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KINETIC DETERMINATION OF CEPHALEXIN IN DRUG FORMULATIONS

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Summary—A kinetic method for the accurate determination of cephalexin has been described. A solution of cephalexin is reacted with $5 \times 10^{-3}M$ cobalt(II) nitrate in $1 \times 10^{-3}M$ sodium hydroxide at 60°C for a fixed time of 6 min, after which the absorbance of the reaction product is measured at 310 nm. The concentration of cephalexin is calculated by using the corresponding calibration equation for the fixed-time method. The method has been applied to proprietary drugs and the results were compared statistically with those given by the BP method. The determination of cephalexin by the fixed-concentration and rate-constant methods is feasible with the calibration equations obtained but the fixed-time method has been found to be more applicable.

Cephalexin is one of the orally active cephalosporins. It exhibits a broad-spectrum of antibiotic activity and may be used to treat a wide range of bacterial infections, particularly sensitive urinary tract infections.[1] Chromatographic methods such as TLC,[2] HPLC,[3–5] are commonly used for its determination, all of which require lengthy treatment and tedious extraction procedures. UV spectrophotometric[6–9] methods have been proposed, some requiring long heating times and others using mercury(II) which is a potential chemical hazard. Many fluorimetric methods[10,11] have been used, not all are specific and require the use of sophisticated equipment (*e.g.* HPLC and GC).

In the method described here, cephalexin monohydrate is assayed kinetically by the fixed-time method in which it is reacted with cobalt nitrate in $1 \times 10^{-3}M$ sodium hydroxide at 60°C for 6 min and the absorbance of the product is measured at 310 nm after cooling. The method has been applied to the determination of cephalexin monohydrate in different proprietary drugs.

EXPERIMENTAL

Apparatus

All spectrophotometric measurements were made on a Perkin Elmer Model 330 Spectrophotometer containing cell programmer using matched sets of LKB 10.00 mm cells throughout.

Reagents

The reagents used were all of analytical or pharmaceutical grade. Working solutions were prepared by appropriate dilution of the following stock solutions.

Cephalexin standard solution, $0.015M$. A 0.0274 g amount of cephalexin monohydrate of drug standard grade, supplied by Chemical Industries Development, Giza, Egypt, was dissolved in about 20 ml of distilled water, stirred for few minutes and diluted to volume in a 100-ml calibrated flask.

Solution from cephalexin tablets. Ten tablets were accurately weighed and then crushed into a fine powder. An amount of this powder equivalent to 500 mg of cephalexin was weighed accurately and dissolved in 50 ml of warm water. The solution was filtered through a Whatman No. 41 filter-paper and then diluted to volume with distilled water in a 100-ml calibrated flask. An aliquot of this solution giving an analyte concentration of 200 μg/ml was transferred into a 50-ml calibrated flask and analysed according to the mentioned procedure.

Cobalt(II) nitrate hexahydrate solution, $0.1M$ was prepared by dissolving 2.9103 g in 100 ml distilled water. Sodium hydroxide ($1 \times 10^{-3}M$) was prepared and used as needed.

Procedure

A 1-ml volume of $0.01M$ sodium hydroxide and 5-ml of $0.05M$ cobalt(II) nitrate solution

were placed in a 10-ml calibrated flask then diluted to the mark with distilled water to give final concentration of $1 \times 10^{-3}M$ NaOH and $5 \times 10^{-3}M$ cobalt(II). Then 3 ml of this mixture was taken and placed in a quartz cuvette (3 ml). The appropriate amount of cephalexin solution was injected into the cuvette (using μl syringe). The cell with its contents was shaken gently and then thermostated at 60°C automatically by the apparatus. The reaction was followed and the absorbance measurements were measured directly at 310 nm. The cephalexin concentration was then calculated by using the corresponding equation for the calibration graph for the fixed-time method. The fixed time at which measurements were made is 6 min. The quartz cuvette used is LKB type of path length 10 mm with maximum volume of 4.2 ml.

