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Research Paper

Data Analysis of Kinetic Modelling Used in Drug Stability Studies: Isothermal *Versus* Nonisothermal Assays

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Purpose. Kinetic modelling was applied to predict the stability of cholecystokinin fragment CCK-4 in aqueous solution, which was analyzed by isothermal and nonisothermal methods using a validated stability indicating HPLC method.
Methods. The isothermal studies were performed in the temperature range 40 to 80°C at pH 12 and ionic strength 0.01 M as constants, whereas nonisothermal stability studies were performed using a linear increasing temperature program, heating rate 0.25°C/h and a temperature interval 40–82°C. The isothermal studies require two-step linear regression to estimate the parameters, resulting in a well-defined confidence interval. Nonisothermal kinetic studies require nonlinear or linear regression by previous transformation of data to estimate the parameters. In this case, the two most popular approaches, derivative and integral, were used and compared.
Results. Under isothermal conditions, an apparent first-order degradation process was observed at all temperatures. The linear Arrhenius plot suggested that the CCK-4 degradation mechanism was the same within the studied temperature range, with quite large uncertainties due to the small number of degrees of freedom based only on the scatter in the plot, and giving an estimated shelf life at 25°C of 35.2 days. The derivative approach yields high variability in the Arrhenius parameters, since they are dependent on the number of polynomial terms chosen, so several statistical criteria were applied to select the best model. The integral approach allows activation parameters to be calculated directly from experimental data, and provides results in good agreement with those of the traditional method, but have the advantage that the uncertainty in the final result directly reflects the goodness of fit of the experimental data to the chosen kinetic model. The application of the bootstrap technique to estimating confidence limits for the Arrhenius parameters and shelf life is also illustrated, and shows there is no difference between the asymptotic and bootstrap confidence intervals.
Conclusions. Nonisothermal studies give us fast and valuable information about drug stability, although their potential for predicting isothermal behaviour is conditioned by the data analysis method applied.

KEY WORDS: Arrhenius parameters; bootstrap; cholecystokinin; isothermal; nonisothermal.

INTRODUCTION

The study of drug-degradation kinetics, the development of a stable dosage form, and establishing an expiration date for the final product are important research activities during the pre-formulation, formulation, and product-development phases of a new pharmaceutical product. The time required for these studies at ambient temperature can be very lengthy because chemical reactions proceed relatively slowly at low temperatures.

Undoubtedly, accelerated testing at high temperatures allows for a significant reduction in testing time; however, preparation and assay of the large number of samples produced for the multiple-temperature accelerated test may offset this benefit.

The nonisothermal method was developed to reduce experimental effort, by allowing kinetic parameters to be estimated from a single set of drug concentration versus time data obtained while the temperature is changed during the time period according to some algorithm (1). However, the complexity of a nonisothermal study primarily arises from the temperature-rise program and the associated data-treatment method. The derivative and integral methods are two possible directions in non-isothermal data analysis (2–8).

An accurate estimate of the uncertainty associated with kinetic parameter calculations is important to avoid misleading inferences. Thus, analysis of isothermal kinetic experiments is traditionally believed to be more reliable because the one variable (T) is held constant during each experiment, thereby reducing the number of kinetic parameters to be determined simultaneously by fitting.

In comparison, nonisothermal runs are more convenient because a sudden initial temperature jump of the sample is not

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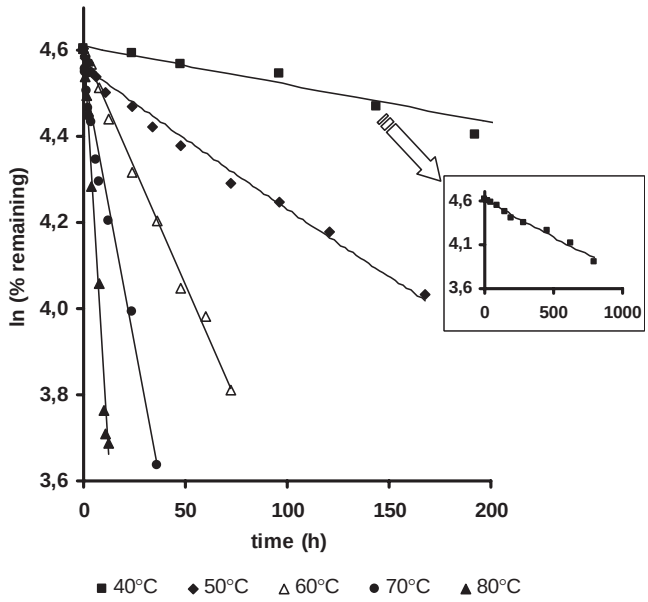

