

## Estimation of the Rate Constants of Second Order Reactions Using Mean Centering of Ratio Spectra

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A new application of the mean centering of ratio spectra method is proposed for estimation of the rate constants of second order reactions, using kinetic-spectrophotometric data. The method is based on the mean centering of the ratio spectra to obtain a kinetic profile of the product. Using computational fitting, the rate constant can be obtained without any ambiguity. An interesting feature of second-order reactions is that the number of steps in the reaction is less than the number of absorbing species, resulting in a rank-deficient response matrix. Through using row mean centering of ratio spectra, the pure response of the product of the reaction could be obtained, and thus an accurate estimation of rate constant would be possible. The applicability of the method was evaluated by using several model data. The reaction of 1,2-naphthoquinone-4-sulfonate sodium (NQS) and 3-nitroaniline (TNA) in ethanol as a real system was also studied applying the proposed method.

**Keywords:** Mean centering of ratio spectra, Kinetic-spectrophotometric data, Rate constant, Second-order reactions

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### INTRODUCTION

Investigating reactions to obtain kinetic information such as rate constants is a useful and common approach in modern chemistry [1-2]. Therefore, reaction rate methods are becoming increasingly important in analytical chemistry. Their present degree of development relies heavily on recent breakthroughs in instrumental design and, especially, on the incorporation of microcomputers into analytical chemical configurations [3].

The on-line spectra recorded from a reaction mixture can form a two-way data matrix that includes substantial chemical information such as the reaction kinetic profile and the pure spectrum of each component, including the unstable intermediate. Such information can be evaluated through an

analysis of the two-way spectroscopic data by means of proper chemometric algorithms. When the individual components in the reaction mixture have a dominating response spectral range, some common methods such as non-linear least-squares fit can be used to extract the kinetic information according to the absorbance data at a single wavelength [4]. Frequently, there is extensive kinetic and spectral overlap between the components [5]. Many studies on solving the kinetic process and extracting the absorption spectra from the two-way data have been published. Most of these studies are oriented towards consecutive first-order reactions. These methods include non-linear least squares fitting [4], rank annihilation factor analysis (RAFA) [6], time shift trilinear models [7,8], target testing [9,10] and curve resolution [11]. The extended Kalman filter has been applied by Mok and Chau [12] to determine kinetic parameters of first-order consecutive reactions. Estimation of the reaction rate constants from a two-

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step reaction and a comparison between the two-way and three-way methods have also been published [13-16]. Recently, a new multivariate analysis tool, namely DECRA (direct exponential curve resolution algorithm), that is based on the generalized rank annihilation method (GRAM), has been developed by Windig *et al.* [17] for resolving consecutive first-order reactions. Vega-Montoto and Wentzell [18] have more recently described the implementation of maximum likelihood parallel factor analysis (MLPARAFAC) in conjunction with DECRA, and have applied this strategy to the resolution of simulated and real experimental data of consecutive first order-reactions.

Some kinetic processes have complex mechanisms and thus are hard to be expressed by simple mathematical models. Furthermore, the apparent reaction order may vary during the reaction. Several methods have been reported to solve such problems, including self-modeling curve resolution [19], hard-soft multivariate curve resolution [20], iterative target transformation factor analysis [5] and non-linear least squares regression [21].

An intrinsic difficulty in the study of kinetic-spectrophotometric data of second-order reactions is the presence of closure rank deficiency. Several papers have been published on solving the kinetic process of second-order reactions from a two-way data. Dunn *et al.* [22] have developed a program for the implementation of a mathematical treatment that corrects for a concentration gradient within the stopped-flow observation cell for reversible second-order reaction kinetics studied by longitudinal absorbance measurements. Polster and Dithmar [23] have presented a method for the spectroscopic kinetic analysis of first and second-order reactions on the basis of multidimensional absorbance diagrams. Typically, these diagrams represent two-dimensional plots. The so-called Mauser space is multidimensional ( $n \geq 2$ ). The axes of this space are established by the absorbances or absorbance differences of  $n$  wavelengths. A reaction system that consists only of one linearly independent reaction step ( $s = 1$ ) leads to a straight line in Mauser space. This line is obtained independent of the reaction order of the system. These authors have also applied the said method to the spectroscopic-kinetic analysis of a non-linear system consisting of two reactions of second order [24]. Thurston and Brereton [25] have discussed five groups of

methods: (i) use of multiple linear regression to obtain concentration profiles and fit kinetics information; (ii) rank augmentation using multiple batch runs; (iii) difference spectra-based approaches; (iv) mixed spectral approaches that treat the reaction as two independent pseudo-species; and (v) principal component regression for two simulated data sets of second-order reactions. A methodology was proposed by Ubide *et al.* [26] employing the reagent profiles obtained in coulometric or volumetric experiments when the titration reaction is slow. The proposal consists of the use of the whole signal-time profile for the reagent to characterize the sample, by using multivariate calibration methods. The reagent profiles have been simulated for a slow second-order reaction and their possibilities for quantitative purposes have been evaluated. A second-order reaction between benzophenone and phenylhydrazine to give benzophenone phenylhydrazone was followed using UV-Vis and mid-infrared spectroscopic probes [27]. Established kinetic (hard) and partial least squares (soft) modeling chemometric methods were applied to both datasets to compare the information acquired with each probe. Carvalho *et al.* [2] also investigated the influence of three sources of error, namely, instrumental noise, error in determining initial concentrations and calibration of pure standards, and the determination of rate constants from the resultant spectra for the second-order reactions. Twelve methods involving a mixture of multivariate and kinetic models for estimating rate constants were compared in five groups (MLR, Rank Augmentation, Difference Spectra, Mixed Spectra and PCR). The behavior of the error surface depends on the information required by each algorithm. In the studies cited above, the best results were obtained using rank augmentation; however, this requires two or more batches run under stable instrumental conditions.

