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ORIGINAL PAPER

KINMODEL (AGDC): a multipurpose computational method for kinetic treatment

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Abstract KINMODEL (AGDC) is a kinetic computational methodology that is valid for the treatment of any reaction mechanism and that allows the determination of different kinetic and non-kinetic parameters from the experimental data acquired by monitoring absorbance at one or several different wavelengths. It is a numerical computational model that can be applied to any reaction mechanism, with the advantage that on changing the treatment from one mechanism to another it is not necessary to modify even a single line of the program code since it automatically establishes and solves the set of differential rate equations. It is able to treat a broad set of reaction mechanisms in the individual and joint determination of the following groups of parameters (***a***) the individual rate constants of the different reaction mechanisms; (***b***) the values of the molar absorption coefficients (which are very valuable in the case of intermediate species and their identification) of all the species involved in the mechanism, and (***c***) the concentrations of the species participating in the mechanism. The program can be used by non-experts in the field and it is able to treat mechanisms involving ambiguities in the solutions and in the identification of parameters when kinetic constants and molar absorption coefficients are optimized together, and it allows a discrimination to be made between the possible mechanisms responsible for the course of the reaction after the residuals have been analyzed statistically, automatically choosing the one that best fits the kinetic data.

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1 Introduction

One of the aims of Computational Kinetics is the development of software that will permit the study of any chemical system, both simple and complex. During the sixties, several papers were published [1,2] that were the precursors of other later contributions that developed computational techniques for kinetic systems [3–7]. The computational techniques developed for the determination of kinetic parameters are very numerous and are of very different natures. They are based on both non-linear regression techniques and on other computational methodologies. Traditional curve-fitting methods (*TCF*) allow the determination of kinetic, analytic and thermodynamic parameters from spectrophotometric data obtained both at a single wavelength (selective or not) and at multiwavelength [8–13]. They use different mathematical optimization algorithms that are implemented in computational programs, readily available as free software [14–19,41–47]. Other methods for data treatment used to determine kinetic parameters are the classic curve-resolution techniques (CCR) and their modifications, Classical Curve Resolution-Hard Modelling (CCR-HM) [20–25], Classical Curve Resolution-Soft Modelling (CCR-SM) [26] or a combination of both, Classical Curve Resolution-Combining Hard-Soft Modelling (CCR-CHSM) [27,28]. The different modifications of classic curve resolution techniques allow the absorption spectra of reacting species to be determined and it is also possible to introduce rate constants as unknown parameters to be optimized. Bijlsma et al. [29] made an interesting comparison between traditional curve fitting techniques (TCF) and classic curve resolution techniques (CCR) for the case of the determination of the rate constants of a biochemical reaction under the treatment of pseudo-first-order and second-order. Other techniques used the so-called Kalman filter algorithm, in its extended version [30–32], which evaluates spectrophotometric data, allowing the prediction of concentrations and rate constants, and there are also methodologies based on Artificial Neural Networks (ANN) techniques [33–37]. In general, the methodologies for kinetic treatment can be found in different text books [38–40], which gather and describe both classic and current methods for data treatment and the determination of different parameters.

Here we propose a new multipurpose computational program (KINMODEL (AGDC)) for the treatment of kinetic data that uses the AGDC mathematical algorithm [41–48] for the determination of different parameters by computing the kinetic data of absorbance monitored at a single and several wavelengths. The AGDC mathematical optimization algorithm has been implemented in several computational programs and has been successfully used in different chemical systems for both kinetic and analytical purposes, such as for the treatment of chemical systems in equilibrium [41,42], allowing the determination of thermodynamic micro and macroconstants of dissociation; the determination of kinetic parameters in different reaction mechanisms

[43–46], and the quantitative analysis of static (SMM) [48] and dynamic (DMM) [46,47] multicomponent mixtures. It is written in C++ language, using JAVA applications, which allows it to be implemented in a WINDOWS environment, facilitating its use by non-experts. It is based on a generalized method that permits the automatic treatment of any reaction mechanism without the need to modify a single line of code when passing from the treatment of one mechanism to another. After it has been provided with the matrix of stoichiometric coefficients, the program itself generates the kinetic model automatically, establishes the set of differential equations, and solves them by applying numerical methods. Application of the AGDC mathematical optimization algorithm allows the KINMODEL (AGDC) program, using spectrophotometric data obtained from absorbance monitoring, to determine a series of parameters corresponding to the following sets of parameters, with a kinetic nature or not: ***1.-*** the kinetic constants (k_r) of the r elemental reactions comprising the mechanism; ***2.-*** the molar absorption coefficients of the j species at different wavelengths ($\varepsilon_{j,\lambda}$), whose values are especially valuable for intermediate species since such values cannot be determined experimentally; ***3.-*** the initial concentrations of the j species ($[B_j]_0$) involved; ***4.-*** the kinetic constants (k_r) and initial concentrations ($[B_j]_0$) jointly and simultaneously from a single optimization process, and ***5.-*** the kinetic constants (k_r) and molar absorption coefficients ($\varepsilon_{j,\lambda}$) simultaneously, according to the following modes: ***5.1***.- by performing a single optimization process; ***5.2.-*** by two specific individual processes of optimization, one of them to optimize k_r and the other for $\varepsilon_{j,\lambda,}$ which in turn can be performed according to several application strategies. In the present paper we did not compute experimental data to obtain concentrations (points 3 and 4) but the program is able to do this once its proper functioning has been checked with previous tests performed with synthetic data.

Among the advantages of the KINMODEL (AGDC), the most important are as follows: (***a***) the program allows the computation of a broad set of data (absorbance at one or several wavelengths) for any reaction mechanism, such that it is of great use in chemical kinetics since it is not necessary to modify the program for each system studied; (***b***) it allows the determination of ($\varepsilon_{j,\lambda}$), values that are of special interest when they belong to intermediate species since they cannot be known with experimental measurements (Beer-Lambert law), owing to their instability, and can thus be used for species identification; (***c***) the program performs an exhaustive statistical analysis of residuals, which allows one to know the reaction mechanism that best fits the kinetic data and which is chosen automatically by the program; (***d***) it affords the joint determination of k_r and $\varepsilon_{j,\lambda}$, and it is possible to detect kinetic systems showing problems of ambiguity in the resolution of the set of parameters and finally (***e***) the program can be used by non-experts users.

2 Theoretical aspects

The absorbance of a mixture, at a wavelength λ and at time i, ($A_{i,\lambda}$), must be the sum of the contribution of all the j species showing absorption at that wavelength. According to the Beer-Lambert law, the total absorbance, $A_{i,\lambda}$, of a mixture formed by N_c chemical species, will be:

$$A_{i,\lambda} = \sum_{j=1}^{Nc} A_{j,i,\lambda} = \sum_{j=1}^{Nc} \varepsilon_{j,\lambda} \left[B_j\right]_i \tag{1}$$

where $A_{j,i,\lambda}$ is the absorbance of the chemical species j at time i and at wavelength λ; $\varepsilon_{j,\lambda}$ is the molar absorption coefficient of the species j at a wavelength λ, and $[B_j]_i$ is the molar concentration of the species j at time i. The sum function of quadratic deviations (SSQ) is the function to be minimized and is given in terms of absorbance:

$$SSQ = \sum_{i=1}^{Nd} \sum_{\lambda=1}^{Nw} \left(\left(A_{i,\lambda}\right)_{calc} - \left(A_{i,\lambda}\right)_{exp} \right)^2 \tag{2}$$

where N_d is the number of experimental data pairs; N_w is the number of working wavelengths; $(A_{i,\lambda})_{exp}$ is the total absorbance value obtained at a wavelength λ, and $(A_{i,\lambda})_{calc}$ is the absorbance value calculated according to Eq. (1). Bearing in mind Eqs. (1) and (2), the SSQ function can be expressed as a function of $[B_j]_i$, according to:

$$SSQ = \sum_{i=1}^{Nd} \sum_{\lambda=1}^{Nw} \left(\sum_{j=1}^{Nc} \left(\varepsilon_{j,\lambda}[B_j]_i\right) - \left(A_{i,\lambda}\right)_{exp} \right)^2 \tag{3}$$

To know $[B_j]_i$ it is necessary take into account a series of considerations concerning the kinetic model under study.

