Published in final edited form as:
Nucl Med Biol. 2008 April ; 35(3): 353–357. doi:10.1016/j.nucmedbio.2007.12.006.

Chemosorption of radiometals of interest to nuclear medicine by synthetic melanins

R. C. Howell[1], **A. D. Schweitzer**[1,2], **A. Casadevall**[3,4], and **E. A. Dadachova**[1,3]

1*Department of Nuclear Medicine, Albert Einstein College of Medicine, Bronx, NY, USA*

2*Howard Hughes Medical Institute - Medical Fellows Program, Chevy Chase, MD, USA*

3*Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA*

4*Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA*

Abstract

Introduction—Melanins are high molecular weight pigments that are ubiquitous in nature and can also be synthesized in the laboratory from the variety of precursors. Melanins possess numerous interesting physico-chemical characteristics including electromagnetic radiation absorption properties and the ability to chelate metals. We have recently reported that melanin has remarkable ionizing radiation shielding properties, possibly because it can interact with photons via Compton scattering. We hypothesized that, if administered internally, in addition to radiation shielding, melanin could play a beneficial role by scavenging various radionuclides.

Methods—Three melanins were synthesized from dopamine, 3,4-dihydroxyphenylalanine (L-Dopa) and from combination of L-Cysteine and L-Dopa. For control synthetic melanin made from tyrosine polymerization (Sigma) was used. Melanins were characterized by elemental analysis. The chemosorption of ^{111}In, ^{225}Ac and ^{213}Bi by melanins was studied at 37°C for up to 48 hrs.

Results—The C to N molar ratios for dopamine, L-Dopa and tyrosine melanins were very close at 7.92, 8.39, and 8.48, respectively, while in mixed L-cysteine/L-Dopa melanin that ratio was much lower at 3.63. This mixed melanin also contained 22.33% sulfur, thus confirming incorporation of S-containing motifs into its structure. Dopamine, L-Dopa and tyrosine melanins were very similar in their ability to decrease the activity of ^{111}In, ^{225}Ac and ^{213}Bi and their radioactive daughters in the supernatants more than 10-fold in comparison with the starting levels while mixed L-cysteine/L-Dopa melanin was able to chemosorb only ^{111}In.

Conclusions—We have demonstrated that synthetic melanins made of diverse precursors can chemosorb ^{111}In, ^{213}Bi and ^{225}Ac with dopamine, L-dopa and tyrosine melanins being the most efficient towards all three of these radionuclides. Such properties of synthetic melanins can contribute to the development of the novel melanin-based radioprotective materials.

Keywords

melanin; chemosorption; 225-Actinium; 213-Bismuth; 111-Indium; radiological attack

*Author for correspondence: Ekaterina Dadachova, PhD, Department of Nuclear Medicine, 1695A Eastchester Road, Bronx, NY 10461 Ph: 718-405-8485; FAX: 718-405-8457; E-mail: edadacho@aecom.yu.edu.

INTRODUCTION

Melanins are high molecular weight pigments that are ubiquitous in nature [1]. These pigments can be also synthesized in the laboratory from a variety of precursors [2]. Melanins possess numerous interesting physico-chemical characteristics including unusual optical, condensed phase electric, electron exchange, paramagnetic, ion exchange, and electromagnetic radiation absorption properties (reviewed in [3]). Melanins are also known to chelate metals [4], a property that might be useful in bioremediation strategies whereby melanized microorganisms such as bacteria or fungi are used to collect and immobilize heavy metal pollutants [5–7].