Fig. 1. First-order degradation of CCK-4 in aqueous solution as a function of temperature. *Solid lines* were obtained by regression linear.

necessary. However, Arrhenius parameters obtained from nonisothermal data are often reported to disagree with the values derived from isothermal experiments. There are various reasons for this disagreement, for example, the prevalent use of kinetic methods that involve force-fitting of nonisothermal data to hypothetical reaction models and that the nonisothermal method would need suitable experimental designs and precise drug content assays to provide reliable parameter estimates. Clearly, the results of nonisothermal experiments are expected to disagree with the isothermal data.

However, not only kinetics parameters value but also the confidence in such values is important, and, consequently, statistical evaluation needs to be considered. To obtain the best possible parameters, the use of appropriate statistics is necessary. The Monte Carlo method, also called the "bootstrap", a randomized re-sampling technique, provides a versatile and reliable statistical method to ensure the accuracy of parameters calculated from experimental data. This technique may be useful for analysing data sets where prior information is sparse, distribution assumptions are unclear, and further data difficult to acquire. It is an empirically based method, in which large numbers of simulated data sets are generated by computer from existing measurements, so that confidence intervals for the derived parameters may be obtained by direct numerical evaluation. This method has no constraint upon the number of times that a datum may be represented in a re-sampled subset, whose size may be fixed arbitrarily, it is independent of the experimental design parameters and may even exceed the total number of data samples (9,10).

The aim of this work was to verify the reliability of a nonisothermal method to produce consistent kinetic results and to explore the potential of predicting isothermal behaviour from nonisothermal data. In this last case, derivative and integral approaches were compared in order to determine the precision and limitations of each. For this, some statistic methods for analysis of experimental data such as *F*-test, Akaike's criterion or bootstrap technique were used to verify the validity of parameters, and hence predictions. As an example, the stability of the cholecystokinin fragment CCK-4 in aqueous solution was evaluated using both isothermal and nonisothermal assays.

MATERIALS AND METHODS

Materials

CCK-4 was purchased from Sigma Chemical Company (St. Louis, MO, USA). Trifluoroacetic acid (TFA; peptide synthesis grade) and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Deionized water was purified in a MilliQ plus system from Millipore (Molsheim, France) prior to use.

HPLC Method

The chromatographic system used was a Waters apparatus (Milford, MA, USA) consisting of a pump (600E Multisolvent Delivery System), an auto sampler (700 Wisp Model) and UV–Vis detector (2487 programmable multi-wavelength Model). Elution was performed at room temperature in a Nova Pack C-18 column (150 × 3.9 mm, 60 Å, 4 μm particle size, Waters). The data collection and analysis were performed using the Millenium32® chromatography program (Waters).

The mobile phase was an acetonitrile–water (30:70, v/v) mixture with 0.05% trifluoroacetic acid, the flow rate 1.0 ml/min, and the injection volume 25 μl. The detection wavelength was set at 280 nm. All solvents were filtered with 0.45 μm (pore size) filters (Millipore) and degassed.

Table I. Comparison of the Arrhenius Parameters and Shelf-Life Obtained by the Isothermal and Nonisothermal Derivative Methods Considering a First-Order Kinetic Model

Derivative Approach	No. of Polynomial Terms				Isothermal Approach
	Number 4	Number 5	Number 6	Number 7	
ln A (h^{-1})	22.83 [12.46–33.21][a]	31.75 [28.84–34.66]	31.06 [30.35–31.78]	30.46 [29.24–31.69]	32.36 [28.46–36.26]
Ea (cal/mol)	18,620 [11,386–25,134]	24,263 [22,327–26,200]	23,796 [23,321–24,271]	23,383 [22,567–24,200]	24,496 [21,918–27,074]
$t_{90\%}$ (days)	13.0 [2.57–65.4]	43.5 [29.1–65.4]	39.4 [35.3–44.1]	35.8 [30.2–42.4]	35.2 [18.9–65.4]
$t_{90\%}$ Error (%)[b]	63.0	23.6	11.9	1.70	Reference

[a] 95% confidence intervals based on typical standard deviation.
[b] $t_{90\%}$ Error = (Isothermal − Nonisothermal)/Isothermal × 100.

