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Binding of chemicals to melanins re-examined: Adsorption of some drugs to the surface of melanin particles

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Abstract

This work presents a first attempt to study the interaction of some drugs with melanins, realistically considered as solid aggregates of primary particles. This situation appears similar to the adsorption of organic molecules onto the surface of colloidal absorbers, as active carbon, zeolites or titanium dioxide. We have applied some of the most popular theoretical models used in technological applications with the aim to give a more realistic picture of the melanin–drug interaction responsible for some observed side effects in vivo. Moreover, this approach can simplify the problem of the search of the physical parameters dominating the binding processes, by reducing the phenomenon to a simple physisorption/chemisorption, at least in a first approximation.

We have studied the binding to melanin of gentamicin, methotrexate and chlorpromazine, molecules with different physico-chemical and structural characteristics. Our study demonstrates the possibility to fit experimental adsorption data with Langmuir, Freundlich, Tempkin and Dubinin–Radushkevich equations. In such a way we obtain binding parameters useful to characterize the drug–surface interaction in terms of energy and of mean affinity.

Keywords: Melanin; Drugs; Adsorption isotherms; Sorption kinetics; Surface interaction; Chemisorption

1. Introduction

The problem of the interaction of drugs with melanins, the natural brown/black pigments present in many different regions of the human and animal body, has attracted a considerable interest since 1962, the year of the publication of the first paper on the binding of phenothiazines to eye melanin [1]. Successively, a noticeable number of works devoted to this topic have appeared and many drugs were studied with the aim to determine the possible toxic effects that their accumulation could cause at the level of pigmented tissues and organs, with particular attention to oculotoxicity and ototoxicity.

Ings, in 1984 [2], reviewed first the biological experiments and the techniques adopted to reveal the localization of drugs in the various pigmented compartments of living organisms. More recently, another important review pointed out that "the binding of drugs to eye melanin is not predictive of ocular toxicity" [3]. This last paper contains a very large number of references, covering drugs of disparate and uncorrelated structure, demonstrating that the affinity of neutral and charged molecules for melanins does not follow a general chemical rule, but melanins behaves as efficient absorber thanks to their physical characteristics.

The data generated by these studies attracted the attention of some scientists interested in physical chemistry and structure of melanins. At those times (from the seventies to the nineties) the pigment was generally considered as a huge insoluble macromolecule whose reactivity could give valuable information about chemical structure. The Scatchard analysis was consequently the method of choice, even if rather poor information could be obtained in terms of number of binding sites and equilibrium constants.

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In more recent times, more sophisticated hypotheses on melanin structure have been proposed and widely accepted, in which the insolubility and the (photo)reactivity found a satisfactory explanation. The presently accepted model, valid for any type of melanin, is based on a hierarchical organization of basic molecules (mainly, but not only, 5,6-dihydroxyindole, indole-5,6-quinone, 5,6-dihydroxyindole-2-carboxylic acid) covalently bound to form the building blocks of protoparticles, further interacting in the form of irregular observable particles, displaying highly complicated surfaces with a large area [4–6].

The adsorption of neutral molecules on such solid aggregates can therefore be analysed by assuming that the binding is analogous to the adsorption on a solid surface, i.e. making use of the classical Langmuir isotherm [7]. This way was followed in the past by some authors [8] with remarkable results.

In the recent years, many new models and theories have been developed to analyse the processes of adsorption of gas and solutes on materials of technological interest and low cost as methods of intervention in environmental contamination or to recover wastes in industrial plants. The interest has been focused mainly on activated carbon, zeolites, colloidal metal oxides and chitosan.

The aim of the present work is to employ such models in a completely different framework in order to achieve results useful for understanding the biophysical behaviour of melanins. The main reason to perform this attempt lies in the surface structure of melanin particles suggesting some parallelisms with activated carbons. In fact, both the BET surface area and the micropore volumes of melanins and amorphous carbon are comparable [9] even if the physical and chemical processes leading to the adsorption can be in principle very different. Moreover, there are experimental indications that the melanin structure can be better described in terms of fractal geometry as in the case of some activated carbons.

The main criticism that can be done to this procedure (but this can be an objection to all the works employing adsorption technologies) is the lack of a strong theoretical justification to apply theories, valid for gas adsorption, to adsorption from solutions on solid surfaces. This is a debate still in course, but, in any case, a number of valid results obtained in the last years encourage such an approach.