In our laboratories we are studying the ability of melanins to interact with the ionizing radiation. We have reported that melanin has remarkable ionizing radiation shielding properties by being able to protect the ionized fungi against very high (up to 8,000 Gy) doses of gamma radiation [8], possibly because it can interact with photons via Compton scattering. Hence, melanin might serve as an energy transducer and/or radioprotector in living cells, with its radioprotective properties being a function of its chemical composition, stable free radical presence, and spherical spatial arrangement [8,9]. Those properties could find applications in protecting bone marrow or gastrointestinal tract of cancer patients undergoing external beam radiation therapy (EBRT) or persons contaminated internally with radionuclides from harmful effects of ionizing radiation by administering melanin systemically in form of nanoparticles or powders [10,11]. Since melanins have the ability to bind different metals [4], we hypothesized that, in addition to shielding, melanin could play a beneficial role by scavenging various radionuclides. Such properties may be particularly relevant for protection against incidents such as the use of “dirty bombs” in a radiological attack. The radionuclides in such accidents may include those used by medical or research facilities. Here we investigated the chemosorption of 111-Indium (^{111}In, $t_{1/2}$ = 67.3 hr), 213-Bismuth (^{213}Bi, $t_{1/2}$ = 45.6 min) and 225-Actinium (^{225}Ac, $t_{1/2}$ = 10 days) (Fig. 1) by four synthetic melanins made of various chemical precursors.

MATERIALS AND METHODS

Syntheses of melanins

Melanins were synthesized as in [12] with some modifications, including using mushroom tyrosinase (Sigma) as a polymerizing enzyme. Melanin precursors were incubated with 8,300 units of mushroom tyrosinase in 40 ml 0.05 M sodium phosphate buffer, pH 6.8, with constant shaking at 37°C overnight. The precursor for dopamine melanin was 0.5 mmol dopamine; for 3,4-dihydroxyphenylalanine (L-Dopa) melanin - 0.5 mmol L-Dopa, and for L-Cysteine/L-Dopa melanin 0.5 mmol L-Dopa and 0.75 mmol L-cysteine. After overnight incubation the oxidation reaction was stopped with the addition of 250 μl 6 M HCl (to pH ca. 3.0). The acidified mixture was kept at 2°C for 1 h. The precipitate was collected by centrifugation, washed three times with 15 ml 1% acetic acid, twice with 15 ml acetone, and then evaporated to dryness. Elemental analysis of lyophilized melanins for carbon, nitrogen and sulfur was performed by Quantitative Technologies, Inc. (Whitehouse, NJ). For control synthetic melanin was purchased from Sigma Aldrich (synthesized by oxidation of tyrosine with hydrogen peroxide).

Radionuclides

^{111}In was purchased from MDS Nordion (Canada). ^{225}Ac was procured from the Curative Technologies (WA, USA). The same batch of ^{225}Ac was used to make ^{225}Ac generator as in [13] from which ^{213}Bi was eluted with 0.1 M hydroiodic acid.

Chemosorption studies

One to six mg of the four synthetic melanins (three synthesized as described above plus commercially available tyrosine melanin from Sigma) were placed into Eppendorf tubes. Approximately 800 μl of ^{111}In-, ^{225}Ac- or ^{213}Bi-containing solution were prepared by spiking normal saline (pH=6.0) with 2–5 μl of the stock solution of the respective radioisotope. Two hundred μl aliquots of each radioisotope were added to separate tubes with each of four melanins. The samples were incubated at 37°C for up to 48 hrs, and the 5 μl aliquots were withdrawn at 0, 10 min, 30 min, 1, 2, 3, and 5 hrs, and counted in a gamma counter. At the pH of normal saline (6.6) ^{213}Bi is present in solution possibly in the form of $^{213}BiI_3$ post elution from the ^{213}Bi/^{225}Ac generator for at least 48 hrs – this was confirmed by incubating ^{213}Bi in normal saline for 48 hrs, passing the solution through 0.02 μ filter to remove possible precipitate and counting the supernatant. The samples were then centrifuged at 5.2 rcf for 90 sec and the supernatant was separated from the melanin pellet and counted again. For ^{225}Ac and ^{213}Bi all samples of supernatant were counted again 24 hr later to take into account the chemosorption of the intermediate daughters. At 24 hr time point the total amounts of supernatants were separated from the respective melanins and counted as a whole in order to increase the counts lost due to the overnight radioactive decay. The supernatants and respective melanins were then recombined and incubated for a further 24 hr and the counting process repeated to obtain a 48 hr time point. The ^{213}Bi samples were not counted at the 48 hr time point because of the short physical half-life of ^{213}Bi and its radioactive daughter ^{209}Pb (Fig. 1). The distribution coefficients K_D was calculated using following formula:

$$K_D = \frac{\text{Radioactivity adsorbed / mass of melanin (mg)}}{\text{Radioactivity remaining in aqueous solution}}$$