RESULTS AND DISCUSSION

Kinetics and optimization

The possibility of the reaction of cobalt(II) nitrate with cephalexin was investigated under various conditions. It was found that the reaction proceeds only in basic media at a relatively elevated temperature. As a result the product species that absorbs at 310 nm was found to depend on the concentration of both reactants, basicity and temperature. Therefore, the effects of these variables were studied.

The reaction rate was found to increase with increasing temperature with a subsequent increase in the slope of the calibration graph (Table 1), indicating higher analytical sensitivity. Above 60°C unwanted chemical changes might occur, such as precipitation, so 60°C was chosen as an adequate temperature.

The influence of pH on the reaction rate was studied between pH 8 and 12.5. It was found that increasing basicity increases the reaction rate with the maximum absorbance being reached in a shorter time. Above pH 11.5 a precipitate was formed. Therefore $1 \times 10^{-3}M$ sodium hydroxide, which corresponds to pH 11, was chosen as the most suitable concentration.

The reaction rate and maximum absorbance also increased with increasing cobalt(II) nitrate concentration, accordingly, higher concentrations are favourable but unwanted chemical changes and the final dilution volume in the flask were considered. As a result, 5 ml of $5 \times 10^{-3}M$ solution in a 10 ml calibrated flask was found to be adequate.

To summarize, the optimum working conditions for the kinetic determination of cephalexin are $1 \times 10^{-3}M$ sodium hydroxide, $5 \times 10^{-3}M$ cobalt(II) nitrate (with a total volume of 3 ml from a mixture solution of hydroxide and cobalt(II)) and heating at 60°C.

The rate of the reaction was also found to be cephalexin dependent. The rates were followed at 60°C with various concentrations of cephalexin in the ranges $1 \times 10^{-4}M$ and $6.25 \times 10^{-6}M$, keeping the other reactants, base and cobalt(II)) constant at the optimum concentrations as above. The graphs shown in Fig. 1 were obtained, from which it is clear that the rate increases as the cephalexin concentration

Table 1. Calibration equations at different fixed times for cephalexin concentration in the range 2.5×10^{-5}–$1.5 \times 10^{-4}M$ keeping NaOH, cobalt(II) nitrate and temperature constant at $1 \times 10^{-3}M$, $5 \times 10^{-3}M$ and 60°C, respectively

Time (*min*)	Calibration equation	Correlation coefficient (r)
2	$A = 0.007 + 0.000864C$	0.968
4	$A = 0.011 + 0.00188C$	0.976
6	$A = 0.025 + 0.00256C$	0.982
8	$A = 0.0705 + 0.00250C$	0.981
10	$A = 0.0912 + 0.00257\ C$	0.980

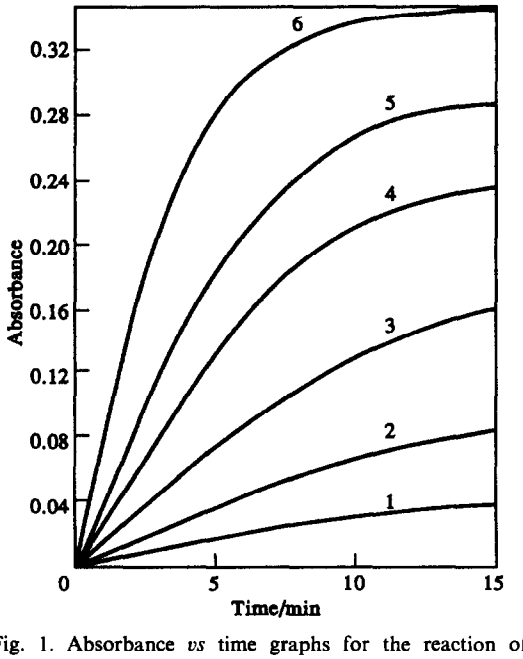


Fig. 1. Absorbance *vs* time graphs for the reaction of cephalexin and cobalt(II) nitrate showing the dependence of the reaction on cephalexin concentration. Concentrations of cephalexin: 1. 6.25×10^{-6}. 2. 1.25×10^{-5}. 3. 2.5×10^{-5}. 4. 5×10^{-5}. 5. 1×10^{-4}. 6. 2×10^{-4}. M.Sod. hydroxide $0.001M$ and Cobalt nitrate $0.005M$. Reactions carried out at 60°C.

increases, following equation.

$$\text{Rate} = k'[\text{cephalexin}]^n \quad (1)$$

where k' is the pseudo-first-order rate constant and n is the order of the reaction.