The present work presents a new application of mean centering of the ratio spectra method for estimation of the rate constants of second-order reactions using kinetic-spectrophotometric data. The method is based on the mean centering of ratio spectra that was recently proposed for simultaneous analysis of binary and ternary mixtures both for thermodynamic and kinetic data analysis [28-31]. Recently, the kinetics of consecutive reactions were investigated by the method of the mean centering of ratio spectra to obtain rate constants and the spectra of the components involved [32].

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Mean centering of ratio spectra was also applied to pre-treat kinetic-spectrophotometric data prior to rank annihilation factor analysis method for the analysis of unknown samples [33].

For the two-way kinetic-spectral data measured in chemical reactions, the pure spectrum of each reactant can be obtained, while that of the intermediate and/or final product (in the case of second-order reactions) usually remains unknown, and the concentration profile of each species in the reaction is not directly available; but they do change according to certain kinetic functions. With the spectra of the reactants available, the rate constants of the second-order reactions are deduced by mean centering of the ratio spectra. In particular, mean centering of ratio spectra is used to entirely remove the contribution of the absorbing reactant (s) from the data matrix, and therefore, their absorbances are eliminated. Here, we present a theoretical formulation of mean centering of ratio spectra to eliminate the contribution of absorbing reactants in second-order reactions. Several two-way kinetic-spectrophotometric data were simulated, and their mean centering of ratio spectra, which gives the pure response of the reaction product during the time of the experiments, were calculated. Then a computational fitting was used to estimate the reaction rate in a simple manner. In addition, the reaction of 1,2-naphthoquinone-4-sulfonate sodium (NQS) and 3-nitroaniline (TNA) was analyzed as a real second-order reaction, and it was proved that this approach could be applied successfully to the analysis of practical systems.

**MATHEMATICAL DESCRIPTION**

In this paper, reactions of the form



are studied, where A and B are the reactants, P is the product and k is the rate constant while O<sub>2</sub> denotes the second-order reaction. For the purposes of this model, it is assumed that no side reactions take place. It should be noted, however, that in the proposed method there is no need to give sufficient time to the completion of the reaction, and, therefore, incomplete reactions can also be studied. In addition, all observed species are assumed to be in solution over the period of monitoring the

reaction and are spectroscopically active. The differential equations for the involved components are as follows:

$$- \frac{d[R]}{dt} = K [A]^1 [B]^1 \tag{2}$$

*R* : A or B or - P

The reactants and product kinetic profiles were generated by numerical integration of the differential equation. For this purpose, the ODE23 function in MATLAB7 was applied as:

```
function,[t,P]=ode23(@kinfun_2ndorder,[t0:inc:tf], [A0 B0 P0],[ ], k);
```

```
function, dCdt = kinfun_2ndorder(t, C, k);
```

$$dCdt = [-k*C(1)*C(2); -k*C(1)*C(2); k*C(1)*C(2)];$$

where [t<sub>0</sub>:inc:t<sub>f</sub>] is the time (t) vector, including starting, increment and final time, [A<sub>0</sub> B<sub>0</sub> P<sub>0</sub>] is the vector of initial concentrations (C), and k is the rate constant of the simulated reaction.

Alternatively, the exact solution of the concentration profiles of the involved components for a second order reaction is given by [1,2,34]:

