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# Partition of polyhydroxy compounds of biological and pharmacological significance between AOT reverse microemulsions and aqueous salt solutions

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ABSTRACT: The partition behavior of shikimic acid, gallic acid, gallotannic acid, rutin and quercetin between reverse microemulsions of the surfactant bis(2-ethylhexyl) sulfosuccinate (AOT) and aqueous salt solutions of LiCl, NaCl and KCl at different salt concentrations and pH was studied. Quantification of the extracted analytes was performed by HPLC on an octadecyl silica column using a methanol–buffer mobile phase at pH 3.0. Clean-up before injection was not needed because the micellar organic phase was compatible with the HPLC mobile phase. A solvent gradient was used to elute all compounds and the surfactant in a reasonable time. The extraction from the aqueous media is fast and reproducible. Different solubilization behavior was observed by changing the salt concentration and the type of cation in the aqueous phase. By proper selection of the experimental conditions, it is observed that shikimic acid and gallic acid remain mostly in the aqueous phase, whereas significant amounts of gallotannic acid and rutin are transferred to the micellar phase in only one contact and quercetin is completely solubilized in this phase. Thus, the different solubilization behavior that these compounds exhibit in the AOT micellar media can be very useful for extraction and preconcentration purposes. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: micellar extraction; AOT; reverse micelles; microemulsions; polyphenols; phase contact method

## INTRODUCTION

Several investigations have been performed to separate, mainly by liquid chromatography (HPLC), plant phenolics of biological and pharmacological significance.[1–5] The antioxidant properties of polyphenols could be related to antiviral activity as well as to prevent cancer, hypertension and heart diseases.[6] Carcinogenic and mutagenic properties have been attributed to hydroxy compounds from plant origin such as shikimic acid[7] or polyphenols such as tannic acid.[8] It was suggested that large amounts of quercetin could cause cancer in animals,[9] although today important antioxidant properties are attributed to this flavonol. Hence, these important reasons justify the isolation and detection, even at trace levels, of this type of compound.

It is known that phenols as well as alcohols can bind via hydrogen bonding to the polar heads (of bis (2-ethylhexyl) sulfosuccinate (AOT).[10] Magid *et al.*[11] have measured the binding constants for a series of phenols to AOT in isooctane; high values were obtained, showing a strong interaction attributed to the polar head of the surfactant, which has carbonyl groups, and the anionic sulfonate group being able to interact with hydrogen-bonding donor compounds. Moreover, hydrogen-bonding interactions between suitable phenols and AOT form the basis for a novel class of organogels;[12–14] therefore it is expected that this surfactant could perform extractions of phenols and polyhydroxy compounds with high efficiencies.

Reverse micelles are self-organized aggregates of amphiphilic molecules in non-polar media. Nanometer-sized aqueous pools are formed by water solubilization in their polar cores. Among other reasons, these systems are of interest for micellar extraction of metals, amino acids and proteins from aqueous salt solutions using an organic solvent in which the surfactant AOT is dissolved.[15–18] The partition behavior of several neutral and charged solutes, such as *p*-nitroaniline, murexide anion, dimidium cation and NaCl, between AOT reversed microemulsions and aqueous salt systems has been reported previously.[19]

Reverse micelles or 'water in oil' (w/o) microemulsions of AOT have been investigated extensively over three decades, mainly because this surfactant does not require the presence of a co-surfactant for stability.[15] By vigorously shaking an aqueous salt solution with an immiscible organic solvent containing the surfactant and letting them separate into two clear phases, a Winsor II system is formed, i.e. reverse micelles of a given size in the upper organic phase in contact with the conjugate aqueous phase at the bottom.[20] This experimental procedure to form

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reverse micelles is named the '*contact method*' or '*phase transfer method*'. The Winsor II system is required to extract polar hydrophilic compounds into the organic phase. The salt concentration needed to obtain these systems varies depending on the salt and the organic phase.[15,21,22] When NaCl is used in AOT–heptane microemulsions, a salt concentration higher than 0.05 mol $dm^{-3}$ is required to obtain the Winsor II system;[23] otherwise, migration of the surfactant to the aqueous phase occurs.

The size of the aqueous pools in reverse micelles depends primarily on $W_o$ ($W_o$ = [water]/[AOT]). This parameter is related to the hydrodynamic ratio of the micelle and is strongly dependent on the salt concentration in the aqueous phase and on the cation type.[24,25] Increasing the salt concentration decreases $W_o$ and hence decreases the water droplet size of the aggregates formed in the upper organic phase. Water uptake and size of the reverse micelles can be predicted by theoretical models, depending on the type and concentration of counter-ions in the water pool.[24,26]

The aim of this study is to explore the ability of the AOT–heptane/water system to separate and concentrate polyhydroxy compounds of biological interest. Thus, the partition behavior of gallic acid, gallotannic acid, rutin, quercetin and shikimic acid (shown in Fig. 1) between AOT–heptane reverse microemulsions and different aqueous salt solutions was studied. Alkaline chlorides (LiCl, NaCl and KCl) at different concentrations and pH have been evaluated.