RESULTS

Table 1 shows the elemental compositions of four melanins employed in this study. Given that it is impossible to dissolve melanins in any solvent – NMR and MS analyses of melanin are not routinely performed with elemental analysis for carbon, nitrogen and, if relevant, sulfur being done instead. The C to N molar ratios for dopamine, L-Dopa and tyrosine melanins were very close at 7.92, 8.39, and 8.48, respectively, while in mixed L-cysteine/L-Dopa melanin that ratio was much lower at 3.63. This mixed melanin also contained very high percentage of sulfur - 22.33% thus confirming incorporation of S-containing motifs into the structure of this melanin during the synthesis.

Fig. 2 displays the chemosorption isoterms for ^{111}In, ^{213}Bi and ^{225}Ac on dopamine, L-Dopa, L-cysteine/L-Dopa and tyrosine melanins. For ^{111}In dopamine, L-Dopa and L-cysteine/L-dopa were almost equivalent in their chemosorption properties with the highest K_D value reaching 30.9 at 24 hr for dopamine melanin (Fig. 2a). Tyrosine (Sigma) melanin was less efficient in chemosorbing ^{111}In at early time points (0–5 hr); however, at 24 hr and 48 hr its chemosorption almost equalized with the other melanins. For ^{213}Bi dopamine, L-Dopa and tyrosine melanins were comparable in their ability to chemosorb this radioisotope and the highest K_D of 11.9 was observed for L-Dopa melanin at 5 hrs (Fig. 2b). This trend continued at 24 hr when all ^{213}Bi has already decayed and the chemosorption of its intermediate daughter ^{209}Pb with 3.3 hr half-life was measured. L-cysteine/L-Dopa melanin was not effective in chemosorbing ^{213}Bi. This mixed L-cysteine/L-Dopa melanin was practically unable to chemosorb ^{225}Ac as well at any time point (Fig. 2c), while L-Dopa and tyrosine melanins showed chemosorption of this radionuclide and its daughters with K_D of 14.3 observed at 48 hrs for tyrosine melanin. Dopamine melanin lagged behind other melanins in chemosorption during early (0–5 hr) time points, but at 24 and 48 hrs its chemosorption became almost equal to that of other melanins.

DISCUSSION

Here we investigated the chemosorption of three radioisotopes of interest to nuclear medicine - ^{111}In, $t_{1/2}$ = 67.3 hr, ^{213}Bi, $t_{1/2}$ = 45.6 min, and ^{225}Ac, $t_{1/2}$ = 10 days, by synthetic melanins of differing compositions as part of our on-going effort to develop melanin-based radioprotective materials suitable for internal administration to cancer patients or individuals internally contaminated with radionuclides [10,11]. In the latter scenario, the scattering of ionizing radiation by melanin particles [8,9] which will be given orally as a powder or via hepatic artery as a suspension in case of liver contamination with radionuclides - would be complimented by chemosorption of radionuclides by melanin. Furthermore, the advantages of synthetic melanin over other radioprotectors or metal chelating agents is its complete chemical inertness (melanins can withstand boiling in 6 M HCl [14]) which would allow its administration orally, locally or intravenously (if melanin is used in form of <30 nm nanoparticles and bone marrow is the target [11]).