2. Binding equations

Though structural features of different melanins differ depending on sources and methods of preparations, some indicative values of their surface characteristics are: the specific surface area varying from 18 m^2/g for synthetic pigments to 25 m^2/g for Sepia pigment and the micropore volume of about 0.01 cm^3/g [9].

Following the Brunauer classification [7], in the case of melanins the adsorption isotherms of drugs from solution are all of type I. Therefore, following the trace of a great number of technical papers, we have performed the analysis of the binding taking advantage of the isotherm equations type Langmuir, Freundlich, Tempkin and Dubinin–Radushkevich. In recent times some more sophisticated models have been added to the list of the classical theories such as those discussed in [10]. Our choice of these few models is due to the poor knowledge of the structure of the adsorber, the melanins, that doesn't encourage at this stage deeper and more quantitative studies and also to the widespread diffusion of these more popular equations.

It should be noticed that the point $(0,0)$, though not experimentally determined, has been included in our graphs. This apparently not rigorous procedure was adopted in order to add a further constraint in the fittings of the data.

Moreover, in the case of the drug gentamicin, we have tested some kinetic models of adsorption.

We will present here a short list of such equations with the information that can be obtained from the analysis of the data. All these equations can be linearized and some authors have used the linear form to better evaluate the parameters. Rigorous theoretical derivations can be found elsewhere [7,10].

2.1. The Langmuir isotherm

It has been widely used to describe many real sorption processes. A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites. Theoretically, a saturation value is reached beyond which no further sorption can take place. The curve is represented by the expression:

$$q = \frac{q_0 K C}{1 + K C}$$

where q is the amount adsorbed ($mmol \cdot g^{-1}$), q_0 and K are related to monolayer adsorption capacity and energy of adsorption, respectively, and C is the equilibrium solution concentration of solute. In the analysis of the results, q_0 is assumed as the total number of binding sites, N_t.

2.2. The Freundlich isotherm

It is often used for heterogeneous surface energy systems. In this equation the concentration of the solute at equilibrium is raised to the power $1/n$ and the semi-empirical expression can then be written:

$$q = q_0 (KC)^{1/n}$$

When the heterogeneity index $1/n < 1$, the adsorption rate decreases with solution concentration as the low-energy sites are occupied. Being all the concentrations expressed with the same units, the Freundlich constant K_F ($=q_0 K^{1/n}$) gives an estimate of the adsorption capacity.

2.3. The Tempkin isotherm

It was proposed with the aim to consider the effects of indirect adsorbate/adsorbate interactions on adsorption isotherms. The Tempkin isotherm has been used in the following form:

$$q = \frac{RT}{b}\ln(AC)$$

where A plays the role of equilibrium constant. In many cases, the equation may be useful mainly for fitting the middle region of the adsorption isotherm.

2.4. The Dubinin–Radushkevich isotherm

This equation has been used to describe adsorption of various substances, including metal ions, on different surfaces. It is generally written in the form:

$$q = q_m e^{-\beta\varepsilon^2}$$

where q_m is the adsorption capacity (in the original treatment, the micropore volume), β is a constant related to sorption energy and ε is the Polanyi potential [10]. The constant β represents the mean free energy E of sorption per molecule of the adsorbate transferred from infinity to the surface that can be calculated by the formula [11]:

$$E = \frac{1}{\sqrt{2\beta}}$$

2.5. Standard deviation

To evaluate the applicability of each isotherm equation, SD was calculated as follows:

$$SD = \sqrt{\frac{\sum (q_{exp} - q_{calc})^2}{N - p}}$$

where N is the number of data points and p is the number of parameters.

