Determination of the association constant of strongly bonded host–guest systems by multivariate regression of infrared spectroscopic data

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A new method applying infrared spectroscopy in combination with multivariate regression for the determination of association constants has been developed to overcome the sensitivity problems arising when strongly associating complexes are investigated by NMR or UV–VIS methods. A concentration profile for the complex is derived by correction of multivariate regression data. Subsequent iterative fitting of the corrected data yields the association constant.

The regression part is not integrated in the process of the association constant determination itself. Separating data treatment from the actual fitting procedure offers a means of evaluating the quality of the data set and the order of association *before* the actual calculation of the association constant. Furthermore, the method of regression can be varied and the applicability of the selected method can be assessed from the data-regression output.

From simulated data, it is estimated that an association constant range of 10^2–10^6 M^{-1} can be determined when measuring at millimolar concentration levels.

Examples are shown for halide complexation of a thiourea compound and a urea-substituted resorcinarene cavitand.

Introduction

In principle, a broad range of spectroscopic techniques can be applied to determine equilibrium constants in host–guest chemistry,[1–3] but for strongly associating complexes, difficulties are often encountered. For an accurate determination of an association constant, it should be possible to monitor adequately the changes in the concentration of components of the sample as a function of the composition of the mixture. For strongly associating systems, this condition generally implies that measurements have to be performed at a (sub)millimolar level, as in that case the changes on variation of initial concentrations are large enough to be observed well. Furthermore, an advantageous side effect of a low concentration level is that interfering effects from dimerisation of host or guest molecules are avoided.

Although current UV–VIS techniques are specifically equipped for use at very low concentrations, their applicability depends on the presence of a suitable extinction coefficient for at least one of the solutes.[4,5] Standard NMR methods suffer from a lack of sensitivity at the (sub)millimolar compound concentrations required when measuring strongly associating host–guest systems.[4,5] Infrared spectroscopy might be a useful alternative, as this technique is applicable at (sub)millimolar concentrations, and is also responsive to conformational changes and bonding phenomena occurring on complexation. A combination of infrared spectroscopy and multivariate data analysis can be used to overcome problems with sensitivity and accuracy, whereas it often enhances its versatility.

Here we describe a method of obtaining concentration profiles of the relevant components partaking in the complexation by multivariate regression analysis of infrared spectroscopic data. As multivariate regression comprises a mathematical correlation of sample-spectra and user-supplied concentration data, the application of regression in the case of association constant determination is not straightforward. Only *initial*, but not the *actual* concentrations of host, guest and complex in solution are known, and an *actual* concentration profile of one or more solutes in a series of solutions has to be determined for association constant calculation.[6–8] Nevertheless, a concentration profile can be obtained by multivariate regression as a function of *initial* concentration data, followed by a data-correction procedure.

The described method is especially suitable when monitoring molecular recognition in hydrogen-bonded systems, an area of current interest.[9,10] Complex formation through hydrogen bonding is easily monitored for alcohol, amino, (thio)amido or (thio)urea hosts as the XH (X = O, N) stretching vibration is very sensitive toward changes in the surroundings of this group. Apart from that, its spectral region is often free of interference from other constituents of the mixture.

The application of the method is illustrated by halide ion association[11,12] with the previously described neutral (thio)urea ligands,[13,14] OFP [*N*-*n*-octyl-*N*′-(*p*-fluorophenyl)thiourea] and NPOE {tetrakis[*N*′-(*o*-nitrophenoxy-*n*-octyl)ureidomethyl]-cavitand}[15] (Scheme 1). Association is achieved through the formation of hydrogen bonds between the (thio)ureido[16,17] moiety and the halide ion. Bonding of the halide to OFP and NPOE[15] is followed by monitoring the NH stretching vibrations of the (thio)urea groups.

