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A chemometric study of amino acid transport through liquid membranes using UV-vis spectroscopy

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The extraction of amino acids at 298 K through hydrophobic liquid membranes is studied using the bulk liquid membrane (BLM) method. The transport of different mixtures of phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) in a buffer solution (pH 1.5) was monitored with a diode array spectrometer by recording their UV-vis spectra at the receiving phase in small time intervals for 30 min. The apparatus used consisted of a Schulmann cell with the buffer solution as the source phase, reverse micelles of Aerosol-OT (AOT) in chloroform as the liquid membrane, and a buffer solution at pH 9.0 as the receiving phase. The ordinary least squares (OLS) method was applied separately to each mixture, using the experimental spectra of pure compounds. The transport rates, which are given by the slope of the kinetic curves, were found to be very small, of the order of 10^{-5}, 10^{-6} and 10^{-7} mol l^{-1} min^{-1} for Phe, Trp and Try respectively. The trilinear decomposition (TLD) method was used to estimate the kinetic profiles for each compound in the ternary mixtures and also their transport rates. They are in fair agreement with those obtained by OLS. Tyr basically does not cross the membrane, so two new experiments were carried out using binary mixtures of Phe and Trp. Their transport rates are of the same order of magnitude as those obtained for the ternary mixtures, and in this case the agreement between TLD and OLS results is excellent. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: multivariate analysis; OLS; TLD; phenylalanine; tyrosine; tryptophan

1. INTRODUCTION

Membrane transport is a fundamental and essential process in many biological systems, and its modelling has been actively studied. This kind of transport can be realized by simple diffusion or more actively by using a carrier [1]. Carrier-mediated transport through liquid membranes, which draws inspiration from the phenomenon of facilitated transport in biology, e.g. oxygen transport through haemoglobin, has frequently been used for separation of ions [2]. In some studies the carrier molecules are added to the non-aqueous (membrane) phase, which is immiscible with both aqueous (source and receiving) phases. In particular, many kinds of synthetic carriers such as crown ethers and cryptands have been used as potential carriers for transporting alkali metal and organic ammonium cations [3]. The carrier molecules used in such studies are invariably surface-active in nature and have both hydrophilic and hydrophobic domains in their structure [1].

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In marked contrast, little attention has been directed towards the transport of anionic species such as amino acids (carboxylate, phenolate and thiolate anions) and ATP (phosphate anion), which are important from the biochemical and medical points of view. Hence the development of a new type of carrier capable of transporting these organic anions is required, not only to simulate many biological systems but also as a new methodology in separation science [3].

In recent years, several transport studies involving a completely new type of mobile carrier, namely micelles or microemulsion globules, have been reported in the literature [4].

The fact that water-in-oil microemulsion-reversed micelles do act as carriers has been demonstrated by Tondre and Xenakis [5]. The authors came to the conclusion that loading and unloading of microemulsion droplets with substances to be transferred through liquid/liquid interfaces do not obey a general mechanism, but may vary from system to system depending upon the nature of the surfactant and liquid membrane. These carriers are complicated entities, consisting of plurimolecular assemblies of surfactant molecules [6]. Two types of surfactants (anionic and cationic) have been commonly employed to form reverse micelles for studies of amino acid and protein extraction from aqueous solutions.

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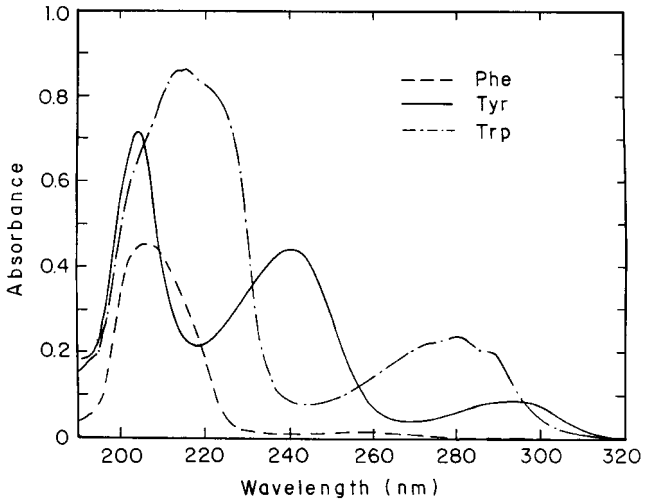

Figure 1. UV-Vis spectra of phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp).

