SHIVJI, A.S.H. 1986. *The determination of drug stability by HPLC assay of degradation products*. Robert Gordon's Institute of Technology, PhD thesis. Hosted on OpenAIR [online]. Available from: https://doi.org/10.48526/rgu-wt-1993300

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SHIVJI, A.S.H.

1986

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THE DETERMINATION OF DRUG STABILITY BY HPLC ASSAY OF DEGRADATION PRODUCTS

A thesis submitted to the Council for National Academic Awards as part fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

by

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Abstract

The Determination of Drug Stability by hplc assay of Degredation Products

A. S. H. Shivji

This work evaluates the advantages in drug stability testing of following the decomposition by analysis of decomposition products. Conventional methods of drug stability testing are criticised and the limitations are shown to be largely a result of analysing for undecomposed drug.

Using simulated analytical results incorporating various levels of precision the use of product concentration is shown to be capable of producing conventional rate constants in shorter times by utilising smaller extents of reaction than use of intact drug analysis. The simulation is applied to single, parallel, consecutive and reversible reactions. The findings of the simulated decompositions are supported by practical decomposition studies on several drugs. Hplc with ultraviolet detection is used as a single analytical method for both reactant and product. Aspirin and diiodoaspirin represent single decomposition product systems, tetracycline is examined as a drug decomposition involving parallel, consecutive and reversible reactions. Nafimidone is studied to establish the advantages and limitations of product and reactant measurements where all decomposition products have not been identified. The oxidation of 5-hydroxymethylfurfural is also examined to indicate the usefulness of product analysis in limit testing and in establishing reaction pathways.

In all cases where product identity is known it is shown that the initial rate method employing product concentrations provides more rapid determination of rate constants. It is suggested that reaction order is overemphasized in shelf life determination of drugs and that the initial rate method with analysis of product can minimise and in certain cases eliminate the need for temperature stressing to determine shelf life. Hplc is shown to be very generally applicable in product measurement. New criteria for stability indicating assays which allow use of the initial rate method are demonstrated for the above drugs and for succinylsulphathiazole, diphenhydramine and chloramphenicol. The separations obtained are described in terms of current ion pairing ideas.

DECLARATION

All the experimental work described in this thesis was carried out by A. S. H. Shivji in the laboratories in Robert Gordon's Institute of Technology, Aberdeen in collaboration with Syntex Research Centre, Research Park, Heriot-Watt University, Riccarton, Edinburgh and as acknowledged.

It has not been accepted in substance or concurrently submitted in candidature for any other degree.

ACKNOWLEDGEMENTS

I am deeply indebted to Dr. R. B. Taylor for his invaluable advice, expert guidance and patience during this project. Grateful acknowledgment is expressed to Dr. D. G. Durham for his help and expertise in various organic syntheses as well as his critical assessment of this project. Appreciation is also extended to Dr. K. E. Kendle and Dr. A. Wilson for their assistance in setting up the computer program.

I would like to thank Mrs. Grace Mills and Mrs. Linda Roy of GM Business Centre (Canada) for their skilled secretarial work.

To my Mum and Dad, my wife, Rosemary, and to Catherine and Charles Gray and Karim.

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INTRODUCTION

1. FORWARD

> "Stability can and does mean different things to, different people or to the same people at different times, even those in the pharmaceutical sciences and industry"[1].

In general to most people stability is synonymous with estimation of shelf life. Indeed most stability determinations have this end in view. The vast majority of kinetic data published has been in terms of the measurement of intact drug. Recently however investigations which have intentionally utilised drug decomposition product analysis have appeared in the literature. This has enabled reactions to be followed at extents of decomposition where the concentration of the intact drug shows little measurable change. This is a fundamentally different approach to the determination of drug stability and is a consequence of the emergence of analytical methods capable of measuring quantitively, low concentrations of such decomposition products.

In this introduction the established methods of estimating shelf life are reviewed. The limitations of these classical approaches to stability testing will be discussed and the advantages and disadvantages of the product measurement approach suggested.

While other authors have recommended the product measurement approach, their criteria of assessment is not apparent from the literature.

The purpose of the present work, therefore, is to

evaluate objectively these different approaches to stability determinations both by kinetic treatment of simulated data and experimentally using hplc with ultraviolet detection as the single analytical method. Currently this analytical technique is the method of choice for stability studies due to its specificity with respect to the intact drug. It is a logical extension of the use of this analytical procedure to exploit its advantages in detecting low concentrations of decomposition products with equal specificity and precision.

CHAPTER 1

1.1 Purpose of Stability Testing

The UK (labelling) Regulations[2] require that any drug preparation with a shelf life of less than three years bears an expiry date. The shelf life of a drug is the time from manufacture during which the preparation retains the properties and characteristics similar to those at the time of manufacture[2]. The expiry date required, provides an unequivocal date after which the medicament must be regarded as suspect[2].

While shelf life may, in certain instances, be limited by a maximum concentration of decomposition product which is known to be toxic or by physical or by microbiological considerations[3], the usual criterion for assigning shelf life and an expiry date is the maintenance of potency in terms of integrity of the active ingredient. The maximal acceptable level of decomposition or potency loss is arbitrarily taken as 10%. Such information is required not only on the raw drug material but also on the finished formulations of the drug[3].

In the course of obtaining data leading to the assignment of shelf life and expiry date, information may be obtained on the effect of parameters affecting the decomposition such as temperature, pH, moisture, light and oxygen. This in turn can provide a basis for the selection of storage conditions which will reduce the rate of decomposition of the drug. A tentative mechanism, or at least the type of reaction occurring during

decomposition, can often be determined in the light of such information.

In view of the number of variables affecting drug decomposition, and the necessarily low rates of decomposition, it is apparent that determination of shelf life requires considerable time. It is proposed in the following section to review the methods which are acceptable to the licencing authorities as shelf life determinations and those which have evolved as attempts to reduce the expenditure of time and effort in such determinations.

1.2 Long Term Storage Tests

The classical method of determining the shelf life of a pharmaceutical preparation requires storing the preparation under ambient conditions and observing any changes in the concentration of the intact drug. Such a method necessitates generation of concentration-time data over a period of at least three years[3]. This requirement makes such an investigation not only time consuming but expensive. In addition this technique makes severe demands upon the analytical methodology used. That is, since measurements are required over a long time scale, the analytical method must be capable of yielding the same concentration on an unchanged drug sample over a period of years. On the other hand, the analysis must be sufficiently precise to detect small changes in concentration of the intact drug. This latter point is recognized within the regulations[4] which point out that

for imprecise analytical methods the early results of a long term investigation will be of limited reliability as an indication of the long term stability trends.

With all its limitations, namely time scale, expense and analytical demands, this remains the only accepted method of unequivocally determining shelf life. Complete data need not be available prior to marketing the preparation. If only limited data is available, three months storage being minimum, six or nine months being preferable, a restricted shelf life may be assigned to the preparation. In such cases, however, an assurance must be provided that on-going stability data, particularly under ambient conditions, will be generated until the end of the proposed shelf life or three years, whichever is the shorter. This further data may then be used to support the claimed shelf life and obtain an extension on the restricted shelf life[2-4].

Because of the limitations outlined, much effort has been devoted to the development of "accelerated" methods of studying drug stability. This has previously been aimed at reducing the overall time scale required to obtain an estimate of shelf life. Another consequence of such accelerated methods is the ability to study larger extents of reaction which is less demanding on both the long and short term precision of the analytical method.

The use of such short term decompositions resulting in higher extents of reaction, however, means that the concentration-time dependence of the drug decomposition is much more important than in the case of long term storage.

That is, when a maximum of 10% decomposition is considered, the concentration time relationship is essentially linear.

The short term or accelerated methods have evolved by applying the ideas of classical chemical kinetics. Before reviewing these methods of shelf life determinations, therefore, the nomenclature and essential basics of chemical kinetics are summarized.

1.3 Basic Chemical Kinetics

For a reaction generalized as

$$A + B \longrightarrow C + D \qquad (1\text{-}1)$$

the rate of reaction may be defined by any one of the following quantities,

$$-\,d[A]/dt;\ -\,d[B]/dt;\ d[C]/dt;\ d[D]/dt$$

where [ ] denotes molar concentration.

It is generally accepted that the rate of a chemical reaction is a function of the concentration of the reactant species

$$\text{Rate} = f([A],[B]) \qquad (1\text{-}2)$$

and this function is usually represented as being of the form

$$\text{Rate} = k.[A]^{n}1.\ [B]^{n}2 \qquad (1\text{-}3)$$

where k is referred to a velocity coefficient or, more usually, as a rate constant and n_1 and n_2 are defined as the order of reaction with respect to that particular reactant. While the form of this expression is empirical[5,6] it is extensively used as a convenient method of expressing the concentration dependence of reaction

rates. The n terms, also, are empirical[5,6] and determined purely by the analysis of experimental data.

The value of n obtained for a given reaction is often taken as a characteristic of the reaction and a reaction may be described as n_1 with respect to A, n_2 with respect to B and overall of $(n_1 + n_2)$ order[5-9]. It is less often appreciated that by defining experimental conditions a given reaction may exhibit different orders of reaction. Where the concentration of a reactant does not change appreciably during a rate measurement, by definition, that concentration is a constant and the reaction order zero. Also the terms order and molecularity are occasionally used synonymously[10]. Molecularity signifies the number of molecules of a reactant species involved in a single elementary reaction step[5]. It cannot be deduced directly from experimental measurements to determine the order of a given reaction under a particular set of experimental conditions.

In the present work the concept of order of reaction is of practical use only in obtaining values of the rate of reaction in terms of rate constants when dealing with appreciably different concentrations of reactant. Classically, in determining reaction rate constants, two distinctly different types of methods have been employed:-

(a) Integral Methods: In this method equation (1-3) is integrated and concentration-time data are substituted in the integrated form of the equation to obtain values of the rate constant. It is usual practice, using the integral method, to first determine the appropriate value

of n for a given reactant that will yield constant values of the rate constant. For values of n = 0, 1, 2 equation (1-3) is integrated for the reactant A to provide the familiar integrated rate equations viz.

$$n = 0 \qquad [A]_t = [A]_0 - k_0 t \qquad (1\text{-}4)$$

$$n = 1 \qquad \ln[A]_t = \ln[A]_0 - k_1 t \qquad (1\text{-}5)$$

and $$n = 2 \qquad 1/[A]_t = 1/[A]_0 + k_2 t \qquad (1\text{-}6)$$

where the subscripts of k indicate the order of reaction being assumed in the integration. The appropriate value of n for a given reaction may be determined by substitution of the experimental data in the above relationships and selecting that which yields the best fit. In general, for integral and non-integral values of n, except n=1, the expression

$$kt = 1/(n-1)(1/([A]_t)^{n-1} - 1/([A]_0)^{n-1}) \qquad (1\text{-}7)$$

may be used to establish n and k, again by trial and error.

The integral methods, thus, allow determination of firstly the order of reaction and subsequently the rate constant by direct use of concentration-time data.

This approach is the most extensively used in drug stability testing and variations of this integral method include determination of the half life period for a reaction. It is a prerequisite for the determination of rate constants by such methods that appreciable concentration changes occur over the time scale of the experiment. Such integral methods have been described as

producing a reaction order with respect to time[6,11].

(b) Differential Method : this method of determining the reaction order, known as the van't Hoff method utilises the rate equation in the form shown in equation (1-3). Two main variations of this method have appeared in the classical literature[5-12].

(i) Differentiation of Data from a Single Experiment: in a single kinetic run d[A]/dt is measured at different values of [A] and the order of reaction and rate constant determined using the relationship

$$\ln(d[A]/dt) = \ln k + n.\ln[A] \qquad (1\text{-}8)$$

for a single reactant species. This allows both n and k to be determined without prior assumption, or indeed separate determination as to the correct reaction order. It requires, however, that appreciable changes in both d[A]/dt and [A] are observed. That is, the reaction be studied over large extents of decomposition.

(ii) Initial Rate Method: an alternative procedure for determining the order of reaction by the van't Hoff method consists of measuring the initial reaction rates for different initial reactant concentrations, viz. during any individual experiment the reactant concentration remains essentially constant. The results of such an experimental procedure can be treated using equation (1-8). It has, however, the added advantage that the variation of decomposition product concentration with time rather than reactant concentration can be used to define the rate. This method does have the apparent restriction in that the extent of reaction studied at each initial

reactant concentration should be as small as possible in order to approximate closely to the condition that reactant concentration is constant during any one experiment viz. the term ln[A] in equation (1-8) approximates closely to $\ln[A]_0$.

The order of reaction determined by such differential methods has been distinguished from that obtained by the integral approach and has been referred to as order with respect to concentration[11].

These differential methods while existing in the chemical literature have been little exploited in drug stability studies[13]. Thus the obvious advantage of confining studies of drug decomposition to levels for shelf life criteria have been ignored.

1.4 Short Term Storage Testing

It is known that chemical reactions including drug decomposition reactions can be accelerated by altering the conditions under which the reaction is performed. This process of accelerating drug decomposition, often termed "stressing" may be accomplished depending on the type of reaction, by altering light intensity, moisture content or temperature[14-16]. Of these the most generally applicable parameter for increasing the reaction rate is temperature and indeed temperature is the only variable which can be related quantitatively to the rate constant.

This has afforded an alternative approach to the determination of shelf life at ambient or storage temperature by making suitable concentration-time

measurements at elevated temperatures.

The almost universally used method of expressing the influence of temperature on decomposition rates is the quantitive relationship put forward by Arrhenius.

$$k = Ae^{-E_a/RT} \qquad (1\text{-}9)$$

Where k is the rate constant

R is the gas constant

T is the absolute temperature

A is the pre-exponential factor

and E_a is the activation energy.

The pre-exponential factor is the integration constant. Equation (1-9) may be expressed logarithmically as:

$$\ln k = \ln A - E_a/RT \qquad (1\text{-}10)$$

or

$$\log k = \log A - (E_a/2.303RT)$$

From equation (1-10) a plot of lnk against 1/T should yield a straight line of slope equal to $-E_a/R$ from which the activation energy may be calculated. The activation energy represents the energy the reactant molecules must achieve to initiate the reaction. While such derived activation energies are often reported in the literature in the case of drug stability studies the numerical value is difficult to interpret and is of little physical significance. (see 1.7)

A more accurate equation relating absolute

temperature to the decomposition rate has been described by Benson[17]

$$\ln k = \ln A + (C/R \ln T) - E_a/RT$$

where C is the parameter with dimensions of heat capacity. As stability data is rarely sufficiently accurate to justify the use of this form of the Arrhenius equation the second term on the right hand side is ignored[18].

The Arrhenius equation has been used in two distinct approaches to predict shelf life.

1.5 Isothermal Accelerated Stability Testing

This involves determining the rate of decomposition of a drug in terms of an appropriate rate constant at several constant elevated temperatures. Under such conditions the decomposition is more rapid. Larger extents of reaction leading to more precise rate constants can be determined over relatively shorter time scales. The rate constants obtained in such experiments are fitted to the Arrhenius equation. Adequacy of fit is taken as indicating the validity of extrapolating data obtained at elevated temperatures to ambient or indeed any desired storage temperature. The rate constant so obtained at the desired temperature is then used to calculate the shelf life of the drug.

Such procedures are essentially predictive and rely largely on the application of the ideas of classical chemical kinetics outlined above. That is, a particular order and thus rate equation is assigned to the drug decomposition. The large variation of rate with time

which forms the basis of this method also means that, if a reasonably constant time scale is used for the determination of each rate constant at different temperatures, the extent of decompostion used to determine order and rate constant will vary with temperature. It is known that this affects precision of the derived rate constants[17]. Thus in the statistical treatment of the extrapolation procedure weighting may be used for the different rate constants at elevated temperatures to obtain a reliable rate constant at the desired temperature[12,19-21].

While such isothermal accelerated methods in general require that absolute values of rate constants are obtained, it has also been pointed out that variation of any property which varies linearly with time during the course of a reaction may be used as a measure of the rate of decomposition[16,19]. The temperature dependence of such essentially relative rate constants can in turn be used to determine the activation energy of the reaction and to test the validity of the Arrhenius equation in that situation. Only in the case of first order reactions, however, where the rate constant is dimensionless in terms of concentration, can the use of a physical property directly be used to determine shelf life at storage temperatures.

Thus, while the use of elevated temperatures allows convenient determination of rate constants by producing high levels of decomposition in reasonable time scales and the Arrhenius equation, shelf life at the desired storage

temperature, a considerable amount of experimental data is required. Normally four such rate constants are regarded as the minimum for reliable extrapolation[19,20].

1.6 Non-isothermal Accelerated Stability Testing

A refinement of the above approach is that of non-isothermal stability testing, where the decomposition is monitored simultaneously with the temperature being increased. In principle, using this method, one experiment yields the activation energy and the rate constant for the decomposition reaction at the desired storage temperature thus allowing calculation of shelf life.

Non-isothermal techniques were first applied to pharmaceuticals by Rogers[22]. The method of Rogers involved raising the temperature of a drug under test with time. The programmed temperature change is so arranged that the reciprocal of temperature varies logarithmically with time as expressed by equation (1-11).

$$1/T_0 - 1/T_t = 2.303B.\log(1+t) \qquad (1\text{-}11)$$

where T_0 is the initial absolute temperature of the programme, T_t is the absolute temperature at time t and B is the program constant which enables the length of the experiment and temperature to be chosen at will. Since

$$d(\log k)/d(1/T) = -E_a/2.303R$$

then [23]

$$\log k_t = \log k_0 + (E_a/2.303R)(1/T_0 - 1/T_t)$$

$$= \log k_0 + (E_a.B/R)\log(1+t)$$

therefore

$$k_t = k_0.(1+t)^{E_a.B/R}$$

If first order kinetics are assumed

$$- d[A]/dt = k.[A]$$

and

$$-\int_{[A]_0}^{[A]_t} d[A]/[A] = \int_0^t k.dt$$

therefore

$$2.303\log([A]_0/[A]_t) = k_0\int_0^t (1+t)^{E_a.B/R}\ .dt$$

$$=k_0((1 + t)^{1+E_a.B/R}-1)/(1+E_a.B/R)$$

$$=(k_0(1 + t)^{1+E_a.B/R}.(1-k_0/k_t)^{1+R/E_a.B})/(1+E_a.B/R)$$

therefore

$$\log(2.303\log[A]_0/[A]_t) = \log k_0 - \log(1+E_a.B/R) + (1+E_a.B/R)\log(1+t) + \log(1-(k_0/k_t)^{1+R/E_a.B})$$

In general, for any order of reaction

$$\log f = \log k_0 - \log (1 + (Ea.B/R))$$

$$+ \quad (1 + (Ea.B/R))\log(1 + t)$$

$$+ \log\ (1 - (k_0/k_t)^{1\ +\ R/E_a.B}) \quad (1\text{-}12)$$

where f is the function of the reactant concentration, k_0 is the rate constant at temperature T_0 and k_t the rate constant at T_t.

For zero order kinetics

$$f = [A]_0 - [A]_t$$

For first order kinetics

$$f = 2.303\log([A]_0/[A]_t)$$

For second order kinetics

$$f = (1/[A]_t - 1/[A]_0)$$

or

$$f = (2.303/[A]_0-[B]_0)\log[A]_t/[B]_t+(2.303/[A]_0 -[B]_0)\log[B]_0/[A]_0$$

where $[A_0]$,$[B]_0$ and $[A]_t$,$[B]_t$ are the reactant concentrations at t = 0 and t = t respectively.

Although the final term on the right hand side of equation (1-12) viz. $\log(1-(k_0/k_t)^{1+R/E_a.B})$ varies with time, it rapidly tends to zero as k_t becomes considerably greater than k_0 i.e. at approximately 10^oC above the initial programme temperature $(T_0)^{22}$. After this period, a plot of logf against log(1+t) should yield a straight line of slope equal to $(1 + E_a.B/R)$ and intercept $\log k_0 - \log(1 + E_a.B/R)$. k_0 can therefore be calculated. Using the values of k_0 and E_a so obtained, the rate constant at storage temperature may be calculated.

Certain criteria must be satisfied when applying equation (1-12). The first is that the reaction studied must occur in a homogeneous liquid or gaseous phase[23]. Secondly, the reaction order must be known before the activation energy and k_0 can be calculated. In addition to these requirements, Rogers' method also suffers from further limitations. The main limitation is the assumption that the final term in equation (1-12) rapidly tends to zero so allowing it to be ignored. The validity of the assumption must be established. Also the error in estimating k_0 may be large. The magnitude of this error is dependent on whether or not the errors in estimating the slope and intercept are additive[23]. Finally a linear equation may not be possible where the decomposition is complex, for example reversible reactions[23].

The above technique has been criticised by Tucker[24] who claims that the method yields activation energies higher than the true values and calculated rate constants lower than the true values.

Since the introduction of non-isothermal stability testing to pharmaceuticals a number of workers have attempted to refine and simplify this technique for the purpose of shelf life prediction. Like Rogers, Cole et.al.[25] and Anderson et.al.[26] too have varied the reciprocal of absolute temperature as a logorithmic function of time. Eriksen et.al.[27] have chosen to vary the reciprocal of absolute temperature linearly with time. Linear relationships of time and temperature [28-30] and a stepwise changing temperature profile [31] have also been

used. Other methods [32-34] involve a comparison of model decomposition curves with experimental data utilising computer techniques. An approach based on slope estimation of the concentration-time temperature curves has also been reported[26].

Yang[35] has described a non-isothermal method that is applicable to both linear and non-linear temperature programming with time that requires no initial estimates of kinetic parameters. Tucker et.al.[36] have developed a method capable of simultaneously determining two rate constants and activation energies for the acid solvent catalysed hydrolysis of p-nitrophenylacetate. Volume changes due to the addition of acid, sampling and thermal expansion can all be accounted for. The rapid continuous non-isothermal method of Zoglio et.al.[34] yields all the parameters necessary for shelf life prediction, including the reaction order. The experimental procedure involves increasing the temperature until degradation is rapid enough to proceed at a convenient isothermal rate for a sufficient number of half-lives with adequate analytical sensitivity to allow unambiguous determination of reaction order.

Pharmaceutically the non-isothermal technique has been focused on decomposition in solution[22-38]. It is apparent from the chemical literature that this method is also applicable to decomposition in the solid state[39-43]. In all these cases, however, decomposition has been followed by monitoring the weight loss of the initial sample involving the use of highly sensitive

thermogravimetric balances.

1.7 Limitations of Accelerated Stability Testing

Both the isothermal and non-isothermal stability testing procedures are dependent upon the applicability of the Arrhenius relationship. In spite of the widespread acceptance and use of this relationship, departures from this relationship may be caused by one or more of the following:

(a) Solvent effects: properties of a solvent often change with temperature. If the reaction is dependent on the solutions oxygen content, the reaction rate may be reduced at elevated temperatures because of the decrease in the solvent oxygen content[44].

Preparations stored in poor moisture resistant containers may lose moisture. In addition moisture may redistribute itself between different tablet components. This will occur to differing extents at different temperatures[45].

(b) Mechanistic effects: usually indicate a complex reaction mechanism or a change in the dominant mechanism over the temperature range studied. Fig. 1.1 and 1.2 provide examples of such departures.

Mechanistic effects can have, in certain instances, alternate pathways for the decomposition of a compound. At low temperatures the reaction with a low activation energy should predominate (E_{a1}); at high temperatures the reaction with a high activation energy (E_{a2}) predominates. (Fig. 1.3). The isorate point ($k_1 = k_2$) describes the same rate of decomposition. Some ergot alkaloids

exhibit such behaviour. They undergo complete decomposition within a year above 45°C but below 35°C their rate of decomposition is less than 1% per year[18]. Thus, unless the temperature chosen for the study are well removed from the isorate point a constant activation energy is unlikely[18].

(c) Formulation Alterations: in addition to the various changes in physical characteristics, elevated temperatures may, for example, induce changes in reaction mechanism dependent upon excipients used or possibly the manufacturing process. Phenylbutazone has been shown to exhibit different mechanisms of decomposition in a number of tablet formulations above 50°C. For this reason it has been recommended that accelerated studies aimed at estimating the shelf life of phenylbutazone tablets should not exceed 50°C[45].

Deviations from the Arrhenius relationship are characterized by curvatures in the Arrhenius plot. Whilst such Arrhenius plots provide information of limited quantitative value, they may provide useful qualitative information. From Fig. 1.3 it is obvious that the departure is due to the complexity of the decomposition mechanism. However often curvatures of this kind are erroneously explained as being due to the wrong choice of reaction order[18].

In addition to the limitations of the Arrhenius relationship accelerated stability studies do have additional limitations:

(i) The predicted shelf life of preparation will only

be valid if the accelerated test is carried out on the final packaged product. This is an essential prerequisite because the pathway or rate of decomposition may depend in some instances upon the containers, e.g. an air-tight container will reduce oxidation.

(ii) The nature of the formulation will dictate the highest temperature that can be used.

(iii) The accelerated testing approach can only be applied to those forms of decomposition which increase with increase in temperature.

(iv) The reaction order must be the same at storage and elevated temperatures.

(v) It may not be possible to extend the prediction to all climatic conditions, especially those encountered in tropical regions.

Accelerated stability testing should, therefore, be viewed in the light of the applicability of the Arrhenius Equation and the above limitations.

1.8 Implications of Reaction Order in Stability Testing

In any accelerated stability study the determination of the reaction order is always attempted. It has been suggested, that where the reaction order cannot be easily determined, a particular order be assumed[46-50]. It has been claimed that assuming the reaction follows first order kinetics will result in minimal errors in the rate constant[46]. Kennon[49] has shown that by ensuring excessive concentrations of all other species present, a second order reaction can be made to approximate first or pseudo-

first order kinetics for approximately 50% decomposition. Woolfe et.al.[47] favour the assumption of first order kinetics for a number of reasons:

(a) Application of first order kinetics to decompositions of a higher reaction order will result in a more favourable shelf life.

(b) The ratio between drug and excipient is such that pseudo-first order conditions would be expected.

(c) Stability predictions are little affected by applying first order kinetics to a zero order decomposition.

(d) Most decompositions in solution follow first order kinetics.

(e) Solid systems that have been studied exhibit first order kinetics[51-56].

(f) Many solid systems decompose topochemically following pseudo-first order kinetics[51].

Some of the above reasons are questionable. Firstly, pharmaceuticals rarely exhibit reaction orders greater than one. Secondly, application of first order kinetics to a reaction of zero order will result in a less conservative estimate of shelf life as is obvious from the examples quoted by these authors[47]. Finally, solid systems exhibiting zero order kinetics have been reported[54] as have those where the actual reaction order could not be determined[53,55-59].

In contrast to the above suggestions, it has been recommended that zero order kinetics be assumed to allow a more conservative estimate of shelf life when the actual

kinetic order is not known[48,60]. It has been shown that the application of first order kinetics to a zero order decomposition results in an over-estimation of activation energy and hence shelf life. The over-estimate in shelf life increases with both increasing activation energy and lowering of storage temperature. The converse is also true in which case both activation energy and shelf life are underestimated. These findings have led Yang[48] to recommend the assumption of zero order kinetics to allow a conservative estimate of shelf life.

It is apparent from the above that the prerequisite of determining the order of a drug decomposition according to the classical chemical principles is not always easily achieved. This has led to differing views being expressed as regards the most useful assumption of reaction order with a view to determining shelf life. It is also evident that, where the reaction order is established for a drug decomposition, it is obtained in the vast majority of cases by integral methods at elevated temperatures[61-63]. Large extents of reaction are measured which are unrealistic in terms of the extent of reaction allowed in the prediction of shelf life. In the light of these facts the relationship between the order of reaction and extent of decomposition is considered in more detail.

1.9 Relationships among Order of Reaction and Extent of Reaction

It is general practice, when studying drug decomposition for stability purposes, to follow the

reaction over appreciable extents of reaction. Various workers have recommended 50-75% decomposition[12,18,23,64-68]. The reasons for this are two fold:

(i) A more precise rate constant is obtained when the concentration variable undergoes large changes during the experimental measurement. This is as a result of the inherent finite precision of any analytical method used to follow a drug decomposition. This will be discussed fully below (section 1.15) concerning the choice to be made between analysis of reactant or product species.

(ii) It is well known that, experimentally, it is difficult to distinguish between zero, first and second order reactions, using integral methods at extents of decomposition below 25%[46-48,68,69]. That is, at such low extents of reaction the concentration-time curves for these various reaction orders will be indistinguishable. Thus it can also be argued that the reason for following reactions to levels of decomposition well beyond that occurring during its shelf life is to determine the reaction order. The purpose of determining the order is to allow the use of an appropriate integrated equation in accelerated stability testing.

As discussed above it may not always be possible to determine an order of reaction[57-59] and it may be adequate to assign an arbitrary reaction order, very often first order to establish shelf life. This difficulty arises mainly as a result of the very extensive use of integral methods in isothermal accelerated testing and the exclusive use of integral methods in non-isothermal

techniques.

The differential methods do not require large extent of reaction and are much more closely related to the real situation of shelf life determination. However, this approach has been almost completely neglected in the field of stability testing. As will be suggested below, this has been as a result of analytical limitations. Given the analytical capability of determining the initial rate of drug decomposition to the required precision, the initial rate method, as described in section 1.3, can be used to determine the order of reaction without recourse to large extents of reaction. In addition it can be readily shown that knowing the initial reaction rate and starting reactant concentration, the conventional rate constant in appropriate units can be calculated for integral and zero orders of reaction i.e. considering any drug decomposition

$$A \longrightarrow \text{products}$$

For zero order kinetics

$$\text{Rate} = -d[A]/dt = k_0[A]^0$$

Integration yields

$$[A]_t = [A]_0 - k_0 t \qquad \text{(i)}$$

On differentiation (i) yields

$$-d[A]/dt = k_0 \qquad (1\text{-}13)$$

where the units of the apparent zero order rate constant are concentration.time $^{-1}$

For first order kinetics

$$\text{Rate} = -d[A]/dt = k_1[A]^1$$

Integration yields

$$[A]_t = [A]_0 e^{-k_1 t} \qquad (ii)$$

On differentiation (ii) yields

$$-d[A]_t/dt = [A]_0 k_1 e^{-k_1 t} \qquad (1\text{-}14)$$

In this case the units of the apparent first order rate constant are time^{-1}

For second order kinetics

$$\text{Rate} = -d[A]_t/dt = k_2[A]^2$$

Integration yields

$$1/[A]_t - 1/[A]_0 = k_2 t$$

rearranging

$$[A]_t = (1/[A]_0 + k_2t)^{-1} \qquad \text{(iii)}$$

Differentiating (iii) yields

$$-d[A]_t/dt = k_2/(1/[A]_0 + k_2t)^2 \qquad \text{(1-15)}$$

where the units of the apparent second order rate constant are concentration^{-1}time^{-1}.

At low extents of decomposition, a single value of $-d[A]_t/dt$ will be obtained whether the particular reaction being studied is zero, first or second order as conventionally defined. That is under these conditions there is no meaning to the term order of reaction. The right hand side of equations (1-13) to (1-15) can be set equal and simplified at the condition of low extents of reaction i.e.

$$k_0 = k_1[A]_0e^{-k_1t} = k_2/(1/[A]_0 + k_2t)^2$$

Under these conditions t tends to zero as does k_2t and

$$k_0 = k_1[A]_0 = k_2[A]_0^2 \qquad \text{(1-16)}$$

Thus by measuring experimentally the initial rate of decomposition of a drug and knowing the order of reaction with respect to concentration from separate measurements or by assumption, conventional zero, first or second order

rate constant values can be determined if the initial concentration of the drug is known.

Similar reasoning has been applied by Carstensen et.al.[70] using series expansion to simplify the rigorous expressions for the different orders of reaction. In the case of these workers the purpose in determining the simple relationship shown in equation (1-16) was to show that at the limiting situation of low extents of reaction zero and first orders were indistinguishable.

1.10 Summary of Disadvantages of Current Methods of Determining Shelf Life

It appears from the above assessment of current pharmaceutical approaches to determining shelf life of drugs that the methods in use suffer several disadvantages:

1. The legally accepted method of long term storage is very time consuming as well as placing very stringent demands upon the analytical techniques used in terms of long and short term precision in the determination of concentration.

2. The isothermal and non-isothermal accelerated methods rely heavily on the ideas of classical chemical kinetics requiring the determination or assumption of a certain reaction order.

3. Both these methods utilize the Arrhenius relationship in a predictive capacity and instances have been cited where the validity of this equation over the temperature range studied is questionable.

4. The accelerated methods have concentrated on the integral approach of determining reaction order and rate constants. Large extents of reaction have had to be studied and this represents an unrealistic situation with respect to the purpose of the method namely to establish the time for 10% decomposition.

1.11 A Possible Alternative Approach to Determining Shelf Life

The differential methods of determining reaction order and rate constants, although amply documented in the chemical literature, are not generally applied in the field of drug stability. Very few reports of the use of such methods appear in the pharmaceutical literature.

Direct differentiation offers little advantage over the integral methods since the reaction must still be studied over a wide change in drug concentration to obtain order and rate constant. The initial rate method, however, potentially offers a much more realistic method of determining drug stability data. That is, using this approach of measuring the initial rate of a decomposition at different initial concentrations, the order of reaction with respect to concentration can be measured without exceeding the realistic levels of decomposition to be expected during the shelf life of a drug. Also, once the reaction order has been established the conventional zero, first or second order rate constant, as appropriate, may be calculated. This approach can be combined with isothermal accelerated testing procedures with advantage

since considerably less time would be required at each elevated temperature. Non-isothermal methods could not be utilized, however, since this approach is dependent on the use of integrated forms of the rate equation.

The classical initial rate method, however, suffers from the same analytical disadvantages described in the use of long term stability studies, namely, high precision is required to measure relatively small changes in concentration required to fulfill the conditions of essentially constant reactant concentration. This disadvantage is however purely experimental and arises because of the almost universal approach to stability analysis of measuring reactant concentration. This and alternative approaches will be discussed in the following section.

1.12 Analytical Requirements

As has been indicated in previous sections a number of procedures are available for the determination of shelf life once reliable concentration-time data for a drug decomposition are available. An indispensable part of any stability study, however, is the developement of an adequate analytical method of following the course of the reaction.

The importance of assay methods used in stability testing is well appreciated and has been the subject of at least four major literature reviews[1,71-73].

The term stability indicating assay has been coined. Such an analytical method is defined as

"A procedure that affords specific determination of a drug substance in the presence of its decomposition products "[71].

This definition emphasizes the need for unique quantitation of the drug i.e. free from interference by decomposition products which must necessarily be present. It may be significant that such a widely accepted definition makes no reference to the requirements of precision. Such precision must be present particularly for the purposes of long term stability testing[4] although less necessary for accelerated methods involving large extents of decomposition.

The main criterion for a stability indicating assay namely that of specificity, however, was not always realized in early drug stability work. This was, in part, a result of the inadequacy of available analytical methods coupled with the molecular complexity of many drug molecules and the diverse routes by which they may degrade.

Numerous types of analytical techniques have been employed in the field of drug stability work ranging from titrimetric to biological methods[1]. Two main groups of methods can, however, be identified. In early work spectrophotometric and colourimetric methods featured prominently in the literature while the later emergence of various chromotographic techniques have resulted in the utilization of these methods to attain the desired specificity.

1.13 Spectrophotometric and Colourimetric Methods

Methods of assaying drug decomposition which depend on ultraviolet spectrophotometry have been very extensively used. In part this was due to the speed of the analytical measurement which is an obvious advantage in time dependent studies. Also the fact that so many drug molecules contain chromophores causing absorption in this region of the spectrum means that the method can be applied to a wide range of drug types.

Unfortunately direct ultraviolet spectrophotometry is a relatively non-specific method for stability determining purposes due to the fact that decomposition products often contain the same or similar chromophores. In the case of the extensively studied acetylsalicylic acid decomposition such interference can be overcome by judicious choice of wavelength of measurement[74]. The literature does, however, contain several examples where the assay methods have been demonstrated to be non-specific.

The decomposition of chloromphenicol (Scheme 1.1) for instance is complex. Assays of chloramphenicol based on ultraviolet absorption are non-specific[18] particularly in decomposition studies since the decomposition products have similar absorption properties. Schwarn[75] has criticised this method. The inadequacy of the method has been demonstrated by Higuchi et.al.[76-79] by employing a separation stage for the intact chloramphenicol using thin layer chromatography prior to analysis by ultraviolet absorption.

Extraction methods have also been used to impart specificity to stability assays based on spectrophotometry. The hydrolysis of atropine (Scheme 1.2) has been studied by extracting the intact atropine from alkaline solution into an organic solvent followed by back extraction into dilute acid solution. The resultant solution is assayed spectrophotometrically[80,81]. This procedure, while appearing satisfactory suffers from the drawback that, under these conditions atropine is converted to apoatropine (Scheme 1.2) by dehydration of the primary alcohol group in the tropic acid residue. This compound is extracted and back extracted with the atropine and has a high absorptivity at the wavelength used for atropine resulting in over estimates of intact atropine[82]. Thus such an assay procedure, designed to yield higher specificity, in practice, by allowing additional decomposition, incurs separate sources of non-specificity.