Determination of the association constant

An association can be described by eqn. (1) in which a host H

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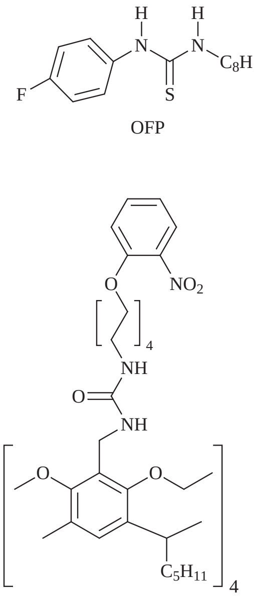

Scheme 1

$$m\mathrm{H} + n\mathrm{G} \overset{K_\mathrm{ass}}{\rightleftharpoons} \mathrm{H}_m\mathrm{G}_n \qquad (1)$$

and guest G associate to form a complex $\mathrm{H}_m\mathrm{G}_n$. The molar K_ass and fractional K' association constants are then given by eqn. (2)

$$K_\mathrm{ass} = \frac{c_\mathrm{complex}}{c_\mathrm{H}{}^m c_\mathrm{G}{}^n}$$

$$K' = \frac{f_\mathrm{c}}{f_\mathrm{H}{}^m f_\mathrm{G}{}^n} = X^{m+n-1}K_\mathrm{ass} \qquad (2)$$

in which solute concentrations are indicated by c_complex, c_H and c_G; f_H, f_G and f_c indicate fractions of host, guest and complex. X is the summed molar concentration of host, guest and complex. The assumption is made that the concentration of organic compounds equals the activity in the concentration range studied.

In order to obtain K_ass, first a concentration profile for one or more solutes has to be assessed. Stipulating that the ith sample spectrum S_i in a specified region can be described as the sum of *two* spectral contributions only (*e.g.* representing host and complex), each multiplied by their appropriate coefficient (score), we write eqn. (3). Here, $\boldsymbol{S}$ is an $N \times L$ matrix whose

$$\boldsymbol{S} = \boldsymbol{CF} + \boldsymbol{E} \qquad (3)$$

rows consist of N sample spectra of length L, $\boldsymbol{F}$ contains two spectral loadings in its rows and $\boldsymbol{C}$ is a scores matrix with two columns. A schematic representation is presented in Fig. 1. The $\boldsymbol{E}$ matrix in eqn. (3) contains the experimental noise and can be omitted for a real two-component system. The condition that $\boldsymbol{S}$ reflects such a system can often be met by careful selection of a spectral window.

If only two of the solutes in samples with different host and guest fractions contribute to the set of spectra $\boldsymbol{S}$, one expects this matrix to be described well by two loading spectra and two scores [eqn. (3)] when spectra are correlated to the *actual* solute concentrations by multivariate regression analysis. These *actual* concentrations of the solutes, however, are not known. Performing regression analysis of spectra by correlating the spectral data to *initial* concentrations (*i.e.* before association) of the host will, in the ideal case, also yield a description by two factors, assuming that eqn. (3) is valid. In practice, for a real set of data, additional loading spectra are introduced to account for noise and residual effects [*i.e.* the $\boldsymbol{E}$ matrix in eqn. (3) is non-zero], but these effects can often be ignored without significantly affecting the results.[18]

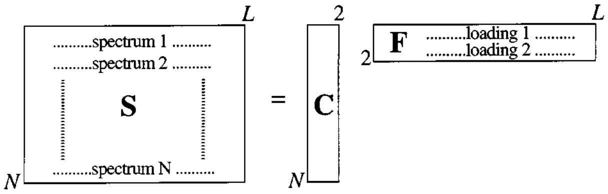

Fig. 1 Graphic representation of eqn. (3). The residual matrix $\boldsymbol{E}$ has been omitted for clarity. The $\boldsymbol{S}$ matrix consists of N spectra of length L.

When performing regression analysis as a function of initial concentrations of the host H for a series of sample spectra, this yields two loading spectra in $\boldsymbol{F}$ and for each spectrum the corresponding scores in $\boldsymbol{C}$. All spectral contributions linear with the initial concentration of the host are reflected in the first loading spectrum. Due to association of the solutes, an additional spectral component is introduced in the spectra, that is not linearly correlated to the initial concentrations of the host. Denoting the two loading spectra as $\boldsymbol{F}_1$ and $\boldsymbol{F}_2$, this deviation of sample spectrum i from the linearly correlated spectral term $C_{i1}\boldsymbol{F}_1$ is then accounted for by spectral contribution $C_{i2}\boldsymbol{F}_2$.