2.1 Generalized establishment of the set of differential equations

According to the IUPAC norms, each of the chemical reactions comprising a mechanism formed by N_r reactions and N_c species [49,50] is expressed thus:

$$0 = \sum_j \nu_{j,r} B_j \qquad j = 1, \ldots, N_c; \quad r = 1, \ldots, N_r \tag{4}$$

and the rate at which reaction r occurs is expressed by:

$$v_r = k_r \prod_{c=1}^{Nc} [B_c]^{|\nu_{c,r}|} \qquad r = 1, \ldots, N_r \tag{5}$$

where k_r is the rate of the reaction r; $\nu_{c,r}$ is the stoichiometric coefficient of the species B_c in the reaction r and $[B_c]$ is the concentration of the species acting as reagents in the reaction r ($\nu_{c,r} < 0$). The variation in the concentration of each species B_j with time is given by the differential equation:

$$\frac{d\left[B_j\right]}{dt} = \sum_{r=1}^{Nr} \nu_{j,r} v_r \qquad j = 1, \ldots, N_c; \quad r = 1, \ldots, N_r \tag{6}$$

The program is equipped with a subroutine (DIFFEQ) that numerically establishes, in an automatic and generalized manner, the set of differential rate equations for the kinetic model under consideration, according to the matrix of stoichiometric coefficients of the model $\nu_{j,r}$, the rate constants of the different reactions integrating the model k_r, and the initial concentrations of the different species involved in the model $[B_j]_0$.

2.2 Numerical solution of the set of differential rate equations

To determine the concentration of all the species in the period of time considered, $[B_j]_i$, the program performs the numerical resolution of the set of differential equations by the method of numerical integration based on the Gear algorithm [51,52], which is efficient and robust and which performs the integration of the system with great accuracy and affords good results even when the system is complex and has stiff characteristics.

2.3 Numerical derivation

The AGDC mathematical optimization algorithm [41–48] is a second-order gradient method that requires calculation of the gradient vector **g** and of the Hessian matrix **H** [53,54], whose terms are those derived from the function to be minimized (SSQ) with respect to each of the parameters to be determined. Calculation of the derivatives is performed numerically by means of the central differences method [51].

2.4 Movement vector. Calculation of the inverse matrix

Determination of the movement vector involves calculation of the inverse of the Hessian matrix. By means of the Gauss elimination method [55] an approximate inverse matrix is determined; then, its accuracy is improved by means of an iterative method of successive approximations [56].

2.5 Optimization

This is carried out by means of the AGDC mathematical optimization algorithm [41–48] for the determination of the parameters whose values one wishes to know. It is a second-order gradient method that minimizes the numerical function (SSQ) by means of an iterative process that first uses as the movement vector the one indicated by the Gauss-Newton method [53,54]:

$$\mathbf{p}^{(m)} = -\mathbf{g}^{(m)} \left[\mathbf{H}^{(m)}\right]^{-1} \tag{7}$$

$\mathbf{p}^{(m)}$, $\mathbf{g}^{(m)}$ and $[\mathbf{H}^{(m)}]^{-1}$ are respectively the movement vector, the gradient vector and the inverse of the Hessian matrix of the iteration m, whose details appear in

Appendix 1. In order to ensure that the minimum of the SSQ function will be obtained [41–48], in each of the iterations it is necessary to perform a rigorous analysis and control of the movement vector and of each of its terms, and suitable modifications can be made if any errors are detected, thereby ensuring successful optimization. Once the optimization process has been achieved, the program determines the errors of the optimized parameters and performs an exhaustive analysis of the residuals, thus allowing the goodness of fit to be checked and at the same time affording the possibility of discriminating between the possible reaction mechanisms. Below we offer a schematic step-by-step view of KINMODEL (AGDC).

1. Select the mechanism of reaction to be studied.
   - 1.1. Select the mechanism of reaction to be studied into the models proposed by the program or,
   - 1.2. Select other mechanisms [47].
2. Select the parameters to be optimizated (**X**).
   - 2.1. Kinetic constants (k_r).
   - 2.2. Initial concentrations of the species involved in the mechanism ($[B_j]_0$).
   - 2.3. Kinetic constants and initial concentrations simultaneously (k_r and $[B_j]_0$).
   - 2.4. Molar absorption coefficients ($\varepsilon_{j,\lambda}$)
   - 2.5. Kinetic constants and molar absorption coefficients (k_r and $\varepsilon_{j,\lambda}$) according to the following modes.
     - 2.5.1. Simultaneously by performing a single optimization process.
     - 2.5.2. Two specific individual processes of optimization, one of them to optimize k_r and the other for $\varepsilon_{j,\lambda,}$
3. m = 0. Input data:
   - 3.1. Experimental data of absorbance/time $(A_{i,\lambda})_{exp}$, convergence criteria CC, known parameter values, matrix of stoichiometric coefficients, $\nu_{j,r}$, if 2.2. is selected,.., etc..
   - 3.2. Initial estimates of the unknown parameters $\mathbf{X}^{(0)}$.
4. Determinate the $SSQ^{(0)}$ function (Eq. 3).
   - 4.1. Establish the rate differential equations system and
   - 4.2. Numerical solution, calculate of concentrations of species $[B_j]_i$.
   - 4.2. Calculate the absorbance at each wavelength $(A_i, \lambda)_{cal}$ (Eq. 1).
5. **AGDC ALGORITHM**
   - 5.1. Calculate the vector of movement $\mathbf{p}^{(m)}$ (Eq. 7).
     - 5.1.1 Compute partial numerical derivatives of $(A_i, \lambda)_{cal}$ with respect to the parameters to be determined $\mathbf{X}^{(m)}$, central differences method.
     - 5.1.2. Compute Gradient vector and Hessian Matrix ($\mathbf{g}^{(m)}$ and $\mathbf{H}^{(m)}$). (Appendix 1)
     - 5.1.3. Compute $(\mathbf{H}^{(m)})^{-1}$ by Gauss elimination method and improvement by sucessive aproximations method.
     - 5.1.4. Calculate the components of the vector of movement $(\mathbf{p}^{(m)} = -(\mathbf{H}^{(m)})^{-1}\mathbf{g}^{(m)})$
   - 5.2. Control and correction of the direction of the vector of movement $\mathbf{p}^{(m)}$
     - 5.2.1. If $\mathbf{H}^{(m)}$ is singular, $\mathbf{p}^{(m)} = -\mathbf{g}^{(m)}$, go to 5.3.
     - 5.2.2. If $\mathbf{p}^{(m)}\mathbf{g}^{(m)} < \varepsilon$ (ε = scalar close to zero), $\mathbf{p}^{(m)} = -\mathbf{g}^{(m)}$ and go to 5.3

5.2.3. If $\mathbf{p}^{(m)}\mathbf{g}^{(m)} > 0$, $\mathbf{p}^{(m)} = -\mathbf{p}^{(m)}$.

5.3. Control the length of the vector of movement $\mathbf{p}^{(m)}$.

5.3.1. Compute the scalar ($\alpha^{(m)}$) by the method of Hartley [53].

5.3.2. $\mathbf{X}^{(m+1)} = \mathbf{X}^{(m)} + \alpha^{(m)}\mathbf{p}^{(m)}$

5.3.3. Determinate the $SSQ^{(m+1)}$ function.

5.3.4. If the Goldstein-Armijo criterium [54] is satisfied go to 5.4.

5.3.5. $\alpha^{(m)} = \alpha^{(m)} / 2$ go to 5.3.2.

