

## Adsorption of Flavonoids on Resins: Cyanidin 3-Glucoside

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Adsorption of cyanidin-3-glucoside in aqueous batch solutions was studied at pH 3.5 and 20 °C using 13 commercial resins with different hydrophobicity, surface areas ( $S_A$ , 330–1200 m<sup>2</sup>/g), and pore radii ( $P_R$ , 20–260 Å). The solute affinity toward different resins was described in terms of Langmuir and Freundlich parameters; experimental data were well-fitted to the two isotherms, which were both utilized to compare resins adsorption capacity. The styrene–divinylbenzene EXA-118 resin ( $S_A$ , 1200 m<sup>2</sup>/g;  $P_R$ , 90 Å) showed the maximum effectiveness among the tested resins; a good efficacy for removing cyanidin 3-glucoside was shown also by EXA-90 ( $S_A$ , 630 m<sup>2</sup>/g;  $P_R$ , 105 Å). Some experiments on a styrene–divinylbenzene resin and an acrylic one demonstrated that adsorption was not influenced by pH variations of the solution within the range of 1.0–4.5. For the 10 resins having  $P_R \leq 105$  Å, correlation analysis evidenced the linear increasing dependence of Freundlich constant  $K_F$  on physical characteristics of surface area and pore radius. The adsorption of cyanidin 3-glucoside at 20 °C was compared with that of hesperidin performed in batch runs on the same resins. Moreover, the best resin (EXA-118) was tested with a sample of pigmented orange juice to assess its performance in terms of selectivity, adsorption, and desorption capabilities on a real matrix.

**KEYWORDS:** Acrylic resins; adsorption; anthocyanins; Freundlich isotherm; Langmuir isotherm; styrene–divinylbenzene resins

### INTRODUCTION

Anthocyanins, glycosylated polyhydroxy/methoxy derivatives of 2-phenyl benzopyrilium (flavilium) salts, are responsible for red, blue, and purple colors of many fruits, vegetables, flowers, and other plant tissues (1). Blood orange juices (Cv. Tarocco, Moro, and Sanguinello) contain several 3-glucosides of the most common anthocyanidins; among them, cyanidin 3- $\beta$ -*O*-glucoside and cyanidin 3-(6''-malonyl)- $\beta$ -*O*-glucoside predominate (2). The antioxidative properties of anthocyanins (3–5) may play a role in preventing or reducing the risk of coronary heart disease (6); because of its biological activities and its absence of toxicity, anthocyanins-based products are widely used by patients suffering, for instance, from diabetes (7) and hypertensive retinopathy (8). Because of the worldwide tendency toward the use of natural pigments to replace synthetic colorants, anthocyanins have received growing importance as European food colorants (E<sub>163</sub>). Because of the applications of anthocyanins as nutraceuticals in food and pharmaceutical preparations, a great deal of product is needed to fulfill the requests. Di Mauro et al. (9) have described a routine procedure for recovering anthocyanins from citrus processing byproducts by concentration on resins, to take aim at highlight products from the residues and balance waste disposal costs. The adsorbent resins are durable nonpolar (styrene–divinylbenzene) or slightly hydrophilic (acrylic) poly-

mers having a high adsorption capacity with possible recovery of the adsorbed molecules, relative low cost, and easy regeneration. Because of their particular physical properties, adsorbent resins are employed by the pharmaceutical industries for the purification of amino acids and peptides (10), antibiotics (11), vitamins (12), prostaglandins (13) and to remove and recover phenolic compounds from products and byproducts of food processing plants (14–20). Adsorbent resins are commonly used also in anthocyanins concentration from diluted extracts for preparative scale isolation and cleanup (21–23). With regard to regulations, these resins are approved for food contact use by the European or Food and Drug Administration code of regulation that provides extractive limitations and general requirements for use. These polymers may be used for the removal of organic substances from aqueous food under prescribed conditions of preparation and should be pretreated overnight with alcohol and water according to the manufacturer's recommendation and good manufacturing practice to guarantee activation and food grade purity at time of use.

Despite the fact that downstream products in food technology commonly pass through purification steps based on adsorption, there is a lack of experimental data for many adsorbate/adsorbent systems that makes empirical the sorption process. The driving force of this process, in which dissolved organic compounds interact with the sorbent, is the distribution of the solute molecules between the sorbent and the aqueous phase. These

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are usually weak hydrophobic interactions due to van der Waals forces, although stronger interactions can sometimes occur, e.g., dipole–dipole and hydrogen bonding. Uptake capacity and sorption kinetic are mainly dependent on the external physico-chemical parameters such as pH, temperature, competing compounds present in solution, and on the resin chemical structure (24) or other characteristics such as particle size, porosity, specific surface area, and pore volume distribution (18). The availability of kinetic and equilibrium data and reliable mathematical isotherm expressions describing experimental systems would help to develop more rational and systematic methods to approach the adsorption process; these very specific curves can be interpreted to obtain information concerning the adsorption mechanism strictly connected with interactions between adsorbent and adsorbate molecules, and they give the opportunity to assess the efficiency of industrial adsorbents applied in separation, purification, and other processes.

In the present paper, we have studied equilibrium and kinetic data of adsorption of cyanidin 3-glucoside chloride from batch solutions at various pH values on 13 commercial resins differing in the chemical and physical properties already tested for the adsorption of standard hesperidin (the dominant flavanone in orange fruit) (25). To compare their adsorption capacity, the experimental equilibrium data at 20 °C and pH 3.5 were fitted to the Langmuir and Freundlich isotherms with the aim to improve the sorption process and to predict the resin performances. Moreover, the adsorption constants  $K_F$  were rationalized in terms of two parameter equations, including surface area ( $S_A$ ) and pore radius ( $P_R$ ) as independent variables. Because of the copresence of anthocyanins and hesperidin in pigmented orange juices, a comparison between the two adsorption systems was done. Moreover, an anthocyanins concentration from pigmented orange juice was carried out in a column filled with the best resin.

