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Polar Interactions in Flavan-3-ol Adsorption on Solid Surfaces

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The adsorption of flavan-3-ol monomers and grape seed procyanidin fractions of different mean degrees of polymerization was studied on three surfaces by means of adsorption isotherms. Reversibility upon dilution was also investigated. These surfaces were three polymeric microfiltration membranes, presenting close Lifshitz−van der Waals components of their surface tension but differing in their surface polar properties. The electron-donor character of the surface was of primary importance for the adsorption of nongalloylated monomers. Increasing the number of phenolic rings above two (galloylated monomers and procyanidins) sharply enhanced flavan-3-ol affinity for surfaces whatever their polarity. However, maximum adsorbed amounts were always much higher on the most polar material. The general trend was a partial reversibility with monomers, whereas an irreversible process was evidenced from the lowest molecular weight tannin fractions. This indicated the formation of multiple bonds with surfaces, in accordance with the high affinity type isotherms. The whole results indicated very different mechanisms in the buildup of the adsorbed layers when the surface electron-donor character varied.

KEYWORDS: Flavan-3-ols; membranes; adsorption; isotherms; polar interactions

INTRODUCTION

Polyphenols in red wines are mainly anthocyanins and condensed tannins (proanthocyanidins) (*1*). Native proanthocyanidins (**Figure 1**), extracted from grape skins and seeds, are oligomers and polymers of flavan-3-ol units primarily linked by C4−C8 bonds, with C4−C6 bonds giving rise to a degree of polymer branching. The constitutive units of seed tannins are (+)-catechin (C), (−)-epicatechin (EC), and (−)-epicatechin-gallate (EC-G). In skin tannins (−)-epigallocatechin (EGC) is also present, accounting for ∼30% of the units (*2*). During wine elaboration and aging, several reactions lead to the modification of the phenolic content, especially by means of anthocyanin−tannin condensation reactions that produce derived pigments (*1*). Condensed tannins and derived pigments are responsible for wine color, bitterness, and astringency, which all play a primary part in the sensory appreciation of the product. In other respects, they can be involved in the formation of hazes and precipitates (*3*) and affect the efficiency of the clarification and stabilization treatments applied to ensure wine limpidity and stability (*4*, *5*). Their ability to coprecipitate with proteins, considered to be responsible for the astringent perception of wine (*6*, *7*), is exploited in fining treatments (*8*, *9*). Most of these properties, positive or not, are related to physicochemical interactions between polyphenols, polyphenols, and macromolecules or polyphenols and surfaces. An improved understanding of these interactions is thus a key point to (i) elucidate the relationship between wine phenolic composition, organoleptic properties, and colloidal stability; and (ii) develop new processes and design low-fouling materials for wine elaboration and stabilization (*10*−*12*).

Research concerning polyphenol interactions has mainly been directed toward protein−polyphenol complexation, due to its importance in many fields (*6*, *8*, *13*−*15*). By contrast, and despite their importance in several technological processes, there are only a few studies dealing with polyphenol interactions with solid surfaces (*4*, *16*−*20*). Thus, the underlying mechanisms implied in their adsorption are still poorly understood. Physicochemical interactions implied in adsorption (or complexation) are mainly Lifshitz−van der Waals, polar Lewis acid/base (electron acceptor/electron donor), and electrostatic interactions (*21*, *22*). In aqueous media, polar forces usually occur in the guise of hydrogen donor/hydrogen acceptor (Brønsted acid/base) interactions. However, the use of the Lewis model, more general, is preferable. When dealing with flavan-3-ols (pK_a values of hydroxyl groups within the range of 8−10), there should be no charged groups in the wine pH range (3−4) and one can neglect electrostatic interactions (*23*). Their adsorption on materials will then be essentially driven by Lifshitz−van der Waals and polar forces. According to several publications, Lifshitz−van der Waals forces between biological macromolecules and polymers immersed in aqueous media are usually attractive and small, and the total interaction is mainly driven by polar forces (*22*). The aims of the present work were (i) to characterize the adsorption of flavan-3-ol monomers and procyanidin fractions of different mean degrees of polymerization (DP) by means of kinetic studies and adsorption isotherms and (ii) to investigate the impact of the solid surface tension on adsorption.

The solid surface tension (γ, mJ/m^2) is an essential parameter in adsorption as it determines the Lifshitz−van der Waals and

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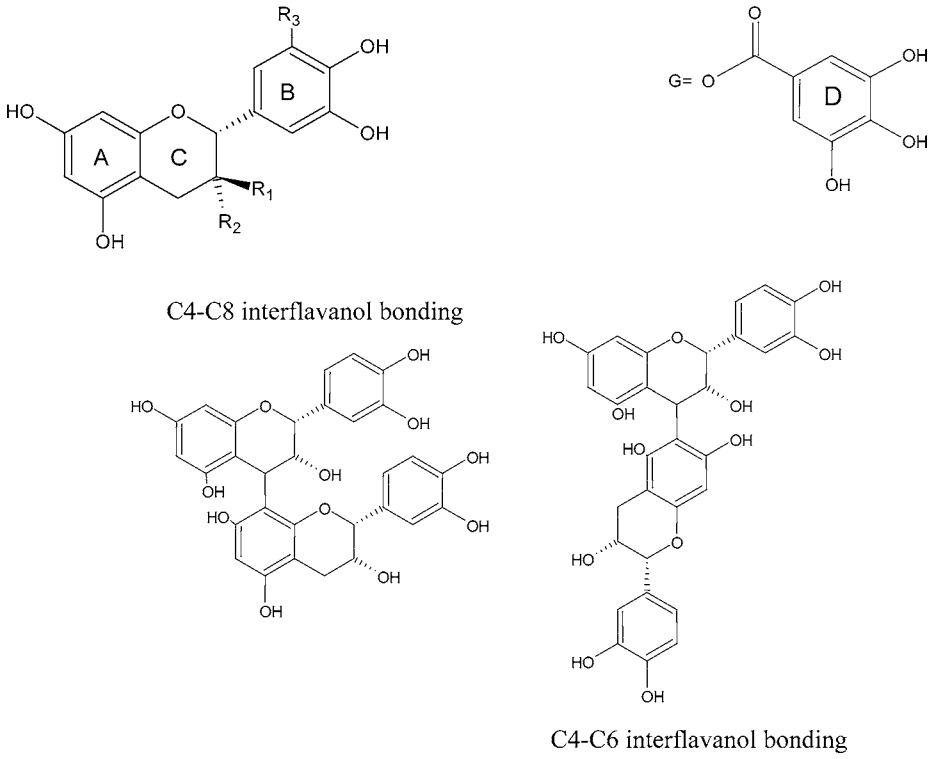