5.4. Calculate $CON = \left|\frac{SSQ^{(m+1)} - SSQ^{(m)}}{SSQ^{(m)}}\right|$

5.5. If convergence is not attained (CON > CC), set m = m + 1 and go to 5.1.

6. $\mathbf{X}^{(m+1)}$ = Optimized Parameters. Calculation of the errors of the parameters [57,58] and performs statistical analysis of residuals (SAR) [59–61].

6.- END.

The computational aspects are offered in Appendix 2.

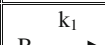
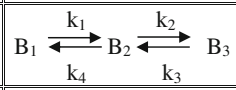
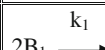
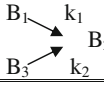
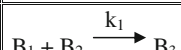
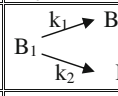
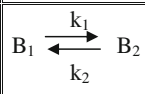
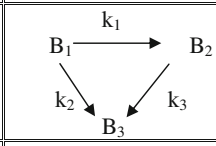
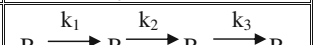
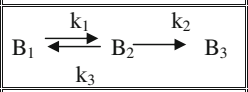
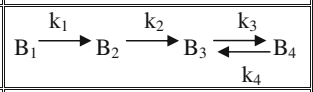
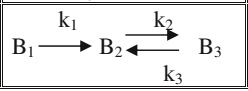
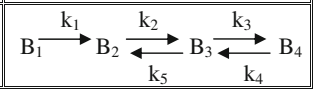
3 Results and discussion

3.1 Determination of individual rate constants

The KINMODEL (AGDC) computational methodology was used for the determination of the individual rate constants (k_r) of each one of the reaction mechanism shown in Scheme 1, considering other parameters known ($\varepsilon_{j,\lambda}$ and $[B_j]_0$). To do so, the treatment of the absorbance data generated at several wavelengths for each reaction mechanism was performed, the data being endowed previously with random error (± 0.0005). Table 1 shows the results obtained in the determination of the individual rate constants for the different reaction mechanisms. It shows the initially estimated values of the different constants (k_{rEST}) and the same values obtained after the optimization (k_r) as well as the value of the SSQ function obtained with the initial estimates of the constants and their values obtained after the optimization process. In all experiments, the values of the rate constants were very similar to the real ones. Additionally, acceptable values were obtained for the SSQ function, for the errors of the parameters and for the standard deviation of fitting (approx. 7. E-05), which were in agreement with the error imposed on the data. Statistical analysis of residuals (SAR) confirmed the goodness of the fitting performed in all the experiments, confirming the validity of the methodology for the determination of the individual rate constants for any reaction mechanism.

3.2 Simultaneous determination of rate constants and molar absorption coefficients

Simultaneous determination of rate constants and molar absorption coefficients is of great interest when there are intermediate species in the reaction mechanism that are difficult to isolate and whose molar absorption coefficients (an important parameter that serves for their chemical identification) cannot be determined experimentally with the Beer-Lambert law. In this type of joint optimization (k_r and $\varepsilon_{j,\lambda}$) an important problem of ambiguity in the solution arises when one is considering reaction mechanisms

Number	*MECHANISM*	*Number*	*MECHANISM*
1		**8**	
2		**9**	
3		**10**	
4		**11**	
5		**12**	
6		**13**	
7		**14**	

Scheme 1 Reaction mechanisms studied with KINMODEL (AGDC)

comprising first-order reactions. The problem lies in the existence of 2 or more solutions that fit different sets of parameters to the experimental kinetic data. Accordingly, for there to be a single solution, or whether by contrast there will be ambiguity in the solution, depends on the kinetic data computed and on the mechanism in question. Thus, if spectrophotometric data (total absorbance of the system *vs.* time) are computed for the system of consecutive reactions ($B_1 \rightarrow B_2 \rightarrow B_3$), considering that the three species involved in the reaction show absorbance and also the working wavelength or wavelengths, the total absorbance ($A_{i,\lambda}$) will be given by:

$$A_{i,\lambda} = \varepsilon_{B,\lambda}\,[B_1]_i + \varepsilon_{B2,\lambda}\,[B_2]_i + \varepsilon_{B3,\lambda}\,[B_3]_i \tag{8}$$

where $\varepsilon_{B1,\lambda}$, $\varepsilon_{B2,\lambda}$, $\varepsilon_{B3,\lambda}$ are the molar absorption coefficients of the species participating in the reaction at the measuring wavelength. Joint optimization of the rate constants and the molar absorption coefficient of the intermediate species, from treatment of the data on total absorbance, can give rise to two types of ambiguities: (***a***) in the parameters (named *Identifiability)*: the solution is not unique when two or more sets of parameters lead to the same absorbance-time curve, and (***b***) ambiguity in the model (named *Distinguishability*): there may be several reaction mechanisms that give rise to the same absorbance-time curve. It is possible to have a priori knowledge of these ambiguities by applying deterministic analysis, using different methods such as the Laplace Transform or the similarity transforms. By applying the Laplace Transform

[62,63] to the differential rate equations of the mechanism and to the response function (absorbance) one obtains a set of polynomial equations whose resolution provides two indistinguishable and equally probable solutions that correspond to two sets of parameters (k_1, k_2, ε_{B2}) and (k'_1, k'_2, ε'_{B2}) related to each other by the equations:

$$k'_1 = k_2; k'_2 = k_1; \varepsilon'_{B2} = \varepsilon_{B1} + k_1(\varepsilon_{B2} - \varepsilon_{B1})/k_2 \tag{9}$$

These solutions satisfy Eq. (8), the optimization process possibly leading to one or the other solution, depending on the closeness of the initial estimates to each solution since both generate identical and indistinguishable minima in the SSQ function. When the kinetic data corresponding to the intermediate species B_2 ($[B_2]$ vs. time or absorbance of B_2 *vs.* time) are computed, a problem of ambiguity arises, although different from the previous one, with two solutions corresponding to the sets of parameters (k_1, k_2, $[B_1]_0$) and (k'_1, k'_2, $[B_1]'_0$) with the following relationships:

$$k'_1 = k_2; k'_2 = k_1; [B_1]'_0 = (k_1/k_2)\,[B_1]_0 \tag{10}$$

In the case of optimizing only k_1 and k_2 there is no ambiguity because the values of $[B_1]_0$ remains fixed. This problem is detected automatically by KINMODEL (AGDC) after a previous test with the optimized parameters from all the mechanisms showing that possibility. There are methods that are able to solve the problem of ambiguity once it has been detected and that have been incorporated into our program. These are as follows: the search for complementary (physico-chemical, structural, etc,...) information about the species involved in the reaction [64]; the inclusion of algorithms available in the literature based on the exchange in the initial estimates between the values of k_1 and k_2; analysis of the dependence on temperature when the values of enthalpies of activation are different for both constants and on pH or any other differentiating property. Its should be noted that when KINMODEL (AGDC) optimizes only k_1 and k_2 (1st part of the program), the ambiguity problem does not exist because the value of the absorption coefficient of the intermediate species (B_2) is constant and unchanging throughout the iterative process.

