Evolving Factor Analysis of Spectrophotometric Titrations: Forget About the Law of Mass Action?**

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Abstract: Evolving Factor Analysis (EFA) is a new and powerful mathematical algorithm for the model-free analysis of any ordered set of multiwavelength data. Applied to spectrophotometric titrations, even strongly overlapping concentration profiles and the spectra of all absorbing species are correctly calculated without making use of the law of mass action and without even defining the stoichiometric composition of the species in question. The basic idea behind EFA is repetitive application of factor analysis to all subsets of 1, 2, ...N measurements and iterative refinement of the eigenvalues thus obtained into concentration profiles with concomitant least-squares estimation of the species spectra. EFA has been tested successfully with a dozen chemical systems as well as with model data for strongly overlapping concentration profiles.

Resolving the measured absorbances Y into molar absorptivities A and concentration profiles C is the obvious goal in the numerical treatment of spectrophotometric titrations and can be expressed as a simple matrix equation: $Y = C \times A$. We wish to introduce Evolving Factor Analysis (EFA) to accomplish this task in a completely model-free way without even implicit use of the law of mass action. Results of this analysis are practically identical with those of a rigorous least-squares treatment (cf. Fig. 1). EFA will find application in many diverse fields of chemistry where a data matrix can be arranged in some well defined order.

Factor Analysis (FA) has been a long-known tool in data reduction with increasing importance for various chemical applications[1]. Recently, several authors have tried a model-free estimation of spectra and/or concentrations of the individual species by appropriate transformation of the corresponding matrices of eigenvectors based on the non-negativity of the calculated spectra[2–4] and sometimes further restrictions such as their minimum envelope[3] or maximum disparity[4]. All of these methods seem to be restricted to rather simple systems and none has claimed to have actually solved the problem.

Evolving Factor Analysis (EFA) is a new mathematical approach. While still based on FA, it does not rely on eigenvectors or on any poorly justified restrictions, but makes full use of all sound information which is available. Completely model-free, EFA does not even need assumptions with respect to the stoichiomet-

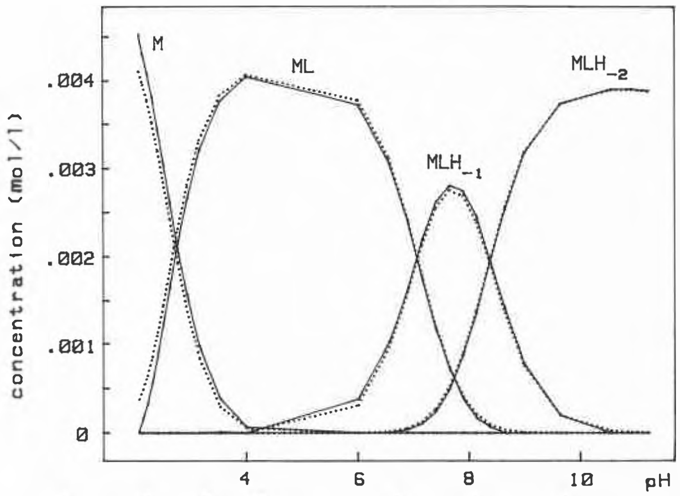

Fig. 1. Concentration profiles of Cu$^{2\oplus}$ (M) complexes with 3,7-diazanonanediamide (L) using EFA (——) or the law of mass action (.....).

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** *Acknowledgement:* This investigation was supported by the Swiss National Science Foundation, Grant No. 2.021-0.83.

ric composition of the absorbing species. Full mathematical description of EFA will be given elsewhere, but the basic steps of the algorithm are the following:

(1) Successively apply classical Factor Analysis to the set of the first 1, 2, 3, ...N spectra (N = total number of spectra).
(2) Plot the eigenvalues calculated in (1) as a function of the progressing titration (e.g. mL of reagent or pH). This is called «forward EFA», and the upsurge of a new factor will in some way reflect the formation of a new absorbing species.
(3) Repeat steps (1) and (2) in the opposite direction to detect the disappearance of a species («backward EFA»).
(4) Combine the i-th forward curve with the (S + 1-i)th backward curve retaining always the smaller of the two respective eigenvalues. S is the number of significant factors and usually equal to the number of absorbing species in the complete set of data. Problems related to that assumption are discussed in[5].
(5) Normalize the curves obtained in (4) to the analytical (total) concentrations of the mixtures («primary EFA»).
(6) Calculate the molar absorptivities A from the normalized concentrations C and from the original data Y by a linear least-squares fit.
(7) Perform iterative refinement of C and A. First, C is recalculated from Y and A, again a least-squares problem. Then all negative concentrations and those corresponding to insignificant eigenvalues in the primary EFA are put to zero. Finally, the iterative cycle is closed by normalization and recalculation of A ((5) and (6)). Convergence is checked in the usual way with the sum of squares of errors.

