	4.79 ± 0.30	2.31 ± 0.05	43.48	0.31 ± 0.05

TABLE 2. Sorption Capacity of Sunflower Husk Melanins and Industrial Manufactured Enterosorbents for Marker Compounds

Sorbent	Sorption capacity for			
	methylene blue, mg/g	methyl orange, mg/g	gelatin, mg/g	I_2, mass%
Sunflower husk melanins	190.9 ± 4.2	302.1 ± 1.8	114.7 ± 2.8	38.4 ± 2.4
Polifepan [21]	72.4 ± 1.7	9.3 ± 1.5	52.5 ± 8.5	
Polifepan, AO Saintekh, R No. 001047/03-2002 [18]	57.1 ± 1.5	…	…	43.2 ± 1.7
Polifepan, Irkutsk R.80.1211.3 [18]	48.9 ± 1.9	…	…	17.9 ± 1.3
Polysorb [21]	82.1 ± 8.9	17.9 ± 2.2	305.8 ± 2.6	…
Polysorb, s. 010598 (Chelyabinsk) [19]	42.4 ± 4.1	…	203.8 ± 9.6	…
Activated charcoal [21]	374.4 ± 0.8	137.2 ± 4.3	41.1 ± 4.1	…
Activated charcoal OU-A, Perm [19]	255.2 ± 9.8	…	46.7 ± 1.2	…
Activated charcoal AU MeKS (Nipiem) [22]		…	…	60

bioavailability of the protein to the sorbent macropores. The surface of activated charcoal is also unavailable to proteins because the molecules are too large to penetrate micro- and mesopores. The sorption capacity for proteins of melanins is determined by the electrostatic component and, presumably, complexing capability [6, 7]. Also, proteins can adsorb to nonpolar surfaces through hydrophobic interactions with nonpolar residues such as amino acids valine, alanine, phenylalanine, etc. [26]. Polysorb features surface silanol groups [27] that are responsible for its high hydrophilicity and protein-binding capability, including for gelatin.

The high sorption capacity of the melanins for gelatin suggested that they were highly effective for absorption of viruses and bacteria with an excess of anionic charge. Differences in the sorption capacities for gelatin of the studied melanins and industrially manufactured sorbents were found to be statistically significant.

The sorption capacity of the melanins for I_2 indicated that the values for I_2 were in the range of those for Polifepan and 1.5 times greater than that for activated charcoal. This indicated melanins were inefficient as low-molecular-mass sorbents. Differences in sorption capacities for I_2 of the studied melanins and activated charcoal were found to be statistically significant.

Thus, an analysis of the results showed that the studied sunflower-husk melanins had high sorption capacity for markers mimicking medium-molecular-mass toxicants and high-molecular-mass pathological proteins. The sorption capacities of the melanins for all markers were high, in contrast with the reference samples. The sorption capacity of the studied samples was found to depend on the charge of the sorbate. Melanins had low sorption capacity for I_2. The results led to the conclusion that melanins could be used a platforms for developing enterosorbents.