2.6. Kinetic studies

The time dependence of the adsorption of gentamicin was studied in order to determine the kind of process involved, such as mass transfer and chemical reactions, that can occur due to the presence of many different functional groups on melanin surfaces. Neglecting, in this stage of the work, mathematically complex models, we have performed the analysis with four relatively simple equations, i.e. [12]:

1.) pseudo-first-order kinetics:
$$\frac{dq_t}{dt} = k_1(q_e - q_t)$$
2.) pseudo-second-order kinetics:
$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$$
3.) intraparticle diffusion model. This is grounded on the rigorous solution of the diffusion equation and, in the case of spherical particles and for the initial tract of the adsorption process, assumes the simple form:
$$q_t = k_p t^{1/2}$$
where the intraparticle rate parameter k_p includes both the diffusion coefficient and geometrical factors [13].
4.) Elovich equation. Its use has been rather popular to study the chemisorption of gases on solid surfaces, but recently interesting results have been obtained also in the description of the sorption rate from solutions when the adsorption energy is supposed to vary with the extent of surface coverage and/or the adsorption sites are heterogeneous and therefore exhibit different activation energies for adsorption. It is normally written [14]:
$$\frac{dq_t}{dt} = a\exp(-bq_t).$$

In all kinetic equations, q_e and q_t (mmol/g) are the amount of drug adsorbed at the equilibrium and at the time t respectively. Integrated (linear) forms of these equations were used in the present work.

Further details on the meaning of these equations and on the information that can be drawn from the parameters will be discussed in the sections below.

3. Analysis of the binding

3.1. Gentamicin

Gentamicin is an aminoglycoside antibiotic with a wide spectrum of antibacterial activity. It is a complex of slightly different molecules (average M.W. 462; freely soluble in water; basic).

Ototoxicity is one of the recognized side effects of the drug and it has been attributed to complexation of the drug with the inner ear melanin. A detailed investigation of the biological mechanisms of such an effect has been performed by Wrześniok et al. [15] and we have based our present study on their adsorption experiments performed with synthetic melanin obtained by autooxidation of L-DOPA (L-dihydroxy-phenil-alanine). The preparations of the complexes with the drug were performed in solutions at pH 7 (0.067 M phosphate buffer).

Fig. 1 shows the fitting of the experimental data with Langmuir, Freundlich, Tempkin and Dubinin–Radushkevich equations. In Table 1 are the summarized values of the parameters calculated, together with the SD and the correlation coefficients. The first result emerging from the table is the good fitting obtained with the Freundlich equation and the very bad one obtained with the Dubinin–Radushkevich theory. It confirms that the surface of the granules is

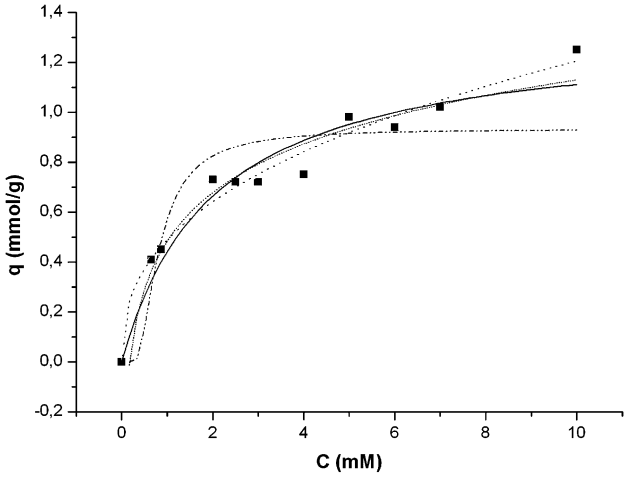

Fig. 1. Equilibrium isotherms for gentamicin adsorption on synthetic DOPA melanin. Fittings of experimental data with different models: —— Langmuir; – – – – Freundlich; ····· Tempkin; –···–··· Dubinin–Radushkevich.

energetically (and consequently structurally) heterogeneous, as pointed out also by the value of the heterogeneity index $1/n$, lying between 0 and 1. Though a comparison between our results and literature data is problematic, due to the unknown but obvious differences with other adsorbents as carbon [12], alunite [16] or ion-imprinted polymers [17,18], it is possible to infer that the adsorption capacity of melanin for gentamicin is significantly high.

