

SOLVERSTAT: a new utility for multipurpose analysis. An application to the investigation of dioxygenated Co(II) complex formation in dimethylsulfoxide solution

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Abstract

A new utility for multipurpose analysis, SOLVERSTAT, taking advantage of the versatility of spreadsheets is here described. By means of this tool advanced statistical tests have introduced in Microsoft Excel Solver thus allowing regression diagnostic and discrimination between different models. The utility is here applied to the determination, by UV-Vis spectroscopy, of the stability constant for the uptake of molecular dioxygen by the 1:2 complex of Co(II) with *N,N'*-dimethylethylenediamine (dmen) in the aprotic solvent dimethylsulfoxide (dmsO) at 298 K and in a medium adjusted to 0.1 mol dm⁻³ with Et₄NClO₄. The reliability of the model and parameters obtained are discussed and the results compared with those obtained by Dynafit, a different software package, and by independent voltammetric measurements. The validity of SOLVERSTAT has been also examined applying it to the discrimination between different models already discussed in the literature.

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1. Introduction

The method of least squares (LS) is the default data analysis tool in most of physical and chemical science [1–6]. In chemical application LS methods are widely used, for instance, for resolving UV-Vis and FT-IR spectra in individual Gaussian-Loren-

tian components and for calculating the concentration of the species by means of mass balance in chemical equilibria or kinetic studies.

Several powerful softwares are now available to solve many chemical problems (Refs. [7,8] and references therein) but they are often designed in order to handle only specific kinds of experimental data and conditions, which means that more programs are to be used for data analysis.

The availability, on the other hand, of current spreadsheet programs, provided with minimization tools, offers the possibility to overcome these

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drawbacks and perform the LS analysis of systems of linear and nonlinear equations defined by the user on the basis of his specific application. At the same time the operator may take advantage of the versatility of spreadsheets in calculation and graphical facilities.

Microsoft Excel Spreadsheets in particular are now more and more used by our group whose research activity deals with determination of equilibrium constants by means of potentiometric, UV-Vis, FT-IR, NMR, calorimetric techniques [9] as well as with the analysis of kinetic problems > [9h]: a flexible system is in fact needed in order to treat all these different data and chemical problems. Nevertheless, as pointed out also in a recent work [10] the Solver of Microsoft Excel yields minimized parameters but provides no estimates of their precision and an incomplete and unprompted statistical analysis of fittings: the statistical analysis of the results is on the other hand, a crucial step to evaluate the reliability of the whole model.

SOLVERSTAT, presented in this work, provides Microsoft Excel of an utility which introduces advanced statistical tests. This utility transforms Microsoft Excel in a powerful tool that yields regression diagnostics and allows to discriminate between different models. At the same time, SOLVERSTAT can be used with small as well as with large data sets.

The utility is here applied to the determination, by UV-Vis spectroscopy, of the stability constant for the uptake of molecular dioxygen by the 1:2 complex of Co(II) with *N,N'*-dimethylethylenediamine (dmen) in the aprotic solvent dimethylsulfoxide (dmsO) at 298 K and in a medium adjusted to 0.1 mol dm^{-3} with Et_4NClO_4 .

The complexes formed in anaerobic conditions, Co(dmen) and Co(dmen)_2 , have been previously investigated and their stability constants reported [9].

The reliability of the model and parameters obtained are discussed and the results compared with those obtained by using the Dynafit package [8], which is not suitable to work directly with spreadsheets. An additional confirmation of the results here obtained from spectrophotometric data comes from independent voltammetric measurements.

The utility has also been tested in order to verify its ability to discriminate between different models already discussed in the literature [11].

2. Experimental

2.1. Chemicals

$\text{Co(ClO}_4)_2 \cdot 6\text{dmsO}$ was prepared and standardized as before [9g]. Dimethylsulfoxide (Fluka > 99%) was purified by distillation according to the described procedures [12], degassed by a pumping-freezing procedure and stored over 4 Å molecular sieves. The ligand dmen (Aldrich > 97%) was purified by fractional distillation [13]. Cobalt(II) perchlorate stock solutions were prepared by dissolving in anhydrous degassed dmsO weighed amounts of the adduct and its concentration was checked by titration with EDTA [14].

The background salt Et_4NCl was recrystallized twice from methanol and dried at 110 °C. Solutions of the ligand were prepared as described [9]. All standard solutions were prepared and stored in a MB Braun 150 glove box under a controlled atmosphere containing less than 1 ppm of water and less than 1 ppm of oxygen. The water content in the solutions, typically 10–20 ppm, was determined by a Metrohm 684 KF Coulometer.

2.2. Spectrophotometric measurements

A total of 10 ml of a $\text{Co(ClO}_4)_2$ stock dmsO solution (0.0115 M) were poured in a three necks flask equipped with the Varian Cary 50 Spectrophotometer UV-Vis optic fiber probe (path of 1 cm), gas inlet and outlet. This solution was conditioned bubbling the gas mixture at a constant flux of 100 ml min^{-1} . The gas outlet was connected, through a trap, to the dmen stock solution (0.07514 M). After 30 min conditioning, 0.5 ml of dmen stock solution was withdrawn and injected into the flask. Once the system reached the equilibrium the UV-Vis spectrum was recorded over the wavelength range 280–500 nm. All the solutions were maintained 0.1 mol dm^{-3} in ionic medium by addition of Et_4NClO_4 as neutral salt.

Seven experiments were performed keeping constant the Co(II) and ligand concentration while varying the gas composition from 15 to 120 torr in O₂.

2.3. Computational methods

2.3.1. Theoretical background

Let's call the data points $(x_1, \dots, x_v, y)_i$ with $i = 1, \dots, n$, and the function $y = F(x_1, x_2, \dots, x_v; b_0, b_1, \dots, b_p)$ where y is the dependent variable, x_j are v independent variables and b_k are p adjustable parameters.