Figure 1. Structures of flavan-3-ol monomers and condensed tannins (dimers): catechin (C), R1 = OH, R2 = R3 = H; epicatechin (EC), R1 = R3 = H, R2 = OH; epigallocatechin (EGC), R1 = H, R2 = R3 = OH; epicatechin-gallate (EC-G), R1 = R3 = H, R2 = G; epigallocatechin-gallate (EGC-G), R1 = H, R2 = G, R3 = OH.

polar interactions this solid will develop with a given solute and the solvent (*21*, *22*). The surfaces used here were three organic microfiltration membranes, chosen for several reasons. First, they possess a sufficiently high surface area, along with large pores, so that there is no limitation in polyphenol accessibility to the surface. Second, these materials, previously characterized in terms of surface tension (*11*) according to the acid/base theory and the approach proposed by van Oss (*22*), were shown to present an interesting set of surface properties for our purpose. According to the acid/base theory (*21*), the surface tension γ is the sum of two components: one related to Lifshitz−van der Waals interactions, γ^{LW}, and the other to the material electron donor/electron acceptor characteristics, γ^{AB}. The polar component γ^{AB} is defined as $\gamma^{AB} = 2(\gamma^+\gamma^-)^{1/2}$, where γ^+ and γ^- are, respectively, the electron-acceptor and electron-donor parameters of the material surface tension. The selected materials mainly differed by their surface electron donor/electron acceptor parameters, which induced variations in their affinity toward tannins when immersed in a red wine (*11*).

Flavan-3-ols were (i) monomers of interest in wine and (ii) three procyanidin fractions of increasing mean degree of polymerization in number (DP 3, 7, and 11), purified from grape seeds. Although very little represented in wines by comparison to condensed tannins, monomers were of interest as they can be obtained as pure molecules and used to check the incidence of the DP and galloylation on adsorption. As we were interested by winelike conditions, adsorption experiments were performed in a 12% ethanol/water buffer (pH 3.4). Ethanol, by decreasing the solvent cohesion, affects interactions (*24*), and there is some evidence in the literature that ethanol content strongly influences procyanidin interactions with biopolymers (*25*).

MATERIALS AND METHODS

Chemicals. Deionized water was obtained with a Milli-Q system (Millipore). Absolute ethanol and acetone were purchased from Prolabo, and sodium hydroxide and tartaric acid were obtained from Labosi. Flavan-3-ol monomers (catechin, epicatechin, epicatechin-gallate, and epigallocatechin-gallate) were supplied by Sigma.

Purification and Characterization of Grape Seed Procyanidin Fractions. One hundred and eight grams of freeze-dried seeds of *Vitis vinifera* were ground in liquid nitrogen with a Büchi mixter B-400 (Büchi), and tannins were extracted with 500 mL of acetone/water (60:40 v/v) under stirring during 8 h. The mixture was centrifuged for 20 min at 7000*g* using a Sorvall model RC5B centrifuge. The supernatant was concentrated under vacuum at 30 °C and stored at 4 °C overnight. The solid phase was extracted again with acetone/water during one night at 4 °C and centrifuged, and the two supernatants were pooled. Lipids were removed by three extractions with hexane, and the extract further concentrated to 57 mL was chromatographed on a 100 mL bed volume column (diameter, 2.7 cm; 18 cm height) of Toyopearl TSK HW-50 (F) gel (Tosoh Corp., Tokyo, Japan). After the tannins extract had been loaded, elution was performed with 1250 mL of ethanol/water/trifluoroacetic acid (55:45:0.05 v/v/v) with a flow rate of 2−3 mL/min to obtain four tannins fractions: F1-1 (360 mL, discarded), F1-2 (185 mL), F1-3 (220 mL), and F1-4 (580 mL). The elution by 6 bed volumes of acetone/water (30:70 v/v) gave fraction F2. Fraction F3 was finally recovered by 1 bed volume of acetone/water (60:40 v/v). Organic solvents were evaporated and the samples freeze-dried. Five tannin fractions, kept under argon in sealed vials and protected from light to avoid oxidation, were obtained. They were analyzed by thiolysis followed by HPLC, as described previously (*26*). This allowed the determination of their mean degree of polymerization in number (DP) and of their percentage of galloylation (**Table 1**). It must be emphasized that thiolysis gave access to a mean molecular weight in the fraction, but does not provide any information about polymer size distribution (*26*). Because fractions F1-2, F1-3, and F1-4 were close, only F1-2 was used in this work. Next, F1-2, F2, and F3 will be designated by their DP (F1-2 = DP 3, F2 = DP 7, and F3 = DP 11).