Sorption kinetic analyses were accomplished on two series of data related to two different concentrations of gentamicin (1.0 and 5.0 mM) on the base of the unpublished experimental data kindly communicated to us by Dr. Jerzy Pałka of the Department of Pharmaceutical Chemistry, University Medical School of Silesia, Poland. In Fig. 2 the plots of the adsorption kinetics are displayed for the models described above and Table 2 summarizes the values of the fitting parameters, and the correlation coefficients R^2 generated by the linear regression programme. No reasonable fitting was possible by adopting the pseudo-second-order model. The best fit has been obtained by Elovich equation, namely by means of a theory that was successfully tested to describe the chemisorption of gas molecules onto various adsorbents and the adsorption of cadmium ions [19] and of dyes and phenols [12] on activated carbon. The increase of the drug initial concentration in the solution causes an increase of the constant a and a decrease of the constant b. This result has suggested a correlation of a to the rate of sorption and of b to the surface coverage, because the concentration increase reduces the available surface for the sorbates [19].

On the other hand, the values of R^2 for the fitting with pseudo-first-order model don't allow excluding such a mechanism whose advantage is to give values of the kinetic constants of straightforward interpretation.

Concerning the analysis with the intraparticle diffusion model, the external surface adsorption (stage 1) is not measurable, as shown in Fig. 2, as well as the final equilibrium adsorption (stage 3). The stage of the intraparticle diffusion control (stage 2) is attained at about 9 min from the start of the process and continues for more than 60 min. The drug is therefore slowly transported via intraparticle diffusion into the melanin granules and is finally retained in specific sites. The diffusion rate constant k_p is comparable with those calculated for adsorption on alunite [16].

3.2. Methotrexate

Methotrexate (MTX, aminomethylpteroylglutamic acid) is a member of the therapeutic class of antineoplastic

Table 1
Binding of gentamicin, methotrexate and chlorpromazine

	Parameters	Synthetic melanin + gentamicin	B16 melanosomes + methotrexate	B16 melanin + methotrexate	Synthetic melanin + methotrexate	Eye melanin + chlorpromazine
Langmuir	SD	0.078	1.81×10^{-3}	1.27×10^{-3}	8.64×10^{-4}	0.18
	R^2	0.946	0.963	0.995	0.997	0.984
	q_0 (mmol·g^{-1})	1.34	0.035	0.062	0.051	1.02
	K (dm^3·mmol^{-1})	0.49	4.80	10.18	20.47	0.33
Freundlich	SD	0.056	3.20×10^{-3}	4.49×10^{-3}	10.58×10^{-3}	0.55
	R^2	0.977	0.918	0.953	0.954	0.939
	$q_oK^{1/n}$ (dm^3·g^{-1})	0.49	0.038	0.071	0.061	0.26
	$1/n$	0.39	0.53	0.38	0.31	0.50
Tempkin	SD	0.101	2.95×10^{-3}	1.84×10^{-3}	1.19×10^{-3}	0.42
	R^2	0.926	0.904	0.983	0.991	0.977
	b	8.57	353.9	172.9	240.3	11.88
	A (dm^3·g^{-1})	5.63	65.93	92.15	244.57	4.21
Dubinin–Radushkevich	SD	0.112	1.47×10^{-3}	1.27×10^{-3}	1.06×10^{-3}	0.60
	R^2	0.664	0.963	0.991	0.997	0.955
	q_m (mmol·g^{-1})	0.97	0.031	0.060	0.052	0.71
	β (mmol^2·J^{-2})	0.24	0.030	0.020	0.013	0.45
	E (J·mmol^{-1})	1.443	4.082	5.0	6.202	1.054

Values of SD, R^2 and of the parameters in isotherm equations for the adsorption of gentamicin on synthetic DOPA melanin, of methotrexate on B16 melanosomes, on melanin extracted from B16 melanosomes and on synthetic DOPA melanin and of chlorpromazine on synthetic DOPA melanin.

