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Revisiting large volume injection in non-miscible diluents: an on-line reversed phase supported liquid extraction/liquid chromatography scenario†

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According to some recently published studies, large volume injection (LVI) of diluents immiscible with the eluents used in reversed phase liquid chromatography (RPLC) appears to be feasible, despite the widely accepted rules governing injection related phenomena. The process was previously described and was successfully applied in practice. The present study describes a simple theoretical model explaining LVI of diluents immiscible with the mobile phase in RPLC; the model relies on the on-line coupling of reversed phase supported liquid extraction (RP-SLE) to the chromatographic separation. The compliance of the theoretical model with experimental observations was tested by using data collected for LVI of a homologous series of *para*-hydroxy alkyl benzoates (methyl, ethyl, propyl, butyl, pentyl, hexyl and octyl congeners) in liquid alkanes (hexane, heptane, iso-octane, decane and dodecane). The SP consisted of an octadecyl chemically modified silica gel eluted with a mixture of acetonitrile : water 4 : 6 (v/v). Although the model mainly focuses on explaining the linear reduction of the retention time with the injected volume, some aspects relating to zone spreading and thermodynamic aspects are also discussed.

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1. Introduction

Large Volume Injection (LVI) in chromatography relates to the continuous challenge of increasing method sensitivity.^{1,2} Injection phenomena arising in liquid chromatography (LC) were extensively studied with respect to the strength³⁻⁷ and viscosity⁸⁻¹⁰ of the sample diluent (D) and their recurrent impact on the chromatographic parameters illustrating retention, band spread, and peak shape. The injection produces a thermodynamic disturbance in the chromatographic system. Column loadability in a LVI complex phenomenon depends directly on its intrinsic characteristics (length, internal diameter, particle size, and phase ratio), mobile phase (MP) composition and elution conditions, D characteristics, and the specific properties of the analytes (A). LC injection phenomena should be also considered in a close relationship with the widely applied sample preparation methods, primarily delivering the target compounds in a wide variety of organic solvents.¹¹ Moreover, removal of solvents producing unwanted phenomena in LC and

their replacement by adequate ones strongly impacts on the characteristics of the sample preparation approaches and directly influences the overall accuracy and precision.

The possibility of using D immiscible with the MP in RPLC was already demonstrated.¹²⁻¹⁴ The process was successfully applied in the pharmaceutical field¹⁵⁻¹⁹ and in bioanalysis.²⁰⁻²⁴ Injection in immiscible D was also used for estimation of the hydrophobic characteristics of a series of pharmaceutical compounds.²⁵ However, determination of the retention corresponding to a hypothetical condition assuming the absence of the D is more related to the intrinsic behavior of the analyte in the chromatographic column, rather than an illustration of injection related phenomena.

The possibility of introduction in the chromatographic column of large volumes of samples in immiscible D to the MP was explained through the competition equilibria between the analyte and the D molecules for the adsorption sites in the stationary phase (SP).¹² Consequently, the process becomes experimentally possible, without affecting peak shapes, if the affinity of the D for the SP is higher than that of the analyte. As a direct consequence, a part of the SP in the column's head becomes unavailable for the compounds contained in the sample, their retention linearly decreasing with the increase of the injection volume. It results that an essential condition for achieving LVI of immiscible D in RPLC is an increased hydrophobic character of the D compared to analytes. The process generally occurs with a loss in the peak efficiency, although band focusing was also reported in some specific conditions.¹³

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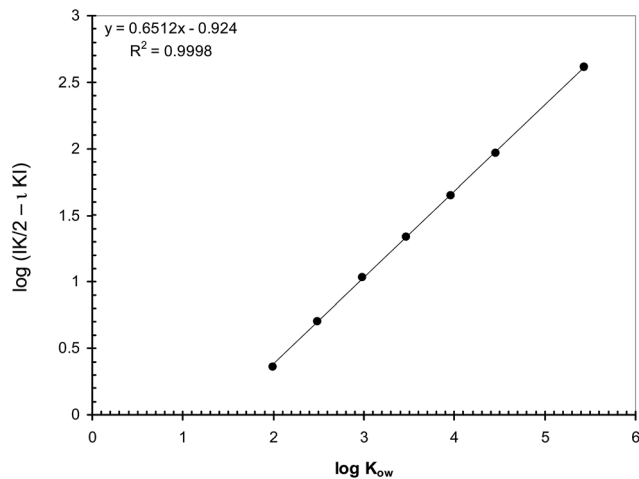