## EQUILIBRIUM MODELS

The adsorption capacity of a resin for a particular solute is a very important parameter when deciding on a separation chromatography step for a purification process. One of the bases for the adsorption process is the distribution of a solute between two phases, the solid and the liquid ones. Sorption equilibrium is established when the concentration of sorbate in the bulk solution is in dynamic balance with that on the interface. The adsorption of solute on solid particles has been extensively studied, and the overall mechanism can be described as the succession of the following steps: (i) solute transport from the bulk solution to the boundary film; (ii) solute transfer from the boundary film to the surface of the adsorbent; (iii) diffusion from the surface to the intraparticle active sites; and (iv) uptake on the active sites, sorption, or precipitation. As agitation speed is maintained at a sufficient rate to avoid a heterogeneous distribution of solute and sorbent in the solution, the first step in the adsorption process can also be disregarded. It is well-known that the fourth step occurs rapidly, so it is also negligible in the overall control of sorption kinetics. External and intraparticle diffusions are seen as the main controlling phenomena. Equilibrium sorption studies provide the capacity of the sorbent, which can be described by a sorption isotherm. An isotherm can be defined as the equilibrium relationship between sorbent and sorbate at a given temperature, and it is characterized by certain constants whose values express the surface properties and the affinity of the sorbent.

The adsorption isotherm data can be determined in batch experiments, isolated from the chromatographic and pseudo-

equilibrium effects encountered in column studies, and can be used to predict the dynamic adsorption behavior of the adsorbent in chromatography process (26). In the present paper, the data are calculated and analyzed in accordance with some of the most frequently used isotherms; these simple isotherms have been widely applied since they give a good description of experimental behavior in a large range of operating conditions and are characterized by a limited number of adjustable variables.

The Langmuir sorption isotherm (27) is the best known and the most often used isotherm for the sorption of a solute from a solution. This model assumes monolayer sorption with a homogeneous distribution of sorption energies and without mutual interaction between adsorbed molecules. The Langmuir isotherm model can be expressed as:

$$q_s = \frac{Q_m a_L C_s}{1 + a_L C_s} = \frac{K_L C_s}{1 + a_L C_s}$$

where  $q_s$  (mg/g) is the adsorbate concentration per unit of weight of adsorbent (solid phase),  $C_s$  (mg/L) is the concentration of adsorbate in solution (liquid phase) at equilibrium, and  $a_L$  (L/mg) and  $Q_m$  (mg/g) are the Langmuir constants;  $a_L$  is the ratio between sorption and desorption rate constants and  $Q_m$  is the maximum sorption capacity corresponding to the complete monolayer coverage. The above equation can be rearranged to the following linear form:

$$\frac{1}{q_s} = \frac{1}{K_L C_s} + \frac{1}{Q_m}$$

in which  $K_L$  and  $Q_m$  can be evaluated from the slope and the intercept by plotting  $1/q_s$  against  $1/C_s$ .

The Freundlich model (28) assumes a heterogeneous distribution among the sorption sites at different energies. It is a two parameter model widely employed for many different adsorbate/adsorbent systems for liquid and gas phases adsorption. The mathematical form of this model is

$$q_s = K_F C_s^{b_F}$$

where  $q_s$  (mg/g) is the sorbate concentration inside the resin sites in equilibrium with  $C_s$  (mg/L) and  $K_F$  (L/g) and  $b_F$  (dimensionless) are temperature-dependent parameters for each adsorption system.  $K_F$  and  $b_F$  indicate the capacity and the intensity of the adsorption process, respectively. In particular,  $b_F$  represents the effect of residual concentration on adsorption capacity. This equation is usually applied in the linear form:

$$\log q_s = \log K_F + b_F \log C_s$$

Plotting the equation on a log–log scale,  $K_F$  and  $b_F$  can be calculated from the intercept and the slope. The Freundlich isotherm was first conceived as an empirical model, although it can be derived from the assumption that the surface is composed of “patches”, following an exponential decay energy distribution with a Langmuir type isotherm behavior on each one. The Freundlich model gives more accurate results than the Langmuir isotherm for a wide variety of heterogeneous adsorption systems, but it can describe adsorption data over a restricted range only because it does not converge to Henry’s law at low surface coverage. Therefore, it fails to describe equilibria as  $q_s \rightarrow 0$  and is thermodynamically inconsistent.

## MATERIALS AND METHODS

Cyanidin 3-glucoside chloride (purity > 99%) and hesperidin (purity 97%) standard used for batch experiments were purchased from Extrasynthèse (Genay, France) and Sigma Aldrich (Milan, Italy),

Table 1. Chemical and Physical Properties of Resins Reported by Manufacturers

trade name	structure	surface area (m <sup>2</sup> /g)	pore radius (Å)	porosity (mL/g)	particle size (mm)	density (g/mL)	code of regulations
XAD-2	SDVB	330	45	0.65	0.25–0.84	1.08	a
XAD-7	Acrylic	450	45	1.14	0.25–0.84	1.24	a
EXA-31	Methacrylic	470	170	1.20	0.35	1.09	b
EXA-117	SDVB	570	80	1.10	0.25		b
XAD-1180	SDVB	600	150	1.68	0.25–0.84	1.04	a
EXA-32	SDVB	600	260	1.30	0.25	1.01	b
EXA-90	SDVB	630	105	1.30	0.25	1.18	b
SP-70	SDVB	700	65	1.10	>0.25	1.01	a
XAD-4	SDVB	725	20	0.98	0.25–0.84	1.02	a
XAD-16	SDVB	800	50	1.82	0.25–0.84	1.08	a
EXA-45	SDVB	1000	38	1.20	0.25	1.01	b
EXA-50	SDVB	1000	57	1.40	0.25	1.01	b
EXA-118	SDVB	1200	90	2.30	0.25	1.04	b