Microfiltration Membranes. The capillary microfiltration membranes used (M1, M2, and M3) were prepared with organic polymers according to the so-called phase inversion technique and provided by X-Flow. M1 and M2, prepared from polyethersulfone (PES) and polyvinylpyrrolidone (PVP) were hydrophilic in nature. PVP, which provides a hydrophilic character to the membrane, is mostly located at the outer surface of pores walls. An adequate post-treatment of the

Table 1. Characterization of Grape Seed Procyanidin Fractions: Mean Degree of Polymerization in Number (DP), Percent of Galloylation, and Molecular Mass

	F1-2	F2	F3
DP	3.3	7.0	10.9
percentage of galloylation[a]	15.6	27.5	27.3
mean molecular mass[b] (g/mol)	≈1020	≈2235	≈3770

[a] Percent of epicatechin gallate units. [b] Calculated from the fraction composition in flavan-3-ol units.

membrane prevents the swelling of PVP while holding its hydrophilic character. M1 and M2 differ from one another in their amount of PVP, larger for M1 than for M2. M3 was prepared with a rather hydrophobic polymer, poly(vinyl chloride). The surface areas of M1, M2, and M3, measured by using the so-called BET technique, were, respectively, 7.72 ± 0.06, 10.63 ± 0.03, and 8.47 ± 0.07 m^2/g.

Membrane Surface Properties and Surface/Solvent Interactions. Membrane surface properties were determined in a previous work by means of capillary rise experiments with several test liquids using the weight time technique and the application of the Washburn equation (*27*, *28*)

$$w_{imb}^2 = \frac{R\rho^2}{\eta}\gamma_L \cos\Theta t \quad (1)$$

where w_{imb} is the increase of weight (g) of the porous media as a function of time t (s) caused by the imbibition; ρ, η, and γ_L are the density (g/cm^3), viscosity (mPa.s), and superficial tension (mJ/m^2) of the test liquid, respectively; Θ is the contact angle between the solid and the liquid; and R (cm) is a constant that is dependent on the structure of the porous medium. R was obtained using dodecane as a totally wetting liquid ($\cos\theta = 1$). Thus, the Washburn equation was used to determine the contact angle between the surfaces and diiodomethan (apolar liquid), water, and formamide (polar liquids). The surface tension components of the membrane surfaces were finally extracted from these contact angles using the acid−base theory, according to the vOGC scale and the calculation procedure proposed by these authors (*22*).

Additional capillary rise experiments were performed in the same conditions with a 12% water/ethanol mixture to evaluate the incidence of ethanol addition on the membrane wettability by the solvent. The physicochemical characteristics of this 12% water/ethanol mixture are $\gamma_L = 48.4$ mJ/m^2 (determined by the Wilhelmy plate method), $\rho = 0.9824$ g/cm^3, and $\eta = 1.48$ mPa.s. The physicochemical properties of dodecane, used as a totally wetting liquid, are $\gamma_L = 25.3$ mJ/m^2, $\rho = 0.749$ g/cm^3, and $\eta = 1.49$ mPa.s. Gravimetric measurements were performed on membrane segments (60 mm) carefully opened lengthwise to avoid capillary rise inside the capillary and by using a processor tensiometer K12 (Krüss) interfaced with Labdesk 2.0 software for data acquisition and treatment.

Adsorption Experiments. Adsorption experiments were realized in model hydroalcoholic solutions composed of 2 g/L tartaric acid and 12% v/v ethanol and in which the pH was adjusted to 3.4 with a 1 M sodium hydroxide solution. Tannin stock solutions were prepared by dissolving each fraction at a concentration of 50 g/L in absolute ethanol. Dilutions of these stock solutions in ethanol were performed to get, by adding 20 μL of the diluted solution to 980 μL of buffer, a range of concentrations between 20 and 1000 mg/L. Buffer consisted of a tartaric acid solution in water/ethanol, tartaric acid and ethanol content in the buffer being calculated so as to obtain the desired final concentration following the addition of the tannin solution. Stock solutions of monomers were directly prepared by dissolution in the model hydroalcoholic solution, at a maximum concentration of 2 g/L.

All adsorption experiments were carried out using 20 mg of membrane soaked in 1 mL of the polyphenol solution, at a given concentration. An aliquot of this solution, serving as control, was used to determine the exact initial concentration. Samples were agitated intermittently. Polyphenol concentration in the bulk before and following adsorption was determined by absorbency measurement at 280 nm, using a Safas mc^2 double-beam spectrophotometer. Calibration curves were first established for each monomer and tannin fraction. Polyphenol concentrations in the solutions were calculated from these calibration curves. The amount of adsorbed polyphenols, Q_{ads} (mg/m^2), was calculated from

$$Q_{ads} = \frac{(C_0 - C_R)V}{WA} \quad (2)$$

C_0 and C_R being the initial and residual bulk concentrations (mg/L), respectively, V the volume of solution (L), and W (g) and A (m^2/g) the weight and the surface area of the adsorbent, respectively. Variations between two separate experiments were determined for several points in the isotherms. Error bars in the results represent the variation determined between two separate experiments.

Table 2. Apparent Contact Angles Θ_{app} between Membranes and Test Liquids and Apparent Surface Tension Components Derived from These Values According to vOGC Theory and within the Acid/Base Scale Proposed by the Authors (*11*, *22*)

	M1	M2	M3
Θ_{app}[a] (deg)			
diiodomethan	66	58	58
water	46	77	nd[b]
formamide	26	59	68
apparent surface tension parameters[a] (mJ/m^2)			
γ^{LW}	25	30	30
γ^+	6.6	0.8	nd
γ^-	25.1	8.5	nd
w^2/t (10^5 g^2/s)			
dodecane	1.75 ± 0.06	1.42 ± 0.04	0.90 ± 0.08
12% water/ethanol solvent	4.14 ± 0.63	3.27 ± 0.30	nd
Θ_{app} (deg)			
12% ethanol/water solvent	44	45	<90

[a] From ref *11*. [b] $\Theta_{app} > 90°$; nd, not determined.

Reversibility upon Dilution. Solutions were brought into contact with 20 mg of membrane during 24 h, and the residual concentration was analyzed by absorbance at 280 nm. Then successive dilutions (*6*) were carried out: 500 μL of supernatant was removed and replaced with 500 μL of buffer, the whole being shaken intermittently. The absorbance was measured 3 h after each dilution (a previous kinetic study showed that the plateau for desorption was obtained before 3 h). The adsorption reversibility was evaluated by comparing the hysteresis observed between the ascending and descending parts of the adsorption isotherm.