The latter term exclusively reflects spectral changes induced by formation of the complex. After regression analysis, however, features due to the associate formed are also observed in the first spectral component $\boldsymbol{F}_1$, in addition to the 'pure' spectral contribution of the uncomplexed host itself. This is due to both the data processing commonly applied before regression (mean centring[19]) and the host fraction range covered by the set of sample spectra. In order to correct this phenomenon, a transformation of $\boldsymbol{C}$ and $\boldsymbol{F}$ has to be performed in order to obtain the 'pure' scores $\boldsymbol{C}'$ and 'pure' loading spectra $\boldsymbol{F}'$ according to eqn. (4) where $\boldsymbol{C}' = \boldsymbol{CR}(\psi)$, $\boldsymbol{F}' = \boldsymbol{R}^{-1}(\psi)\boldsymbol{F}$ and

$$\boldsymbol{S} = \boldsymbol{C}'\boldsymbol{F}' \qquad (4)$$

$\boldsymbol{R}(\psi) = \begin{pmatrix} \cos\psi & \sin\psi \\ -\sin\psi & \cos\psi \end{pmatrix}$. Matrix $\boldsymbol{R}(\psi)$ is a rotation operator. The transformation applied consists of a rotation through angle ψ. Rescaling all elements of our new $\boldsymbol{C}'$ matrix by multiplication with a factor $\cos^{-1}\psi$ leads to scores $\boldsymbol{C}''$ [eqn. (5)].

$$\boldsymbol{C}'' = \boldsymbol{C}\cdot\begin{pmatrix} 1 & x \\ -x & 1 \end{pmatrix} \text{ with } x = \tan\psi \qquad (5)$$

A closer inspection of eqn. (5) reveals that, in order to obtain a corrected set of scores $\boldsymbol{C}''$ containing values linearly related to the *actual* concentrations of the solutes, we have to correct the scores $\boldsymbol{C}$ for all samples i according to eqn. (6), where C_{ij} and C''_{ij} are matrix elements of $\boldsymbol{C}$ and $\boldsymbol{C}''$. Eqn. (6*b*) indicates, for

$$C''_{i1} = C_{i1} - xC_{i2} \qquad (6a)$$

$$C''_{i2} = C_{i2} + xC_{i1} \qquad (6b)$$

instance, that in order to obtain a 'pure' score for the associated complex in solution, linearly related to its actual concentration, we need to correct the original score for the second loading spectrum from regression analysis with a factor x times the score for the first loading spectrum. The most convenient way to obtain the value of x is to refine it together with the other parameters in an iterative process (*vide infra*).

Assuming that x is known, the relevant corrected scores in matrix $\boldsymbol{C}''$ are linearly related to concentration of complex $[\mathrm{H}_m\mathrm{G}_n]$ in sample i according to eqn. (7). Constant y_0 is an

$$c_{\mathrm{complex},i} = (C''_{i2} + y_0)y_1 \qquad (7)$$

intercept term, introduced as a result of mean centring[18,19] of the spectra prior to regression. In this data-pretreatment step, an average of all sample spectra is subtracted from each spectrum. As a result of this, the calculated scores shift such that their average becomes zero. The variable y_1 is a proportionality constant that bears analogy to the extinction coefficient of the complex. The value of y_1 is obtained as a result of an iterative fitting process, the value of y_0 need not be known (*vide infra*).

Given the initial concentrations for sample *i*, a stoichiometry (*m*, *n*) and an association constant K_{ass}, concentrations $c_{solute,i}^{pred}$ of the solutes can be predicted from eqn. (2). The set of 'pure' scores $\boldsymbol{C}''$ can be calculated according to eqn. (6) on insertion of matrix $\boldsymbol{C}$ and *x*, and *e.g.* a complex concentration $c_{complex,i}^{calc}$ can be calculated according to eqn. (7). From a combination of eqns. (2), (6) and (7), we derive that K_{ass}, y_1 and *x* can now be obtained by minimisation of the 'relative fit residual' *R* as a function of the latter variables [eqn. (8)], where $\Delta_{i,j} = c_{complex,i} - c_{complex,i-j}$.

$$R(K_{ass},y_1,x) = \left| \sum_{i=1+q}^{N} \sum_{j=1}^{q} [(\Delta_{i,j}^{calc})^2 - (\Delta_{i,j}^{pred})^2] \right| \qquad (8)$$

Residual *R* effectively amounts to a comparison of squared data point differences of a concentration with its neighbour ($q = 1$), with its neighbour and next-nearest neighbour ($q = 2$) and so on. Assessment of the y_0 value is avoided here as difference values Δ rather than absolute values $c_{complex,i}$ are used. Application of *q*-values higher than 1 sometimes leads to better results with noisy sets of calculated scores, as a 'window' of *q* datapoints is applied in the comparison of calculated and predicted data point differences Δ.