KINMODEL (AGDC) was applied, to determine simultaneously k_r and $\varepsilon_{j,\lambda}$, to the consecutive first-order reaction mechanisms (reversible and irreversible) indicated in Scheme 1 (numbers 5, 6, 7 and 8) in which three species are involved and in which initially only the reagent (B_1) is present. The absorbance ($A_{i,\lambda}$) to which the three species contributed was monitored at one or several wavelengths (Eq. 8). Using the Laplace Transform [61,62], below we discuss the identifiability problem of the parameters leading to the above-mentioned problem of ambiguity in the solution in the systems of consecutive reactions, 5, 6, 7 and 8. The following situations were observed: model 8 is an identifiable system that has a single set of parameters that give rise to the total absorbance-time curve; models 5 and 7 are identifiable systems, but with two solutions: i.e., two sets of parameters that afford the same total absorbance-time curve. Regarding system 6, this is a non-identifiable system because there may be infinite solutions that will give the same value of total absorbance. In the four models studied, we assessed the influence of a series of factors on the optimization process: (***a***) initial estimates of the rate constants and the molar absorption coefficients; (***b***) number

Table 1 Rate constants optimized for different reaction mechanisms (Scheme 1) k_r / min^{-1}

Mechanism	k_{1EST}	k_{2EST}	k_{3EST}	k_{4EST}	k_{5EST}	SSQ_{INIT}	k_1	k_2	k_3	k_4	k_5	SSQ_{FINAL}
1	1.0E00					5.437	2.002E-2					1.712E-7
2	5.0E0					2.636	1.008					1.307E-7
3	1.0E-3					0.162	2.007E-1					5.317E-7
4	1.0E-5	1.0E-5				1.196	0.999E-2	0.499E-2				2.691E-8
5	5.0E-2	1.0E-1				0.156	1.001E-1	4.993E-2				2.369E-7
6	1.0E-3	1.0E-3	1.0E-3			4.366	0.999E-1	0.499E-1	0.999E-2			1.683E-7
7	1.0E-2	2.0E-2	2.0E-2			1.775	1.000E-1	0.499E-1	1.002E-2			1.681E-7
8	1.0E-2	2.0E-2	2.0E-2	2.0E-2		2.366	1.000E-1	0.499E-1	1.002E-2	0.994E-2		1.259E-7
9	1.0E-3	1.0E-3				7.559	1.000E-1	5.060E-2				2.302E-7
10	1.0E-4	1.0E-4				1.729	1.000E-2	0.499E-2				6.952E-8
11	1.0E-3	1.0E-2	1.0E-3			0.236	1.000E-2	0.499E-2	0.999E-4			7.987E-8
12	1.5E-1	5.5E-2	2.5e-2			0.1377	1.014E-1	5.771E-2	1.241E-2			1.291E-4
13	1.5E-1	5.5E-2	2.5e-2	1.5e-2		0.3981	1.000E-1	5.005E-2	1.997E-2	9.978E-3		6.370E-9
14	1.5E-1	5.5E-2	2.5e-2	1.5e-2	1.5e-3	0.4684	1.001E-1	5.063E-2	2.051E-2	9.888E-3	1.940E-3	1.704E-6

and type of parameters to be optimized, and (***c***) the precision of the results obtained as a function of the error assigned to the absorbance data. In the system of consecutive, irreversible first-order reactions (number 5), there are two sets of parameters (k_1, k_2, ε_{B2}) and (k'_1, k'_2, ε'_{B2}) that lead to the same total absorbance-time curve. We performed the joint and simultaneous optimization of (***a***) three parameters (k_1, k_2 and $\varepsilon_{B2,\lambda}$) from the data obtained at a single selective wavelength λ; (***b***) five parameters (k_1, k_2, $\varepsilon_{B2,\lambda1}$, $\varepsilon_{B2,\lambda2}$ and $\varepsilon_{B2,\lambda3}$) from the data obtained at one and three selective wavelengths; λ_1, λ_2 and λ_3. In case (***a***) it may be seen from Table 2 and Fig. 1 that two sets of parameters afford the same absorbance-time curve. The value of the SSQ function decreases considerably in the two solutions obtained, from the first iteration to the last one, the final value being very low in both cases. The two sets of parameters determined are mathematically acceptable; it is not possible to distinguish between the two solutions, and according to mathematical criteria both are acceptable. The Fig. 2 shows the 3D graphical representation with reduction of the subinterval in the proximities of the two identical and mathematically indistinguishable minima of the SSQ function obtained. In case (***b***) Table 2 shows the results obtained in the optimization process, observing the two possible solutions. We increased the number of parameters to optimize through joint determination of rate constants and molar absorption coefficients of all reacting species. When the absorbance data were obtained at a single wavelength, the number of parameters to be optimized was 5 and when three wavelengths were used it was 11. Table 3 shows the results obtained, the optimization process was carried out correctly, despite the appreciable increase in the number of parameters, and mainly depended on the initial estimates of the coefficients. Two equally possible mathematical solutions were obtained, leading to two minima of the SSQ function. In all the experiments performed, a Statistical Analysis was made of the residuals with the observation of no appreciable differences in the two solutions obtained, pointing to the ambiguity detected by the program in the solution for this kinetic system.

3.3 Discrimination among reaction mechanisms

We consider a mechanism formed by two first-order consecutive reversible reactions (4 rate constants) $B_1 \leftrightarrow B_2 \leftrightarrow B_3$. The kinetic absorbance data were generated for this mechanism considering the following parameters: initial concentrations, rate constants and molar absorption coefficients. We imposed random noise on these data similar to the experimental error (± 0.005) and they were then computed with KINMODEL(AGDC) for the determination of the individual rate constants considering the mechanisms depicted in Scheme 2, which considers the three species participating in the reaction. After treating these systems, we found that for mechanisms 10, 11, 16, 19, 21, 25, 26 and 27 the statistical results concerning the Statistical Residuals analysis were unacceptable and they did not allow the individual rate constants to be obtained. Table 4 shows the results obtained when treating the kinetic data of absorbance generated for mechanism 1 and fitted to the rest of the reaction mechanisms where the optimized parameters (individual rate constants) and the values resulting from the Statistical Residuals analysis were consigned. In some

Table 2 Simultaneous optimization of individual rate constants and absorption coefficient of the intermediate species for the reaction mechanism $B_1 \rightarrow B_2 \rightarrow B_3$. (***a***) Absorbance data obtained at a single wavelength (Exp. 1–6). (***b***) Absorbance data obtained at three wavelengths (Exp.7–10). k_r/min^{-1} $\varepsilon_{Bj}/mol^{-1}cm^{-1}dm^3$

