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*Article*

# Evaluation of the Effects of Hofmeister Series on Salting Out in the Determination of Organophosphorous Pesticides and Pyrethroids by LDS/DLLME

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Various greener microextraction procedures have been proposed with low limits of detection to control of pesticides present in the environment, including the low-density solvent dispersive liquid-liquid microextraction (LDS/DLLME) technique. The addition of electrolytes is important in order to induce the salting-out effect. Thus, it is possible to use the Hofmeister series and the Voet lyotropic number to study the effect of different ions on pesticide extraction in LDS/DLLME technique. Different anions were tested and a strong correlation was observed between the lyotropic number of the anion and the recovered volume of extraction solvent. The lyotropic numbers of the anions were successfully compared with the chromatographic peak areas normalized by the recovered solvent volumes, and similar empirical cubic relationships were obtained for all the pyrethroids evaluated. In the extraction of the organophosphorous pesticides, chlorpyrifos presented similarity with the pyrethroids, while correlation was observed between methyl parathion and profenofos.

**Keywords:** microextraction, Hoffmeister series, pesticides, lyotropic number

## Introduction

Pesticides have been used in food production since the early 20th century.[1] More recently, there has been increasing concern about their effects on humans and other organisms. Efforts are being made to control the concentrations of these substances in different environmental compartments (including water, soil, and biota), as well as to identify less toxic compounds.[2,3] Because of the need for better control of pesticides in the environment, there is also a requirement for analytical methods that offer lower limits of detection and greater sensitivity. In addition to advances in terms of instrumentation, a variety of pre-extraction techniques have been proposed, such as solid phase microextraction (SPME),[4] single drop microextraction (SDME),[5,6] dispersive liquid-liquid microextraction (DLLME),[7] dispersive liquid-liquid microextraction method based on solidification of floating organic drop (DLLME-SFO),[8,9] and low-density solvent dispersive liquid-liquid microextraction (LDS/DLLME).[9,10] These methods complies with the principles of green chemistry, because it minimizes the use of organic solvents as well as the amounts of waste generated[11,12] and they can even help to extend the useful life of older and less sensitive instruments.

In particular, LDS/DLLME was first proposed in 2009[10] and it is based on the dispersion of a small volume of a low-density organic solvent (extraction solvent), which has low miscibility with water, in an organic solvent (dispersive solvent) that is miscible with both water and the extraction solvent.

The presence of electrolyte is an important parameter to evaluate during the optimization of techniques based on solvent extraction, due to a salting-out effect.[13] In the case of LDS/DLLME, only a limited number of electrolytes have been studied, such as NaCl.[10,14,15]

The characterization of the salting-out effect, which is induced by increasing the ionic strength of the medium, is not trivial, especially considering the possibility of salting-in effects. The effects of increased ionic strength have been treated theoretically in studies of activity coefficients in aqueous solution,[16] an example being the specific ion theory (SIT), proposed by Brønsted,[17] and colloid models such as the DLVO model.[18] In another way, the ideas of Hofmeister concerning the influence of electrolytes on the solubility of proteins led to the development of the widely used empirical Hofmeister series, whereby ions are qualitatively classified according to the intensity of their influence on certain phenomena.[19] The lyotropic number, proposed by Voet in 1936,[20] is an empirical scale used to quantify the Hofmeister effect, based on the concentration of an ion

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required to cause flocculation of gelatin and agar-agar. Voet reported the correlation between the lyotropic number and various physicochemical properties, and demonstrated that this parameter can be used to quantify the Hofmeister series.

No systematic experimental studies of these effects have been reported for LDS-DLLME. The objective of the present work was therefore to evaluate the influence of different anions on the determination of pesticides in water, using the DLLME technique, and compare the results with the Voet lyotropic numbers.