<sup>a</sup> Council of Europe, Resolution AP (97) 1 on Ion Exchange and Adsorbent Resins used in the Processing of Foodstuffs, adopted by the Committee of Ministers on 30 September 1997. <sup>b</sup> U.S. Food and Drug Administration, Code of Federal Regulation Title 21—Food and Drugs—Revised as of April 1, 2002. Part.173.65: divinylbenzene copolymers. Part 173.5: acrylic resins.

respectively. Adsorption experiments were carried out using citric acid/potassium citrate monohydrate buffer at pH 3.5 (Sigma Aldrich); the solute concentration was measured after dilution with HCl/KCl buffer at pH 1.0 prepared with analytical grade hydrochloride (Merck, Milan, Italy) and potassium chloride (Panreac Quimica, Barcelona, Spain). Ethanol (96%) for resins activation was by Panreac Quimica. Acetonitrile, acetic acid, and methanol were from Sigma Aldrich.

Commercial food grade resins Sepabeads SP-70, Relite EXA-31, EXA-32, EXA-45, EXA-50, EXA-90, EXA-117, and EXA-118 were kindly supplied by Resindion, Mitsubishi Chem. Co., Milan, Italy; Amberlite XAD-2, XAD-4, XAD-7, XAD-16, and XAD-1180 were purchased from Sigma Aldrich. EXA-31 and XAD-7 are methacrylic and acrylic polymers, respectively; all other resins are macroporous styrene–divinylbenzene (SDVB) copolymers, with no functional groups. The resins are characterized by a wide range of surface areas (330–1200 m<sup>2</sup>/g), pore radii (20–260 Å), and porosities (0.65–2.30 mL/g). According to the manufacturers, chemical and physical properties of resins, together with the European or FDA code of regulation, are shown in Table 1.

**Kinetics of Adsorption.** All adsorption experiments were carried out utilizing weighed quantities of resins based on the dry weight of each commercial lot. Such a quantity was previously determined by drying the beads at 70 °C up to a constant weight in an electric oven for over 24 h and putting it in a desiccator to cool. Because of their hydrophobic properties, the resins contain essentially no water; they can be wetted for use in an aqueous system by initial contact with a water soluble alcohol. Before they were used, glass columns (120 mm × 20 mm) were packed with weighed quantities of resins, which were soaked overnight in 96% ethanol and then washed with 5 BV of distiller water.

Experiments leading to determination of the adsorption equilibrium isotherms were conducted in batches in 250 mL glass flasks, where the cyanidin 3-glucoside chloride was put in contact with the corresponding resin. The aqueous phase was prepared by dissolving 2.6 ± 0.3 mg of cyanidin 3-glucoside chloride in 100 mL of buffer solution at pH 3.5. A fixed amount of activated resins (0.05–0.4 g of dry weight) and the anthocyanin solutions were stirred at 300 rpm for 200 min using a thermostated bath (20 °C). Preliminary runs showed that the adsorption equilibrium was achieved after 180 min of contact time for all tested resins. Small aliquots were withdrawn at regular intervals and diluted in buffer solution at pH 1.0, and the residual concentration of anthocyanin in the solution was quantitated by UV/vis spectrophotometry at 510 nm (Shimadzu UV 2401) using a calibration curve. The equilibrium between colored and colorless forms of anthocyanins depends on hydrogen ion concentration, shifting to the red flavilium cation in highly acidic medium; therefore, absorbance must be measured in a pH 1.0 solution where anthocyanin is present only as a flavilium salt to detect latent red color stored in the colorless chromenol. The resin phase concentration of cyanidin 3-glucoside chloride was estimated

by the following mass balance equation:

$$q_t = \frac{(C_0 - C_t)V}{m}$$

where  $q_t$  (mg/g) is the concentration of anthocyanin on the adsorbed phase at time  $t$ , expressed as the amount of solute per weight unit of resin (dry basis),  $C_0$  and  $C_t$  are the initial and  $t$  time liquid phase concentrations (mg/L),  $V$  is the volume of the solution (L), and  $m$  is the weight (g) of dry resin.  $q_t$  and  $C_t$  values at equilibrium are the  $q_s$  and  $C_s$  ones used in the Langmuir and Freundlich isotherms.

The kinetic and equilibrium data of cyanidin 3-glucoside chloride on some resins were determined varying the pH value of the solution from 1.0 to 4.5 at 20 °C; a citric acid/potassium citrate buffer was used for the runs at pH 3.5 and 4.5, while for the buffer at lower pH values the pH 3.5 buffer was corrected with 6 N HCl. Control samples were employed to monitor any change in the initial concentration values, to exclude effects on measured absorbance. The equilibrium batch runs at every pH value were conducted at least in duplicate.