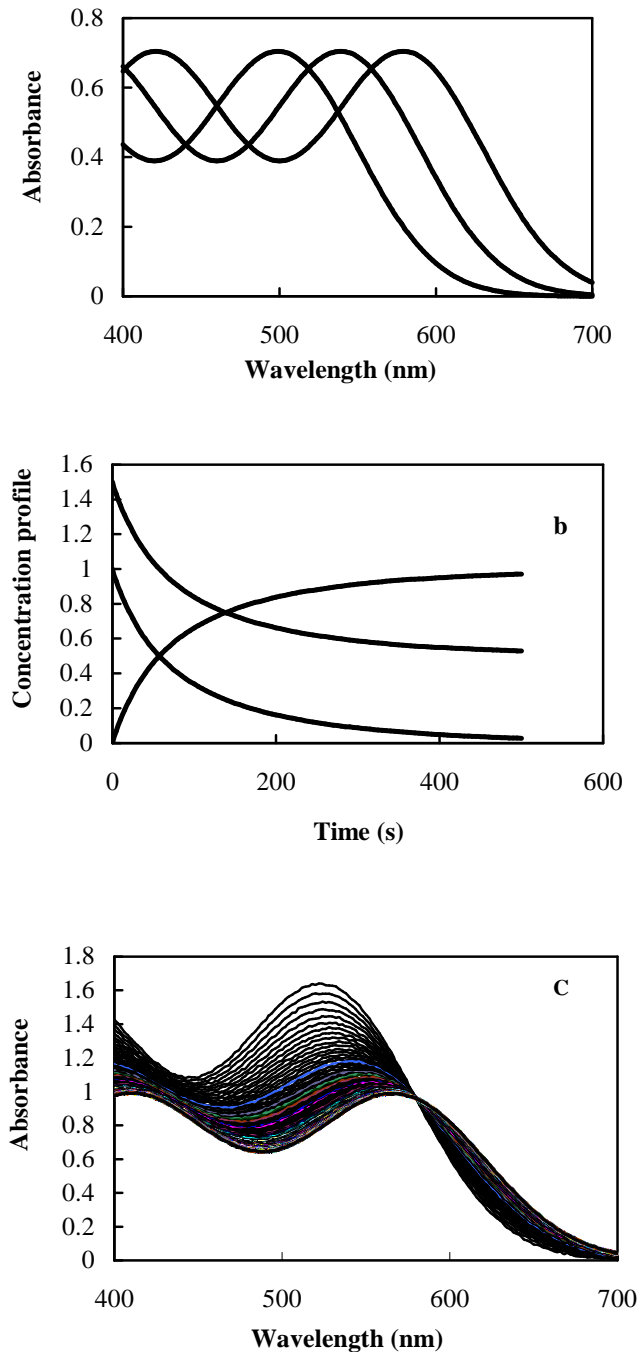
$$[A]_i = \frac{([B]_0 - [A]_0)[A]_0}{[B]_0 \exp(k ([B]_0 - [A]_0)t_i) - [A]_0}$$

$$[B]_i = [B]_0 - ([A]_0 - [A]_i)$$

$$[P]_i = [P]_0 + ([A]_0 - [A]_i)$$

where [A]<sub>0</sub>, [B]<sub>0</sub> are the initial concentrations of the two reactants A and B, [P]<sub>0</sub> is the initial concentration of the product P, and k is the rate constant. It should be noted that the only restriction of exact equations is that the initial concentration of reactants should be non-equal ([A]<sub>0</sub> ≠ [B]<sub>0</sub>).

The data set (D<sub>1</sub>) in Fig. 1 was simulated from artificially constructed UV-Vis spectra and kinetic profiles (presented in Figs. 1a, b). It is formed by 101 spectra (0-500 s with 5 s increments) of 201 absorbance values (400-600 nm with increment 2 nm). This data set belongs to the simulated reaction with initial concentrations of 1 and 1.5 of reactants A



**Fig. 1.** A case simulated overlapped spectra of species A, B and P (a), the kinetic profiles of the reactants and product for  $k = 0.024 \text{ s}^{-1}$  (b) and the resulting data set (c).

and B, respectively, with  $k = 0.024 \text{ s}^{-1}$ . The other data sets (Table 1) were simulated in the same manner as  $D_1$ , but using different initial concentrations of A, B and different rate constants,  $k$ .

The relative concentration  $\mathbf{q}_i$  at different reaction times can form a column vector  $\mathbf{q}_i$ , which is referred to as the kinetic profile of  $i$ -th species (indices  $i$  refers to A, B or P). Assuming that each species is absorptive in the measured wavelength range, a two-way data matrix  $\mathbf{Y}$  with rank 2 can be formed by measuring the absorbance at different wavelengths at a series of chosen times. Apart from the residual error matrix, one can write:

$$\mathbf{Y} = \mathbf{q}_A \mathbf{e}_A^T + \mathbf{q}_B \mathbf{e}_B^T + \mathbf{q}_P \mathbf{e}_P^T = \mathbf{Q} \mathbf{E}^T \quad (3)$$

where  $\mathbf{q}_i$  is the kinetic profile (column vector),  $\mathbf{e}_i^T$  is the absorption spectrum (row vector, and the superscript T denotes the transpose of a matrix or vector). The bold letters  $\mathbf{E}$  and  $\mathbf{Q}$  represent matrices formed by the pure spectrum and the kinetic spectrum of each species, respectively. Most of the time, the pure spectra of reactants ( $\mathbf{e}_A$ ) and ( $\mathbf{e}_B$ ) are known prior to the start of the reaction, while the spectrum of the product ( $\mathbf{e}_P$ ) in a second-order reaction is usually unknown. By using ternary mean centering (MC) of ratio spectrum analysis [29-31], the kinetic profile of product ( $\mathbf{q}_P$ ) can be easily resolved.