Discussions are focused on the factors that influence the partition process of the studied solutes (such us surfactant concentration, and type salt concentration in the aqueous phase and pH) and the physicochemical effects on the molecular interactions between the micelles and the studied solutes. By elucidating how these experimental variables influence the partition process, it is possible to predict how to change them to favor the extraction process and to obtain some selectivity of the system useful for preparative or analytical separations of polyhydroxyphenols present in complex matrices.

## EXPERIMENTAL

### Chemicals

The AOT obtained from Sigma was dried in a vacuum oven at 40 °C for two days and used without further purification. Organic solutions were prepared by dissolving the surfactant in *n*-heptane of reagent grade (Baker). All salts and inorganic acids were reagent grade or better. Phosphate buffer was 100 mM and it was prepared by mixing appropriate amounts of phosphoric acid and sodium hydroxide. The Karl-Fischer reagent was purchased from Tetrahedron (Acquasol Monocomponent 5, Ind. Arg.). Water was purified with a Milli-Q water purification system (Millipore Co.). Methanol was obtained from Mallinckrodt. Shikimic acid, quercetin and gallotannic acid were obtained from ICN, gallic acid from Merck and rutin from Fluka and used as received.

### Apparatus

Chromatographic separations were made on an HP 1100 liquid chromatograph, equipped with a binary pump,

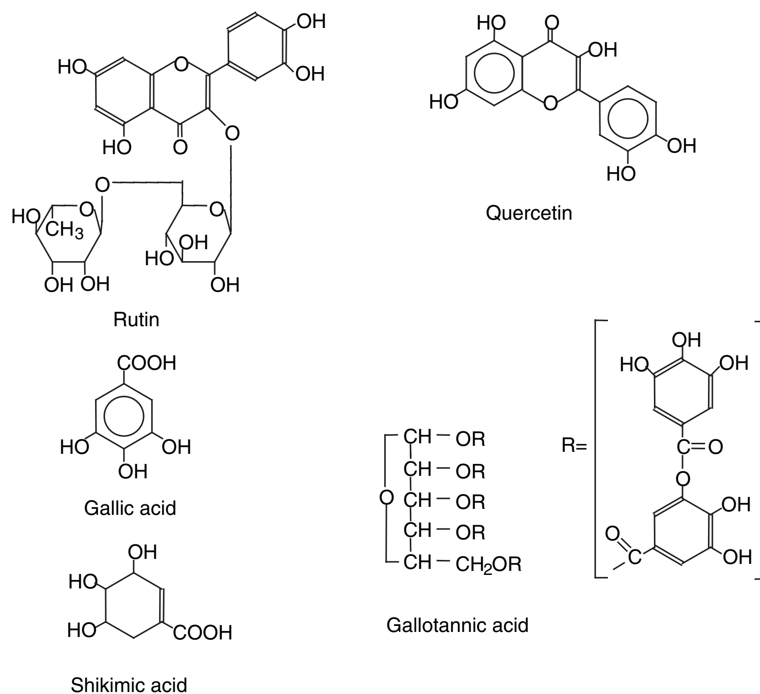


**Figure 1.** The polyhydroxy compounds studied in this work

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manual injector, variable wavelength detector and degasser. Detection for each compound was performed at their UV–Vis absorption band maxima. A Zorbax SB-$C_{18}$ column ($150 \times 4.6$ mm, i.d.) was used. Chromatograms were acquired by the Chromatography Station for Windows by Data Apex Ltd. The same software was used to construct the calibration curves and to perform the quantification of the analytes.

### Methods

A reverse-phase HPLC method able to separate the surfactant from the mixture and detect all the compounds in the same chromatographic run was developed. The mobile phase was prepared by mixing methanol with phosphate buffer of pH 3.0. Reservoir B consisted of 85% methanol–15% buffer and reservoir A of 5% MeOH–95% buffer. The chromatographic analysis was made in 6 min for each sample after 3 min of equilibration with the initial mobile phase. A gradient was used during the first 3 min from 0 to 85% B, followed by 3 min with isocratic elution using the latter composition. The gradient was necessary to elute all the analytes in a reasonable period of time and to clean the surfactant from the column, which elutes at 7.2 min with a broad peak. The high gradient steepness was necessary also to minimize the band tailing for gallotannic acid.

