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Study of the stability of erythromycin in neutral and alkaline solutions by liquid chromatography on poly(styrene-divinylbenzene)

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Abstract

A liquid chromatography method using poly(styrene-divinylbenzene) as the stationary phase was used as the analytical method to investigate the stability of the macrolide antibiotic erythromycin in neutral and alkaline solutions (pH 7.5–11). Decomposition of erythromycin A was monitored up to 20% of degradation. Three degradation products were detected: pseudoerythromycin A enol ether, pseudoerythromycin A hemiketal and a hydrolysis product of erythromycin A, i.e., the product with an opened lactone structure. The decomposition kinetics have been studied as a function of the ionic strength, the concentration of the buffer, the amount of methanol in the solvent and the temperature.

Keywords: Erythromycin; Stability; Neutral solution; Alkaline solution

1. Introduction

In a previously published paper, the degradation of the macrolide antibiotic erythromycin A (EA) in acidic aqueous solutions was discussed (Cachet et al., 1989). EA is converted to anhydroerythromycin A (AEA), while it is in equilibrium with erythromycin A enol ether (EAEN) (Vinckier et al., 1989). In neutral media (pH 6–8) small amounts of pseudoerythromycin A enol ether (psEAEN) are formed. psEAEN is the substance obtained by translactonization of erythromycin (Kibwage et al., 1987), resulting in a ring contraction of the aglycone. In the literature no detailed information about the degradation products of erythromycin formed in alkaline solutions is available. Papers dealing with the stability of erythromycin in alkaline solution only describe the disappearance of the starting material, but do not give information about the degradation products formed (Pluta and Morgan, 1986; Allwood, 1990, 1992). It is known that erythromycin A is converted to psEAEN or pseudoerythromycin A hemiketal (psEAHK) by reaction in alkaline solution (Kibwage et al., 1987). By heating erythromycin in sodium hydroxide solution, the lactone is hydrolysed, forming more polar products (Flynn et al., 1954; Waddell and Blizzard, 1992), e.g., the 10,11-dehydroseco acid. Previously, we

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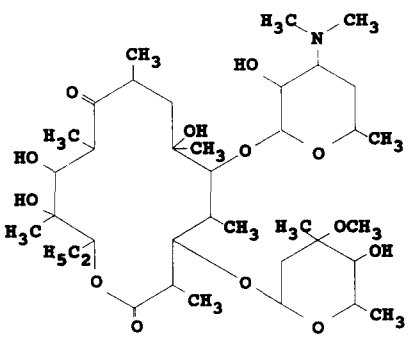
described a liquid chromatography (LC) method for the analysis of erythromycin A and related substances (Paesen et al., 1991, 1993), in which the samples were dissolved in a mixture of methanol and phosphate buffer (1:1). Mixtures of methanol and phosphate buffer were also used as the solvent in this study. In order to monitor the decomposition an LC method, able to separate EA from its degradation products formed in alkaline solution, had to be developed.

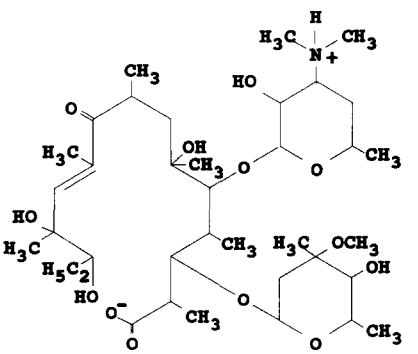
2. Materials and methods

2.1. Reference substances and chemicals

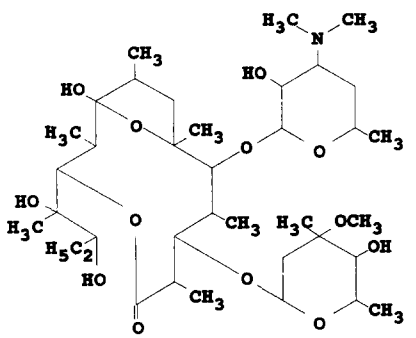
A house standard of erythromycin A (EA-HS) was prepared by five consecutive crystallisations from acetone/water (1:1). EA-HS was assigned to contain 94.7% of erythromycin A (Cachet et al., 1991). House standards for psEAEN and psEAHK were prepared from EA as described (Kibwage et al., 1987). The assigned content was 98.0% for psEAHK and 98.7% for psEAEN. The hydrolysis product of EA (EAHP) was prepared following the method of Waddell and Blizzard (1992) by storing a solution of 11.1 g of commercial erythromycin base in 500 ml of 0.04 N NaOH for 24 h at room temperature. After reaction, the solution was extracted with chloroform (300 ml), the pH was adjusted to 7.0 with 0.1 N HCl and the water was evaporated under reduced pressure. The residue was dissolved in 50 ml of dry ethanol and the solution was again evaporated under reduced pressure. The residue was dissolved in chloroform, the solution was filtered