Primary EFA (steps (1) to (4)) has shortly been reported in a previous communication[6]. Here we show that including steps (5) to (7) the analysis can be carried much further, yielding in effect a perfect picture of species distribution curves and individual spectra on a completely model-free basis. In Fig. 1 we show the concentration profiles for the complexation of Cu$^{2\oplus}$ (M) by 3,7-diazanonanediamide (**1**, L), a well defined system with 4 absorbing species, namely M, ML, MLH$_{-1}$, and MLH$_{-2}$[7,8]. After iterative refinement, the agreement between the concentrations calculated with EFA and by using the law of mass action[9] is essentially perfect and also the standard errors of the residuals are nearly the same with the two methods. The performance of EFA has been successfully checked with roughly a dozen chemical systems that had been studied previously in our laboratory[10].

For additional tests of EFA under completely controlled conditions we also did some calculations using semi-synthetic data from the spectra calculated for ligand **1** and a concentration matrix obtained with different sets of stability constants. A random noise of $4 \cdot 10^{-4}$ absorbance units was superimposed in order to mimic the performance of our experimental set-up[8]. As shown in Fig. 2a, the fundamental features, such as the formation of additional species at certain pH values and the ranges of species coexistence, are already nicely reproduced by the primary non-iterative EFA (steps (1) to (5))[11]. After iterative refinement (Fig. 2b and Fig. 3), the agreement between classical and EFA calculations is again essentially perfect despite the rather serious overlap of the concentration profiles. It may be added that after assigning stoichiometric coefficients to the individual species, the values for all stability constants can be obtained simply by reading the pH values at the intersections

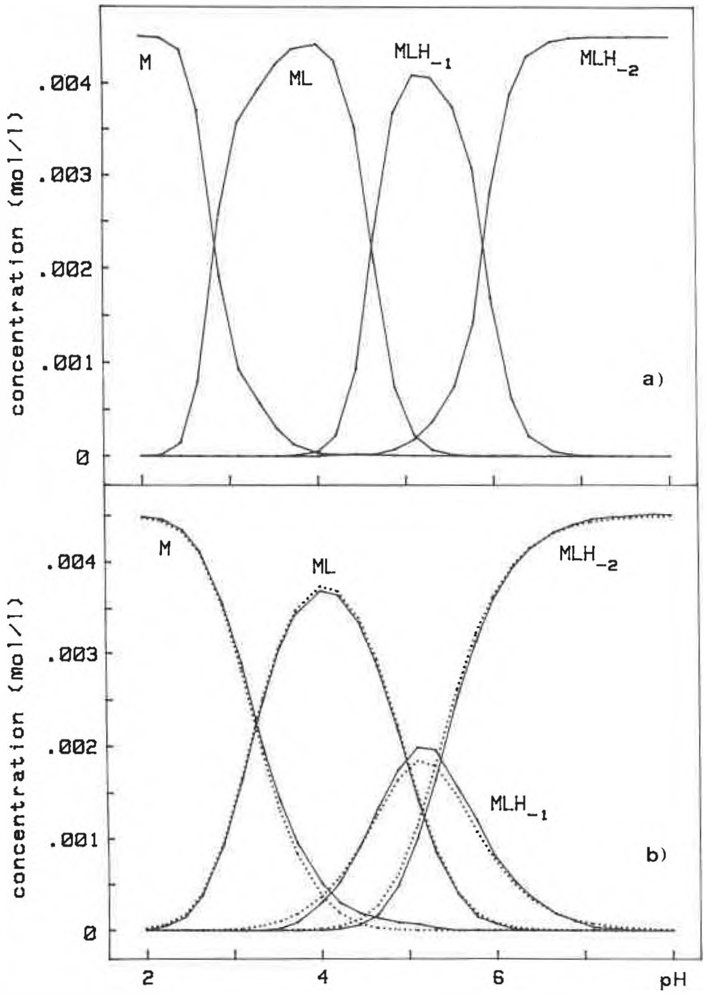

Fig. 2. Concentration profiles of complexes based on model data using EFA (——) or the law of mass action (.....). a) Primary EFA without iterative refinement, b) after least-squares refinement.