RESULTS

Membrane Surface Properties and Surface−Solvent Interactions. Contact angles between the membranes and the test liquids, obtained by capillary rise experiments, and surface tension components extracted from these values are reported in **Table 2**. As discussed previously (*11*), and due to the complexity of flow patterns for liquids in porous media of irregular pore structure such as membranes, this method does not give access to "true" intrinsic contact angles between solids and liquids. These contact angles are in most case overestimated, thus leading to anomalously low values of the surface tension components when compared with those obtained from contact angle measurements on dense substrata (*28*−*30*). Although relative, these values were of great interest because they allowed us to classify materials and to compare their properties with those of their constitutive polymers. It was concluded from these experiments that the Lifshitz−van der Waals components of the M1, M2, and M3 membranes were close, as are the Lifshitz−van der Waals components of polyethersulfone (PES), PVP, and PVC (*22*, *31*). In contrast, significant differences were observed between the three membranes when using polar liquids,

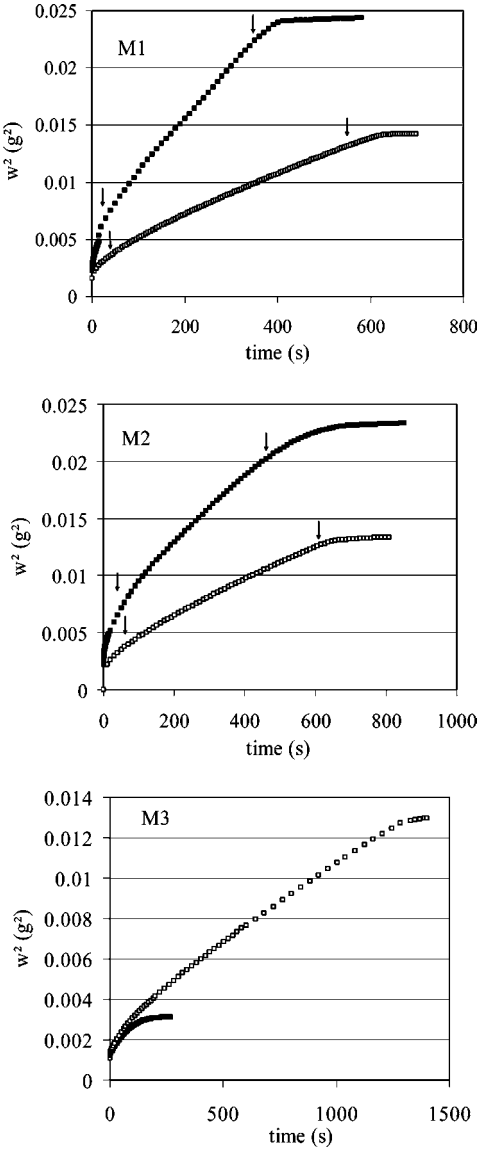

Figure 2. Results of capillary rise experiments performed with dodecane (□) and a 12% water/ethanol hydroalcoholic solution (■) on M1, M2, and M3. The sharp increase in the measured weight observed within the first seconds of the experiment is related to the contact between the solid and the liquids. w^2/t values were calculated from the regular increase in weight observed after, as indicated by the arrows.

differences that agreed with the properties of their constitutive polymers. The M1 and M2 surfaces were classified as polar and mainly basic, in accordance with the properties of PES and PVP (*22*, *32*). The higher γ^- parameter found for M1 well correlated with its higher amounts in PVP, N—C═O groups in PVP being considered as strong electron-donor sites (*15*). With the PVC-based M3 membrane, no spontaneous penetration of water was observed, indicating contact angle values on the order of (or higher than) 90°. This also agrees with literature data (*33*): PVC is usually considered as an only slightly polar polymer, with a predominantly acidic (electron-acceptor) character related to the presence of methylene—chlorine groups.

In the present study, we were interested by adsorption experiments in winelike conditions and so in a 12% v/v ethanol/water solvent. Interactions between the membrane surfaces and the solvent were evaluated by means of capillary rise experiments, as with the test liquids (**Figure 2** and **Table 2**). As expected from the total decrease of γ_L (*24*), ethanol addition increased membrane wettability by the solvent and reduced

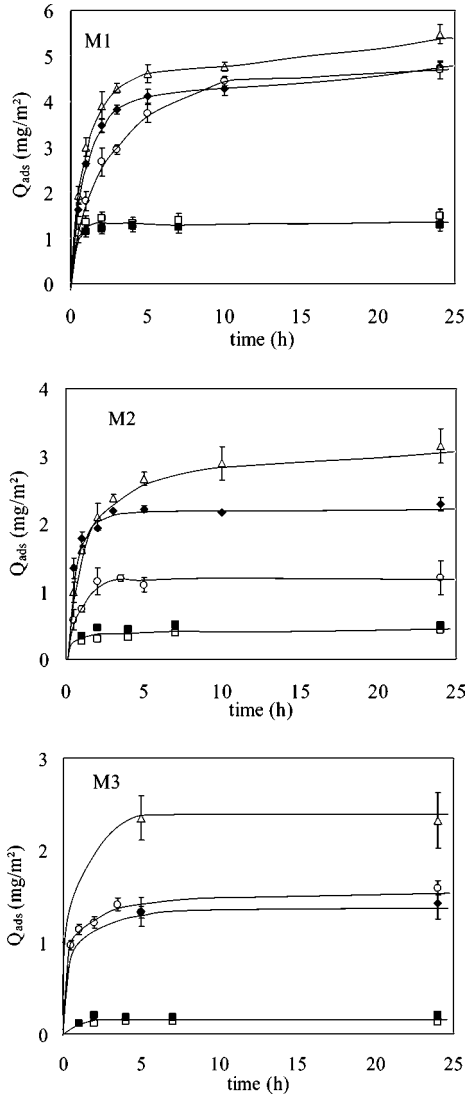

Figure 3. Adsorption kinetics of catechin (■), epicatechin (□), and DP 3 (○), 7 (◆), and 11 (△) procyanidin fractions on M1, M2, and M3. The initial flavan-3-ol concentration was 1 g/L. Errors bars represent the deviation between two separate experiments.

differences noted with water. Very close contact angle values were obtained with the M1 and M2 materials and the 12% ethanol solution, indicating close membrane/solvent interactions for these two materials. In contrast, surface/solvent interactions were much lower with M3, in relation with its low polarity. Spontaneous penetration of the ethanol solution was observed, indicating that this latter wets the polymer, but the observed capillary rise was not high enough to allow quantitative evaluation of the contact angle.