The structures of melanins are uncertain (reviewed in [3]) due to the amorphous, heterogeneous and insoluble nature of these pigments, which preclude their structural solution given the currently available analytical tools. It is generally accepted that there are two major types of melanin: eumelanin and pheomelanin. Eumelanin is a dark-brown to black pigment composed of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) monomer units with 6–9% nitrogen (Fig. 3a) which does not contain sulfur while a mixed reddish-brown pigment called pheomelanin is composed of benzothiazine monomer units with 8–11% nitrogen and a variable percentage of sulfur [12]. At the secondary structural level there is no generally accepted model of melanin polymers with this uncertainty resulting from the multiple binding configurations that functionalized indoles can adopt. Two most prevalent models of melanin structure are: 1) heteropolymeric model which postulates that the melanin polymer forms via random bonding between monomers; 2) stacked oligomer model which suggests that melanins are composed of stacked oligomeric "proto-molecules" consisting of no more than 5–6 indolequinone units (reviewed in [3]). In this study the dopamine, L-Dopa and tyrosine melanins belong to the eumelanin subclass while the sulfur-containing L-cysteine/L-Dopa melanin belongs to the pheomelanin subclass.

The highest chemosorption values were observed for ^{111}In. This can be explained by small ionic radius of this nuclide (0.081 nm) when compared to those of ^{213}Bi and ^{225}Ac (0.096 and 0.118 nm, respectively) resulting in more stable complexes with melanins. Dopamine, L-dopa and Sigma (tyrosine) melanins acted as efficient chemosorption agents with regard to all 3 radionuclides studied. This can be explained by the structural similarity of these melanins reflected in their close elemental composition (Table 1). Lower chemosorption values at early time points for ^{111}In and ^{225}Ac by tyrosine and dopamine melanins, respectively, may be explained by slower chelation kinetics for these radionuclides. L-cysteine/L-dopa melanin was ineffective in chemosorbing both ^{213}Bi and ^{225}Ac, and, conversely, quite effective in chemosorbing ^{111}In, which can be explained by the high percentage of sulfur atoms in is chemical structure resulting in more affinity towards amphoteric nuclides like In and practically none – towards ^{213}Bi and ^{225}Ac. Eumelanins contain carboxyl, amine, hydroxyl (phenolic), quinone and semiquinone groups and pheomelanins also contain benzothiazine units, all of which can serve as potential sites for metal chelation. While the interaction of alkali metals, some divalent metals such as Ca, Mg, Zn, Cu and Mn(II) with melanin has been studied (reviewed in [4]) – not much is known about chelation of trivalent metals by melanin with the exception of Fe(III). We concentrated our study on trivalent metals with very different chemical properties – In, Bi and Ac. We propose that all three of these metals are possibly chelated by ortho-phenolic groups in eumelanins (L-Dopa, dopamine and tyrosine melanins) while benzothiazine units in pheomelanin (L-Dopa/L-cysteine melanin) also contribute to chelation

of In. Based on our results (Fig. 2) and published observations [15] - it seems unlikely that benzothiazine units can contribute to chelation of Bi and Ac.

In addition to their very different physical half-lives, the radioisotopes used in the study have also dissimilar decay schemes (Fig. 1). ^{111}In decays through Electron Capture to stable daughter; ^{213}Bi decays via α,β decay to ^{209}Pb which subsequently undergoes β decay to stable ^{209}Bi; and ^{225}Ac undergoes three α decays leading to ^{213}Bi. Encouragingly, for each of these radionuclides at least three melanins were able to decrease the radioactivity of the supernatant 10-fold or more in comparison with the starting levels and – in case of ^{225}Ac and ^{213}Bi – also to chemosorb its radioactive daughters which was verified by measuring the activity of the supernatants 24 hr later. The ability of synthetic melanins to chemosorb ^{225}Ac is particularly important as ^{225}Ac represents the group of actinides that are extremely radiotoxic even in minute amounts and could potentially represent the radionuclides of choice for a radiological attack.