## Experimental

### Reagents and standards

Toluene (99%) was obtained from Merck (Rio de Janeiro, Brazil), and acetonitrile (99.5%, HPLC grade) was from Sigma-Aldrich (Saint Louis, USA). The anions of ammonium salts used were $SO_4^{2-}$, $HPO_4^{2-}$, $Cl^-$, $NO_3^-$, obtained from VETEC (Rio de Janeiro, Brazil) and $SCN^-$ from ISOFAR (Duque de Caxias, BRAZIL). The pesticide standards cypermethrin (92.4%), λ-cyhalothrin (86.5% m/m), deltamethrin (99% m/m), and methyl parathion (99% m/m) were acquired from Syngenta (Basel, SWISS). Profenofos (92% m/m) was from Dr. Ehrenstorfer (Augsburg, GERMANY), and bifenthrin (92.2% m/m) was from FMC (Rio de Janeiro, Brazil). Stock standard solutions were prepared at concentrations of 1000.0 mg $L^{-1}$ in acetonitrile, from which working standard solutions were prepared at 10.0 mg $L^{-1}$ in acetonitrile. Dilutions of the working standards were performed as required, and all solutions were stored at approximately –20 °C.

### Apparatus and materials

Determination of the pesticides was performed using a Model 17-A gas chromatograph (GC) (Shimadzu, Kyoto, Japan) fitted with a split/splitless injector and an electron capture detector (ECD). Separations were carried out with a ZB-5 fused silica capillary column (5% phenyl methyl siloxane, 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 µm). Nitrogen was used as the carrier gas (99.999%, Air Products, Rio de Janeiro, Brazil) at a flow rate of 1.2 mL $min^{-1}$. The injection port was used in split mode (1:5) and maintained at 280 °C. The oven was programmed with an initial temperature of 150 °C, held for 1 min, followed by a ramp at a rate of 30 °C $min^{-1}$ to 210 °C, held for 1 min, and then a ramp at a rate of 30 °C $min^{-1}$ to 250 °C, held for 2 min. Finally, the temperature was increased to 290 °C at a rate of 20 °C $min^{-1}$, and held for 5 min. The total time required for a single GC run was 14.3 min. The injected sample volume was 1.0 µL. The nitrogen (99.999%) used as the makeup gas for the ECD was passed through sequential molecular sieve and oxygen traps (CRS, Inc.), at a pressure of 55 kPa. The extraction process employed a vortex mixer (Model CERTOMAT MV, B. Braun Biotech International, Melsungen, Germany) and a centrifuge (Model 222-T28, Quimis, Rio de Janeiro, Brazil). The chromatographic data were managed by software Shimadzu GC Solution.

### Sample preparation

Samples of distilled water were fortified with working standard solutions (10.0 mg $L^{-1}$) in volumetric flasks, to give final concentrations of 5 µg $L^{-1}$. Each experiment employed a 5 mL volume of sample.

The extraction protocol was the rapid injection of an extraction mixture consisting of 100 µL of toluene and 500 µL of acetonitrile into a test tube with screw cap with 5.0 mL of the aqueous solution fortified with the pesticides and containing an electrolyte at a concentration of 2.0 mol $L^{-1}$. After vortex agitation for 1 min (to guarantee a high repetitivity of response) of and centrifugation at 3200 rpm (2290 g) for 5 min, the organic phase was removed with a syringe and 1 µL was injected onto the column of a gas chromatograph fitted with an electron capture detector (GC/ECD).

## Results and Discussion

### Volume recovered in the DLLME procedure

The volume of toluene recovered after the addition of 100 µL in the DLLME procedure was influenced by the type of anion present in the aqueous solution. There was a strong correlation between this recovered volume and the lyotropic number (Figure 1), with a coefficient of determination value of 0.952 (significant at the 95% confidence level). This result suggests the influence of the anion on the partitioning of acetonitrile between toluene and water, with the ratio between the recovered and added volumes of toluene varying from 0.92 (in the presence of thiocyanate) to 1.75 (in the presence of sulfate).

The regression model obtained was also used to determine the lyotropic numbers for the hydrogen phosphate ion (3.26) and for water in the absence of electrolytes (10.3).