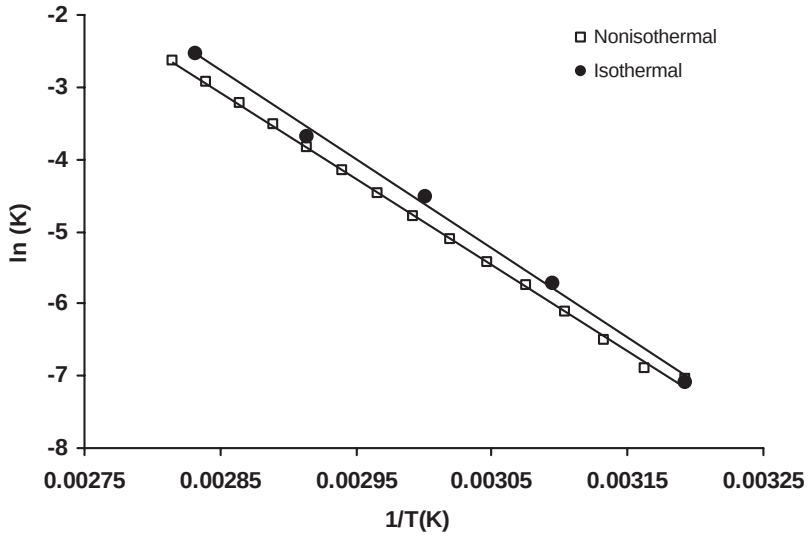

Fig. 2. Arrhenius plot for the degradation of CCK-4 in aqueous solution obtained by the classical isothermal method (*filled circle*) and by a nonisothermal derivative method (*open square*), fitting the data to a six-term polynomial model.

The RP-HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines (11). The results obtained in the validation process indicate that the method is specific, linear over a range of concentrations of 2–12 μg/ml, accurate (recovery mean = 100.2 ± 2.03%), precise (repeatability = 0.66%), and reliable (inter-assay precision = 2.74%). Limit of detection was established at 0.35 μg/ml and limit of quantitation at 1.06 μg/ml. Acceptable robustness indicates that the analytical method remains unaffected by small but deliberate variations, which are described in the ICH-Q2B guidelines (11).

Stability Studies

– Isothermal study: The oven (BR-UT 6000 Model, Heroes Instruments, Germany) temperature was pre-set and maintained at the desired temperature for isothermal studies. A 10 mg/ml peptide solution in dimethyl sulfoxide (DMSO) was transferred to a 10 ml volumetric flask, and the pH was adjusted to 12 with NaOH solution. Aliquots of this bulk solution were stored in the oven at a fixed temperature with variations less than ±0.1°C. Aliquots were removed from the oven at various time intervals, diluted with the mobile phase to obtain concentration values within the calibration range and analyzed the same day in triplicate.

– Nonisothermal study: The temperature of the oven was controlled by a loop control program written in Test Point® (version 4.0) designed to run from 40 to 82°C, the total reaction time was 7 days to obtain a linear heating rate of 0.25°C/h. Samples were prepared and analyzed as under isothermal studies, but were removed from the oven every 12 h until all data points were collected.

Nonisothermal Studies Data Analysis

Both integral and derivative methods were used to analyze the nonisothermal stability data, methods well documented and described in the pharmaceutical literature (1–8).

1. Integral method: For a first-order reaction, a combination of the integrated rate expression and the Arrhenius equation yields:

$$C = C_0 \cdot \exp^{-A \cdot \int_0^t \exp[-Ea/RT(t)]dt} \quad (1)$$

This method involves direct evaluation of the integrated expression on the right of Eq. (1). For this, the R® statistical program (12) was used, which allows a direct nonlinear estimation of the activation energy (*Ea*), the frequency factor (*A*) and the initial drug concentration (C_0) expressed as percentage of drug remaining.