In conclusion, we have demonstrated that synthetic melanins made of diverse precursors can chemosorb ^{111}In, ^{213}Bi and ^{225}Ac radiometals with dopamine, L-dopa and tyrosine melanins being the most efficient towards all three of these radionuclides. Such properties of synthetic melanins can contribute to the development of novel melanin-based radioprotective materials.

ACKNOWLEDGEMENTS

R.C. Howell and E. Dadachova are supported by the NIH grant AI60507, A. Casadevall – by NIH grant AI52733. A. Schweitzer is a Howard Hughes Medical Institute Medical Research Training Fellow.

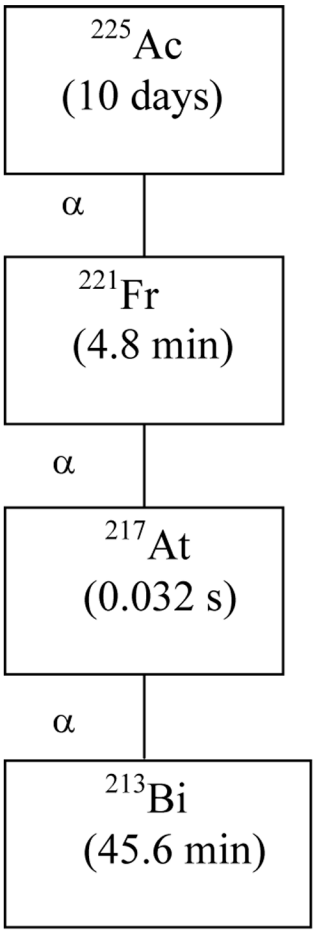
REFERENCES

1. Hill HZ. The function of melanin or six blind people examine an elephant. Bioessays 1992;14:49–56. [PubMed: 1546980]
2. Nicolaus, RA. Melanins. Paris: Hermann; 1968.
3. Meredith P, Sarna T. The physical and chemical properties of eumelanin. Pigment Cell Res 2006;19:572–594. [PubMed: 17083485]
4. Hong L, Simon JD. Current Understanding of the Binding Sites, Capacity, Affinity, and Biological Significance of Metals in Melanin. J. Phys. Chem. B 2007;111:7938–7947. [PubMed: 17580858]
5. Turick CE, Caccavo F Jr, Tisa LS. Electron transfer from Shewanella algae BrY to hydrous ferric oxide is mediated by cell-associated melanin. FEMS Microbiology Letters 2003;99:104.
6. Zhdanova NN, Vasilevskaya AA, Sadnovikov YuS, Artyshkova LA. The dynamics of micromycete complexes contaminated with soil radionuclides. Mikologia i Fitopatologiya 1990;24:504–512.
7. Mahmoud Y. A-G Uptake of radionuclides by some fungi. Mycobiology 2004;32:110–114.
8. Dadachova E, Bryan RA, Howell RC, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A. Radioprotective properties of melanin are a function of its chemical composition, free stable radical presence and spatial arrangement. Pigment Cell Melanoma Res. in press
9. Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One 2007;5:e457. [PubMed: 17520016]
10. Dadachova, E.; Casadevall, A. Melanin nanoshells for protection against radiation and electronic pulses. US Patent Application. # 60/819,992. PCT Patent Application PCT/US2005/035707
11. Pazo V, Revskaya E, Howell R, Nosanchuk JD, Casadevall A, Dadachova E. Melanized nanoparticles lodged in the bone marrow have protective effect against external gamma radiation. J. Nucl. Med 2006;47:220P.
12. Ito S, Fujita K. Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical degradation and liquid chromatography. Anal Biochem 1985;144:527–536. [PubMed: 3993914]
13. Boll RA, Malkemus D, Mirzadeh S. Production of actinium-225 for alpha particle mediated radioimmunotherapy. Appl Radiat Isot 2005;62:667–679. [PubMed: 15763472]