The standard solutions of the analytes for the calibration curves were prepared in water. The concentrations of the analytes in the stock solution used for extraction were as follows: 150 μM for gallic acid, 120 μM for shikimic acid, 35 μM for rutin, 45 μM for tannic acid and 0.4 μM for quercetin, which is the most insoluble compound. All the solutions were flushed with gaseous nitrogen for 15 min, protected from light and stored at 5 °C until used.

The 'phase transfer method'[15] was applied as follows: 4-ml aliquots of the stock solution containing the analytes were placed into different stoppered $16 \times 98$ mm glass test tubes, different volumes of concentrated solutions containing the different salts were added to each of them and the aqueous mixtures were diluted to 5 ml with deionized water. The aqueous phase was brought into contact with 5 ml of the organic solution containing the surfactant (AOT in heptane). The tubes were then placed in a vibrating shaker for 15 min, which is the optimum time for maximum solubilization in the organic phase. The tubes were centrifuged at 4000 rpm for 15 min to obtain clear phases and placed in a temperature-controlled bath at 25 °C for 2 hours prior to analysis.

The extraction efficiency (%$E$) is defined and calculated as shown in Eqn (1):

$$\%E = 100\frac{(C_{\rm o}V_{\rm o})}{(C_{\rm i}V_{\rm i})} \quad (1)$$

where $C$ and $V$ are molar concentration and volume, respectively. Subscript '**o**' refers to the organic phase after it was brought into contact with the initial ('**i**') aqueous phase.

Concentrations of the analytes in the organic phase after extraction ($C_{\rm o}$) are determined by HPLC through direct injection without previous separation of the surfactant. Calibration curves were made for each solute using the external standard procedure. Five levels of calibration were made and each level was an average of three replicates. Linearity of the calibration curves was confirmed by the Fischer test.

The amount of dissolved water in the micellar phase was determined three times for each sample by Karl–Fischer titration and the averaged parameter $W_{\rm o}$ and their respective standard deviations were calculated.

## RESULTS AND DISCUSSION

### Solubility behavior of the studied compounds in different solvents and in the micellar system

The selected compounds shown in Fig. 1 have different solubilities in water and cover a wide lipophilic range. Shikimic acid, gallic acid and gallotannic acid are very water-soluble compounds, rutin is slightly soluble and quercetin is almost insoluble.

None of the studied compounds are soluble in pure heptane. However, the presence of the surfactant AOT allowed their solubilization in the organic phase. As expected, solubility increases with the amount of surfactant. Nevertheless, in the 'phase transfer' experiments performed at concentrations higher than 0.1 M AOT the aqueous phase becomes quite turbid after 15 min of centrifugation, independently of the cation type and concentration. Thus, further studies were performed at that concentration of surfactant.

### Chromatographic behavior of the studied compounds

Because the studied compounds were eluted under reverse-phase conditions, their elution order is directly related to their hydrophobicities or water solubilities. Thus, from the chromatograms obtained (not shown) it is possible to conclude that shikimic acid and gallic acid are the most hydrophilic compounds (they are the first eluted solutes) whereas gallotannic acid has an intermediate hydrophilicity (mainly due to its big molecular volume) and rutin and quercetin (the last eluting compounds) are hydrophobic compounds. This behavior agrees with their water solubility, as explained before. These conclusions will be useful to interpret the extraction efficiencies obtained with the micellar system.

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## Salt effect on the formation of the microemulsions and aqueous pool size

The effect of $Li^+$, $Na^+$ and $K^+$ as chloride salts was studied by varying their concentrations in the range 0.1–1 M in aqueous solution. With $Li^+$ concentrations lower than 0.2 M the organic phase separates into two phases: a bottom turbid phase with the corresponding chromatograms showing that it includes high amounts of surfactant as well as almost all of the initial analytes, and a top phase that consists practically of pure heptane (no peaks of the analytes were detected). Probably a Winsor III system is formed in this case,[27] i.e. oil and water phases co-existing with a surfactant-rich phase. It should be mentioned that Leodidis and Hatton[24] observed that reversed micelles are not formed when $Li^+$, $Be^{2+}$ and $Mg^{2+}$ are present in the aqueous phase and produce other different phases. This could be due to the strong hydration of these cations, which precludes stabilization of the microemulsion.

Figure 2 shows the $W_o$ values obtained by manual Karl–Fischer titration (open symbols; see caption). Each $W_o$ value is an average of three replicates. Standard deviations were low for NaCl and KCl (SD between 1.2 and 2.3, depending on the salt concentration). However, the standard deviations for LiCl were higher than for the other salts. Typical values were 5.1 for low salt concentrations and 2.5 for a salt concentration of around 1 M. The $W_o$ data obtained by other authors for NaCl and KCl are also shown for comparison (filled symbols). It can be seen that the agreement is quite good.