REFERENCES

1. V. G. Nikolaev, S. V. Mikhalovskii, and N. M. Gurina, *Efferentnaya Ter.*, **11**(4), 3 – 17 (2005).
2. S. A. Kuznetsova, M. L. Shchipko, B. N. Kuznetsov, et al., *Khim. Rastit. Syr'ya*, No. 2, 25 – 29 (2004).
3. E. V. Veprikova, M. L. Shchipko, S. A. Kuznetsova, et al., *Khim. Rastit. Syr'ya*, No. 1, 65 – 70 (2005).
4. E. S. Kokh, A. S. Gavrikov, A. A. Tumashov, et al., *Nauchn. Vedom. BelGU Ser.: Med. Farm.*, **201**(4), 160 – 166 (2015).
5. M. G. Ismailov, Kh. M. Makhkamov, and P. L. Ismailov, *Khim.-farm. Zh.*, **34**(12), 38 – 40 (2000).
6. O. Yu. Kuznetsova, *Vestn. Kazan. Tekhnol. Univ.*, **23**(16), 136 – 138 (2013).
7. N. V. Sushinskaya, T. A. Kukulyanskaya, N. V. Gavrilenko, et al., *Usp. Med. Mikol.*, **3**(3), 192 – 195 (2004).
8. N. V. Ikonnikova, I. A. Goncharova, and N. M. Rovbel', *Usp. Med. Mikol.*, **5**, 187 – 190 (2005).
9. D. A. Novikov, *Tr. BGU Ser.: Fiziol., Biokhim. Mol. Osnovy Funktsion. Biosistem*, **1**, 105 – 114 (2006).
10. E. A. Prutenskaya, A. S. Vasil'ev, E. Yu. Lebedeva, et al., *Simvol Nauki*, **11**, No. 3, 11 – 13 (2016).
11. V. A. Baraboi, *Usp. Sovrem. Biol.*, **12**, 1 – 12 (2001).
12. N. V. Gracheva, V. F. Zheltobryukhov, and V. F. Kablov, in: *Proceedings of the IInd All-Russian Scientific-Practical Internet-Conference with International Participation* [in Russian], Petrozavodsk, 2016, pp. 19 – 22.
13. G. Britton, *The Biochemistry of Natural Pigments*, Cambridge Univ. Press, New York, 1983, 366 pp. [Russian translation, Mir Moscow, 1986, pp. 92 – 99, 259 – 279].
14. N. V. Gracheva, *Vestn. Kazan. Tekhnol. Univ.*, **19**(15), 154 – 157 (2016).
15. S. P. Lyakh, *Microbial Melaninogenesis and Its Function* [in Russian], Nauka, Moscow, 1981, pp. 22 – 270.
16. L. A. Kazitsina and N. B. Kupletskaya, *Applications of UV, IR and NMR Spectroscopy in Organic Chemistry* [in Russian], Vysshaya Shkola, Moscow, 1971, pp. 23 – 60, 235 – 257.
17. V. S. Chuchalin and N. V. Kelus, *Razrab. Regist. Lek. Sredstv*, **12**(12), 86 – 91 (2015).
18. E. V. Veprikova, S. A. Kuznetsova, G. P. Skvortsova, et al., *SibFU J. Chem.*, **1**(3), 286 – 292 (2008).
19. V. I. Reshetnikov, *Khim.-farm. Zh.*, **37**(5), 28 – 32 (2003).

20. D. N. Olennikov, Author's Abstract of a Doctoral Dissertation in Pharmaceutical Sciences, Ulan-Ude, 2012.
21. N. V. Kelus, V. V. Sheikin, A. E. Gundareva, et al., *Byull. Sib. Med.*, No. 1, 121 – 125 (2011).
22. V. M. Mukhin, P. V. Uchanov, and N. I. Sotnikova, *Sorbtsionnye Khromatogr. Protsessy*, **13**(1), 83 – 90 (2013).
23. E. P. Gernikova, A. I. Luttseva, T. N. Bokovikova, et al., *Vedom. NTsESMP*, No. 4, 48 – 50 (2013).
24. V. N. Panfilova and T. E. Taranushenko, *Pediatr. Farmakol.*, **9**(6), 34 – 39 (2012).
25. V. A. Solov'ev and A. M. Kutnevich, *Higher Fungi and Their Physiologically Active Compounds* [in Russian], Nauka, Leningr. Otd., Leningrad, 1973, pp. 35 – 39.
26. D. A. Markelov, O. V. Nitsak, and I. I. Gerashchenko, *Khim.-farm. Zh.*, **42**(7), 30 – 33 (2008).
27. A. A. Chuiko (ed.), *Medicinal Chemistry and Clinical Application of Silicon Dioxide* [in Russian], Naukova Dumka, Kiev, 2003, pp. 12 – 18, 131 – 144.