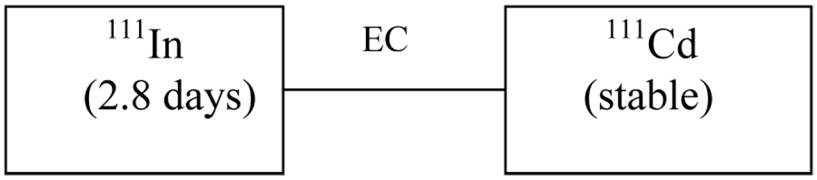
14. Wang Y, Aisen P, Casadevall A. Melanin, melanin "ghosts," and melanin composition in Cryptococcus neoformans. Infect Immun 1996;64:2420–2424. [PubMed: 8698461]

15. Alexander V. Design and synthesis of macrocyclic ligands and their complexes of lanthanides and actinides. Chem. Reviews 1995;95:273–342.

a)



b)



c)

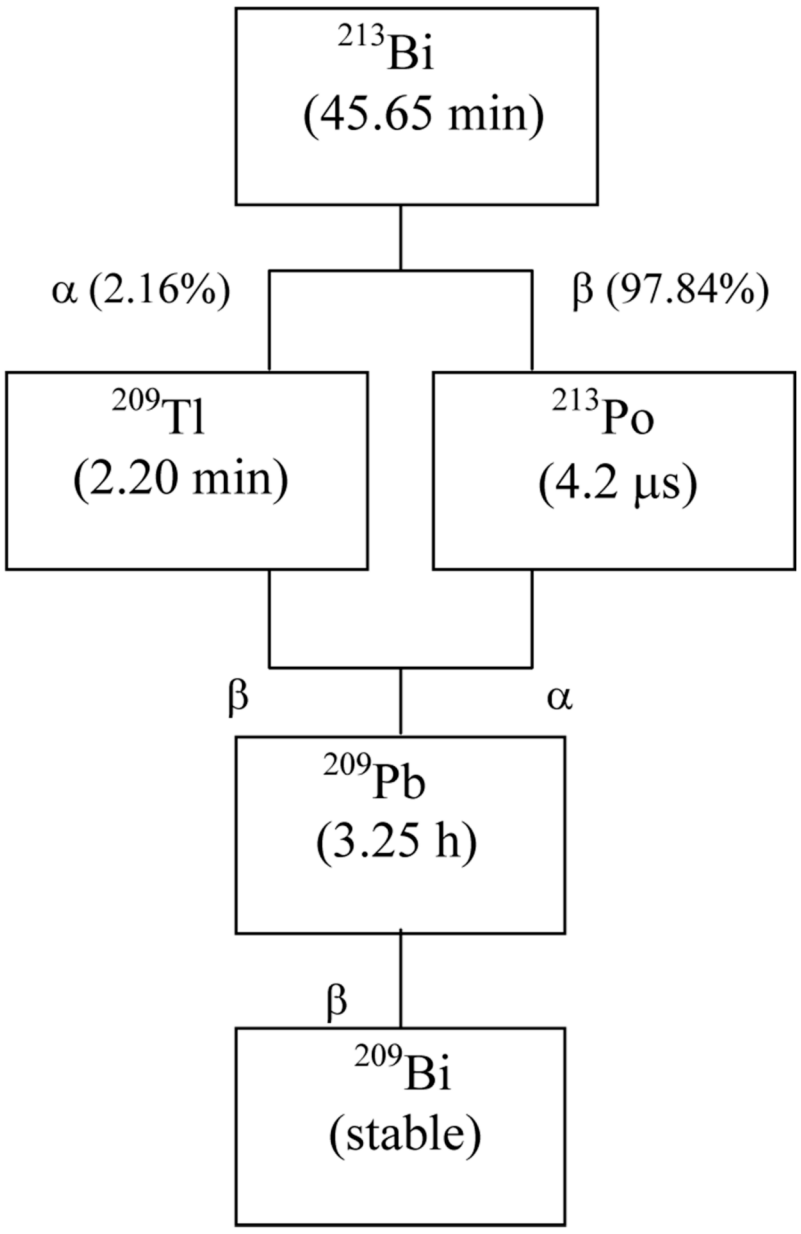


Fig. 1.
Decay schemes for ^{111}In, ^{213}Bi and ^{225}Ac. EC – electron capture.