Adsorption Kinetics. Adsorption kinetics, performed with catechin, epicatechin, and the three procyanidin fractions at a concentration of 1 g/L, are reported **Figure 3**. These kinetics depended on both the membrane polar surface properties and the flavan-3-ol DP. Maximum adsorption was obtained within the first 2 h with monomers, whereas longer contact times were needed to achieve the maximum concentration of adsorbed tannins. An equilibrium was obtained within 2—5 h on the less polar materials M2 and M3, whereas no clear plateau value could be obtained on M1, even for contact times as long as 24 h. Such long times for equilibrium suggest that the adsorption process may involve structural rearrangements within the adsorbed layer, the extent of which varies depending on the membrane surface

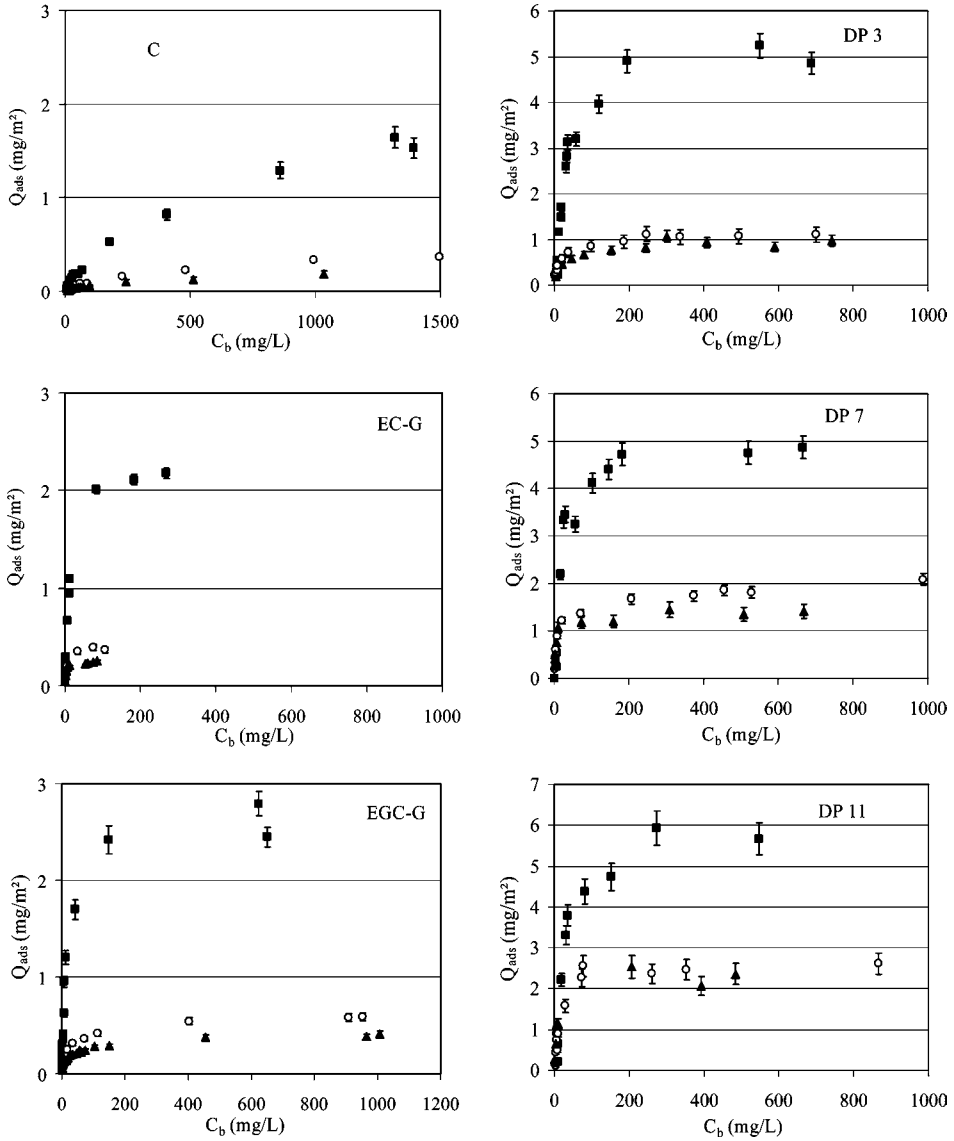

Figure 4. Adsorption isotherms of flavan-3-ol monomers and procyanidin fractions on M1 (■), M2 (○), and M3 (▲). The isotherms found with nongalloylated monomers being close, only those obtained with catechin are shown here.

properties (*34*, *35*). Kinetic studies also evidenced a strong incidence of membrane surface properties on adsorbed amounts. For a given flavan-3-ol and at the tested concentration, adsorbed amounts increased with the membrane polarity.

Adsorption Isotherms. From these kinetic data, contact time for adsorption isotherms was set at 24 h, with the understanding that when the most polar surface and procyanidins were of concern, some time dependency could persist for longer times. Adsorption isotherms (adsorbed amounts Q_{ads} versus the bulk concentration C_b) are reported **Figure 4**. With epicatechin-gallate, available in low amounts, only the initial parts of the isotherms were determined. Whatever the flavan-3-ol, and in accordance with kinetic results, adsorbed amounts were always higher on the most polar material, which confirmed the determinant part of polar acid−base interactions in the adsorption process. Furthermore, very different behaviors were observed between nongalloylated monomers, on the one hand, and galloylated monomers and procyanidin fractions on the other.