Colorimetric methods have also been employed in stability determinations either to confer absorption properties on poorly absorbing drug species or to confer specificity in a decomposition system. One such method involves the reaction of acyl derivatives with hydroxylamine to form hydroxamic acid derivatives. The latter forms coloured complexes with ferric salts.

The decomposition of pilocarpine (Scheme 1.3) has been studied in this way[83-87]. Subsequent measurement[88,89], however, have shown that isopilocarpine is also formed during the reaction indicating that the

derivatisation procedure is inadequate in terms of specificity of measurement.

While ultraviolet spectrophotometry has been the most extensively used spectroscopic technique for the study of stability, others have been used. Fluorescence methods[90,91] can impart useful additional specificity and nuclear magnetic resonance spectroscopy[92,93] confers a high degree of specificity under suitable conditions.

1.14 Chromatographic Methods

With the advent of chromatographic techniques in analysis it has become possible to design assays for drug stability studies having inherently higher specificity than that afforded by ultraviolet absorption methods.

Paper chromatography has been used in stability studies on tetracycline[94] and chlorpromazine[95]. The more rapid and highly resolving thin layer methods were a somewhat later development and have been very extensively used. Examples include tetracycline[96], atropine sulphate[97], androgenic hormones[98] and chloramphenicol[99]. These chromatographic methods together with classical or open column chromatography suffer however from the major disadvantage for stability work, in that, quantitation is poor. Direct quantitation using some property of sample spot is usually subjective and imprecise[100]. For quantitative stability work such methods are usually accepted as representing refined separation stages. That is, they impart high specificity in conjunction with ultraviolet spectrophotometry. Such combinations of

techniques, in general leads to increased analysis time which is an obvious disadvantage in kinetic investigations. Also, variable levels of precision are encountered due to the transfer stages involved.

Such chromatographic methods have, however, contributed greatly to the overall field of stability study by allowing elucidation of products formed during the reaction. Thin layer methods in particular, have achieved a permanent place in the wider context of stability by allowing ready detection of low levels of impurity produced during a drug decomposition[101,102].

Gas liquid chromatography while representing an advance on previous chromatographic techniques in terms of speed, resolution and the integral ability to quantitate molecular species, has been, in practice, relatively little used in drug stability studies. This is undoubtedly due to the higher temperatures required for the vaporization of most drugs with the consequent risk of decomposition. For non-volatile compounds derivatisation may be used to achieve volatility. Derivatisation however, needs to be carried out on a quantitative basis. A few examples however appear in the literature of stability studies utilizing gas liquid chromatography as the analytical method. These include promazine[103] and meprobamate[104].

The relatively modern chromatographic method of high pressure liquid chromatography (hplc) has, to a very considerable extent, overcome the inadequacies of previous analytical methods for the purpose of stability testing.

This is as a result of combining the separation or resolving ability of thin layer or gas liquid chromatography with the generality and precision of quantitation associated with spectrophotometric measurements. In the field of drug analysis in general and the area of stability testing in particular it can be argued that hplc is the analytical method of choice. This contention is supported by the exponential rise in the number of publications appearing in the pharmaceutical and biomedical literature utilising this method[105].

Martin and Synge[106] in 1941 suggested the possibility of using very small particle size stationary phase and high pressure differential to obtain improved chromatographic resolution. It was not until the late 1960s' that Horvath[107], Kirkland[108] and Huber[109] independently built the first liquid chromatographs and it was considerably later in the early 1970s' that commercial equipment became readily available.

Initially separations were obtained by applying the ideas of thin layer chromatography using uncoated silica as adsorbent or silica coated with an additional liquid layer as a medium for partition chromatography. A major advance was the production of chemically bonded silica stationary phases usually incorporating alkyl groups of various chain lengths by Halasz[110]. These chromatographic systems termed reverse phase and utilizing mixed organic-aqueous mobile phases were quickly appreciated as being very applicable to the analysis of drugs in formulations and in biological systems. The usefulness of the

developing hplc method was enhanced by the almost universal use of ultraviolet detection systems. This allowed work on drug investigation to exploit the wealth of information available from direct ultraviolet spectrophotometry. It has been possible to analyse a large number of drug compounds by hplc with a high degree of specificity and with a precision approaching that of direct spectrophotometric methods.

A limitation in drug analysis existed in the use of highly ionic type drugs, in particular those with predominently basic character. It was found difficult to control retention and also to obtain resolution of such compounds comparable with that obtained for neutral drugs. The emergence of ion-pairing techniques in particular those involving hydrophobic pairing ions have made considerable advances in overcoming these difficulties[111].

Although the exact mechanisms operating in liquid chromatography are still the subject of considerable work, particularly in the case of ion-pairing systems, it is now possible to attempt development of stability indicating assays for drug decomposition.

In view of the widely accepted advantages of hplc together with the limitations of previously used analytical methods it is understandable that the literature contains many stability investigations which re-examine drug decomposition systems with improved specificity[112-120]. The numerous reviews appearing in the literature are indicative of the importance of this technique in pharmaceutical analysis[121-133]. In addition,

novel investigations of drug decompositions both in academic research and industrial development are by choice attempted using this technique[134].

1.15 Choice of Chemical Species to be Monitored for Stability Studies

A study of assay methods appearing in the literature shows that the species most often measured in stability studies is the parent drug[135-140] rather than the decomposition product. In other words, it is the concentration of the intact drug that is used as a measure of decomposition. It may be that this represents the most pragmatic approach, since the primary purpose of determining shelf life is to ensure maintenance of potency. Another factor may be that the integrated rate equations so extensively used in accelerated stability testing are expressed in terms of unreacted drug. It has been indicated in section 1.3 that the initial rate method offers some advantages over integral methods in determining shelf life. Thus the decision on which kinetic approach to adopt is associated with the feasibility of analysis of reactant compared with decomposition product.

A number of authors have suggested that monitoring the decomposition product(s) is a better approach to stability studies[16,19,65,71,141,142]. In most cases such comments are unsubstantiated. The utility of this approach is dependent on the reaction being sufficiently characterised to assure that the quantity of decomposition

product determined can be properly related to the extent of drug decomposition. Carstensen[141] has stressed the importance of following the increase in decomposition products rather than evaluating the decrease in intact drug concentration for short term stability studies. Hudson[65] makes the point that the product measurement approach is more sensitive and can also be applied to those situations where the decomposition products have not been identified. It has also been suggested, that this method is advantageous in those situations where there is little drug decomposition[12] or where accelerating the decomposition by increasing temperature is not practicable. In such instances, monitoring the decomposition product allows determination of the initial rate of decomposition.

Contrary to the above suggestions, other workers[47] have criticised such an approach, arguing that the measurement of the appearance of decomposition products should be avoided on the basis that many drugs decompose by more than one route and many decomposition products undergo further decomposition. They emphasize the need for analytical specificity in the intact drug method. They do, however, emphasize the need to measure the rate of formation of any decomposition product that has a greater toxicity than the pure drug.

Even in the light of such controversies, as to which chemical species to monitor, a number of examples are available in the literature where decomposition products have been measured[142-146]. Such examples include

dexoxadrol[142] and hydrochlorthiazide[144]. In both these cases the authors have measured the decomposition products not through choice, but the lack of it. The decomposition products afford better analytical characteristics. The remaining intact drug concentration has been determined from such decomposition product data and the conventional kinetic methodology applied. Some other examples exist, however, where the decomposition product has been measured through choice, for example morphine[145] and noxythiolin[146]. It is perhaps significant that these represent recent investigations.

1.16 Purpose and Organisation of Present Work

It is the purpose of the present work to examine the initial rate method using decomposition product concentration-time data and to compare this with the integral method as conventionally applied using reactant concentration-time data.

Included in such a comparison will be the relative precisions of the resulting rate constants, the time or extent of reaction required, the applicability of the different methods to different types of drug decomposition and the experimental feasibility of obtaining adequate analysis. The implications of the initial rate approach using analysis of the decomposition product in the related areas of reaction mechanism and the determination of levels of impurity arising from decomposition will also be considered.

In order to evaluate the initial rate method three

main aspects will be considered. Firstly, in Chapter 2, a comparison will be made of the rate constants obtained by the initial rate method and integral method using simulated concentration-time data. Use of such data will enable more general conclusions to be reached as to the kinetic merits of the two methods. It appears that current workers are applying the initial rate method to more complex reactions than simple decompositions involving only one product. It is generally accepted, certainly in the chemical literature, that product or reactant can equally well be measured to determine rate constants in simple decompositions. It is not clear, however, how useful the initial rate method is in the commonly encountered systems of parallel and consecutive reactions frequently experienced in drug stability work. Simulated data having an in-built level of variance such as would be encountered in experimental measurements, will allow rapid examination of such systems in general without the need for identifying such systems and for the demanding quantitative analysis required to obtain such data practically. This chapter will allow the effect of precision of analysis, time and extent of reaction to be considered.

Chapters 3-7 are concerned with the practical stability determinations of different drug decompositions to determine if the conclusions of Chapter 2 are borne out in practice in as far as they affect the particular decomposition when a common analytical method is applied.

In Chapter 3 the decomposition of aspirin is studied

as an example of a single decomposition product system because of the extensive literature which exists on the stability of this drug. Such a system is further examined in Chapter 4 using the di-iodo analogue of aspirin mainly because of current interest in this compound in the treatment of sickle cell anemia[147] since no stability data could be located in the literature.

In Chapter 5 the initial rate method is applied to the acid decomposition of tetracycline. This represents a decomposition system embodying all the possible complexities of drug decomposition viz. parallel, consecutive and reversible reaction types.

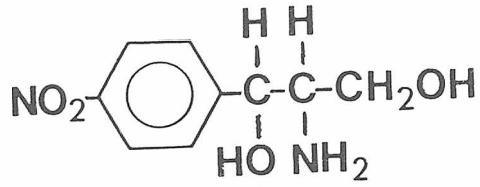
In the above examples, the main decomposition products and established reaction pathways are known with a fair degree of certainty. In Chapter 6 this information obtainable from the alternative methods is assessed by the study of the decomposition of Nafimidone (1-(2-naphthoylmethyl)imidazole hydrochloride) a potential drug compound kindly made available by Syntex Research Centre. This chapter indicates the difficulties and limitations encountered in assessing the stability of a novel compound for which no literature data is available.

In the light of comments made on the importance of measuring potentially toxic decomposition products as an integral part of stability testing[147], the decomposition of 5-hydroxymethylfurfural, itself a decomposition product of D-glucose, is examined to highlight the importance of the initial rate method in obtaining information on levels of impurity. This work is undertaken in this context

because of clinical interest[148] in the nature and rate of appearance of decomposition products arising during the autoclaving of D-glucose in dialysis fluids.

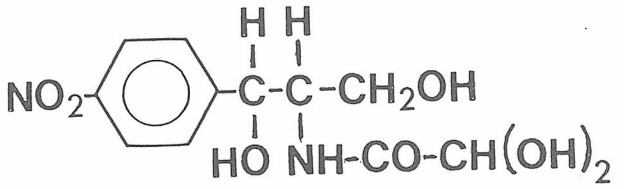
Chapter 8 is a concluding chapter where, in the light of theoretical and experimental information, an overall assessment of the potential of the initial rate method utilising product concentration measurement is made. In addition a third aspect of the work is considered, namely, the generality of hplc as applied to stability testing. A general approach is outlined to the problem of obtaining resolution among reactant and product(s) in drug decomposition on the basis of their possibly differing ionic character. This involves using current ideas on the use of ion-pairing in hplc to control resolution and selectivity[149].

Scheme 1.1 Showing the decomposition of Chloramphenicol.



1-(4'-nitrophenyl)-2
-amino-1, 3-propandiol

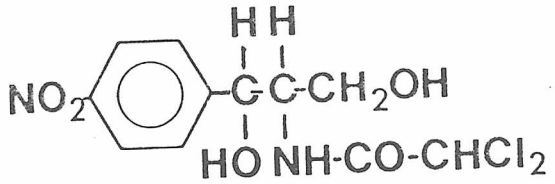
pH 6



1-(4'-nitrophenyl)-2-dihydroxy-
acetamido-1,3-propandiol

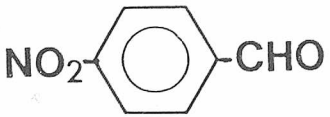
pH 6

photochemical

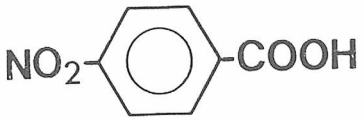


chloramphemical

photochemical

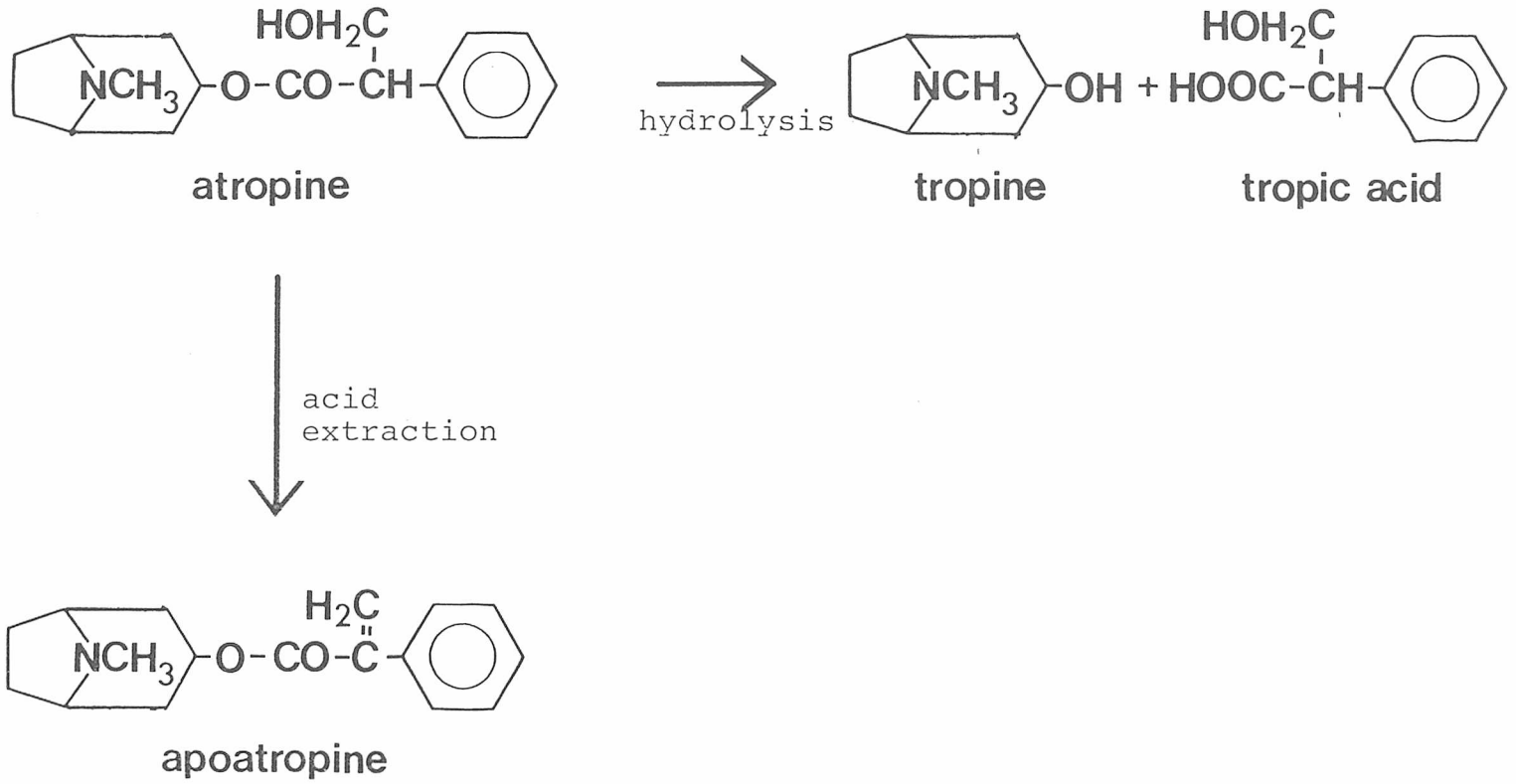


p-nitrobenzaldehyde



p-nitrobenzoic acid

Scheme 1.2 Showing the decomposition of Atropine.



Scheme 1.3 Showing the decomposition of Pilocarpine

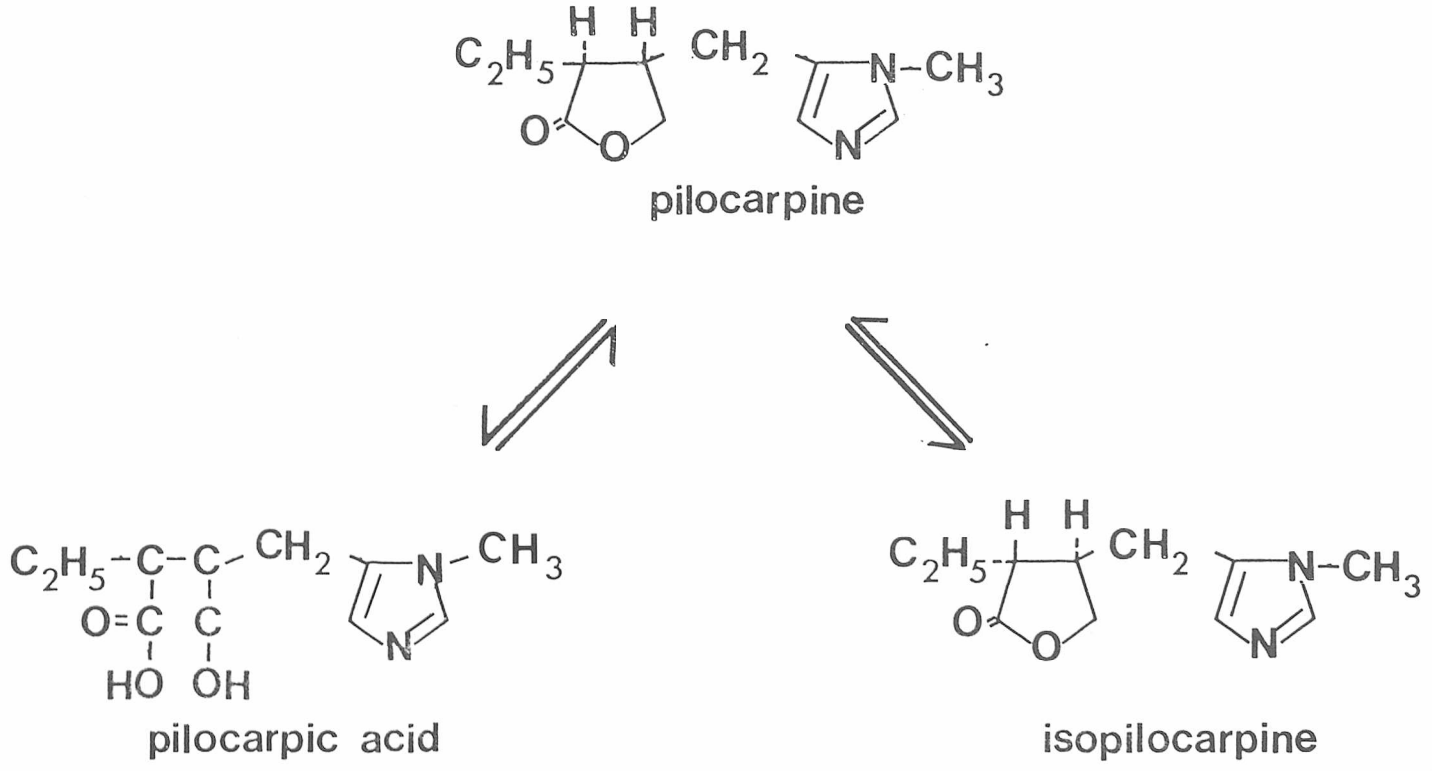


Figure 1.1
Arrhenius plot typical of catalyst breakdown with increasing temperature.

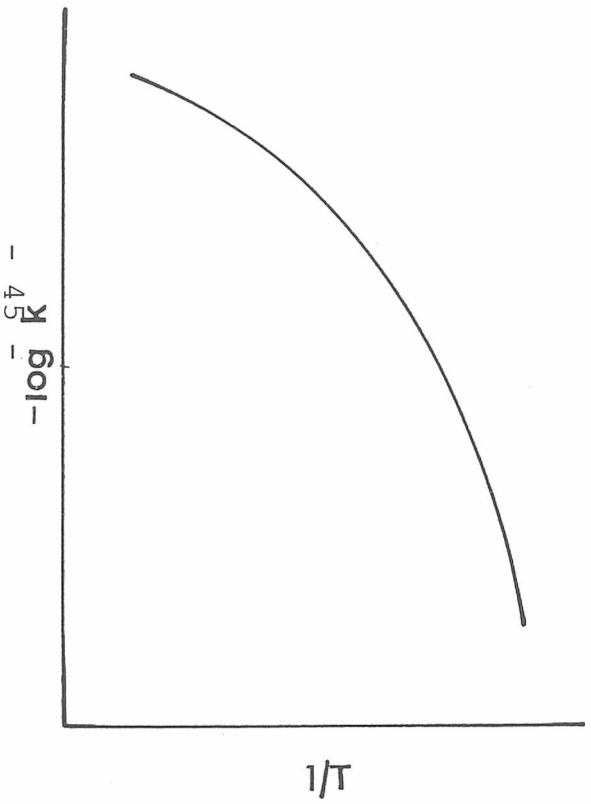


Figure 1.2
Arrhenius plot for two concurrent reactions with differing activation energies.

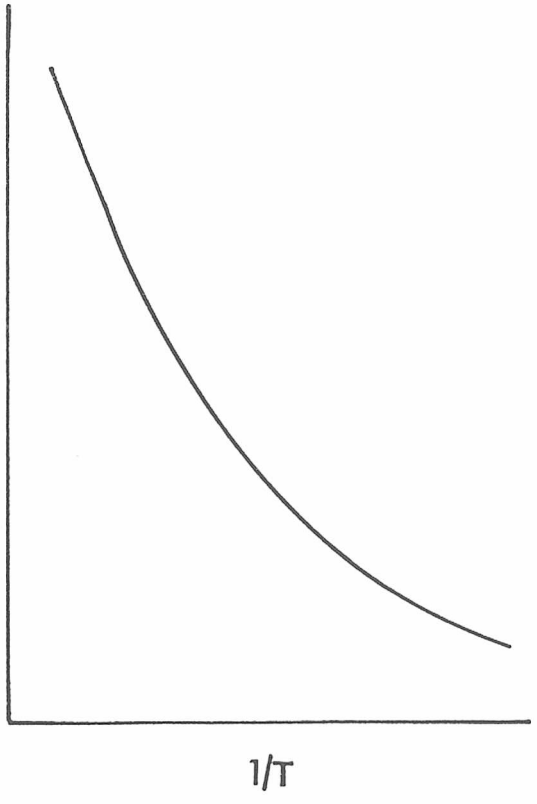
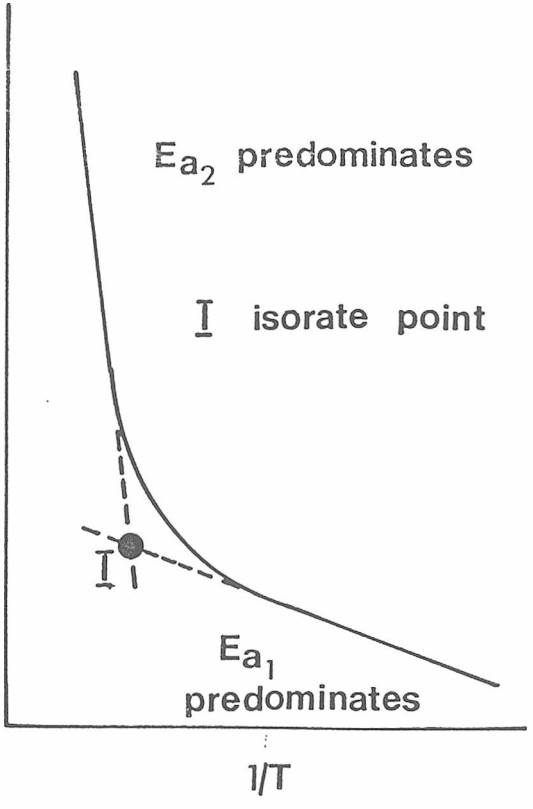


Figure 1.3
Arrhenius plot for Figure 1.2 showing on isorate point where the two constants are equal.



Chapter 2

2.0 Computer Simulation

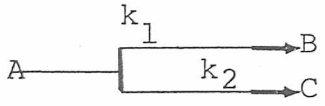
2.1 Introduction

The precision of the rate constant for a drug decomposition obtained by the integral method outlined above depends upon the inherent precision of the analytical measurement of drug concentration and the extent of reaction studied[17]. To compare the alternative methods for rate constant determination, integral and initial rate, for different analytical precision levels and different extents of reaction is a formidable experimental task. This is particularly so when different pathways may be involved. In order to demonstrate the effect of analytical error and extent of reaction followed in terms of both intact drug remaining and decomposition product produced, simulated concentration-time data will allow more complete examination of the consequences of measuring each species. In addition, simulation will allow study of alternative types of drug decomposition, viz.:

(a) Simple $A \xrightarrow{k_1} B$

(b) Consecutive $A \xrightarrow{k_1} B \xrightarrow{k_2} C$

(c) Parallel



(d) Reversible $A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$

Such reaction pathways are frequently encountered in drug

decomposition, for example procaine[150] represents a simple reaction, hydrocortisone[151] a consecutive, sulphisoxazole[152] a parallel and the epimerisation of epichlortetracycline[153] a reversible reaction. The decomposition of benzylpenicillin[154] serves as an example involving parallel, consecutive and reversible steps.

2.2 Simulation

Since the great majority of drug decompositions have been shown to follow (or can be made to follow) first order kinetics, the integrated first order equation is used to generate exact concentration-time data for reactant and product(s). To this concentration data is added a normally distributed random error of any desired magnitude. Figure 2.1 shows the normal distribution of error produced in this simulation for a single concentration value. It is assumed in the simulation that the same relative standard deviation will be appropriate for both reactant and product. This is the situation observed experimentally using chromatographic methods of analysis[155]. Sets of reactant and product concentrations as a function of time such as would be expected from experimental measurements of the stated precision are thus obtained. Rate constants are then derived by linear regression analysis using the simulated concentration-time data for the reactant or product as desired. Such linear regression procedures assume consistency of standard deviation within the data set[156]. The assumption of a constant relative standard deviation represents an

inadequacy in the model. In view of the uncertainty of choosing a concentration on which to base calculation of the required constant standard deviation from the chosen relative value, this limitation in the model is accepted. It is shown in Table 2.1, that by choosing the midpoint concentration on which to base calculation of standard deviation, rate constants very close to those calculated on the basis of the proposed model are obtained.

The information required for the program is as follows.

(a) The rate constants for the decomposition reaction(s)
    (i) for a simple reaction k_1
    (ii) for consecutive, parallel or reversible reactions k_1 and k_2.

(b) The zero time molar concentration of all species; reactant and product(s). For decomposition products this may be taken as zero. The effects of finite concentrations of decomposition products are not considered in the present work.

(c) The extent of reaction to be considered.

(d) The number of concentration-time values to be calculated.

(e) The relative standard deviation (RSD%) to be incorporated in the concentration data.

From the accuracy, in terms of agreement of derived rate constants with input values and the precision in terms of the calculated RSD% the consequences of reactant

and product analysis can be compared as a function of extent of reaction for different levels of analytical precision for the various types of reaction pathways. In the present simulation work a reliable rate constant is taken arbitrarily as one in which the derived value has a RSD% of less than 2%. It is realized that these are stringent conditions since a survey of the literature indicated that, in general, rate constants are quoted as having a relative standard deviation obtained by linear regression analysis of some 6%.

The results obtained from the simulation are shown below for the different types of decomposition pathways.

2.3 Results

2.3.1 Simple A ⟶ B Reaction

Classically two methods have been used to determine the concentration of reactant remaining.

Method I — The concentration of A is measured as a function of time. Direct application of the appropriate rate equation yields the rate constant.

Method II — In those cases where the reactant cannot be analysed directly the concentration of B, the decomposition product, is measured and the amount of A calculated from the initial concentration of reactant. That is

$$[A]_t = [A]_o - [B]_t \qquad (2\text{-}1)$$

Method II is essentially a modification of the integral method where it is experimentally necessary to determine product concentration in order to follow the reaction.

Method III This method represents the initial rate approach where the concentration of B is measured as a function of time during the initial stages of the reaction. The initial rate is calculated assuming a linear relationship between concentration of B and time. First order rate constants are calculated as indicated in Equation (1-16) knowing the initial reactant concentration.

To compare the rate constants obtained by these methods the following parameter values have been used:

(i) The input first order rate constant is 9.6×10^{-4} min.$^{-1}$, the value obtained in preliminary experiments for aspirin decomposition at 50°C.

(ii) Twenty concentration-time points are generated for each extent of decomposition studied.

(iii) The extent of reaction is varied from 0.5 to 90%.

(iv) The initial reaction concentration is taken as 0.1M.

(v) The error incorporated into the concentration

data ranges from 0.5 to 8% RSD.

2.3.1.1 Reliability of Rate Constant at different extents of reaction for an Analytical Precision of 1%.

The results of rate constants obtained by the above methods are shown in Table 2.2 for 1% analytical precision. Using Method I reliable rate constants are only obtained above 30% decomposition. Below this extent of reaction the RSD of the rate constant is greater than 2%. Method II yields reliable rate constants at an extent of decomposition up to 90%. Above this value the error in measuring the concentration of A by difference becomes large. It can also be seen from Table 2.2 that Method III yields reliable rate constants at very much lower extents of reaction than does Method I. In principle, using the criteria outlined above, reliable rate constants may be obtained using product measurement after only 0.5% decomposition. Table 2.2 also shows that, when the limiting conditions required for Equation (1-16) are exceeded, inaccurate i.e. low values of the first order rate constant are obtained by this method.

Method II can only be used when the appropriate st oichiometry existing between A and B is known. In this simulation, this has been taken as a one to one molar correspondence. In an experimental situation this correspondence must be demonstrated and Figure 2.2, using simulated concentration for reactant and product demonstrates the constancy of total molar composition which must be observed. This restriction also applies to

Method III and, if the stoichiometries observed are other than one to one equation (1-16) i.e. $k_0 = k_1[A]_0$ must be suitably modified to obtain conventional first order rate constants.

2.3.1.2 Reliability of Rate Constant at different levels of Analytical Precision

So far the effects of various extents of reaction for a single 1% analytical error has been described. The consequences of altering the analytical error in the range 0.5% - 8% are shown in Figures 2.3 - 2.5 for the different methods outlined above.

Figure 2.3 shows the results for Method I. As the analytical error increases the extent of reaction which must be studied increases. At an analytical eror of 0.5%, 20% decomposition will produce precise values of k_1, while for an 8% error 90% of reaction must be studied. These results are as would be expected qualitatively and confirm literature data[17] .

In the case of Method II, Figure 2.4 shows that at analytical errors of 0.5% and 1% reliable rate constants are obtained up to 90% decomposition. However, as the analytical error is further increased rate constants obtained by this method are precise only when measured over successively lower extents of reaction. With an analytical error of 8% no reliable rate constants can be obtained at any extent of decomposition.

Figure 2.5 shows the results obtained for different analytical errors using Method III. As the analytical

error increases from 0.5 to 8% RSD lower extents of reaction must be followed if the rate constants produced are to be reliable. For an analytical error below 2% reliable rate constants are obtained up to 10% decomposition. As the extent of decomposition increases the RSD% exceeds 2%. At an analytical error of 4%, 5% decomposition represents the maximum extent of reaction which can be studied. As in the case of Method II an 8% analytical error does not provide reliable rate constants at any level of decomposition.

2.3.2 Consecutive Reactions

So far a simple reaction has been considered where the decomposition product B is stable. In a consecutive reaction the intermediate B is unstable and undergoes further decomposition to C.

$$A \longrightarrow B \longrightarrow C$$

In general consecutive reactions obeying first order kinetics will yield concentration-time curves for each species according to the following equations[6].

$$[A]_t = [A]_0 e^{-k_1 t} \qquad (2\text{-}2)$$

$$[B]_t = [A]_0 (k_1/(k_2-k_1))(e^{(-k_1 t)}-e^{(-k_2 t)}) \qquad (2\text{-}3)$$

$$[C]_t = [A]_0((1+k_2 e^{(-k_1 t)}-k_1 e^{(-k_2 t)})/(k_1-k_2)) \qquad (2\text{-}4)$$

The rate constant k_1 can be obtained in the usual way using equation (2-2). The value of k_2 is obtained by

fitting the concentration-time data to these equations by some form of iterative curve fitting procedures. If, however, the decomposition is to be followed by measuring the intermediate B by say Method III described above, then the value of k_1 obtained will be affected by the magnitude of k_2. Should k_2 be considerably greater than k_1 the reaction simplifies to A $\longrightarrow$ C and the same conclusions as to the reliability of measuring C will apply as shown above. In this case some B will be formed but may experimentally be below the level of detection. Alternatively if k_1 is very much greater than k_2, C may be ignored.

The simulation procedure has been applied to situations where neither of the above situations prevail. To examine the effect of the relative magnitudes of k_1 and k_2, k_1 is maintained constant at 9.6×10^{-4} min.$^{-1}$ and k_2 is varied such that the ratio k_1/k_2 ranges from 1000 to 10^{-4}. The rate constant variation with extent of reaction has been examined using Methods I - III as previously described.

Using Method I, the change in precision of k_1 with increasing extents of reaction for a 1% error in analysis is shown in Figure 2.6 for different values of k_2. As expected, the pattern observed is the same as that for the simple A $\longrightarrow$ B reaction (Fig. 2.3). Again, more than 30% decomposition must be followed before reliable rate constants are obtained.

Application of Method II necessitates quantitation of both B and C. The concentration of A remaining is

calculated using equation (2.5) below

$$[A]_t = [A]_o - ([B]_t + [C]_t) \qquad (2\text{-}5)$$

The results obtained resemble that for the simple A ⟶ B reaction. Reliable rate constants are obtained for all ratios of k_1/k_2 ranging from 1000 to 10^{-4} over any extent of decomposition for a 1% analytical error.

The results obtained by following the intermediate B are summarised in Table 2.3. It shows that the precision and accuracy of k_1 values obtained depend markedly on the ratio of k_1/k_2. As this ratio decreases reliable rate constants are obtained only at progressively lower extents of decomposition. B remains a reliable measure of k_1 until the ratio $k_1/k_2 = 2$ at levels of decomposition not exceeding 10% and when $k_1/k_2 = 0.1$ at levels of decomposition not exceeding 1%. At smaller values of k_1/k_2 it is impossible to obtain k_1 values by measuring B.

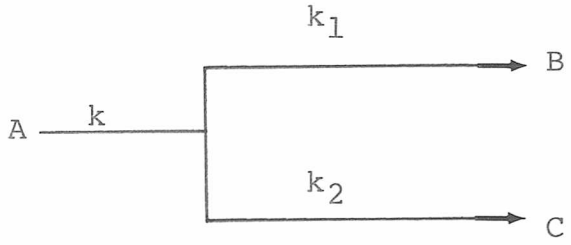
Table 2.3 also summarises the conditions under which measurement of C may be used to determine k_1 values. It is seen that precise estimates of k_1 are obtained only when k_1/k_2 is less than 5×10^{-3} and the extent of reaction confined to below 10%.

The accuracy of k_1 values obtained by monitoring intermediate B or C to smaller extents of reaction is represented in Figure 2.7. Here the variation of k_1 with k_1/k_2 ratio is shown for both B and C over 5% decomposition. It is seen that if the very small extents of reaction are studied B may be used above a k_1/k_2 ratio

of 0.1 while C may be used below a k_1/k_2 value of 5×10^{-3}.

2.3.3. Parallel Reactions

In many instances a parent drug may undergo decomposition via more than one pathway, i.e.



where k represents the overall rate constant.

In this case measuring the disappearance of A will determine the overall rate constant k which only has the physical significance of being the sum of the rate constants for the two individual reactions and is of practical use only for the purpose of determining the shelf life of the drug.

Using Methods I and II produces identical results to those obtained in both the previous situations. When Method III is applied similarly identical results are obtained to those observed when treating the simple A ⟶ B reaction with the separate rate constants k_1 and k_2 being determined individually. From Method III, therefore, the overall rate constant can be obtained by adding k_1 and k_2. Table 2.4 shows that in this case within the limits of extent of reaction and precision of analytical method the same results are obtained as were previously described.