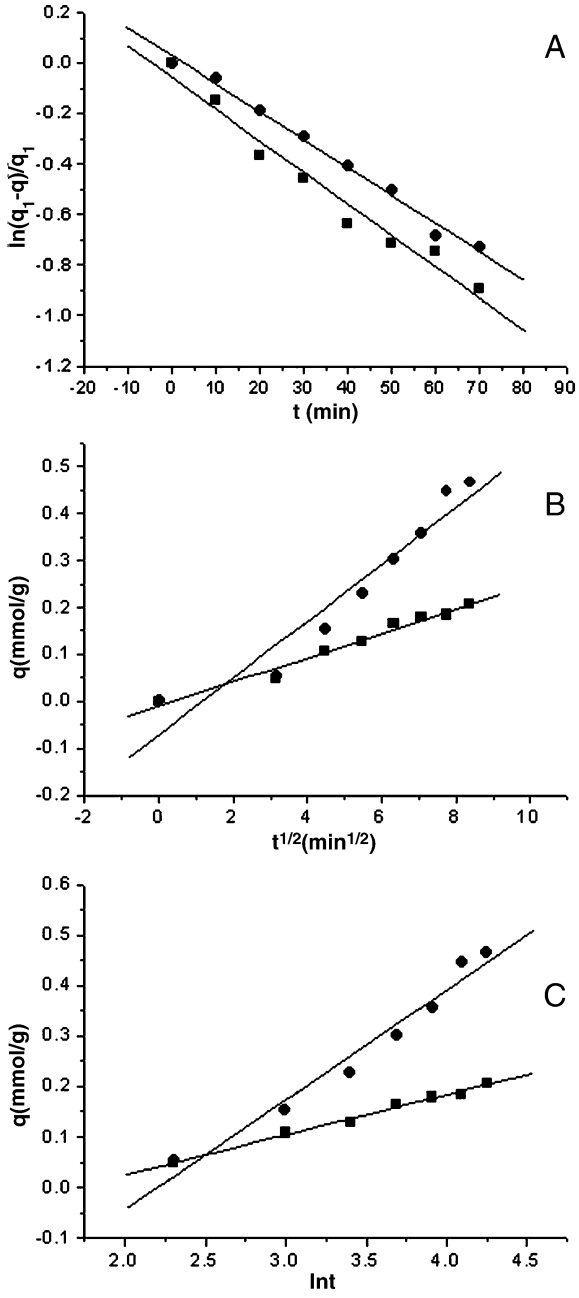

Fig. 2. Test of kinetic models for the adsorption on synthetic DOPA melanin of gentamicin at different concentration: ■ C=1 mM; ● C=5 mM. A: pseudo-first-order equation; B: intraparticle diffusion model; C: Elovich equation.

and antirheumatic drugs (M.W. 454.46; water solubility 0.045 mg/ml, pK_a=5.60). The behaviour of this molecule is described in a paper of Wilczok et al. [20] where

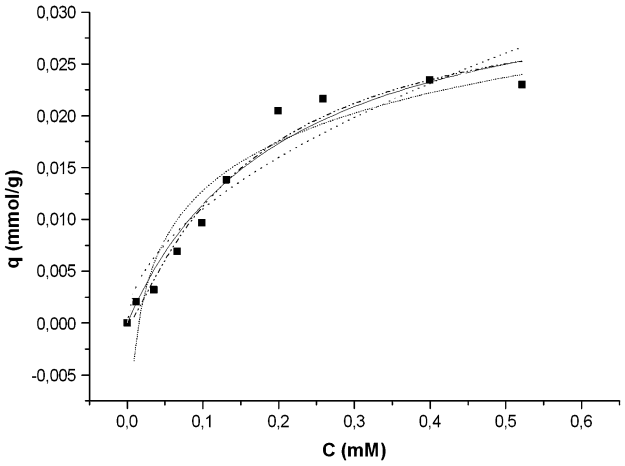

Fig. 3. Equilibrium isotherms for methotrexate adsorption on intact B16 melanosomes. Fittings of experimental data with different models: —— Langmuir; – – – – Freundlich; ····· Tempkin; –·–·– Dubinin–Radushkevich.

binding curves of MTX to intact B16 melanosomes, protein-free melanin from B16 melanosomes and synthetic L-DOPA melanin are reported and compared, together with the corresponding Scatchard analysis. In all cases, the binding was performed in 0.067 M phosphate buffer (pH 7). The adsorption isotherms show saturation values that are approximately double for melanins with respect to the entire melanosomes. Moreover, the Scatchard plot suggests the existence of a single class of independent binding sites for melanins and the possible presence of another class or of cooperativity for melanosomes.