Langmuir and/or Freundlich isotherms are often used to describe molecular adsorption. Even if the fundamental assumptions related to these models are not fulfilled, curve fitting provides convenient mathematical constants that can be used to compare results between solutes and surfaces. Such approaches have been applied to tannin adsorption on polymeric adsorbents (*19*) and on apple cell walls (*18*). Neither the Langmuir nor the Freundlich equations allowed here an adequate description of the isotherms. The plots of C_b/Q_{ads} versus C_b (Langmuir) and Ln Q_{ads} versus C_b (Freundlich) were not straight lines, except for the galloylated units and procyanidin fractions on M3 (Langmuirian type isotherms). It must be noted that deviations are often observed when the concentration range is large, as in the present study. At low molecular concentrations, lateral interactions between the adsorbed species are low and the initial part of the isotherm reflects the solute or polymer affinity toward the surface (*36*). Maximum adsorption also depends on this affinity but may greatly vary with several other parameters, among which important ones are the conformation and the orientation of the polymer, the possibility of structural rearrangements after adsorption, and the lateral interactions between the adsorbed species (*37*−*39*). The initial slopes of the isotherms, calculated from the curves for all of the studied systems, are reported in **Table 3** along with the plateau values. To make the comparison between the galloylated units and the different procyanidin fractions easier, plateau values are also

Table 3. Initial Slopes Q_{ads}/C_b Deduced from the Initial Part of the Adsorption Isotherms and Plateau Values

	M1			M2			M3		
	Q_{ads}/C_b (10^3 L/m^2)	Q_{max} (mg/m^2)	Q_{max}[a] (μM/m^2)	Q_{ads}/C_b (10^3 L/m^2)	Q_{max} (mg/m^2)	Q_{max}[a] (μM/m^2)	Q_{ads}/C_b (10^3 L/m^2)	Q_{max} (mg/m^2)	Q_{max}[a] (μM/m^2)
C	5.0	nd[b]	nd	1.6	nd	nd	0.7	nd	nd
EC	2.9	nd	nd	0.9	nd	nd	0.6	nd	nd
EC-G	83.6	nd	nd	48.9	nd	nd	33.0	nd	nd
EGC-G	87.0	2.8	6.1	18.6	0.6	1.3	26.3	0.4	0.9
DP3	82.0	5.1	5.1	69.0	1.1	1.1	35.4	0.9	0.9
DP7	130.8	4.8	2.2	102.6	1.9	0.8	109.9	1.4	0.6
DP11	102.5	5.8	1.5	86.0	2.5	0.7	130.6	2.3	0.6

[a] For comparison only. [b] nd, not determined.

given in micromoles per square meter. It is important to note that with procyanidins, values in micromoles are only mean ones, calculated from the fraction composition (**Table 1**).

Nongalloylated units exhibited low-affinity adsorption isotherms, with an initial slope that increased with the surface polarity. No plateau values were obtained within the tested concentration range (0−2 g/L). A rough estimation of the surface coverage could be performed with these monomers, on the basis of their molecular dimensions. According to Martinez (*40*), a projected molecular area on the order of 0.8 nm^2 can be expected for a flavan-3-ol unit such as catechin or epicatechin and for a horizontal orientation of the molecule upon adsorption (phenolic rings oriented parallel to the surface). For a vertical orientation, this projected surface area would vary between 0.2 and 0.3 nm^2, depending on the axial or equatorial conformation of the heterocyclic ring C (*40*, *41*). A monolayer of these monomers would then represent ∼2 μmol/m^2 (≈0.6 mg/m^2) for a horizontal orientation and from 5 to 8 μmol/m^2 (≈1.5−2.4 mg/m^2) for a vertical orientation of the adsorbed species. Adsorbed amounts thus were well below the supposed monolayer value on M2 and M3, whereas on M1 the Q_{ads} values at the highest bulk concentrations suggested either a vertical orientation of the adsorbed species or multilayer coverage.

Increasing the number of phenolic rings (galloylated units and procyanidin fractions) sharply enhanced polyphenol affinity for surfaces, whatever their surface tension (**Table 3**). In that case, the general trend was a rapid increase of the level of adsorption at low concentrations, followed by a gradual leveling off until the so-called pseudo-plateau level was reached. This plateau value indicated the formation of an adsorbed layer that was not, within the tested concentration range, strongly influenced by the bulk concentration. On M1, galloylated units and procyanidin fractions all exhibited high initial adsorptions. The initial adsorption of EC-G, EGC-G, and DP 3 still decreased with the surface polarity, as observed with catechin and epicatechin. The two highest molecular weight procyanidins (DP7 and 11) exhibited high initial adsorption whatever the surface. The highest plateau values were always found for M1. With this material, plateau values in weight were close for the three procyanidin fractions and were about two times higher than those obtained with epigallocatechin-gallate. A large drop in maximum adsorbed amounts was observed between the M1 surface and the other two. However, this drop was significantly decreased with the DP 7 and 11 fractions. Considering procyanidins, the comparison of the maximum adsorbed amounts in weight and in moles emphasized the different building of the tannin adsorbed layers between the materials. The close plateau values in weight found for M1 with DP 3, 7, and 11 were related to a large decrease in moles, whereas on M2 and M3 the increasing adsorbed mass when the procyanidin molecular weight increased was related to rather close values in moles.