In addition, simultaneous adsorption of cyanidin 3-glucoside and hesperidin at 20 °C on XAD-4 was carried out utilizing the same initial concentration of the two flavonoids ( $5.36 \times 10^{-5} \pm 8.35 \times 10^{-7}$  M) and different resin amounts from 1 to 2.5 g/L. The equilibrium concentrations of the two compounds were determined by UV/vis spectrophotometry. Because in aqueous solutions cyanidin 3-glucoside has a characteristic absorbance at 510 nm and hesperidin does not absorb at this wavelength but otherwise both substances absorb at 283 nm ( $\lambda_{\max}$  hesperidin), the following equations are suitable for determining the concentrations:

$$\begin{cases} C_{\text{cyan}} = \frac{\text{Abs}_{510}}{\epsilon_{510\text{cyan}}} \\ C_{\text{hesp}} = \frac{\text{Abs}_{283} - C_{\text{cyan}}\epsilon_{283\text{cyan}}}{\epsilon_{283\text{hesp}}} \end{cases}$$

$C_{\text{cyan}}$  and  $C_{\text{hesp}}$  are the anthocyanin and the flavanone concentrations,  $\text{Abs}_{283}$  and  $\text{Abs}_{510}$  are the absorbance at 283 and 510 nm, respectively.  $\epsilon_{283\text{cyan}}$  and  $\epsilon_{510\text{cyan}}$  are the slopes of the calibration curves of cyanidin 3-glucoside at 283 and 510 nm, and  $\epsilon_{283\text{hesp}}$  is the slope of the calibration curve of hesperidin at 283 nm. The relative amount of solute adsorbed on the resin at equilibrium was calculated from the mass balance equation.

**Statistical Analyses.** To quantify the agreement between the experimental observations and the model predictions ( $q_s$ ) of the studied system, three different statistical error formulas were used (29) as follows:

$$\text{MSR (mean square of residuals)} = \frac{\sum [(q_s)_{\text{predicted}} - (q_s)_{\text{experimental}}]^2}{N}$$



$$E_i \text{ (square normalized error)} = \sum_{j=1}^N \left[ \frac{(q_s)_{\text{experimental}} - (q_s)_{\text{predicted}}}{(q_s)_{\text{experimental}}} \right]^2$$

$$\text{RPE\% (relative percentage error)} = \frac{\sum [(q_s)_{\text{predicted}} - (q_s)_{\text{experimental}} / (q_s)_{\text{experimental}}] \times 100}{N}$$

where  $N$  is the number of observations.

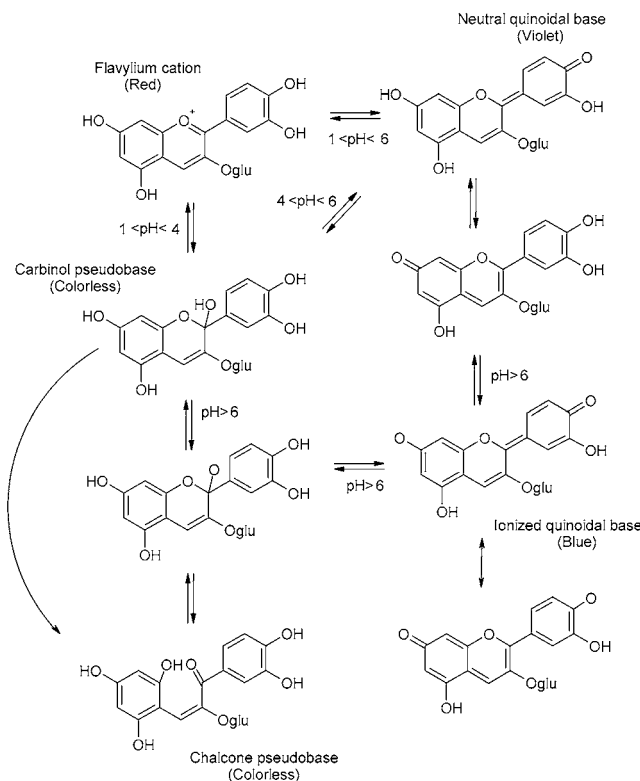
Simple and multiple regressions of  $K_F$  values (the dependent variable) against the  $S_A$  or/and  $P_R$  values (the independent variables) were performed using "Statgraphic Plus" software for Windows 98 (Manugistic Inc., Rockville, MD).

**Concentration of Anthocyanins in Column.** A sample of pigmented orange juice (cv. Moro) was obtained in our laboratory using a domestic squeezer. The juice was centrifuged at 13 000 rpm for 20 min at 4 °C (Braun Biotech GMBH DR 15, Melsungen, Germany), and then, the supernatant was filtered through a Whatman No. 1 filter paper by vacuum suction using a Buchner funnel. The juice (5.1 L) was loaded onto a glass column (length, 52.5 cm; i.d., 3.5 cm), filled with 200 mL of activated EXA-118 resin, with an outflow of 10 mL/min. The saturated resin was washed with distilled water (5 BV) to remove the sugars and other water soluble compounds, and anthocyanins were desorbed using methanol (2 BV). The collected fraction was concentrated until a final volume of 100 mL. The juice and the concentrate were characterized by the measurements of anthocyanin and hesperidin contents, performed using spectrophotometry and high-performance liquid chromatography (15, 16, 30).

## RESULTS AND DISCUSSION

**Effect of pH.** The equilibrium between colored and colorless forms of anthocyanins depends on the pH (31). The prevalent form of anthocyanins at pH values <2.0 is the polar red flavylium cation, which undergoes various structural transformations with increasing pH values (Figure 1). Solvation of the flavylium salt in a slightly acidic or neutral aqueous solution results in the immediate formation of the neutral and/or ionized quinonoidal bases. Moreover, at pH values ranging from 4 to 6, the common 3-glycosides and 3,5-diglycosides change rapidly to the more stable colorless carbinol for complete hydration of the 2-position of the flavylium cation. This in turn can equilibrate, at a slower rate, to an open form, the chalcone pseudobase, which is also colorless. Because of the different polarity of equilibrium forms, some experiments of adsorption of cyanidin 3-glucoside at 20 °C were performed at six different pH values ranging from 1.0 to 4.5; the SDVB EXA-90 (1.5 g/L) and the methacrylic EXA-31 (2.0 g/L) polymers were tested, and the initial concentration of cyanidin 3-glucoside was  $27.7 \pm 1.4$  mg/L. Because of the presence of polar carboxylic ester groups, slightly hydrophilic acrylic resins were suggested from the manufacturers for recovering anthocyanins instead of strongly hydrophobic SDVB ones, characterized by a hydrocarbon structure. The equilibrium data summarized in Table 2 show that the mean value for adsorbed cyanidin 3-glucoside at equilibrium is 14.77 mg/g ( $\pm 1.04$ ) for EXA-90 and 6.13 mg/g ( $\pm 0.43$ ) for EXA-31. Kinetics appear to be superimposable for both resins, and equilibrium data show no significant effect on adsorption on changing pH, demonstrating that adsorption does not depend on the different equilibrium forms of cyanidin 3-glucoside neither for the partially hydrophilic resin.