Fits are performed for different stoichiometries (*m*, *n*). The best fit of calculated and predicted regression data yields the desired association constant K_{ass} and stoichiometry (*m*, *n*).

Experimental

Ligands

The ligands OFP [*N*-*n*-octyl-*N*′-(*p*-fluorophenyl)thiourea] and NPOE (tetrakis[*N*′-(*o*-nitrophenoxy-*n*-octyl)ureidomethyl]-cavitand) were prepared as previously described.[15]

Infrared spectroscopy

Infrared spectra were recorded on a Perkin-Elmer System 2000 Spectrometer equipped with a DTGS detector and a sample shuttle. Scanning conditions: resolution 4 cm^{-1}, scans 16, apodisation medium Norton–Beer. Solutions were measured in cells with KBr, NaCl or CaF_2 windows and path length 0.5, 1 or 2 mm. Freshly prepared solutions in water- and ethanol-free chloroform (Merck, p.a.) with host fractions covering the range 0.1–0.9 and constant total molar content (<1 mM) were measured for hosts OFP and NPOE with tetra-*n*-butylammonium halide salts (TBA–X, with X = I, Br, Cl) (Aldrich, p.a.) as guests. No dimer formation of the host was observed in the used concentration range.

Simulated data

In order to assess the validity of the algorithm, normalised simulated data were generated for a 1 : 1 complexation model; band positions, relative band intensity and band width were chosen to be similar to data from the experiments. The host band maximum height was normalised to 1.0 at host fraction 1, the maximum complex band height was set to 0.5 at host fraction 0.5; sets of nine spectra were generated with host fractions ranging from 0.1 to 0.9, at constant total molar content (1 mM), for association constants $K' = 10^n$ ($n = -1, 0, 1, 3, 5$).

Data treatment

Simulated data were subjected to Partial Least Squares-1 (PLS-1) analysis[20] to provide insight into the performance of the algorithm. Measured data: in order to monitor the association for OFP and NPOE, the NH stretching vibrations of the (thio)urea moiety were used as a probe and the wavenumber region was chosen such that all bands of interest were included (3550–3200 cm^{-1}, or smaller). In this region, the contribution of the guest was found to be negligible.

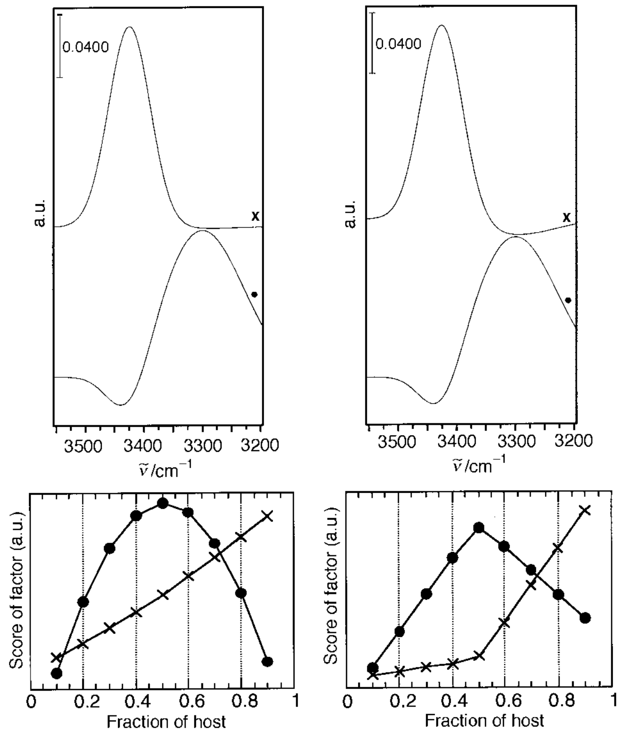

Fig. 2 Calculated PLS-1 factors (top) and scores (bottom) for first (x) and second (●) factor. Simulated spectra for $K' = 1$ (left) and $K' = 1000$ (right).

PLS-1 was used to build a model by correlation to initial host concentration. At least five samples were prepared with a constant molar content of host and guest. The built-in baseline correction was applied during the process if necessary.