If only one decomposition product were measured for the determination of k, the major product, the reliability

of the result obtained would depend upon the relative magnitudes of k_1/k_2. The simulation programme was used to investigate the accuracy in the derived k from the measurement of the major rate constant k_1 at various values of the k_1/k_2 ratio. The results are shown in Figure 2.8 where it is seen that the derived value lies within 5% of the overall k input when the ratio of k_1/k_2 is greater than about 40 for a reaction followed to 5% decomposition.

2.3.4 Reversible Reactions

Unlike consecutive reactions, where the unstable intermediate B undergoes further reaction, the decomposition product B in a reversible reaction reverts back to the starting material i.e.

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$$

By measuring the change in intact reactant concentration as a function of time, the overall rate of reaction k may be calculated provided the concentration of A at equilibrium ($[A]_{eq}$) can be determined[6,10] i.e.

$$\ln([A]_0 - [A]_{eq}/[A]_t - [A]_{eq}) = kt \qquad (2\text{-}6)$$

A plot of $\ln([A]_0 - [A]_{eq}/[A]_t - [A]_{eq})$ against t should yield a slope equal to k. To calculate the rate of forward and reverse reaction requires a knowledge of K, the equilibrium constant. K may be calculated from

$$K = [B]_{eq}/[A]_{eq}$$

It is thus necessary to follow the reaction to equilibrium to enable determination of reactant and product concentrations at equilibrium.

From the knowledge of the equilibrium constant and the overall rate constant the rate constant for the reverse reaction is given by equation(2-7)

$$k_2 = k/(K + 1) \qquad (2\text{-}7)$$

and the rate constant for the forward reaction determined by difference i.e.

$$k_1 = k - k_2 \qquad (2\text{-}8)$$

Table 2.5 shows the extent of decomposition that must be followed to obtain reliable rate constants using Method I for different values of K. It is seen that at values of K ranging from 100 to 1.0 the precision requirements are the same as for a simple A ⟶ B reaction. As K decreases below 1.0 a larger extent of decomposition must be followed. At K=0.1 the reaction behaves as though B were the reactant (B ⟶ A), equilibrium being attained at 9% decomposition. Method I therefore cannot be applied under such conditions. Method II on the other hand is applicable up to various levels of decomposition for any value of K.

For K values greater than 10, the reaction behaves as a simple A ⟶ B decomposition and Method III will yield reliable estimates of k_1 and k_2 up to 10% decomposition.

However for K values below 10, B must be followed at lower extents of decomposition.

2.3.5 Determination of Order of Reaction

In order to compare the initial rate and integral methods of determining the order of reaction for a simple A $\longrightarrow$ B decomposition a set of simulated concentration-time data were generated over 10% extent of decomposition. An analytical error of 4% was chosen. These data which must conform to first order kinetics by definition were treated using zero, first and second order integrated equations. The results are shown in Table 2.6. It is seen that little difference exists in the standard deviation and correlation coefficient of the slope for any of the equations used. The same data taken over 75% decomposition shows that a clear distinction can be made.

Using the same procedure product data for a simple A $\longrightarrow$ B reaction was generated over 10% decomposition for different initial concentrations of A. The reaction order was determined according to Equation (1-8). The slope is seen to be very close to unity and good standard deviation and correlation coefficient are obtained. When this procedure is repeated using initial rates by measuring reactants the precision of determining the order in terms of standard deviation and correlation coefficient is seen to be very much less. (Table 2.7)

2.4 Conclusion

These results confirm, without reference to any

particular drug decomposition system, that the reliability of a decomposition reaction rate constant depends upon the precision associated with the analytical method used to determine concentration and on the extent of reaction studied. This is so whatever kinetic method is used to obtain rate constant values from the concentration-time data. In addition they indicate the magnitude of the uncertainty in rate constant values at several different levels of precision and extents of reaction. They demonstrate the errors which may be expected in rate constants obtained by application of the integral methods when low extents of reaction only are studied. Most importantly they allow direct comparison of the various methods of estimating rate constants depending on whether reactant or decomposition product is measured.

For a simple reaction involving only one product clear advantages are apparent in the use of product measurement; either by relating it to the concentration of reactant remaining with consequent increase in precision, or by applying the initial rate method. The reaction need be studied to much smaller extents of reaction compared with reactant determination at comparable levels of analytical precision. Alternatively, for a given level of precision of rate constant a larger error may be tolerated in the measurement of product when the study of the reaction is confined to the early stages. This latter point is of relevance when the purpose of the rate constant measurement is the determination of shelf life.

The calculation of first order rate constants using

product measurement, however, requires that the stoichiometry of the reaction be known. The necessity of obtaining absolute values of rate constants will be discussed in subsequent chapters.

Drug decompositions which involve more than one simultaneously occurring reaction follow the same generalisations as indicated above for the simple case when each of the reactions is treated separately. That is, the rate constants for each of the reactions are subject to the advantages and limitations outlined above. Conventional first order rate constants can be obtained by summing individual values.

For such types of decomposition the purpose of the stability measurement must be considered in comparing the alternative methods. The integral method in this case provides a rate constant which is useful only in the prediction of shelf life. It has no direct significance to any decomposition reaction. This aspect will be dealt with fully in later chapters. Where one product only is taken as characterising the decomposition process it has been shown that alternative routes should constitute less than 5% of the total.

In the case of consecutive reactions it has been shown that product measurement may be used with advantage using either, the intermediate B if the rate constant of the first reaction is greater than 0.1 times the second reaction, or the final product C if the second rate constant is more than 200 times that of the first. In the intermediate region of rate constant ratio adequate rate

constants for the decomposition of the initial drug cannot be obtained using product measurements.

For reversible reactions integral methods, as well as requiring that the reaction be carried out to the usual large extents of reaction, also require knowledge of the equilibrium constant for the reaction. This is required in order to separate the individual forward and reverse rate constants from the overall reaction rate constant which is the quantity experimentally measured. It is also required to estimate equilibrium concentrations of reactant species which otherwise would involve following the reaction essentially to completion. This disadvantage applies also to the use of product concentration to calculate more precise values of remaining reactant concentration.

The initial rate method is advantageous in the study of such reactions, however, since the rate constant of the forward reaction is obtained directly from the initial rate of appearance of product and knowledge of the initial reactant concentration. Equilibrium concentrations are not required nor is the value of the equilibrium constant. Rate constants obtained by the initial rate method will be larger than those obtained by integral methods but the error incurred in the first 10% of reaction required for shelf life determination is very small. If the equilibrium constant is known then the rate constant for the reverse reaction and the overall rate constant can be readily calculated.

It also appears from this simulated work that the

initial rate method using product measurement affords a very adequate method of determining the reaction order should this be required. This can be achieved within an extent of reaction where integral methods cannot distinguish betwen zero, first and second orders.

Table 2.1 A comparison of the different simulation models.

	SD calculated from mid point concentration data point	Present Model
5% decomposition of reactant		
k_1	6.96×10^{-4}	6.80×10^{-4}
RSD(%)	46.02	41.60
R^2	0.208	0.240
90% decomposition of reactant		
k_1	9.67×10^{-4}	9.62×10^{-4}
RSD(%)	1.01	0.66
R^2	0.998	0.999
5% formation of decomposition product		
k_0	9.23×10^{-5}	9.41×10^{-5}
RSD(%)	0.71	0.83
R^2	0.999	0.999
90% formation of decomposition product		
k_0	3.23×10^{-5}	3.20×10^{-5}
RSD(%)	6.82	7.39
R^2	0.923	0.910

Table 2.2 A comparison of rate constants ($min.^{-1}$) obtained at varying extents of decomposition using Methods I, II and III. Input $k_1 = 9.6 \times 10^{-4}$ $min.^{-1}$, C_0 =0.1M and analytical error equals 1% RSD.

	Method I			Method II			Method III			
% decomp.	Rate constant ($min.^{-1} \times 10^4$)	RSD	R^2	Rate constant ($min.^{-1} \times 10^4$)	RSD	R^2	Initial Rate ($mol.dm^{-3}$ $min.^{-1} \times 10^5$)	RSD	R^2	Rate constant ($min.^{-1} \times 10^4$)
0.5	18.00	87.53	0.068	9.60	0.45	0.999	9.57	0.44	0.999	9.57
1.0	19.00	36.24	0.297	9.59	0.42	0.999	9.54	0.41	0.999	9.54
2.0	69.40	55.55	0.153	9.62	0.42	0.999	9.52	0.42	0.999	9.52
3.0	12.20	16.82	0.663	9.65	0.52	0.999	9.49	0.50	0.999	9.49
4.0	9.51	17.75	0.638	9.66	0.42	0.999	9.46	0.43	0.999	9.46
5.0	12.00	12.44	0.782	9.62	0.40	0.999	9.37	0.38	0.999	9.37
10.0	10.70	5.76	0.944	9.60	0.46	0.999	9.09	0.56	0.999	9.09
20.0	10.40	3.17	0.982	9.64	0.53	0.999	8.58	0.72	0.999	8.58
30.0	9.92	2.33	0.990	9.57	0.38	0.999	7.96	1.21	0.997	7.96
40.0	9.77	1.64	0.995	9.62	0.42	0.999	7.40	1.62	0.995	7.40
50.0	9.51	1.29	0.997	9.63	0.62	0.999	6.77	2.18	0.992	6.77
75.0	9.62	0.41	0.999	9.53	0.40	0.999	4.84	4.36	0.967	4.84
90.0	9.55	0.39	0.999	9.48	1.26	0.998	3.26	6.97	0.920	3.26

Table 2.3 The reliability in k_1 as a function of k_1/k_2 by measuring either the intermediate B or final product C for a consecutive A ► B ► C reaction.

	Intermediate B followed		Final Product C followed	
k_1/k_2	Precision in k_1 obtained up to % decomposition	Accuracy in k_1 obtained up to % decomposition	Precision in k_1 obtained up to % decomposition	Accuracy in k_1 obtained up to % decomposition
1000	50	$\leq$10	-	-
100	46	"	-	-
10.0	41.5	"	-	-
2.0	33	"	-	-
0.5	18	$\leq$3	-	-
10^{-1}	5.5	$\leq$1	-	-
10^{-2}	$\leq$0.5	-	-	-
10^{-3}	-	-	-	-
5×10^{-3}	-	-	3-40	-
4×10^{-4}	-	-	40	$\leq$10
2×10^{-4}	-	-	50	$\leq$10

Table 2.4 A comparison of rate constants ($min.^{-1}$) obtained at varying
Input $k = 9.6 \times 10^{-4}$, $k_1 = 8.64 \times 10^{-4}$, $k_2 = 9.6 \times 10^{-5}$ min.

	Method I			Method II		
% decomp.	Rate Constant ($min.^{-1} \times 10^4$)	RSD	R^2	Rate Constant ($min.^{-1} \times 10^4$)	RSD	R^2
0.5	29.40	55.19	0.154	9.59	0.34	0.999
1.0	8.64	91.13	0.063	9.63	0.30	0.999
2.0	1.97	205.40	0.013	9.71	0.39	0.999
3.0	8.76	20.71	0.564	9.58	0.49	0.999
4.0	10.20	15.27	0.704	9.62	0.43	0.999
5.0	9.52	12.01	0.794	9.54	0.37	0.999
10.0	9.55	8.22	0.891	9.60	0.31	0.999
20.0	9.31	2.76	0.986	9.61	0.42	0.999
30.0	9.40	2.38	0.990	9.59	0.38	0.999
40.0	9.41	1.44	0.996	9.59	0.50	0.999
50.0	9.70	0.98	0.998	9.63	0.46	0.999
75.0	9.66	0.63	0.999	9.68	0.54	0.999
90.0	9.65	0.33	0.999	9.44	0.95	0.998

extents of decomposition for a parallel reaction using Methods I, II and III.
$^{-1}$, C_0 = 0.1M and analytical error equals 1% RSD.

Method III Product B				Method III Product C			
Initial Rate (mol.dm^{-3} min.$^{-1}$ x10^5)	RSD	R^2	Rate Constant (min.$^{-1}$ x 10^4)	Initial Rate (mol.dm.$^{-3}$ min.$^{-1}$x10^6)	RSD	R^2	Rate Constant (min.$^{-1}$ x 10^5)
8.59	0.49	0.996	8.59	9.59	0.29	0.999	9.59
8.61	0.57	0.999	8.61	9.56	0.41	0.999	9.56
8.53	0.41	0.999	8.53	9.44	0.48	0.999	9.44
8.45	0.54	0.999	8.45	9.50	0.39	0.999	9.50
8.47	0.49	0.999	8.47	9.42	0.36	0.999	9.42
8.39	0.38	0.999	8.39	9.39	0.31	0.999	9.39
8.15	0.59	0.999	8.15	9.15	0.50	0.999	9.15
7.68	0.77	0.999	7.68	8.54	0.76	0.999	8.54
7.20	1.13	0.998	7.20	7.97	1.13	0.998	7.97
6.67	1.46	0.996	6.67	7.36	1.55	0.996	7.36
6.12	2.05	0.993	6.12	6.75	2.23	0.991	6.75
4.36	4.23	0.969	4.36	4,82	4,16	0.970	4,82
2.93	6.90	0.921	2.93	3.27	6.77	0.924	3.27

Table 2.5 The reliability of k_1 as a function of k_1/k_2 by measuring the decomposition product B for a reversible reaction $A \rightleftharpoons B$.

Equilibrium Constant (K)	Method I applicable at	Method II applicable up to	Method III applicable up to
100	>30% decomp.	up to 90%	<10% decomp.
50	"	"	"
10	"	"	<5% decomp.
5	"	"	"
1.0	>40% decomp.	<40% decomp.	"
0.1	N/A	up to 8%	<0.5% decomp.

Table 2.6 A comparison of rate constants obtained by treating reactant concentration-time data at 10% and 75% decomposition using zero, first and second order integrated equations for a simple A⟶B reaction. Analytical error equals 4%.

% Decomp.	Reaction Order	Rate Constant	RSD (%)	R^2	Calculated first order rate constant ($\times 10^4$ $min.^{-1}$) using Equation (1-16)
10	0	1.18×10^4 $mol.dm.^{-3}min.^{-1}$	23.81	0.495	11.80
	1	1.26×10^3 $min.^{-1}$	24.58	0.479	12.60
	2	1.36×10^2 $mol.^{-1}dm.^{3}min.^{-1}$	25.41	0.680	13.60
75	0	5.07×10^5 $mol.dm.^{-3}min.^{-1}$	5.66	0.945	5.07
	1	9.90×10^4 $min.^{-1}$	2.32	0.990	9.90
	2	2.15×10^2 $mol.^{-1}dm.^{-3}min.^{-1}$	4.42	0.983	21.50

Table 2.7 Comparison of the reaction order as calculated by measuring the initial rate for reactant and product at different initial reactant concentrations over 10% decomposition at an analytical error of 4% RSD.

Species Followed	Initial Reactant conc. ($mol.dm.^{-3}$)	Initial Rate ($mol.dm.^{-3}min.^{-1}$)	RSD	R^2	Calculated Reaction Order n	RSD (%)	R^2
React.	0.05	$4.17x10^{-5}$	26.77	0.437			
	0.08	$6.33x10^{-5}$	27.90	0.417			
	0.10	$1.18x10^{-4}$	23.81	0.495			
	0.30	$2.76x10^{-4}$	24.73	0.476			
	0.50	$1.60x10^{-4}$	58.41	0.140	0.67	34.50	0.858
Product	0.05	$4.67x10^{-5}$	1.15	0.998			
	0.08	$7.41x10^{-5}$	1.93	0.993			
	0.10	$9.14x10^{-5}$	2.24	0.991			
	0.30	$2.72x10^{-4}$	1.55	0.996			
	0.50	$4.48x10^{-4}$	2.05	0.993	0.98	0.34	0.999

Figure 2.1 Plot showing the normal distribution of errors generated by the computer program for a single concentration (N=100) 64 points lie within ± 1 SD, 95 points within ± 2 SD and 100 points within ± 3 SD.

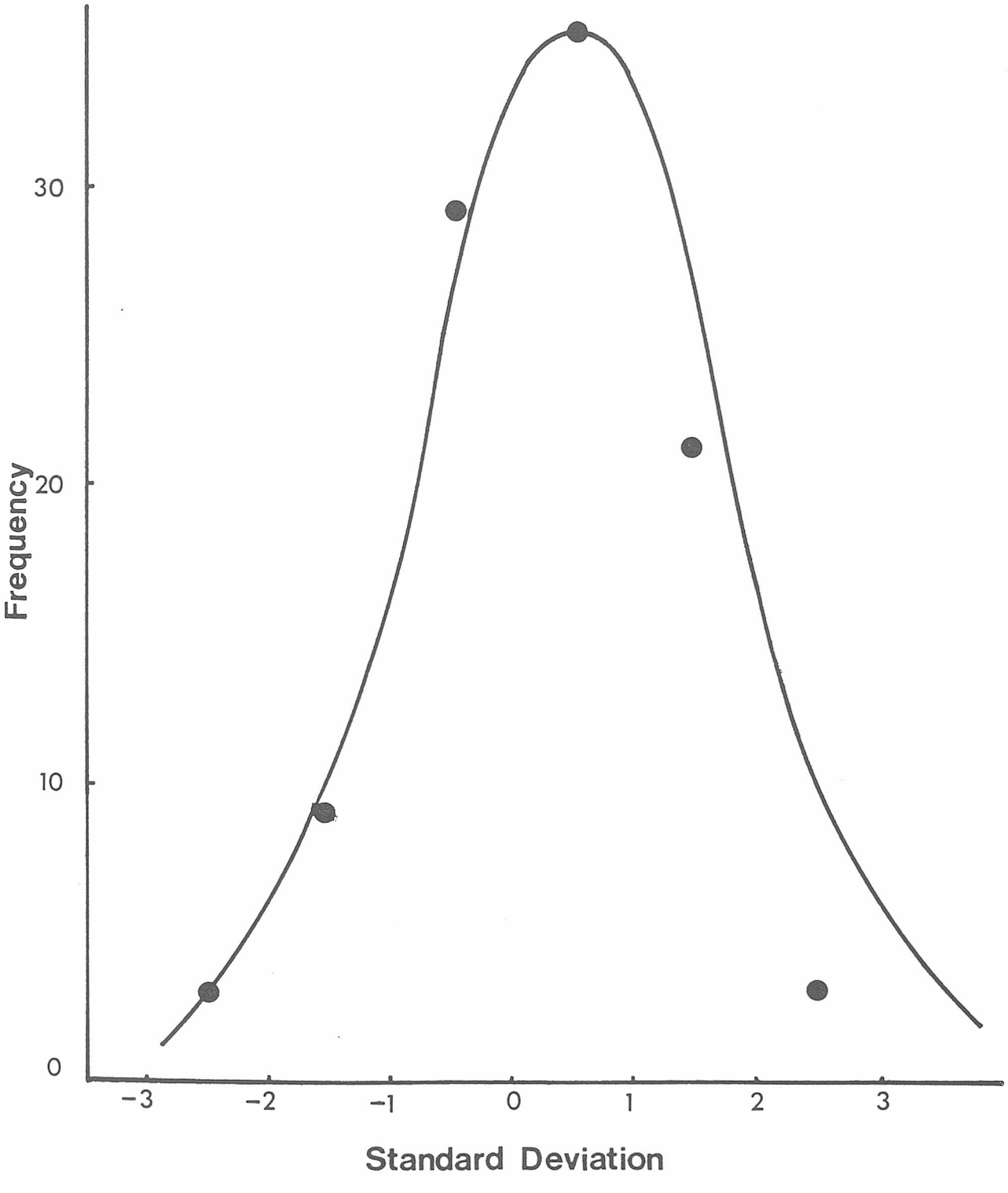


Figure 2.2 Showing the mole for mole correspondence between reactant and product for the simple A⟶B reaction. All concentration terms include an analytical error of 1% RSD as generated by the computer program.

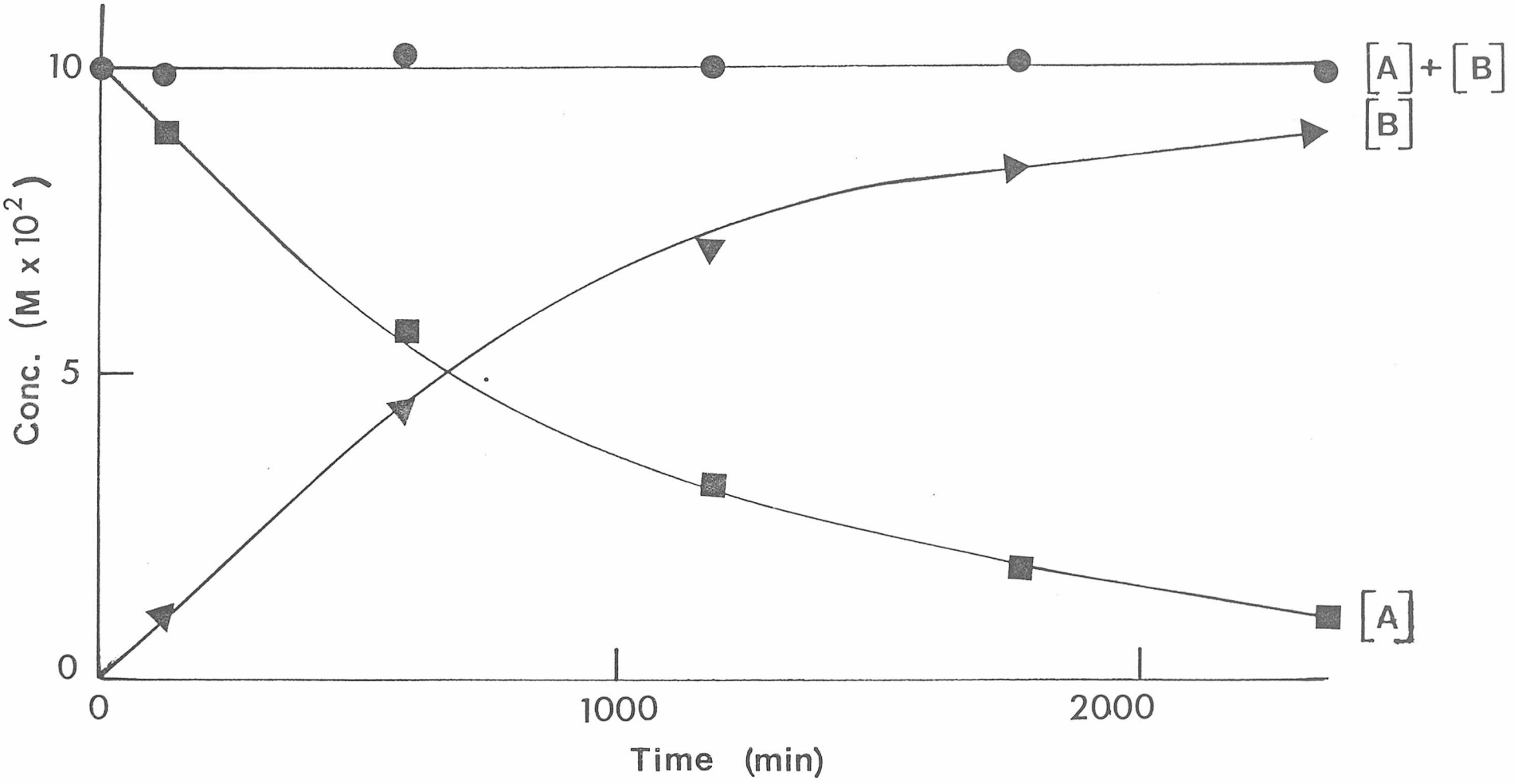


Figure 2.3 Showing the variation in the precision (% RSD) obtained on the rate constants with the extent of decomposition for Method I for differing analytical errors. (% RSD)

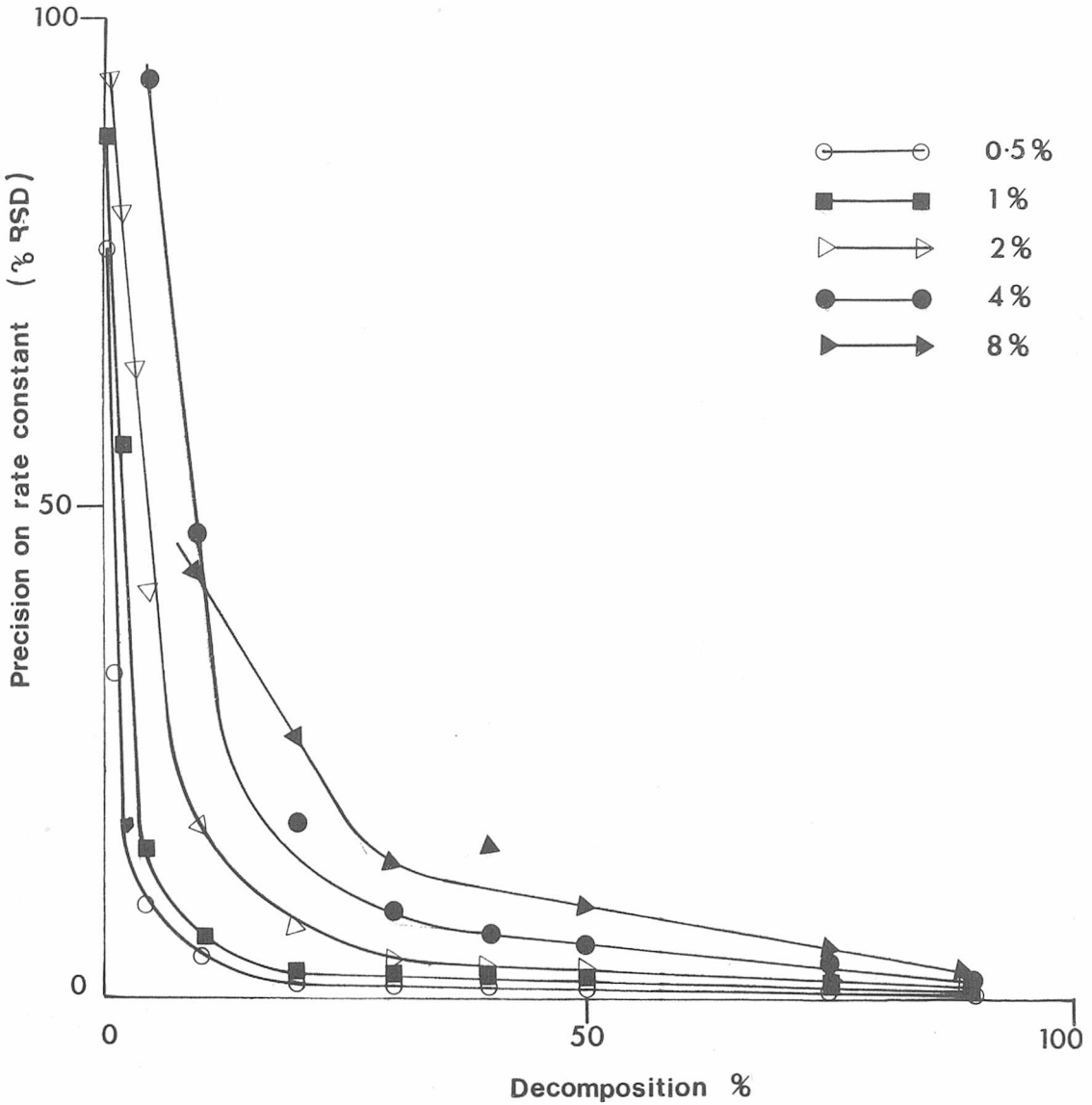


Figure 2.4 Showing the variation in precision (% RSD) obtained on the rate constant with the extent of decomposition for Method II for differing analytical errors (% RSD).
Key as for Figure 2.3

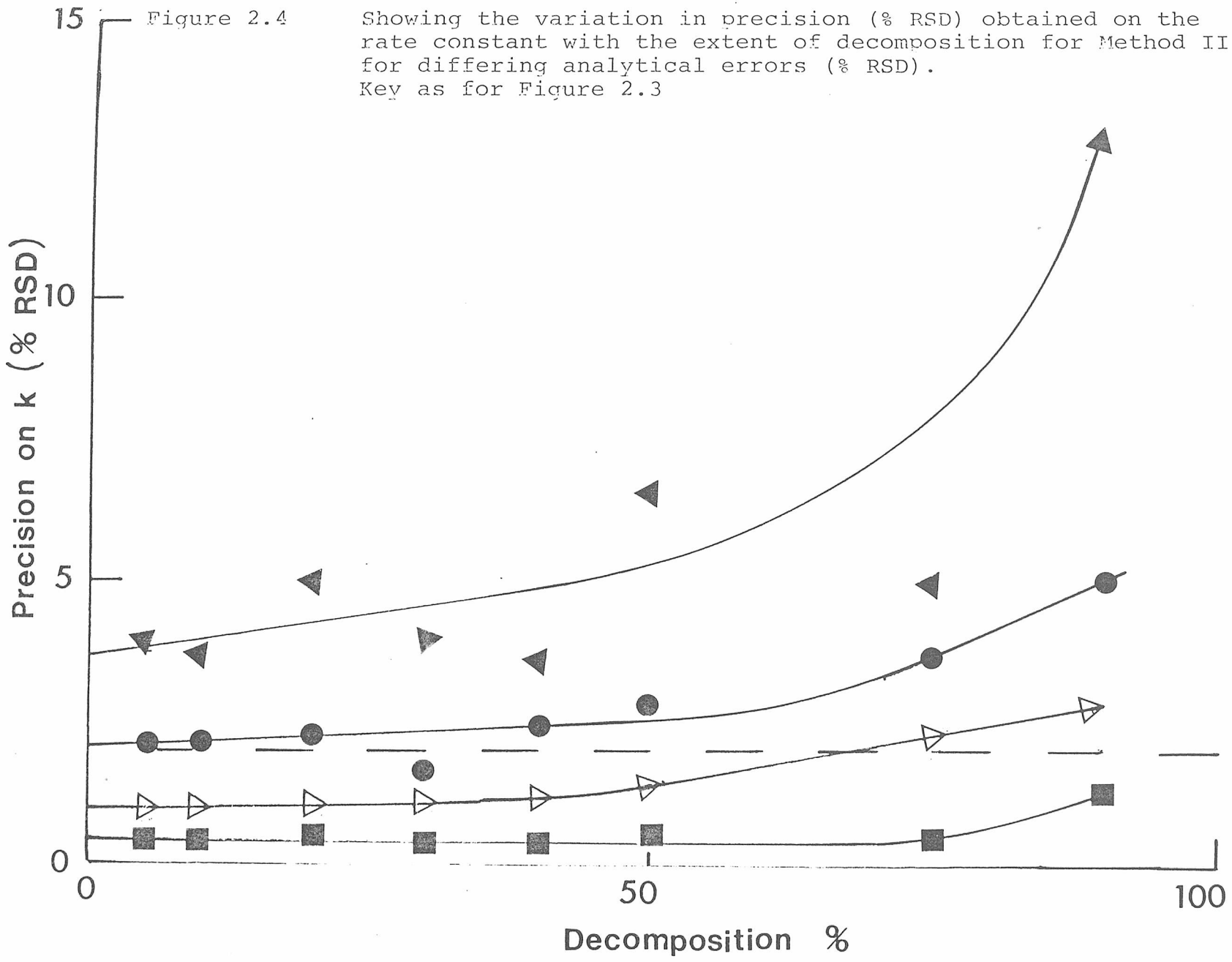


Figure 2.5 Showing the variation in precision (% RSD) obtained on the rate constant with the extent of decomposition for Method III for differing analytical errors (% RSD). Key as for Figure 2.3.

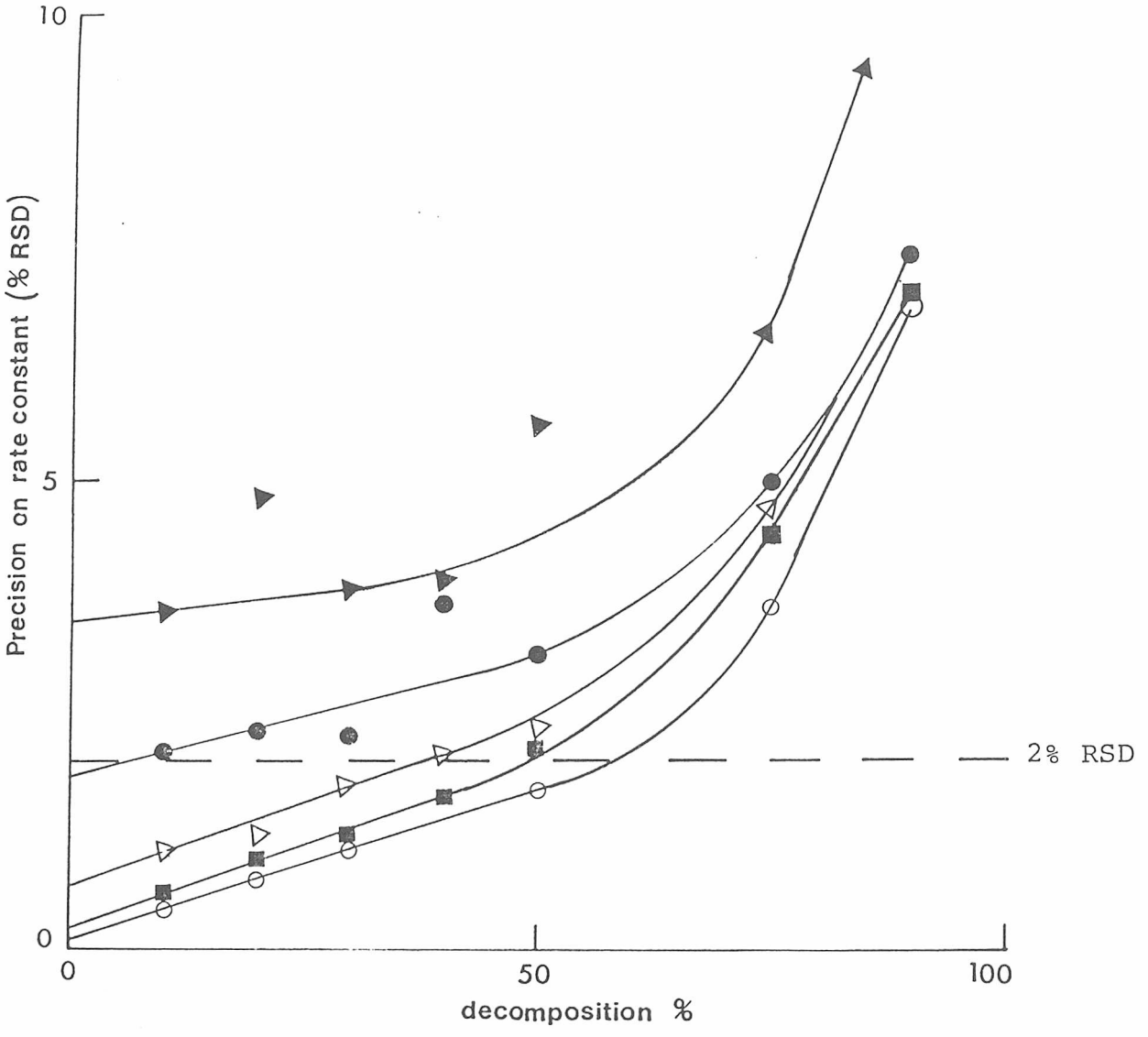


Figure 2.6 Showing the variation in precision (% RSD) obtained on the rate constant with the extent of decomposition for different k_2 values in a consecutive $A \xrightarrow{k_1} B \xrightarrow{k_2} C$ reaction using Method I.