For the two-way kinetic spectral data of  $\mathbf{Y}$  if the elements of rows ( $\mathbf{Y}(i,:)$ ) are divided by the elements of  $\mathbf{e}_A$ , corresponding to the spectrum of reactant (A), the first ratio spectra are obtained in the form of Eq. (4) (to enable proper division, the zero values of  $\mathbf{e}_A$  should not be used in the divisor):

$$\mathbf{D} = \mathbf{Y}(i,:) / \mathbf{e}_A = \mathbf{q}(i)_A + \frac{\mathbf{q}_B \mathbf{e}_B^T}{\mathbf{e}_A} + \frac{\mathbf{q}_P \mathbf{e}_P^T}{\mathbf{e}_A} \quad (4)$$

If the matrix  $\mathbf{D}$  is row mean centered, Eq. (5) would be obtained:

$$\text{MC}(\mathbf{D}) = \text{MC} \left[ \begin{array}{c} \mathbf{q}_B \mathbf{e}_B^T \\ \mathbf{e}_A \end{array} \right] + \text{MC} \left[ \begin{array}{c} \mathbf{q}_P \mathbf{e}_P^T \\ \mathbf{e}_A \end{array} \right] \quad (5)$$

By dividing each row of  $\text{MC}(\mathbf{D})$ , shown as  $\text{MC}(\mathbf{D})(i,:)$  in Eq.

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**Table 1.** The Results for Applying the Proposed Method on Several Simulated Second Order Reactions with Different k and Different Initial Concentration of Reactants

Simulated concentration and rate constants				Estimated rate constant	
Exp. No	[A]	[B]	k (s <sup>-1</sup> )	k (s <sup>-1</sup> )	RSE (%) <sup>a</sup>
1	2.0	1.0	0.01	0.0101	1.0
2	2.0	1.0	0.06	0.0604	0.7
3	2.0	1.5	0.001	0.00105	5.0
4	2.0	1.5	0.007	0.0071	1.4
5	1.5	0.5	0.014	0.0138	-1.4
6	1.5	0.5	0.019	0.0188	-1.1
7	1.0	0.25	0.1	0.1025	2.5
8	1.0	0.25	0.15	0.1546	3.1
9	1.0	0.5	0.01	0.0102	2.0
10	1.0	0.5	0.055	0.0545	-0.9
11	1.0	1.5	0.024	0.0241	0.4
12	1.0	1.5	0.07	0.0071	1.4
13	0.75	1.0	0.06	0.0591	-1.5
14	0.75	1.0	0.1	0.0993	-0.7
15	1.5	0.75	0.055	0.0559	1.6
16	1.5	0.75	0.2	0.1949	-2.6
17	2.0	0.75	0.18	0.1866	3.7
18	2.0	0.75	0.024	0.0241	0.4
19	1.25	0.5	0.095	0.0969	2.0
20	1.25	0.5	0.2	0.2008	0.4

<sup>a</sup>Relative standard error.

(6), by  $MC(e_B/e_A)$ , element by element, (for calculating  $MC(e_B/e_A)$ , the spectrum of the component B is divided by the spectrum of component A, element by element, and the obtained ratio profile is mean centered), the second ratio spectra are obtained:

$$U = MC(D)(i,:) / MC(e_B/e_A) = \frac{MC(q_p e_p^T / e_A)}{MC(e_B/e_A)} + q_B(i) \quad (6)$$

Now, if Eq. (6) is row mean centered, Eq. (7) would be obtained:

$$L = MC(U) = MC \frac{MC(q_p e_p^T / e_A)}{MC(e_B/e_A)} \quad (7)$$

Each row of matrix **L** depends on the concentration of the product ( $q_p$ ), therefore, plotting every column of **L** versus time will give a pure kinetic profile of the product. Finally, the rate constants of the reactions can be simply reached by curve fitting. For this purpose, the *fminsearch* command of *MATLAB* was applied. The *fminsearch* finds the minimum of a scalar function of several variables, starting at an initial estimate. This is generally referred to as unconstrained nonlinear optimization. *Fminsearch* uses the simplex search method [35]. This is a direct search method that does not use numerical or analytic gradients. Alternatively, the other commands such as *Nlinfit* or *lsqnonlin* could be applied. These commands use the Gauss-Newton Method or Levenberg-

Marquardt algorithm. The *lsqnonlin* command not only gives the estimated parameter (s) but also provides the estimated error in the fitted parameters.

## SOFTWARE

All calculations in the computing process were done in Matlab7.0.1. The programs for simulation of data and mean centering of ratio spectra were written in Matlab7.0.1. In addition, the *ODE23* and *fminsearch* commands of MATLAB were applied for simulating and fitting the kinetic profiles.

## RESULTS AND DISCUSSION

### Simulated Data

To evaluate the performance of the method, several sets of kinetic-spectral data were created. Simulated overlapped spectra of species A, B and P (matrix **E**) were produced by a Gaussian function in the range of 400-600 nm (Fig. 1a). Kinetic profiles of reactants and the product (matrix **Q**) in the second-order reactions were produced as Eqs. (1-2) in the range of 0-500 s (Fig. 1b). By multiplying **Q** by **E**, the two-way data matrix (**Y**) with dimension  $n_t \times n_w$  can be calculated ( $n_t$  and  $n_w$  denote the number of time and wavelength, respectively). Random noise was added to the generated set of artificial data to test the method more rigorously. In this step, after creation of the kinetic profiles of the binary mixtures, random noise of  $\pm 0.005$  absorbance unit was added to each profile, the procedure was followed as explained above, and the rate constants of the modeled reaction in the noisy signal was predicted (Fig. 1c).