Aerosol-OT, an anionic surfactant, has been used in the low-pH range for the extraction of proteins and amino acids [5] in their cationic state.

In this work we determine the transport rates of mixtures of three amino acids, phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp), when being transported in a bulk liquid membrane system using reverse micelles of the surfactant Aerosol-OT.

The ordinary least squares (OLS) method is applied separately to each transport experiment, using pure spectra to estimate the kinetic profiles. The kinetic profiles are also estimated by using the trilinear decomposition (TLD) method for different sets of experiments. The transport rates, which are given by the slope of the kinetic curves, are then calculated from OLS and TLD results and compared to each other.

2. CHEMOMETRICS

Modern analytical chemistry has benefited from the development of second- and higher-order instrumentation, i.e. instruments capable of providing bi- and multidimensional

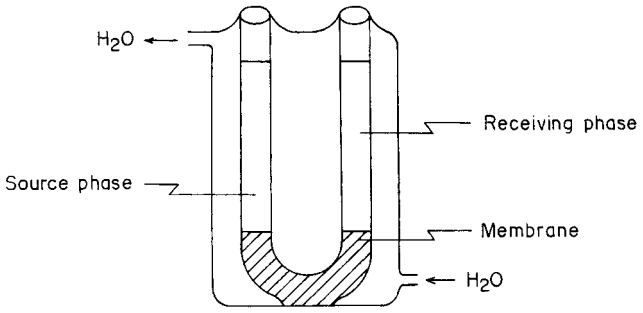

Figure 2. Schulmann's cell.

data arrays, which usually result in a substantial improvement in analytical capability. Second-order instrumentation relies on two separate analytical mechanisms linked in series such that the signal of the latter is modulated by the former, generating a second-order tensor or a data matrix for each single experiment. This is the case for the so-called bilinear hyphenated techniques such as UV-vis DAD, LC/UV-vis, LC/MS, GC/MS, GC/FTIR and other techniques such as two-dimensional excitation–emission fluorometry, multichannel detection spectroscopy titration and flow optical sensors [7]. This way, the data matrix for a single experiment contains information on both spaces and can be written as

$$\mathbf{R} = \mathbf{X}\mathbf{Y}^{\mathrm{T}} \tag{1}$$

where $\mathbf{R}$ is the bilinear matrix and $\mathbf{X}$ and $\mathbf{Y}$ are matrices whose columns correspond to different analytes in both spaces.

In our previous work it was shown that UV-vis diode array spectrophotometers are ideal for following experiments on transport through membranes [8]. In that work the transport of potassium through a chloroform membrane was studied using ternary mixtures of the isomeric anions 2,4-dinitrophenolate and 2,5-dinitrophenolate together with 2-nitrophenolate, using a crown ether as the carrier. In that example, $\mathbf{X}$ and $\mathbf{Y}$ were three-column matrices containing the pure UV spectra and the kinetic profiles of the three compounds respectively. Using the three-way method TLD [9], the spectra of these pure components could be resolved

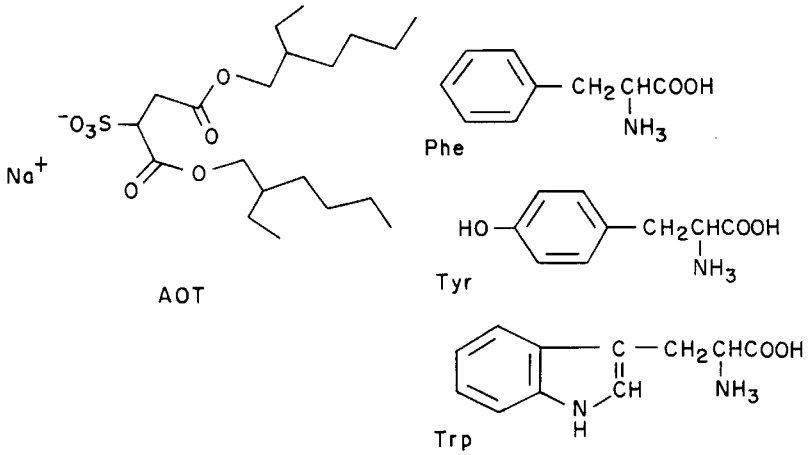

Figure 3. Chemical structures of AOT and amino acids.