In the Least Squares fitting [11,15], the optimized values of the adjustable parameters can be obtained by minimizing the sum of the weighted squares differences r between the dependent variable y_i and their calculated value F_i :

$$S = \sum w_i r_i \quad (1)$$

S is called residual sum of squares (RSS) or sum of squared errors (SSE) Therefore in matrix formulation it can be introduced [16–18]:

$$\mathbf{S} = \mathbf{r}^t \mathbf{W} \mathbf{r} \quad (2)$$

where \mathbf{W} is the square $n(\text{rows}) \times n(\text{columns})$ matrix of weights, \mathbf{r} is the residual vector with n elements and the upperscript t indicates a *transpose* matrix, \mathbf{W} is the reciprocal of the variance–covariance matrix and is frequently diagonal. If (and *only if*) the variance of the observed data points is known independently from the points themselves, the quantity defined in Eq. (1) is distributed as a *Chi Square Distribution* χ^2 with $n-p$ degrees of freedom (DF): this can be properly applied for statistical checks.

The variance in y can be estimate as:

$$\sigma_y^2 \cong s_y^2 = S/(n-p) \quad (3)$$

In *ordinary least squares* (OLS) unitary weights are assigned to each data point.

The linear (the term linearity is inherent to the parameters not to the variables) LS (LLS) solution using the first order Taylor expansion for the estimated parameters is:

$$\mathbf{b} = (\mathbf{J}^t \mathbf{W} \mathbf{J})^{-1} \mathbf{J}^t \mathbf{W} \mathbf{y} \quad (4)$$

where \mathbf{y} is the dependent variable column vector

with n element: \mathbf{b} the parameter column vector with p elements and \mathbf{J} is a $n \times p$ matrix called *Jacobian Matrix* whose elements are the derivatives of function F_i with respect to the parameters.

The matrix:

$$\mathbf{A} = \mathbf{J}^t \mathbf{W} \mathbf{J} \quad (5)$$

has great importance as the variance of the parameters are the diagonal elements of the *variance–covariance* matrix \mathbf{V} which is proportional to \mathbf{A}^{-1} :

$$\mathbf{V} = k \mathbf{A}^{-1} \quad (6)$$

Another important relation is:

$$\mathbf{j} = \mathbf{J}(\mathbf{J}^t \mathbf{W} \mathbf{J})^{-1} \mathbf{J}^t \mathbf{W} \mathbf{y} \quad (7)$$

where \mathbf{j} is the calculated value for \mathbf{y} . The $n \times n$ matrix:

$$\mathbf{H} = [\mathbf{J}(\mathbf{J}^t \mathbf{W} \mathbf{J})^{-1} \mathbf{J}^t] \mathbf{W} \quad (8)$$

is called *Hat Matrix* and its diagonal elements can be used in statistical analysis of the LLS residuals since various tests are based on this quantity.

The weights given to the single experimental points have great importance [17]: the OLS method can be used only if the variances are constant (*Homoscedasticity*). In the case of non constant variance (*Heteroscedasticity*) weights must be used to resolve this situation.

Let us now consider the variance of parameters which can be obtained from the \mathbf{V} Matrix as:

$$\sigma^2(b_j) = k v_{jj} \quad (9)$$

where v_{jj} indicates the j -diagonal elements of \mathbf{V} (Eq. (6)). If the variances of observed data points are known and are used in a WLS, then $k = 1$, otherwise if they are known only in a relative mode (for example $\sigma(y_i)^2 \propto y_i^2$) or are unknown then $k = s_y^2$ [16,18].

In *Nonlinear Least Squares* (NLLS) the quantity minimized is again SSE [17] and an equation similar to Eq. (4) must be solved iteratively. The iterations sequence is terminated when the difference between the new and the old estimated value is sufficiently small for all the parameters. To improve convergence rate various methods are applied (Gauss–Newton, Levenberg–Marquardt [19]).

The use of NLLS enormously increases the possibility of experimental data elaboration and allows better results. *Linearization* of the model is often applied to simplify the calculation, but it is a bad practice as it introduces biases in statistical weight of data [16,18]; in these cases NLLS fitting is a better choice [11].

A critical point in nonlinear fits is the initial selection of parameter values. If the initial values are far from the true ones, the calculation can diverge or converge to a local minimum [19]. Another great limit of NLLS is the estimation of the errors on parameters which is very complex [20], but often good values can be obtained using Eq. (9) if n are large and the error values are small [16]. The errors obtained with this way are called *Asymptotic Errors*. A *Monte Carlo Study* (MC) can often validate the reliability of data treatment [1,21].

In spite of these problems, the use of NLLS displays great attractive for its simplicity to be set up as a spreadsheet and for its direct connection between experimental data and theoretical model.

2.3.2. Statistical evaluation of fitting

In this field Billo [22] proposed a pioneering application in the evaluation of parameters precision in NLLS with Excel. Some other authors presented the use of Excel with LLS and NLLS [3,15,23] and some of them [9,10,24] used various methods to obtain error estimates of the parameters but for an accurate and selective diagnostic an adequate statistical treatment of data and result of fitting is of paramount importance.

In order to use Excel Solver for minimizing our experimental data, a column vector was written in the worksheet with n cells for the value weights, one column (or more) for independent variable(s), one for dependent variable, one for the appropriate formula to be applied to calculate dependent variable and one for squared residuals. A cell which contains the sum of weighted residuals was also inserted. In a row vector with p cells the initial estimate values of parameters were reported.

Then Excel Solver was used and the minimized LLS or NLLS parameters obtained.

The statistical evaluation of the model is now crucial and this is provided by SOLVERSTAT, a

Routine based on well defined statistical tests [17,21,25], written in Excel *VBA* (*Visual Basic for Application*), a simple and powerful Excel programming language [26,27].

This routine analyzes: (a) the *regression triplet* [6], i.e. the *data quality* for a proposed model, the *model quality* for a given data set, the *regression method quality*, that is the fulfilment of all LS assumption and (b) can discriminate between different models allowing the selection of the best. The routine calculates the corresponding statistic and when available the corresponding critical value for selected probability and/or the computed probability.