Figure 2 shows the steps of mean centering of ratio spectra explained in the previous section. The pure response of the product (kinetic profile of **P**) is also shown in Fig. 2e. The result of *fminsearch* fitting for a case data is also presented in Fig. 3.

Provided that the rate constant or the kinetic profiles of the reactants ( $\mathbf{q}_A$  and  $\mathbf{q}_B$ ) are available, the pure absorption spectrum of the product component can be reached by subtracting the contribution of reactants (A and B), so that:

$$\mathbf{q}_p \mathbf{e}_p^T = Y - \mathbf{q}_A \mathbf{e}_A^T - \mathbf{q}_B \mathbf{e}_B^T$$

where  $\mathbf{q}_p \mathbf{e}_p^T$  is a matrix that represents the pure spectra of the

product of the reaction versus monitored times. Figure 3 represents the spectrum of the product of this reaction obtained by the procedure in question, which is fully in agreement with the simulated one.

The results of all the simulated data sets are presented in Table 1. There is good agreement between the synthesized and predicted rate constants, showing the applicability of the method to noisy systems.

### Experimental Data

The proposed algorithm was applied to investigate a particular second-order reaction. The reaction between 1,2-naphthoquinone-4-sulfonate sodium (NQS) and 3-nitroaniline (TNA) was monitored spectrophotometrically. It was found [36] that 1,2-naphthoquinone-4-sulfonic sodium (NQS) could react with amino group of primary amine derivative. Amino group of 3-nitroaniline (TNA) displays nucleophilicity. So, TNA can react with sodium 1,2-naphthoquinone-4-sulfonate in a nucleophilic substitution reaction. The probable second-order reaction between 1,2-naphthoquinone-4-sulfonate sodium and 3-nitroaniline in ethanol is represented in Scheme 1.

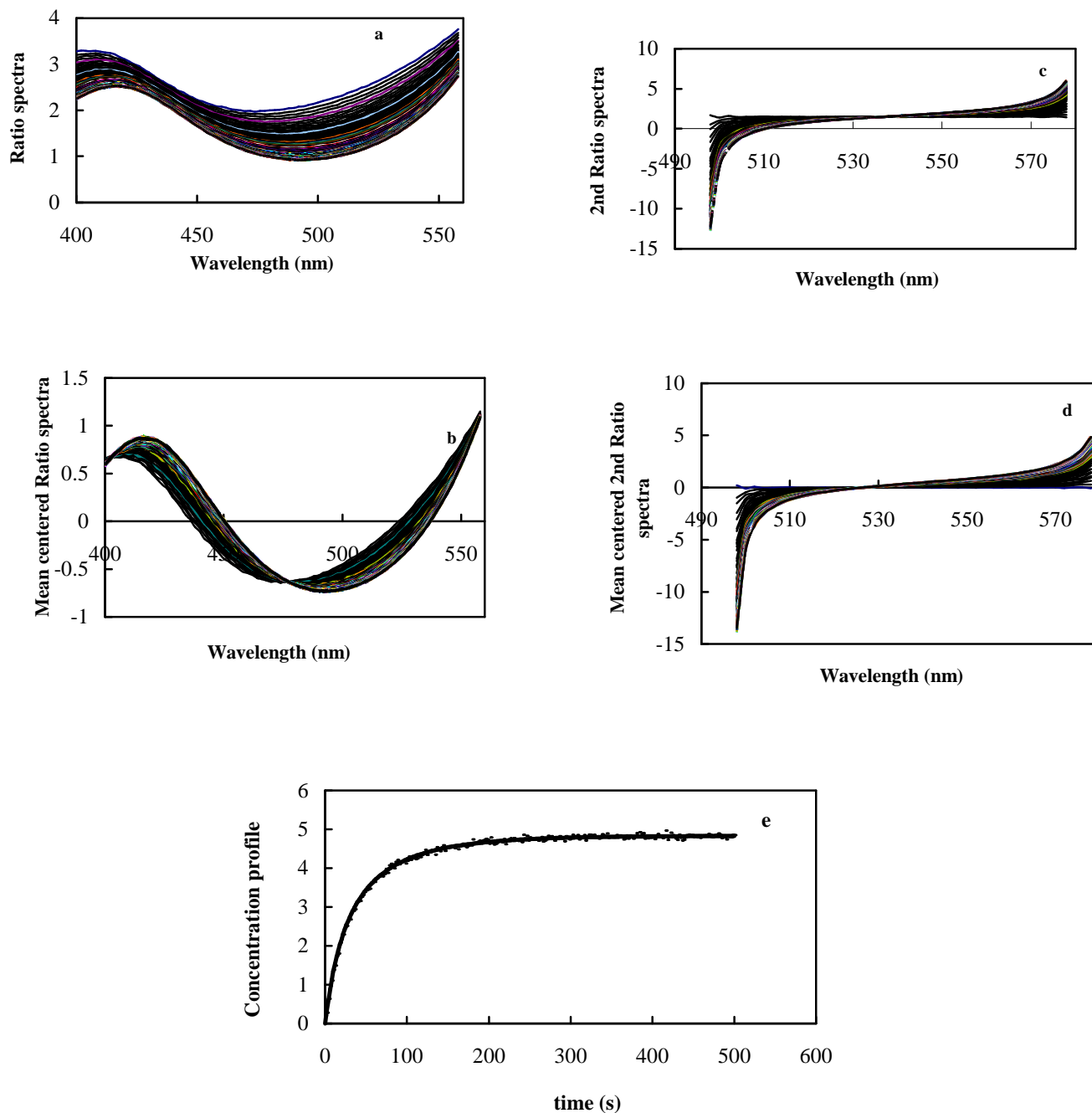
In this study,  $2.8 \times 10^{-4}$  M of NQS and  $3.36 \times 10^{-4}$  M of TNA were monitored in ethanol as the solvent at 29 °C. The spectra of the reactants, NQS and TNA, are presented in Fig. 4, which also shows the monitored spectra of this reaction at different times.

