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THE KINETICS OF DEGRADATION OF ETHACRYNIC ACID IN AQUEOUS SOLUTION

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ABSTRACT

The degradation of Ethacrynic Acid has been investigated as a function of temperature and concentration. Degradation produced a dimer, the concentrations being measured using an HPLC analysis. Degradation data were fitted to the Arrhenius equation using a least squares regression analysis. Data were fitted separately for each temperature and simultaneously for all temperatures. Arrhenius parameters were calculated. A statistical assessment of the influence of experimental design on the estimation of Arrhenius parameters has been made.

INTRODUCTION

From the results of a polarographic study, Cohen (1) indicated that the principal route of degradation for Ethacrynic Acid is by a Diels-Alder type condensation leading to the formation of a dimer. This report is an investigation of the aqueous solution stability of Ethacrynic Acid carried out prior to a more detailed project related to the stability of various physical forms of Ethacrynic Acid. The kinetics of the degrad-

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ation process have been investigated as a function of concentration of Ethacrynic Acid and temperature.

EXPERIMENTAL

Apparatus

The high performance liquid chromatographic (HPLC) system was equipped with a variable flow pump and an automatic loop injector. Additional components consisted of a column heater, autosampler, variable wavelength ultraviolet-visible detector[i], and a single channel plotting - reporting integrator[ii].

Reagents and Materials

Ethacrynic Acid[iii], 1M Sodium Hydroxide Solution, 0.05M Tham Perchlorate Buffer pH 3.5[iv], Methanol[v], and Distilled Water were used.

Chromatographic Conditions

A 250 x 5 mm I.D. Zorbax ODS packed column[vi] was used at 50° C. The mobile phase consisted of Methanol - 0.05M Tham Perchlorate Buffer pH 3.5. The solvents were filtered through 10μm filters, mixed and pumped through the HPLC system at a flow rate of 1.5 ml/min. All sample and standard injection volumes were 10μl. Linear gradient elution was employed from 65% to 95% Methanol. The detector employed a wavelength of 279 nm. The average Ethacrynic Acid peak area was recorded and the assay determined with reference to the standard solution results.

RESULTS AND DISCUSSION

Concentrations of Ethacrynic Acid $[c]$ as a function of time and temperature have been fitted to the rate equation

$$\frac{d[c]}{dt} = -k[c]^n$$

where t denotes time, k is the apparent rate constant and n is the apparent order of reaction. The data were fitted

(a) separately for each temperature and concentration

(b) separately for each temperature at all concentrations

(c) simultaneously for all temperatures and concentrations using the Arrhenius equation

$$k = \exp(A-E_a/RT)$$

to relate k to the absolute temperature T. exp(A) is the frequency factor and E_a is the activation energy. Consistent with the mechanism described above for the degradation process, a value of n = 2 was used. The fits were obtained by least squares regression analysis using the statistical package NONLIN (2). Deviations between observed and calculated concentrations were weighted by a factor inversely proportional to the magnitude of the observed concentration.

Table 1 lists values of k for individual concentrations and temperatures. Table 2 lists the values of the estimated values of k for each temperature and the corresponding coefficients of variation. Table 3 lists the estimated Arrhenius parameters. Figure 1 shows the linear relation between $\log_{10}$ k and 1/T calculated from the estimated Arrhenius parameters listed in Table 3. The values of $\log_{10}$ k from which the Arrhenius parameters were calculated are also plotted to allow direct visual comparison.

From an inspection of Table 2 and Figure 1 it can be seen that the errors associated with the estimation of k are proportionately greater at 20° C than they are at the higher temperatures. That is the coefficient of variation is greater and the 95% confidence limits are wider at the lowest temperature investigated. This observation is doubtless due to the experimental design since the study was conducted over approximately the same period of time at all temperatures with the result that the effects of storage were markedly different and were in fact slight at 20° C. However the treatment of data in the estimation of the Arrhenius parameters allows the lesser effects

TABLE 1

RATE CONSTANT (DAYS^{-1}) FOR DEGRADATION OF INDIVIDUAL ETHACRYNIC ACID SOLUTIONS CONTAINING DIFFERENT CONCENTRATIONS OF ETHACRYNIC ACID AT INDIVIDUAL TEMPERATURES