The hydrogen phosphate ion value was similar to the value reported for orthophosphate (3.2),[18] and the water value was in agreement with the value obtained by Voet (9.8).[19] The increase in the volume of the organic phase

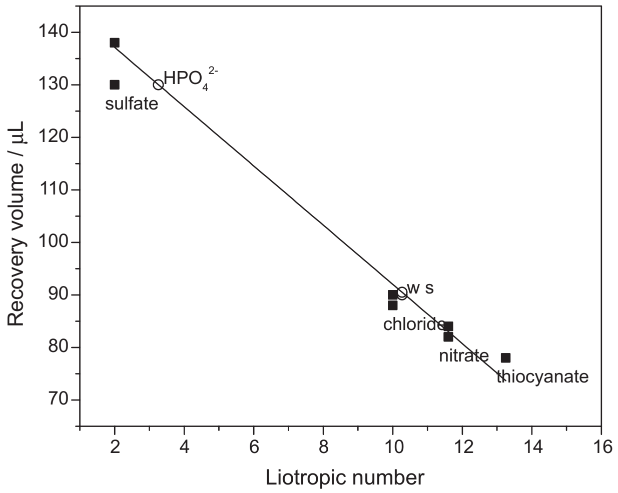

**Figure 1.** Influence of anion lyotropic number on the recovered volume of toluene. The lyotropic numbers of $HPO_4^{2-}$ and water (WS) were obtained from the regression model.

could have been due to reduced solubility of acetonitrile in water (a partitioning effect), and suggested greater interaction of the water with the anions sulfate and hydrogen phosphate, compared to acetonitrile. On the other hand, anions with lyotropic numbers smaller than that of water influenced the solubility of toluene in water (albeit to a lesser extent), hence explaining the reduction in the volume of the organic phase.

## Extraction of analytes

### Pyrethroids

The influence of the different anions on extraction of the analytes was studied by comparing the chromatographic peak areas (normalized by dividing the areas by the recovered volumes) with the lyotropic numbers. In the case of the pyrethroids (Figure 2), these parameters showed similar behavior, with an increase in the corrected chromatographic area corresponding to an increase in the lyotropic number.

For all the analytes (Table 1), the data could be fitted using an empirical cubic model (equation 1), with coefficients of determination greater than 0.86. Differences between the values obtained for the analytes were related to differences in the sensitivity of the detector for the individual pyrethroids. In equation 1, Y is the corrected peak area and x is the lyotropic number.

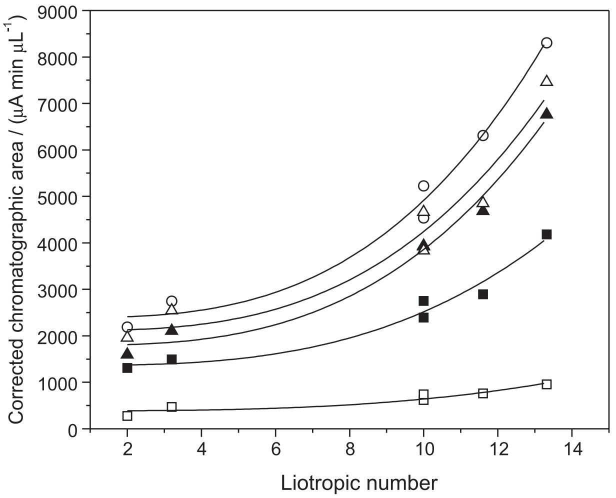

**Figure 2**. Influence of the anion lyotropic number on the corrected chromatographic peak areas for the pyrethroids λ-cyhalothrin (○); bifenthrin (■); permethrin (□); cypermethrin (▲); and deltamethrin (△). Samples volume: 5.0 mL (5 µg L$^{-1}$); extraction mixture: 100 µL of toluene and 500 µL of acetonitrile; electrolyte concentration: 2.0 mol L$^{-1}$.

$$Y = a + bx^3 \quad (1)$$

This relationship shows the way in which the Voet lyotropic number can be used to describe the Hofmeister series. The results obtained can be partially explained by changes in the polarity index of the organic phase (which is a weighted average of the polarity indices of its components). The polarity index increased for higher concentrations of acetonitrile in the organic phase because the polarity index of acetonitrile (6.2) is greater than that of toluene (2.4), hence lowering the partitioning coefficients of the analytes.[21]

The similarity in behavior of the different pyrethroids (Figure 1) was supported by the fact that the same regression equation could be used for all the compounds. Further confirmation was provided by constructing a Pearson correlation matrix using the corrected peak areas obtained for each pesticide. Statistically significant correlations were obtained between all the pyrethroids ($0.92 < r < 0.99$; $p < 0.05$).[22]