Table I. Concentrations (10^{-3} mol l^{-1}) of amino acids used for each transport experiment

Exp.	[Phe]	[Tyr]	[Trp]
1	4.0	2.0	2.0
2	4.0	2.0	4.0
3	2.0	4.0	4.0

All solutions were at pH 1.5.

and the kinetic profiles obtained. From the kinetic profiles the transport rates could be calculated.

TLD performs a triadic decomposition of the data set, obeying the trilinear model described by the equation [9]

$$R_{ijk} = \sum_{n=1}^{N} X_{in} Y_{jn} Z_{kn} + E_{ijk} \quad (2)$$

where R_{ijk} is an element of the third-order array which represents the intensity from the kth experiment taken at the jth time and for the ith frequency, and N is the number of factors in the model. **X**, **Y** and **Z** are matrices describing the pure spectra, the kinetics and the relative contributions of each constituent in the sample respectively. The trilinear model can be set as an eigenvalue–eigenvector problem (solved by the QZ algorithm) if each slab $\mathbf{R}_k$ follows a bilinear model. As a bilinear device, the response profiles in both domains arising from each chemical compound must be unique and independent of the presence of other species [9]. In this case the problem has a unique solution and the rank of each pure component matrix is one in the absence of noise, making the number of pure components equal to the total rank (N). In the presence of spectral non-linearities and noise the rank of each pure component might no longer be one, but some deviations can be accounted for, using extra factors. In this work, each slab contains spectral data from a mixture taken at time j. The pure spectra are linearly

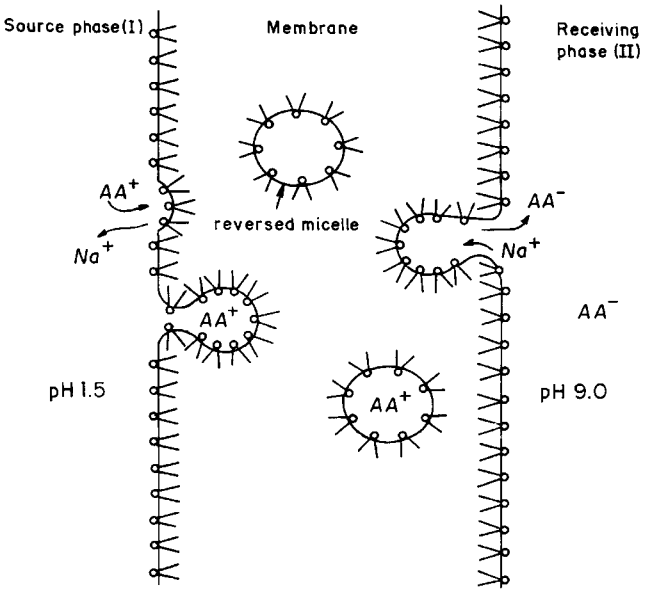

Figure 4. Mechanism of transport of amino acids through a liquid membrane.

Table II. OLS transport rates (10^{-5} mol l^{-1} min^{-1}) for amino acids

Mixture[a]	Phe	Tyr	Trp
1	0.73	0.05	0.31
2	0.85	0.05	0.38
3	0.61	0.06	0.30

[a] k = 1, 2, 3 (see Equation (3) in text).

independent (**X** space), because the species are stable during the time of detection and the spectral profile for each does not change with time, since no reaction or decomposition occurs during the experiment. The experiment is processed in the low-concentration range where, in principle, there is no interaction among the species. In the temporal domain (**Y** space) the transport behaviour of each compound is expected to be independent of the others, since they are transported in different regions of the reverse micelles.

Since the spectra of the pure components to be transported were also recorded, it was possible to obtain their kinetic profiles by applying the OLS method for quantification. For the pure spectrum **r** of a solution of one compound in a well-defined concentration y it is possible to determine its values of molar absorptivity **x** at all wavelengths by Beer's law $[\mathbf{r}] = [\mathbf{x}]y$, which is, in fact, one column of the **X** matrix.