## Stability of the microemulsions

Figure 3 shows $W_o$ values of the microemulsion versus the salt concentration and cation type of the aqueous solution determined immediately (open symbols; see caption), and after 1 week (filled symbols) the aqueous and micellar phases were contacted. Considering the standard deviations of the $W_o$ values obtained by manual titration, it can be observed that for sodium and potassium concentrations higher than 0.2 M, typical microemulsions were obtained. The same can be concluded for high lithium concentrations (0.8 M and more). However, a dramatic instability of the formed aggregates is observed for low salt concentrations (0.1 M for NaCl and KCl and lower than 0.8 M for LiCl) and, as a consequence, a 'true', thermodynamically stable, microemulsion could not be formed. In these cases, water initially incorporated in the organic phase is almost completely expelled after 1 week. However, even for these low salt concentrations, the aggregates are stable for at least 2 days.

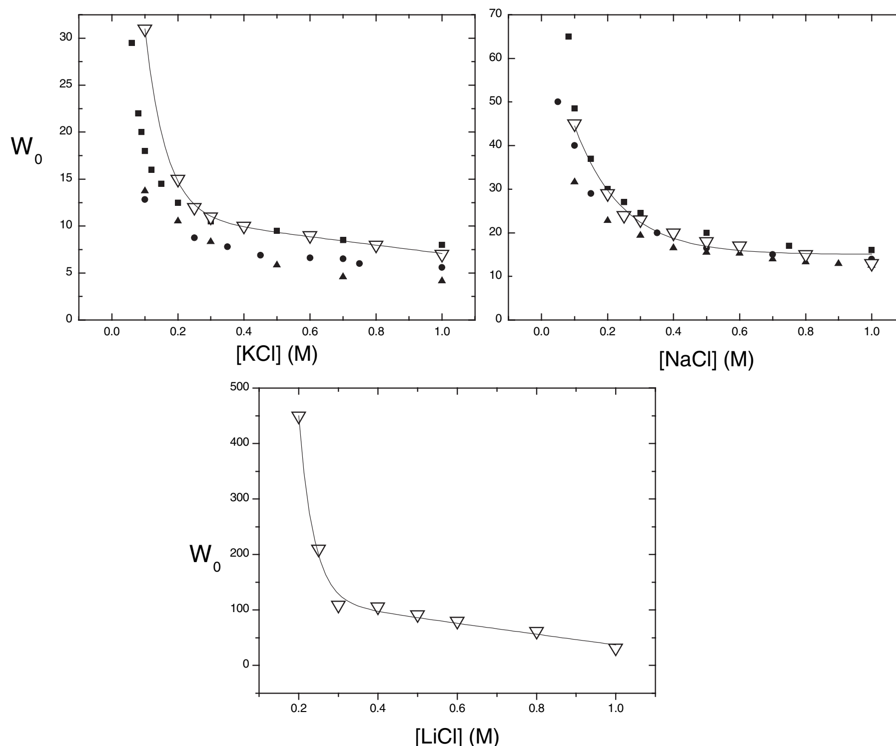


**Figure 2.** The $W_0$ value as a function of the salt concentration for KCl, NaCl and LiCl (▽). Initial organic phase: 0.1 M AOT in heptane. Comparison with values obtained by Rabie and Vera[25] (●), Nitsch and Plucinski[28] (▲) and Leodidis and Hatton[24] (■)

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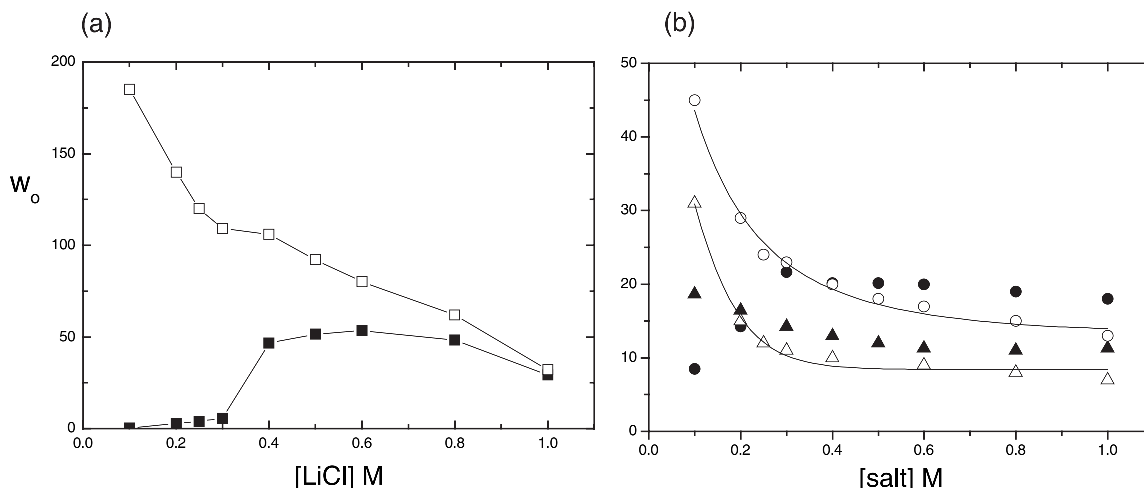