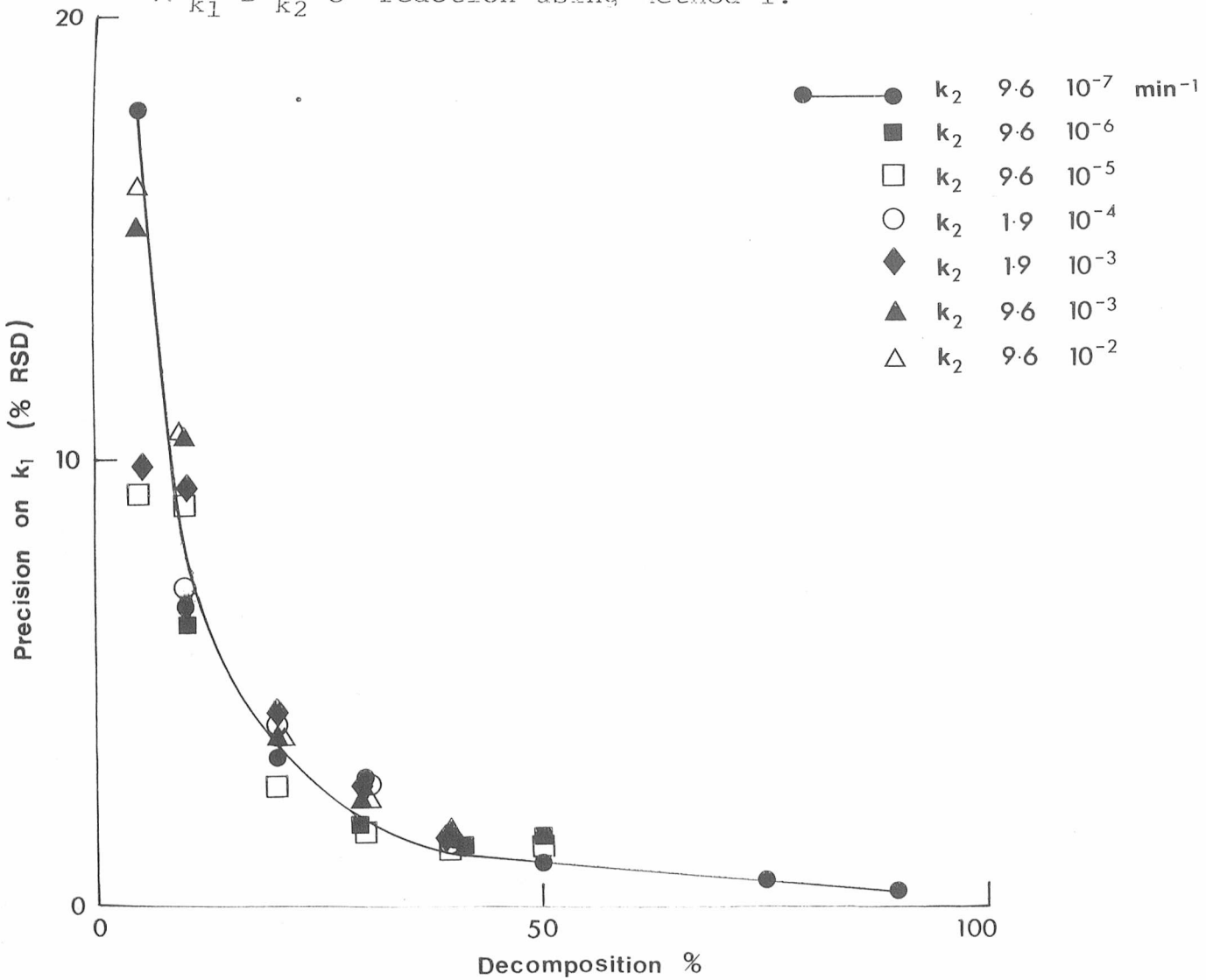


Figure 2.7 Showing the accuracy in k_1 obtained by following the increase in intermediate (B) and final product (C) for a consecutive A $\xrightarrow{k_1}$ B $\xrightarrow{k_2}$ C reaction for different ratios of k_1/k_2.

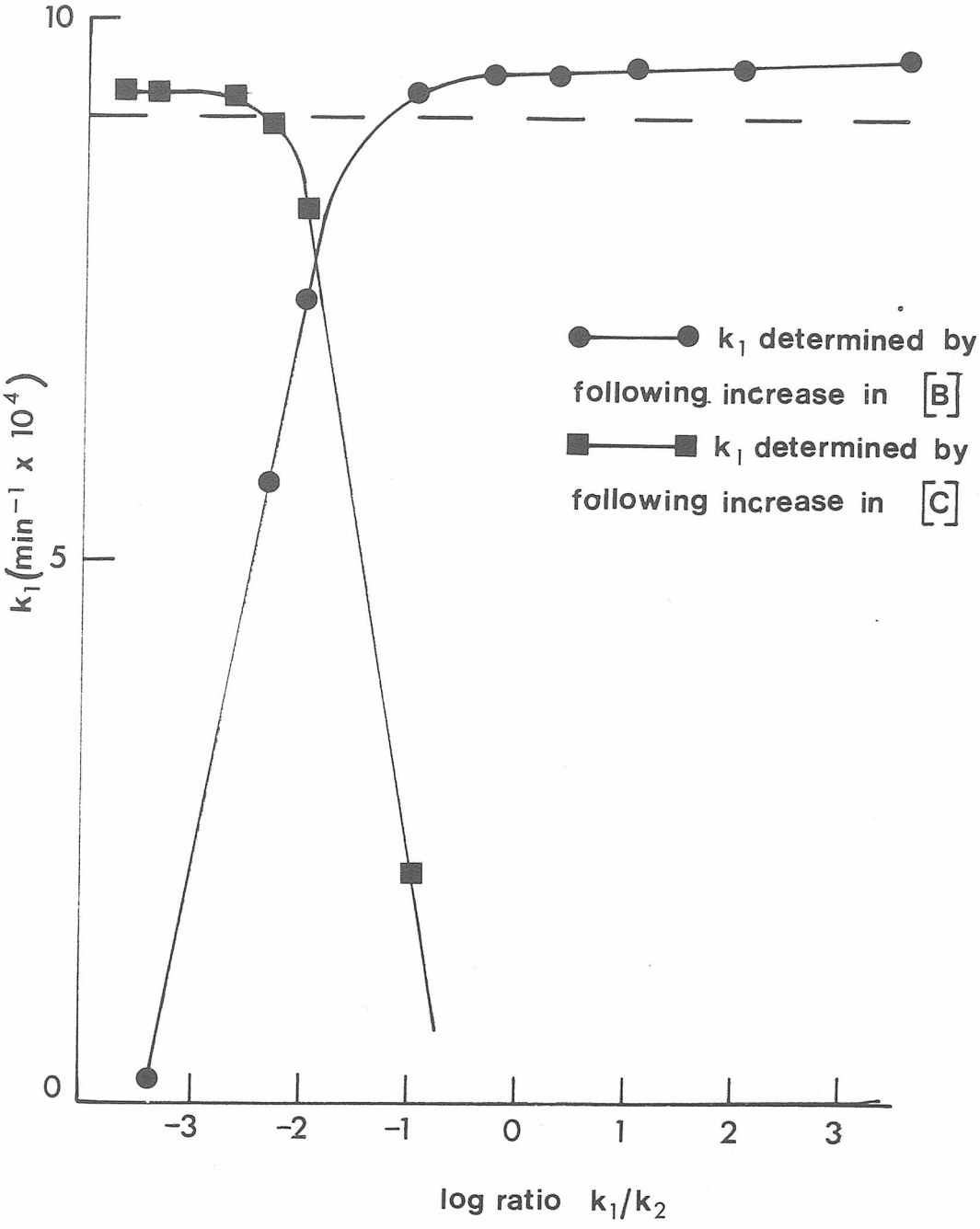
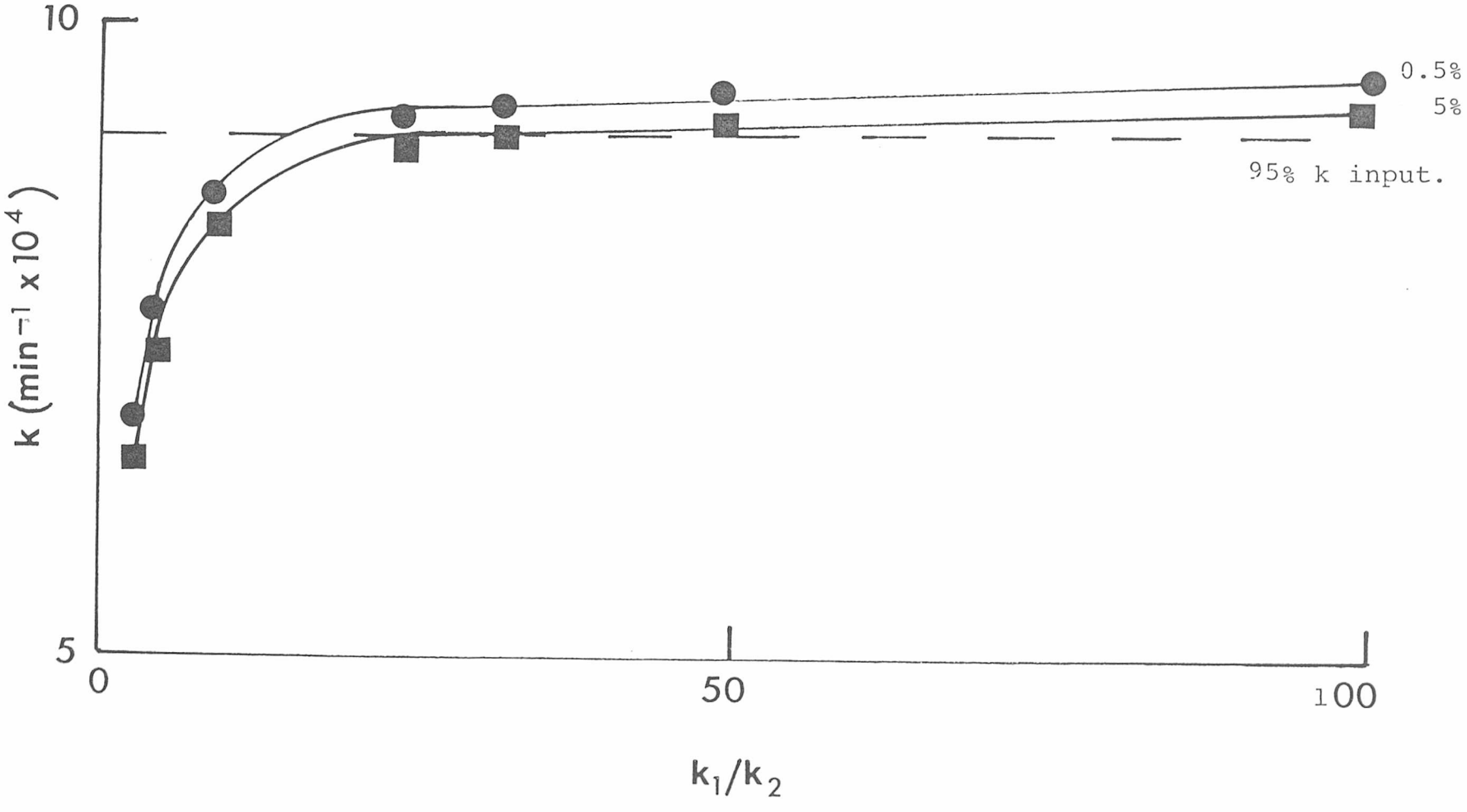


Figure 2.8 Showing the accuracy in k for different k_1/k_2 ratios in a parallel reaction. k is estimated by following the major product of decomposition at two different levels of decomposition.



Chapter 3

3.0 The Decomposition of Aspirin

3.1 Introduction

Aspirin, acetylsalicylic acid (ASA) is probably one of the most extensively studied drugs in the pharmaceutical literature due to its widespread use as an antipyretic and analgesic.

The literature on the stability of this drug is extensive and the list of references encompasses mechanism of reaction[74,157-160], kinetic and stability studies [161-171] and impurity limit testing [172]. Such studies carried out under a variety of conditions have been performed using analytical methods ranging from ferric iron complexation [173-175] to spectrophotometry[74,157,158,176] through classical column chromatography[177,178] to modern hplc methods. Although the first report on the hydrolysis of ASA was published in 1908[179] new analytical methods were still being presented in 1984[180]. The GLC method reported [181,182] suffers from the disadvantage in that the elevated temperatures necessary for separation of ASA from its decomposition product salicylic acid (SA) induces decomposition. The earlier hplc methods reported are based on normal phase chromatography[182-185]. In these reports, with one exception[185], SA was eluted before ASA. Separation using reverse phase chromatography utilise ion suppression in many cases[186-190]. However in these cases

SA was eluted after ASA. Although ,many have reported the separation of ASA from SA[186-190] these have been designed for quality control[187,190], examination of alternative decomposition reactions[188] and for assay of SA in multicomponent formulations containing ASA[190]. None of these hplc methods have been used practically for stability studies. Thus, the order of elution has not been considered important.

As will be discussed more fully in Chapter 8, if the initial rate method is to be employed by following the appearance of product it is very advantageous in determining the rate in the initial stages of reaction to elute the product before the main reactant which is present in large excess[111]. Since a reverse phase procedure is obviously more convenient for study of decomposition in aqueous solution none of the previously published methods is suitable for this purpose.

Unlike the stability reports on the vast majority of drug compounds where the analysis is by choice of the undecomposed drug, the stability literature using analytical methods predating hplc on ASA reveals that for the majority of stability investigations presented, the chemical species analysed was the decomposition product SA[161-171]. This is in direct contradiction of the accepted definition of stability indicating assay[71]. In the case of ASA, spectrophotometric measurement at 270-280nm, the wavelength of maximal absorption for ASA is not specific to ASA. SA also shows some absorption in this region. However, by employing the wavelength of maximal SA

absorption, 300nm, ASA absorption is negligible; the assay is rendered stability indicating[74]. That SA should have been the species monitored in the majority of literature stability investigations[192] supports the contention that the choice of species for analysis has been dictated by the relative ease of analysis of reactant and product. That this is the case is supported by the treatment of such analytical data by previous workers[192]. It has been used to calculate the concentration of undecomposed drug[74,186], that is, the conventionaal large extents of reaction and the kinetics applied have been those appropriate to ASA having been quantified. Indeed in several stability reports the decomposition is represented as ASA-time curves when in effect SA was the species measured[74,167,169,170].

The hydrolysis of ASA was chosen as a model reaction in the present work to represent a simple system as defined in Chapter 2, that is, one in which a single stable chemical species is formed by the decomposition of a drug by a unique reaction. This can be represented as shown in Scheme 3.1.

Although other investigations concerning alternative decompositions have been reported[188] it has been established that these are very minor[188] and Scheme 3.1 adequately fulfills the single product type.

In this chapter, this extensively studied reaction is used as a model system to test practically the findings of Chapter 2 which used only simulated data. An assay method is demonstrated employing reverse phase hplc which allows

sensitive detection of SA in the presence of excess ASA. This assay is used to obtain stability data on the hydrolysis reaction by both conventional and initial rate methods. This will allow comparison of the alternative methods of obtaining rate constants, activation energies and logk-pH profiles using a single analytical method. It will be argued that both the analytical methodology and the kinetic treatment of the concentration data will be general for all reactions of this type.

3.2 Experimental

3.2.1 Materials and Equipment

The chromatographic system consisted of a Waters Associates M6000A pump and Model 441 fixed wavelength ultraviolet detector (254nm). Injection was by means of a Rheodyne 7125 valve fitted with a 20ul loop. The columns used were 100 x 4.6mm. slurry packed with 5μm. ODS Hypersil (Shandon). Acetonitrile was obtained from Rathburn Chemicals and ASA and SA from Fisons. All other chemicals were of AnalaR grade or equivalent. Water was purified by a Millipore MilliQ system and stability studies were carried out using a Tecam constant temperature water bath.

3.2.2 Procedure

3.2.2.1 Chromatographic Separation

Reverse phase hplc was chosen as the analytical

method to enable direct injection of aqueous solutions. In such reverse phase systems SA was found to elute after ASA when both species were either completely ionised or unionised. In order to ensure adequate sensitivity for low concentration detection of SA it was necessary to reverse this elution order [111]. While many variables can be modified on an hplc assay to control retention the control and alteration of selectivity is much more difficult. While ion pairing techniques have recently been employed as a method of altering selectivity among compounds where the nature of the ionic charge may differ, this is unhelpful here, where both compounds are monoprotic acids. Ion suppression, on the other hand, can produce a change in elution order if the pK_a values of two such acids are appreciably different. Retention order does not appear to have been manipulated in any of the previously published hplc methods for this system[186-190]. The variation of column capacity factor (k') with pH for ASA and SA are shown in Figure 3.1. It is seen that marked changes are observed in the retention of ionised and unionised forms and the order of elution changes with pH. Selective ion suppression over the range pH 3-5 produces the desired elution order.

A chromatographic solvent of acetonitrile-buffer (10/90), 0.02M in sodium dihydrogen phosphate adjusted to pH 3.5 using 88% orthophosphoric acid resulted in the chromatographic separation shown in Figure 3.2. The retention time of 8 minutes for ASA is considered suitable for routine stability work.

3.2.2.2. Chromatographic Quantitation

The reproducibility of the assay method was determined by nine replicate injections of a partially decomposed ASA solution containing a low concentration of SA. The relative standard deviations of ASA and SA peaks were found to be 0.73% and 0.34% at appropriate detector sensitivities, that is, 0.2AU for ASA and 0.01AU for SA. Standards of each compound were found to produce linear peak height changes with concentration over the ranges used (R^2>0.995) allowing estimation of concentration from peak height measurement. Calibration lines are shown in Figures 3.3. and 3.4 for ASA and SA respectively. Changes in solvent strength at this pH indicated no other products of decomposition. Figure 3.5 shows a chromatogram for the separation of ASA and SA using a solvent of acetonitrile-buffer (5/95), 0.02M in sodium dihydrogen phosphate adjusted to pH 3.5. No additional peaks are seen in the partially decomposed ASA sample used.

3.2.2.3 Stability Measurements

An appropriate concentration of ASA (8×10^{-3}M) to give a peak absorbance of approximately 0.2 on injection of a 20ul sample were prepared in phosphate buffer of various pH values. The solutions were stored in a thermostatted water bath and sampling was carried out at short time intervals to obtain data on the initial rate of SA formation. Sampling was continued to extents of reaction in between 30% and 80% decomposition in order to obtain reliable rate constants for the decomposition of the parent ASA. Data on both product increase and

reactant decrease were thus obtained under identical conditions.

3.3 Results and Discussion

Although the majority of stability investigations concerning the decomposition of ASA have been performed by measurement of SA, such studies have in almost all instances assumed a molar correspondence between ASA and SA under the various experimental conditions used. Before determining the effect of temperature and pH on the hydrolysis of ASA, the molar relationship betwen ASA and SA concentrations was established. Figure 3.6 shows the correspondence between ASA and SA concentrations over 75% decomposition at 60^{o}C in phosphate buffer pH 6.0. The total concentration of ASA and SA at any given time is found to be essentially constant verifying a one to one stoichiometry under the present experimental conditions.

From the above data it is found that the ASA concentration-time data is adequately represented by the first order integrated rate equation when large extents of reaction are followed. The reaction order is confirmed by the initial rate method using initial ASA concentrations ranging from 3.4×10^{-3}M to 3.4×10^{-2}M. Figure 3.7 shows the initial rates of SA formation at these different initial ASA concentrations. A log initial rate against log initial ASA concentration results in the following regression equation

$$\log (\text{Rate}) = 0.94[\text{ASA}] - 3.49$$

This initial rate method has the advantage in that the

reaction order is established over a 10 fold range in ASA concentration which is greater than the highest extent of reaction studied. In addition there exists no necessity to accelerate the decomposition by employing elevated temperatures, nor the need to follow the reaction to unrealistic extents, viz. in excess of 50%. Even when using lower temperatures and allowing less than 5% decomposition the reaction order as determined by the initial rate method requires a short experimental time of some two hours.

Application of the appropriate integrated rate equation, that is, the first order equation, to the ASA concentration-time data obtained during the initial stages of the reaction results in imprecise rate constants. Methods II and III, on the other hand, when applied to the same level of decomposition results in rate constants of acceptable precision. This is demonstrated by the scatter on the various concentration-time points in Figure 3.8. Such data shows the need to follow the reactant concentration over much longer periods of decomposition than is necessary when measuring product concentration.

Having established the stoichiometry and order of reaction, the rate constants at various temperatures and pH values were determined by Methods I, II and III, Method I being followed in excess of 30% decomposition and II and III over the first 10%. The effect of temperature has been determined at pH 6 using temperatures ranging from $30^{o}C$ to $60^{o}C$. Table 3.1 shows the rate constants obtained by the three methods together with their derived activation energies. Table 3.1 also shows the rate

constants calculated from data reported by Edwards[46] at 17°C. The activation energy used in these calculations is the mean of those reported by the same author[46,157]. It can be seen from these results that the rate constants determined by the three methods are in good agreement as are their activation energies. The accuracy of these values is indicated by the adequate agreement with independent literature values[46] (maximum difference 16%). The activation energy are also in good agreement with the many previously reported values, mean = 71.38kJmol^{-1}, n = 5 standard deviation = 5.02[46,158,162,163,169].

The effect of pH on ASA decomposition at 50°C is shown in Table 3.2 in terms of rate constants, their associated relative standard deviation and correlation coefficient. Comparable rate constants are obtained by all three methods. No comparable data was located in the literature. The most comprehensive logk-pH profile was determined at 17°C[46]. The results of that investigation were used to calculate rate constants at 50°C at the corresponding pH values using the mean activation energy. These calculated values are shown in Figure 3.9 to be in agreement with those in the present investigation. Thus the present work shows the same general features of the logk-pH profile as obtained by Methods I, II and III. Optimum stability is observed at pH 2.5. Below this pH the rate of decomposition increases markedly with small pH changes. Between the pH range 4.5-8.5 the rate of hydrolysis is pH independent, a dramatic increase

occurring above pH 8.5.

3.4 Conclusions

The reverse phase chromatographic method developed allows the elution of SA before ASA for greater analytical sensitivity in measuring SA concentration. Using this chromatographic assay method it has been possible simultaneously to monitor the increase in SA and decrease in ASA concentrations by appropriate choice of sensitivity. In this way the applicability of Methods I, II and III have been practically assessed.

The results show that in the case of a simple A⟶B decomposition comparable rate constants with similar precision can be obtained by any of the three methods. The conventional integrated approach, however, in this case required some two to six hours for the determination of a single rate constant. Both Methods II and III, on the other hand, allowed the determination of equally precise rate constants within twenty to thirty minutes. Method II, however, suffers from the need to determine the undecomposed ASA concentration with no additional advantage over Method III.

The simple decomposition of ASA ⟶ SA shows the advantage in applying Method III, that is, measuring the product increase over much lower extents of decomposition and confirms the simulated findings for a simple non-reversible reaction discussed in Chapter 2.

Table 3.1 Comparison of the rate constants and activation energies obtained from ASA decomposition using Methods I, II and III.

Temperature ^{o}C	Rate Constants ($x10^{3}min.^{-1}$) Method I	Method II	Method III	Literature Values*
30	0.27	0.29	0.28	0.34
40	0.72	0.87	0.72	0.84
50	2.00	2.14	2.04	2.26
60	4.67	4.90	4.40	5.55
E_a $kJmol^{-1}$	79.34	77.92	76.79	-
RSD(%)	5.30	3.16	6.15	-
R^2	0.999	0.999	0.998	-

* Calculated from data presented by Edwards[46] at $17^{o}C$ using an activation energy of 78.0 $kJmol^{-1}$.

Table 3.2 Variation in rate constants obtained with pH for ASA decomposition using Method

	Method I			Method II	
pH	Rate constant ($min.^{-1} \times 10^3$)	RSD	R^2	Rate constant ($min.^{-1} \times 10^3$)	RSD
1.20	8.50	2.24	0.998	8.75	4.87
2.00	0.54	36.4	0.750	0.53	5.70
2.50	0.60	10.0	0.978	0.51	1.21
2.90	0.77	6.41	0.984	0.68	2.49
3.45	1.21	2.39	0.996	1.30	2.00
4.00	1.83	2.08	0.999	1.64	4.53
5.00	2.10	4.03	0.989	2.11	1.42
6.00	2.01	4.68	0.985	2.14	3.20
7.50	2.38	4.20	0.995	2.03	2.35
8.50	2.27	4.10	0.993	2.61	5.25
9.70	2.88	4.58	0.986	2.98	0.96
11.10	78.20	1.98	0.997	82.3	1.14

I, II and III at 50° C.

			Method III	
R^2	Initial Rate (mol.dm.$^{-3}$ min.$^{-1}$x10^5)	RSD	R^2	Rate constant (min.$^{-1}$x10^3)
0.993	4.45	7.12	0.975	6.32
0.985	0.64	5.68	0.991	0.54
0.999	0.39	1.08	0.999	0.51
0.997	0.52	3.00	0.995	0.67
0.998	0.97	2.40	0.997	1.24
0.992	1.29	3.76	0.993	1.66
0.999	1.56	1.90	0.998	2.03
0.995	1.60	3.05	0.995	2.04
0.998	1.51	3.41	0.994	1.90
0.987	1.81	4.50	0.995	2.43
0.999	2.18	1.79	0.998	2.77
0.999	44.10	2.01	0.992	60.2

Scheme 3.1 Showing the decomposition of ASA to SA.

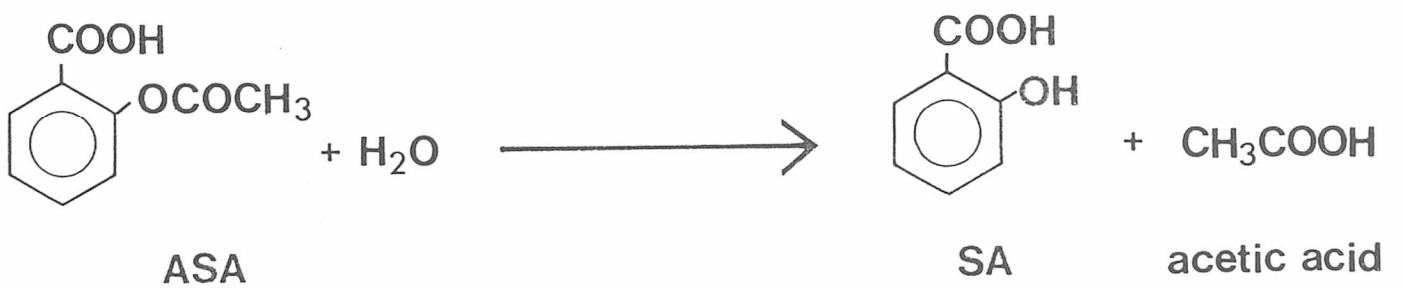


Figure 3.1 Plot showing the variation in capacity factor (k') with pH of the solvent for ASA and SA.
Solvent: acetonitrile : water (10/90) containing 0.02M phosphate buffer.

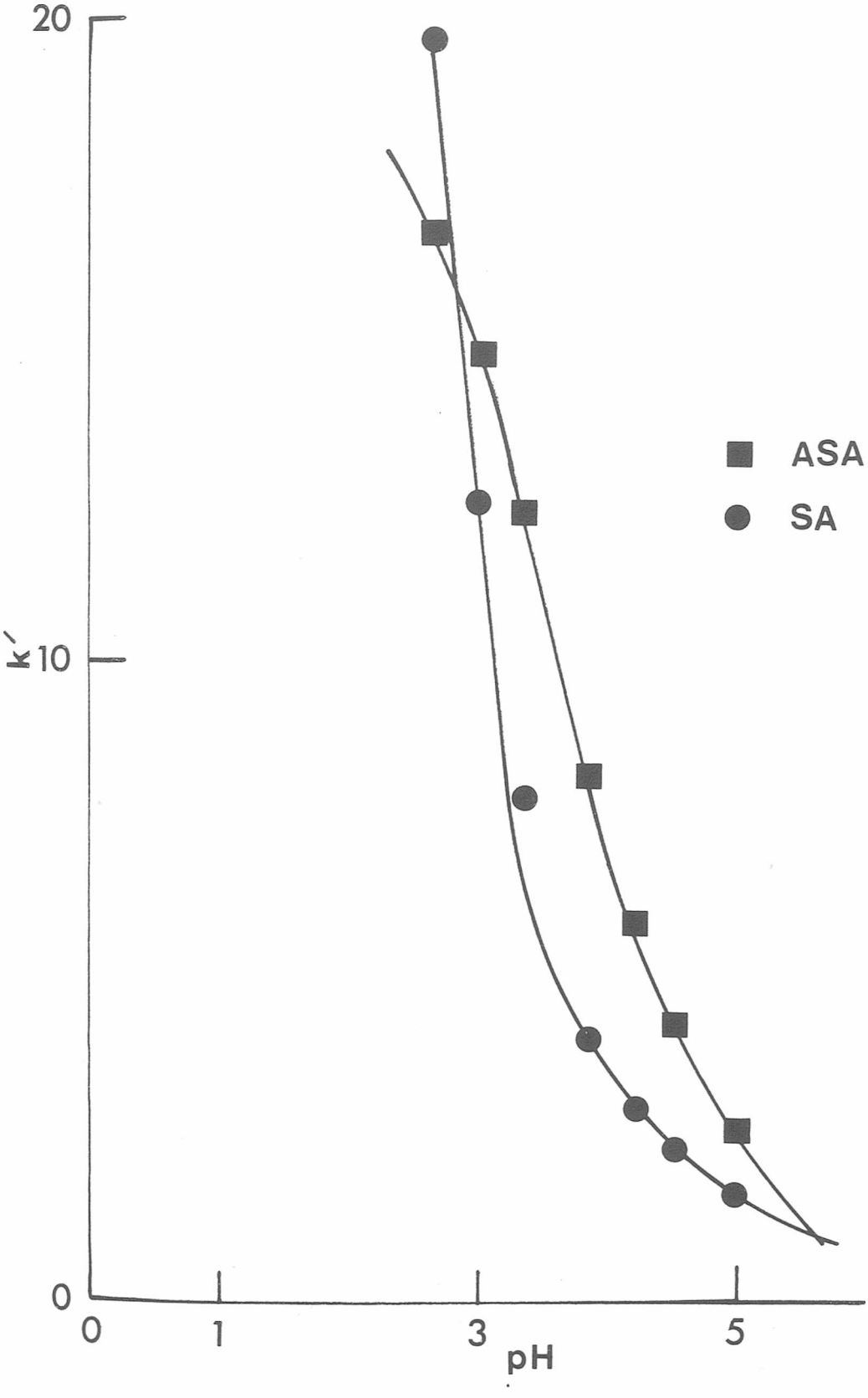


Figure 3.2 Showing the optimum chromatogram for the separation of ASA and SA. A represents undecomposed ASA. B represents a partially decomposed sample. ASA measured at 0.2 AU and SA measured at 0.01AU.
Solvent: acetonitrile : water (10/90) containing 0.02M phosphate buffer at pH 3.5

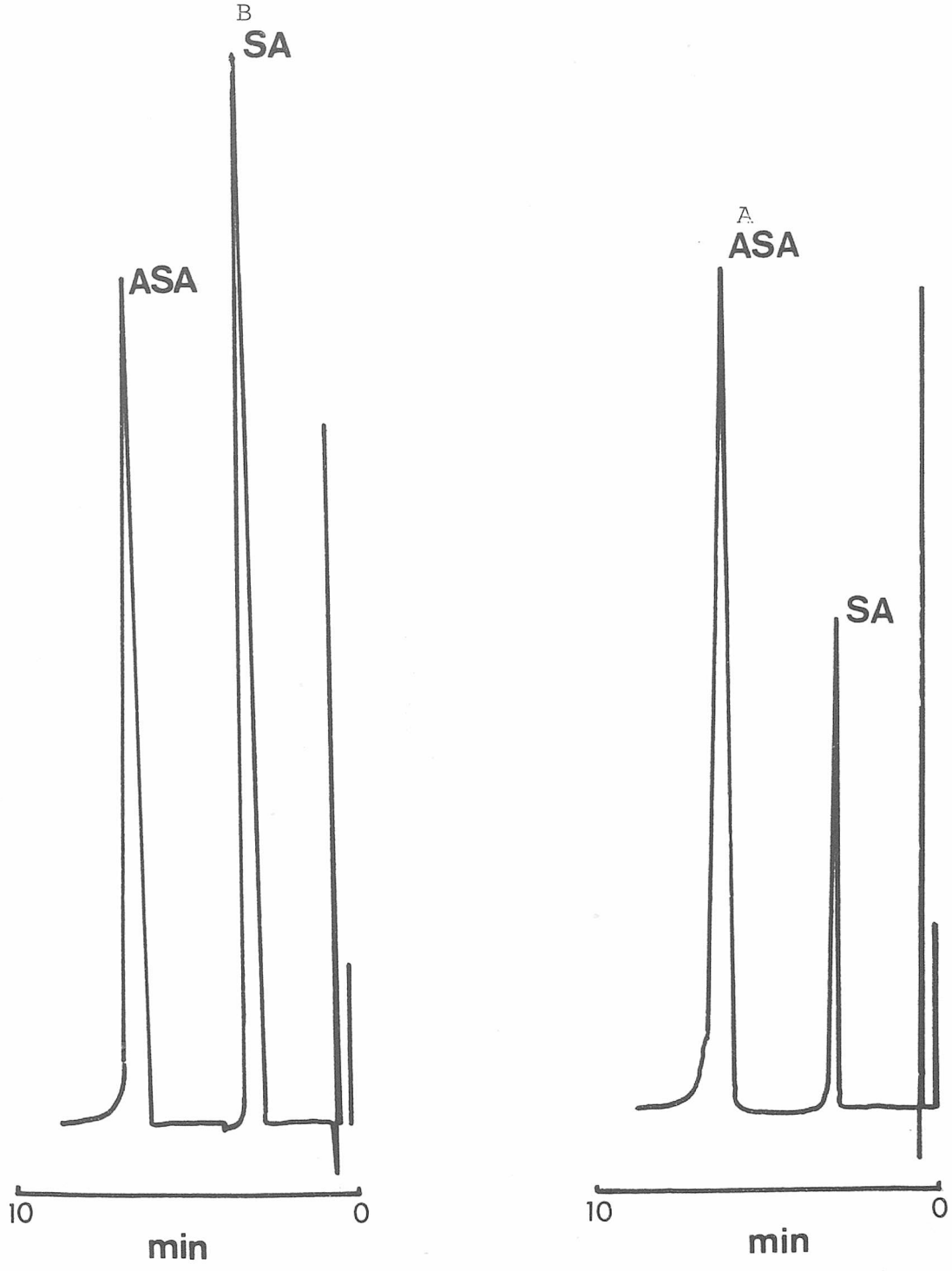


Figure 3.3 Showing the variation in peak height for various ASA concentrations at 0.2AU.

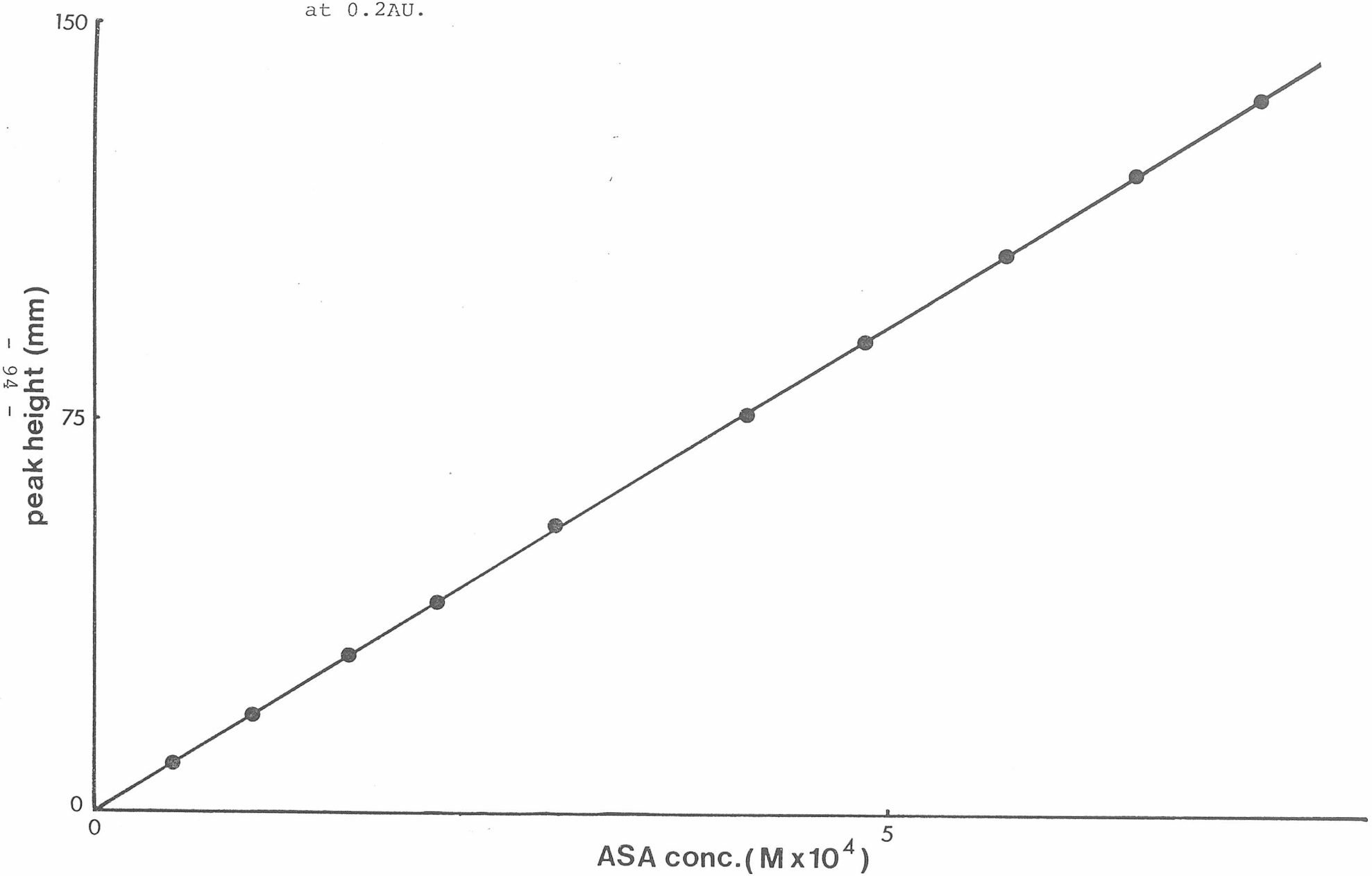


Figure 3.4 Showing the variation in peak height for various SA concentrations at 0.01AU.