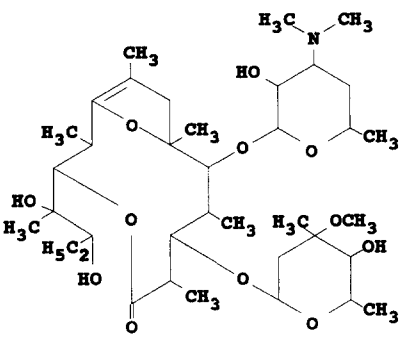
Erythromycin A (EA)



Erythromycin A hydrolysis product (EAHP)



Pseudoerythromycin A hemiketal (psEAHK)



Pseudoerythromycin A enol ether (psEAEN)

Fig. 1. Structures of erythromycin A and its decomposition products.

and the filtrate was evaporated. The powder obtained was dried at 60°C in vacuo over silica gel for 8 h. Titration of the product as an acid gave a content of 97.6% w/w on dry. The structures of EA, psEAEN, psEAHK and EAHP are shown in Fig. 1.

Chemicals used for the preparation of buffers were of analytical grade (Merck, Darmstadt, Germany). Water was distilled twice from glass apparatus. 2-Methyl-2-propanol (Janssen Chimica, Beerse, Belgium) was distilled before use. Acetonitrile, LC grade S, and methanol, LC grade, were from Rathburn (Walkerburn, U.K.). Tetrabutylammonium hydrogen sulphate (TBA) was from Janssen Chimica.

2.2. Preparation of buffers and sample solutions

0.05 M potassium phosphate buffers of pH ranging from 7.5 to 11.0 were prepared by mixing suitable amounts of 0.05 M solutions of potassium dihydrogen phosphate, dipotassium hydrogen phosphate and tripotassium phosphate. EA-HS (3.0 mg/ml) was dissolved in 1:1 mixtures of methanol and 0.05 M potassium phosphate buffer of the desired pH. In some experiments designed to examine the influence of the amount of methanol, ratios other than 1:1 were used. Methanol had to be used as part of the solvent since erythromycin does not dissolve in pure buffer solutions at pH above 7. The sample solutions were filled in glass vials (2 ml), closed with aluminium crimp caps, fitted with a natural rubber disc and a PTFE film. Vials were stored in incubators (20, 40 and 60°C) and withdrawn at appropriate time intervals. Sample vials were briefly stored at −15°C until analysis.

2.3. Chromatographic method

The liquid chromatographic (LC) system was composed of a Milton Roy Minipump (Laboratory Data Control, Riviera Beach, FL, U.S.A.), a Marathon autosampler (Spark Holland, Emmen, The Netherlands) equipped with a 100 μl injection loop, a Merck-Hitachi L-4000 variable UV detector (Darmstadt, Germany) set at 215 nm, and a Hewlett-Packard integrator model 3396 A (Avondale, PA, U.S.A.). The autosampler was connected to an F10C circulator (Julabo, Seelbach, Germany) at 20°C. A 25.0 × 0.46 cm i.d. column was home-packed with PLRP-S 8 μm 1000 Å (Polymer Laboratories, Church Stretton, Shropshire, U.K.). The mobile phase was 2-methyl-2-propanol/acetonitrile/0.2 M potassium phosphate buffer pH 6.5/0.2 M TBA pH 6.5/water (10.5:3:5:5:76.5% v/v). The 0.2 M TBA solution was adjusted to pH 6.5 using 40% m/v sodium hydroxide in water before making up to the final volume. The mobile phase was degassed by ultrasonication. The flow rate was 1.5 ml/min. The column was maintained at 35°C by immersion in a water bath.