**Application of the Langmuir and Freundlich Adsorption Isotherms.** The adsorption capacity of the 13 commercial resins was compared in terms of Langmuir and Freundlich parameters obtained from the linearized equations using  $q_s$  and  $C_s$  values at 20 °C and pH 3.5. In Table 3 are listed the  $Q_m$  (maximum adsorption capacity) and  $a_L$  (adsorption energy) values obtained



**Figure 1.** Structural transformations of cyanidin 3-glucoside in aqueous solution at varying pH.

**Table 2.** Adsorption of Cyanidin 3-Glucoside<sup>a</sup> on EXA-90 and EXA-31 Resins at Various pH and 20 °C

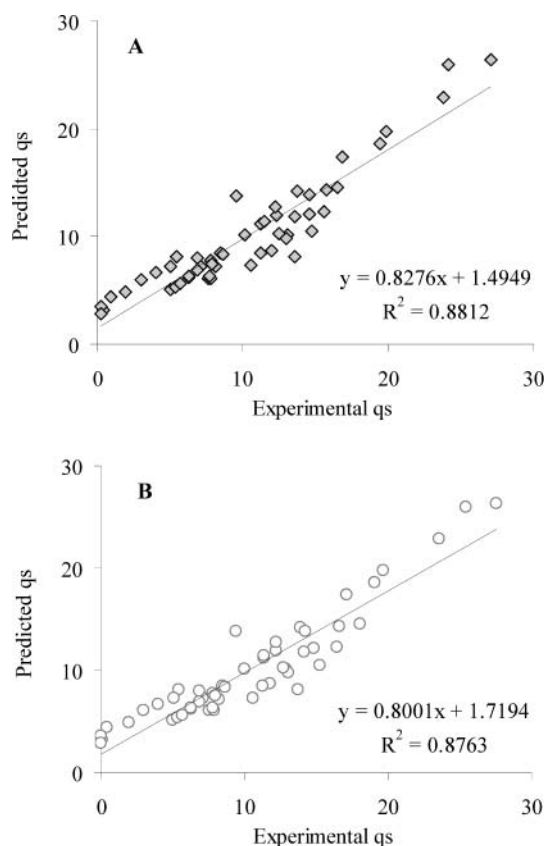
pH	EXA-90 <sup>b</sup>	EXA-31 <sup>c</sup>
	$q_s^d$ (mg/g)	$q_s^d$ (mg/g)
1.0	15.87 <sup>e</sup>	6.94 <sup>e</sup>
1.5	15.55	6.07
2.0	15.53	6.04
2.5	13.47	5.63
3.5	14.60	6.03
4.5	13.62	6.05
mean <sup>f</sup>	14.77 (1.04)	6.13 (0.43)

<sup>a</sup>  $C_0$ ,  $27.7 \pm 1.4$  mg/L. <sup>b</sup> Resin, 1.5 g/L. <sup>c</sup> Resin, 2.0 g/L. <sup>d</sup> Mean value of duplicate runs. <sup>e</sup> Mean value of four runs. <sup>f</sup> Standard deviation in parentheses.

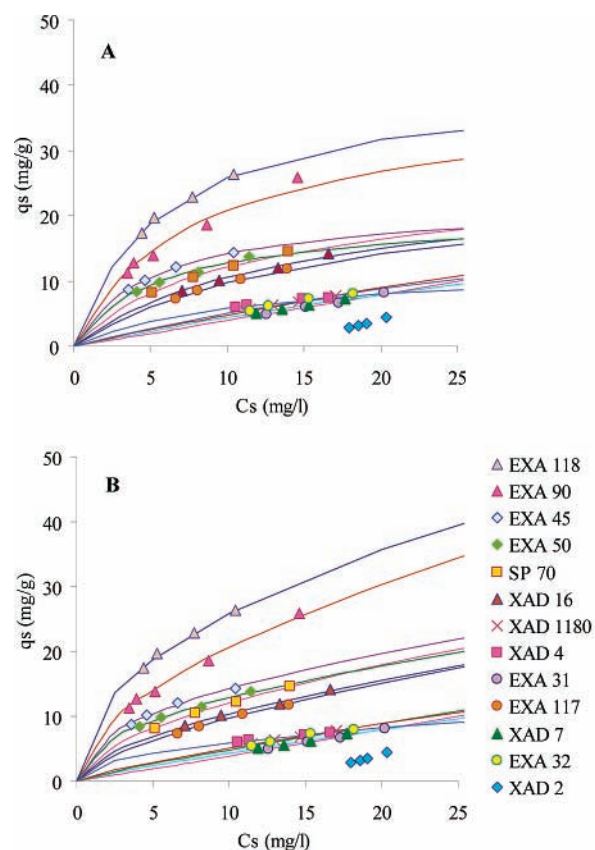
from the Langmuir isotherm together with the Freundlich values of  $K_F$  and  $b_F$ , which are correlated with adsorption capacity and adsorption intensity, respectively. Table 3 also reports, for both models, the errors between experimental and predicted  $q_s$  values calculated for each resin; the model giving the smallest error values agrees better with the experimental data. The comparison of the MSR,  $E_i$ , and RPE values proves that both models are practically equivalent in the prediction of the studied adsorbent/adsorbate system. The errors between the experimental and the predicted  $q_s$  values obtained in most cases are superimposable, quite apart for the utilized isotherm. Among the resins showing the smallest errors (XAD-7, EXA-90, and XAD-16), the best fit is observed by using the Freundlich isotherm; on the contrary, the Langmuir equation provides a best correlation for the resins having the biggest error values (EXA-31, XAD-2, and SP-70). Figure 2 shows the good linear correlation between all of the experimental  $q_s$  values and the predicted ones on the basis of the two isotherms for the 13 tested resins. The values of the slope and the regression coefficients are the same for the two models, but both isotherms overestimate the adsorption capacity of the less effective resins (XAD-2, XAD-7, and EXA-31) as