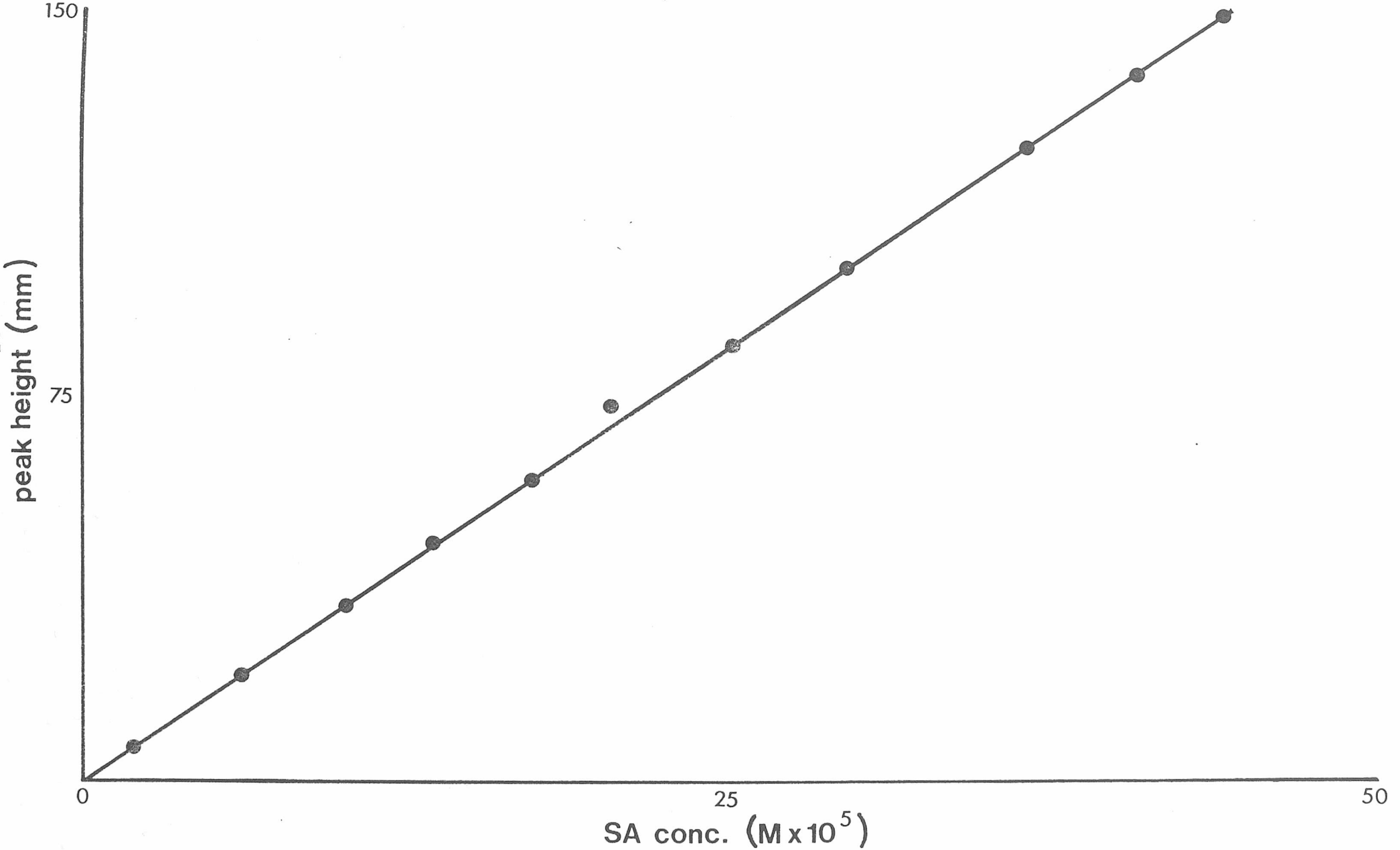


Figure 3.5 Showing the absence of additional products during the decomposition of ASA. Chromatogram obtained at reduced solvent strength. Solvent: acetonitrile : water (5/95) containing 0.02M phosphate at pH 3.5. Sensitivity of detection equals 0.01AU.

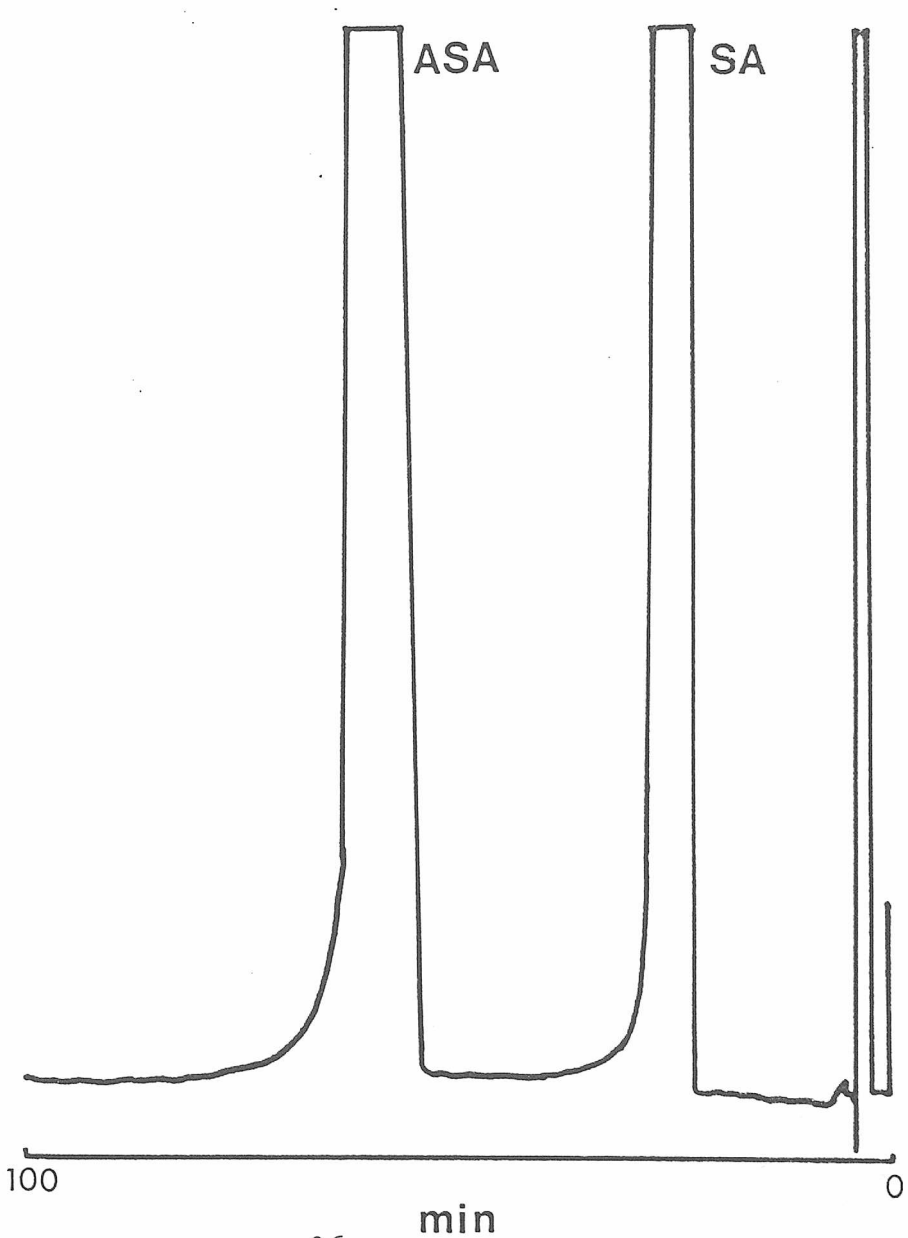


Figure 3.6 Showing the stoichiometric correspondence between ASA and SA during ASA decomposition.

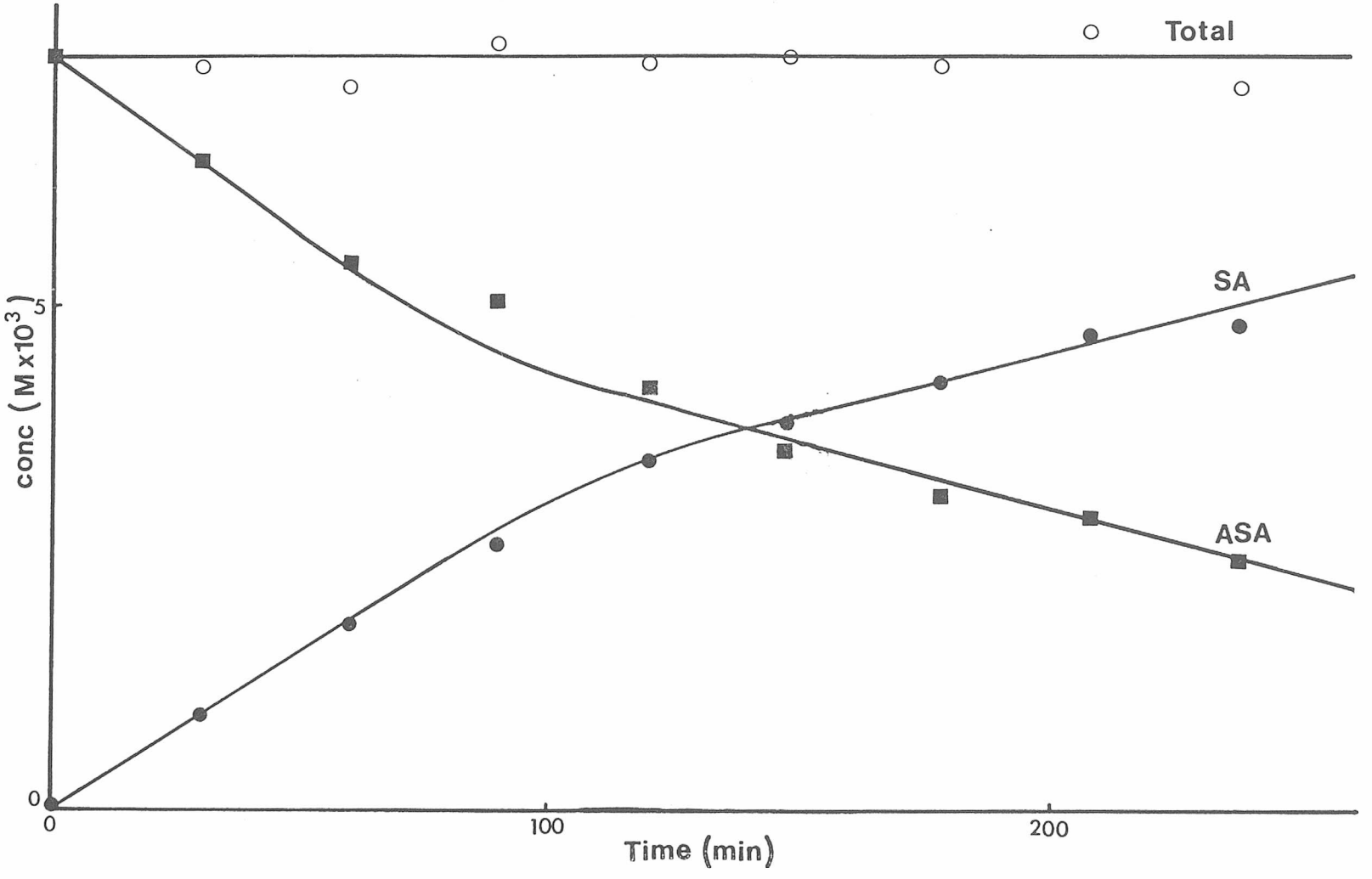


Figure 3.7 Showing the initial rate of SA formation for different initial concentrations of ASA. Initial molar ASA concentrations shown in parenthesis.

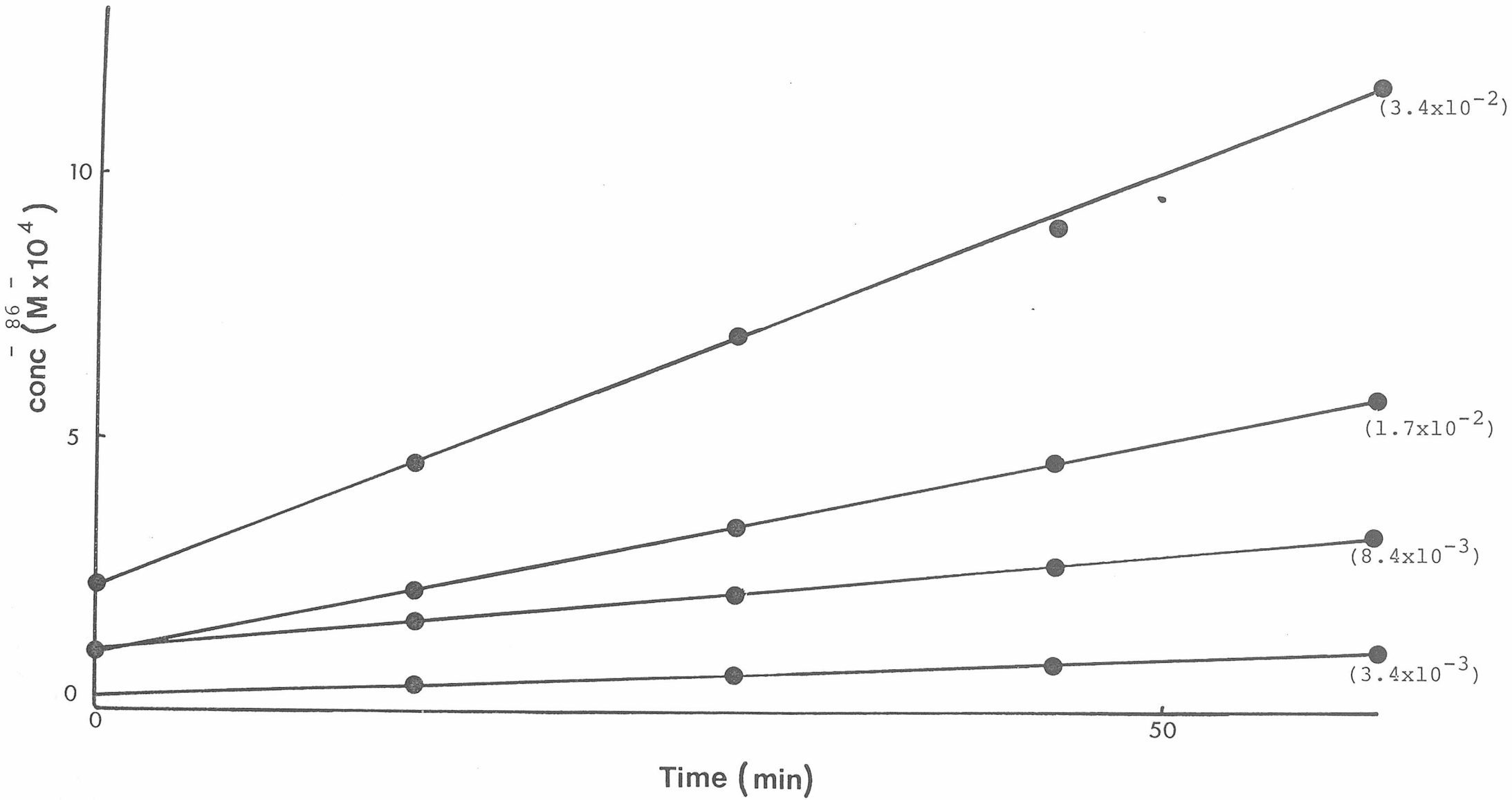


Figure 3.8 Showing the precision in determining rate constants by Methods I, II and III during the initial stages of ASA decomposition.

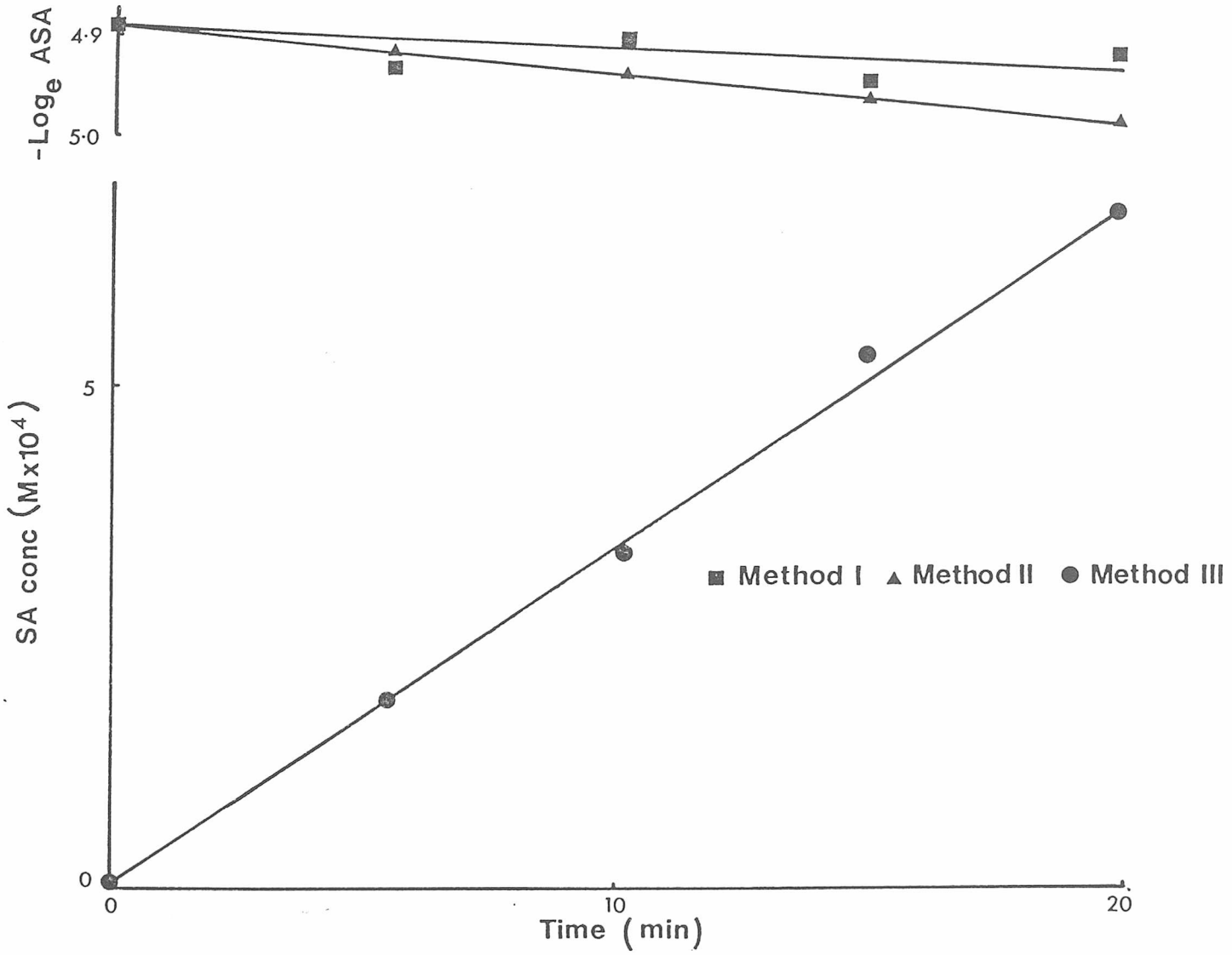
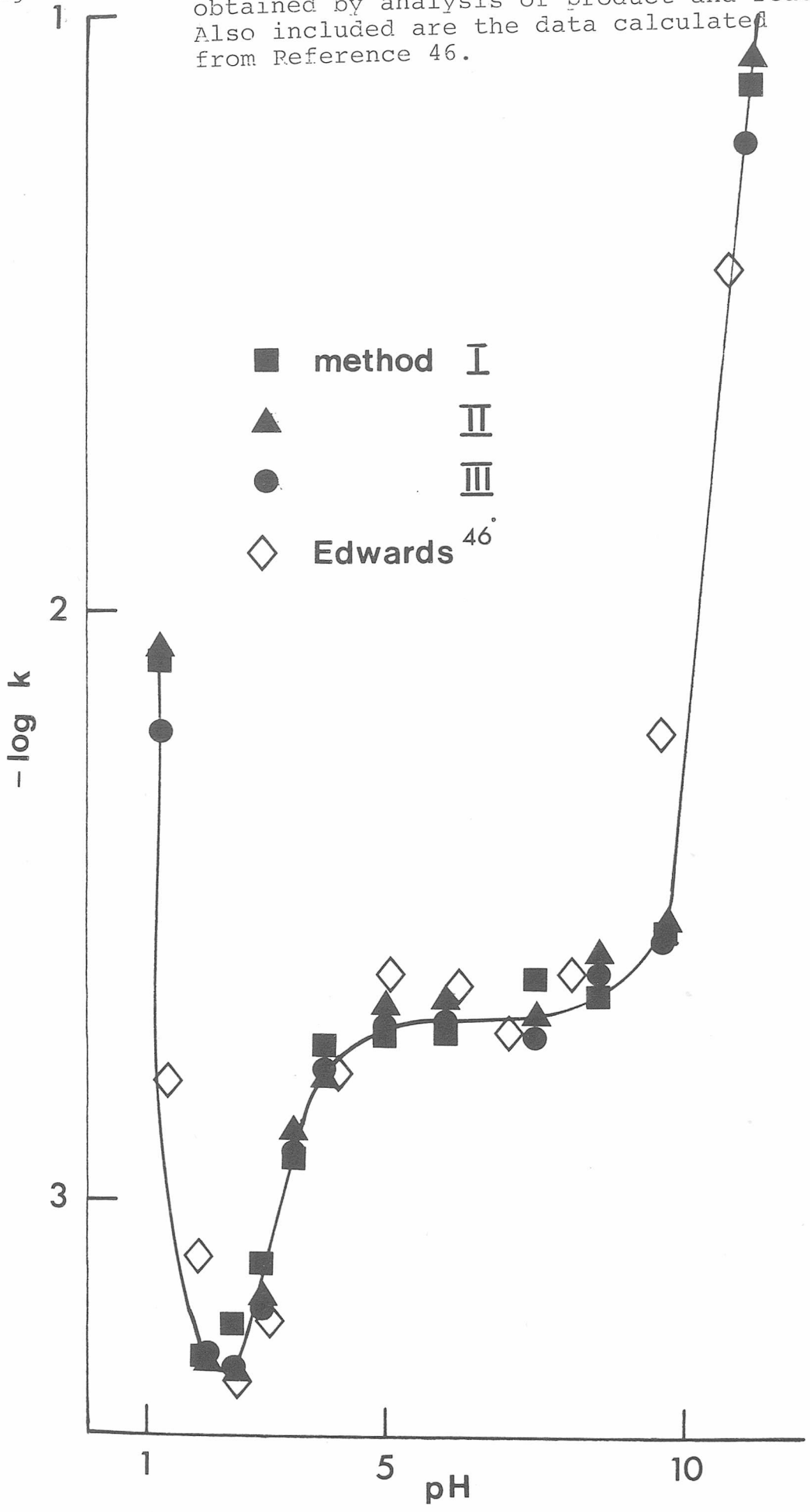


Figure 3.9 Showing the logk - pH profile for ASA at 50°C obtained by analysis of product and reactant. Also included are the data calculated from Reference 46.



Chapter 4

4.0 The Decomposition of Diiodoacetylsalicylic Acid

4.1 Introduction

Although ASA is more renowned for its antipyretic and analgesic activity, more recently it has been shown to possess antisickling activity in the treatment of sickle cell anemia[193]. Sickle cell anemia is a haematological disorder in which the erythrocytes undergo transformation from the normal spherical shape to the abnormal sickle shape. The fundamental abnormality is the presence of an abnormal form of haemoglobin (Hb.S) leading to cell aggregation. Such aggregates are readily trapped in the microvasculature, the blockage leading to tissue damage and haemolytic anemia[194]. In vitro acetylation of Hb.S has been found to reverse or prevent such aggregation by acetylation of the amine groups in Hb.S although sickling may not be completely inhibited[195]. A number of compounds have been shown to possess such antisickling activity, these including urea and substituted alkylureas [196], ethanol[197], mono and dihalo-substituted aspirins[197] and double headed aspirins or bis-salicylates[198]. The beneficial effects of ASA therapy in sickle cell anemia patients has recently been demonstrated[193].

It has been suggested that the dihalogen analogues of ASA may be more effective than ASA[199], monohalo derivatives showing decreased haemoglobin modification in

the order I > Br > Cl > F[194] leading to the suggestion that the dihalo analogue 3,5-diiodoacetylsalicylic acid (DIASA) may be most effective[200].

While the literature contains numerous reports of investigations on the solution stability of ASA, no data regarding the solution stability of DIASA or any other dihalo derivatives has appeared in the literature. During the course of this work it became necessary to evaluate the stability of DIASA in aqueous solution before investigations into the potential of this new aspirin derivative could be initiated[200]. In addition, the decomposition of DIASA to a single decomposition product, 3,5-diiodosalicyclic acid (DISA) represents a novel system on which to evaluate the proposed approach of determining stability by measuring the increase in decomposition product. Stability determinations have been carried out in a similar manner to that for ASA (Chapter 3).

4.2 Experimental Procedure

4.2.1 Synthesis of DIASA

This compound was not available commercially and was prepared by acetylation of DISA (Aldrich). To one gram of DISA was added 40ml. acetic anhydride and a few drops concentrated sulphuric acid. The resultant mixture was gently heated in a water bath (40^{o}C) until all the DISA had dissolved. The reaction mixture was rapidly poured into ice cold distilled water and the product was precipitated as a white solid. The precipitate was washed

with 300ml. of ice cold water and dried in vacuo. The spectral data were consistant with the expectant structure of DIASA. Spectral data: n.m.r. ($CDCl_3$) δ 4.76 (S,3), δ 8.05 (d,1,$J_{4,6}$ = 2.5Hz), δ 8.13 (d,1,$J_{4,6}$ = 2.5Hz), i.r. ν (cm^{-1}), 1685 (Acid C=O), 1765 (Ester C=O), m.p. 152-154°C, literature value = 153°C[201]. Figures 4.1 and 4.2 show the n.m.r. and infrared spectra respectively. Figure 4.3 shows the ultraviolet characteristics of both DIASA and DISA. The purity of DIASA was determined, using the chromotographic system described below, as 97%.

4.2.2. Chromatographic Separation

Chromatographic separation of DISA from DIASA using reverse phase hplc was achieved in a manner similar to that described for SA/ASA (Chapter 3) using selective ion suppression. Figure 4.4 shows the variation in column capacity factor for each compound with pH. Over the pH range 2 to 4, DISA is eluted prior to DIASA, that is the desired elution order is obtained within this pH range.

A chromatographic solvent of acetonitrile-buffer (30/70), 0.06M in sodium dihydrogen phosphate adjusted to pH 3.0 with 88% orthophosphoric acid resulted in the chromatographic separation shown in Figure 4.5.

4.2.3. Chromatographic Quantitation

The reproducibility of the assay was determined by nine replicate injections of a partially decomposed DIASA solution containing a low concentration of DISA. The relative standard deviations of DIASA and DISA peaks were

found to be 2.80% and 1.54% respectively. In following the decomposition reaction appropriate detector sensitivities were used and standards of each compound were found to produce linear peak height changes with concentration over the ranges used as shown in Figures 4.6 and 4.7. Peak height measurements were thus adequate for estimation of concentration. Figure 4.8 shows the absence of any decomposition products at high sensitivity other than DISA when the solvent strength was reduced.

4.2.4 Stability Measurements

Appropriate concentrations of DIASA to give peak absorbance values of approximately 1.0 an injection of a 20µl sample were prepared in buffer solutions of various pH values. The solutions were stored in a thermostatted water bath and sampling was carried out as described for the decomposition of ASA. Data on both product increase and reactant decrease were thus obtained under identical conditions.

4.2.5 Material and Equipment

The chromatographic system used was the same as that for ASA with the exception that a Pye LC871 detector operated at 220nm was used to maximise sensitivity for both species, although use of 270nm would have allowed the use of higher initial DIASA concentrations.

4.3 Results and Discussion

Prior to determining the effect of temperature and pH, the stoichiometry of the reaction was determined. As shown in Figure 4.9, the decomposition of DIASA to DISA was found to occur on a mole for mole basis over 50% decomposition at pH 6 and 60°C. The DIASA concentration-time data were found to be adequately fitted by the first order integrated rate equation, this being confirmed by the initial rate method using initial DIASA concentrations ranging from 2×10^{-4} to 5×10^{-4}M. The initial rate of formation of DISA was found to vary with DIASA concentration as

$$\log(\text{Rate}) = 0.92[\text{DIASA}] - 3.45$$

Table 4.1 shows the first order rate constants obtained at different temperatures at pH 6 using temperatures ranging from 30°C to 70°C together with their activation energies, relative standard deviations and correlation coefficients. The variation in rate constant with temperature is shown in Figure 4.10. Comparison with data shown in Table 3.1 indicates that DIASA is more stable than the non-iodinated compound. The activation energy, estimated at 73 $kJmol^{-1}$ is slightly lower than that determined for ASA of 78 $kJmol^{-1}$ (Chapter 3).

The effect of pH on the rate constants obtained at 60°C is shown in Table 4.2. The logk-pH profile over the range studied shown in Figure 4.11 is similar to that obtained for ASA. Values below pH 2 could not be measured because of the inadequate solubility of this drug at very low pH. This, presumably, is as a result of increased hydrophobicity on halogenation.

Tables 4.1 and 4.2 also show in the case of this drug decomposition system, that like for the ASA/SA system, comparable rate constants can be obtained with similar precision by application of Methods I, II and III. Method I however required three to six hours for determination of a single rate constant. The initial rate method, on the other hand, allowed the determination of rate constants within thirty minutes.

4.4 Conclusions

Chromatographic and stability data for a new drug, DIASA, are presented. The proposed method of determining the stability of a simple decomposition system indicates that equally precise results can be obtained by following product increase in a shorter time scale. DIASA is found to be approximately twice as stable as ASA. The hydrolysis is affected in much the same manner by pH and it would appear that a similar reaction mechanism as that for ASA is operative.

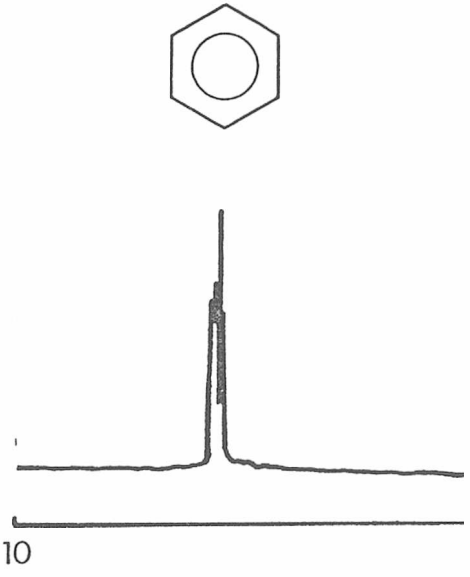
Table 4.1 Comparison of the rate constants and activation energies obtained from DIASA decomposition using Methods I, II and III.

Temperature ^{o}C	Method I Rate Constant ($x10^3$ $min.^{-1}$)	Method II Rate Constant ($x10^3$ $min.^{-1}$)	Method III Rate Constant ($x10^3$ $min.^{-1}$)
30	0.18	0.22	0.20
50	0.99	1.29	1.28
60	2.64	2.73	2.62
65	3.70	3.78	3.55
70	5.44	6.18	5.82
E_a $kJmol^{-1}$	74.80	70.50	71.70
RSD (%)	2.3	5.70	3.0
R^2	0.999	0.997	0.999

Table 4.2 Variation in rate constants obtained with pH for DIASA decomposition using Methods I, II and III at 60° C.

	Method I			Method II			Method III			
pH	Rate constant ($min.^{-1} \times 10^3$)	RSD	R^2	Rate constant ($min.^{-1} \times 10^3$)	RSD	R^2	Initial Rate ($mol.dm.^{-3} min.^{-1} \times 10^5$)	RSD	R^2	Rate constant ($min.^{-1} \times 10^3$)
1.9	2.30	8.26	0.984	2.48	0.81	0.999	1.45	0.85	0.999	2.16
2.3	2.94	2.04	0.999	3.16	2.22	0.999	1.82	2.79	0.998	3.01
3.7	2.65	1.89	0.991	2.71	2.21	0.999	1.46	2.00	0.999	2.61
5.0	3.02	2.65	0.998	2.83	2.47	0.998	1.42	0.48	0.994	2.59
6.0	2.64	3.03	0.997	2.73	5.46	0.993	1.08	5.18	0.993	2.62
6.9	3.00	5.00	0.994	3.01	3.33	0.997	1.71	0.38	0.997	2.88
8.0	2.73	4.41	0.994	3.66	2.73	0.998	1.29	2.74	0.998	3.47
9.5	5.84	3.60	0.997	9.84	2.24	0.999	3.45	3.30	0.998	9.16
10.0	12.03	5.74	0.995	22.4	4.64	0.996	7.53	0.62	0.992	12.00

Figure 4.1 Showing the ^{1}H-NMR spectrum for DIASA.



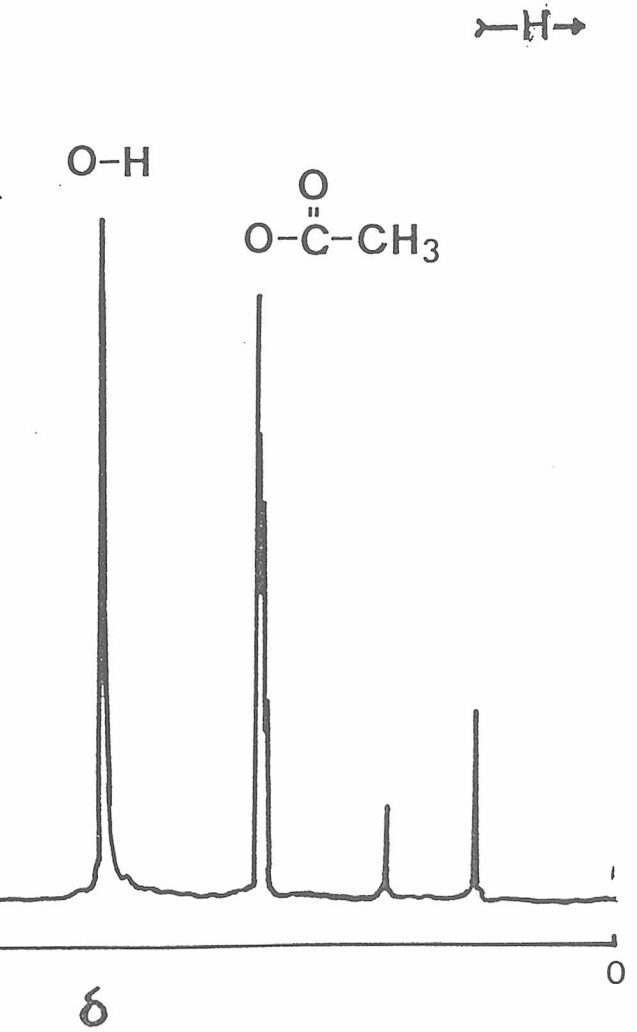

H
O–H
O
O–C–CH3
0
δ

Figure 4.2 Showing the infrared spectrum for DIASA in Nujol Mull.