2.3.3. Regression triplet

2.3.3.1. Data quality. As far as linear parameter models are concerned the following useful statistics for observations diagnostic are available in SOLVERSTAT and are based on residuals r (Eq. (2)) and Hat Matrix of Eq. (8).

One of the main goals of the observation diagnostics [28] is the identification of the *outliers* (points whose residuals are so large that they indicate that either the model or the response for the respective data point are erroneous) and the *influential points* (the points which remarkably affect the model parameters). If an outlier is influential, it will cause serious distortions in the regression calculations. Once an observation has been determined to be an outlier, it must be checked to see if it resulted from a mistake. If so, it must be corrected or omitted. However, if no mistake can be found, the outliers should not be discarded just because are outliers as they often suggest that the model is inadequate. For a good review on outliers and influential points statistic application in chemistry see Refs. [5,6].

A first diagnostic test is based on the value of the hat diagonal h_{jj} (*leverage*) [25]. Cut point for this statistic is $2p/n$ or $3p/n$. A high-leverage observation is not a bad observation but exerts extra influence on the final results.

Studentized Deleted Residual (called also *Jack-knife Residuals*) [28] is calculated by scaling the residuals using a computed variance omitting a certain point, j , from the regression. The idea is

that, excluding this point, a better estimate of the variance will be obtained if the point is an outlier. This statistic has a t distribution with $(n-p-1)$ degrees of freedom [5]. Other suggested cutoff are values of 2 or 3 [25].

A good diagnostic plot that helps in simultaneous visualization of outliers and influential points is the *Williams Graph* [25] in which Studentized Deleted Residuals is plotted versus leverages value (see for example Fig. 2b).

DFFITs is the standardized difference between the predicted value with and without that observation [28]. If the absolute value of DFFITS is larger than 1, the observation should be considered to be influential. Suggested cut point for this statistic are $2\sqrt{p/n}$ or $3\sqrt{p/n}$ [25].

Another statistic test is the *Cook's Distance* [28] which measures the influence of each point on all fitted data. Values greater than one indicates a point that has large influence. Suggested cutoff value was 1 or $4/(n-2)$ [25] or the 50th percentile of the F -distribution with $(p, n-p)$ DF [5].

The COVRATIO [17] describes the influence of each j point on the variance of estimated parameters. A value exceeding 1 implies that the observation j provides an improvement in the fitting quality; a value of CovRatio less than 1 flags an observation that decreases the precision of the model. A suggested guideline is that if $\text{CovRatio} > 1 + 3p/n$ then omitting this observation significantly damages the precision of at least some of the regression estimates. If $\text{CovRatio} < 1 - 3p/n$ then omitting this observation the precision of at least some of the regression estimates significantly improves.

The DFBETAS criterion measures the standardized change in a regression parameters when an observation is omitted [28]. Common cutpoint was $2\sqrt{n}$ or $3\sqrt{n}$. when n is greater than 100. When n is less than 100, some authors have suggested to use a cutoff of 1 or 2 for the absolute value of DFBETAS [25].

2.3.3.2. Model quality. The first test applied is Model ANOVA (analysis of the variance, an F -Test) [17] that gauges the contribution of the independent variables in predicting the dependent

variable. If the F ratio is around 1, the model association between the variables described in the model are not statistically significant.

If in the model fitted, the weights of the points are based on independently known variance, the sum of weighted residual squared S in Eq. (1) is tested with the *Chi Square Statistic* [21]. This tests if the variance of the checked model is compatible with the true known variance. If this test fails, the model is inadequate to describe the data or the known variance is not the true.

Runs-of-Sign Statistic test has also been introduced to evaluate if runs of identical signs in residuals occur by chance [28]. The test fails when the calculated curve deviates systematically from the data points: in that case the model is likely inadequate.

Parameters error computation is based on Eqs. (5), (6) and (9). As usual [16,17,25] a *Student Test t* with $n-p$ degree of freedom checks if the single parameter is statistically different from zero. If the test is negative then the parameter is not significant to describe the model or the data scattering of the points is too wide. In the case of use of independent known variance for data point, SOLVERSTAT uses the Normal Statistic Z for this test [16].

Correlations between parameters are calculated from the matrix \mathbf{V} :

$$R_{\text{Corr}_{lm}} = V_{lm} / \sqrt{(V_{ll} V_{mm})} \quad (10)$$

where subscripts l and m refer to different parameters. When the function parameters are correlated, the confidence intervals (CI) calculated using the usual t statistic will be significantly underestimated [20]. More reasonable values are the joint confidences intervals (JCI) that can be calculated using an F Statistic with $(p, n-p)$ degrees of freedom. When independent known variance is used this Statistic must be replaced with a χ^2 Statistic with p degrees of freedom [20].

The errors and the confidence intervals calculated in this way, are corrected only for linear parameters; for NLS CI can be more wide, sharp or asymmetric [20]. Nevertheless their reliability is good if the relative standard error is better than 1/10 [16].

An additional tool introduced in SOLVERSTAT and called MCSOLVERSTAT, based on *Monte Carlo Simulation* [21], can be invoked to check the correct goodness of parameters error in the nonlinear case. The limit of this optional utility is that it takes a very long time to end the calculations (typically 0.1–1 second for each single simulation).

The central idea behind MC [1,21,30–32] sampling is that the finite-sized data sets collected in the laboratory are drawn from a distribution of possible experimental results (due to random measurements errors). MC sampling allows a quantitative determination of precision by generating a large number of data sets consistent with the experimentally measured data (i.e. drawn from a normal distribution with the same mean observed in the laboratory and a standard deviation equal to the error estimate for each data point). After carrying out the fitting procedure on each member of the synthetic data set, a distribution of parameter values is obtained whose standard deviations are the uncertainty in the fit parameters. MC simulation is one of the most precise method for the evaluation of CI of determined parameters [20]. For this purpose the validity of the method is dependent on how realistically the set of pseudorandom uncertainties models the uncertainties in the actual experimental data.