Fig. 5 Functional relationship established between the absolute values of the slopes of the linear regressions relating the apparent retention factor (k_{app}) to the reduced injected volume ($V_{inj}V_0^{-1}$) and the log K_{ow} of the studied compounds.

injection of 1 μ L solution in methanol (accounted only for the chromatographic separation process) is added to the plot, the correlation coefficient is reduced to 0.9609.

One can conclude that the thermodynamic study revealed the existence of the distinctive LLE and the RP chromatographic stages. As both stages are based on similar interactions of the analyte distributed between a hydrophobic phase (D and SP) and the MP, van't Hoff plots remain linear. The slight increase of the enthalpy on increasing the D volume is well compensated by the entropy change. Through analyzing the compensation plots, the small difference between the chromatographic separation taken alone and the on-line coupling between RP-SLE and RPLC resulted in a reduction of the correlation coefficients. A large entropy change indicates that the solute molecules are retained with less random movement in the SP than they were in the D layer.

3.5. Hydrophobicity indicating scale

From eqn (19) it results that the slope of the linear regression $k_{app} = f(\alpha)$ depends on the retention factor of the model compound in the chromatographic column and its partition constant from the MP to the D (K). Consequently, the slope of the linear regression should globally express the affinity of the analyte towards hydrophobic media (the D and the SP), or towards the hydrophilic MP. Thus, a correlation between the log K_{ow} of the analytes and the slope of the linear regression $k_{app} = f(\alpha)$ should be observable. Fig. 5 illustrates the correlation between the calculated log K_{ow} values of the target compounds and the logarithms of the slopes of the linear regressions $k_{app} = f(\alpha)$, taken as absolute values, when using heptane as D on injection. One can observe the excellent correlation between the two sets of data.

Similar correlations were established between log K_{ow} and the logarithms of the absolute slopes resulting from the injection of all analytes in the other D (see the ESI,† Part 7). In fact,

the data generated in all D are similar, as long as their hydrophobic characteristics are similar with respect to the hydrophobic characteristics of the SP. It appears that the slope of the linear relationship between k_{app} and α may successfully act as a hydrophobicity descriptor.

4. Conclusions

Large volume injection (LVI) of immiscible D in reversed phase liquid chromatography (RPLC) may be successfully described by means of a reversed phase supported liquid extraction process (RP-SLE) on-line coupled to the chromatographic separation. The simple mathematic model being developed fully explains the linear relationship experimentally established between the apparent retention factor (k_{app}) and the reduced injected volume ($\alpha = V_{inj}V_0^{-1}$). The model is based on the immobilization of the D plug immediately after its transfer in the column's head, followed by the formation of MP channels. The formation of MP channels leads to inflation of the D plug and allows liquid-liquid extraction of the analytes. The chromatographic separation follows the SLE process in the part of the column remaining after inflation of the D plug up to its maximal extent. The model allows the calculation of the column cross-section occupied by the D. The model was verified on a homologous series of *para*-alkyl hydroxybenzoates, using hexane, heptane, iso-octane, decane, and dodecane as D. A rapid liquid-liquid extraction from the D in the MP leads to analyte focusing and improves band spreading in the case of rapidly eluting compounds, as compared to the situation of injection of large volumes of samples dissolved in the MP. For analytes poorly extracted from the D by the MP, band spreading is limited by re-focusing in the SP. It was experimentally proven that D saturation of the MP is not responsible for the retention factor reduction under the given conditions. The SLE and RPLC processes consecutively acted according to the proposed mechanism, and are observable in the enthalpy-entropy compensation plots, if compared to the chromatographic separation conditions taken alone. It also results that the logarithm of the slope of the linear relationship between k_{app} and α , taken as the absolute value, may successfully act as a hydrophobicity descriptor. LVI of immiscible D in RPLC is also one of the most direct proofs that the chromatographic retention mechanism is based on adsorption. Under the experimental conditions, the adsorption based chromatographic retention is "quenched" by the D.

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