2. Derivative method: The drug concentration–time relationship can be expressed as a power series with time:

$$C(t) = a_0 + a_1 t + a_2 t^2 + a_3 t^3 + \ldots\ldots + a_n t^n \quad (2)$$

Table II. Application of Several Statistical Criteria for Determining the Best Model in the Derivative Approach

No. of polynomial terms	Kipp	Akaike	Model comparison	Test *F*		
				Degree of freedom	*F* calculated	*F* tabled
7	0.9984	8.586	7 vs 6	8	−3.95	5.32
6	0.9994	−3.626	7 vs 5	9	−0.66	4.46
5	0.9920	1.894	6 vs 5	10	5.86	5.12
4	0.8467	37.05	–	–	–	–

Differentiating Eq. (2) with respect to time gives the first derivative, which provides a rate constant at each temperature, and the *Ea* of the reaction is subsequently calculated with the help of the Arrhenius equation. The Excel® spreadsheet from Microsoft Office was used to fit the nonisothermal data and obtain the first derivative at each time point for each reaction order, and hence, corresponding to temperature as well.

RESULTS AND DISCUSSION

Isothermal Studies

An apparent first-order degradation process was observed at all temperatures studied (40 to 80°C). Higher temperatures were excluded since it was not possible to follow the degradation process. To verify the validity of the kinetic model and to measure the linearity, correlation coefficient (r) and standard errors were calculated. The rate constants were obtained from the slopes of the semi-log plots of concentration versus time by linear regression analysis, the correlation coefficient being greater than 0.95 (Fig. 1). The residual plots showed the absence of trends or correlations, the signs test (13) confirmed the validity of proposed model and therefore, the residuals represent only the experimental error.

A linear Arrhenius plot was observed (r = 0.998), suggesting the same CCK-4 degradation mechanism within the studied temperature range. The *Ea* and *A* obtained were equal to 24.5 kcal/mol and 1.21×10^{14} h^{-1}, respectively, similar to that reported for degradation of various peptides in aqueous solution (14–17). The uncertainties given as 95% confidence intervals were calculated from the residual standard deviation by the standard expression (13) are shown in Table I. The uncertainties are quite large due to the small number of degrees of freedom ($\upsilon = 3$) based only on the scatter in the Arrhenius plot.

The estimated shelf life ($t_{90\%}$) at 25°C was 35.2 days. This calculation was made on the assumption that the activation energy remains constant over the temperature range of 25 to 80°C. In order to estimate the uncertainty in the estimate of $t_{90\%}$, the 95% confidence intervals were calculated to ln K, obtaining the values of 18.9 and 65.4 days, respectively. These confidence intervals are very large since there is a coupling of the uncertainty of the prediction due to fitting the rate constants at each temperature and the temperature dependence for extrapolation to storage temperature.

Nonisothermal Studies

The principal challenges of nonisothermal studies are the generation of a programmed temperature rise and the subsequent data analysis. In the first case, the difference between the theoretical (0.250°C/h) and experimental (0.251°C/h) lineal heating-rate constants was less than 1.2%, therefore the temperature control was excellent throughout the experiment.

For data analysis, two common methods, derivative and integral approaches, were used to calculate the Arrhenius parameters. In the first, the concentrations–time data were