**Table 3.** Isotherms Parameters and Statistical Errors for the Adsorption of Cyanidin 3-Glucoside on Resins at 20 °C

resins	Langmuir			errors			Freundlich			errors		
	$N^a$	$Q_m$ (mg/g)	$a_L$ (L/mg)	MSR	Ei	RPE%	$N^a$	$K_F$ (L/g)	$b_F$	MSR	Ei	RPE%
XAD-2	4	-1.385	-0.038	9.277	3.066	87.400	4	0.000098	3.557	11.457	3.753	96.790
XAD-7	4	42.553	0.011	0.006	0.001	1.229	4	0.584	0.872	0.006	0.001	1.110
EXA-31	4	-188.329	-0.002	7.881	0.869	45.435	4	0.350	1.040	8.099	0.895	46.080
EXA-117	4	26.455	0.058	6.607	0.376	28.527	4	2.212	0.639	7.476	0.402	30.313
XAD-1180	4	33.445	0.018	0.001	0.000	0.542	4	0.811	0.797	0.002	0.000	0.511
EXA-32	4	65.789	0.008	2.088	0.173	17.311	4	0.645	0.876	2.055	0.168	17.103
EXA-90	5	37.594	0.123	0.775	0.011	4.159	5	5.666	0.560	0.127	0.003	2.488
SP-70	4	25.562	0.091	15.800	0.709	37.158	4	3.200	0.574	20.770	0.844	42.867
XAD-4	4	12.610	0.088	1.526	0.153	17.441	4	2.061	0.460	1.723	0.171	18.768
XAD-16	4	25.773	0.069	0.092	0.002	2.000	4	2.695	0.585	0.045	0.001	1.450
EXA-45	4	21.882	0.184	7.059	0.286	24.615	4	4.885	0.466	7.594	0.281	25.578
EXA-50	4	20.284	0.169	7.000	0.199	16.813	4	4.283	0.476	7.422	0.213	17.383
EXA-118	4	40.984	0.170	0.426	0.003	2.717	4	8.894	0.464	0.492	0.003	2.31

<sup>a</sup> Number of experiments.**Figure 2.** Linear correlation between the experimental  $q_s$  values and the predicted ones on the basis of the Langmuir (A) and Freundlich (B) models at 20 °C.

compared with the experimental observations, making the overall predictive values of the models worse. For these reasons, both Langmuir and Freundlich parameters may be considered suitable for describing the tested adsorption system in the studied concentration range and for comparing the adsorption capacity of the different resins at 20 °C. The amount of cyanidin 3-glucoside adsorbed (mg/g) at different  $C_s$  was calculated on the basis of the constants given in **Table 3** for each resin; **Figure 3** shows the resulting curves obtained for the two models and the experimental equilibrium data. To compare different resins, usually the one with the highest amount of solute adsorbed ( $q_s$ ) at a specified residual concentration ( $C_s$ ) would be preferred for the particular application. Results show that the SDVB EXA-118 resin, with the greater  $q_s$  values than the other resins at all

**Figure 3.** Adsorption curves calculated on the basis of Langmuir (A) and Freundlich (B) constants and experimental equilibrium data at 20 °C.

anthocyanin residual concentrations, is the most suitable for the adsorption of cyanidin 3-glucoside from aqueous solutions. The Freundlich model describes better than the Langmuir one the experimental behavior of the SDVB EXA-90 resin, which has a good adsorption capacity. The remaining resins could be divided into two groups whose behavior is similarly described by Langmuir and Freundlich equations: the resins EXA-45, EXA-50, SP-70, XAD-16, and EXA-117 with an intermediate adsorption capacity and the resins EXA-32, XAD-1180, XAD-4, XAD-7, and EXA-31, which show low efficiency for removing anthocyanins. Among them, the XAD-4 resin shows a better efficiency for removing trace amounts of cyanidin 3-glucoside in regard to higher  $C_s$  values. The prediction of the experimental behavior of the SDVB XAD-2 is unsatisfactory with both of the used isotherms; probably, it is due to its scarce