Exp	Estimated values			Optimized values			SSQ
	k_1	k_2	ε_{B2}	k_1	k_2	ε_{B2}	
1	2.0E-1	2.0E-2	800	0.1013	0.0493	897.0101	1.0272E-6
2	1.0E-2	1.0E-1	1000	0.0996	0.0502	900.9315	9.2124E-8
3	2.0E-2	2.0E-1	800	0.1000	0.0501	900.00	6.9819E-18
4	5.0E-1	1.0E-2	1500	0.0500	0.1002	1200.00	3.6320E-17
5	2.0E-2	2.0E-1	1500	0.0502	0.1001	1200.00	2.2173E-17
6	2.0E-2	2.0E-1	1100	0.0500	0.0999	1199.998	1.6988E-10

	λ_1 λ_2 λ_3					λ_1 λ_2 λ_3					SSQ
	k_1	k_2	ε_{B2}	ε_{B2}	ε_{B2}	k_1	k_2	ε_{B2}	ε_{B2}	ε_{B2}	
7	0.10	0.05	1000	1000	1000	0.10	0.05	900	1000	1100	4.2820E-17
8	0.10	0.05	1300	1300	1300	0.05	0.10	1200	1300	1400	5.7853E-16
9	0.05	0.10	900	900	900	0.10	0.05	900	1000	1100	4.2820E-17
10	0.05	0.10	1300	1300	1300	0.05	0.10	1200	1300	1400	3.7030E-16

Table 3 Simultaneous optimization of individual rate constants and molar absorption coefficients of all species for the reaction mechanism $B_1 \rightarrow B_2 \rightarrow B_3$. (***a***) Absorbance data obtained at a single wavelength (Exp. 1–4). (***b***) Absorbance data obtained at three wavelengths (Exp. 5–8). k_r/min^{-1} $\varepsilon_{Bj}/mol^{-1}cm^{-1}$ dm^3

Exp	Estimated values					Optimized values					SSQ
	k_1	k_2	ε_{B1}	ε_{B2}	ε_{B3}	k_1	k_2	ε_{B1}	ε_{B2}	ε_{B3}	
1	0.10	0.05	700	600	700	0.10	0.05	900	600	700	2.3223E-10
2	0.10	0.05	800	300	600	0.04	0.10	900	298	700	7.1025E-10
3	0.05	0.10	700	600	700	0.10	0.05	900	600	700	4.5893E-11
4	0.05	0.10	800	300	600	0.04	0.10	900	295	700	1.2002E-08
5	0.10	0.05	700	1000	800	0.10	0.05	600	900	700	3.1454E-17
			700	1000	800			700	1000	800	
			700	1000	800			800	1100	900	
6	0.10	0.05	600	1200	700	0.05	0.10	600	1200	700	6.4764E-17
			600	1200	700			700	1300	800	
			600	1200	700			800	1400	900	
7	0.05	0.10	600	1000	700	0.10	0.05	600	900	700	2.0658E-17
			600	1000	700			700	1000	800	
			600	1000	700			800	1100	900	
8	0.05	0.10	700	1200	800	0.05	0.10	600	1200	700	1.6632E-17
			700	1200	800			700	1300	800	
			700	1200	800			800	1400	900	

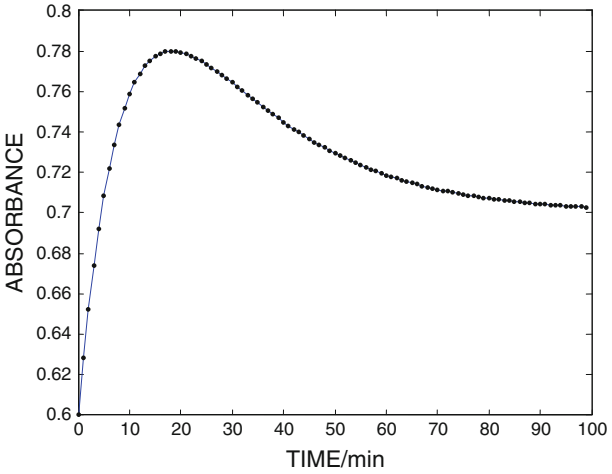


Fig. 1 Curve absorbance/time for groups of parameters $\boldsymbol{k}$ ($k_1 = 0.1\,min^{-1}$, $k_2 = 0.05\,min^{-1}$, $\varepsilon_A = 600\,mol^{-1}cm^{-1}dm^3$, $\varepsilon_B = 900\,mol^{-1}cm^{-1}dm^3$, $\varepsilon_C = 700\,mol^{-1}cm^{-1}dm^3$) (continuous line) y $\boldsymbol{k'}$ ($k'_1 = 0.05\,min^{-1}$, $k'_2 = 0.1\,min^{-1}$, $\varepsilon_A = 600\,mol^{-1}cm^{-1}dm^3$, $\varepsilon'_B = 1200\,mol^{-1}cm^{-1}dm^3$, $\varepsilon_C = 700\,mol^{-1}cm^{-1}dm^3$) (line of points)

of the reaction mechanisms assayed (8, 13 and 22), negative values of the individual rate constants were obtained, which is not acceptable from the chemical point of view, even though mathematically this would be possible since one is dealing with an optimization without restrictions. Evidently, these results completely ruled out the mechanisms that originated these negative values. In other cases, (model 7), we observed that the errors calculated for the constants were one order of magnitude greater than their own optimized value, indicating that the fit had not been performed properly and that the mechanism should be rejected. Statistical analysis of the residuals for the remaining mechanisms revealed a normal distribution [60,61], the standard deviation (SD) of the fit of mechanisms 2, 4 to 6, 9, 12, 17, 22 and 28 to 33 being greater than the experimental error, such that they were also rejected. In light of the results obtained for the statistical analysis of residuals for the remaining mechanisms it may be concluded that the one that best fits the kinetic data is model 1, since it had the lowest values both for the SSQ function and for the quadratic error (QE) (which considers the sum of the random errors and the systematic errors) and for the measurement of the statistical fitting (MF) (which takes into account the quadratic error due to bias and the estimate of the instrumental variance) [60,61]. In sum, the results obtained allowed us to conclude that the mechanism that best represents the kinetic data of absorbance was precisely one involving two first-order irreversible consecutive reactions $B_1 \leftrightarrow B_2 \leftrightarrow B_3$ (mechanism 1) with four rate constants. The statistical parameters for this mechanism, once the rate constants had been determined, revealed a clear difference with those obtained for the other mechanism that we considered as being responsible for the course of the reaction.