3. Results and discussion

3.1. Analytical method

The LC method was derived from a procedure described previously for analysis of erythromycin in bulk (Paesen et al., 1991). Some modifications had to be made. A buffer of pH 6.5 was used instead of that described at pH 9.0, since it allowed better separation between EA and psEAHK. The temperature of the column was reduced from 70 to 35°C, because at pH 6.5 psEAHK is converted on the column into psEAEN at temperatures higher than 35°C. A lower amount of 2-methyl-2-propanol (10.5% instead of 16.5%) was used due to the lower pH of the mobile phase, causing less retention of erythromycin. With a flow rate of 1.5 ml/min the back pressure did not exceed 2000 lb/inch^2. Using the mobile phase described previously (Paesen et al., 1991), EAHP was eluted closely after the solvent peak, which made proper integration difficult. Therefore, TBA was added, to increase the retention of EAHP. TBA had practically no effect on the retention of EA, psEAHK and psEAEN. This effect can be explained by the fact that EAHP forms an ion pair with TBA. Using this LC system, EAHP, EA, psEAHK and psEAEN are sufficiently separated. A typical chromatogram of a solution of EA-HS at pH 8.0, stored at 40°C for 60 h, is shown in Fig. 2.

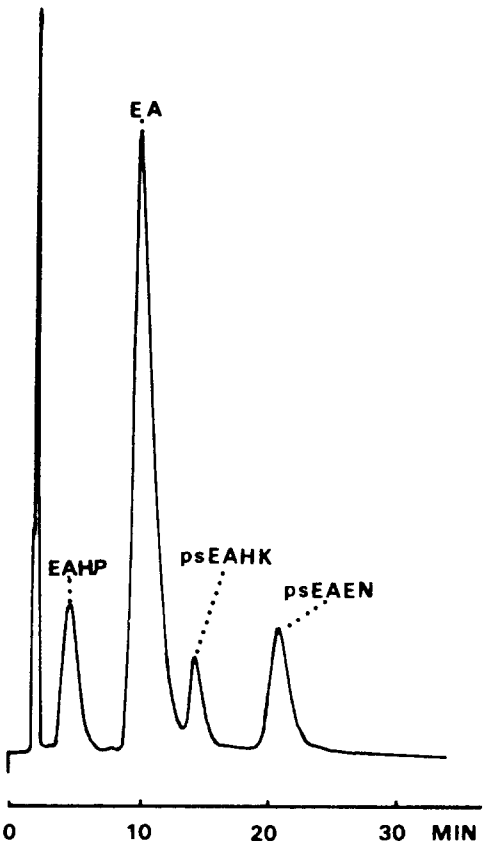


Fig. 2. Typical chromatogram of a solution of erythromycin A at pH 8.0 stored at 40°C for 60 h.

Quantitation of EA and the decomposition products EAHP, psEAHK and psEAEN, was based on peak area measurements. Calibration curves for EA, EAHP, psEAHK and psEAEN were obtained using solutions of the house standards in a 1:1 mixture of methanol/0.05 M potassium phosphate buffer pH 7.0. The relationship between area y and the amount of product injected x (in μg) is given in Table 1, where r is the correlation coefficient, $S_{y,x}$ denoted the standard error of the estimate and MR is the range of injected mass (μg) examined. The total number of analyses per calibration curve was 20. The repeatability of the method, expressed as the relative standard deviation (RSD) on the area of EA for six injections, was 1.0%.

Table 1
Calibration curves for EA, psEAHK, EAHP and psEAEN

	Intercept	Slope	r	$S_{y,x}$	MR (μg)
EA	−445	73	0.9952	291	225–330
EAHP	240	564	0.9984	735	3–60
psEAHK	15	57	0.9977	86	3–60
psEAEN	−97	1135	0.9999	199	1.5–30

$S_{y,x}$, standard error of estimate; MR, range of injected mass examined.