Once **X** is available, the concentrations of all compounds present in the unknown interferent-free mixtures can be estimated by standard linear algebra:

$$\mathbf{Y}_k^{\mathrm{T}} = (\mathbf{X}_k^{\mathrm{T}}\mathbf{X}_k)^{-1}\mathbf{X}_k^{\mathrm{T}}\mathbf{R}_k \quad (3)$$

where $(\mathbf{X}_k^{\mathrm{T}}\mathbf{X}_k)^{-1}\mathbf{X}_k^{\mathrm{T}}$ is the pseudoinverse of **X**. For the kth transport experiment, $\mathbf{Y}_k^{\mathrm{T}}$ is the concentration matrix where each row contains the kinetic profile of one pure compound.

3. EXPERIMENTAL

Phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) employed in this kinetic study are the only amino acids which present absorbance in the UV-vis region. Tyr and Trp show spectral differences in this range of wavelengths, in spite of a strong overlap, as shown in Figure 1. On the other hand, the Phe spectrum is completely overlapped.

In these experiments a Schulmann cell (U-tube, shown in Figure 2) was connected to a continuous flow system consisting of an HP 8453 diode array spectrophotometer, a peristaltic pump, a magnetic stirrer and a thermostatted water bath. The liquid membrane was a 1.0×10^{-3} mol l^{-1} solution of sodium bis[2-ethylhexyl(sulphosuccinate)] (Aerosol-OT (AOT)) in chloroform. An aqueous solution of pH 1.5 was the source phase containing the mixtures of amino acids to be transported. Spectra of the receiving phase

Table III. Concentrations (10^{-3} mol l^{-1}) used for binary mixtures of amino acids

Mixture	Phe	Trp
4	2.0	4.0
5	4.0	4.0

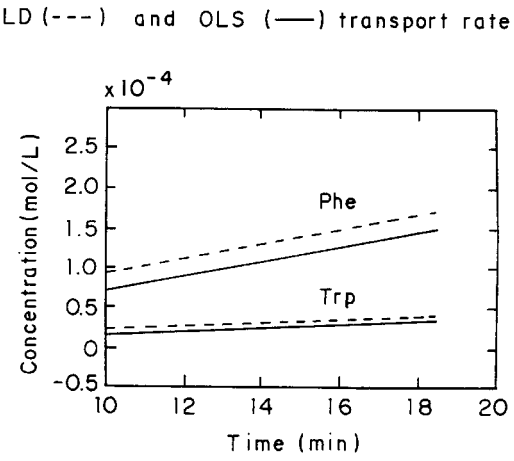

Figure 5. OLS and TLD estimated kinetic profiles for binary mixtures. (a) $k = 4$ for OLS and $k = 4,5$ for TLD (see Equations 2 and 3 in text).

(an aqueous solution of pH 9.0) were recorded at 30 s intervals for 30 min, in the range 217–308 nm with 1 nm increments, and arranged as a 91×60 data array. In this work, experiments involving simultaneous transport of all three amino acids and of binary mixtures containing only Phe and Trp were carried out. The spectra of pure components were also recorded.

The calculations were done with MATLAB (Math Works) version for MS-Windows. The TLD algorithm used in this work was based on the one given by Sanchez and Kowalski [9].

4. RESULTS AND DISCUSSION

The chemical structures of all compounds used are shown in Figure 3. Table I lists the concentrations of ternary mixtures of these amino acids in each of the solutions employed as source phase.

The transport mechanism is the following (see Figure 4). At the interface between the aqueous source phase (I) and the organic phase (membrane) an anionic and lipophilic surfactant such as AOT binds the cationic species of an amino acid (AA^+) via electrostatic interaction, extracting it into the organic phase. AOT is a typical surfactant that aggregates in the apolar medium to form reverse micelles [10–12], as can be seen in Figure 4. Hydropathy is a parameter that combines hydrophobicity and hydrophilicity [13] and allows one to predict which amino acids will be

Table IV. OLS and TLD transport rates (10^{-5} mol l^{-1} min^{-1}) for binary mixtures of amino acids

Mixture	Phe	Trp
OLS		
4	0.92	0.22
5	0.90	0.22
TLD		
45[a]	1.06	0.22

[a] TLD applied to experiments 4 and 5 ($k = 4,5$ see Equation (2) in text).