Figs. 3–5 show the non-linear fittings of the experimental data with the above quoted theories and Table 1 summarizes the values of the calculated parameters, of the correlation coefficient and of the SD for the different adsorbers. The analysis of the results leads to the following observations:

- all the fittings executed on B16 melanosomes are not satisfactory. On the contrary, melanoma melanin and synthetic melanin follow rather well various models. This is not surprising considering the complex chemical structure of the melanosomes, including the presence of the melanosomal membrane and of structural and functional proteins;
- the SD and R^2 values suggest that Langmuir and Dubinin–Radushkevich are the equations most suitable to analyse the experimental isotherms for the two

Table 2
Gentamicin adsorption: kinetic models

Gentamicin concentration	1st order kinetic model		Intraparticle diffusion		Elovich equation		
C_0	k_1 (dm^3/min)	R^2	k_p (mmol/g·min$^{1/2}$)	R^2	a (mmol/g·min)	b (g/mmol)	R^2
1 mM	0.012	0.985	2.59×10^{-2}	0.988	1.5×10^{-2}	12.6	0.995
5 mM	0.011	0.996	6.06×10^{-2}	0.961	2.4×10^{-2}	4.6	0.982

Values of the calculated parameters for different kinetic models for two initial gentamicin concentrations.

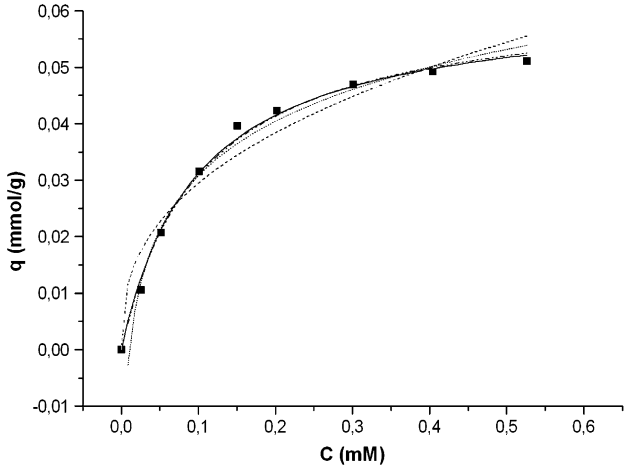

Fig. 4. Equilibrium isotherms for methotrexate adsorption on protein-free melanin from B16 melanosomes. Fittings of experimental data with different models: —— Langmuir; – – – – Freundlich; ····· Tempkin; –··–·· Dubinin–Radushkevich.

melanins. The same can be affirmed also for the melanosomes, despite the poor quality of the fittings;

- the constants K that are related to adsorption energy in the Langmuir theory indicate an increase of the adsorption energy for melanins with respect to melanosomes. A possible explanation could be an increase of the interaction potential due to a better correspondence between pore dimensions and adsorbate molecules.

In order to better investigate this last hypothesis, the binding of methotrexate has been studied through the application of fractal models, but, as also in the cases of gentamicin and chlorpromazine, no results have been obtained demonstrating the presence of surfaces with fractal properties that can be revealed by the application of isotherm models to the binding of such relatively large molecules (as the fractal version of the Frenkel–Halsey–Hill theory [21]).

Other noticeable results are: 1) the low value of the monolayer adsorption capacity for the melanosomes (q_0=0.035 mmol·g^{-1}), confirming that the presence of proteins and membranes reduces the binding capacity for the drug, 2) the concomitant reduced value of K_F with respect to those obtained with the two kinds of melanins, 3) the low values of the heterogeneity index $1/n$ suggesting the highly irregular geometry of the surface of the melanin particles, and, 4) thanks to the applicability of the Dubinin–Radushkevich theory, the mean free energy E of sorption per molecule of the adsorbate that can be evaluated, and the obtained values compared with those of other absorbing systems, showing smaller values but similar order of magnitude. As the term chemisorption is used in presence of adsorption energies comparable to the chemical bond energies, in our case we are in an energy range where chemical reactions can probably be heavily contributing to or predominating in the process.