Sample spectra for a complexation series were described with a model incorporating two factors. Factors were checked by eye for anomalies. Scores of second factors were corrected using the above-stated algorithm and resulting data were fitted in order to obtain association constants.

Results and discussion

Sets of simulated spectra with association constants K' in the range 0.1–10^5 were correlated to the initial host fractions by PLS-1. Results for sets of spectra representing complexation reactions with $K' = 1$ and $K' = 1000$ are shown in Fig. 2. As is apparent from the figure, the first factor mainly reflects a host contribution. The second factor shows a positive contribution of the complex at 3300 cm^{-1} combined with a negative contribution at 3450 cm^{-1} in the host region. Especially for a stronger association, a contribution of the complex to the first factor is observed. Fig. 3 shows the result (dotted lines) of a correction of the scores for the second factors following the aforementioned procedure. As expected, the corrected C''_{i2} scores now resemble a symmetric Job plot[5] for a 1-to-1 association. Consecutive iterative fitting of the corrected scores yields the association constants (Table 1). It is observed that association constants are reproduced well by use of the proposed algorithm.

For measured data, the influence of noise can be drastic as it influences the regression process. Nevertheless, it is normally still possible to select a minimal number of factors that predicts well the largest variation in the spectral series.[18] On complexation of halide by OFP, a new NH stretching vibration band emerges at a wavenumber lower than the original, non-bonded

Table 1 Association constants reproduced from simulated data. K'_{sim} was used to generate data. K'_{fit} is the fitted association constant.

K'_{sim}	K'_{fit}	y_1	x
10^{-1}	0.115	2.09	0.017 1
10^{0}	0.998	0.0542	−0.008 57
10^{3}	1.000×10^{3}	0.0541	−0.117
10^{5}	1.001×10^{5}	0.0541	−0.123

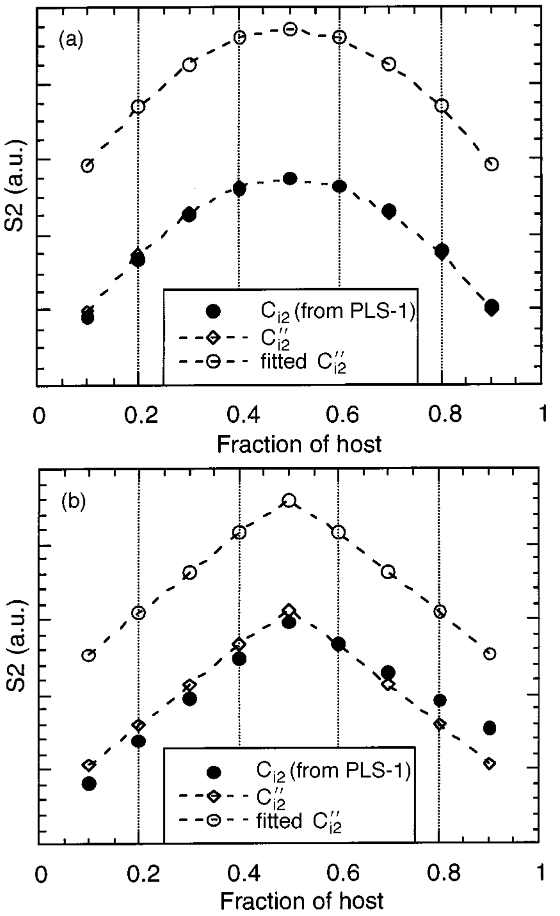

Fig. 3 Original, corrected and fitted C_{i2} scores for simulated data of a first-order association with $K' = 1$ (a) and $K' = 1000$ (b). Fitted scores have been shifted for clarity.

ν_{NH} vibration. We assign this new band to a NH···halide hydrogen bond.[21] This is clearly reflected in the second factor (factors 1 to 3 obtained from PLS-1 analysis are shown in Fig. 4), which accounts for both an increase of the $\nu_{NH\text{-bonded}}$ band and a decrease of the ν_{NH} band. The first factor closely resembles the band profile as observed for the unassociated host. Results for the association of chloride, bromide and iodide with OFP are shown in Table 2. Associations are described best by a 1 host to 1 guest model for chloride and bromide guests (K_{ass} 2.2×10^3 and 8.6×10^3 M^{-1}, respectively). The value for the complexation of chloride with OFP is in excellent agreement with the value obtained by ^{1}H NMR spectroscopy (2.0×10^3 M^{-1}).[15] The stoichiometry of the association of iodide with OFP was found to be 2:1 host to guest with a K_{ass} value of 1.6×10^3 M^{-2}. This change in stoichiometry might be explained by the larger volume of the iodide ion.