**Table 1.** Parameters of the nonlinear regression model (equation 1) obtained for the pyrethroid pesticides bifenthrin (BFT); λ-cyhalothrin (CLT); permethrin (PMT); cypermethrin (CPT); and deltamethrin (DMT)

| | BFT | CLT | PMT | CPT | DMT |
|---|---|---|---|---|---|
| $a \pm s_a$ | 1363.7 ± 1.2 | 2391.1 ± 2.5 | 387.01 ± 0.25 | 1793.8 ± 2.1 | 2112.9 ± 2.1 |
| $b \pm s_b$ | 132.88 ± .10 | 195.02 ± 0.15 | 56.46 ± 0.04 | 161.98 ± 0.13 | 320.57 ± 0.25 |
| $R^2$adjusted | 0.9609 | 0.9821 | 0.8649 | 0.9815 | 0.9347 |

$s_a$, $s_b$: standard deviation of the parameter a and b of the regression model, respectively; $R^2$: coefficient of determination.

### Organophosphorous pesticides

The behaviors of the organophosphorous pesticides in the DLLME procedure also differed according to the anion used. Pearson correlation analysis revealed a significant correlation of chlorpyrifos with the pyrethroids ($r > 0.84$). For methyl parathion and profenofos, the effects of the presence of the ions chloride and thiocyanate in the solution differed from the predictions of the cubic model (Figure 3).

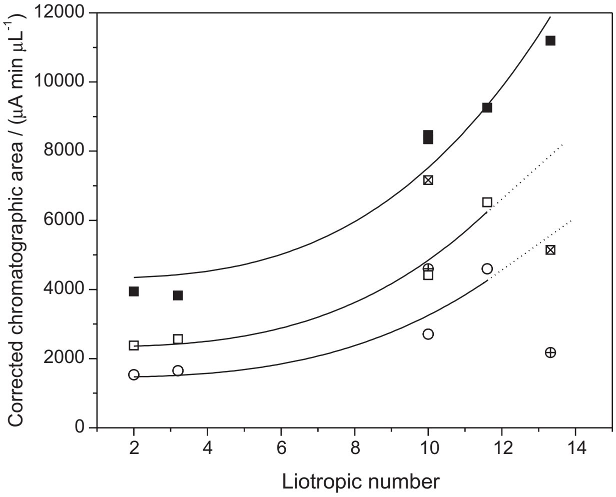

**Figure 3.** Effect of the anions in aqueous solution on DLLME for the organophosphorous pesticides chlorpyrifos (■); methyl parathion (☐); and profenofos (○). Points marked with ☒ (methyl parathion) and ⊕ (profenofos) were not included in the cubic model. Samples volume: 5.0 mL (5 µg L$^{-1}$); extraction mixture: 100 µL of toluene and 500 µL of acetonitrile; electrolyte concentration: 2.0 mol L$^{-1}$.

Strong correlation between methyl parathion and profenofos ($r > 0.92$) suggested that there was also similarity between the behaviors of the ions chloride and thiocyanate. The findings related to the behavior of these two ions are important, because they show that other factors, in addition to those involved in the Hofmeister series, can also exert an influence on analyte behavior. This could help to explain behaviors reported previously, where, for example, the salting-out effect of chloride during extraction procedures has been observed for some analytes, but not for others.[23,24]

## Conclusions

Ion-specific effects exert an important influence on the efficiency of pesticide extraction using DLLME. The partitioning of acetonitrile between an aqueous solution and toluene could be explained by a linear model, enabling estimation of the Voet lyotropic numbers for hydrogen phosphate and for water. A relationship was observed between the lyotropic number of the anion and the efficiency of extraction of the pyrethroids and organophosphorous pesticides, using a cubic model applied to the corrected chromatographic peak areas. The data obtained for most of the ions and pesticides could be fitted by the model, with the exception of chloride and thiocyanate used in the extraction of methyl parathion and profenofos. The findings demonstrate the importance of evaluating the electrolytes used in DLLME, as well as the value of employing the Hofmeister series during optimization of this preconcentration technique.

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