**Figure 3.** Comparison of $W_o$ obtained 2 h (empty symbols) and 1 week after (filled symbols) mixing the organic phase containing AOT with the aqueous salt solution: (a) LiCl (□,■); (b) NaCl (○,●) and KCl (△,▲)

## Partition constants as a function of the initial phase in which the compounds are dissolved

In order to check if the partitioning equilibrium for the studied compounds was achieved, the results obtained from experiments in which the solutes were initially dissolved in the water phase containing LiCl at different concentrations and 0.1 M AOT were compared with those in which the solutes at the same salt concentrations were initially dissolved in the organic phase at the same surfactant concentration. The experimental procedure was the same as that explained in the Experimental section. Partition constants $K_p$ for both 'phase transfer' experiments were calculated by determining the solute ($S$) concentrations in the micellar phase, $[S_{org}]$, and in the aqueous phase, $[S_{aq}]$, according to:

$$K_p = \frac{[S_{org}]}{[S_{aq}]} \quad (2)$$

The results are shown in Fig. 4. The agreement between $K_p$ at each salt concentration from both experiments is good only at high salt concentrations except for gallotannic acid. This means that equilibrium was reached for all solutes except for this acid. Fletcher[19] previously observed this anomalous solute behavior for murexide ion at $Na^+$ concentrations higher than 1 M. The reason for this phenomenon is not clear to us, but it could be related to the transfer kinetic process at the interface, as was observed for other solutes.[28] Nevertheless, even when no complete equilibration between both phases was reached for gallotannic acid, the results of Fig. 4 are reproducible and they did not change for at least 2 days. For gallic acid and shikimic acid, differences between $K_p$ values obtained from the experiments in which the analytes were dissolved initially in the aqueous phase and those in which they were dissolved initially in the micellar phase are only important at low salt concentrations. For rutin, $K_p$ values show almost no dependence on the starting phase in which the solute was dissolved.

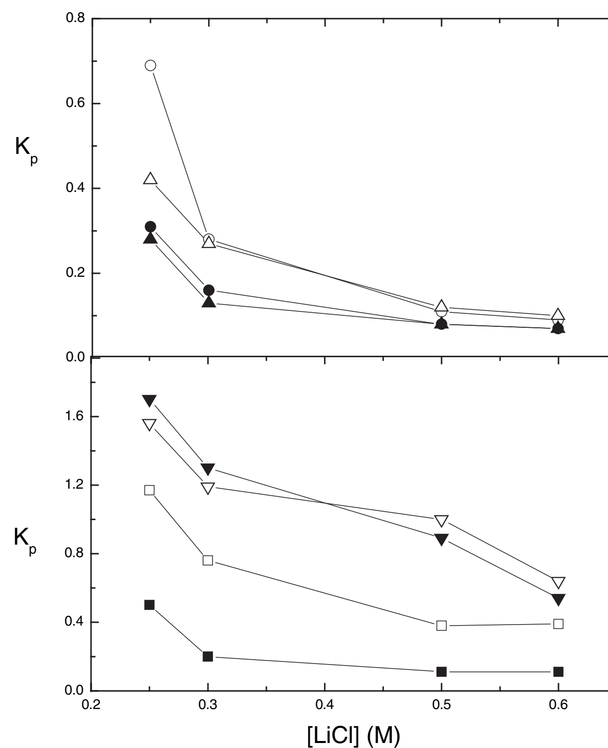


**Figure 4.** Partition coefficients, $K_p$, obtained after solubilizing the analytes initially in the organic phase (*open symbols*) or initially in the aqueous phase (*filled symbols*) as the $Li^+$ concentration is varied: Shikimic acid (○,●), gallic acid (△,▲), rutin (▽,▼) and gallotannic acid (□,■). [AOT] = 0.1 M in the organic phase

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## Extraction efficiencies versus cation type, salt concentration and pH

Figure 5 shows the $\%E$ values obtained with Eqn (1) versus $W_o$ for $Li^+$, $Na^+$ and $K^+$ at pH 3.7. The $\%E$ values obtained were an average of three replicates. Standard deviations were low for NaCl and KCl (SD around 2.3, depending on the salt concentration) and higher for LiCl (SD around 7.2). The higher standard deviations observed in $\%E$ and $W_0$ when using LiCl in the aqueous phase could be attributed to the instability of these aggregates, as discussed before.