	Storage Temperature °C			
mM/litre	20	40	50	60
22.1	0.2071×10^{-2}	0.1826	0.3399	0.5645
46.6	0.9136×10^{-2}	0.1175	0.3065	0.4199
76.4	0.1518×10^{-1}	0.1291	0.2671	0.4545
96.3	0.1804×10^{-1}	0.1304	0.2825	0.4261
122.1	0.2540×10^{-1}	0.1176	0.2571	0.4246
149.2	0.2621×10^{-1}	0.1223	0.2474	0.4155

TABLE 11

RATE CONSTANTS FOR DEGRADATION OF SOLUTIONS CONTAINING DIFFERENT CONCENTRATIONS OF ETHACRYNIC ACID AT INDIVIDUAL TEMPERATURES

	Storage Temperature °C			
	20	40	50	60
k (days^{-1})	0.2436×10^{-1}	0.1226	0.2599	0.4254
coefficient of variation %	2.929	1.462	1.254	1.661

TABLE 111

ARRHENIUS PARAMETERS FOR DEGRADATION OF ETHACRYNIC ACID ESTIMATED FROM RATE CONSTANTS AT ALL TEMPERATURES AND CONCENTRATIONS

Frequency factor exp(A) (days^{-1})	exp(19.7466)
Activation energy E_a (J/°C/mole)	56,869

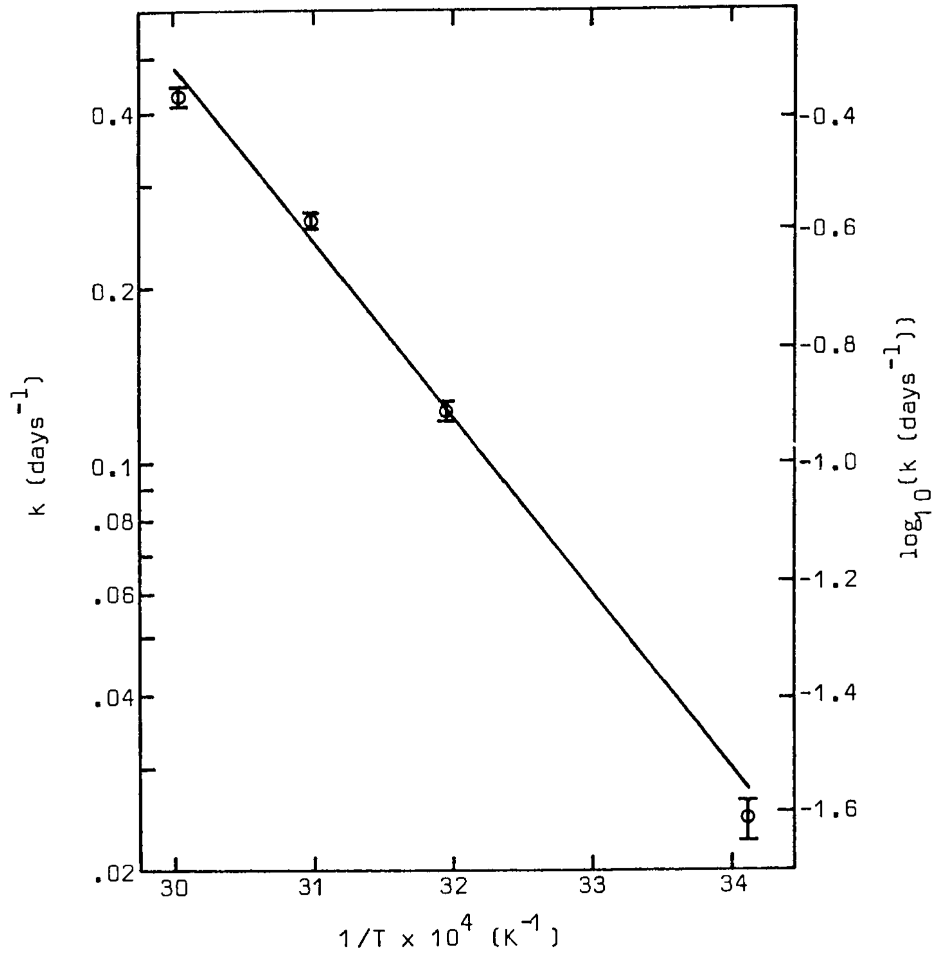

FIGURE 1

THE DEPENDENCE OF RATE CONSTANT k ON RECIPROCAL ABSOLUTE TEMPERATURE 1/T

— the relationship according to the Arrhenius parameter listed in Table 3.

o the rate constants k given in Table 2.