Table 4 Rate constants optimized for different reaction mechanisms (Scheme 2) and statistical parameters. k_r/mim^{-1}

Mechanism	k_1	k_2	k_3	k_4	SSQ	SD	QE	MF	χ^2
1	0.996±0.005	0.48±0.01	0.238±0.006	0.51±0.02	2.543E-5	1.122E-3	1.271E-6	1.586E-6	1.6
2	1.15±0.08	0.154±0.008	1.3±0.1		2.834E-3	1.149E-2	1.417E-4	1.747E-4	4.0
3	0.90±0.02	0.76±0.02	0.32±0.01		7.894E-4	5.304E-3	3.947E-5	4.650E-5	8.8
4	0.42±0.05	0.29±0.02			8.685E-2	6.436E-2	4.342E-3	5.378E-3	3.2
5	1.2±0.5	6. ±3.			4.072E-2	4.496E-2	2.036E-2	2.541E-3	8.8
6	1.6±0.8	3. ±2.			4.088E-2	4.521E-2	2.044E-3	2.555E-3	8.8
7	0.9±0.2	0.7±2.	0.3±2.	0.2±0.2	3.386E-2	1.912E-2	1.693E-3	1.784E-2	20.
8	1.2±0.1	1.6±0.2	-0.007 ± 0.01		4.524E-3	1.482E-2	2.262E-4	2.81E-4	8.0
9	1.12±0.07	1.3±0.1	0.13±0.01		2.650E-2	1.151E-2	1.325E-4	1.656E-4	4.8
12	2.1±0.3	0.09±0.02	0.04±0.05		6.225E-3	1.761E-2	3.112E-4	3.888E-4	3.2
13	0.40±0.01	1.6±0.2	0.14±0.01	-0.3 ± 0.2	2.833E-4	2.788E-3	1.416E-5	1.610E-5	6.4
14	0.42±0.03	0.62±0.04	1.1±0.2		1.979E-3	9.936E-3	9.899E-5	1.236E-4	6.4
15	0.41±0.01	1.43±0.03	0.125±0.005		4.259E-4	3.071E-3	2.129E-5	2.365E-5	4.0
17	1.1±0.1	0.14±0.01	1.3±0.1		2.597E-3	1.139E-2	1.298E-4	1.623E-4	5.6
18	0.86±0.02	0.63±0.01	0.095±0.003		1.021E-3	7.135E-3	5.107E-5	6.380E-5	6.4
20	0.43±0.02	0.63±0.02	1.1±0.1		1.977E-3	9.926E-3	9.889E-5	1.235E-4	4.8
22	0.51±0.01	0.5±0.1	0.13±0.01	-0.07 ± 0.1	4.819E-4	2.293E-3	2.409E-5	2.623E-5	3.2
23	0.53±0.03	0.21±0.01	0.6±0.1		1.657E-3	9.084E-3	8.286E-5	1.034E-4	7.2
24	0.5±0.1	0.5±0.1	0.13±0.05		2.566E-4	1.704E-3	1.283E-5	1.356E-5	8.8
28	2.0±0.5	0.082±0.003			6.699E-3	1.788E-2	3.349E-4	4.149E-4	5.6
29	0.26±0.01	0.85±0.01			2.620E-2	3.429E-2	1.310E-3	1.604E-3	4.0
30	0.43±0.05	0.29±0.02			8.677E-2	6.465E-2	4.338E-3	5.384E-3	3.2
31	0.26±0.02	0.84±0.05			2.609E-2	3.489E-2	1.304E-3	1.608E-3	4.8
32	0.38±0.03	0.32±0.01			1.814E-2	3.011E-2	9.074E-4	1.134E-3	3.2
33	2.0±0.5	0.082±0.003			6.690E-3	1.787E-2	3.345E-4	4.143E-4	5.6

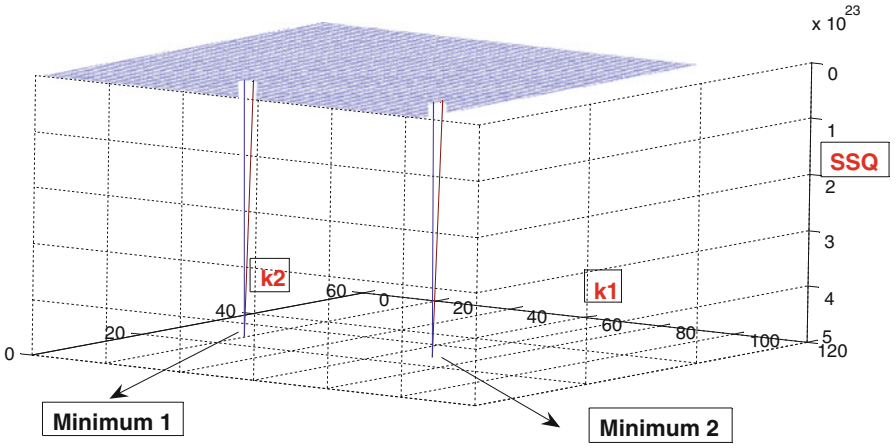


Fig. 2 Graphical representation (3D) of the two identical and mathematically indistinguishable minima of the SSQ function. *Minimum 1:* $k_1 = 0.1$, $k_2 = 0.05$, $\varepsilon_A = 600$, $\varepsilon_B = 900.0$, $\varepsilon_C = 700$ *Minimum 2:* $k'_1 = 0.05$, $k'_2 = 0.1$, $\varepsilon_A = 600$, $\varepsilon'_B = 1200$, $\varepsilon_C = 900$

3.4 Treatment of experimental data

We applied KINMODEL (AGDC) for the treatment of experimental data from the kinetics of the isomerization of the steroid 5-cholesten-3-one (5CHOL) to 4-cholesten-3-one (4CHOL), in presence of sodium hydroxide [65,66]. In neutral medium, the isomerization occurs very slowly, but when a base is added the transformation occurs rapidly through a reversible first-order kinetic process with respect to the concentrations of both isomers and to that of the base. There is experimental evidence [68] to suggest that the mechanism of the process comprises two consecutive reversible steps (mechanism 1, Scheme 3), with the presence of the corresponding ionic compound in enolic form (E^-). If the concentration of sodium hydroxide is in excess, the concentration of OH^- remains constant along time and the concentration of the solvent (H_2O) also remains constant, such that it may be considered that mechanism 1 would be reduced to one involving 3 chemical species (mechanism 2, Scheme 3), comprising 4 pseudo-first-order rate constants ($k'_{12} = k_{12}[OH^-]$, $k'_{23} = k_{23}[H_2O]$, $k'_{32} = k_{32}[OH^-]$ and $k'_{21} = k_{21}[H_2O]$).

For the experimental study of this reaction, a series of kinetic experiments was performed with an initial concentration of the 5CHOL isomer equal to 8.659×10^{-5} mol dm^{-3} and different initial concentrations of NaOH. The evolution of the reaction was followed by monitoring the total absorbance at 242 nm to which the 3 species involved in the mechanism contribute, such that at a given time i the total absorbance measured would be given by:

$$A_i = \varepsilon_{5CHOL}[5CHOL]_i + \varepsilon_{4CHOL}[4CHOL]_i + \varepsilon_E[E^-]_i \tag{11}$$

The molar absorption coefficients of the species 5CHOL and 4 CHOL (ε_{5CHOL} and ε_{4CHOL}) were determined experimentally at 242 nm by applying the Beer-Lambert