2.3.3.3. Regression method quality. Some conditions are to be observed for the applications of LS fit [17]: (i) absence of multicollinearity (the presence of high or near-perfect intercorrelations between or among the independent variables) in the case of multiple regression analysis; (ii) residuals are normally distributed; (iii) residuals have constant variance (*Homoscedasticity*); (iv) residuals are independent from each other.

The problem of multicollinearity (i) is analyzed with the *variance inflation factor* (VIF), a test based on the R^2 obtained when each variable is regressed on the remaining independent variables. Collinearity arises from sampling methodology, over-defined model, model choice and outliers. Multicollinearity determines inaccurate estimates of the regression parameters, alters the result of various tests and degrades the predictability of the

model. A VIF of 10 or greater indicates a multicollinearity problem [17].

Point (ii) is tested with *Shapiro–Wilk Statistic* SW (for large samples SOLVERSTAT uses *Shapiro–Francia* modification) [29]; if the calculated SW value is less than a selected critical value, the residuals are not normally distributed and the model might not be adequate. Another test is the *Jarque–Bera Statistic* JB based on the coefficient of *skewness* and *kurtosis*. JB is distributed as a *Chi Square Distribution* χ^2 with 2 DF. JB test is more useful with large data sets, SW is a better choice with small data sets [29].

The constant variance of residuals (iii) is examined with *Levene F Test* [17]: if calculated F value is less than a critical value, residuals can be considered to have constant variance; if the test fails, either the model chosen is inadequate or it is necessary to use non constant weighting of the data. In this case a review of the background of model choice and of the experimental procedure are essential.

Independence of residuals (iv) can be tested with *Durbin–Watson Statistic* DW [23] for first order autocorrelations. The presence of autocorrelation arises from time sequenced data or model misspecification and causes underestimation of confidence intervals and distortion of various statistical tests. If the residuals are not correlated, the Durbin–Watson statistic will be around 2.

If violation of regression assumption are detected, it is necessary to take opportune corrective action such as the deletion of outliers for the violation of residuals normality or the use of WLS for the case of *Heteroscedasticity*. See Refs. [17,28] for more details.

2.3.4. Models discrimination

When different models pass previous checks for regression triplet analysis, the comparison can be performed by means of various statistical *criteria*. The classical, not fully satisfactory, approach is based on the evaluation of the residuals and error magnitude on minimized parameters and selecting the model with the lowest values. Other information can be obtained from *Multiple Correlation Coefficient* R , *Coefficient of Multiple Determination* R^2 and *Adjusted Coefficient of Multiple*

Determination R_{adj}^2 [17]. This statistic evaluation indicates the fraction of total variability in the data shown by the regression model. The R_{adj}^2 is superior to the common R^2 value as it is sensitive to addition of irrelevant variables (R_{adj}^2 decreases whereas R^2 increases or at least stays constant).

When the differences within more models are low, SOLVERSTAT helps in the selection of the more reliable one with two type of *F-test*. The first is called *Extra Sum of Squares F-Test*, F_{ExSS} [17] and is based on the ratio between the differences of SSE for the *full* model minus SSE for the *reduced* model and SSE for the *full model*:

$$F_{\text{ExSS}} = [(S_{\text{Full}} - S_{\text{Reduced}})/S_{\text{Full}}] \times [DF_{\text{Full}}/(DF_{\text{Full}} - DF_{\text{Reduced}})] \quad (11)$$

Only if F_{ExSS} is large, the addition of one or more terms to the reduced model significantly improves the model.

The second available *F-test* [25] (*F* value for models comparison in Table 1) compares the SSE of the two examined model and determine if the differences are statistically significant ($S_2 > S_1$):

$$F_{\text{RSS}} = (S_2/DF_2)/(S_1/DF_1) \quad (12)$$

If calculated *F* is larger than selected critical value at desired confidence level, then the two models are different and the model with low variance is really the best.

Another available *decision tool* is the prediction error sum of squares (PRESS) [25] a test based on h_{jj} . The smaller PRESS is, the better the predictability of the model.

The PRESS value can be used to compute the $R_{\text{Prediction}}^2$, which reflects the prediction ability of the model. It is possible to have a high R^2 and a low $R_{\text{Prediction}}^2$. In a similar way as R_{adj}^2 this statistic shows a maximum for the best predictive model. The difference is that $R_{\text{Prediction}}^2$ is based on the predictive power of the model.

The last subset selection tool available is the *Akaike Information Criteria* (AIC) [28]. It is based on measuring the fit given by SSE and correcting it for the number of regressors. The best model will show low value of AIC.

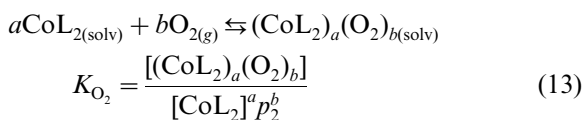
2.3.5. Procedure for regression model building

The next procedure can therefore be used for the regression model building:

- i) Proposal of a model from theory and preliminary exploration of data
- ii) Make the regression model
- iii) Run regression and statistic
- iv) Conduct the triplet analysis
- v) If after examination of *data quality*, some outliers are found and deleted, go to (iii)
- vi) If after examination of *model quality* some parameters are omitted go to (ii)
- vii) If the examination of regression method evidences the necessity of corrective action, take opportune remedial measures and go to (ii)
- viii) If more than one model is selected and not discarded by the regression triplet, then conduct a *model discrimination*.

3. Results and discussion

The equilibrium constant for the dioxygen addition:



was determined by spectrophotometric measurements at various dioxygen pressure at 25 °C in dmso (see Section 2 for details).