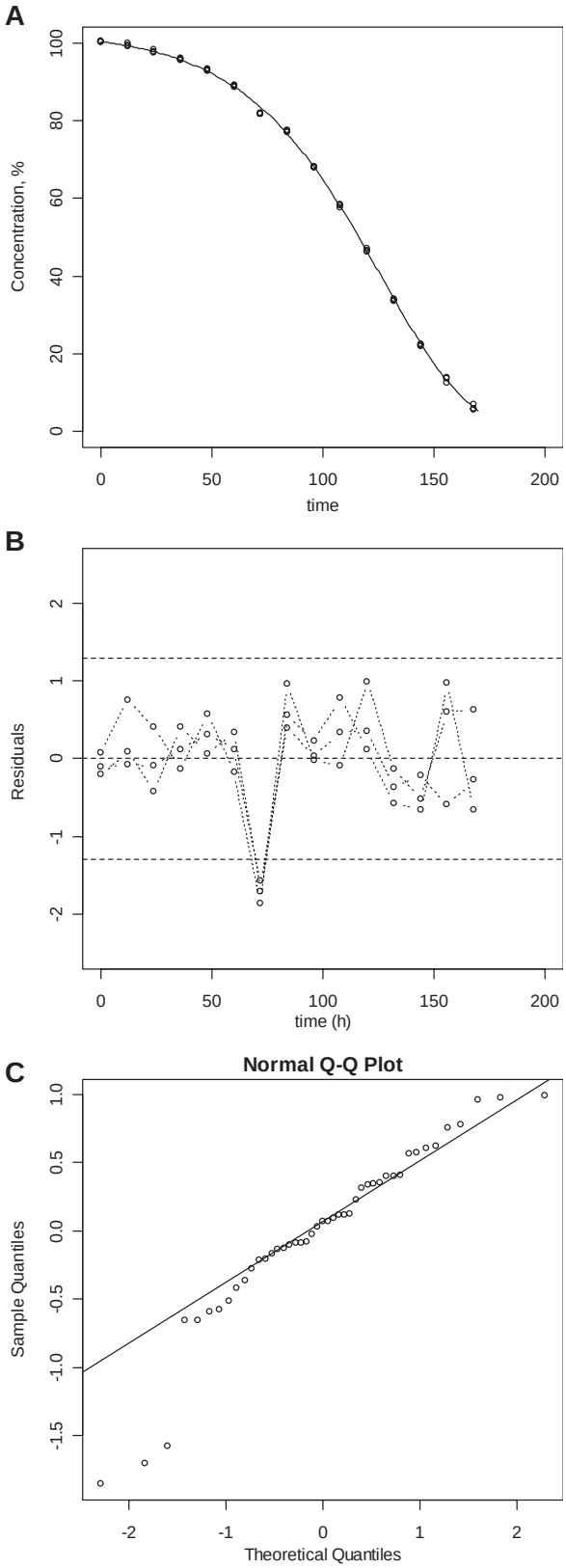

Fig. 3. (A) Variation of the CCK-4 concentration expressed as percentage remaining (*open circle*) *versus* time, considering an experimental linear heating rate of 0.251°C/h. Data were fitted using the R® program. (B) Plot of residuals against predicted values. (C) Q–Q plot (i.e., quantile–quantile) of the residuals showing a normal distribution line.

fitted using polynomials in the forms described by Eq. (2). Rate constants were then obtained by differentiation of Eq. (2) at various values of t, and the corresponding temperatures calculated from the temperature–time lineal relationship. The resulting temperature-dependent rate constants were then fitted to the Arrhenius equation (Fig. 2), but the estimated parameter values depend on the number of terms and the kinetic model used (Table I). Once again, the uncertainties are 95% confidence intervals based only on the scatter in the Arrhenius plot, although they are narrower (except for the four-term polynomial model) than in the isothermal assays, due to the difference in the number of experimental points (15 *versus* 5). However, the *Ea* values and their uncertainties do not overlap the estimated value of 24.5 kcal/mol, except for the five-term polynomial model.

Quite apart from the problem of obtaining realistic error limits, there is the problem of choosing an appropriate order of polynomial to fit the concentration–time curve. To determine the correct number of terms, there are a variety of criteria that can be used to select the best model. These may appear to be superior to graphical and parameter variability criteria because they are less subjective, however all these criteria should be used together in making the decision about the best model. At first, the criterion established by Kipp (18) was chosen, where the goodness of fit is customarily estimated by the coefficient of linear correlation (r) for the Arrhenius plot. For this, the data were treated using zero, first, and second-order reaction model, the first-order kinetic model yielded the highest correlation in the Arrhenius plot and the smallest residual sum of squares (*RSS*). Thus, a single pair of *Ea* and *A* is then commonly chosen as that corresponding to a reaction model that leads to the maximum absolute value of the correlation coefficient. In this case, a six-term polynomial, assuming first-order kinetics, was the best model (r = 0.9994). The correlation coefficients for the two other reactions order, however, were comparatively poor and negative, and highly varied values for the activation energies were obtained (data not shown).

This model gives activation energy of 23.8 kcal/mol, the shelf life being 39.4 days, similar to values obtained from isothermal data (Table I). However, the seven-term polynomial model gives a value of $t_{90\%}$ that is fairly consistent with isothermal data, with an error below 2.0%, but data analysis does not allow this model to be chosen without the exclusion of the other.