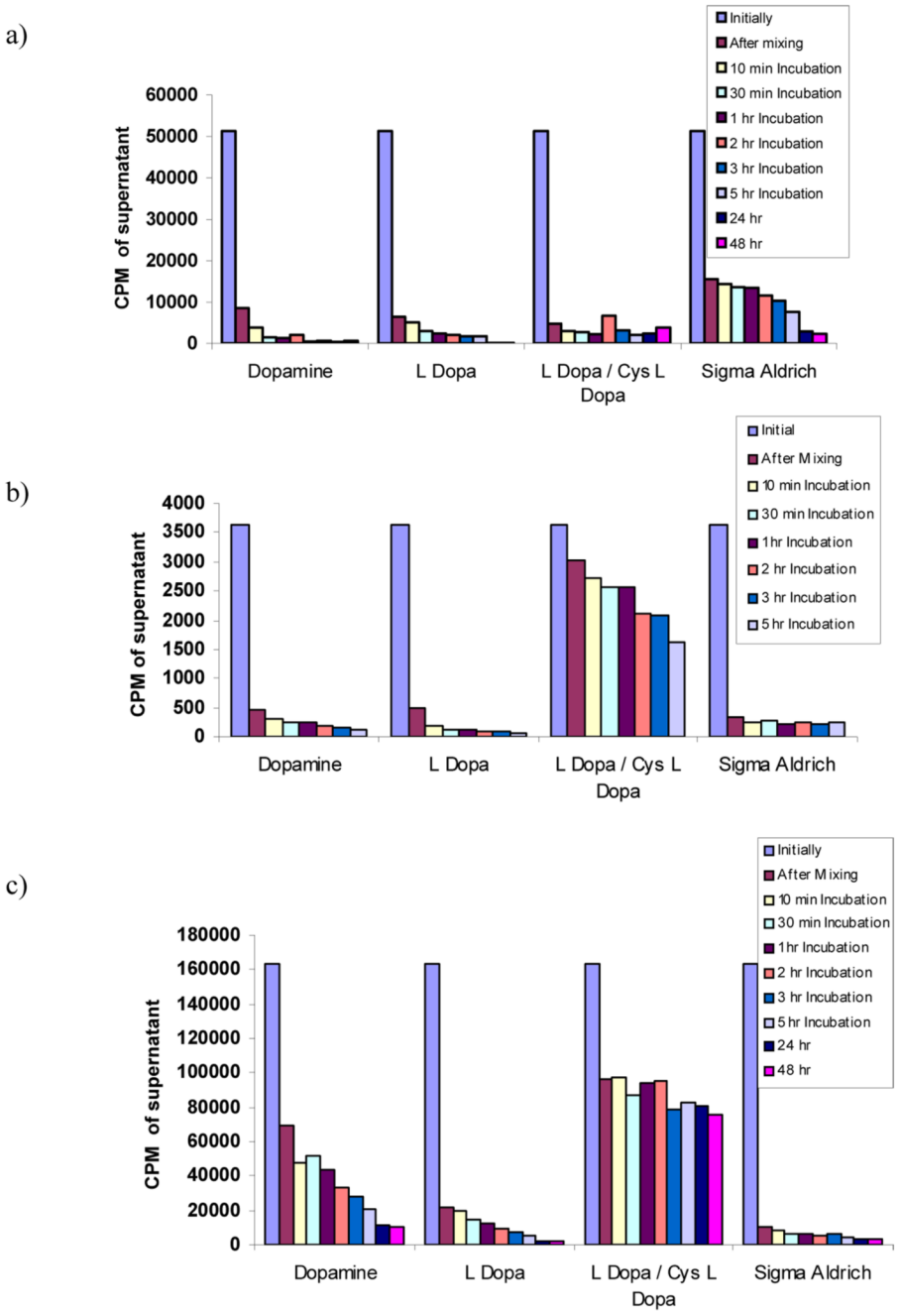


Fig. 2.