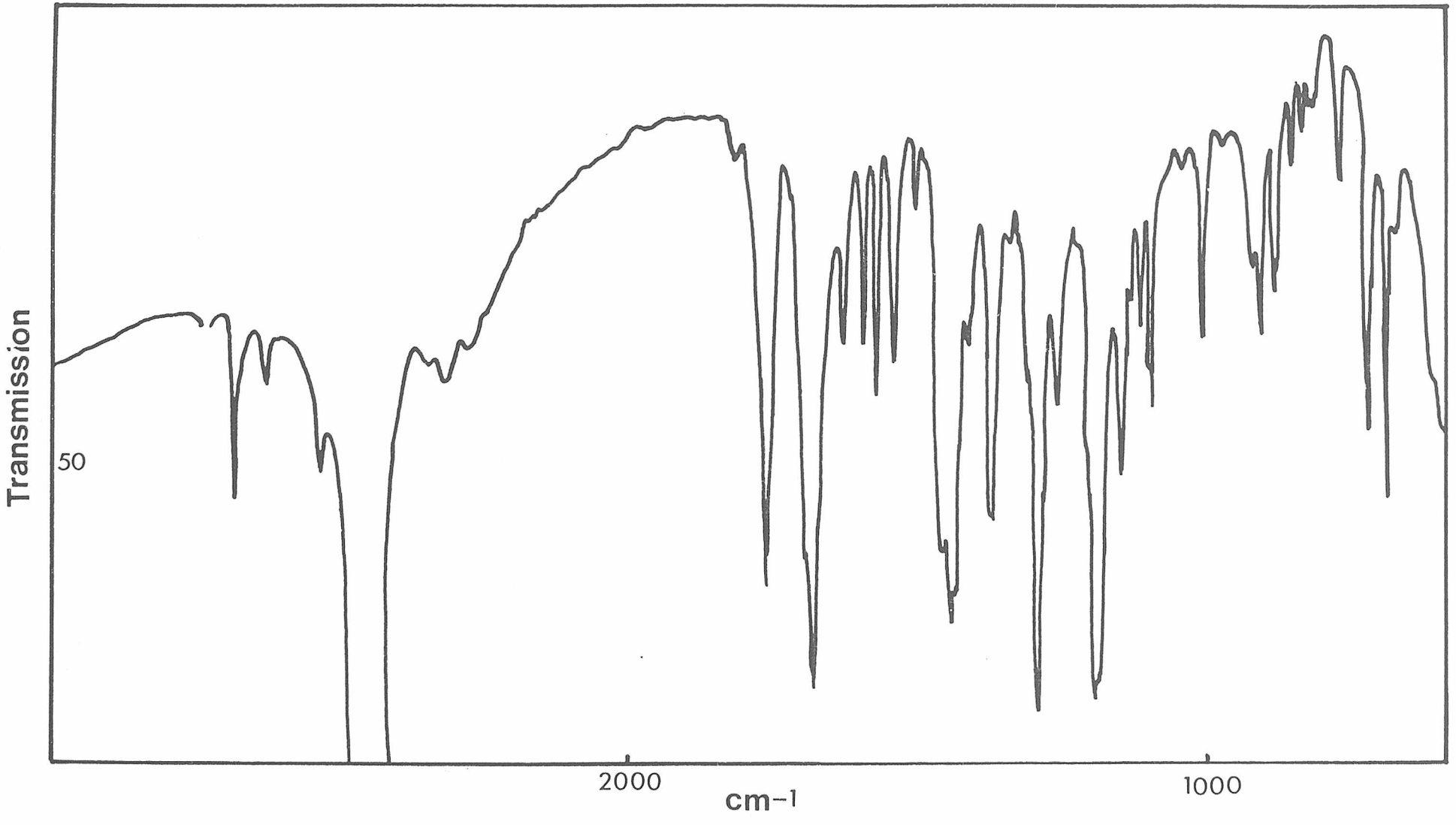


Figure 4.3 Showing the ultraviolet spectra for DIASA and DISA in methanol.

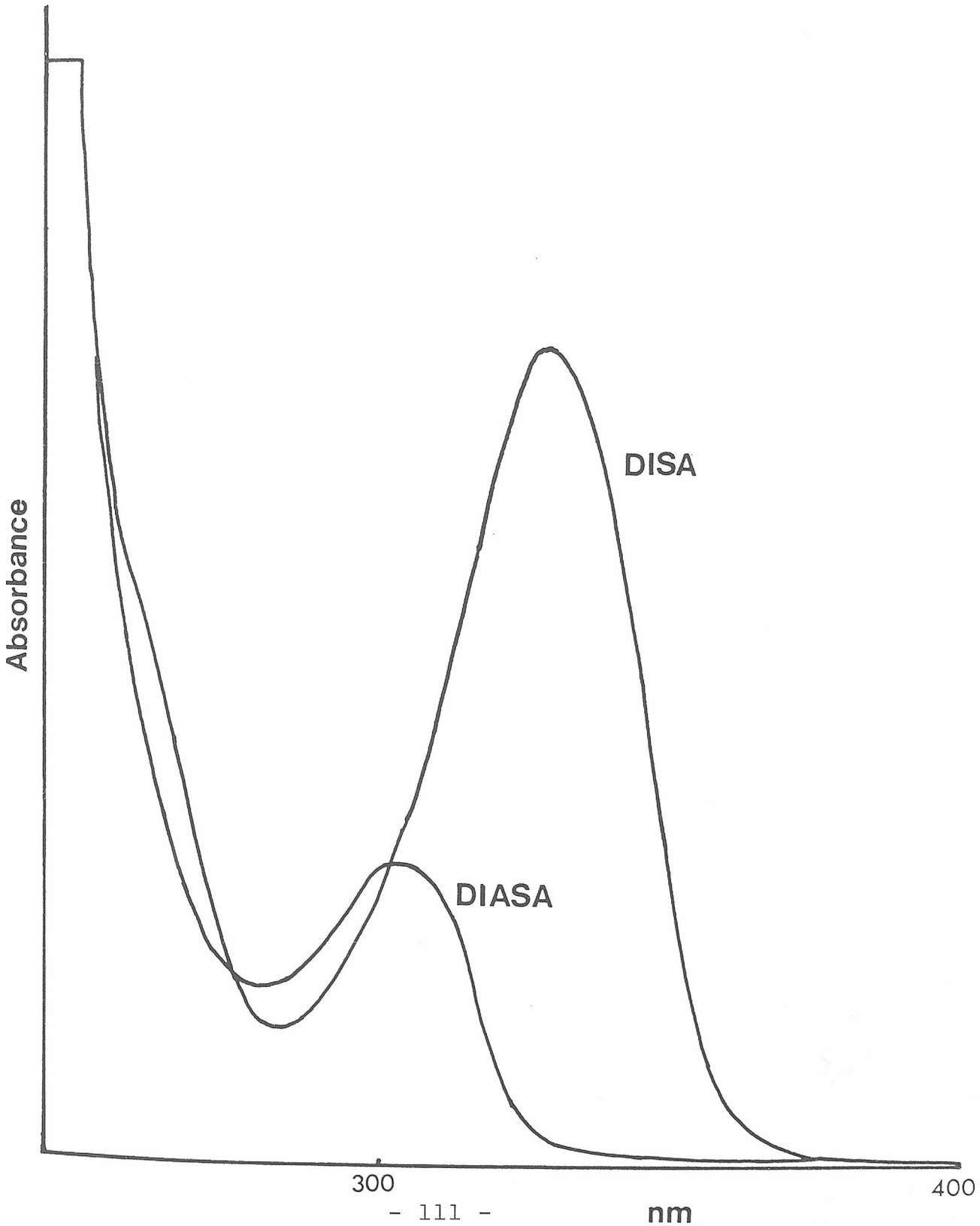


Figure 4.4 Plot showing the variation of capacity factor (k') with pH of the solvent for DIASA and DISA. Solvent comprises acetonitrile : water (30/90) containing 60mM phosphate buffer.

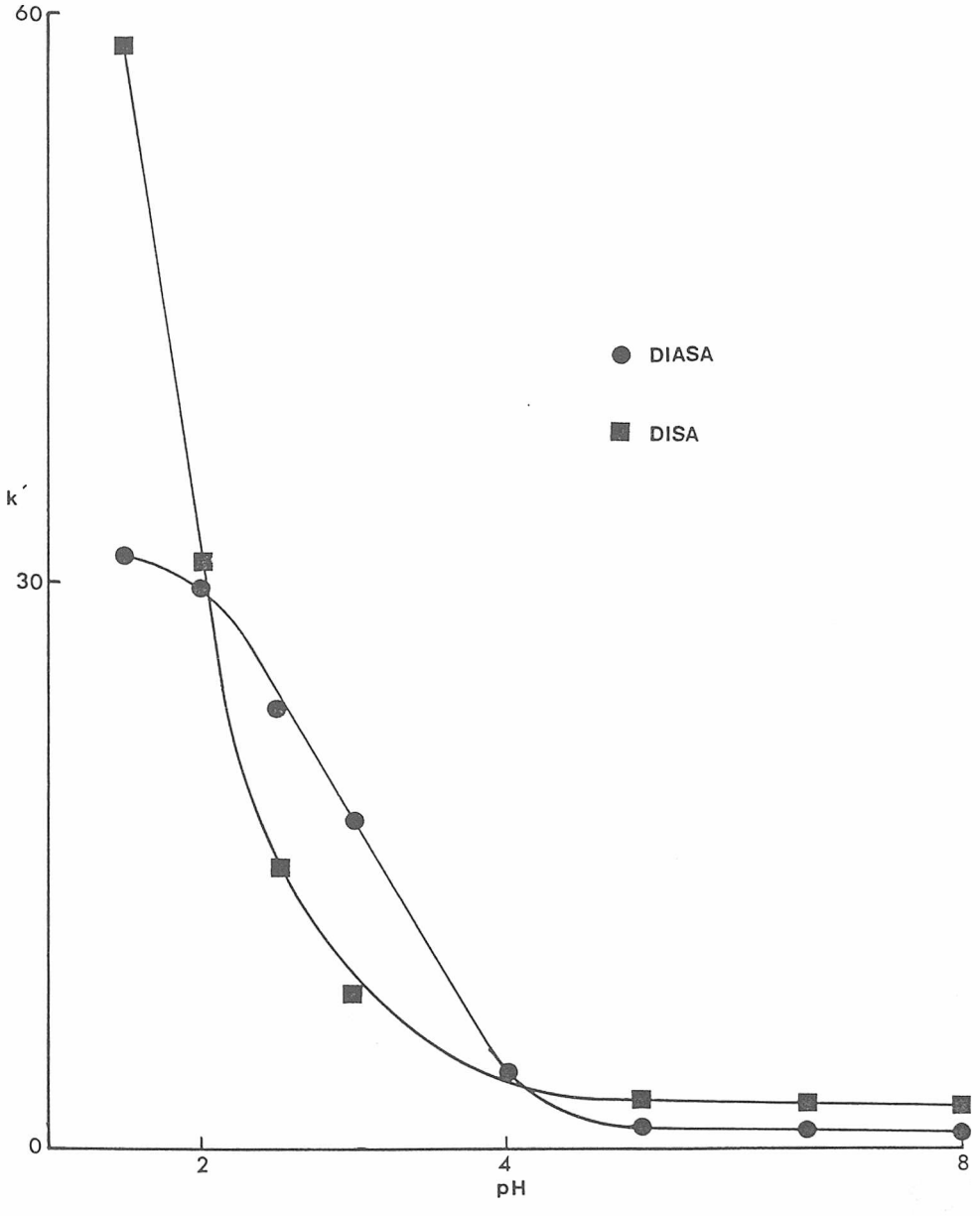


Figure 4.5 Showing an optimum chromatogram for the separation of DIASA and DISA in a partially decomposed DIASA sample at 0.1AU. Solvent: acetonitrile : water (30/90) containing 60mM phosphate buffer at pH 3.

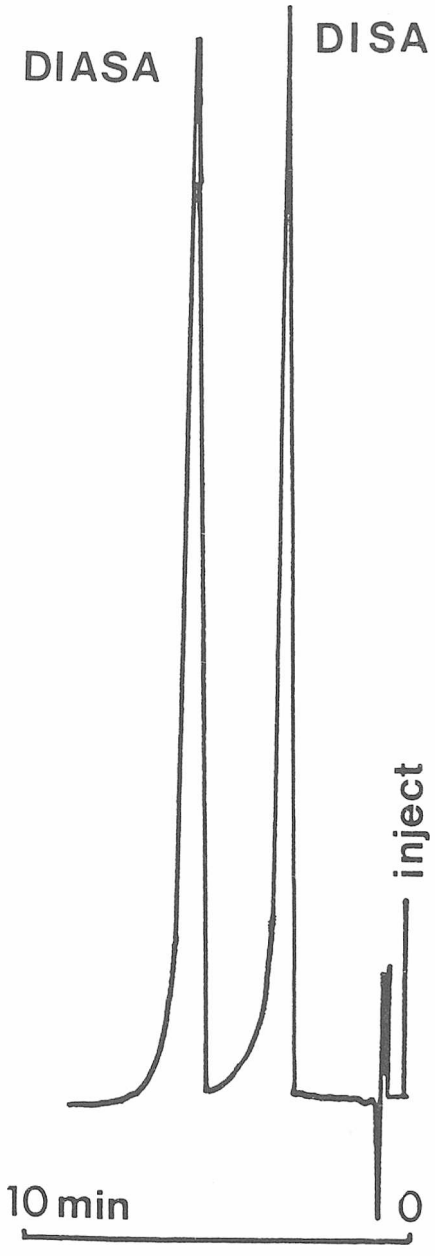


Figure 4.6 Plot showing the variation in peak height as a function of DIASA concentration at 0.64AU.

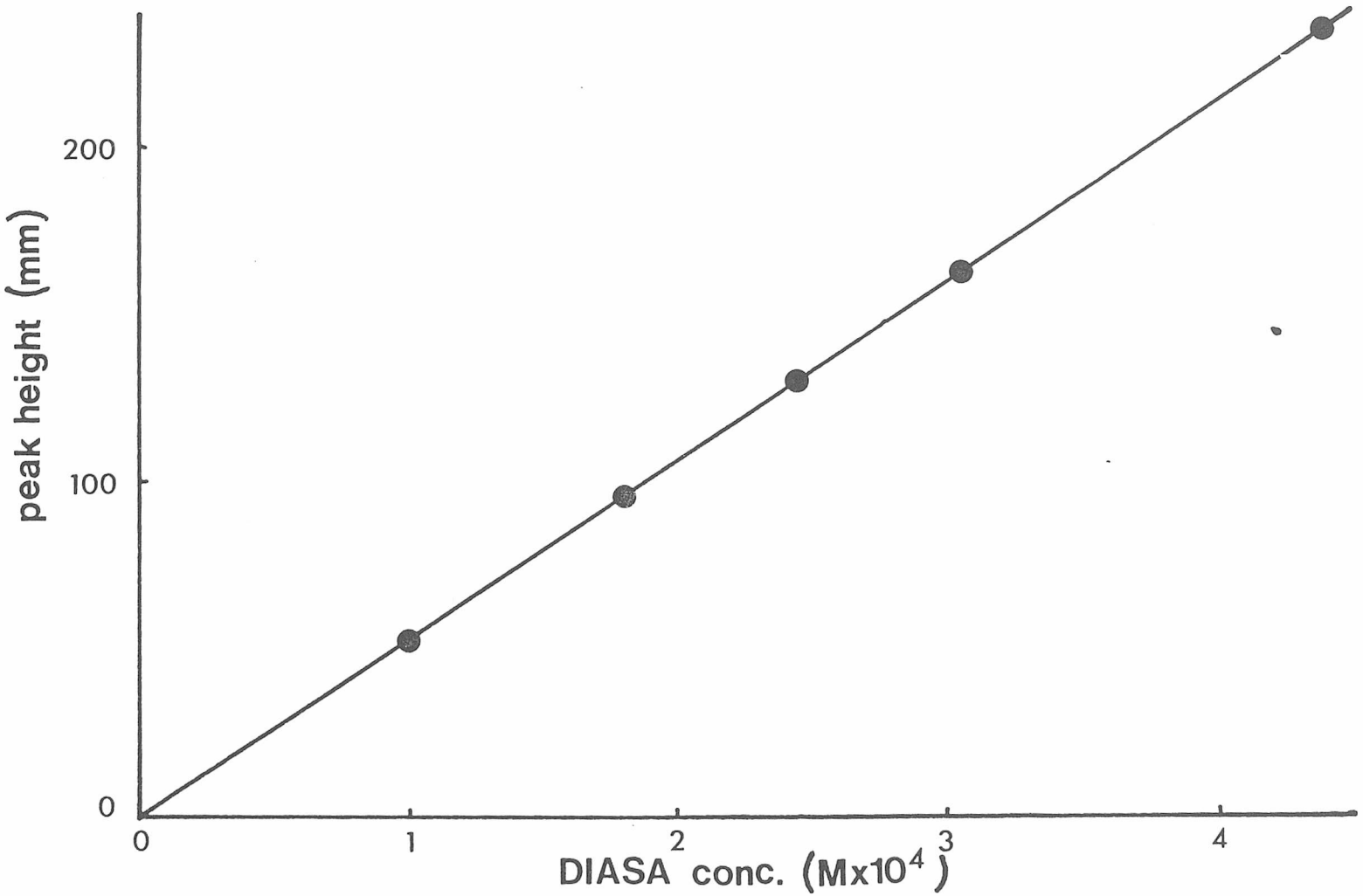


Figure 4.7 Plot showing the variation in peak height as a function of DISA concentration at 0.04AU.

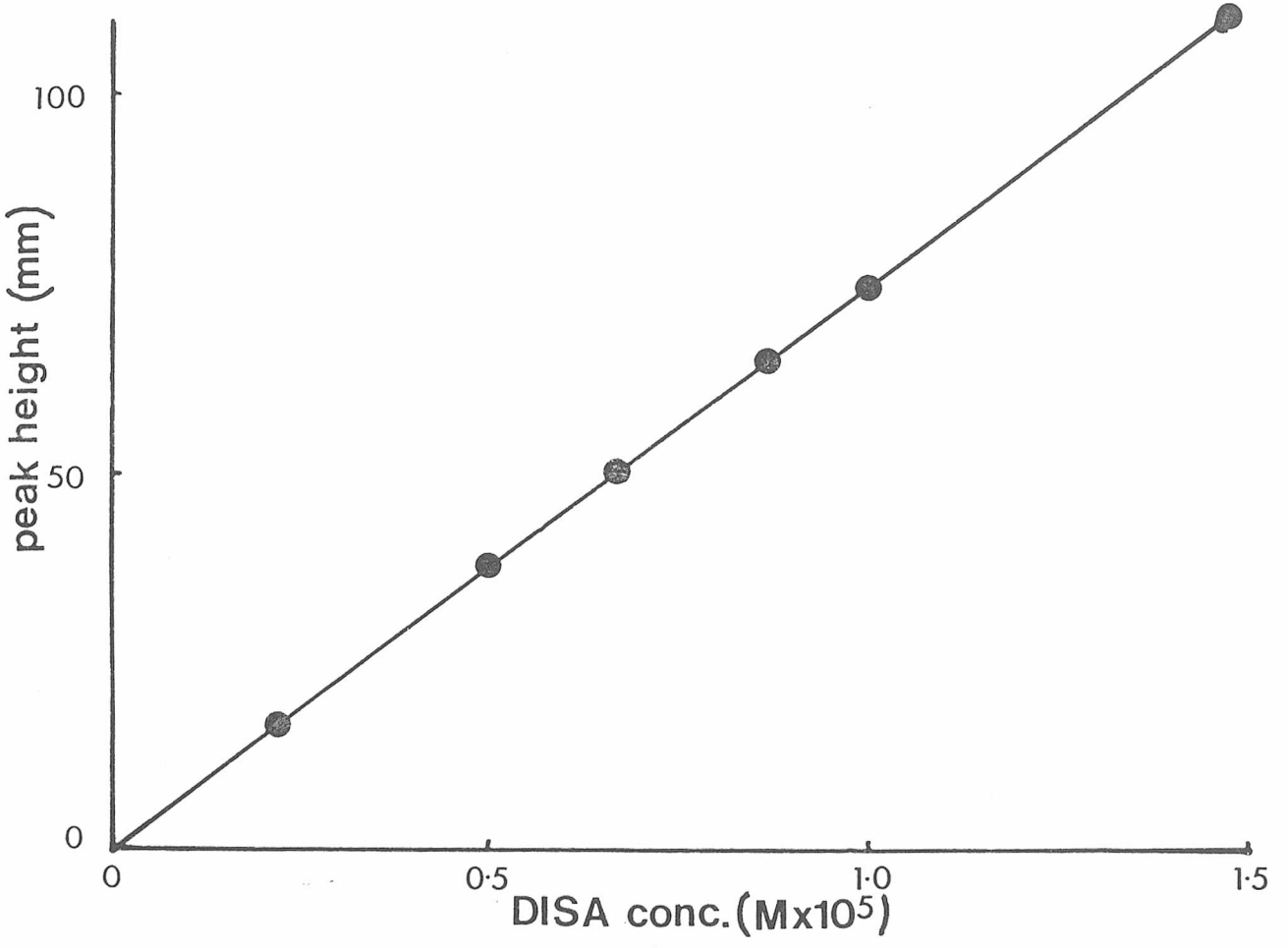


Figure 4.8 Showing the separation of DIASA and DISA at reduced solvent strength. No additional peaks observed indicating DISA as the only product. Solvent: acetonitrile : water (20/80) containing 60mM phosphate buffer at pH 3.0.

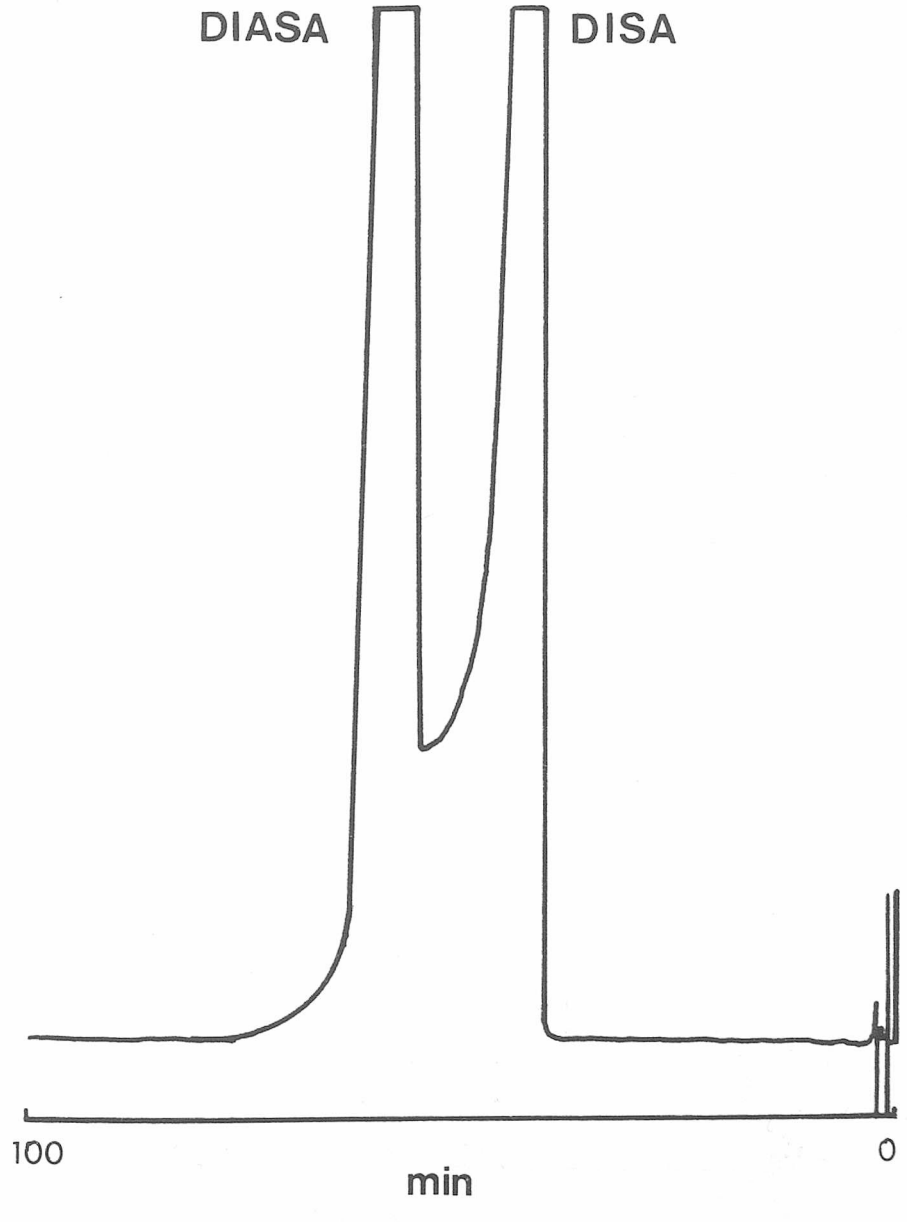


Figure 4.9 Showing the stoichiometry between DIASA and DISA during the decomposition of DIASA to DISA.

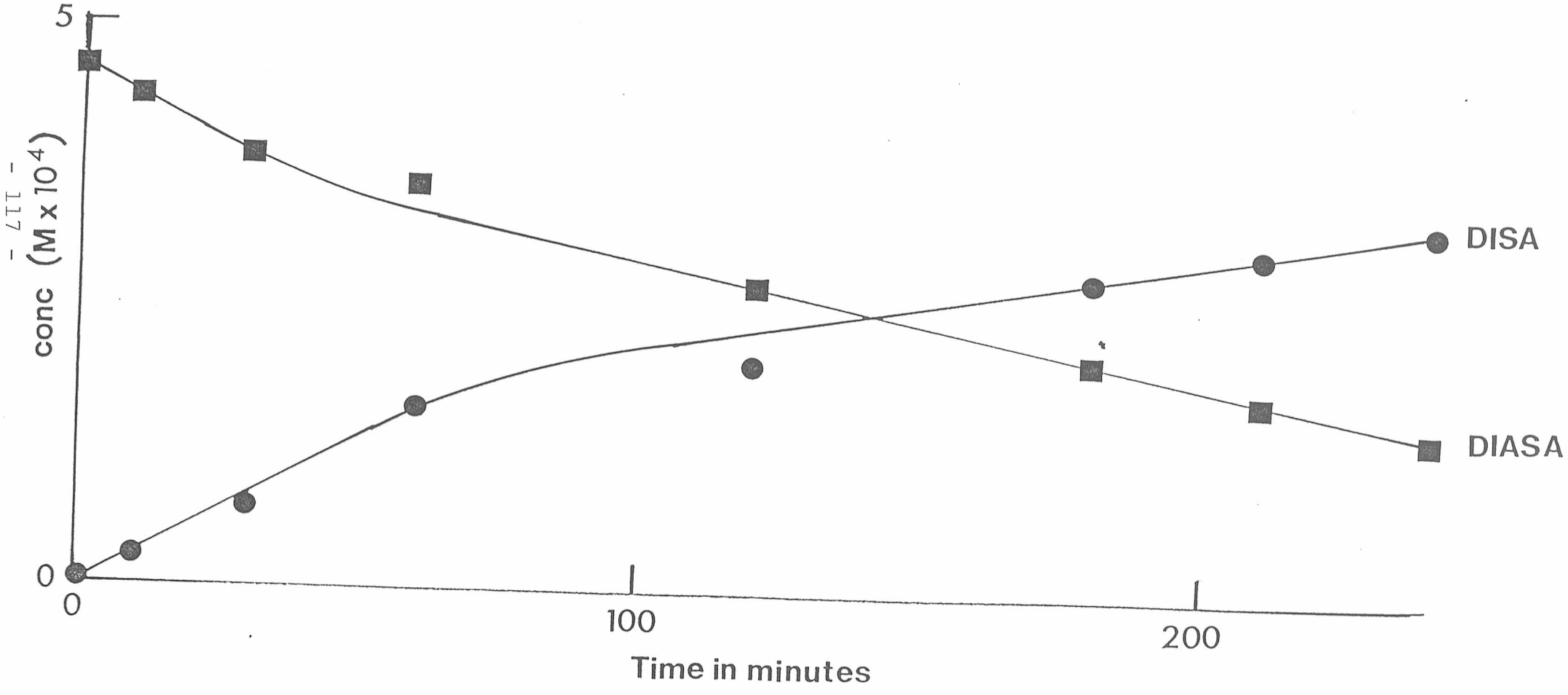


Figure 4.10 Arrhenius plots as obtained using the data in Table 4.1.

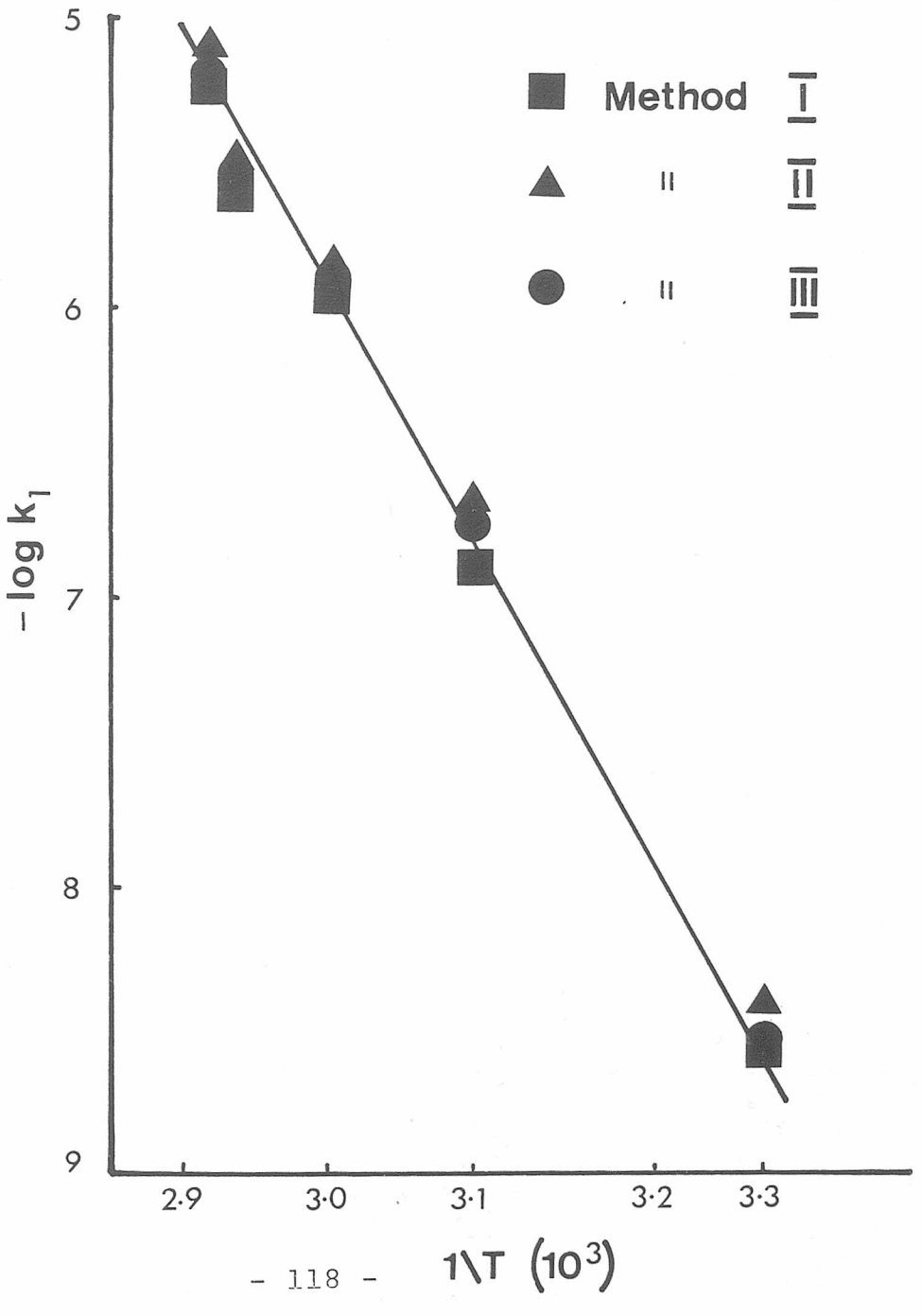
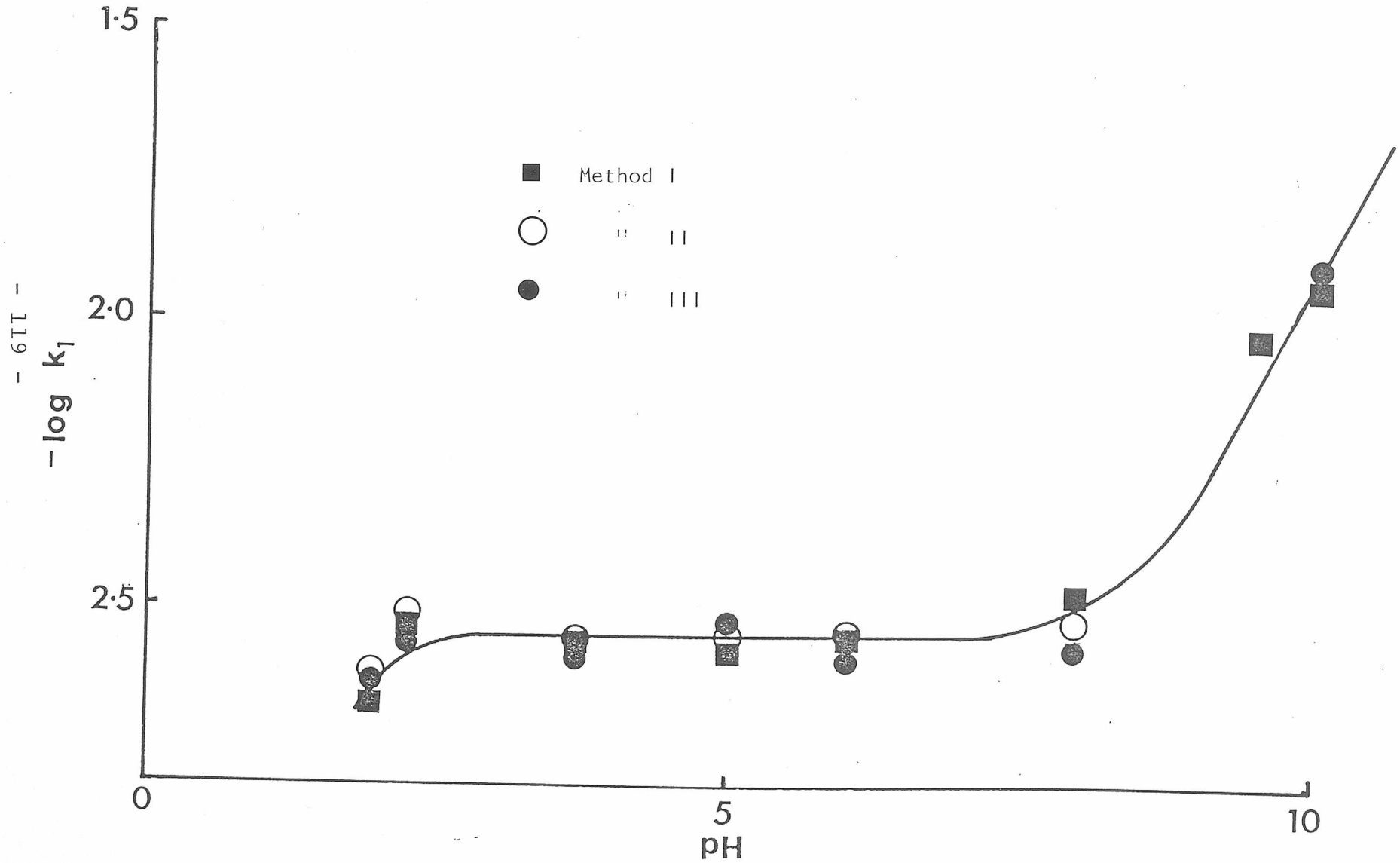


Figure 4.11 Plot showing the log k - pH profile for DIASA at 60°C obtained using the data in Table 4.2.



CHAPTER 5

5.0 The Decomposition of Tetracycline

5.1 Introduction

The tetracyclines have been defined as a family of closely related compounds which have a common perhydronaphthacene skeleton[202]. Their broad spectrum antibiotic activity has led to the discovery of a number of compounds including chlortetracycline, oxytetracycline and tetracycline, itself considered as the parent. The tetracycline compounds have been the topic of numerous investigations concerned with the structure determination of both the drugs themselves[203-207] and their products of decomposition[208-211].

Tetracycline (TC) can undergo decomposition by at least four different pathways[212], that is, epimerisation, dehydration, hydrolysis and oxidation, the first two being the most commonly encountered routes of decomposition. The ease with which TC appears to decompose has led to investigations showing the presence of decomposition products as impurities in commercially available TC products[213-216].

Epimerisation of TC is said to occur as a reversible process, particularly in the pH range 2-6[211,217-219] leading to the formation of the inactive and non-toxic 4-epitetracycline (ETC). On the other hand dehydration of both TC and ETC to anhydrotetracycline (ATC) and

epianhydrotetracycline (EATC) respectively is said to occur predominantly at or below pH 2[212,220]. Scheme 5.1 represents the accepted decomposition of TC to EATC via ETC and ATC.

It has been observed that administration of aged TC preparations leads to renal impairment[221] and the toxic effects have been attributed to the decomposition product EATC[222,223] formed as a result of improper storage of the preparation.

While a number of reports have appeared in the literature concerned with the stability of TC[212,216-220,224,225] the various investigators have assumed that, by adjusting the reaction conditions, one or the other decomposition pathway, namely epimerisation or dehydration, can be minimised and the rate constants obtained for the predominant route. Only two reports were located in the literature where all components of decomposition were estimated. The first concerned with the thermal decomposition of TC in the solid state[216] shows no decomposition at 37°C and 50°C after 27 months. The low level of decomposition observed at 70°C could not be accounted for by increase in the three decomposition products. Indeed at this temperature the low ETC concentration present at the start is seen to decrease, the toxic EATC content remaining virtually unchanged. Only ATC showed a small increase.