At this point, the polynomial models in x are naturally ordered in that most workers would require evidence justifying a higher-order model before proposing it in preference to a lower order one. Thus, linear models are preferred to quadratic, quadratic models to cubic, and so on. In this situation, Akaike's (19) information criterion (*AIC*) can be used to choose among models that are hierarchical (i.e., one is the full model, and all the rest can be expressed as restricted cases of that full model), but for which there is no a priori reason to prefer one over another. An *AIC* value can be calculated from the final data fit:

$$AIC = n \ln (RSS/n) + 2p \tag{3}$$

where n is the number of data points and p is the number of adjustable parameters.To discriminate between two or more models, the lowest *AIC* value is used. The results shown in Table II indicate it is −3.626 for the six-term polynomial.

A second statistical criterion is the *F*-test. The idea is to test whether the increase in the number of parameters has produced a significant improvement in the fit. The *F*-value is calculated and compared with tabled values, usually at the 5% significance level.

$$F = \left(\frac{RSS_j - RSS_k}{RSS_k}\right) \times \left(\frac{df_k}{df_j - df_k}\right) \tag{4}$$

where the indices j and k refer to the two models being compared and *df* are the degrees of freedom for each model. The model with the highest number of parameters is indexed as k (17). The *F*-test results suggest that polynomial models with seven and six terms offer no significant improvement over the five-term one, since the calculated values are lower than the tabled value (Table II). On comparison, the six-term model is better than the five-term because the calculated value (F = 5.86) is larger that the tabled value (F = 5.12).

All statistical criteria point to the six-term polynomial as the best. In spite of the obvious viability of the seven-term model, we have to stress that statistical analysis of data does not allow this model to be preferred over the other, whose use results in satisfactory predictions of the isothermal measurements.

An explanation consistent with this could be the high correlation between the estimates of *Ea* and *A*, attributed to the nature of the Arrhenius equation. This fact can be verified if the asymptotic correlation matrix is analysed. Thus, small variations in one parameter will considerably affect the other; therefore the $t_{90\%}$ will change. We can find a situation where the *Ea* and *A* parameters may differ greatly for two given models, whereas the $t_{90\%}$ estimates are very similar, hindering the selection of an appropriate model. In general, this can be considered a specific problem of non-isothermal studies, but not of the model-fitting method.

The second data treatment method utilized in this study was proposed by Yoshioka *et al.* (5), which involves a direct

Table III. Comparison Between Asymptotic and Bootstrap Confidence Intervals for the Nonisothermal Integral Approach

Parameter	Estimates	CI Asymptotic[a]	Basic	95% CI Bootstrap	
				Percentile	BCa
C_0 (%)	100.3	99.92–100.7	100.0–100.7	99.90–100.7	99.90–100.7
ln A (h^{-1})	31.38	30.72–32.04	30.73–31.98	30.7–32.02	30.79–32.04
Ea (cal/mol)	24,009	23,566–24,455	23,576–24,417	23,602–24,443	23,613–24,445
$t_{90\%}$ (days)	41.3	37.7–45.0	37.7–44.7	38.0–45.0	38.2–45.2

[a] 95% confidence interval based on asymptotic standard error.