3.3. Chlorpromazine

Chlorpromazine (M.W. 318.88; water solubility 0.4 g/ml; pK_a=9.30) is a phenothiazine antipsychotic drug that shows side effects at ophthalmologic and central nervous system levels, both attributed to some kind of interaction with the melanins in such compartments of the body. Many works have been devoted to this problem, starting with the above quoted study of Potts [1], who in a successive paper suggested the involvement of an electron-transfer process between the electron-acceptor melanin and the strong electron-donor chlorpromazine [22]. Moreover, chlorpromazine exists mainly as a mono-protonated cation at physiological pH, and the calculation of association constants by Scatchard analysis is indicative of van der Waals forces

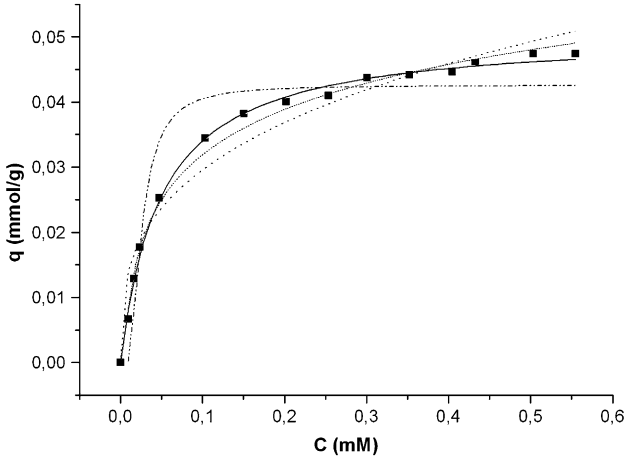

Fig. 5. Equilibrium isotherms for methotrexate adsorption on synthetic DOPA melanin. Fittings of experimental data with different models: —— Langmuir; – – – – Freundlich; ····· Tempkin; –··–·· Dubinin–Radushkevich.

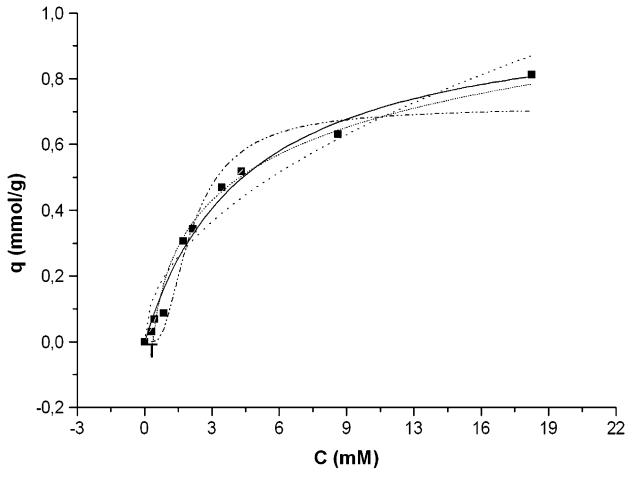

Fig. 6. Equilibrium isotherms for chlorpromazine adsorption on eye melanin. Fittings of experimental data with different models: —— Langmuir; – – – Freundlich; ····· Tempkin; –··–·· Dubinin–Radushkevich.

occurring between the aromatic rings in the drug and the aromatic indole-nuclei of melanin [23]. The data used by us were obtained in phosphate buffer at pH 6.

In Fig. 6 are reported the fitting curves of the experimental data related to melanin from beef eyes [22] with the various theoretical models, and Table 1 resumes the values of the calculated parameters. Also in this case the best fitting has been obtained with the Langmuir equation, but a reasonably good fit can be observed also with the Tempkin equation. On the contrary, the model of Dubinin–Radushkevich doesn't work well. Neither the calculated value of the binding sites number N_t nor the Langmuir and the Dubinin–Radushkevich adsorption capacities are indicative of a peculiar behaviour of chlorpromazine.

4. Discussion

Though the procedures adopted in this work are rigorously derived from the adsorption theories as developed in applied sciences, it is nevertheless useful to remember once again the starting problem and the nature of the biological system on which we have performed our calculations. The intrinsic and poorly understood complexity of melanins and the structural differences among natural and synthetic pigments have been discussed in various papers [5,6,24]. On the other hand, to limit the comparisons to synthetic pigments only doesn't guarantee we are speaking of a substance with uniform characteristics, due to the differences in the preparation procedures. Moreover, it is interesting that the binding of different drugs are best analysed by different models. This means that also the chemico-physical and/or the geometrical characteristics of the interacting molecules play a subtle but essential role in the mechanisms of interaction.

For the sake of clarity, we shall comment the results by points, following when possible the above sequence of results.