The experimental curves, in terms of absorbances vs. wavelengths, are reported in Fig. 1: their trend indicates that reasonably only one oxygenated species is formed in our experimental conditions. On the basis of chemical knowledge [33] two possible models should be selected, i.e. the model where the stoichiometric coefficients *a* and *b* are both assumed equal to 1 (Model A) and the model where *a* = 2 and *b* = 1 (Model B), corresponding respectively to the formation of a mononuclear superoxo or a dinuclear μ -peroxo complex: the dinuclear μ -superoxo is not considered, as the maximum of the charge-transfer band is here localized only at 325 nm whereas normally the

Table 1

Most significant statistical parameters calculated by SOLVERSTAT and parameter values (K_{O_2} and molar extinction (ϵ_{λ}) values at the selected wavelength λ (nm)) as given by the least square fit of Excel Solver for Models A and B

Statistic results	Critical value ^a	Model A	Model B				
Runs-of-signs statistic	> 0.025	0.802	0.098				
F value for Levene test	< 4.08	3.43	1.90				
Jarque–Bera statistic	< 5.991	0.847	1.849				
Shapiro Wilk statistic	> 0.942	0.969	0.894				
ANOVA F value	> 2.37	1655.00	141.62				
R		0.9982	0.9800				
95% CI for R		0.9966–0.9991	0.9612–0.9898				
R ²		0.9965	0.9604				
SSE		1.516 × 10 ⁻⁴	1.560 × 10 ⁻³				
R ² _{Adj}		0.9959	0.9537				
PRESS		0.0002050	0.001777				
R ² _{Prediction}		0.9953	0.9588				
AIC		-509.0	-419.9				
F value for models comparison	> 1.757	8.359					
Calculated parameters	t _{Critical}	Value	$\sigma(b_j)^b$	t _{Calculated}	Value	$\sigma(b_j)^b$	t _{Calculated}
ϵ_{327}	> 2.030	186.9	2.4	77.22	835.4	211.3	3.954
ϵ_{333}	> 2.030	190.3	2.4	77.71	850.3	215.1	3.954
ϵ_{338}	> 2.030	184.6	2.4	76.87	825.1	208.7	3.953
ϵ_{340}	> 2.030	175.2	2.3	75.35	782.9	198.1	3.952
ϵ_{346}	> 2.030	151.5	2.1	70.82	677.2	171.6	3.946
ϵ_{350}	> 2.030	136.3	2.0	67.24	609.1	154.6	3.940
K_{O_2}	> 2.030	0.01694	0.00042	39.87	0.01571	0.0046	3.449
95% CI for K_{O_2}		0.01595–0.01794	0.005045–0.02638				
95% JCI for K_{O_2}		0.007164–0.02657	-0.002507–0.03394				

K_{O_2} in torr⁻¹; $\epsilon_{\lambda}/\text{nm}$ in mol⁻¹ dm³ cm⁻¹.

^a Critical value selected for probability 5% and computed by SOLVERSTAT.

^b Asymptotic standard deviation for calculated parameters: $\sigma(b_j) = \sqrt{\sigma^2(b_j)}$.

electronic spectrum of the green μ -superoxo complex shows an additional band around 710 nm.

The procedure for determining the equilibrium constant for the reaction (13) was based on the treatment of the experimental data by an ordinary NLLS method of the Excel Solver as described in Section 2.3.

It is to be remembered that the high correlation between molar absorbances and equilibrium constant, which is a central problem in the equilibrium constants determination from spectrophotometric data, can only be resolved by proper experimental design and selection of wavelengths to be considered. For this reason, the wavelengths selected have been chosen around the maximum absorption value.

In Fig. 2a the experimental points, in terms of absorbances *vs.* the partial dioxygen pressures, are

reported together with the curves calculated by using the K_{O_2} and the molar absorbances values as given by the least square fit and summarized in Table 1. In the same table the most significant statistical parameters computerized by SOLVERSTAT are also entered.

From the data in Table 1 one can notice that Excel Solver converges to a solution when both Models are considered, so showing the typical situation of a difficult choice in absence of significant statistical analysis, as both models have been regarded as chemically significant.

Therefore the points (iv) and (v) of the procedure described in Section 2.3.5 and provided by SOLVERSTAT have been followed.

Examination of data quality. For both models the outliers statistics show that 2 points are suspected outliers, nevertheless they are very close

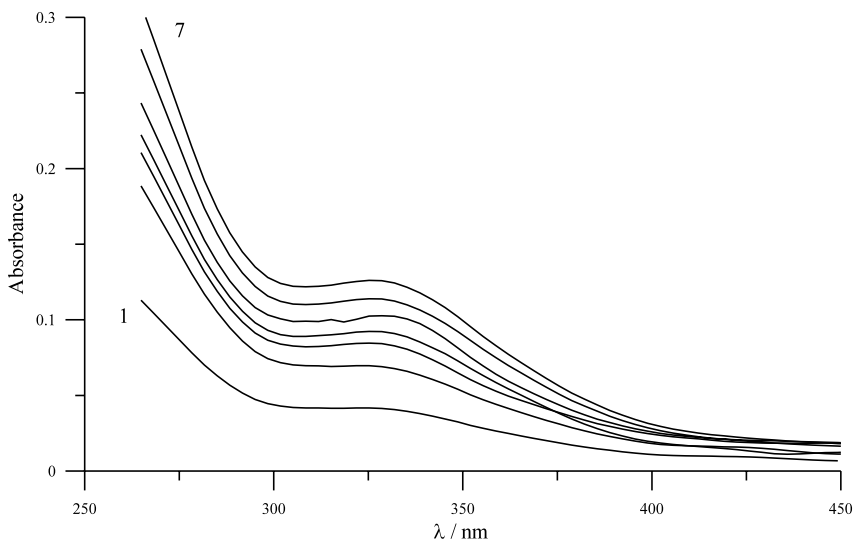


Fig. 1. Spectral changes of Co(dmen)_2^{2+} at various dioxygen partial pressures in dmsol at 25 °C: (1) 15.1 torr, (2) 30.4 torr (3) 45.6 torr, (4) 60.8 torr, (5) 76 torr, (6) 91.2 torr, (7) 120.7 torr. The relative maximum occurs at 325 nm. The spectrum at $p_{\text{O}_2} = 0$ torr is not reported: its maximum occurs at 505 nm with an extinction molar coefficients $\epsilon = 9.20 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$.

to the cut-off value. The influential statistics indicates that some influential points are present, but none of them are suspected outliers. Williams graphs reported in Fig. 2b highlight the situation and support the choice of not removing data from sets.