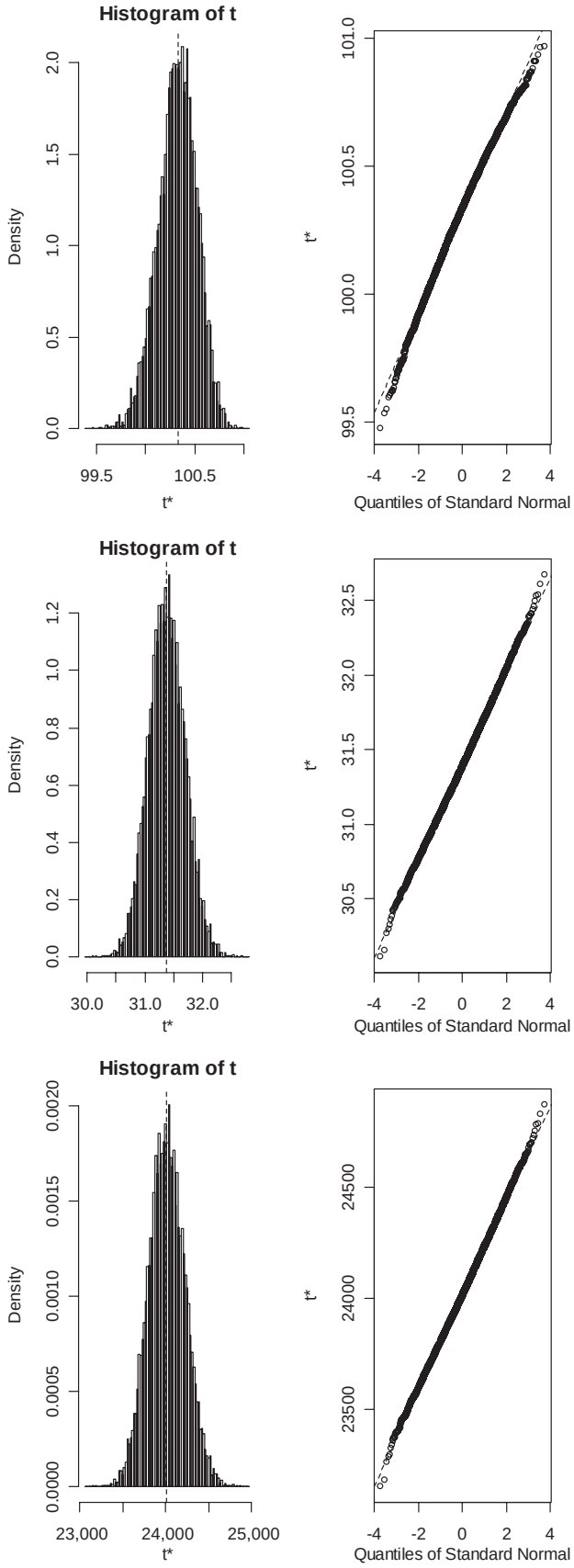

Fig. 4. Histograms of 9,999 bootstrap iterations and the quantiles of standard normal plots for the C_0 (*upper*), ln A (*medium*) and Ea (*lower*) parameters estimated by the integral approach.

nonlinear estimation of the Arrhenius parameters from Eq. (1) without preliminary mathematical treatment; providing reliable estimates with smaller deviations and biases. In our cases, the R® statistical program (version 2.1.1) was used to fit the degradation profile of CCK-4. Both Ea and A were determined by an iterative nonlinear least-square method (Fig. 3A). The output from the R® program generated an optimal value both for the activation energy of 24.0 kcal/mol and 4.25×10^{13} h^{-1} for the frequency factor.

The application of the numerical integration method is free from bias because it fits the values of Ea and A directly to the data in a single step, rather than fitting the data to some functional form and then obtaining Ea and A separately. In addition it takes into account the scatter in the original data, which is inherently impossible using the two-step isothermal and derivative approaches. Thus, this method gives a reliable estimate of all parameters, e.g. the energy activation differs by only 0.5 kcal/mol from the isothermal data, and the most realistic estimate of the uncertainties involved (Table III) due to the higher number of degrees of freedom. These results are reflected in the goodness of fit between experimental values and the kinetic model chosen. Also, the assumption of normally distributed errors was checked by examining the residuals and Q–Q (quantile–quantile) probability plots (Fig. 3B, C). The plot of residuals shows that one data set (time = 72 h) has a large residual value, probably an anomaly (no assignable cause can be found), a fact not clearly evident from Fig. 3A, but confirmed by viewing Fig. 3B, C. In any case, the normality and independence of the errors is preserved.

For nonlinear models, estimation of confidence intervals is not straightforward, and they are not necessary symmetrical; the extent of asymmetry depends on the nonlinearity of the function and the quantity of data. As the subject is too complex to deal with here, some procedures for deriving confidence intervals for nonlinear parameter estimation are analysed without going into detail (20). The first and easiest method is to use asymptotic standard errors, but this may significantly underestimate the confidence intervals. A more reliable method would be to search for values of each parameter causing the objective function to be greater than its minimum by the amount of some critical value given by χ^2 or F-distribution. This is also very tedious. By far the best method to evaluate confidence intervals applies the Monte Carlo technique and is called the "bootstrap". Today the lowest-cost computers and software have free statistical packages such as Solver® in Excel® or R® program, which have gained acceptance and popularity in the field of applied statistics.

Table IV. Analysis of Data Published by Mu-Lan and Stavchansky Using the Integral Approach

Parameter	Reported Value[a]	Calculated Value[b]	Percent Difference[c] (%)
Ea (kcal/mol)	21.11	20.99 [20.94–21.04]	0.57
ln A (h^{-1})	26.43	26.01 [25.94–26.08]	1.59
$t_{90\%}$ (days)	45.9	54.0 [53.5–54.5]	17.6

[a] Uncertainties were not given for these reported values.
[b] Estimated value with 95% confidence intervals based on BCA bootstrap method.
[c] Error = (Isothermal − Nonisothermal)/Isothermal × 100.