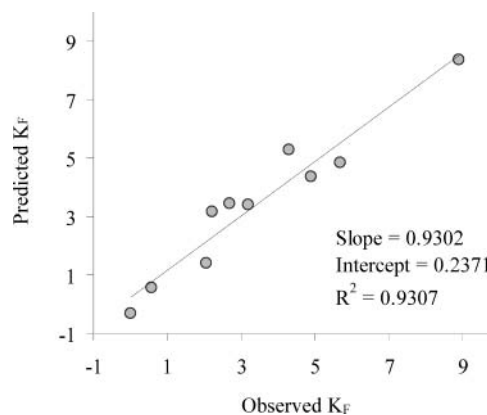
**Table 4.** Regression Analysis<sup>a</sup> of the Equation  $K_F = aS_A + bP_R + c$ 

regr. no.	resins	cases no.	FD	$a$	SE <sub>a</sub> (%)	$P_a$ value	$b$	SE <sub>b</sub> (%)	$P_b$ value	$c$	SE <sub>c</sub> (%)	$P_c$ value	R <sup>2</sup> adj	F test
1	all	13	1	0.0089	19.1	0.0002				-3.415	35.8	0.0175	69.8	28.7
2	$P_R \leq 105 \text{ \AA}$	10	1	0.0082	23.2	0.0024				-2.623	55.9	0.1115	66.9	19.2
3	$P_R \leq 90 \text{ \AA}$	9	1	0.0088	14.8	0.0002				-3.427	29.4	0.0114	85.6	48.7
4	all	13	2	0.0087	20.7	0.0006	0.0024	270	0.7222	-3.099	51.3	0.0721	67.2	13.3
5	$P_R \leq 170 \text{ \AA}$	12	2	0.0089	20.2	0.0008	0.0048	208	0.6422	-3.669	45.2	0.0545	67.6	12.5
6	$P_R \leq 150 \text{ \AA}$	11	2	0.0085	21.1	0.0017	0.0122	106	0.3757	-3.821	44.1	0.0531	66.9	11.0
7	$P_R \leq 105 \text{ \AA}$	10	2	0.0075	13.3	0.0001	0.0486	21.0	0.0020	-5.001	18.2	0.0009	91.1	47.0
8	$P_R \leq 90 \text{ \AA}$	9	2	0.0080	11.3	0.0001	0.0353	34.0	0.0258	-4.726	17.4	0.0012	93.1	55.4
9	$P_R \leq 80 \text{ \AA}$	8	2	0.0068	10.3	0.0002	0.0184	48.9	0.0977	-3.149	22.2	0.0064	93.5	51.0
10	$P_R \leq 65 \text{ \AA}$	7	2	0.0068	11.8	0.0009	0.0175	77.1	0.2676	-3.124	29.7	0.0193	93.0	40.7

<sup>a</sup> FD, freedom degrees; SE, standard error of estimate.  $P$  value < 0.01 indicates significance >99% confidence level.

adsorption capacity, the lowest among the tested resins as shown by the experimental equilibrium data of **Figure 3**. This hypothesis is confirmed for the Freundlich isotherm by the fact that it does not converge to Henry's law at low surface coverage and it fails to describe equilibria as  $q_s \rightarrow 0$ .

**Correlation Analyses of Freundlich Constant  $K_F$ .** Regarding the hesperidin adsorption from aqueous solutions (25), recently, a linear dependence of the Freundlich constant  $K_F$  on the physical characteristics of the resin as surface area ( $S_A$ ) and pore radius ( $P_R$ ) was demonstrated. The  $K_F$  constant linearly increases with the increase of the surface area and the pore radius of resins up to a limit value of 90 Å, while beyond this limit the contribution of  $P_R$  is slightly negative. In this paper, this procedure was applied to the adsorption of cyanidin 3-glucoside by testing the same polymeric matrixes. To rationalize the data set and establish the relative importance of each parameter, a series of simple and multiple regressions was performed using  $S_A$  and/or  $P_R$  as independent variables. The values of the coefficients of the variables and the constant terms together with the corresponding standard errors, the regression coefficients,  $P$  values, and  $F$  test values are reported in **Table 4**. No linear correlation exists between the Freundlich constant and the resin pore radius. Regressions 1–3 show a rough linear trend of  $K_F$  with  $S_A$  that gets better in term of standard errors of variable coefficient and constant term if we consider few resins, eliminating the ones with greater pore radii and minimizing the  $P_R$  variation; the values of the variable coefficient and the constant term do not change considering either 13 (regression 1) or nine resins (regression 3). In particular, the regression of  $K_F$  with  $S_A$  for the resins having  $P_R \leq 90 \text{ \AA}$  (regression 3) shows very small standard errors and  $P$  values; this regression explains 50.8% of  $K_F$  variance. The regressions 4–10 refer to the introduction of the  $P_R$  variable in the previous correlations, to evaluate its possible contribution on determining the  $K_F$  value. Results explain a Gaussian trend of the values of the standard errors of the  $P_R$  coefficient and the constant term ones, together with the  $P$  value and  $F$  test values, excluding the resins with greater  $P_R$ . The best statistical parameters in terms of  $P_R$  coefficient errors are obtained for the 10 resins having  $P_R \leq 105 \text{ \AA}$  (regression 7). Considering all regressions performed, it is clear that the  $S_A$  coefficient and the constant term, even if associated to different standard errors, are almost the same quite apart from the number of the variables and the resins considered; more significant variations are ascribed to the  $P_R$  coefficient. The introduction of this parameter into the linear equations improves the significance of the correlations, minimizing the standard errors of the corresponding single parameter equations and increasing the regression coefficients. For the tested resins having  $P_R \leq 105 \text{ \AA}$ , it is possible to demonstrate a positive linear dependence of the Freundlich constant  $K_F$  on both physical



**Figure 4.** Plot of observed and predicted  $K_F$  values at 20 °C for the 10 resins with  $P_R \leq 105 \text{ \AA}$ .

parameters, according to the following equation:

$$K_F = 0.0075 S_A + 0.0486 P_R - 5.001$$

**Figure 4** shows the plot of the observed  $K_F$  against the calculated  $K_F$  on the basis of the above proposed two parameter equation, pointing out the high accuracy and the strongly predictive value of the proposed model for cyanidin 3-glucoside adsorption. The lack of a significant number of resins having large pore radius makes us unable to find a statistical valid correlation for the ones with  $P_R \geq 105 \text{ \AA}$ . Although this study has been focused on the relationship between the resin properties and only one of the two Freundlich constants, in the investigated concentration range at greater  $K_F$ , values correspond an higher adsorption capacity.