Figure 1 clearly illustrates that it is a two-step reaction, the first step being fast and the second slow. The latter, being the rate-determining step, could be calculated by the differential initial rate method of measurement[12] as $\Delta A/\Delta T$, where A is absorbance and t is time in sec. Taking logarithms of rates and concentrations (Fig. 2), equation (1) becomes

$$\log(\text{rate}) = \log\frac{\Delta A}{\Delta t} = \log k + n\ \log[\text{cephalexin}]. \quad (2)$$

Regression of log(rate) *vs* log[cephalexin] gave the calibration equation

$$\log(\text{rate}) = -3.70 + 0.95 \log C \quad (3)$$

with a correlation coefficient (r) = 0.992. Hence $k' = 2.02 \times 10^{-4}$/sec and the reaction is first-order ($n \simeq 1$) with respect to cephalexin.

Mechanism

Cephalosporins have been known to undergo remarkably facile cleavage of their β-lactam bonds in aqueous solution.[13] Cephalexin is considered an α-aminobenzyl cephalosporin in which α-aminobenzyl group in the 7-position and unsubstituted methyl group in the 7-position. Therefore, Indelicato *et al.*[14] suggested that the increase in reactivity of cephalexin in the alkaline solution is due to an additional reaction which competes with hydroxide attack on the β-lactam. Furthermore, they suggested

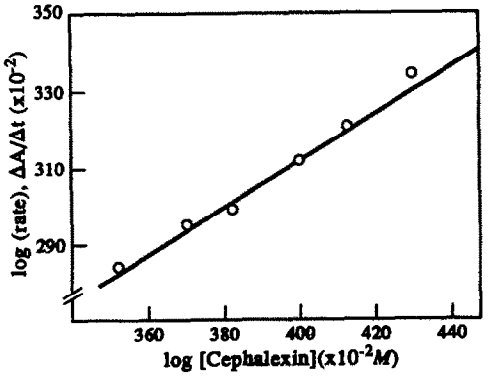
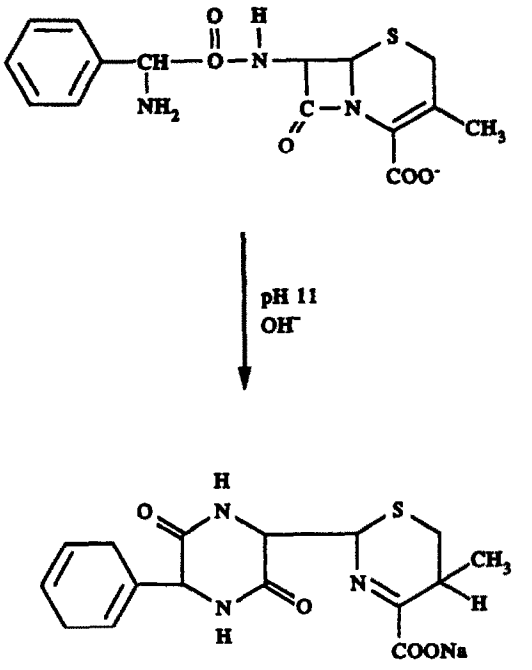


Fig. 2. Logarithms of rates for different concentrations of cephalexin at constant concentrations of $1 \times 10^{-3}M$ NaOH and $5 \times 10^{-3}M$ cobalt(II) nitrate at 60°C.



Diketopiperazine derivative

(I)

Scheme 1. Basic hydrolysis of cephalexin.