In this case, the software R® program was used for deriving 95% confidence interval bootstraps i.e., basic, percentile and bias-corrected and accelerated (BCa), for the following parameter estimates: C_0, *Ea* and *A*. In total, 9,999 bootstraps were performed on these parameters, and the different bootstrap confidence intervals are shown in Table III. The bootstrap replicates are displayed in Fig. 4. The symmetry of the parameter estimates indicates a normal distribution. When the values of the individual bootstrap replicates are examined, they show narrow dispersions: $-C_0 = 99.5$ to 101.0, ln $A = 30.0$ to 32.5 and $Ea = 23{,}000$ to 25,000. The length and shape of the different confidence intervals show no differences between them, since all approaches give closely similar uncertainty estimates and the results from the different runs lie within one another's uncertainty limits. These confidence intervals are very close to those obtained using the asymptotic standard error method and, therefore, the assumption of normally distributed errors and the kinetic model chosen are correct.

The $t_{90\%}$ was calculated by the standard procedure assuming first-order kinetics at 298.15 K using Eq. (5):

$$t_{90\%} = 0.1054 \Big/ \left(e^{A - \frac{Ea}{RT}}\right) \tag{5}$$

The *Ea* and *A* estimates were substituted in Eq. (5), obtaining a value of 41.3 days. The bootstrap technique was again used for deriving confidence intervals for $t_{90\%}$, and confirms that its distribution is also normal. The asymptotic and bootstrap confidence intervals were very similar. Also, this approach give closely similar estimates of the uncertainty to those obtained using the derivative approach assuming a six-term polynomial model, but with the additional advantage that the calculated uncertainties in the estimated parameters reflect the real scatter in the experimental data. The estimated shelf life was quite close to the value derived from isothermal data, a difference of 6 days was observed, similar to value obtained by Mu-Lan and Stavchansky (8), but significantly more accurate. This fact can be observed in the length of the 95% confidence interval for the estimated parameter. This variation is due to the differences in the estimation method and the nature of the experimental errors.

At first, this integral approach can be applied to any kinetic experiment carried out under nonisothermal conditions. For this, kinetic data published by Mu-Lan and Stavchansky (8) were analysed. The results are summarized in Table IV.

The results obtained indicate that the estimates kinetic parameters were comparable to those reported values, but the estimated shelf life was higher, 54 *versus* 45.9 days. This discrepancy could be due to the differences in the data analysis method. These authors used the integral approach, but a five-term polynomial was previously used for the concentration–timer curve fitting, whereas in our case, the kinetics parameters are determined in a single-step performing non-linear regression analysis on response values and to provide a realistic estimate of the confidence associated with the parameters calculated.

However, the difference with respect to the reported shelf life at isothermal conditions was similar, although omitting the confidence interval (the uncertainties were not given in the original paper), makes the parameter estimate uninterpretable.

CONCLUSIONS

The linear Arrhenius plot seems to indicate that the degradation mechanism and kinetics do not change with temperature. At first, the similarity of *Ea* values obtained under each set of conditions with a difference lower than 2.5% seems to confirm this despite a marked difference in the frequency factors.

The nonisothermal results suggest that first-order kinetics give a better description of the process, confirming the result obtained under isothermal conditions.

In contrast, the Arrhenius parameters obtained in nonisothermal conditions are highly variable, exhibiting greater dependence on the reaction model chosen and the data analysis method, especially with the derivative approach. In this case, the application of different statistical criteria indicates that a six-term polynomial model is the best. The results indicate that the integral approach seems to be superior to the derivative method in that both *Ea* and *A* can be estimated directly without previous data treatment, and the model must be chosen on the basis of statistical criteria. The uncertainties reflect the fit of the original data to the kinetic model and not simply the scatter in the Arrhenius plot. Also the bootstrap technique provides a way to obtain accurate realistic estimates of experimental uncertainties in the data.

In summary, the integral approaches used in this paper provide a fast and low-effort method for estimation of the activation parameters and shelf life in comparison with traditional methods. This approach can be recommended as a trustworthy way of obtaining reliable and consistent kinetic information form isothermal data.

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