Examination of model quality. The runs-of-signs statistic probability values (see Table 1) indicate that no systematic deviation of the data with respect to the model is observed in the two cases. For K_{O_2} CI and JCI likely indicates a larger significativity of the Model A parameters, whereas JCI for Model B seems to point out some drawbacks about the reliability of its parameters.

Examination of regression method quality. Levene test indicates that both Models show constant variance. The Jarque–Bera Statistic accepts the hypothesis of normality of the residual for both models whereas Shapiro–Wilk Statistic shows that the distribution of the residuals is normal for Model A but not for Model B. This can be seen as a suspected violation of regression validity assumptions (see Section 2.3.2) for Model B.

As evident in the graphical results of the LS fit reported in Fig. 2a, the suspected nonnormality of Model B is likely due to an unsatisfactory fitting of data, which is reflected in the parameter errors

reported in Table 1. On the other hand the theoretical fitting model is in very good agreement with the experimental data for Model A.

In Fig. 2c the residuals of the data versus absorbance are also reported: the constant variance is evident and accounts for the choice of data analysis using unitary weights for the experimental data.

From the above analysis the discarding of one of the two models is still ambiguous. A glance to the statistical criteria described in Section 2.3.3 and provided by SOLVERSTAT is therefore determinant in order to distinguish them.

The ANOVA F values for regression significance are both higher than the critical one: this indicates that both regressions are significative and not fortuitous.

The R , R^2 , and R_{adj}^2 values are high for both fitting but seem to indicate that Model A is more suitable to explain the experimental data: in fact taking into account also the 95% CI for calculated R [34], the differences from 0.9982 to 0.9800 should be regarded as statistically significative.

The SSE value is lower for Model A by about 1 order of magnitude and this is an additional indication playing for higher reliability of the Model.

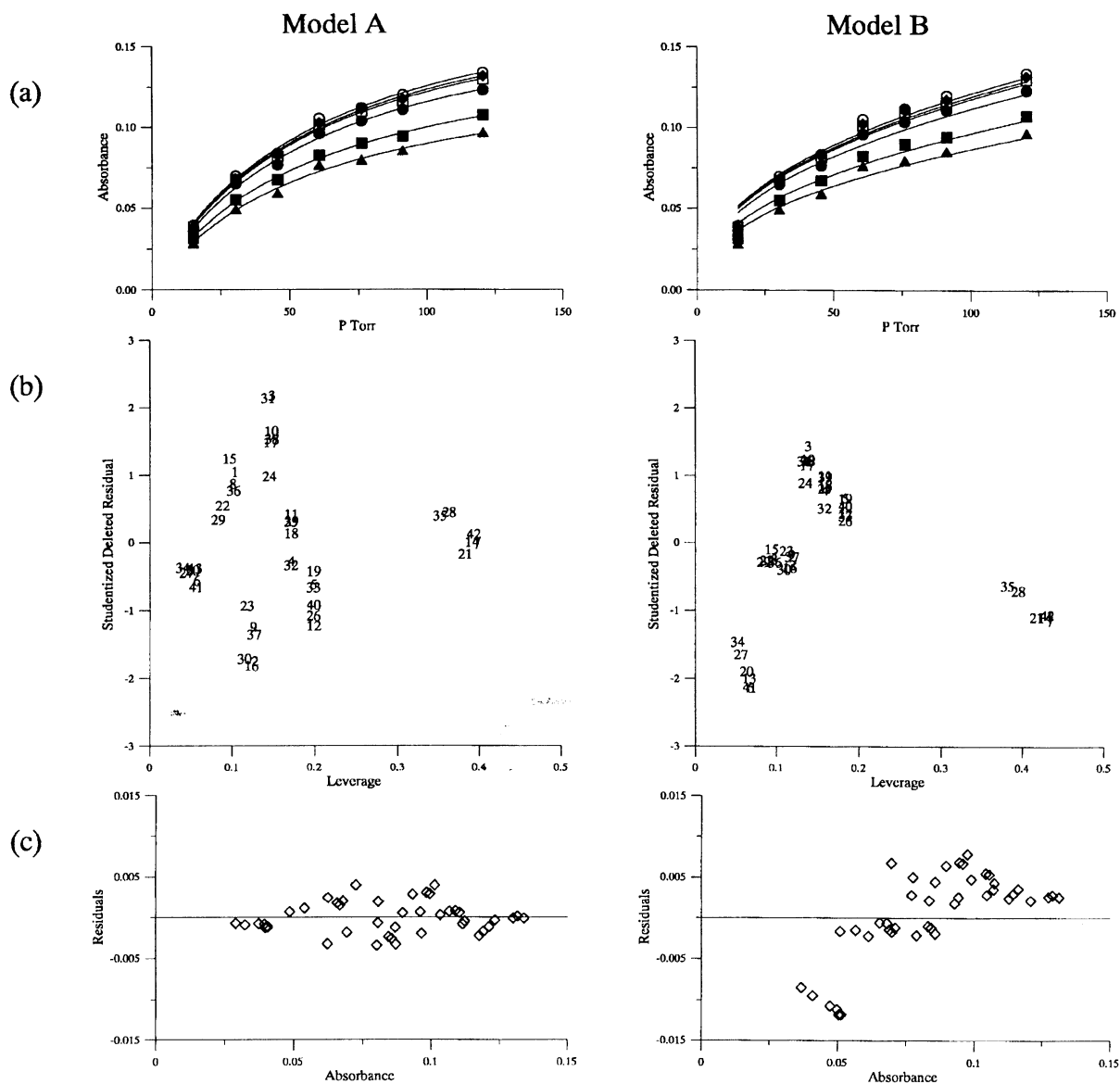


Fig. 2. (a) Graphical results of the LS fit for Model A and B in terms of absorbances vs. dioxygen partial pressure for dmsO solutions containing $1.05 \times 10^{-3} \text{ mol dm}^{-3}$ of $\text{Co}(\text{dmen})_2^{2+}$ complex. The points refer to the following λ values: (○) 333, (◆) 327, (□) 338, (●) 340, (■) 346, (▲) 350 nm. (b) Williams Graph for Models A and B. Cut-off values are 0.333 for leverages and 2.08 (t distribution with $P = 0.95$ and $DF = 34$) for Studentized deleted residuals. (c) Residuals of the least squares fit for Models A and B.