Table V. TLD transport rates (10^{-5} mol l^{-1} min^{-1}) for amino acids

Mixture	Phe	Tyr	Trp
12[a]	1.93	0.004	0.16
13	1.06	0.01	0.12
123	1.09	0.01	0.13

[a] TLD applied to experiments 1 and 2 ($k = 1, 2$; see Equation (2) in text).

found in an aqueous environment (negative values) and which will be found in a hydrophobic environment (positive values) of micelles. Its values for the amino acids used in this work are 2.8 (Phe), −0.9 (Trp) and −1.3 (Tyr), indicating that they tend to occupy different regions in the micelles and that little competition takes place in the process of transport. Thus, depending on its electric charge and hydrophobicity, the amino acid can be solubilized preferentially in the water core, or at the interface between water and the surfactant layer, or right in the amphiphile palisade [10–12]. The amino acid, which is in cationic form inside the reverse micelles, is released to the receiving phase (II) by an exchange reaction with a cation through an antiport mechanism.

The kinetic profiles **Y** of the three pure components were obtained by the OLS method from each 91×60 data matrix using Equation (3). The first few measurements were discarded, since the concentrations were too low to be accurately detected.

The concentration at the receiving phase is expected to vary linearly with respect to time [14]. The rates of transport, given by the slope of the kinetic curves, were calculated and the results for these three experiments are given in Table II. Comparing the numerical values, it can be seen that the transport rates obtained are very low and have the same order of magnitude for each compound. Phe is transported fastest, having a rate of the order of 1.0×10^{-5} mol l^{-1} min^{-1}. Trp is about 10 times slower and Tyr about 100 times slower than Phe.

The variations in the estimated rates of transport are mostly due to the type of carrier used. For instance, the carrier for phenol transport, a case previously studied [8], had a well-defined cavity size, while for amino acids there are micelles with size following a Boltzmann distribution. At the interfaces a dynamic process of micelle formation and breakage occurs. It is impossible to control this size distribution and, if there is more availability of a certain amino acid, it is possible that micelles may become greater, making the transport faster. As a consequence, variations of the order of 15% in the transport rate are commonly expected for such experiments when performing replicates [1].

Note that (Table II) Tyr is always transported at a very low rate in the presence of Phe and Trp in the source phase. Despite the fact that Tyr is carried in a different region within the reverse micelles (preferably in the aqueous bulk), it is highly insoluble in water owing to dimer formation. Since solubilization is essential for amino acid transport, it is clear why Tyr has such a low rate of transport among the studied amino acids [12].

Two new experiments were carried out using binary mixtures of Phe and Trp, the amino acids that have higher rates of transport through the membrane. Their concentra-

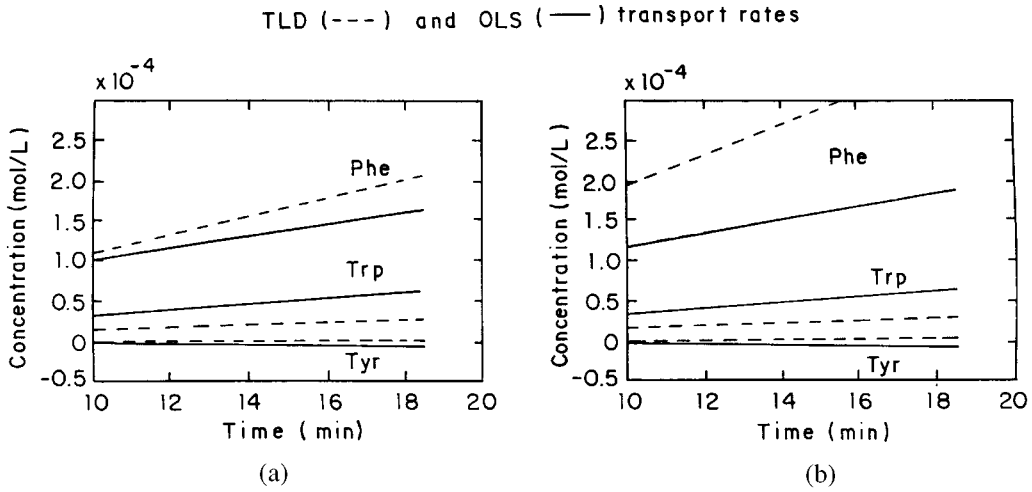

Figure 6. OLS and TLD estimated kinetic profiles for ternary mixtures, (a) $k = 1,2,3$ (see Table V, Equations 2 and 3 in text), (b) $k = 1,2$.

tions are listed in Table III. The obtained kinetic profiles are shown in Figure 5 and the transport rates for both experiments are given in Table IV. The rates changed somewhat in the absence of Tyr. There was a slight decrease in the transport rate of Trp and an increase for Phe.