The $\%E$ data were obtained at pH 2.4 also, but results were almost the same as those at pH 3.7, showing that not all compounds are ionized even at pH 3.7. Working at this pH prevents negatively charged hydroxy and carboxylate groups from decreasing the $\%E$ due to repulsion with the negatively charged AOT sulfonate groups.

Quercetin is completely solubilized in the micellar phase, independently of the cation type and concentration. Considering the lack of solubility both in heptane and in water, it seems that this compound is hosted at the micellar interface. For the other solutes, partition behavior varies with salt concentration as observed in Fig. 5. Shikimic acid and gallic acid show a small increase of extraction (except for $Li^+$ salts) as more water is solubilized in the organic phase, i.e. as salt concentration decreases. The large amounts of water solubilized in the $Li^+$ system produce a proportional increase of the solubilized analyte. For gallotannic acid and rutin, changes in $\%E$ are not linear and 'S'-shaped curves are obtained; this behavior was observed previously with some proteins.[29] These curves can be interpreted as follows: initially there is some dissolved solute at the interface of the micelle; as the water concentration increases the 'micelles' become a 'microemulsion', i.e. there is some free water inside the aggregate.[17] Solute concentration inside the aqueous pool should be the same as in the aqueous phase. Finally, a 'plateau' is observed because there is a maximum amount of water that can be incorporated inside the pool, which is controlled by the salt concentration.

The observed decrease in efficiency with $W_o$ can be attributed to a decrease of the micellar aqueous pool, which produces a decrease in the amount of solubilized water and analytes. Moreover, cations can be competing with the hydroxy groups of the analytes both for the negatively charged sulfonate groups and for the carbonyl groups of the surfactant. As cation concentration increases, the interaction with the analytes decreases and thus the extraction efficiency is lower. The extraction behavior for gallotannic acid when $K^+$ salts were used in the aqueous phase was dissimilar from the other solutes. Different phenomena can be cooperating to produce this final result. Although micellar size decreases with salt concentration and $K^+$ competes with the analyte for the sulfonate groups

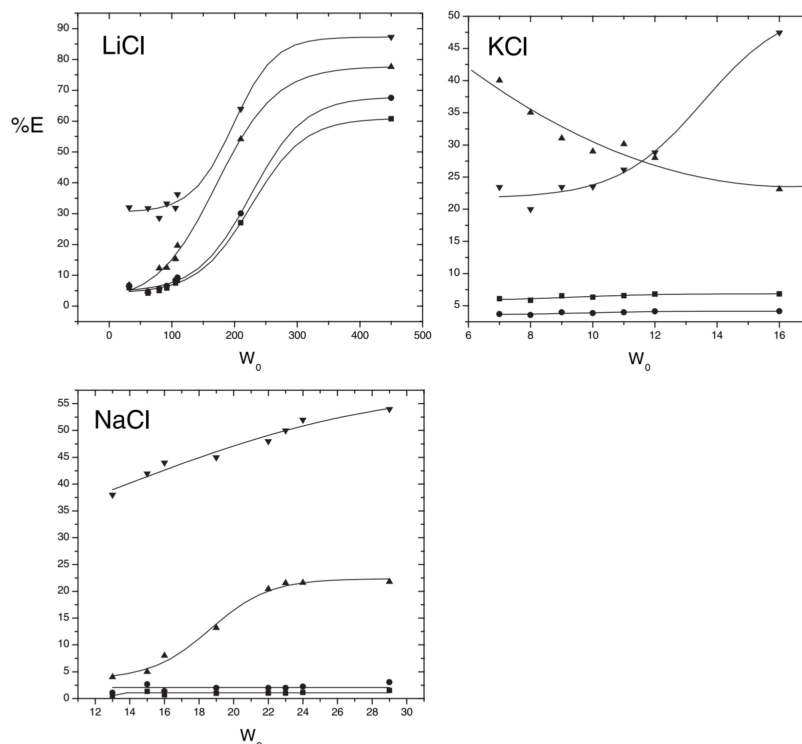


**Figure 5.** Extraction efficiencies ($\%E$) at different $W_o$ values for shikimic acid (■), gallic acid (◆), gallotannic acid (▲) and rutin(▼)

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of the surfactant, for gallotannic acid the salting-out effect (expulsion of the solute from the aqueous to the organic phase) must be contributing to a greater extent.

Although high %$E$ values are obtained at low concentrations of LiCl, this salt does not interact selectively with these very different solutes. As can be observed in Fig. 5, parallel curves are obtained, i.e. the amounts of all analytes increase or decrease in proportion with the salt concentration. When $Na^+$ and $K^+$ are used, a quite different solubilization behavior is observed. After only one contact between the aqueous and the micellar phase, 20–40% gallotannic acid is extracted into the organic phases at 0.1 M AOT, depending on the type of cation and concentration, whereas rutin, which is a very hydrophobic compound, is extracted in amounts of 30–50%. However, small hydrophilic solutes such as shikimic acid and gallic acid remain almost completely in the aqueous phase.