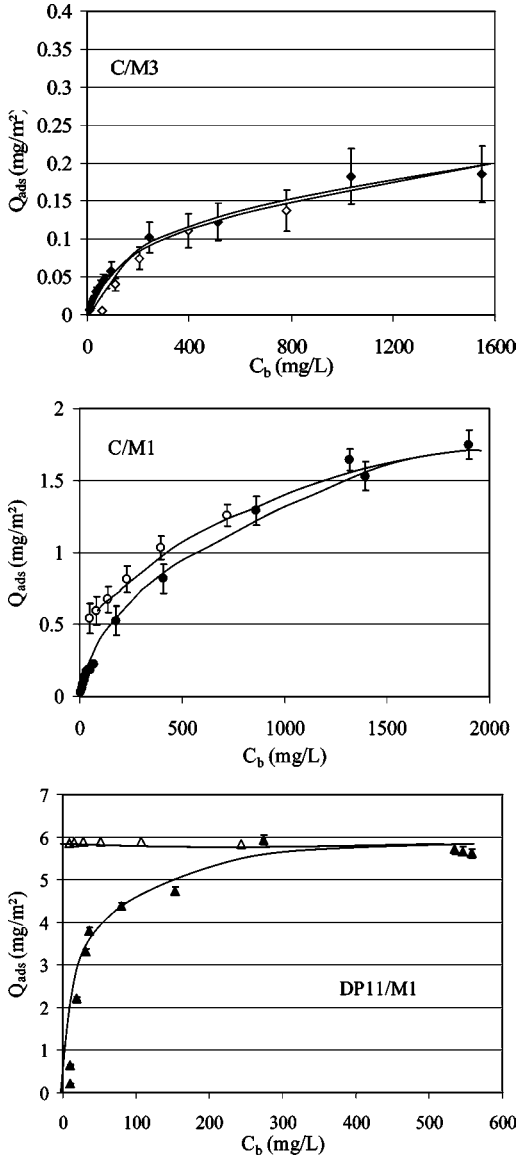

Figure 5. Example of complete adsorption isotherms: (solid symbols) ascending part of the isotherms; (open symbols) descending part of the isotherms (desorption upon dilution).

Unfortunately, there is no information concerning the exact conformation and molecular dimensions of procyanidins in aqueous solvents, so the surface coverage could not be calculated in this case. According to Helfer and Mattice (*41*) this conformation depends on several factors, among which are the type of linkage between units (C4−C6 or C4−C8); the stereochemistry at the interflavan bond, which is either α or β; the conformation of the heterocyclic ring C; and the proportion

Table 4. Hysteresis Observed during Desorption Experiments by Dilution, Performed Following 24 h of Exposure Times and from the Highest Surface Coverage[a]

	M1	M2	M3		M1	M2	M3
C	+	+	–	DP3	++	++	++
EC	+	–	–	DP7	++	++	++
EGC-G	+	+	+	DP11	++	++	++

[a] ++, large hysteresis as in the case of the DP11/M1 system shown in **Figure 5**; +, slight hysteresis, corresponding to the C/M1 system; –, no hysteresis, as in the C/M3 system.

of the different monomers (*41*). With EGC-G, plateaus on M2 and M3 were on the order of 0.58 mg/m^2 (1.3 μmol/m^2) and 0.4 mg/m^2 (0.9 μmol/m^2), respectively. Considering the molecular dimensions of the nongalloylated monomers, a monolayer (eventually incomplete) with the units adsorbed parallel to the surface is reasonable. The much higher surface coverage found for M1 suggested, as with catechin and epicatechin, either a vertical rather than horizontal orientation of the monomer or a multilayer coverage.

Reversibility upon Dilution. Reversibility upon dilution was evaluated for the studied flavan-3-ol/surface systems from the highest obtained surface coverage. Examples of complete adsorption isotherms (ascending and descending branches) are shown **Figure 5**. Hysteresis was observed in most cases, as often in adsorption (*34*, *36*). Such hysteresis is attributed to irreversibility, and its extent greatly varied depending on the flavan-3-ol DP. The whole results are summarized in **Table 4**. In our experimental conditions (long adsorption times), adsorption was partly or mostly reversible with monomers, whereas a strong hysteresis, indicating enhanced irreversibility, was observed from the lowest molecular weight procyanidins and on the three studied surfaces.

DISCUSSION

Adsorption from solution results from a complex interplay between solute/surface, solute/solvent, surface/solvent interactions and solvent cohesion (*21*, *22*, *37*, *39*). These interactions, along with the surface implied in adsorption (contactable surface area or number of contact points) (*22*, *36*), determine the solute affinity for the surface. As stated before, maximum adsorbed amounts depend not only on this affinity but also on solute/solute interactions within the adsorbed layers, on their orientation upon adsorption, and on the possibility of structural rearrangements. Our aim here was to investigate flavan-3-ol adsorption on surfaces and to study the incidence of surface polarity and polyphenol mean degree of polymerization on it. To this end, the adsorption kinetics and isotherms obtained for different flavan-3-ol monomers and procyanidin fractions on various membranes were compared. Membrane surface properties and flavan-3-ol DP strongly influenced the adsorption kinetics, isotherms (initial adsorption and plateau values), and reversibility upon dilution.

The membranes used in the present study possess close Lifshitz−van der Waals components of their surface tension. Thus, for a given flavan-3-ol and solvent, Lifshitz−van der Waals interactions will be close and differences in adsorption will be related to polar flavan-3-ol/surface and surface/solvent interactions (**Table 2**). Surface/solvent interactions were close for M1 and M2 and higher than for M3. Increasing the surface polarity enhanced the initial adsorption of the flavanol monomers and of the lowest molecular weight procyanidins, as well as maximum adsorbed amounts when a plateau value was reached. This was observed despite close (M1 with regard to M2) or intensified (M1 and M2 with regard to M3) surface/solvent interactions that hinder attraction. With the highest molecular weight procyanidins, close initial slopes were found whatever the surface properties; however, plateau values clearly differed. These results indicate that polar acid/base attractive forces between the membrane and the macromolecules play a determinant part in the association. The increasing surface polarity between M3, M2, and M1, being associated with an enlarged electron-donor character, denotes that polyphenols primarily react with surfaces as electron acceptors. These results are in accordance with the strong interactions observed between polyphenols and proline-rich proteins, polyvinylpolypyrrolidone (PVPP) or nylon (*15*−*17*, *42*), usually attributed to the formation of H-bonds between the H-acceptor sites of these proteins or basic polymers and the hydroxyl groups carried by polyphenols. They also agree with the results of Li et al. (*19*), who studied the thermodynamic aspects of tannin sorption on different hypercross-linked polymeric adsorbents. However, the tannin extract used in this later work was not described. Finally, the involvement of polar interactions in the association of apple procyanidins with cell wall material has been evidenced by studying the effect of several environmental parameters (*25*).