Third-order arrays of intensity values ($91 \times 60 \times s$, where s is the number of mixtures) were obtained by stacking the sample matrices of spectra taken along the time axis. Theoretically, for a perfect trilinear model the rank should be $N = 2$ for binary mixtures and $N = 3$ for ternary mixtures. Singular value decomposition was used to inspect the rank of the binary systems (experiments 4 and 5), showing that in all combinations (each individual matrix, and both matrices when juxtaposed row-wise or column-wise and analysed as a single matrix) the rank is two. The TLD method was applied to estimate the kinetics, followed by determination of the transport rate, and the results are given in the last row of Table IV. The agreement between the results obtained by both methodologies is within the experimental error of 15% expected for such experiments. The kinetic profiles obtained using TLD and OLS methodologies are plotted in Figure 5, showing that they are in excellent agreement. The estimated kinetic profiles stay quite stable when fitting different subsets generated from the original three-way array. However, in the present work it was not possible to have a good estimate of the true spectral profiles as obtained in our previous work [8]. As shown in Figure 1, the spectra of pure compounds are strongly overlapped, especially Phe, and that could have affected the spectral resolution.

The results obtained in SVD analysis by juxtaposing different combinations of ternary mixtures (first column in Table V) row-wise and analysing as a single matrix indicate a rank-three system (spectral domain). On the other hand, when the slabs are juxtaposed column-wise (temporal domain), a rank-two system is indicated. For ternary mixtures the pure spectra are linearly independent; no interaction among species inside the micelles is expected, but the kinetic profiles are not linearly independent, since one of them (for Tyr) is approximately zero (very low concentrations in the receiving phase and high absorptivities in the spectral domain). Therefore there is a lack of fit of data to a perfectly linear model. Even in this case not warranted by the conditions mentioned above, we still used the TLD method. The kinetic profiles obtained by TLD for each species are shown in Figure 6 together with the results obtained from OLS, showing that there is fair agreement between estimated profiles for both methods. Table V shows the rate of transport calculated using TLD results obtained by stacking different slabs (first column of Table V). Comparing these results with those obtained by the OLS method, it can be seen that, except for one TLD analysis (experiments 1 and 2), the results are on average 50% higher for Phe and 50% lower for Trp. The transport rates are very small in magnitude, being in the range of 10^{-5}–10^{-7} mol l^{-1} min^{-1}, and these differences are well tolerated. In summary, both results, from TLD (Table V) and OLS (Table II), indicate the same trend, i.e. Phe is transported faster having the rate of order 1.0×10^{-5} mol l^{-1} min^{-1} while Trp is about 10 times slower and Tyr 100 times slower than Phe.

5. CONCLUSIONS

The transport of amino acids through liquid membranes is not a simple process and is strongly dependent on the type of species being transported and on the bulk conditions. Each compound has a preferential solubilization in a specific region within the reverse micelles, and this behaviour is reflected in its specific transport rate through the membrane. The transport rates were estimated using ordinary least squares and trilinear decomposition methods. Both results can be considered similar, since the experimental values obtained for these systems are very small in magnitude, i.e. of the order of 10^{-5}–10^{-7} mol l^{-1} min^{-1}, compared to the range of values for the rate of transport (frequently of the order of 10^{-3} mol l^{-1} min^{-1}). Therefore in this case it can be considered that the condition of linear independence is a sufficient condition for the success of the method, but not a necessary condition. For binary mixtures the transport rates obtained with OLS and TLD results are in excellent agreement, while for ternary mixtures the results obtained

from these methods differ by approximately 50%. This leads to the conclusion that TLD is a good alternative method to describe the transport kinetics when the spectra of pure compounds are not available. Better agreement between OLS and TLD results might be obtained by using another three-way methodology, dealing with different ranks on different domains. Studies in this direction are presently being considered.

Acknowledgements

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