3.2. Concentration-pH profiles

Fig. 3 shows the degradation profiles at 20 and 40°C of EA as a function of time at pH 7.5, 8.0, 9.0, 10.0 and 11.0. Each point of the profile was the mean of two replicate analyses. The profile at pH 7.5 and 20°C was not determined, since

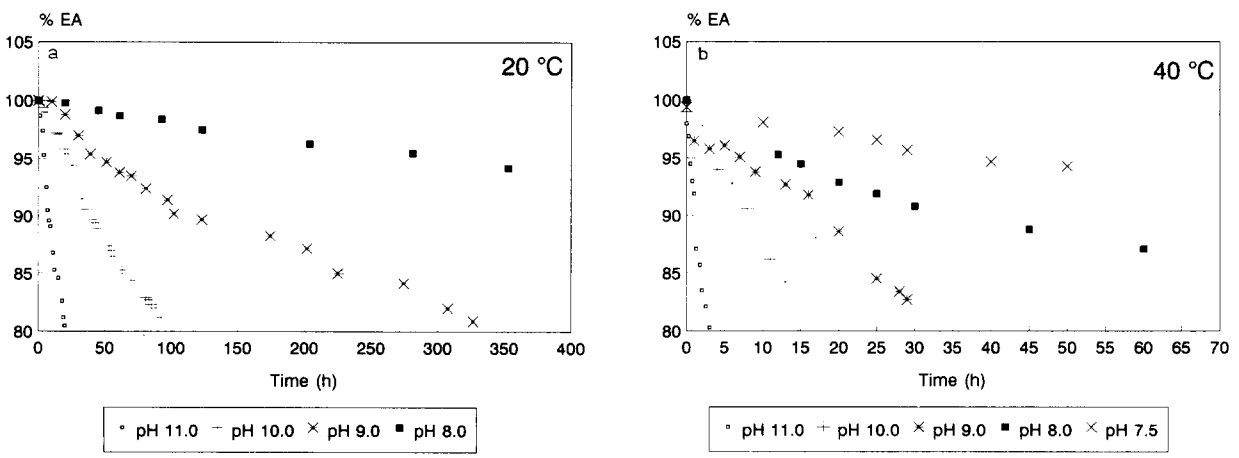


Fig. 3. Concentration-pH profile for EA at (a) 20°C and (b) 40°C.

Table 2
Pseudo first order rate constants (h^{-1}) for the degradation of EA as a function of pH, in 0.05 M potassium phosphate buffer/methanol (1:1) at 20 and 40°C

pH	$k_{20°C}$	$k_{40°C}$
7.5	not determined	$(7.37 \pm 0.83) \times 10^{-4}$
8.0	$(1.61 \pm 0.046) \times 10^{-4}$	$(2.60 \pm 0.33) \times 10^{-3}$
9.0	$(5.76 \pm 0.270) \times 10^{-4}$	$(6.01 \pm 0.48) \times 10^{-3}$
10.0	$(2.16 \pm 0.064) \times 10^{-3}$	$(1.31 \pm 0.04) \times 10^{-2}$
11.0	$(1.07 \pm 0.050) \times 10^{-2}$	$(6.84 \pm 0.36) \times 10^{-2}$
Slope	0.604 ± 0.02	0.508 ± 0.05
Intercept	-8.65 ± 0.05	-6.82 ± 0.15

degradation was very slow under these conditions. The pseudo first order rate constants (h^{-1}) obtained from these experiments are listed in Table 2. In every case, the observed rate constants are lower than those mentioned in the literature (Pluta and Morgan, 1986). However, comparison with those results is difficult, because they were obtained by microbiological assay using very diluted aqueous solutions.

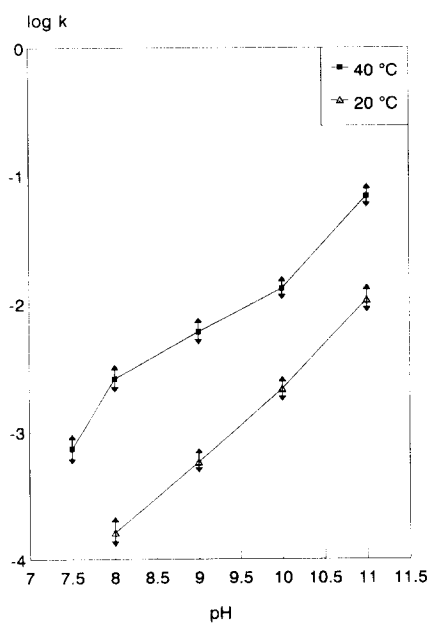


Fig. 4. Log k-pH profile for the degradation of EA in 0.05 M potassium phosphate buffer/methanol (1:1) at 20 and 40°C. Standard deviations on measured values are indicated with arrows.