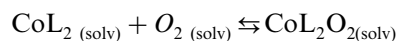
The analysis of PRESS, $R_{\text{prediction}}^2$, and AIC values also shows that Model A is better. The F value for Models comparison reported in Table 1 is the definitive proof that Model B can be discarded on the basis of the whole of the statistical analysis ($8.359 \gg 1.757$, probability $\ll 0.001$).

In order to build the MC confidence intervals for the estimated parameters found with Model A, 1000 MC numerical simulations were also performed using a scattering factor chosen on the basis of an estimate of experimental uncertainties as given by SOLVERSTAT. As shown in Fig. 3, where the K_{O_2} distribution is reported as an

example, normality test of the parameters fitted shows normal distribution. The values of MC parameters reported in Table 2 indicate a good agreement with those obtained from LS fitting, further confirming the reliability of the parameters found and of their errors.

To sum up SOLVERSTAT utility evidences that the most trustworthy model, which best overcomes all the statistical checks, is undoubtedly the one for which $a = 1$ and $b = 1$. The calculated value of the stability constant for reaction (13) is therefore $K_{O_2} = 0.01694(0.00042)$ torr⁻¹ (standard state is 1 torr at 25 °C).

The value K'_{O_2} for the oxygenation reaction in solution:



$$K'_{O_2} = \frac{[(\text{CoL}_2)(\text{O}_2)]}{[\text{CoL}_2][\text{O}_2]} \quad (14)$$

which will be useful for the aim of comparison, can be calculated by using the Henry Constant $K_H^0 = 2.76 \times 10^{-6}$ mol dm⁻³ torr⁻¹, being the solubility of O₂ at 1 atm pressure and 25 °C in dmsO 0.1 mol dm⁻³ with Et₄NClO₄ equal to 2.1 mmol dm⁻³ [35]: this value results in $\log K'_{O_2} = 3.788(0.011)$ mol⁻¹ dm³.

A detailed discussion on the nature of the complex formed, its possible application and the meaning of the value of its stability constants is out of the aim of this work and therefore will be considered elsewhere [36].

It is to be pointed out that the results here presented have been compared (i) with the results obtained by minimizing the same spectroscopic data by the program Dynafit [8], which is provided by excellent statistical tests but is not suitable for treating Excel worksheets directly and (ii) with the results obtained by using independent voltammetric measurements on the same Co(dmen)₂ system [36].

The analysis of the spectrophotometric data by Dynafit gives indications similar to those obtained by the application of Solver Excel–SOLVERSTAT: an inadequate fit of data is obtained when Model B is considered whereas a good fit characterizes Model A treatment. The values found for K'_{O_2} (the Dynafit program works in this case with the concentrations expressed in mmol dm⁻³) and ϵ values are in excellent agreement with those obtained with Excel Solver and statistically checked with SOLVERSTAT (Table 2).

The same indications come from the electrochemical measurements and from the relative

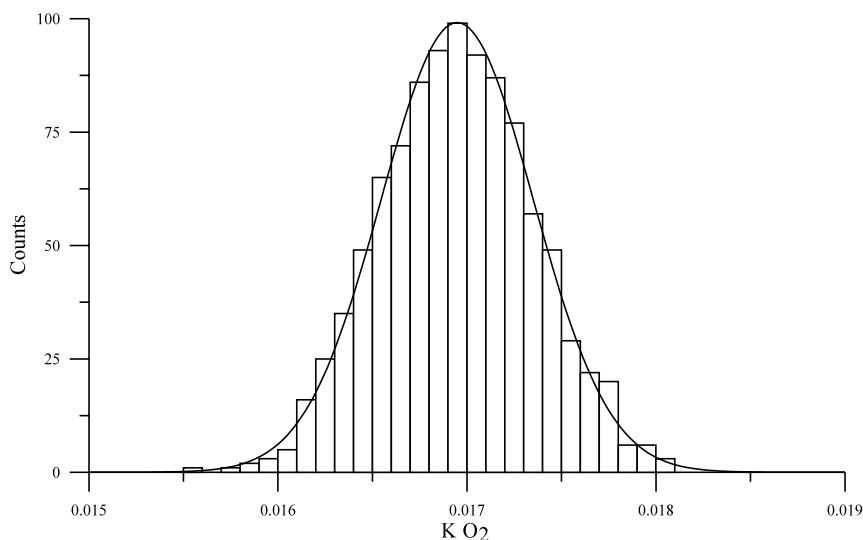


Fig. 3. Histogrammed results of Monte Carlo simulation for K_{O_2} derived from Model A from 1000 fits of 42 data sets. The smoothed curve is a Gaussian fitted to the K_{O_2} distribution.