The obtained data ( $n_t \times n_w$ ) were handled by the proposed method, as explained above. The pure spectra of the reactants (NQS and TNA) were used as divisors and their contributions were eliminated from the data set.

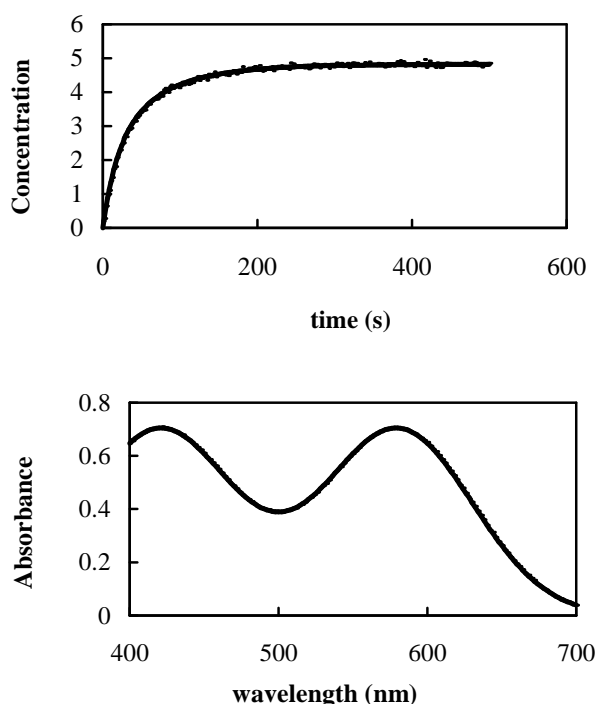
For the two-way kinetic spectral data of NQS and TNA reaction (**Y**), if the elements of rows ( $\mathbf{Y}(i,:)$ ) are divided by the elements of  $e_{NQS}$ , corresponding to the spectrum of 1,2-naphthoquinone-4-sulfonate sodium (NQS), the first ratio spectra would be obtained according to Eq. (4) which is called **D**.

If the matrix **D** is row mean centered according to Eq. (5), MC(**D**) would be obtained. By dividing each row of MC(**D**), shown as MC(D)(i,:) in Eq. (6), by  $MC(e_{TNA}/e_{NQS})$ , element by element, the second ratio spectra are obtained which is called **U** (for calculating  $MC(e_{TNA}/e_{NQS})$ , the spectrum of the TNA is divided by the spectrum of NQS, element by element, and the

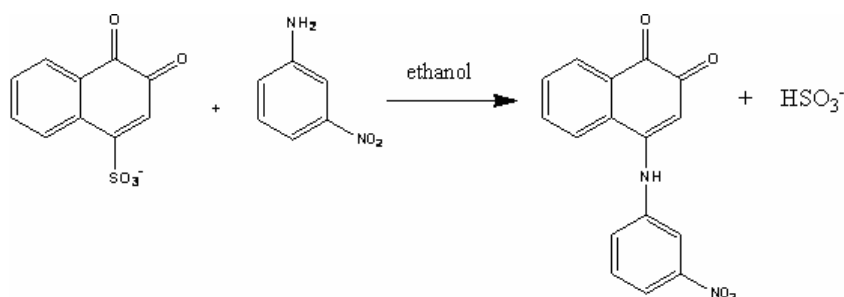
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**Fig. 2.** (a): The ratio profiles which obtained by dividing the simulated data by reactant spectrum ( $e_A$ ), (b): the mean centering of ratio profiles, (c): second ratio profiles were obtained by dividing by  $MC(e_B/e_A)$ , (d): Mean centering of these vectors and (e): the concentration profile of the product.



**Fig. 3.** The results of applying the proposed method on simulated data (the calculated kinetic profile and spectrum ( $\blacklozenge$ ) and the simulated ones ( $\text{—}$ )). The kinetic profile was estimated by fitting and the spectrum of product of reaction was calculated by subtraction the contribution of reactants from data.



*Scheme 1.* The probable second order reaction between 1,2-naphthoquinone-4-sulfonate sodium and 3-nitroaniline in ethanol

obtained ratio profile is mean centered). Now, if the matrix  $\mathbf{U}$  is row mean centered according to Eq. (7), the matrix  $\mathbf{L}$  would be obtained each row of which depends on the concentration of the product ( $\mathbf{q}_p$ ), and, therefore, plotting every column of  $\mathbf{L}$  versus time will give a pure kinetic profile of the product. Finally, the rate constants of the reactions can be simply reached by curve fitting.