**Adsorption of Cyanidin 3-Glucoside and Hesperidin on Resins: A Comparison.** Anthocyanins and hesperidin are two valuable compounds of pigmented orange juices, which find application in the food and pharmaceutical industries. The commercial value of the anthocyanins is higher than the hesperidin one. The copresence of both compounds in orange juices and especially the great amount of hesperidin present in citrus byproducts such as pulp wash could be relevant for the use of these kind of adsorbents in anthocyanins recovery. Comparing the adsorption in batch solutions of cyanidin 3-glucoside and hesperidin (25), the sequence of the adsorption resin capacities for the two flavonoids is on the whole similar but the removal of the flavanone at 20 °C is greater than the anthocyanin one. The same results are reported in the literature about the column adsorption of anthocyanins (9) and hesperidin (15, 16) from citrus processing byproducts and confirm the high affinity of these kind of matrixes for strongly hydrophobic compounds. Among the 13 tested resins, only the XAD-4 shows



**Table 5.** Equilibrium Data of the Simultaneous Adsorption of Cyanidin 3-Glucoside and Hesperidin on XAD-4 Resin at 20 °C and pH 3.5

resin (g/L)	cyanidin 3-glucoside <sup>a</sup>	hesperidin <sup>b</sup>
	percentage of adsorption <sup>c</sup>	percentage of adsorption <sup>c</sup>
1.0	31.02	28.30
1.5	41.97	40.67
2.0	52.84	49.13
2.5	58.01	59.71

<sup>a</sup>  $C_0$ ,  $5.31 \times 10^{-5} \pm 8.5 \times 10^{-7}$  mol/L. <sup>b</sup>  $C_0$ ,  $5.42 \times 10^{-5} \pm 2.8 \times 10^{-7}$  mol/L.  
<sup>c</sup> Mean value of two runs.

in terms of Freundlich parameters the same adsorption capacity for both compounds and it is supposable that in the presence of an unitary ratio of the two flavonoids it should adsorb the same amount of each of them. This hypothesis is confirmed by the results of simultaneous adsorption of the same initial concentration of hesperidin and cyanidin 3-glucoside ( $C_0$ ,  $5.36 \times 10^{-5}$  M) performed using different resin amounts within the range of 1–2.5 g/L at 20 °C. The equilibrium percentages of adsorption reported in **Table 5** show that the adsorbed amounts of the two flavonoids are approximately the same at all investigated resin concentrations.

**Adsorption of Anthocyanins from Pigmented Orange Juice on Resin EXA-118.** Because the real food matrix is an important factor that could influence adsorption on resins, the SDVB EXA-118 was tested on a pigmented orange juice containing 162 mg/L of anthocyanins and 269 mg/L of hesperidin. A 200 mL amount of resin was saturated after 5.1 L of juice loaded; it corresponded to about 825 mg of adsorbed anthocyanins. After desorption with methanol, the collected fraction contained 6.60 g/L of anthocyanins that were the 80% of the anthocyanins loaded, while the content of hesperidin was only 1.50 g/L. Moreover, the anthocyanin profile was identical before and after the treatment. The results demonstrated a good selectivity of the adsorption/desorption process for recovering anthocyanins.

## CONCLUSIONS

The equilibrium experimental data of the adsorption of cyanidin 3-glucoside on 13 commercial resins at 20 °C were well-fitted to Langmuir and Freundlich isotherms, describing the adsorbent/adsorbate behavior and predicting the capacity of the resins with high accuracy in the studied concentration range. The SDVB EXA-118, having the highest surface area ( $S_A$ , 1200 m<sup>2</sup>/g) and a pore radius of 90 Å, proved to be the most efficient among the tested resins for removing cyanidin 3-glucoside from aqueous solutions at all residual equilibrium concentrations; a good adsorption capacity was demonstrated also by the SDVB EXA-90 ( $S_A$ , 630 m<sup>2</sup>/g;  $P_R$ , 105 Å). Contrary to all expectations, the acrylic resins (EXA-31 and XAD-7) recommended from the manufacturers for recovery anthocyanins because of their partially hydrophilic nature have shown a low efficiency with regard to the other polymers. Moreover, experimental data on EXA-31 and EXA-90 resins have demonstrated that the cyanidin 3-glucoside removal is not affected by pH variations and probably it does not depend on the different equilibrium species due to pH values. Correlation analysis has demonstrated that the Freundlich constant  $K_F$  depends on the physical characteristics of the resin. For the resins having  $P_R$  ≤ 105 Å, the  $K_F$  constant linearly increases with the increase of the surface area and the pore radius therefore increasing

adsorption capacity. A similar correlation was already found for the adsorption of hesperidin (24) on the same commercial resins, pointing out the strongly predictive value of the proposed method in the adsorption of flavonoids. This relationship can be helpful in the selection of suitable adsorbents for various processes and makes easier the study of the adsorption profile of a specific adsorbent/adsorbate system. The comparison of the resin adsorption capacities at 20 °C with the results of the hesperidin removal (25) has confirmed that this kind of matrix has a stronger affinity with hydrophobic compounds such as hesperidin and therefore its removal is higher than the cyanidin 3-glucoside one. Only XAD-4 resin has shown the same adsorption capacity in terms of Freundlich parameters; this result may be interesting being the higher commercial value of anthocyanins with respect to hesperidin, but probably, it is not industrially advantageous due to resin low adsorption capacity.

The most effective resin (EXA-118) was successfully applied to obtain a highly concentrated extract of anthocyanins from a sample of pigmented orange juice. The results evidenced a good adsorption and cleanup potential of the resin, together with a noticeable selectivity for the pigments.

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