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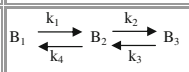
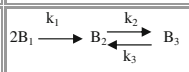
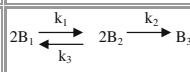
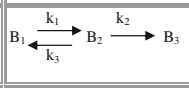
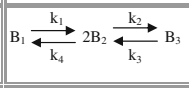
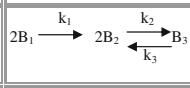
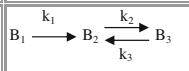
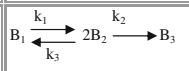
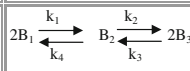
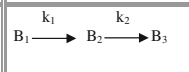
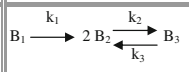
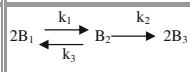
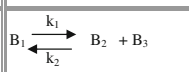
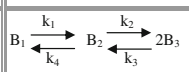
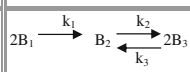
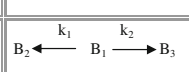
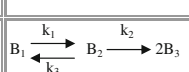
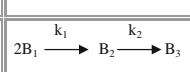
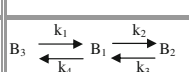
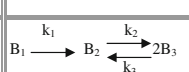
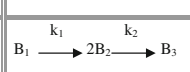
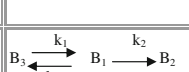
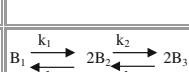
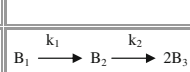
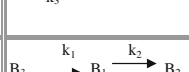
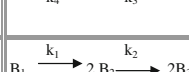
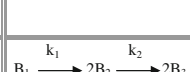
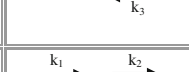
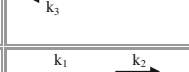
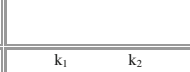
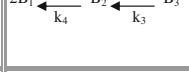
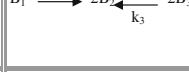
1		12		23	
2		13		24	
3		14		25	
4		15		26	
5		16		27	
6		17		28	
7		18		29	
8		19		30	
9		20		31	
10		21		32	
11		22		33	

Scheme 2 Models tested for discrimination between reaction mechanisms by applying KINMODEL (AGDC)

law, obtaining the following values: $\varepsilon_{5\mathrm{CHOL}} = 236.1\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}\ \mathrm{dm}^3$ and $\varepsilon_{4\mathrm{CHOL}} = 16414\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}\ \mathrm{dm}^3$. The enolate anion, E^-, is an intermediate species whose great instability prevents it from being studied at the laboratory, such that its molar absorption coefficient (ε_{E}) cannot be determined experimentally. Its value can be determined computationally by applying KINMODEL (AGDC), or, bearing in mind that the group chromophore, in the zone of the spectrum corresponding to the UV, is the group of conjugated double bonds that have both the E^- like 4CHOL, it may be considered that the values of the molar absorption coefficients of both species are equal [66,67].

Kinetic study of this reaction was performed using the Steady-State approach [68], since the enolate anion (E^-) is a very reactive intermediate. Application of the

Number	*MECHANISM*
1	$5CHOL + OH^- \underset{k_{21}}{\overset{k_{12}}{\Leftrightarrow}} E^- + H_2O \underset{k_{32}}{\overset{k_{23}}{\Leftrightarrow}} 4CHOL + OH^-$
2	$5\mathrm{CHOL} \underset{\mathrm{k'_{21}}}{\overset{\mathrm{k'_{12}}}{\Leftrightarrow}} \mathrm{E}^- \underset{\mathrm{k'_{32}}}{\overset{\mathrm{k'_{23}}}{\Leftrightarrow}} 4\mathrm{CHOL}$
3	$5\mathrm{CHOL} \overset{\mathrm{k'_{12}}}{\rightarrow} \mathrm{E}^- \overset{\mathrm{k'_{23}}}{\rightarrow} 4\mathrm{CHOL}$
4	$5\mathrm{CHOL} \underset{\mathrm{k'_{21}}}{\overset{\mathrm{k'_{12}}}{\Leftrightarrow}} \mathrm{E}^- \overset{\mathrm{k'_{23}}}{\rightarrow} 4\mathrm{CHOL}$
5	$5\mathrm{CHOL} \overset{\mathrm{k'_{12}}}{\rightarrow} \mathrm{E}^- \underset{\mathrm{k'_{32}}}{\overset{\mathrm{k'_{23}}}{\Leftrightarrow}} 4\mathrm{CHOL}$
6	$5CHOL \underset{k'_{21}}{\overset{k'_{12}}{\Leftrightarrow}} 4CHOL$

Scheme 3 Reaction mechanisms studied with KINMODEL (AGDC) for the isomerization of the steroid 5-cholesten-3-one (5CHOL) to 4-cholesten-3-one (4CHOL), in presence of sodium hydroxide

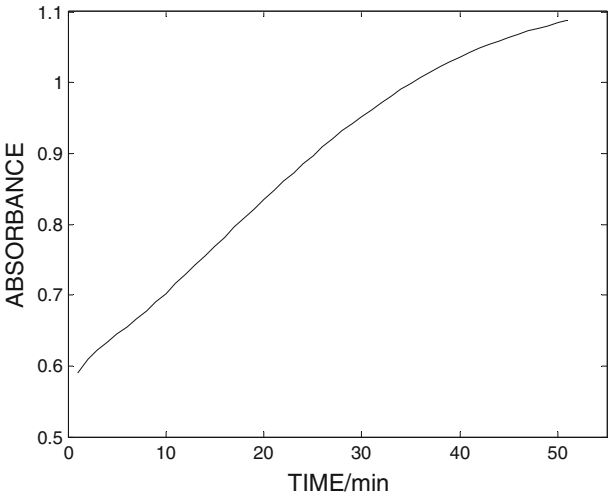


Fig. 3 Experimental data absorbance/time, from the kinetics of the isomerization of the 5-cholesten-3-one ($[5\mathrm{CHOL}] = 8.659 \times 10^{-5}\,\mathrm{mol\,dm^{-3}}$) to 4-cholesten-3-one (4CHOL), in presence of sodium hydroxide ($[\mathrm{NaOH}] = 2.581 \times 10^{-3}\,\mathrm{mol\,dm^{-3}}$)

KINMODEL (AGDC) computational program permitted a rigorous study of the isomerization reaction to be made without having to perform any type of approximation, allowing the values of the rate constants of the individual steps ($\mathrm{k'_{12}}$, $\mathrm{k'_{21}}$, $\mathrm{k'_{23}}$ and $\mathrm{k'_{32}}$) to be obtained, which was not possible with application of the Steady-State

Table 5 Rate constants optimized for different reaction mechanisms (Scheme 3)

Mechanism	$k'_{12}/\,min^{-1}$	$k'_{21}/\,min^{-1}$	$k'_{23}/\,min^{-1}$	$k'_{32}/\,min^{-1}$	SSQ
2	1.181	1.735	5.141×10^{-2}	6.101×10^{-4}	3.9788×10^{-3}
3	4.6911×10^{-2}		1.2857×10^{4}		1.6609
4	1.1509	1.8167	5.2270×10^{-2}		4.1843×10^{-2}
5	3.7759×10^{-2}		4.4285×10^{3}	5.9801×10^{2}	1.5789
6	9.5329×10^{-2}	4.2005×10^{-2}			0.4339

approach. When the isomerization reaction was conducted with an NaOH concentration of 2.581×10^{-3} mol dm^{-3}, treatment of the experimental absorbance data (Fig. 3) with KINMODEL (AGDC), considering mechanism 2 of the Scheme 3 to be applicable, provided the values for the constants indicated in Table 5. Along the optimization process a clear decrease in the value of the SSQ function was observed until a minimum value of 3.9788×10^{-3} was reached, which afforded a value for the standard deviation of the fit of the order of the error in the experimental data. The results obtained in the statistical analysis of residuals confirmed the applicability of KINMODEL (AGDC) for the determination of the individual rate constants of the isomerization reaction. Additionally, these results suggest that the reaction would occur through mechanism 2. In order to discriminate among the mechanisms that might be responsible for the course of the reaction, we carried out the treatment of the experimental absorbance data considering other possible mechanisms in which the three species are involved, with different possibilities of transformation into one another and hence a different number of kinetic constants. Application of KINMODEL (AGDC) to the experimental absorbance data considering that mechanisms 3, 4 and 5, shows in Scheme 3, may be responsible for the course of the reaction, afforded the values indicated in Table 5 for the rate constants. On comparing the results obtained considering mechanisms 2, 3, 4 and 5, it may be concluded that the one that best represents the data is mechanism 2, as confirmed by the SSQ values and the statistical analysis of residuals.