Chemosorption isoterms at 37°C for (a) ^{111}In, (b) ^{213}Bi and (c) ^{225}Ac on dopamine, L-Dopa, L-cysteine/L-dopa and Sigma (tyrosine) melanins. The counts per minute (CPM) of supernatants were normalized per mass of melanin in the samples.

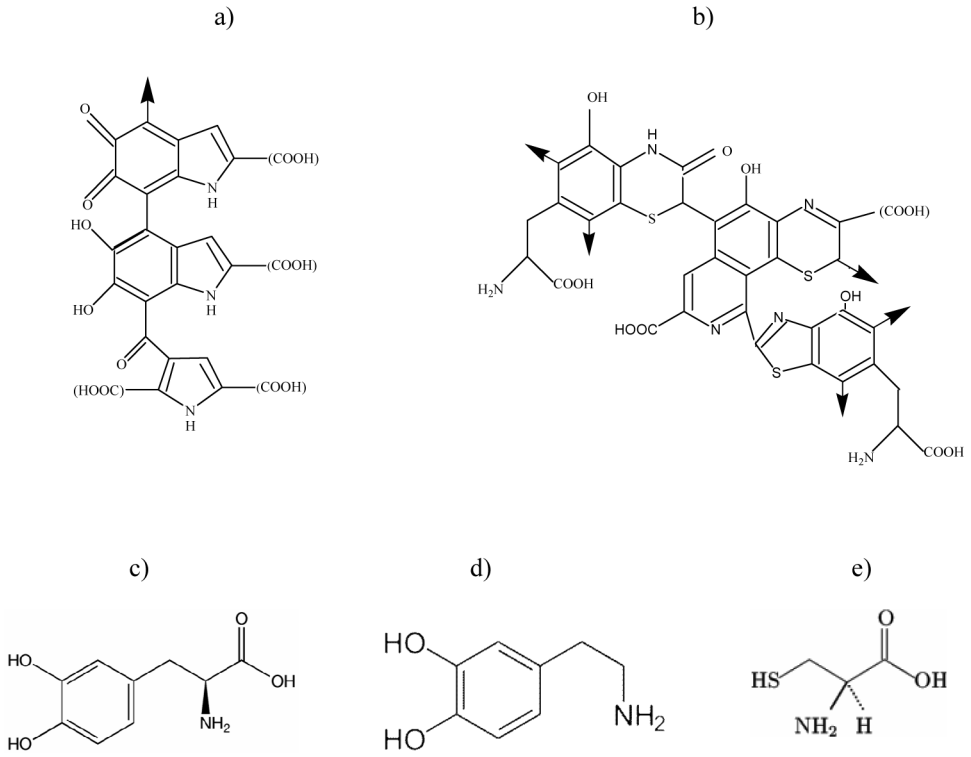


Fig. 3.
Chemical structures of melanin and its precursors: a) structure of eumelanin oligomer; b) structure of pheomelanin oligomer; c) L-Dopa; d) dopamine; e) L-tyrosine.

Table 1

Elemental composition of synthetic melanins

Precursors	Method of Oxidation	Percent Carbon	Percent Hydrogen	Percent Nitrogen	Percent Sulfur	C:N, C:N:S Molar Ratio
Dopamine	Tyrosinase	48.16	3.42	7.09	N/D	7.92:1.00
L-DOPA	Tyrosinase	52.05	3.87	7.24	N/D	8.39:1.00
L-Cysteine, L-DOPA	Tyrosinase	33.50	4.87	10.78	22.33	3.63:1.00:0.90
Tyrosine (Sigma)	H_2O_2	48.00	N/D	6.60	N/D	8.48:1.00

N/D – not done