The second of these two reports[224], the only one available in the literature to calculate all rate constants shown in Scheme 5.1, utilised acid phosphate

buffer solution and elevated temperatures to ensure complete reaction. Employing a single pH of 1.5, rate constants k_1 to k_4 were obtained by curve fitting of the data according to the kinetic equations derived from Scheme 5.1. These results will be discussed in the light of the present findings later in this chapter.

The knowledge that TC decomposition involves the formation of the nephrotoxic EATC has led to the development of a number of analytical methods capable of assaying TC in the presence of its decomposition products[212,217-219,226] or after separation from these impurities[214,225,227-234]. While some of these methods have been claimed to be stability indicating[226,231,234] not all have been employed for stability purposes. Other analytical methods have been developed for the purpose of impurity limit testing, that is, ensuring the level of EATC in a TC preparation is within the stated acceptable limits laid down in the various monographs. These analytical methods range from spectrophotometry[212,217-219,226], classical chromatography[227-234], nuclear magnetic resonance[219], polarography[235], the more modern GLC[236] requiring derivatisation, and hplc[216,224,237-241]. The reverse phase hplc methods reported may be divided into four groups, those employing buffer/organic modifier[237], those incorporating ethylenediaminetetracetic acid (EDTA)[238-241], those requiring gradient elution[216] and those employing ion pairing[242] techniques. Unlike the more generally used hydrophobic pairing ions, the latter report [242] has utilised a hydrophilic pairing ion, namely

perchlorate. No reports, involving the separation of TC from its three accepted decomposition products employing the more commonly used hydrophobic pairing ion, could be located in the literature. Although separation may be achieved at low pH, when using pH ranging from 3-5, the addition of EDTA is claimed to be essential[242].

Although a number of hplc methods have been developed for the separation of TC, ETC, EATC and ATC not all have been utilized in stability studies. Of the two that have, one requires gradient elution and the other requires maintaining the column temperature at 36°C. Both methods require some 35 to 40 minutes for elution of the four compounds.

Tetracycline decomposition was chosen as a model system because this decomposition has been shown to be complex involving reversible epimerisation, parallel dehydration and consecutive decomposition to EATC via ETC and ATC (Scheme 5.1). Such a reaction scheme offers a searching test for the practical evaluation of the merits and limitations of the proposed initial rate method. It also offers the potential of evaluating the simulated findings in Chapter 2 for the individual complex reactions. In addition, the instability of TC is important in that, as is well known, one of its products of decomposition namely EATC is toxic. Thus the rate of production of this impurity is arguably more relevant medically than is the loss of the TC potency[243].

5.2 Materials and Equipment

Chromatographic measurements were carried out using the equipment described in Chapter 3.

Tetracycline and its decomposition products were supplied by Lederle Laboratories, cetrimide was obtained from Thornton and Ross Ltd. and sodium lauryl sulphate from Fisons. Acetonitrile was obtained from Rathburn Chemicals and water was purified using Millipore MilliQ system. All other chemicals were of AnalaR or equivalent grade.

5.3 Results and Discussion

5.3.1 Chromatographic Separation

Previously reported reverse phase solvent systems involving buffer/organic modifier alone[216], those incorporating EDTA[238-240] and those employing hydrophilic pairing ion[242] were found to give inadequate resolution, in particular between TC and ETC in times short enough to provide adequate sensitivity for the longer retained ATC. It was decided, therefore, to use existing ideas of hydrophobic ion-pairing to develop an adequate hplc method which would allow the rapid quantitation of the parent drug and its accepted breakdown products. This assay method would allow quantitation of TC concentration decrease and decomposition product increase allowing direct comparison of the two approaches and also comparison with limited stability data available in the

literature.

The four compounds of interest are amphoteric exhibiting three ionisation constants. In strongly acidic pH the molecule exists in the fully protonated form as a singly charged cation. Under alkaline conditions loss of a proton from 12-position hydroxyl group results in the formation of an anion. At intermediate pH values the molecule exists predominently in the zwitterion form with the dimethylamine group protonated and the hydroxyl group in the 3-position ionised. Thus while the four compounds are not highly hydrophobic and retention on reverse phase C-18 chromatographic systems is low they should be amenable to control of retention by the addition of anionic hydrophobic pairing ion at low pH or cation hydrophobic pairing ion at high pH. Figures 5.1 and 5.2 show the variation in column capacity factor (k') with sodium lauryl sulphate (SLS) at pH 2 and cetrimide (CTAB) at pH 7 respectively. At pH 2 in the presence of SLS all four compounds exhibit maxima, ATC and EATC showing a much steeper increase in k' with increasing SLS concentration than do TC or ETC (Figure 5.1). Figure 5.2 shows the k' for ATC increasing more sharply than that for EATC with increasing CTAB concentration. Both EATC and ATC exhibit a maxima while TC and ETC show little or no change in k'.

Optimal chromatograms obtained using these pairing ions are shown in Figure 5.3 and 5.4. The anionic SLS pairing ion produced the better overall resolution as shown in Figure 5.3. If, however, quantitation of EATC only were required say for impurity limit testing, the

CTAB system produces adequate resolution for this compound in a much shorter analysis time as shown in Figure 5.4. The solvent system of acetonitrile/water (40/60), 0.05M in phosphate buffer and 0.05M in SLS, at pH2 was used for subsequent stability analysis.

5.3.2 Chromatographic Quantitation

The calibration lines of peak height against concentration for the four compounds were determined to be linear (R^2>0.99) over the different concentration ranges used. The precision of the peak height quantitation method was determined by repeated injection of a partially decomposed TC sample containing all species. For eight replicates, the following relative standard deviations were obtained: TC (1.3%), ETC (0.91%), EATC (1.6%), ATC (1.3%). During decomposition runs, standards of concentration appropriate to the absorbance range of the detector were used.

5.3.3 Stability Measurements

5.3.3.1 Determination of individual rate constants

In order to evaluate the individual rate constants defined in Scheme 5.1, the appropriate compound was used as the reactant at an accurately prepared concentration of approximately 5 x 10^{-3}M in pH 1.5 phosphate buffer prepared as described by Yuen et. al.[224]. These solutions were stored in a constant temperature bath at 30°C. The solutions were analysed for decomposition products over

time scales such that the extent of reaction did not exceed 5% of the initial reactant concentration. Under these conditions the decomposition product concentration-time lines were found to be linear (R^2>0.99). The concentration-time lines for TC, ETC, ATC and EATC are shown in Figures 5.5 to 5.8. Under these conditions, these results indicate that TC decomposition follows zero order kinetics. First order rate constants were calculated using Equation (1-16) derived in Chapter 1. The calculated values of the various rate constants so obtained are shown in Table 5.1 together with their relative standard deviations. Using TC as the reactant initial formation of ETC and ATC allowed determination of k_1 and k_3 respectively. ATC and EATC as reactant enabled k_2 and k_{-2} to be determined by measuring the increase in EATC and ATC respectively. When ETC was used as the reactant, however, k_4 could be determined by measuring EATC increase but resolution between TC and ETC was inadequate in the situation where, TC present as the minor component, was eluted immediately after ETC which was present in much larger quantities. Thus direct determination of k_{-1} was not possible. The value of k_{-1} shown in Table 5.1 was calculated indirectly after measuring the conventional first order rate constant for ETC decomposition over larger extents of decomposition, that is, $(k_4 + k_{-1})$. Previous knowledge of k_4, enabled calculation of k_{-1}. The various rate constants shown in Table 5.1, with the exception of k_{-1} were readily determined in an overall time of two hours at 30°C. Also

shown, for comparison, in Table 5.1 are the values of rate constants extrapolated from literature data obtained at higher temperatures[224].

Comparison of the corresponding values show that in the present work consistently lower rate constants are obtained for the epimerisation processes but the rate constants for the dehydration reaction are higher. The present work is considered more reliable because direct measurements are involved. Also previous literature data[217] show that the rate of ETC epimerisation is faster than that for TC as does the present study. Epimerisation of ATC is approximately 30% faster than that of TC[240]. The results of Yuen et.al.[224] indicate that the epimerisation of TC to ETC is more rapid than the dehydration of TC to ATC contradictory to the previous literature findings.

Other investigations regarding the dehydration reactions[212] indicate that TC dehydrates more rapidly than does ETC. These findings are not borne out by the present study where the dehydration of TC and ETC are found to occur at approximately the same rate. Previous studies regarding the dehydration of TC and ETC have, however, assumed that at or below pH2 epimerisation is negligible, the dehydration pathway predominating. From Table 5.1, however, it can be seen that at the most there is only an eight-fold difference between the two reactions and that epimerisation occurs at a readily measurable rate. Epimerisation at low pH values is, therefore, not negligible. Although the same argument is used in the literature regarding epimerisation as the predominant

route of decomposition above pH 2, the validity of this assumption is confirmed by the present work in that above pH 2 dehydration occcurs at least ten times more slowly above pH 3. (See pH data later).

These results indicate that the initial rate method affords a more reliable approach to estimating the individual rate constants than the previously used curve fitting method[224]. Also it is capable of measuring all species and therefore requires no assumption regarding the predominant route of decomposition.

5.3.3.2 Temperature and pH dependence of TC decomposition

The decomposition of TC has hitherto been studied over a limited pH range[212,217,220]. To provide more complete logk-pH data and allow estimation of activation energies for the individual reactions involving TC as reactant, the initial rate method was applied to TC at 40, 50, 60 and 70°C at pH 7.0 and at a single temperature of 75°C over a pH range of 2.3 to 8.0. McIlvaine's buffer was used to maintain a constant ionic strength of 0.5μ. Under the same conditions TC decomposition was allowed to proceed to higher extents of reaction (30-70%) in order to obtain rate constants by the conventional integrated approach.

Figures 5.9 and 5.10 show representative results at 50 and 70°C respectively measured over approximately the same time scale. Measurable concentrations of the decomposition products are present at the start of the reaction and all are seen to increase as the reaction

proceeds. In both cases TC concentration is seen to decrease. At high extents of reaction, as shown in Figure 5.10, ATC and EATC reach steady state while ETC concentration passes through a maximum. At lower extents of decomposition (Figure 5.9) only the increasing parts of the ETC, ATC, EATC curves are seen. These observations regarding the presence of impurities in the starting material are consistent with literature findings[213-216] regarding the decomposition pathway, as are the curves[224].

Using the TC data of Figure 5.10(A), the first order integrated rate equation is found to represent the decomposition reaction adequately. First order kinetics have been confirmed using the initial rate method by measuring the increase in ETC concentration for initial TC concentrations ranging from 5.1×10^{-4}M to 4.7×10^{-3}M. The regression equation so obtained is

$$\log(\text{Rate}) = 1.1[\text{TC}] - 4.53$$

The initial rate of ETC and ATC formation should allow estimates of the initial rate of TC decomposition to be made, viz.

$$-d[\text{TC}]/dt = d[\text{ETC}]/dt + d[\text{ATC}]/dt$$

Thus the TC to ETC reaction can be approximated to zero order at low extents of reaction and k_1 calculated from

$$k_1 = k_{0\,[\text{ETC}]}/\text{TC}_0$$

Similarly k_3 may be calculated from

$$k_3 = k_{0\,[\text{ATC}]}/\text{TC}_0$$

where $k_{0[ETC]}$ and $k_{0[ATC]}$ are the initial slopes of ETC and ATC concentration-time data and TC_0 the initial concentration of TC. The resultant rate constants, namely, k_1, k_3 and k_{TC} the overall rate constant obtained by following TC concentration are shown in Table 5.2 (R^2>0.99) together with their associated activation energies. Also shown in Table 5.2 are the values of k'_{TC} = k_1 + k_3. At all temperatures the dehydration reaction is slow at pH 7 and large errors are associated with the determination of k_3 which are reflected in the uncertainty in the activation energy quoted for this rate constant. As can be seen from Table 5.2, the value of the activation energy obtained are close to within 1% and are equally precise. This is consistent with previous literature findings where the activation energy for both the forward and reverse epimerisation reactions have been found to be the same between 71.5 and 98.7 kJmol^{-1} [214]. The activation energy for the dehydration reaction is seen to be higher than that for epimerisation and is in agreement with literature values of 107.9 kJmol^{-1} [224].

The logk-pH profiles are shown in Figure 5.11 for the various rate constants. In all cases there is an increase with decreasing pH. This is most marked with k_3 and is consistent with the higher rate of dehydration compared with epimerisation at pH 1.5 shown in Table 5.1. Good correspondence is obtained between k_{TC} and k_1 and k'_{TC} over the pH range 2.6 to 5.5 as seen in Table 5.3. In this pH range the dehydration reaction is on average 30 times slower than the epimerisation confirming that

epimerisation is the predominent pathway under these conditions.

As epimerisation is the predominant reaction the reversible first order equation may be applied to TC data to calculate both the forward and reverse rate constants. This calculation has been carried out for TC decomposition at pH 3.9. The equilibrium constant for the reaction has been calculated from literature data[217] at pH 4 and 23°C as 0.61. From this value it is possible to calculate the percent TC unchanged at equilibrium using the equation

$$(100 - TC_{eq})/TC_{eq} = K \quad \text{or} \quad (100/K + 1) = TC_{eq}$$

where K is the equilibrium constant and TC_{eq} the percentage of TC at equilibrium. Using the above equation TC_{eq} was calculated as 62%. Applying Equation (2-6) shown in Chapter 2 the overall rate constant was calculated as $0.024 \pm 1.4 \times 10^{-3}$ min^{-1}. The forward and reverse rate constants were calculated as ranging from 8.6×10^{-3} to 9.4×10^{-3} and 1.4×10^{-2} to 1.6×10^{-2} $min.^{-1}$ respectively using Equations (2-7) and (2-8) also shown in Chapter 2. The forward rate constant obtained at pH 3.9 by the initial rate method of $1.02 \times 10^{-3} \pm 2.8 \times 10^{-4}$ $min.^{-1}$ compares favourably with that calculated by the integrated reversible first order equation above indicating that ETC, in this case the major decomposition product, can be used to determine the stability of TC under those conditions where epimerisation predominates.

At pH values above pH 5.5 Table 5.2 shows that the

rate of TC decrease exceeds that of ETC and ATC emergence suggesting other possible reactions. This is supported by the appearance of additional peaks in the chromatograms obtained at higher pH values and results in k'_{TC} values lower than k_{TC}. Figure 5.12 shows these additional peaks in relation to the three accepted products of decomposition. These additional peaks are inadequately resolved for quantitation purposes but do not interfere with any of the compounds shown in Scheme 5.1. The presence of unidentified decomposition products at such elevated temperatures has previously been reported in the literature[216]. This report is based on the lack of reasonable correlation between the concentration of TC lost and concentration of ETC, ATC and EATC gained. The chromatographic system used in that study showed no additional decomposition products.

5.4 Conclusion

The reverse phase ion-pairing system suggested for TC stability studies is adequate to follow the kinetics of decomposition by the initial rate method except in the situation where ETC is the reactant. It also demonstrates the existence of other reactions at high pH. The additional compounds produced do not interfere with the quantitation of any compounds shown in Scheme 5.1.

The initial rate method has been shown to be capable of producing realistic rate constants for this complex reaction scheme. While it is required that the reaction pathway be known, this method allows evaluation of rate

constants at realistic extents of decomposition, that is, below that normally accepted as limiting shelf life.

While it has been suggested that the dehydration of TC to ATC predominates at or below pH 2 this study has shown that at pH 1.5 epimerisation does occur at a measurable rate. It is more likely that dehydration does predominate at pH levels well below 1.5. Epimerisation is the predominant route of decomposition above pH 3 and the results obtained by applying the reversible first order integrated equation to data at pH 3.9 are in good agreement with results of the initial rate method at this pH. They show that the major product of decomposition may be followed by the initial rate method to yield reliable rate constants so enabling stability evaluation of a complex reaction more readily than the integral method. These results confirm the simulated findings in Chapter 2.

Table 5.1 Rate constants for the decomposition of Tetracycline according to Scheme 5.1 obtained over low extents of decomposition by the initial rate method at 30°C in pH 1.5 buffer.

Rate Constant	Present Work k ($\times 10^4 min.^{-1}$)	RSD %	R^2	Literature[224] k($\times 10^4 min.^{-1}$)
k_1	0.65	2.3	0.999	3.51
k_{-1}	1.98	By Difference *		4.63
k_2	1.80	2.5	0.998	15.0
k_{-2}	0.85	3.6	0.996	12.7
k_3	4.40	1.7	0.999	2.83
k_4	4.31	2.4	0.997	0.71

* The value for k_{-1} quoted was obtained indirectly as indicated in the text.

Table 5.2 Rate constants for the decomposition of TC obtained by the integrated and initial rate methods at different temperatures in solution at pH 7.0.

Temp. °C	$k_{TC} \times 10^3$* ($min.^{-1}$)	RSD %	$k_1 \times 10^3$* ($min.^{-1}$)	RSD %	$k_3 \times 10^4$* ($min.^{-1}$)	RSD %	$k'_{TC} \times 10^3$* ($min.^{-1}$)
40	0.22	6.6	0.33	2.0	0.02	5.2	0.33
50	0.43	12.0	0.67	1.6	0.03	2.8	0.67
60	0.93	15.0	1.65	2.7	0.40	2.7	1.66
70	2.10	2.0	3.01	14.0	0.61	1.6	3.07
Act. Energy $kJmol^{-1}$	66.3 RSD=4.3		66.6 RSD=2.1		98.0 RSD=19		69.0 RSD=2.6

* Rate constants as defined in the text.

Table 5.3 Rate constants for the decomposition of TC obtained by the integrated and initial rate methods at 75°C in solutions of different pH.

pH	$k_{TC} \times 10^3$* ($min.^{-1}$)	RSD %	$k_1 \times 10^3$* ($min.^{-1}$)	RSD %	$k_3 \times 10^4$* ($min.^{-1}$)	RSD %	$k'_{TC} \times 10^3$* ($min.^{-1}$)
2.3	7.60	3.4	11.5	1.9	22.2	4.0	13.7
2.6	9.32	4.9	10.3	4.2	9.75	2.7	11.3
3.1	11.2	1.1	12.1	4.4	5.46	1.5	12.6
3.3	8.72	1.3	8.65	2.0	4.74	1.6	9.12
3.6	9.82	4.4	10.0	5.0	2.90	1.6	10.3
3.9	7.21	3.4	10.2	2.8	2.32	1.2	10.4
4.2	7.70	5.5	8.11	1.7	3.30	2.4	8.44
5.0	6.86	7.2	6.64	2.7	1.59	2.7	6.80
5.5	6.50	4.6	5.89	3.8	1.28	3.8	6.02
6.0	5.49	9.3	4.59	0.7	0.61	1.9	4.65
6.2	5.20	4.8	3.40	5.0	0.95	5.9	3.50
7.3	5.24	5.9	2.19	1.1	0.62	5.1	2.25
8.0	4.52	3.3	1.14	2.1	0.15	7.0	1.29

* Rate constants as defined in the text.

Scheme 5.1 Showing the complex decomposition of Tetracycline.

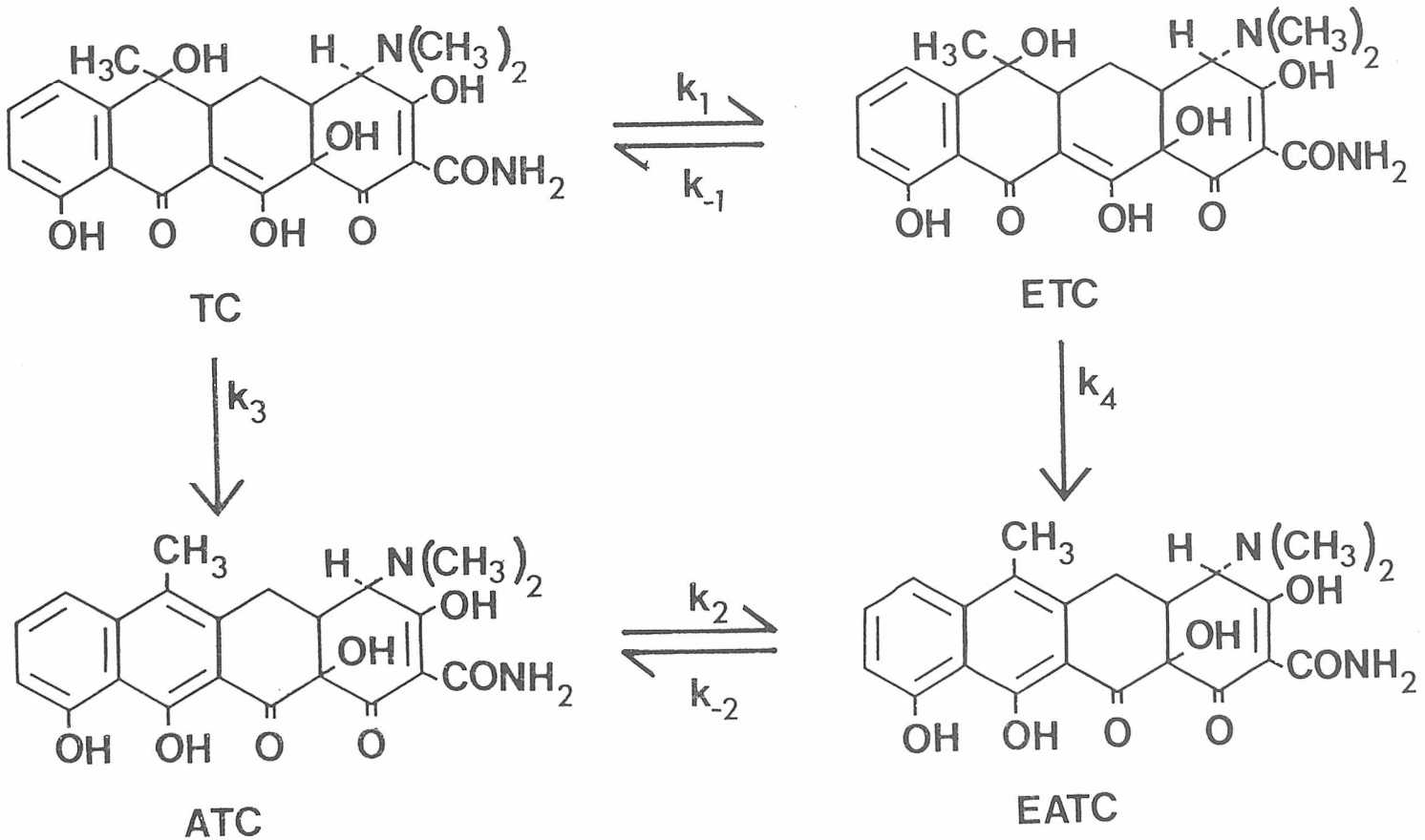


Figure 5.1 Plots showing the variation in capacity factor (k') with anionic pairing ion concentration for TC and its decomposition products. Solvent: acetonitrile : water (40/60) containing 50mM phosphate buffer at pH 2.

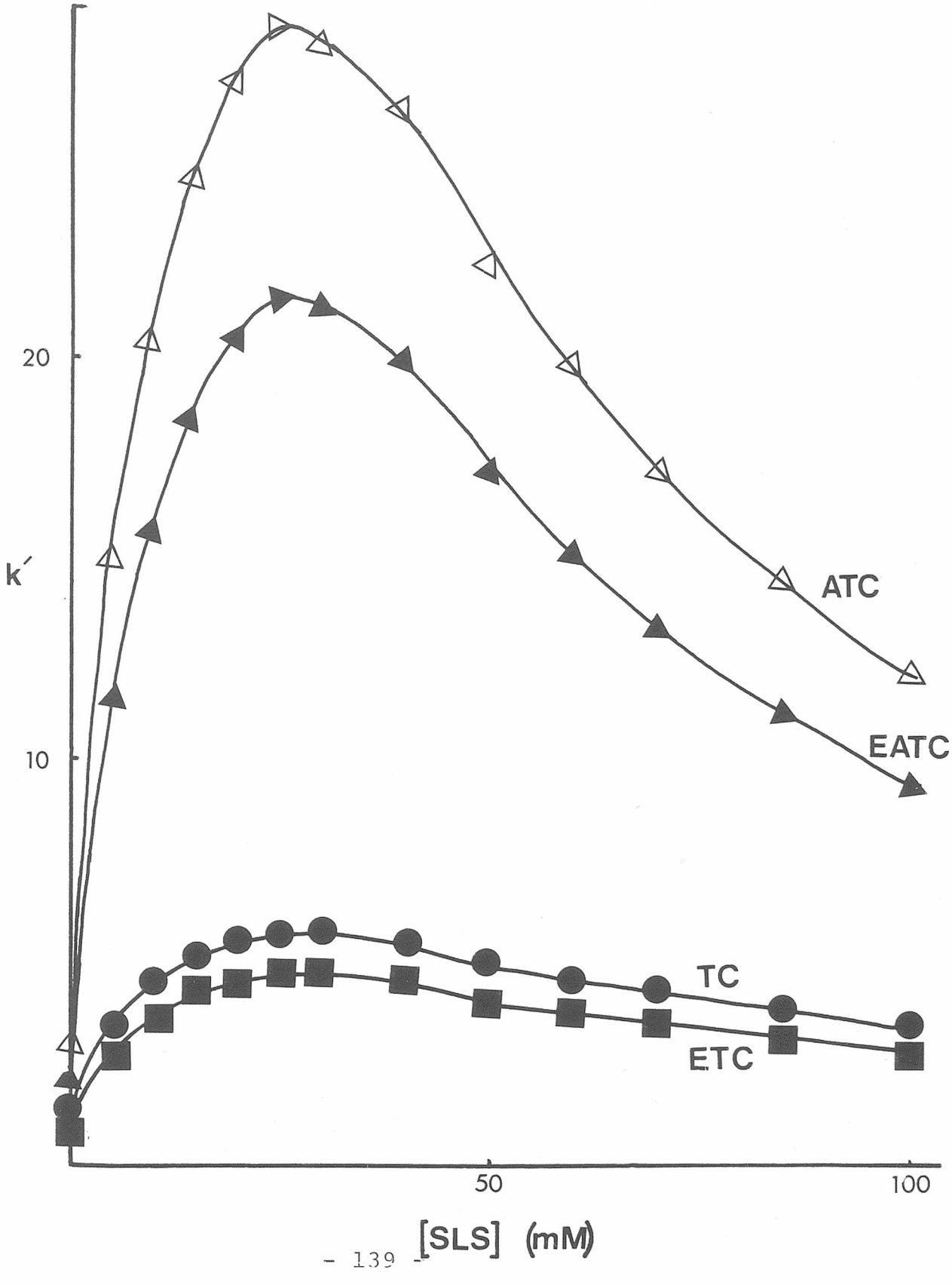


Figure 5.1 Plots showing the variation in capacity factor (k') with anionic pairing ion concentration for TC and its decomposition products. Solvent: acetonitrile : water (40/60) containing 50mM phosphate buffer at pH 2.

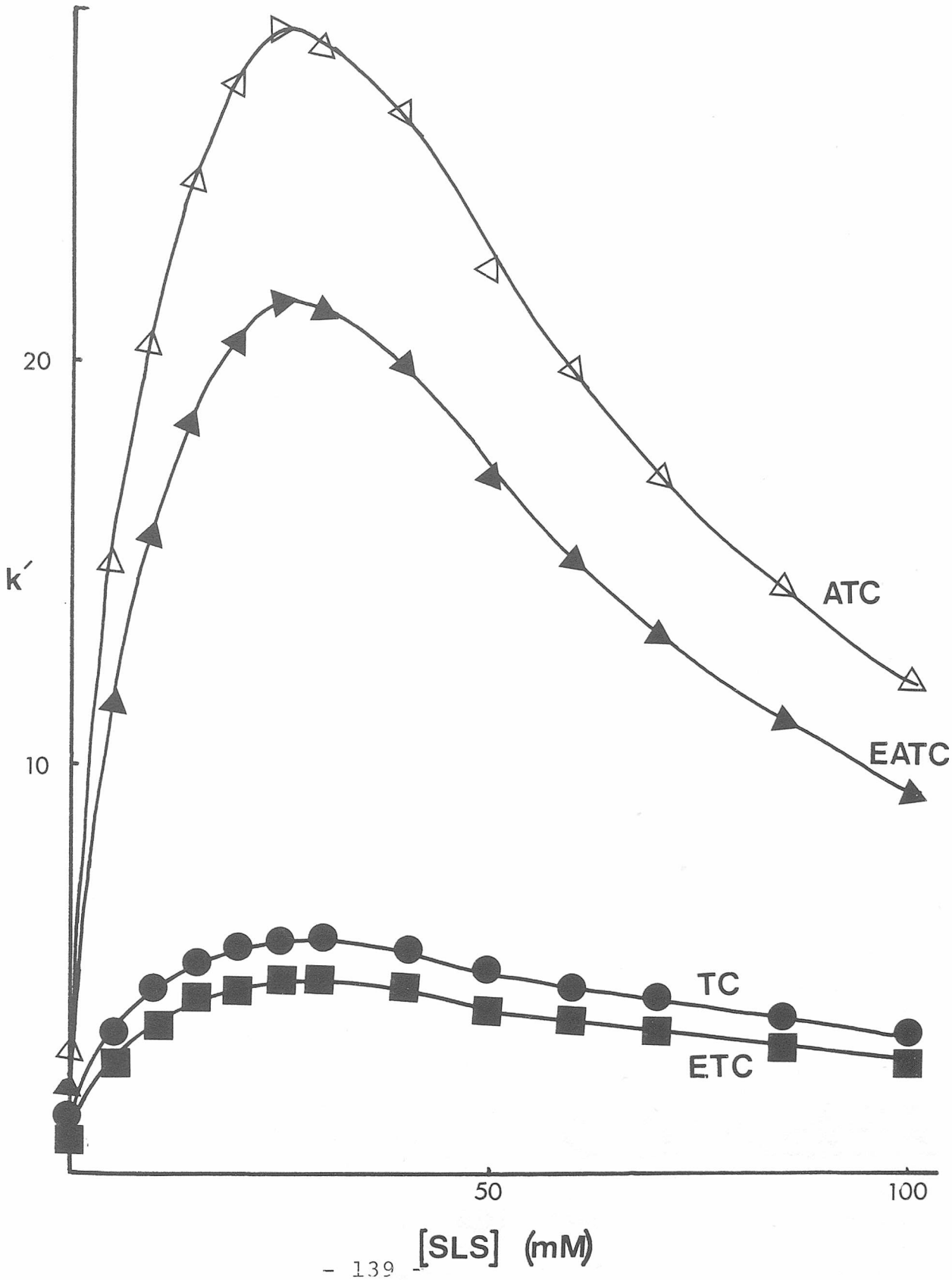


Figure 5.2 Plots showing the variation in capacity factor (k') with cationic pairing ion concentration for TC and its decomposition products. Solvent: acetonitrile : water (40/60) containing 50mM phosphate buffer at pH 7.

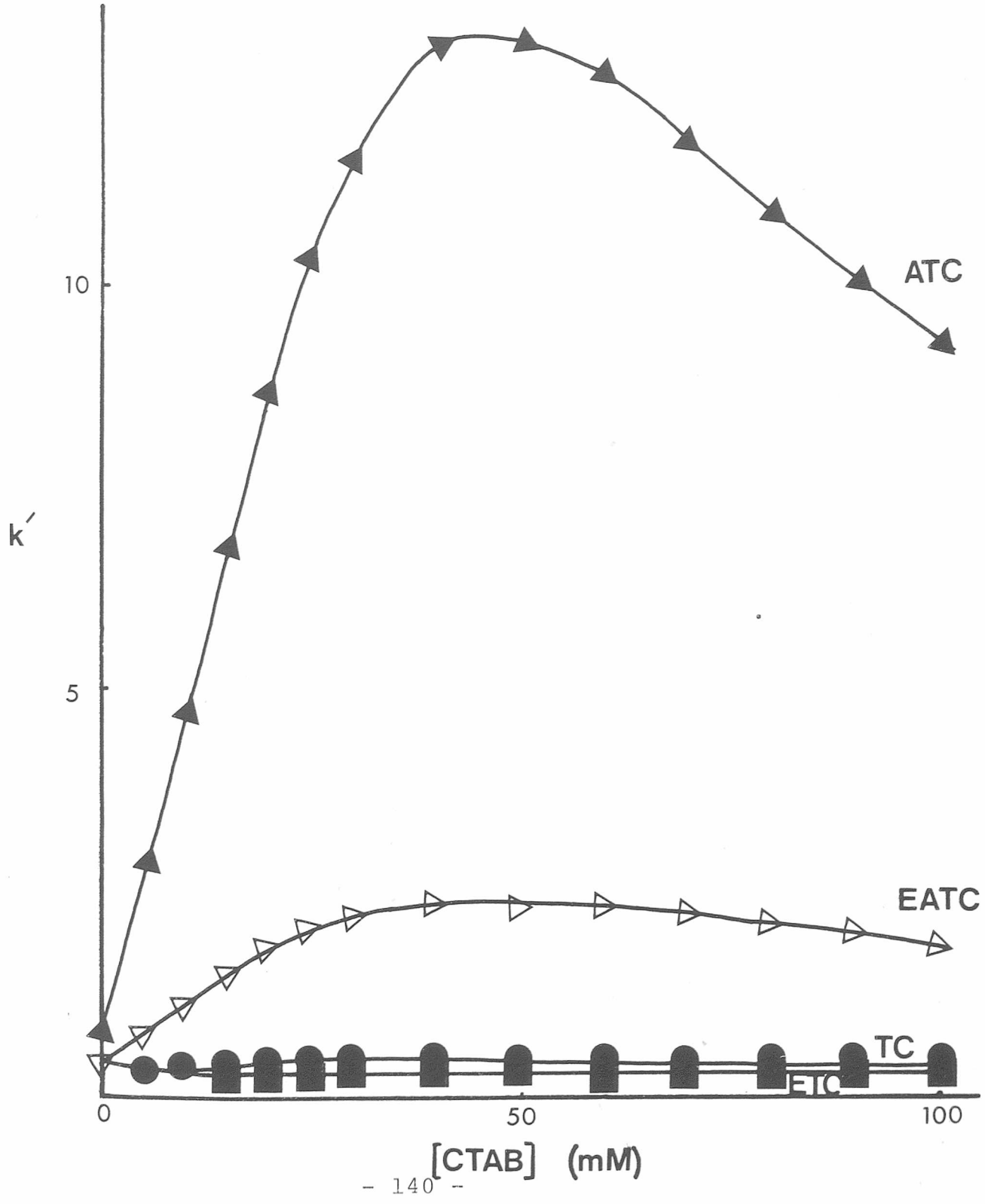


Figure 5.3 Optimum chromatogram showing the separation of TC from its decomposition products using anionic pairing ion. Solvent: acetonitrile : water (40/60) containing 50mM phosphate buffer and 50mM SLS at pH 2 at 0.2AU.

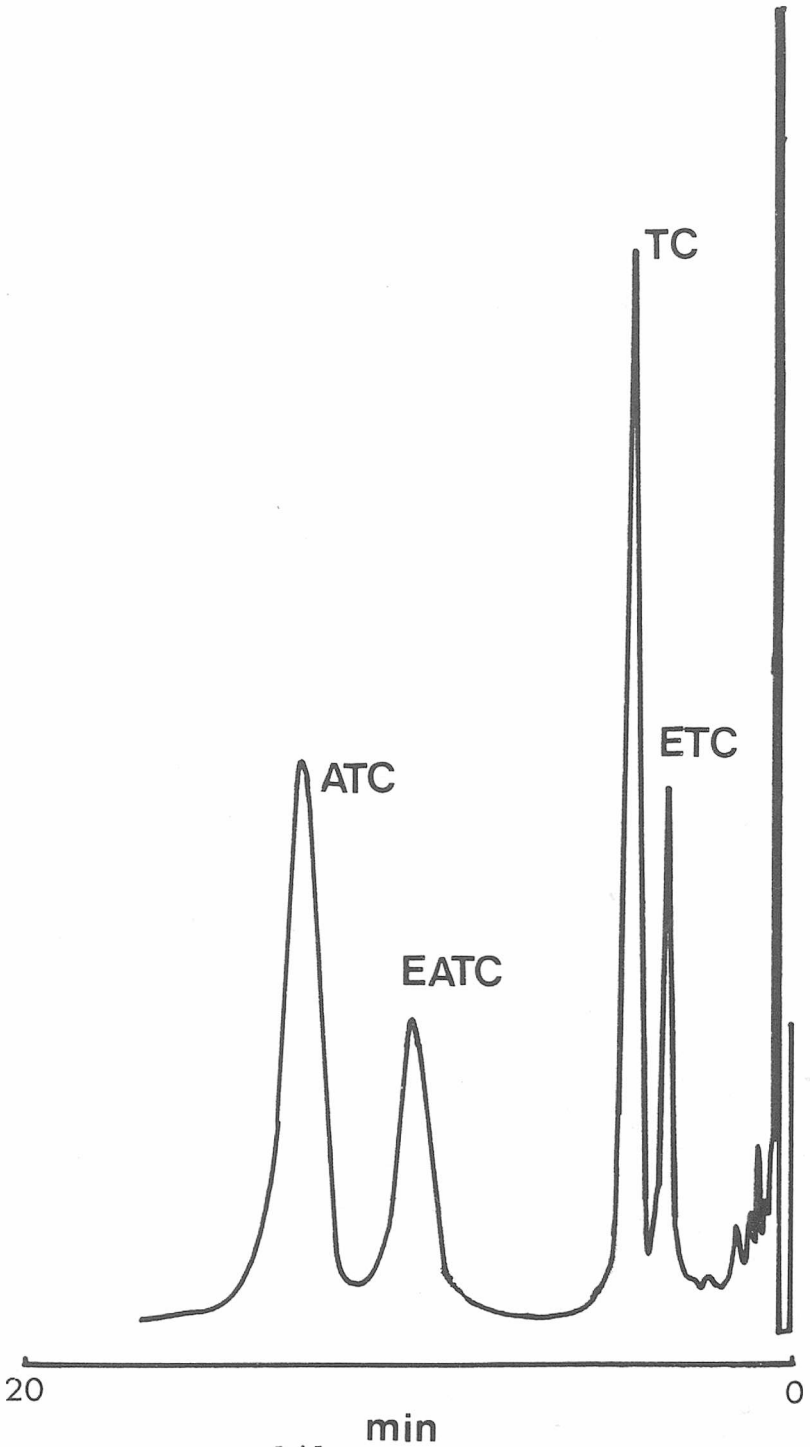


Figure 5.4 Sample chromatogram showing the separation of TC from its decomposition products using cationic pairing ion. Solvent: acetonitrile : water (40/60) containing phosphate 50mM and CTAB 2.5mM.