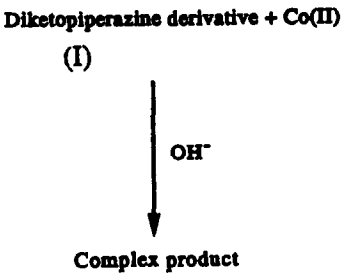
the formation of a 2,5-diketopiperazine(I) derivative as a product of the intramolecular α-amino attack on the β-lactam ring, but they were unsuccessful in attempts to isolate the diketopiperazine derivative from the aqueous medium of the reaction.

From the kinetic data above, the general base catalytic effect on the degradation of cephalexin can be represented by the proposed mechanism as shown in Scheme 1.

Since the reaction is mainly between the cephalexin in the basic medium and Co(II) and to prove that the reaction between cephalexin and Co(II) could be complex-formation reaction, several spectra were recorded (between 380 and 200 nm) of basic solutions which contained cephalexin ($5 \times 10^{-5}M$); cobalt(II) nitrate ($5 \times 10^{-3}M$). The spectra obtained are shown in Figs 3–6. The repetitive scan (6 min/cycle) for the reaction of cephalexin with Co(II) confirms the existence of a product complex which could be shown as in the Scheme (2).

Evaluation of kinetic methods

The determination of cephalexin under the optimum experimental conditions mentioned above, in which the cobalt(II) nitrate and sodium hydroxide concentrations were at least



Scheme 2. Complex-formation reaction between (I) and Co(II).

40 times the initial concentration of the analyte, would result in pseudo-zero-order conditions with respect to their concentrations and the rate will be directly proportional to cephalexin concentration in a pseudo-first-order rate equation as follows:

$$\text{Rate} = k' \text{ [cephalexin]} \qquad (4)$$

where k' is the pseudo-first-order rate constant.

Several experiments were then carried out to obtain cephalexin concentrations from rate data according to equation (4). Initial-rate, rate constant, constant concentration and constant-time

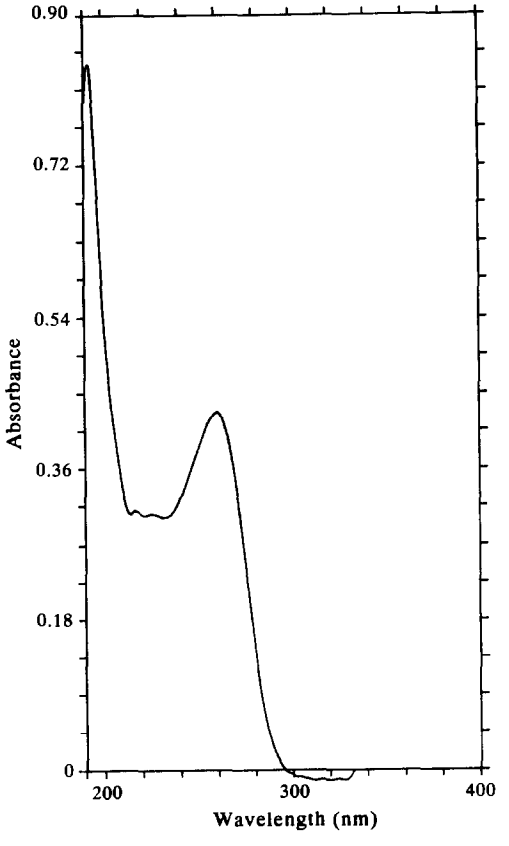


Fig. 3. Absorption curve for cephalexin ($5 \times 10^{-5}M$) in distilled water.