Whatever the membrane surface properties, a sharp rise in initial adsorption was observed between nongalloylated and galloylated monomers, in line with that observed for their complexation with proteins (*43*). This can be attributed to the decrease in the molecule's affinity for the solvent, brought about by the additional phenolic ring D (**Figure 1**) (*44*, *45*), and/or to the high flexibility of the latter, which allows the formation of multiple bonds with other substances. A proposed approach in the literature to classify polyphenolic structures with regard to their affinity for water is to compare their partition coefficients between water and octan-1-ol. In this way, Spencer et al. (*45*) demonstrated that galloylation decreases the relative affinity of flavan-3-ol monomers for water. In other respects, galloylation allows the formation of several bonds between galloylated flavanol units and proteins (*13*, *46*). Such multiple bonding or, generally speaking, the formation of several contact points is also likely to occur with surfaces, leading to a stronger interaction than that observed with nongalloylated monomers. The adsorption of flavan-3-ol units remained mostly reversible, and the much higher surface coverage found for M1 by comparison to the two others can be attributed either to a different orientation of the molecule upon adsorption (the presence of numerous electron-donor sites allowing a vertical orientation of the molecules) or to "multilayer" coverage. NMR studies of the complexation between several polyphenols (procyanidin B2, penta- and trigalloylglucose, epicatechin, and propyl gallate) and a proline-rich protein fragment showed that these molecules may self-associate when bound (*46*). The much higher surface coverage found for M1 could thus be related to polyphenol/polyphenol interactions within the adsorbed layer, favored by high local concentrations.

The number of contact points between flavan-3-ols and surfaces, and thus initial adsorption, is expected to increase with increasing number and/or increasing conformational flexibility of phenolic rings (*14*, *15*). This was well illustrated here by comparing the different procyanidin fractions, among themselves and with their constitutive monomers (catechin, epicatechin, and epicatechin-gallate) (**Table 3**). Moreover, with procyanidins, a sharp hysteresis was observed between the ascending and descending parts of the adsorption isotherms. This can be attributed to an enlarged number of contact points between the

macromolecules and the surfaces (*36*), which largely enhances and strengthens their interaction with materials by comparison to monomers, even when only small oligomers (DP3) are of concern. This conclusion is in accordance with that observed for procyanidin adsorption on apple cell wall. In that case, desorption was studied by several rinsing cycles (between 12 and 20) with fresh adsorption buffer (*18*). It was shown that the percentage of desorption decreased when the procyanidin molecular weight increased, in relation with enlarged multiple binding between the macromolecules and the cell wall material. In the present study, we did not evidence differences between procyanidin desorption as a function of their molecular weight. There are several possible reasons for this. First, the substrata were of very distinct nature (cell wall polysaccharides in one case, synthetic polymers in the other); second, the procedures adopted were quite different. Tannins adsorption on apple cell wall was completed after 0.5 h, and reversibility was studied after a contact time of 30 min, whereas this contact time in the present study was set at 24 h. Irreversibility may increase with increasing exposure time, as shown for protein adsorption on both hydrophilic and hydrophobic surfaces (*34*). This is attributed to the strengthening of the bonds formed between macromolecules and surfaces during the aging of the adsorbed layer and may also play an important role when procyanidins are of concern. Moreover, in the present study, it is reversibility upon dilution that was studied, whereas in the work of Renard et al. (*18*), desorption was checked by means of successive washing steps with fresh buffer. The driving force for desorption was thus much higher in the last case. It will be of importance to further investigate these points. With the surfaces studied in the present work, the application of successive washing steps could evidence differences between the procyanidin fractions and/or the surfaces, indicative of differences in the interaction strength. Likewise, the incidence of the exposure time on reversibility, which is an important point in the understanding of adsorption phenomena, deserves to be further investigated.

Maximum adsorbed amounts in mass on M1 did not depend on the procyanidin DP, and kinetic studies showed that the adsorbed layer evolved during a much longer time scale than on less polar materials. After a given surface coverage was attained, the adsorption rate decreased, but adsorption still proceeded. It can then be supposed that on the most polar material, the presence of numerous sites suitable for the formation of electron-donor/electron-acceptor interactions favors rearrangements of the adsorbed molecules that closely pack to optimize their interaction with the surface. This leads to the adsorption of a close number of phenolic rings. As hypothesized with monomers, another possible explanation for this differential behavior of the M1 surface could be the occurrence of polyphenol/polyphenol interactions, favored by high surface concentrations. However, in that case, different adsorbed amounts in mass should be observed for the three fractions. Moreover, the initial adsorption of the DP 7 and 11 procyanidins was close whatever the membrane, so that high surface concentrations can be expected in all cases, which should lead to multilayer coverage on the three surfaces. When the surface polarity decreases (M2 and M3), there are fewer sites for polar interactions between the molecules and the surface. Equilibrium was reached much more readily, suggesting fewer rearrangements leading to lower surface coverage (in mass or moles). Adsorbed amounts in moles only slightly decreased with the flavanol molecular weight, leading to very different adsorbed amounts in mass. This indicates quite different mechanisms in the buildup and the configuration of the adsorbed layer. Further investigations will be needed to explain the differences noted between the surfaces. Research will have to be directed not only toward the elucidation of the structures of the adsorbed layers formed by procyanidins but also toward the elucidation of their conformation in solution.

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