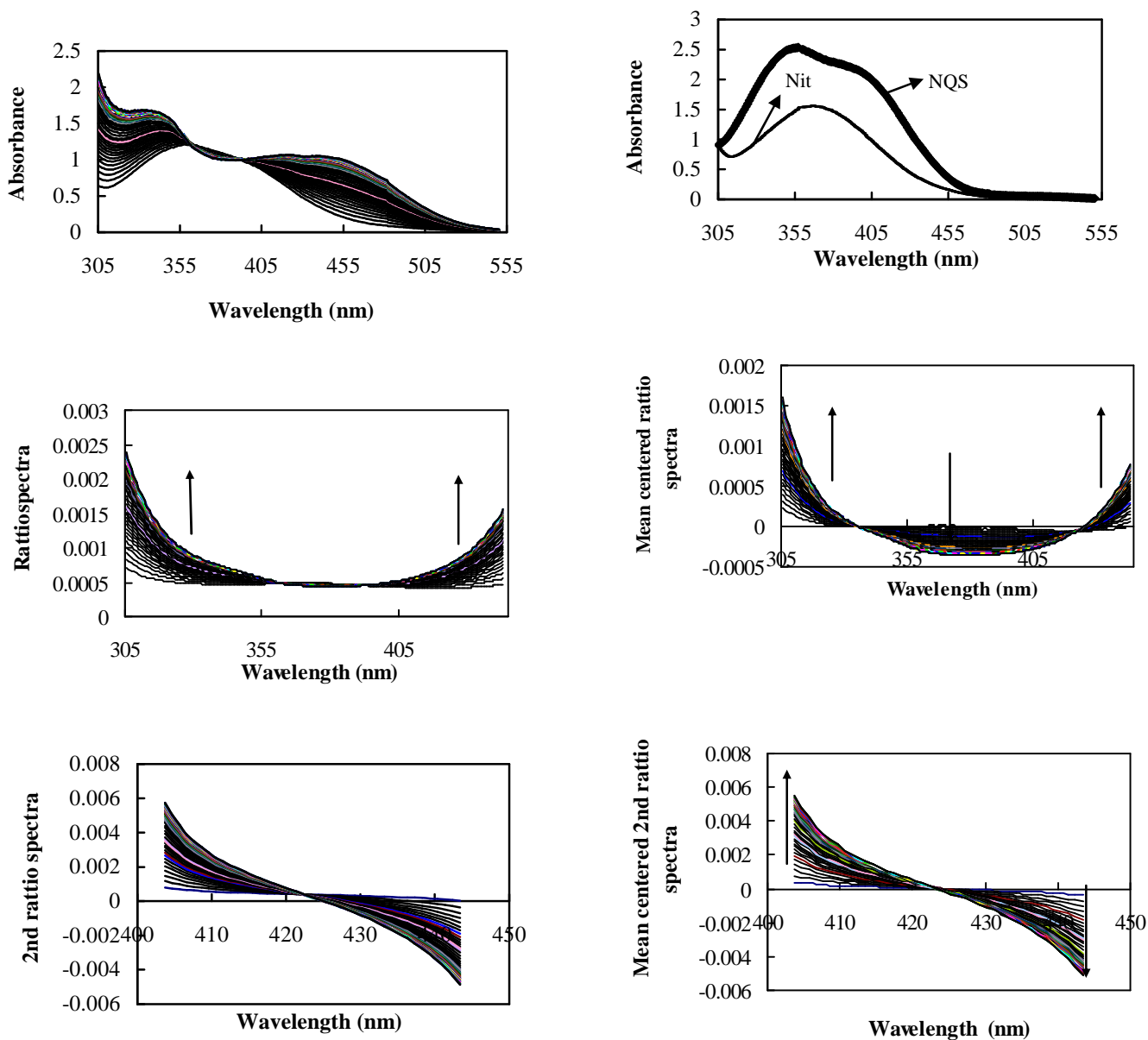
The ratio and the mean centered ratio profiles of the experimental data are represented in Fig. 4. The singular value decomposition of this data set and those of mean centered ratio spectra ( $\mathbf{D}$  and  $\mathbf{L}$ ) are presented in Table 2. These indicate that the rank of the original data ( $\mathbf{Y}$ ) and the first mean centered ratio spectra ( $\mathbf{D}$ ) is 2, but the singular value decomposition of the 2<sup>nd</sup> ratio spectra ( $\mathbf{L}$ ) revealed that there was only one component. Therefore, it can be concluded that the data set belongs to a one-step, second-order reaction, because the contributions of the reactants (NQS and TNA) have been totally eliminated by the proposed method.

Accordingly, by eliminating the contribution of both reactants from this data set, the pure response for the product of the reaction could be reached (Fig. 4). The kinetic profile of the product of this second-order reaction was obtained by plotting the amplitude of the resulting spectra at the maximum or minimum wavelength shown in Fig. 4 versus time.

In addition, Fig. 5 represents the result of curve fitting for this data set. The rate constant for this reaction was estimated as  $213 \text{ min}^{-1}$ .