The log k vs pH graphs at 20 and 40°C are depicted in Fig. 4. The profile in the pH range 8.0–11.0 obtained at 20°C shows good linearity. This indicates the occurrence of base catalysis in the decomposition reaction. Since the slope was not close to +1, there is no specific base catalysis. At 40°C, there is a deviation from linearity at pH 7.5. This can be explained by the fact that at this pH one may expect some influence of acid catalysis in the decomposition reaction. Specific acid catalysis has been reported previously (Cachet et al., 1989).

3.3. Degradation products

In this study the decomposition of EA up to 20% of degradation was monitored. In neutral and alkaline solutions (pH 7.5–11.0) of EA, stored at 20 or 40°C, three main degradation products were detected: EAHP, psEAHK and psEAEN. Fig. 5 shows plots of the formation (%) of degradation products as a function of time. At every pH, psEAHK is the most important degradation product. psEAEN is not detected in solutions of pH > 9.0. EAHP is present in all solutions, but in a smaller amount than psEAHK. The mass balance (i.e., the sum of the percentages of EA, EAHP, psEAHK and psEAEN) was always close to 100%, which indicates that all the degradation products formed were detected.

3.4. Influence of the ionic strength

In order to investigate the influence of the ionic strength (μ) on the decomposition of EA, various amounts of potassium chloride were added to a solution of EA in 0.05 M potassium phosphate buffer pH 11.0/methanol (1:1) stored at 20°C. In the range examined ($\mu = 0.16$–0.26) the ionic strength had a negligible effect on the pseudo first order rate constant: $k = 1.077 \times 10^{-2}$ h^{-1} at $\mu = 0.16$ and $k = 0.953 \times 10^{-2}$ h^{-1} at $\mu = 0.26$.

3.5. Influence of the buffer concentration

The effect of the buffer concentration was examined using 0.05, 0.1 and 0.2 M potassium