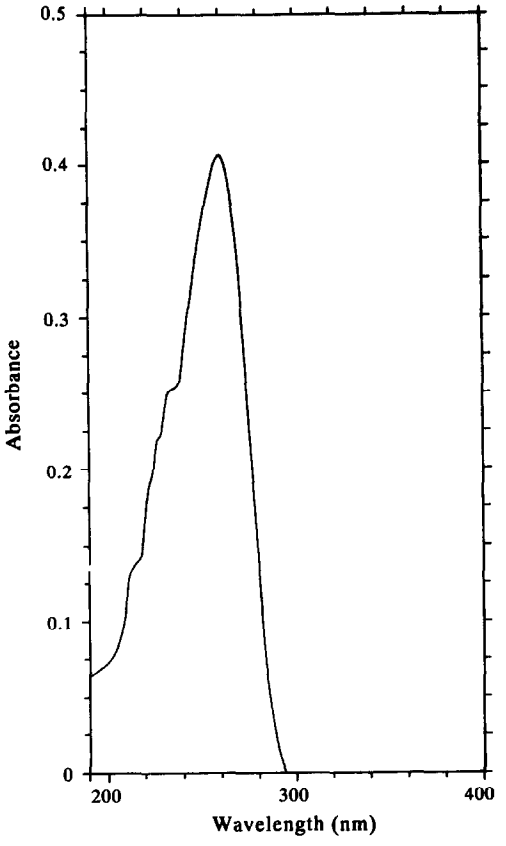


Fig. 4. Absorption curve for cephalexin ($5 \times 10^{-5}M$) with Co(II) ($5 \times 10^{-3}M$) in distilled water.

methods[15,16] were used and the best method was chosen on the basis of applicability, the slope(s) of the calibration graph, intercept and correlation coefficient (r).

Initial-rate method (pseudo-zero-order method)

In this method, graphs of rate (at the beginning of the reaction) *vs* cephalexin concentration were not easy to obtain because the first step of the reaction is not rate determining and is too fast to follow, so tangents to the curves at zero time are not easy to draw. This method was therefore abandoned.

Rate-constant method

Graphs of log(absorbance) *vs* time for cephalexin concentrations in the range 2.5×10^{-5}–$3.0 \times 10^{-4}M$ were plotted by a computer and all were straight lines. Pseudo-first-order rate constants (k') corresponding to different cephalexin concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in Fig. 7. Regression of k' *vs* C gave the equation:

$$k' = 0.00033 - 45.52C \ (r = 0.996)$$

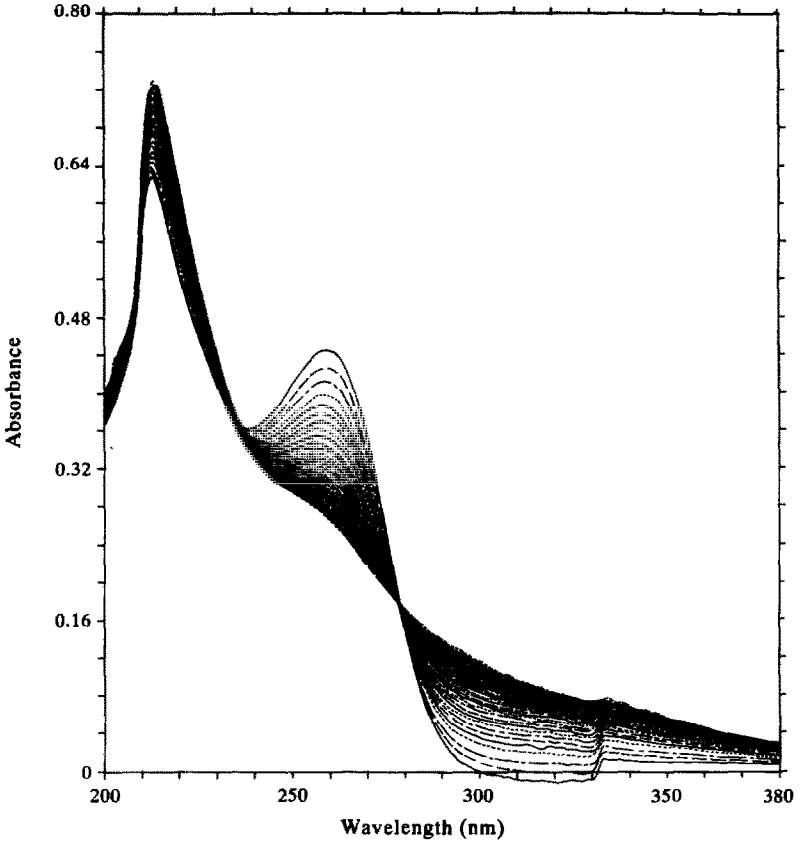


Fig. 5. Absorption curves for cephalexin ($5 \times 10^{-5}M$) with NaOH ($2 \times 10^{-2}M$) only.