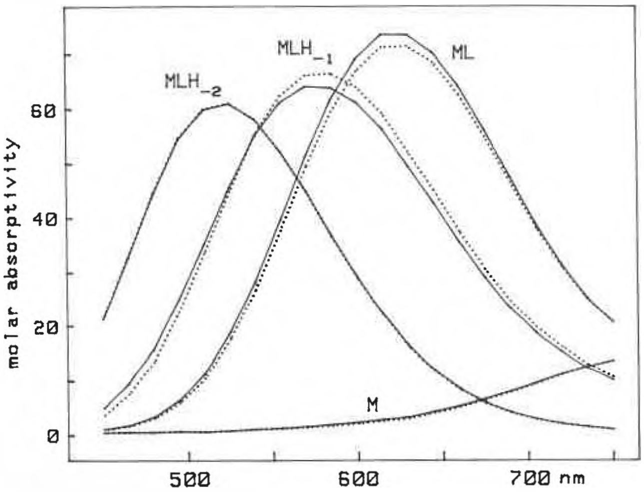

Fig. 3. Absorption spectra of species corresponding to Fig. 2.

of the EFA curves without bothering about least-squares calculations using the law of mass action.

While the total scope of EFA may not be finally judged today, the following conclusions are appropriate:

- Noniterative EFA is a new and most powerful method to obtain the number of species formed in the course of spectroscopic titrations and to estimate their range of existence as well as their equilibrium constants.
- Using iterative EFA it is possible even in cases with strong overlap of concentration profiles and spectra to perform a complete analysis without ever using the law of mass action and without defining the stoichiometric composition. It seems that earlier attempts of such abstract analyses have essentially failed because two simple points have been overlooked: the information based on knowing the analytical (total) concentrations and the ordered nature of the data matrix.
- Obviously, the law of mass action cannot really become obsolete even for the analysis of spectrophotometric titrations. On the other hand, EFA will in many cases give results of equal quality without any assumptions with respect to the chemical model, and comparison between these two completely independent approaches offers a unprecedented possibility for selecting the correct model.
- Looking at the mathematical algorithm of EFA we conclude that this method should neither be restricted to spectrophotometry nor to titrations. An identical or closely similar approach should be most promising for the analysis of any kind of spectroscopic titrations, for peak resolution in chromatography, for GC-MS analysis, and perhaps even for multiwavelength kinetics.

Received: September 23, 1985 [FC 35]

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[8] Details of the experimental set-up have been described previously: G. Hänisch, T. A. Kaden, A. D. Zuberbühler, *Talanta 26* (1979) 563. 2.30 mL of 4.50 mM $CuSO_4$ and 5.00 mM **1** · 2HCl ($I = 0.5$ M, KCl) were titrated with 0.20 M NaOH. 28 spectra were recorded at 21 wavelengths between 750 and 450 nm. Using the law of mass action, the following stability constants, which closely correspond to the literature values, were obtained: $\lg K^{\mathrm{H}}_{\mathrm{LH}} = 8.40$, $\lg K^{\mathrm{H}}_{\mathrm{LH_2}} = 6.55$, $\lg K^{\mathrm{M}}_{\mathrm{ML}} = 12.11$, $\lg K^{\mathrm{H}}_{\mathrm{ML}} = 7.09$, $\lg K^{\mathrm{H}}_{\mathrm{MLH_{-1}}} = 8.36$.

[9] All calculations were done on a HP 9000/216 computer with a modified version of SPECFIT: a) H. Gampp, M. Maeder, C. J. Meyer, A. D. Zuberbühler, *Talanta 32* (1985) 95; b) *ibid. 32* (1985) 257. All data are available upon request from the authors.

[10] Some of the data used for testing EFA have been analyzed previously using the law of mass action[7c, 9b]; see also H. Gampp, D. Haspra, M. Maeder, A. D. Zuberbühler, *Inorg. Chem. 23* (1984) 3724.

[11] A set of data constructed with $\lg K^{\mathrm{M}}_{\mathrm{ML}} = 11.00$, $\lg K^{\mathrm{H}}_{\mathrm{ML}} = 5.00$, and $\lg K^{\mathrm{H}}_{\mathrm{MLH_{-1}}} = 5.30$ (other values as for ligand **1**) is discussed in the following.