Similarly, the association of NPOE with chloride, bromide and iodide was studied. Optimised constants are summarised in Table 3. PLS data, corrected scores and fitted results are shown for complexation of chloride, bromide and iodide in Figs. 5–7. For urea NPOE, data could only be fitted well assuming a 1-to-1 complexation model for the halide ions studied (K_{ass} values are 8.6×10^4, 1.3×10^4 and 1.4×10^4 M^{-1}, respectively, for chloride, bromide and iodide). For the complexation of chloride with NPOE in CDCl$_3$, a value of 1×10^5 M^{-1} was found using ^{1}H NMR.[22]

Table 2 Association of host OFP and TBA–X in chloroform. PLS-1 data analysis was followed by an iterative optimisation for the stoichiometries given, yielding the association constant K_{ass} and constants x and y_1 (see text for explanation of symbols). Solutions with a linear correlation of calculated scores C'_{i2} *vs.* predicted scores < 0.95 have been omitted. The best stoichiometry was selected by comparison of correlation of actual and fitted data points, and is printed in bold typeface.

Stoichiometry *m* host : *n* guest	ν/cm^{-1}	K_{ass}/ M^{-1-m-n}	y_1	x
OFP:TBA–Cl	3550–3200			
1:1		$\mathbf{2.2 \times 10^3}$	**0.190**	$\mathbf{-0.522 \times 10^{-3}}$
2:1		2.8×10^4	1.297	0.104
OFP:TBA–Br	3525–3250			
1:1		$\mathbf{8.6 \times 10^3}$	**0.676**	**−0.004 54**
2:1		6.4×10^5	0.545	0.065 0
OFP:TBA–I	3500–3200			
1:1		0.010	772	0.117
1:2		1.4×10^3	0.426	0.061 1
1:3		1.1×10^5	0.357	0.028 3
2:1		$\mathbf{1.6 \times 10^3}$	**2.19**	**0.221**
3:1		7.2×10^5	2.56	0.357

Table 3 Association (1-to-1) of NPOE with chloride, bromide and iodide in chloroform. See text for explanation of symbols.

	ν/cm^{-1}	K_{ass}/M^{-1}	y_1	x
NPOE + TBA–Cl	3550–3230	8.6×10^4	1.55	−0.0509
NPOE + TBA–Br	3550–3230	1.3×10^4	3.07	−0.0660
NPOE + TBA–I	3550–3230	1.4×10^4	3.22	−0.0940

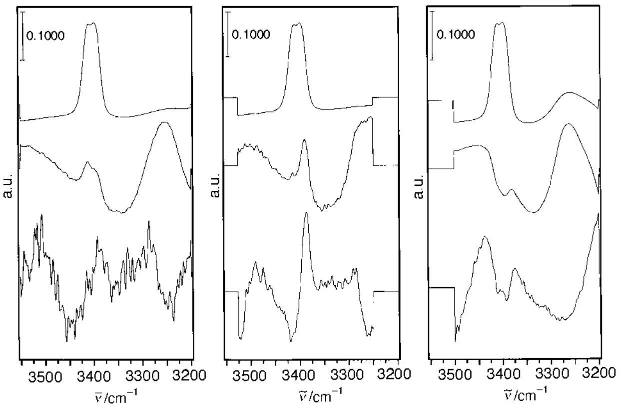

Fig. 4 Factors 1 to 3 (in descending order) from PLS-1 calculated for the association of OFP with TBA–Cl (left), TBA–Br (middle) and TBA–I (right)

Each sample set for NPOE and OFP complexation of chloride, bromide and iodide was described well by two factors using PLS-1, as judged by application of the common criteria.[18] Therefore, the spectral information present in the third factor (see Fig. 4) is not significant, and we ascribe this effect to small sampling errors and noise in the spectra.