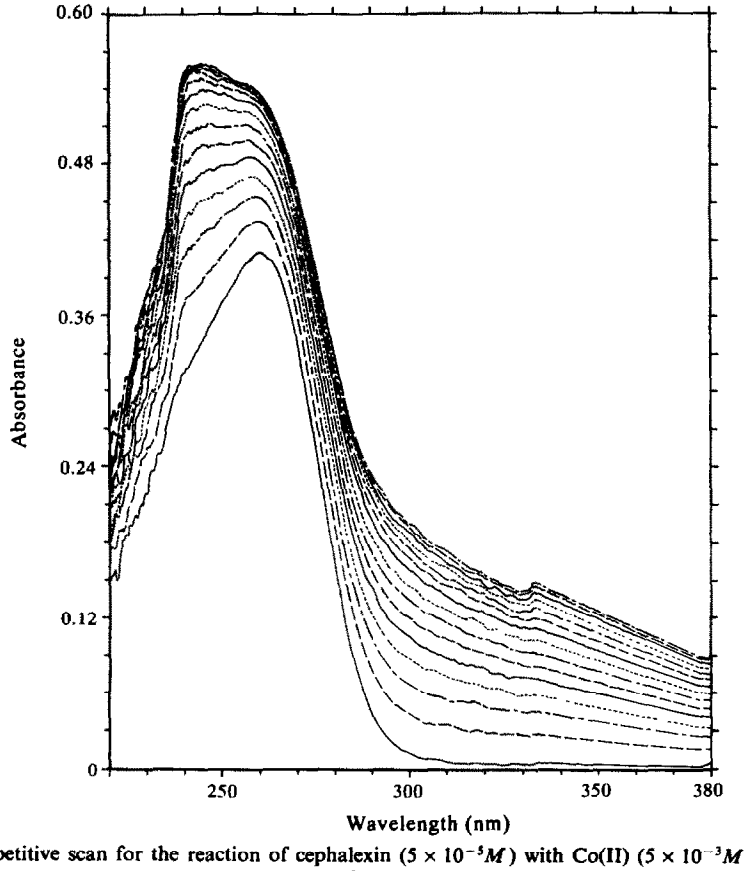


Fig. 6. Repetitive scan for the reaction of cephalexin ($5 \times 10^{-5}M$) with Co(II) ($5 \times 10^{-3}M$) in NaOH ($1 \times 10^{-3}M$) medium.

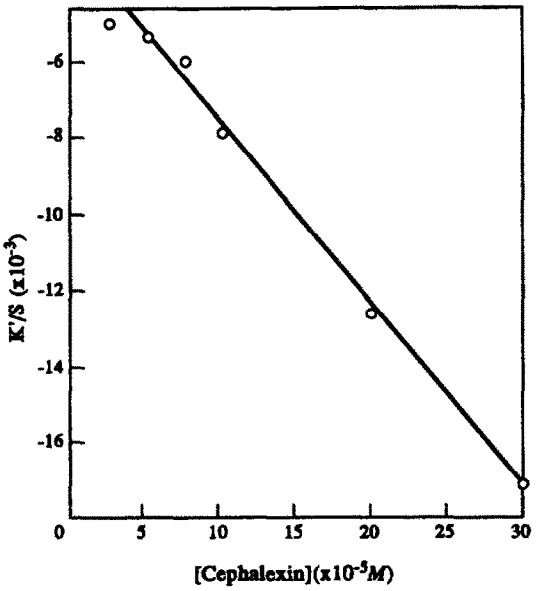


Fig. 7. Values for k' calculated from slopes of log A *vs* t graphs multiplied by -2.303 for different concentrations of cephalexin at constant concentrations of $1 \times 10^{-3}M$ NaOH and $5 \times 10^{-3}M$ cobalt(II) nitrate at 60°C.