In the reaction mechanisms analyzed, three species are involved (5CHOL, E^- and 4CHOL), it could be speculated that because of the very low values of the concentration of the enolate (E^-) the experimental data could also be fitted to the mechanism in which only the two isomers 4CHOL and 5CHOL would be involved (mechanism 6, Scheme 3), where k'_{12} and k'_{21} are pseudo-first-order constants ($k'_{12} = k_{13}[OH^-]$ and $k'_{21} = k_{31}[OH^-]$). Treatment of the experimental data on absorbance considering this mechanism to be applicable afforded the values of the constants indicated in Table 5, the results obtained (SAR, SSQ) confirm that a better fit is achieved with mechanism 2.

4 Experimental

The experimental measurements were performed on a SHIMADZU 240 diode array spectrophotometer, whose cell was thermostatted at 25.0 °C by exterior circulation forced into the cell-holder from a cryostatic bath (± 0.1 °C). Analytical-reagent 5-cholesten-3-one and 4-cholesten-3-one (SIGMA, St. Louis MO) were used.

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Appendix 1

$$\mathbf{g} = 2 \begin{pmatrix} \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw} RES_{i,\lambda}\frac{\partial f_{i,\lambda}}{\partial PI} \\ \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw} RES_{i,\lambda}\frac{\partial f_{i,\lambda}}{\partial P2} \\ \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw} RES_{i,\lambda}\frac{\partial f_{i,\lambda}}{\partial P3} \\ \vdots \\ \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw} RES_{i,\lambda}\frac{\partial f_{i,\lambda}}{\partial PNp} \end{pmatrix}$$

$$\mathbf{Hs} = 2 \times \begin{bmatrix} \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_1}\right)^2 & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_1}\frac{\partial f_{i,\lambda}}{\partial P_2}\right) & \sum_{i=1}^{Nd}\sum_{\lambda=l}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_1}\frac{\partial f_{i,\lambda}}{\partial P_3}\right) & \cdots & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_1}\frac{\partial f_{i,\lambda}}{\partial PNp}\right) \\ \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_2}\frac{\partial f_{i,\lambda}}{\partial P_1}\right) & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_2}\right)^2 & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_2}\frac{\partial f_{i,\lambda}}{\partial P_3}\right) & \cdots & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_2}\frac{\partial f_{i,\lambda}}{\partial P_{Np}}\right) \\ \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_3}\frac{\partial f_{i,\lambda}}{\partial P_1}\right) & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_3}\frac{\partial f_{i,\lambda}}{\partial P_2}\right) & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_3}\right)^2 & \cdots & \sum_{i=1}^{Nd}\sum_{\lambda=l}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_3}\frac{\partial f_{i,\lambda}}{\partial P_{Np}}\right) \\ \vdots & \vdots & \vdots & \cdots & \vdots \\ \sum_{i=1}^{Nd}\sum_{\lambda=l}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_{Np}}\frac{\partial f_{i,\lambda}}{\partial P_1}\right) & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_{Np}}\frac{\partial f_{i,\lambda}}{\partial P_2}\right) & \sum_{i=1}^{Nd}\sum_{\lambda=l}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_{Np}}\frac{\partial f_{i,\lambda}}{\partial P_3}\right) & \cdots & \sum_{i=1}^{Nd}\sum_{\lambda=1}^{Nw}\left(\frac{\partial f_{i,\lambda}}{\partial P_{Np}}\right)^2 \end{bmatrix}$$

$P_1, \ldots, P_{Np}$ are the parameters to optimize, $(A_{i,\lambda})_{calc} = f_{i,\lambda}$ and the residual is given by:

$$RES_{i,\lambda} = \left((A_{i,\lambda})_{calc} - (A_{i,\lambda})_{exp}\right) = \left(f_{i,\lambda} - (A_{i,\lambda})_{exp}\right)$$

Appendix 2

The general program **KINMODEL (AGDC)** is written in C++, using JAVA applications, apply in the Windows environment. The computational application comprises a main program and a series of subroutines, in which the different treatments and calculations necessary for the optimization process of AGDC are carried out.The program has been structured in the following parts:

1. ***Main program*** Denominated **KINMODEL**; performs the following functions:

- Input data:
  - Reaction mechanism will be studied: Select the mechanism into the models proposed by the program or introduce other mechanisms, in this case be provided the number of reactions that comprise the mechanism, number of species involved and the matrix of stoichiometric coefficients.
  - Parameters to be optimizated and strategy: Kinetic constants (k_r); initial concentrations of the species involved in the mechanism ($[B_j]_0$); kinetic constants and initial concentrations simultaneously (k_r and $[B_j]_0$), molar absorption coefficients ($\varepsilon_{j,\lambda}$); kinetic constants and molar absorption coefficients (k_r and $\varepsilon_{j,\lambda}$), simultaneously by performing a single optimization process or two specific individual processes of optimization.
  - Estimates of the unknown parameters whose value is to be determined.
  - Parameter values whose value is known
  - Convergence criteria.
  - Experimental data absorbance/time, ...
- Automatically generates the model to study taking into account the data entered by the user: number of reactions that comprise the mechanism, number of species involved and matrix of stoichiometric coefficients.

2. Subprograms:

2.1- OTIMAGDC, Collection of subroutines that perform the optimization process by applying the AGDC algorithm. It consists of the following parts:**KINAGDC**, optimizes the rate constants (k_r); **CONCAGDC**, optimizes the initial concentrations, $[B_j]_0$; K**INCONAGDC**, joint and simultaneous optimization of the rate constants and initial concentrations (k_r and $[B_j]_0$); **COEFAGDC,** optimizes the molar absorption coefficients, $\varepsilon_{j,\lambda}$; **SIMULAGDC**, joint and simultaneous optimization into a single optimization process of the molar absorption coefficients and rate constants; **SEQAGDC**: sequential optimization of the molar absorption coefficients and rate constants, through two possible processes, **SEQ1** (first determines k_r and then $\varepsilon_{j,\lambda}$) and **SEQ2** (in each iteration first determines k_r and then $\varepsilon_{j,\lambda}$).

2.2- GEARAGDC, A package of subroutines that generates and solves the set of differential rate equations according to the model considered. It contains the following subroutines: **DIFFEQ**, establishes the set of differential rate equations; **RESECDF**, solves the set of differential rate equations and determines the concentration of each species in the time interval considered, $[B_j]_i$.

2.3- DERIVAGDC, A set of subroutines that calculate the numerical partial derivatives of the total absorbance of the sample with respect to the parameters to be optimized: **DERCONC**, derivatives with respect to the initial concentrations; **DERIVK,** derivatives with respect to the rate constants; **DERIVCOEF,** derivatives with respect to the molar absorption coefficients.

2.4- INVERAGDC, Calculates the determinant and performs the inversion of the Hessian matrix.

2.5- ESTADAGDC,Subprogram that determines the errors of the parameters and performs statistical analysis of the residuals.

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