Gallotannic acid and rutin show greater dependence of %$E$ on $W_o$ and also on the type of cation in the aqueous phase than for the other solutes. It is expected that these relatively more hydrophobic solutes are located in the micellar interface whereas the much more hydrophilic gallic acid and shikimic acid reside in the water pool. Because the micellar interface is strongly affected by the salt concentration and cation type,[15] the solute localization in this part of the micelle will be more sensitive to those variables. In the case of the more hydrophobic compound, quercetin, this behavior is not observed because it is completely bound to the micelle interface and, as a consequence, it is not sensitive to these variables.

## Partition constants at infinite dilution as a measure of the analyte-micellar interface interactions

In order to have a quantitative measure of the interactions between the micellar interface and the analytes, Leodidis and Hatton[15] developed a solubilization model that considers the different solubilization sites in the two-phase system. Considering that the aqueous phase is always more diluted in the solutes, they obtained an expression (Eqn 3) for the partition coefficient at infinite dilution, $K^\infty$, between the micellar interface and the micellar water pools for hydrophilic solutes (amino acids) that are insoluble in the organic solvent:

$$K^\infty = \frac{55.5V_{\mathrm{ai}}}{N_{\mathrm{s}}}\left(\frac{C_{\mathrm{ai}} - C_{\mathrm{af}}}{C_{\mathrm{af}}}\right) \quad (3)$$

where $V_{\mathrm{ai}}$ is the volume of the initial aqueous phase, $N_{\mathrm{s}}$ is the number of moles of surfactant, $C_{\mathrm{ai}}$ is the concentration of solute in the initial aqueous phase, $C_{\mathrm{af}}$ is the concentration of solute in the final aqueous phase and 55.5 is the water molarity. Rearranging Eqn (3) gives:

$$C_{\mathrm{af}} = \frac{1}{(1 + K^\infty N_{\mathrm{s}}/55.5V_{\mathrm{ai}})} C_{\mathrm{ai}} \quad (4)$$

By plotting $C_{\mathrm{af}}$ versus $C_{\mathrm{ai}}$ it is easy to detect those compounds that effectively associate with the micellar interface ($K^\infty \neq 0$). If that association does not exist ($K^\infty = 0$), a unit slope straight line should be observed. Because the solutes studied in this work are not soluble in heptane and the aqueous phase containing the solutes is much diluted, Eqn (4) can be used to obtain $K^\infty$. Figure 6 shows the plots obtained when $Na^+$ and $K^+$ are used in the aqueous phase. Good straight lines are obtained except for gallotannic acid with NaCl, for which curvature is observed at high $C_{\mathrm{ai}}$. The calculated $K^\infty$ values obtained for each solute are gathered in Table 1. Because quercetin remains completely in the organic phase, $K^\infty = \infty$. As can be observed in Table 1, the association between these solutes and the interface depends upon the type of cation used in the aqueous phase. It is clear that $K^\infty$ increases in the same order as their hydrophobitities (or elution times in the octadecyl silica column), i.e. shikimic acid < gallic acid < gallotannic acid < rutin < quercetin. Thus, shikimic acid shows no interaction with the micellar interface and quercetin is strongly retained at the interface. The other compounds partition between the aqueous pool and the interface.

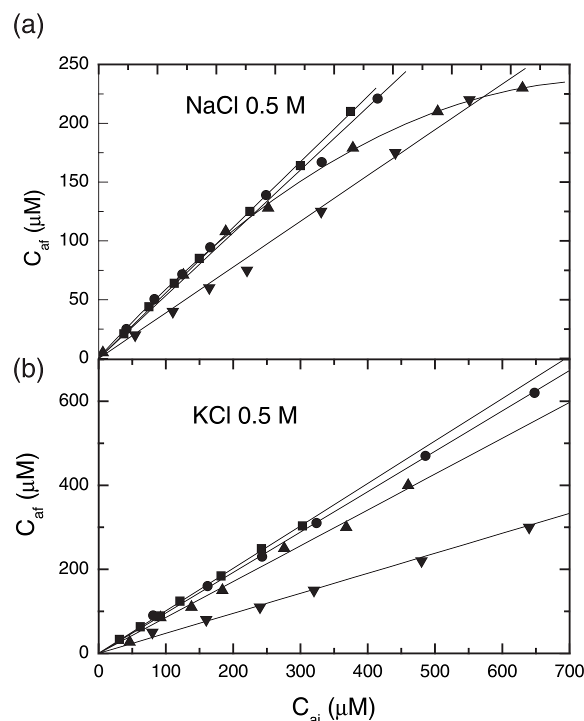


**Figure 6.** Initial concentration ($C_{\mathrm{ai}}$) versus final concentration ($C_{\mathrm{af}}$) in the aqueous phase after extraction with 0.1 M AOT in heptane: (a) 0.5 M NaCl in the aqueous phase; (b) 0.5 M KCl in the aqueous phase. Shikimic acid (■), gallic acid (●), gallotannic acid (▲) and rutin (▼)