Conclusions

The association constants obtained from multivariate analysis of simulated data followed by iterative fitting of the corrected scores correspond well with the expected values. We estimate that a K_{ass} range of 10^2–10^6 M^{-1} can be attained for solutions at the millimolar level. By shifting the concentrations of the solutes to a submillimolar level, a shift of the above-mentioned association constant window to higher values is effected. In

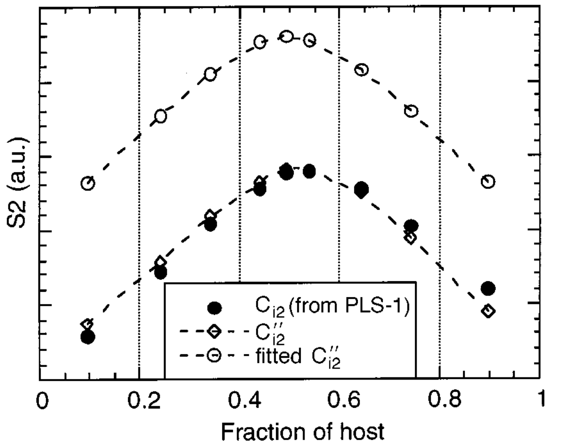

Fig. 5 Original scores C_{i2} (●), corrected scores C''_{i2} (◇) and fitted scores (○) for the complexation of chloride ion by NPOE. The fitted data have been shifted for clarity.

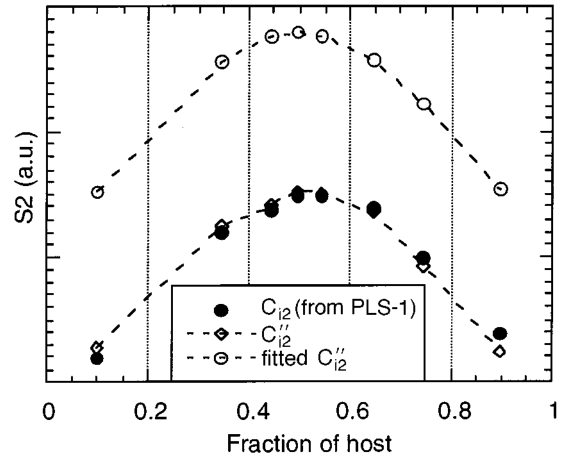

Fig. 6 Original scores C_{i2} (●), corrected scores C''_{i2} (◇) and fitted scores (○) for the complexation of bromide ion by NPOE. The fitted data have been shifted for clarity.

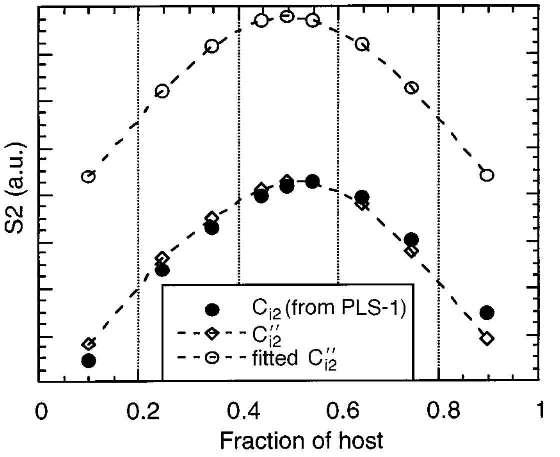

Fig. 7 Original scores C_{i2} (●), corrected scores C''_{i2} (◇) and fitted scores (○) for the complexation of iodide ion by NPOE. The fitted data have been shifted for clarity.

practice, the upper boundary of the range is limited only by the sensitivity of the spectroscopic method at very low concentrations, assuming that an appropriate spectral window with a suitable set of absorptions can be found.

The results of the association constant analysis for both compounds OFP and NPOE indicate 1-to-1 complexation with chloride and bromide guests. The stoichiometry of association of OFP and iodide was found to be 2 host molecules to 1 guest, but for NPOE, again, 1-to-1 complexation was found.

Application of multivariate regression of data in a separate stage offers a means of outlier detection *before* the actual calculation of the association constant. Furthermore, for the best results, the method of regression can be varied and the applicability of the selected spectral region can be assessed from the data-regression output.

For association processes involving hydrogen bonding, infrared spectroscopy is particularly suitable, due to both the sensoric properties of the ligating groups and the sensitivity towards conformational changes. In the case of complexations involving alcohols, amines, (thio)amide or (thio)urea compounds, the high wavenumber region where vibrations of these functionalities are being observed is often free from interfering absorptions. Both the possibility of measuring at a low concentration level and the sensitivity of infrared spectroscopy offer advantages over the currently-used NMR techniques.

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