I standard deviation of the rate constants k listed in Table 2.

at the lower temperatures to contribute to the estimation of the Arrhenius parameters without undue weight. It can be seen that the value of k estimated only from the data obtained at 20° C deviates appreciably from that calculated from the estimated

Ethacrynic Acid Solution Preparation and Storage

25 g of Ethacrynic Acid were dispersed in approximately 300 ml of Distilled Water using a paddle stirrer. The pH of this suspension was 3.5. 1M Sodium Hydroxide solution was added slowly until a constant pH of 7.0 was obtained. The solution was then made up to 500 ml, filtered through a 0.22μm Millipore membrane filter previously washed with Distilled Water and adjusted to pH 7.0 with 1M Sodium Hydroxide solution. Aliquots of this solution were diluted with Distilled Water and the final solutions adjusted to pH 7.0 with 1M Sodium Hydroxide solution. The resultant concentrations of Sodium Ethacrynate in the solutions were 22.1, 46.6, 76.4, 96.3, 122.1 and 149.2 mM/ litre.

These solutions were transferred to containers, stoppered and stored at 20, 40, 50 and 60^{0} C for sampling at appropriate intervals.

Preparation and Assay of Standard Solution

Approximately 100 mg of Ethacrynic Acid was accurately weighed into a 100 ml volumetric flask and 50 ml of Methanol added. The flask was agitated to dissolve the Ethacrynic Acid and the resulting solution made up to volume with Methanol. This solution was found to be stable for up to 24 hours under ambient conditions. At least three standard samples were chromatographed under the described HPLC conditions and the average Ethacrynic Acid peak area determined.

Preparation and Assay of Sample Solutions

The stored solutions were allowed to cool to 20^{0} C. For each sample 5 ml was pipetted into an appropriately sized volumetric flask and made up to volume with Distilled Water to yield a final concentration of ≃ 1 mg/ml. These samples were chromatographed in duplicate under the described HPLC conditions. Arrhenius parameters. The validity of the use of the Arrhenius equation to express the relation between concentration of

Ethacrynic Acid, time and temperature has been tested by using the Fisher F test

$$F_{2,166} = \frac{(\Sigma WSS_1 - WSS_2)/\ \Sigma DF_1 - DF_2)}{(WSS_2/DF_2)} = 24.3$$

where DF denotes degrees of freedom WSS denotes weighted sum of squares, subscript 1 denotes the separate, and subscript 2 denotes the simultaneous, fits of data at the different temperature levels. The tabulated (3) value of F corresponding to $F_{2,166}$ is approximately 3.0, (P=0.05). F tabulated < F calculated and it therefore is concluded in the case of the data reported here that there is a significant (P=0.05) improvement in the representation of the data by reporting the rate constants k for the dimerisation of Ethacrynic Acid at the four separate temperatures rather than simultaneously describing the time and temperature dependencies using the Arrhenius equation.

REFERENCES

(1) E.M.Cohen, J. Pharm. Sci. Vol 60, 11, 1702-04, 1971.

(2) Metzler, C.M, Effring, G.L., McEwan A.J. (1974).

(3) Fisher R.A. and Yates F., Statistical tables for biological agricultural and medical research. 6th edition. Edinburgh Oliver and Boyd, 1970.

i) Varian Associates Inc., Palo Alto, California.

ii) Model 3390A, Hewlett Packard, Avondale, Pennsylvania.

iii) B.P. Grade, Merck Sharp and Dohme, Hoddesdon, England.

iv) Prepared from Analytical Reagent grade, BDH Chemicals Ltd. Poole, England.

v) HPLC Grade, Fisons Scientific Apparatus, Loughborough, Leics., England.

vi) HPLC Technology Ltd., Wilmslow, Cheshire England.