Table 2

Parameter values and their errors as found by Excel Solver-SOLVERSTAT, MC simulation and Dynafit [8]

	Solver value	Asymptotic $\sigma(b_j)^a$	MC ^b value	MC $\sigma(b_j)^a$	Probability for Shapiro–Wilk statistic	Dynafit value	Dynafit $\sigma(b_j)^a$
Log K_{O_2}	3.788	0.011	3.788	0.010	0.77	3.813	0.015
ε_{327}	186.9	2.4	186.9	2.3	0.05	185.2	2.5
ε_{333}	190.3	2.4	190.3	2.3	0.47	188.4	2.5
ε_{338}	184.6	2.4	184.7	2.2	0.11	182.8	2.5
ε_{340}	175.2	2.3	175.2	2.2	0.72	173.6	2.4
ε_{346}	151.5	2.1	151.5	2.2	0.37	150.1	2.2
ε_{350}	136.3	2.0	136.3	1.9	0.78	135.0	2.1

K_{O_2} in mol⁻¹ dm³; ε_{λ}/nm in mol⁻¹ dm³ cm⁻¹.

^a Asymptotic standard deviation for calculated parameters: $\sigma(b_j) = \sqrt{\sigma^2(b_j)}$.

^b MC simulation was carried out using for absorbances a σ value of 0.00192 as estimated from experimental data.

Hill's plot [37]. The cyclic voltammeteries, carried out with O₂ partial pressures ranging from 8 to 36 torr in 0.02 and 0.1 mmol dm⁻³ solutions of Co(dmen)₂, showed the formation of a Co(dmen)₂:O₂ complex of 1:1 stoichiometry for which $K_{O_2} = 0.018(0.002)$ torr⁻¹. The whole of the experimental procedure and of the results obtained with this method is discussed elsewhere [36]: within this context the value of the resulting K_{O_2} is reported only for the aim of comparison.

In addition the reliability of SOLVERSTAT has been tested by application to systems where the problem of discrimination between different models had already been discussed in the literature: in particular the kinetic data reported in details in Ref. [11] have been minimized taking into account the two different Models proposed by the authors.

In a first step the data have been analyzed as done by Zielinsky et al. [11]. The results obtained agree quite well with those published earlier: a second order reaction pathway is in fact preferred as can be seen by analyzing the parameters and the F value for Models comparison reported in Table 3, Step 1.

As the data quality analysis carried out on all data evidenced the presence of almost two outliers, these have been removed and a second regression has been run. From the statistic results reported in Table 3, Step 2, it can be seen that the regression triplet analysis is satisfactory for both models, whereas the statistical criteria described in Section 2.3.3 unambiguously discard the first-

order fitting, being the calculated parameters substantially the same found after Step 1.

4. Conclusions

The proposed utility provides Microsoft Excel Solver of a powerful statistical method of analysis of the results thus allowing to discriminate between different chemical significative models.

For both A and B, Excel Solver in fact runs to convergence and only the statistical analysis provided by SOLVERSTAT allows the selection of the best one, Model A, whose parameters and errors reliability have also been tested by MC numerical simulations. The application of Dynafit package to the same experimental data, the additional analysis of independent electrochemical measurements and of data available in the literature further confirms the reliability of SOLVERSTAT utility.

SOLVERSTAT is available free of charge upon request by e-mail to PP (pierluigi.polese@dstc.uniud.it).

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Table 3

Parameter values and their errors as found by Excel Solver-SOLVERSTAT for first- and second- order nonlinear fitting of the data reported in Ref. [11] and as found in [11]

Step 1							
	SOLVERSTAT			Ref. [11]			
	First order	Second order		First order	Second order		
Rate constant k	3.5×10^{-6}	6.6×10^{-4}		3.5×10^{-6}	6.6×10^{-4}		
Asymptotic $\sigma(k)^a$	2×10^{-7}	2×10^{-5}		2×10^{-7}	2×10^{-5}		
C_0	0.0090	0.0099		0.0090	0.0099		
Asymptotic $\sigma(C_0)^a$	0.0002	0.0002		0.0002	0.0002		
SSE	3.79×10^{-6}	1.18×10^{-6}		3.62×10^{-6}	1.04×10^{-6}		
F value for models comparison	3.214			3.480			
Step 2							
	Critical value ^b	First order		Second order			
Runs-of-signs statistic	> 0.025	0.080		0.505			
F value for Levene test	< 4.45	0.105		0.010			
Shapiro Wilk statistic	> 0.901	0.950		0.926			
Jarque–Bera statistic	< 5.99	0.986		3.01			
ANOVA F value	> 4.45	449.58		2968.14			
R		0.983		0.997			
95% CI for R		0.956–0.994		0.992–0.999			
R^2		0.967		0.994			
SSE		1.859×10^{-6}		4.72×10^{-7}			
R^2_{Adj}		0.965		0.994			
PRESS		2.29×10^{-6}		5.72×10^{-7}			
$R^2_{Prediction}$		0.959		0.993			
AIC		–301.9		–327.9			
F value for models comparison	> 2.37	10.87					
Calculated parameters	$t_{Critical}$	Value	$\sigma(b_j)^a$	$t_{Calculated}$	Value	$\sigma(b_j)^a$	$t_{Calculated}$
Rate constant k	> 2.13	3.56×10^{-6}	2×10^{-7}	22.23	6.91×10^{-4}	2×10^{-5}	44.99
C_0	> 2.13	0.0090	0.0002	40.44	0.0099	0.0001	81.86
95% CI for k		3.23×10^{-6} – 3.90×10^{-6}			6.58×10^{-4} – 7.23×10^{-4}		
95% JCI for k		3.13×10^{-6} – 3.99×10^{-6}			6.50×10^{-4} – 7.32×10^{-4}		
95% CI for C_0		0.0085–0.0095			0.0097–0.0102		
95% JCI for C_0		0.0084–0.0096			0.0096–0.0103		

Step 1 refers to the treatment of all the data reported in [11], in Step 2 two outliers are not taken in to account (see Section 3). C_0 in mol dm⁻³, k in s⁻¹ or dm³ mol⁻¹ s⁻¹.

^a Asymptotic standard deviation for calculated parameters: $\sigma(b_j) = \sqrt{\sigma^2(b_j)}$.

^b Critical value selected for probability 5% and computed by SOLVERSTAT.

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