1. The amount of gentamicin bound to synthetic melanin is the largest among the three drugs studied. Moreover the relative adsorption isotherm is best fitted by the Freundlich equation. In the present case, the value of the heterogeneity index $1/n$ is typical of highly heterogeneous surfaces and the constant K_F , related to sorption capacity, is only about two orders of magnitude smaller as compared with literature values for powerful technical adsorbers, specifically activated carbon.
2. Methotrexate sorption curve is best fitted by Langmuir and Dubinin–Radushkevich equations, so it is worthwhile to discuss only the parameters evaluated by these models. The first observation concerns the values of the constants K, greater than that obtained for the other drugs. It is correlated to the absorption energy. The Langmuir theory assumes that sorption takes place at specific homogeneous sites. This assumption suggests that methotrexate strongly interacts only with a specific chemical configuration on the melanin surface and this could explain the relatively low value of the theoretical monolayer saturation capacity q_0. This hypothesis agrees with the conclusions derived from Scatchard plots and with the amount of bound methotrexate determined by Wilczok et al. [20].
3. Similar inferences can be put forward when considering the parameters of the fitting with the Dubinin–Radushkevich equation. Besides a strict concordance regarding the parameters q_m and q_0, high values of the mean free energy E seem to support such an interpretation. The lower values calculated for melanosomes reflect the presence, in significative percentage, of other components of the organelle, not interacting with the drug.
4. Chlorpromazine has been often considered as emblematic between the drugs that, quantitatively bound to melanins, are responsible of toxic side-effects. However, our study doesn't show any peculiar behaviour regarding this binding. Langmuir and Tempkin equations fit the experiments better than the others. Monolayer adsorption capacity and adsorption energy are comparable with those calculated for gentamicin, while the validity of the Tempkin theory, based on chemisorption on energetically heterogeneous surfaces [25], introduces the possibility of a lateral (repulsive) interaction [26]. The parameters b, correlated to the heat of adsorption, and A, the adsorption equilibrium constant, can only be compared with very different systems. For example, the interaction of proteins with metal ions [27] leads to A values of the same order of magnitude, and the same happens for the adsorption of Acid Red 114 by activated carbon, a chemical system somewhat more similar to ours [28].
5. The analysis of the adsorption kinetics, though limited to gentamicin, allows gaining some deeper insight to the binding mechanism. As shown in Table 2, the equation of Elovich appears as the more suitable model to describe the process, which therefore can be considered as a chemisorption process by active sites with different activation energies. The pseudo-first-order kinetics seems to be another good model. On the other hand, also the intraparticle diffusion model appears to be operating. When the rate of adsorption is controlled by a diffusion mechanism, the calculation of the effective diffusion coefficient for particle adsorption (assuming as valid the 2nd Fick's law) can be performed through the Vermeulen approximation [29] in which the fractional approach to the equilibrium $F(t)$ can be written as:

$$F(t) = \left[1 - \exp(-Dt\pi^2/r_0^2)\right]$$

Putting $F(t)=0.5$, the half time for adsorption is given by:

$$t_{50} = 0.030 r_0^2 / D$$

where r_0 is the radius of the particles assuming spherical geometry and D is the effective particle phase diffusivity. Assuming for melanin aggregates (the more probable form of the particles [24]) an average radius of $100 \div 200$ μm, the effective diffusivity for gentamicin can be evaluated as about 1.8×10^{-13} m^2 s^{-1}, to compare with figures calculated for resorcinol and catechol on activated carbon. Analogous considerations can be done for the values of k_p, quite reasonable and once more confirming the high adsorption capacity of melanins (see [16]).

In conclusion, our work has put in evidence some general points. Firstly, the analysis of the binding of drugs to melanins can be usefully performed in the more realistic picture considering particles with a more or less complex surface able to adsorb organic molecules, through a physisorption or a chemisorption process. The adsorption isotherms allow classifying such surfaces as microporous (pores with a diameter not larger than 2 μm). Secondarily, almost all the calculated parameters have values comparable with classical absorbers as activated carbon, namely melanins behaves as good drug-binding substances. A further observation concerns the true physical situation involving the presence of water, whose molecules easily and strongly bind to melanins [30]. This means that the adsorption of drugs takes place on hydrated particles, i.e. the real surface seen by the sorbate has been subjected to a possible "smoothing effect" by water molecules.

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