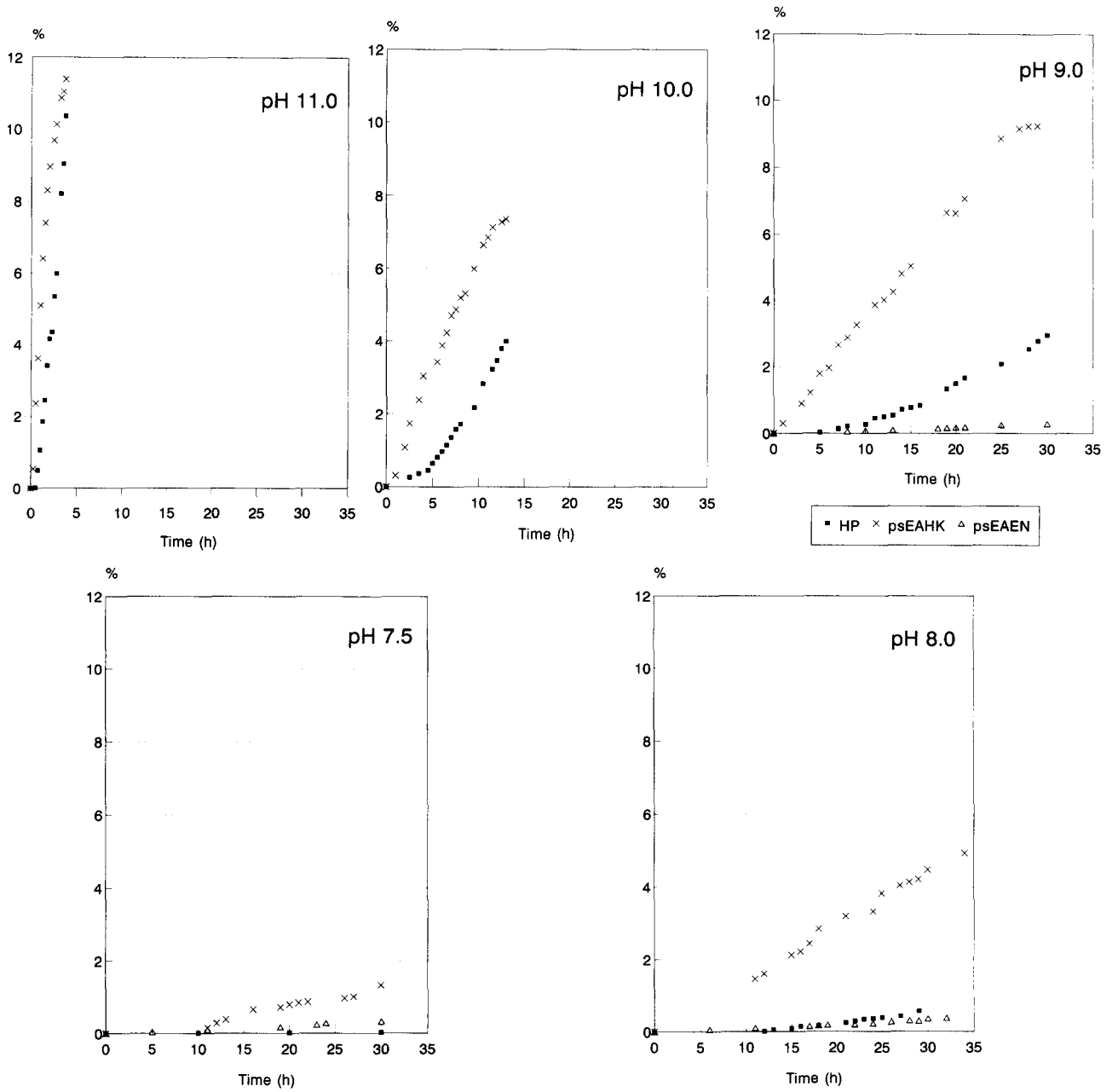


Fig. 5. Plots showing the formation (%) of degradation products as a function of time at 40°C and at different pH values.

phosphate buffer pH 11.0/methanol (1:1) mixtures as the solvent. The relationship between the observed rate constants k (1.077×10^{-2}, 1.411×10^{-2} and 1.483×10^{-2} h^{-1}) and the buffer concentrations (0.05, 0.1 and 0.2 M, respectively), at pH 11.0 and 20°C was not perfectly linear.

Table 3
Pseudo first order rate constant (h^{-1}) for the decomposition of EA as a function of the amount of methanol in the sample solvent at pH 11.0 and 22°C

Ratio methanol/buffer	k ($\times 10^2$) (h^{-1})
2:1	1.996 ± 0.094
1:1	1.996 ± 0.057
1:2	2.077 ± 0.066
1:3	2.120 ± 0.085

3.6. Influence of the amount of methanol in the solvent

Methanol and potassium phosphate buffer pH 11.0 were mixed in ratios of (2:1), (1:1), (1:2) and (1:3) and used as the solvent. The overall buffer concentration was kept constant at 0.1 M. The influence of these different amounts of methanol on the degradation rate was investigated at 22°C. The results, expressed as pseudo first order rate constants k (h^{-1}) are summarized in Table 3. It is clear that the amount of methanol in the solvent does not significantly influence the degradation rate.

3.7. Influence of the temperature

The influence of the temperature was investigated at 20, 40 and 60°C using a solution in 0.05 M potassium phosphate buffer pH 8.0/methanol (1:1). When the natural logarithm of the rate constant k was plotted vs $1/T$, a straight line was obtained. From the slope, the apparent activation energy was calculated, as shown in Table 4. The activation energy at pH 7.0 mentioned in the literature was 77.8 kJ/mol (Connors et al., 1986), determined in pure aqueous medium containing no methanol.

Table 4
Pseudo first order rate constant (h^{-1}) and Arrhenius parameters for the decomposition of EA as a function of temperature, in 0.05 M potassium phosphate buffer pH 8.0/methanol (1:1)

Temperature (°C)	k ($\times 10^4$) (h^{-1})
20	1.61 ± 0.046
40	26.0 ± 3.3
60	190.9 ± 2.4
Slope ($-E_{obs}/R$)	−11675 ± 679
E_{obs} (kJ/mol)	97.1 ± 5.6

4. Conclusion

The analytical method developed separates EA from its decomposition products formed in neutral and alkaline medium. The degradation rate of EA in neutral and alkaline medium increases with pH. The presence of methanol in the solvent does not significantly influence the decomposition rate. The results demonstrate base catalysis of the decomposition reaction in the pH range 8.0–11.0. The decomposition products formed are psEAHK, EAHP and psEAEN.

Acknowledgement

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