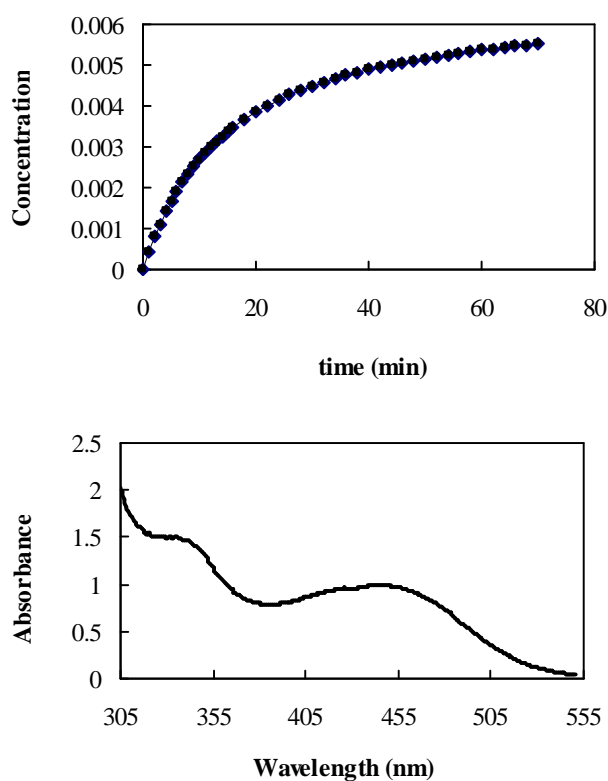
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**Fig. 4.** Absorption curve of two-way kinetic-spectra of reaction between reaction of 1,2-naphthoquinone-4-sulfonate sodium (NQS) and 3-nitroaniline (TNA) in the ethanol solvent, the pure spectra of reactants (NQS and TNA), the ratio profiles that obtained by dividing the data by reactant spectrum ( $e_{NQS}$ ), the mean centering of ratio profiles, second ratio profiles were obtained by dividing by  $MC(e_{TNA}/e_{NQS})$ , mean centering of these vectors and the concentration profile obtained for the product of the reaction.

**Table 2.** Singular Value (SV) Decomposition of Experimental Data and Mean Centered Ratio Spectra

No. of SV	Data	Mean centered ratio spectra 1 ( <b>D</b> )	2 <sup>nd</sup> Mean centered ratio spectra ( <b>L</b> )
1	97.8515	0.024664	0.080804
2	10.1502	0.0010031	0.000244
3	0.3142	1.93E-04	0.000124
4	0.0968	7.56E-05	9.31E-05
5	0.0685	5.97E-05	9.03E-05
6	0.0668	5.18E-05	8.23E-05
7	0.0615	3.97E-05	7.54E-05
8	0.0583	3.75E-05	7.19E-05
9	0.0542	3.50E-05	6.86E-05
10	0.0496	3.28E-05	6.21E-05



**Fig. 5.** The results of applying the proposed method on experimental data (the calculated data (♦) and the fitted ones (—)). The kinetic profile was estimated by fitting and the spectrum of product of reaction was calculated by subtraction the contribution of reactants (NQS and TNA) from data.

Provided that the rate constant or the kinetic profiles of the reactants ( $\mathbf{q}_{\text{NQS}}$  and  $\mathbf{q}_{\text{TNA}}$ ) are available, the pure absorption spectrum of the product component can be reached by subtracting the contribution of reactants (NQS and TNA), so that:

$$\mathbf{q}_p \mathbf{e}_p^T = Y - \mathbf{q}_{\text{NQS}} \mathbf{e}_{\text{NQS}}^T - \mathbf{q}_{\text{TNA}} \mathbf{e}_{\text{TNA}}^T$$

where  $\mathbf{q}_p \mathbf{e}_p^T$  is a matrix representing the pure spectra of the product of the reaction versus the monitored times. Figure 5 represents the spectrum of the product of the reaction obtained by this procedure.

## CONCLUSIONS

In this research we used the mean centering of ratio spectra to estimate the rate constants of second order reactions without any ambiguity. The proposed algorithm was simple, very sensitive and easy to understand and apply. Several simulated data, as well as the reaction between 1,2-naphthoquinone-4-sulfonate sodium (NQS) and 3-nitroaniline (TNA) in ethanol as a real system were evaluated by the proposed method. The results were all satisfactory. The proposed method could also be applied to the investigation of the reactions with reaction orders other than 1 with respect to each reactant. The sampling time intervals may be equal or not. Although the spectra of the reactants must be known for the application of the proposed method (and this may be a disadvantage of the method), an

important advantage of the method, as mentioned above, is that it can be used for resolving the kinetic profiles of unknown reaction models, *i.e.*  $O_2$  in Eq. (1) does not have to be 2. In other words, the method is suitable not only for the proposed method could also be applied to reaction systems in which one of the reactants, or even the product itself, is a non-absorbing species.

In these cases a binary mean centering of ratio spectra [29-32] could be applied instead of a ternary second-order reactions, but also for reactions with fractional orders, 1<sup>st</sup> order or higher order, with respect to the reactant (s).

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