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**Table 1.** Partition coefficients at infinite dilution, $K^{\infty}$, with 0.5 M NaCl and 0.5 M KCl

| | $K^{\infty}$ (KCl) | $K^{\infty}$ (NaCl) |
|---|---|---|
| Shikimic acid | 0 | $2.51 \pm 0.07$ |
| Gallic acid | $4.4 \pm 0.1$ | $8.0 \pm 0.2$ |
| Gallotannic acid | $18.7 \pm 0.5$ | (13.4)[a] |
| Rutin | $120 \pm 3$ | $51 \pm 1$ |

[a] Obtained with points corresponding to the linear portion of the curve (see Fig. 6a).

### Optimizing experimental conditions for separation and maximum extraction efficiencies

In every 'phase transfer' experiment reported until now we have used the same volumes for the organic and aqueous phases (i.e. 5 ml for each one). Considering the previous results, we conclude that a salting-out effect and higher surfactant concentrations favor solute transfer to the micellar phase. Because a good preconcentration factor (= [organic phase]/[aqueous phase]) is pursued, the following experiment was performed: 9 ml of an aqueous phase containing the analytes and 3 M $Na^{+}$ and 1 ml of an organic phase containing 0.30 M AOT were mixed. With these experimental conditions, small micelles will be formed and the most hydrophobic compounds should be extracted with better efficiencies. Because the aqueous phase has a high salt content, a clear phase is obtained after centrifugation even at this high surfactant concentration. Figure 7 shows the chromatograms for both phases. It can be seen that almost all shikimic acid and gallic acid remains in the aqueous phase, whereas about 30% of the gallotannic acid and 50% of the rutin are transferred to the micellar phase in only one contact. Because this amount is present in a small volume, a good chromatographic signal is observed. In light of this experiment, it seems that separations of polyhydroxy compounds of different hydrophobic nature can be achieved by changing salt type and salt concentration, as well as optimizing the micellar to aqueous volume ratio.

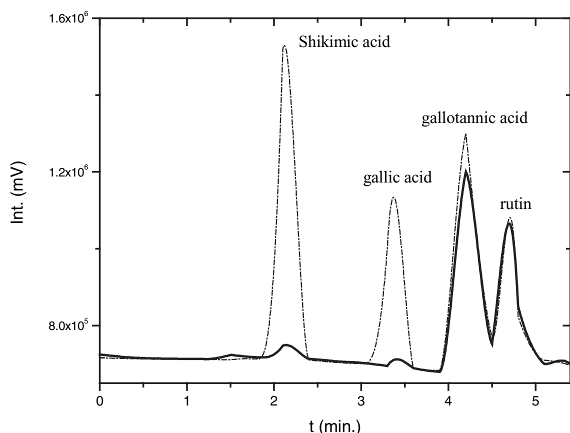


**Figure 7.** Initial aqueous phase containing the analytes and 3 M NaCl (dashed line); final organic phase (filled line) after extraction with 1 ml of 0.30 M AOT from 9 ml of the initial aqueous phase

## CONCLUSIONS

The partitioning behavior of polyphenols with very different molar volumes, polarities and hydrophobicities between an organic phase containing the anionic surfactant AOT and aqueous phases containing different salts at different salt concentrations was studied. The results show that by using 0.1 M AOT and salt concentrations higher than 0.25 M two clear phases can be obtained immediately after vigorous shaking and centrifugation. The amount of analytes in each phase was quantified by HPLC. Reproducible results can be obtained after 2 h of contact between the aqueous and micellar phases. It should be noted that the micellar phase is compatible with the HPLC mobile phase, allowing direct injection in the column without cleaning the surfactant. A solvent gradient allowed elution of all analytes in a short time and clean-up of the surfactant from the HPLC column.

The AOT allowed significant amounts of these heptane-insoluble analytes to be solubilized in the organic phase. Clear evidence of interaction between gallotannic acid, rutin and quercetin with the micellar interface was observed. Interaction with the interface, which enhances solubilization caused only by the micellar aqueous pool, was described through the partition constants at infinite dilution.

Different extraction efficiencies were observed by changing the type of cation and salt concentration. Thus, by proper selection of the extraction conditions, this method can be used to separate and preconcentrate polyhydroxy compounds and polyphenols in complex mixtures for which a typical extraction using an organic solvent alone could not be enough for complete sample resolution.


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