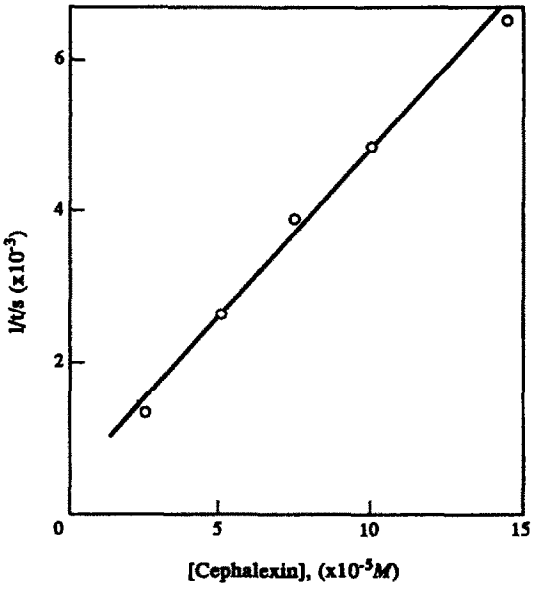


Fig. 8. Values of reciprocal of time taken at fixed absorbance for different rates of variable concentrations of cephalexin at constant concentrations of $1 \times 10^{-3}M$ NaOH and $5 \times 10^{-3}M$ cobalt(II) nitrate at 60°C.

The poor linearity is probably due to changes in the rate constant (k') with the inevitable slight changes in the elevated temperature of the reaction.

Fixed-concentration method

Reaction rates were recorded for different cephalexin concentrations in the range of 15×10^{-5}–$2.5 \times 10^{-5}M$. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time *vs* the initial concentrations of cephalexin (Fig. 8) was plotted and the following equation of the calibration graph was obtained:

$$1/t = 0.00065 + 39.11C \ (r = 0.99)$$

The range of cephalexin concentrations giving the most acceptable calibration graph with the above equation was very limited, which could be a disadvantage.

Fixed-time method

Reaction rates were measured for different concentrations of cephalexin. Calibration graphs of absorbance *vs* initial concentrations of cephalexin were obtained at fixed times of 2, 4, 6, 8, and 10 min with the calibration equations shown in Table 1. It is clear that both the slopes and intercepts increase with time. The best correlation coefficient and more complex formation (indicated by higher absorbance readings as shown in Fig. 1) were obtained for a fixed time of 6 min. Therefore, a fixed time of 6 min was chosen as the most suitable time for measurements.

Applications

The fixed-rate method was applied to the determination of cephalexin in commercially available drug formulations supplied in different dosage forms. The concentration of cephalexin

Table 2. Statistical comparison of the results obtained by the fixed-time method using the equation $A = 0.0670 + 0.000476C$ with those obtained by the official method

Proprietary name	Nominal mass of cephalexin per tablet (*mg*)	Recovery s.d. (%)		
		Kinetic method	Official method	t
Keflex	500	99.7 ± 0.4	99.8 ± 0.2	0.46
	250	100.5 ± 0.9	100.1 ± 0.7	0.16
Cephalexid	250	100.1 ± 0.2	99.9 ± 0.3	1.12

Average of five determinations.

was calculated using the corresponding calibration equation shown in Table 1 at a fixed time of 6 min.

The results obtained for the analysis of the proprietary drugs were compared statistically with those obtained by the official BP[17] method (Table 2). The Student t-test values at the 95% confidence level did not exceed the theoretical value of 2.776, indicating no significant difference between the two methods.

This method is general for all cephalosporins (*e.g.* cephalexin, cephradine, cephaloglycin, . . ., *etc.*) and ampicillin, which contains α-aminobenzyl group in the 7-position and unsubstituted methyl group in the 7-position. But the method is being selective for cephalosporins in the presence of penicillins, because penicillins give different degradation products in alkaline medium.[18]

The procedure described in this paper is simple and does not need the elaborate treatment and tedious extractions required in chromatographic methods.

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