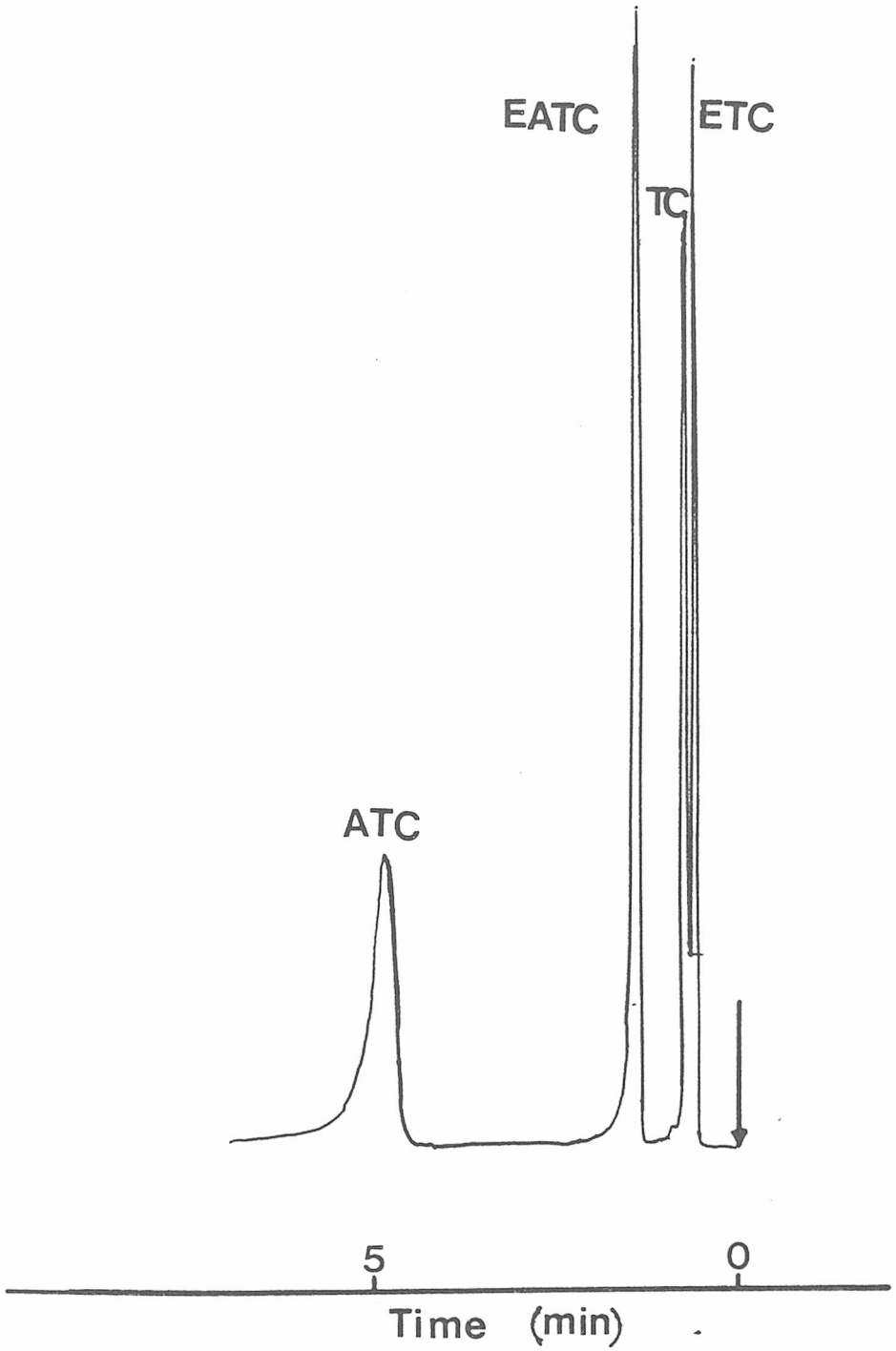


Figure 5.5 Plots showing the initial rate of ETC and ATC formation using TC as reactant at 30°C in buffer pH 1.5[224].

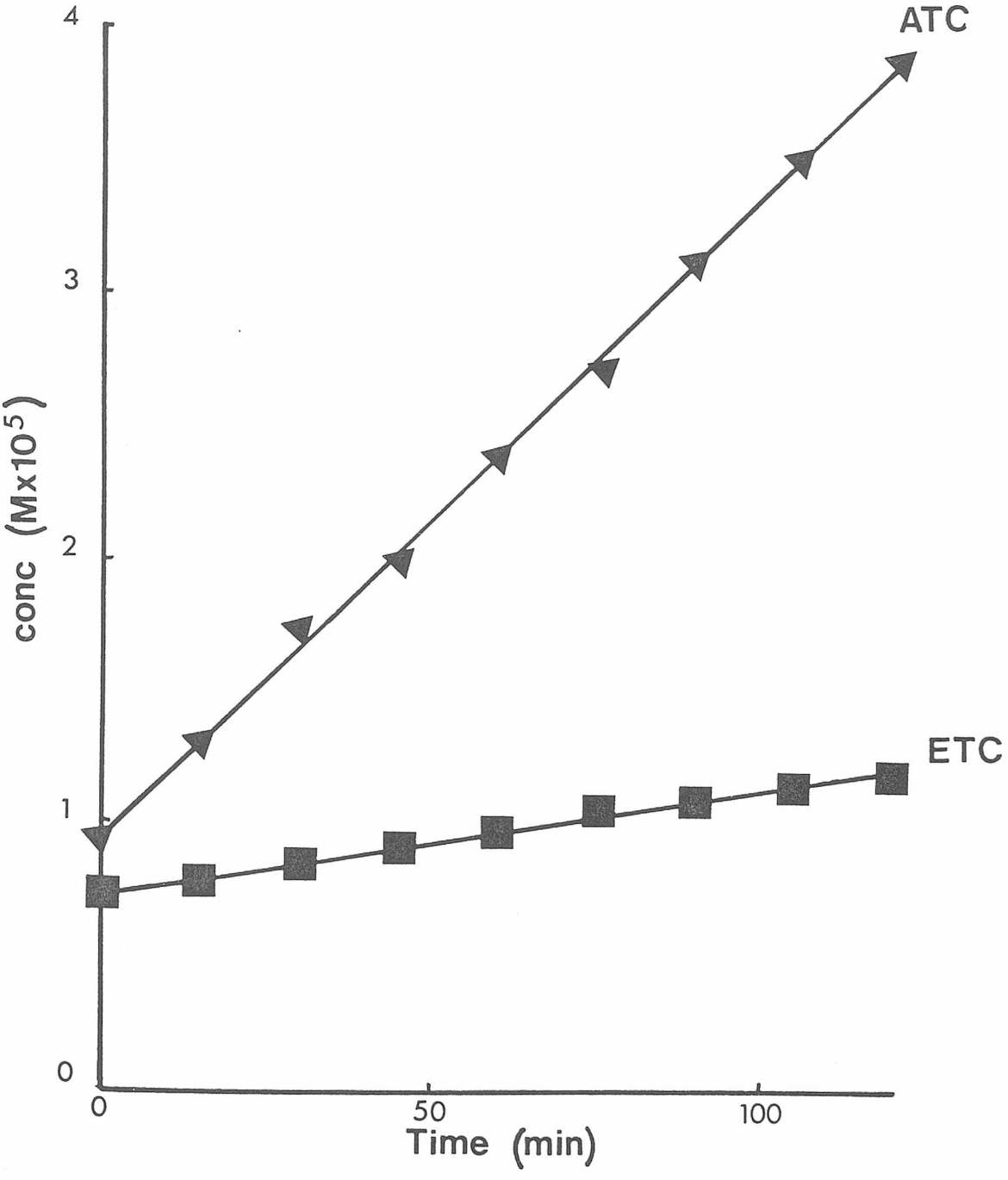


Figure 5.6 Plot showing the initial rate of EATC formation using ETC as the reactant at 30°C in buffer pH 1.5[224].

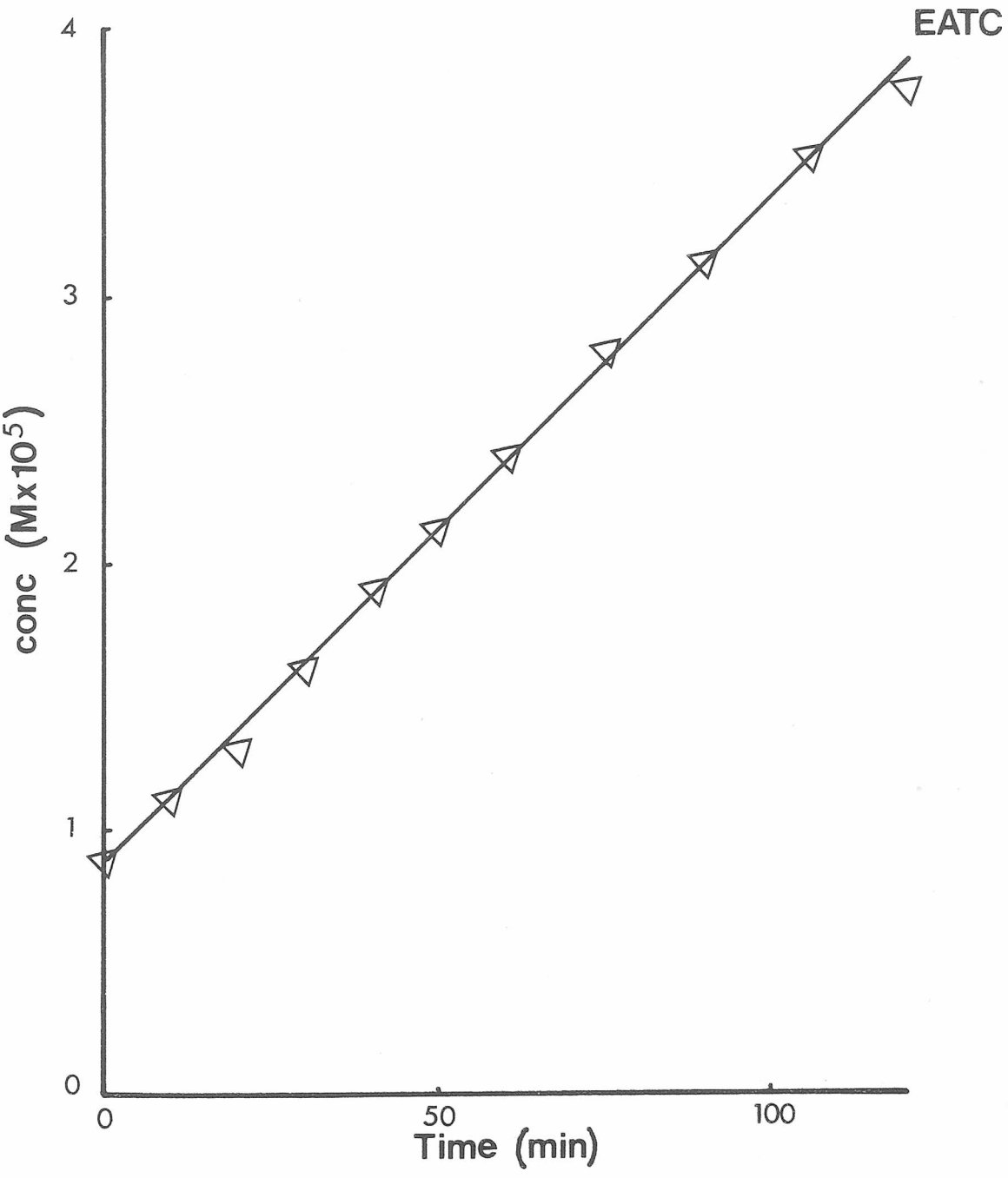


Figure 5.7 Plot showing the initial rate of EATC formation using ATC as reactant at 30°C in buffer pH 1.5[224].

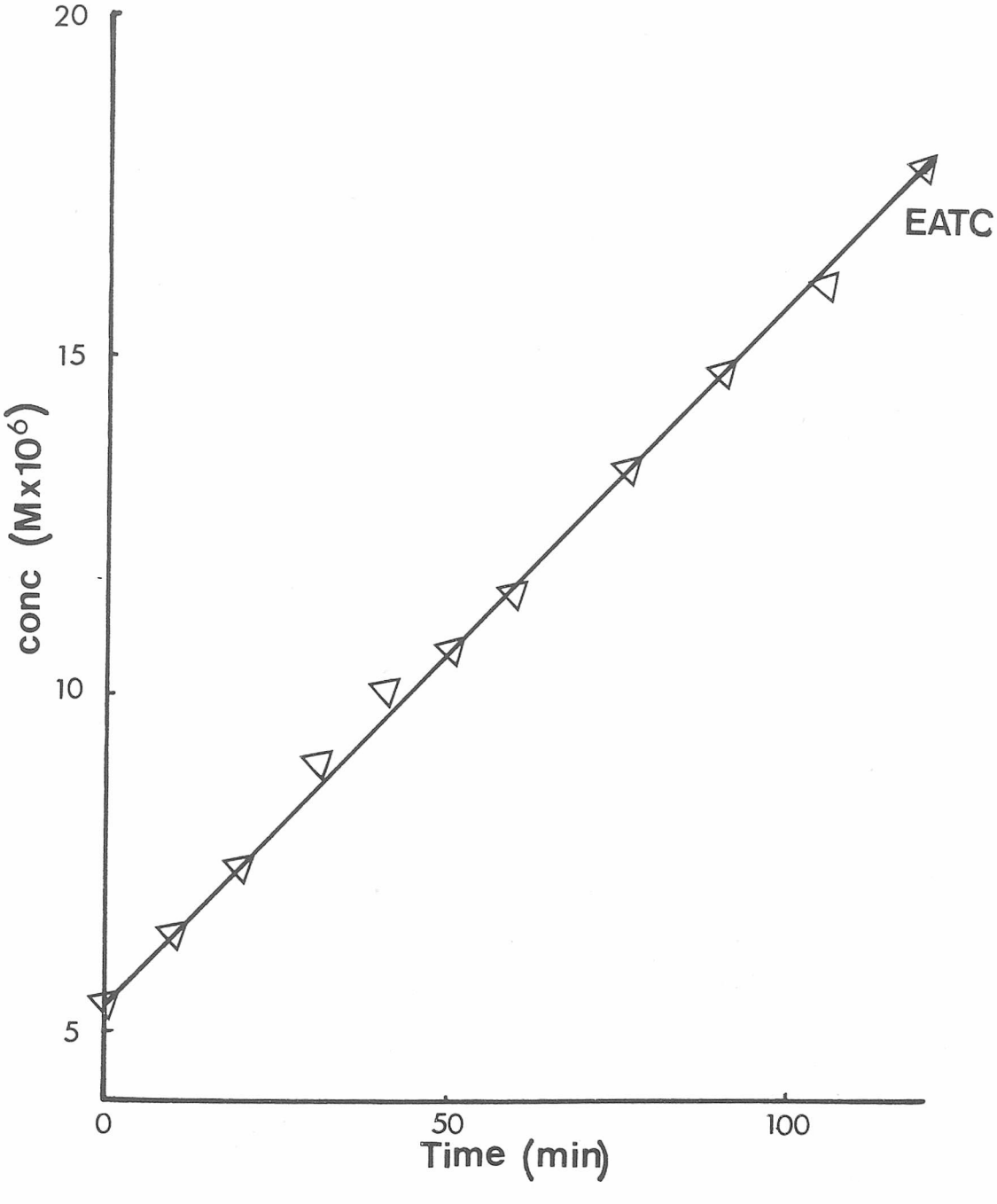


Figure 5.8 Plot showing the initial rate of ATC formation during EATC decomposition at 30°C in buffer pH 1.5[224].

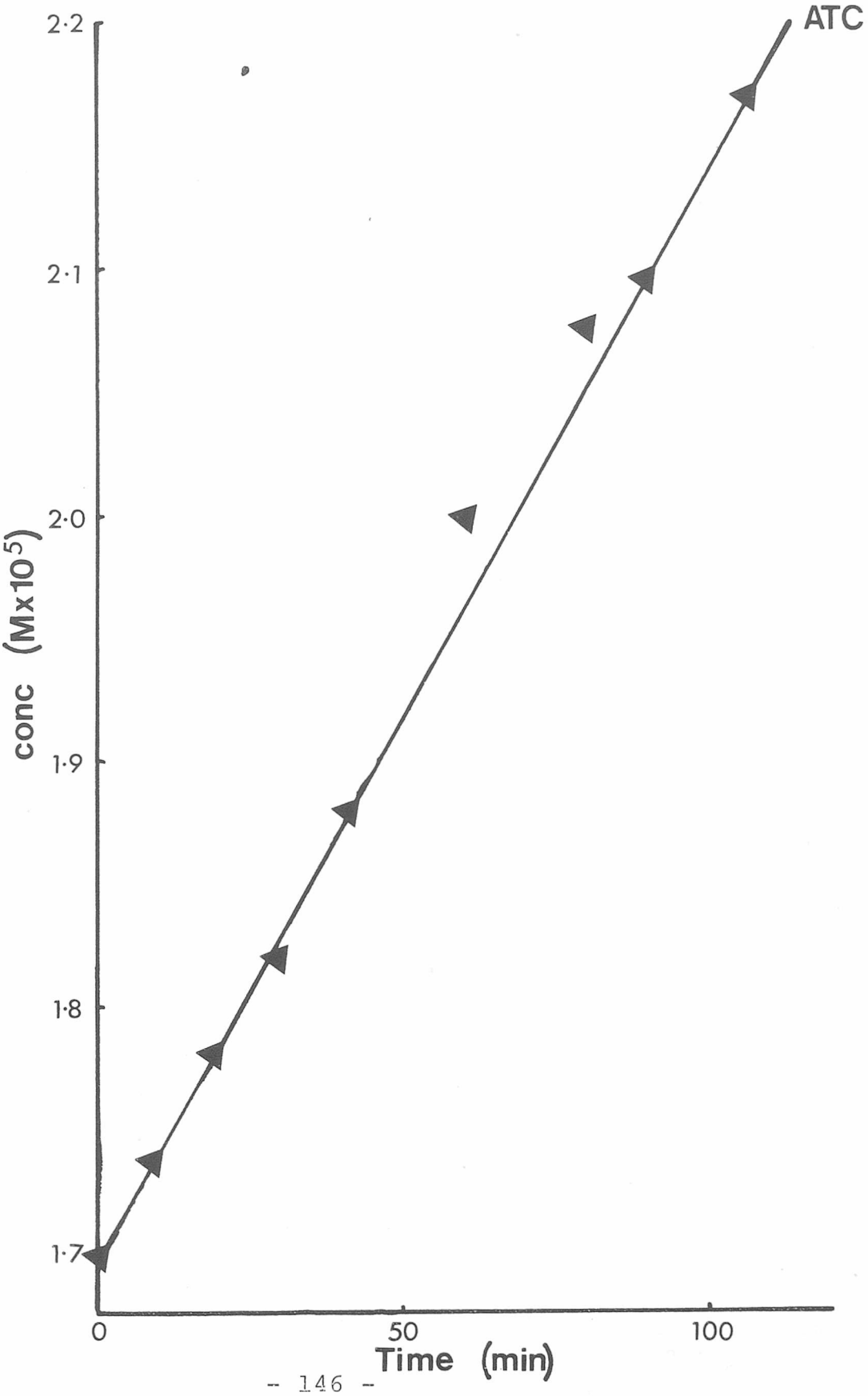


Figure 5.9 Plots showing the variation in concentration with time for (A) TC, (B) ETC, and (C) ATC and EATC at 50°C.

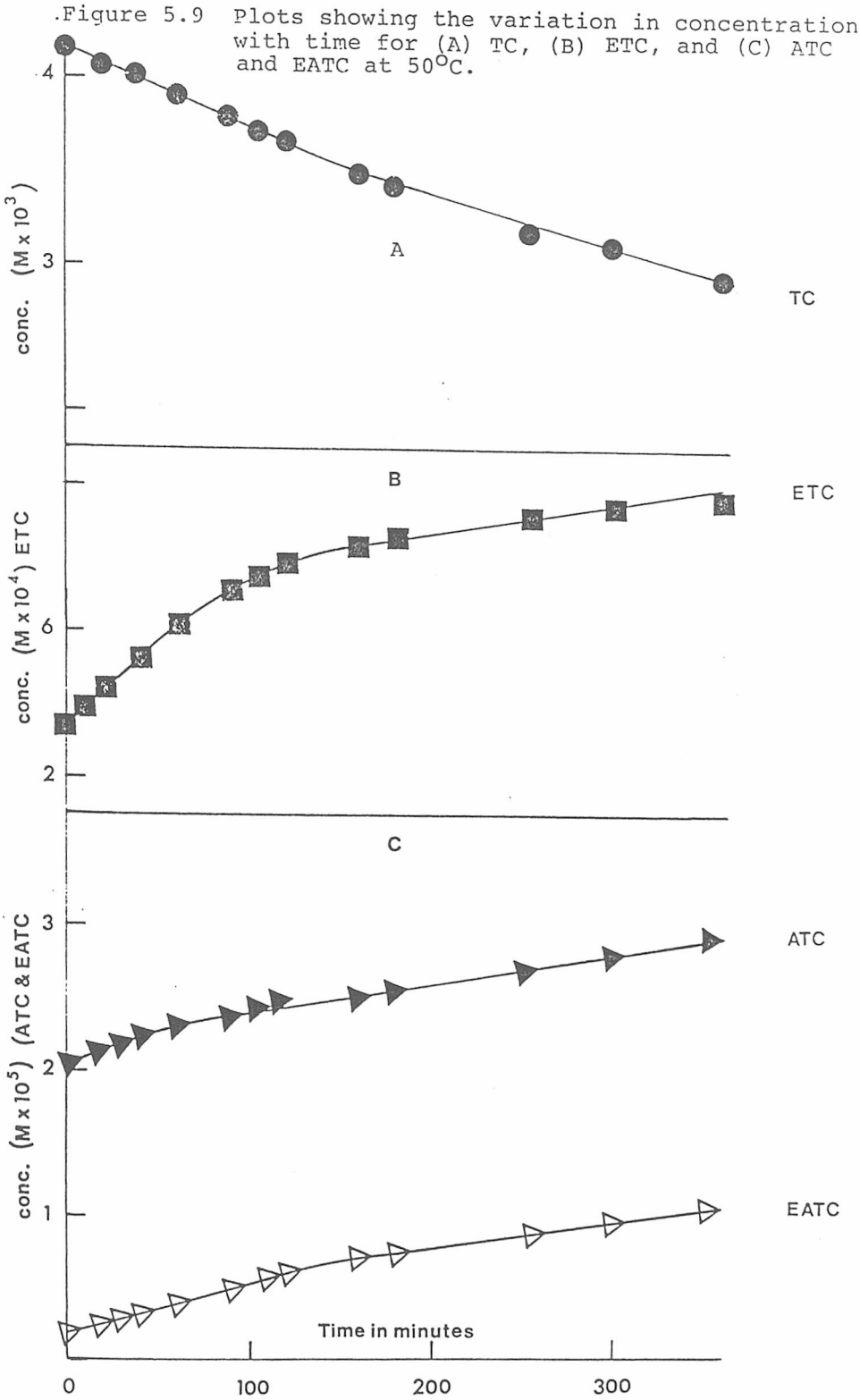


Figure 5.10 Plots showing the variation in concentration with time for (A) TC, (B) ETC and (C) EATC at 70°C.

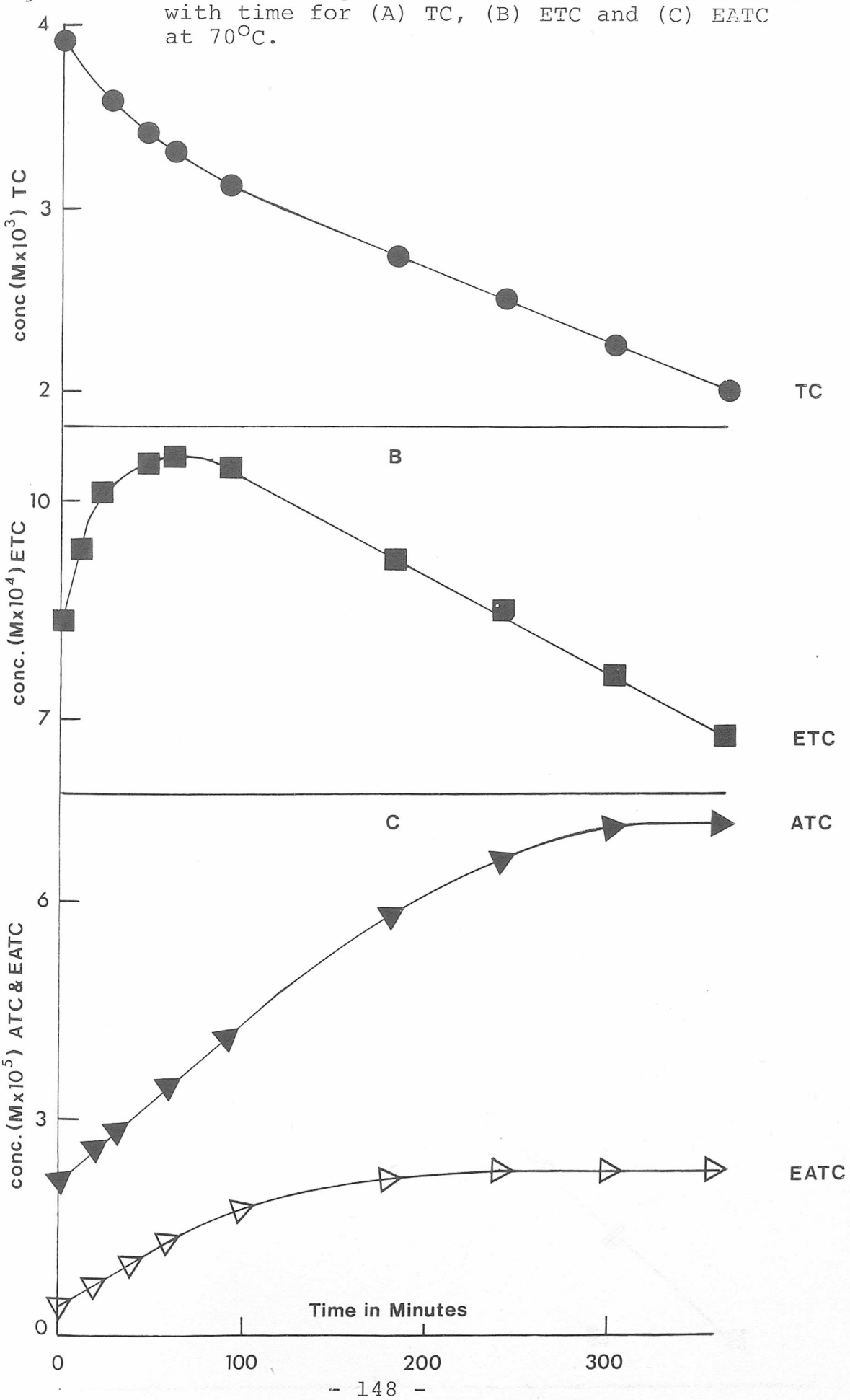


Figure 5.11 Showing the logk - pH profile obtained from data contained in Table 5.3.

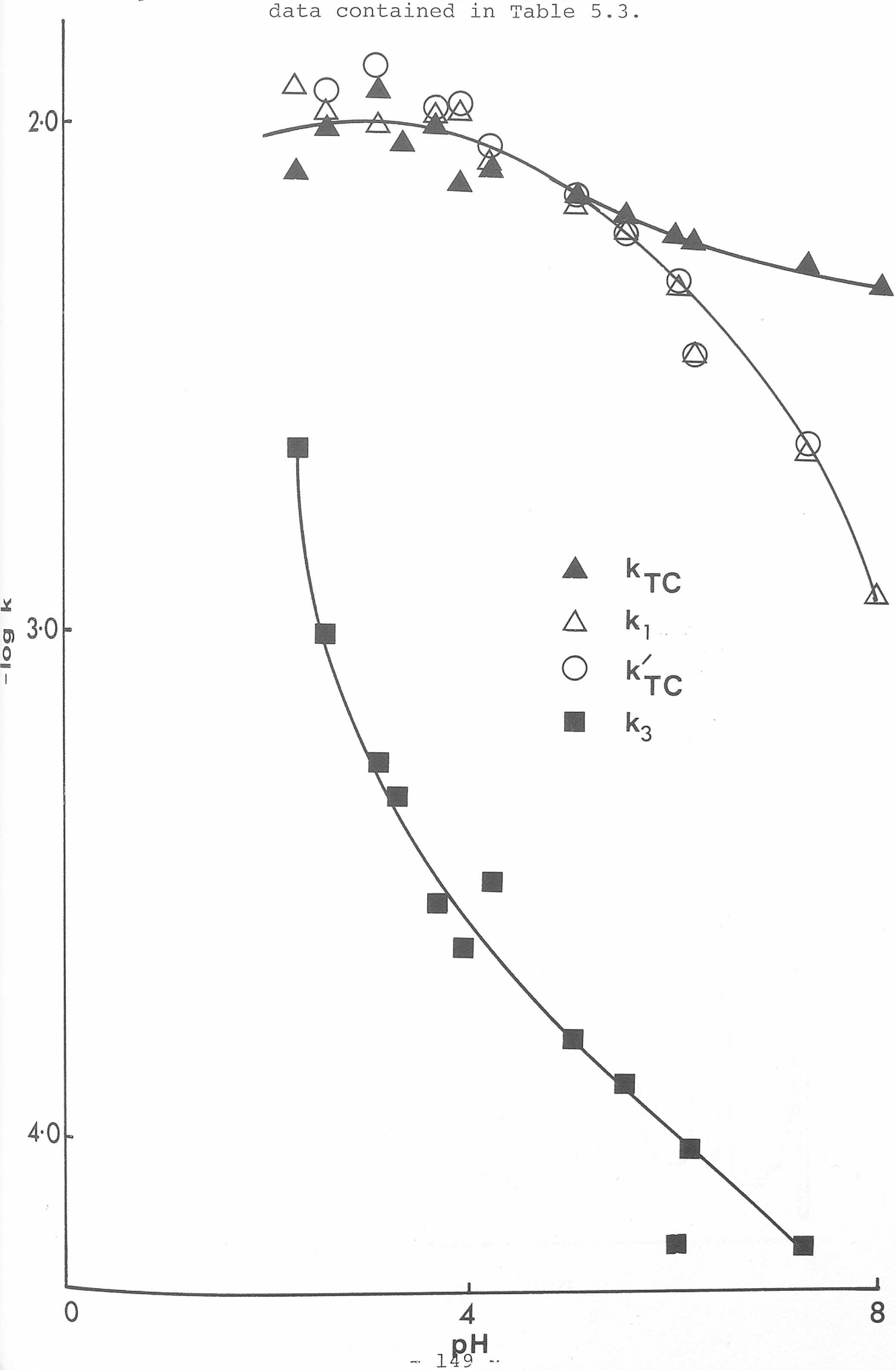
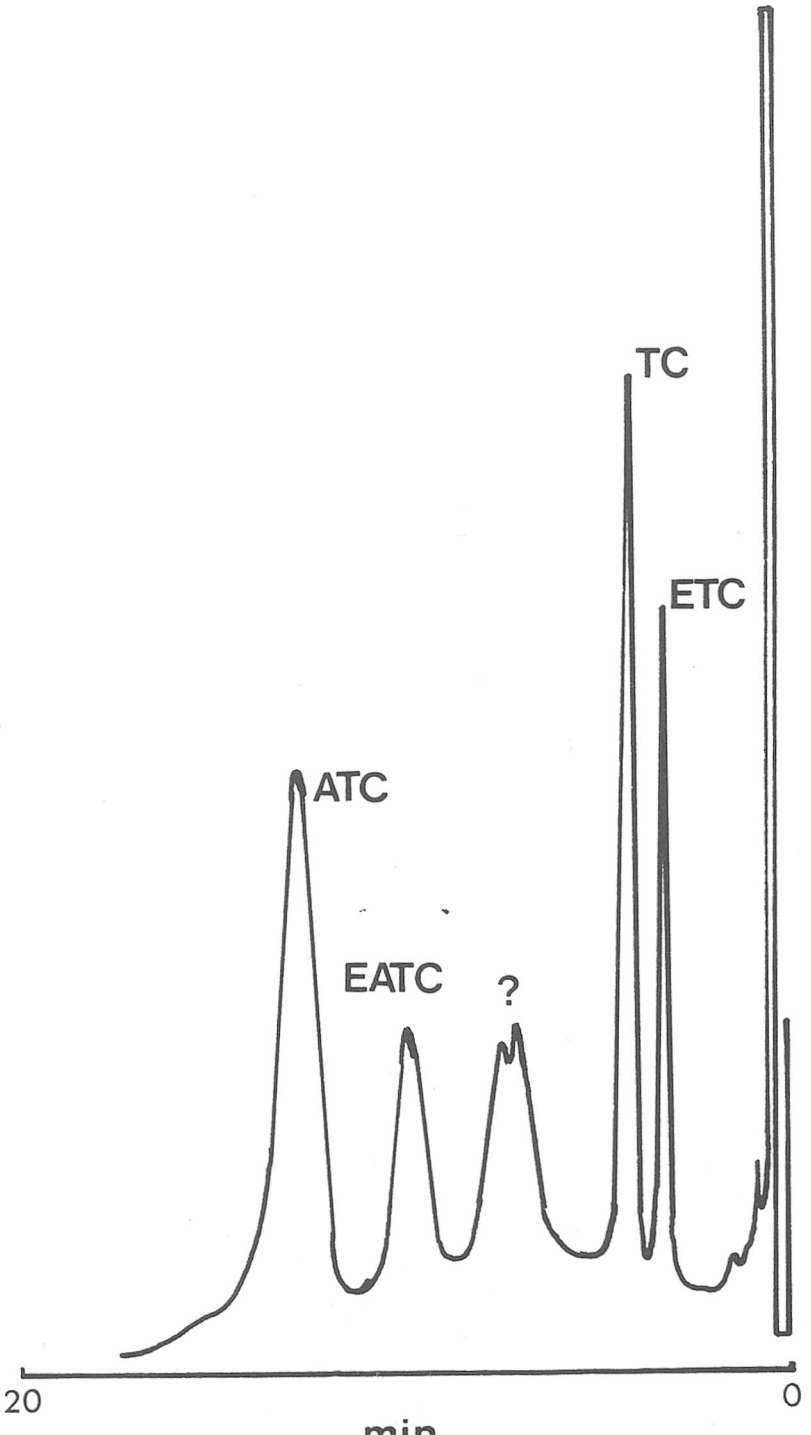


Figure 5.12 Sample chromatogram showing the presence of additional peaks during the decomposition of TC in McIlvaine's buffer at pH 8.0.



Chapter 6

6.0 The Decomposition of Nafimidone

6.1 Introduction

Nafimidone represents a potential drug compound which has been inferred to possess anti-convulsant activity in grand-mal epilepsy[244]. It has been proven to be an antagonist of stunning psycho-motor seizures suggesting that it would be useful for various other epileptic conditions besides grand-mal with the exception of petit-mal epilepsy.

Nafimidone (ND) comprising of fine needle like crystals contains a naphthalene skeleton. The formulae of ND are shown in Scheme 6.1. It is a non-hygroscopic compound with a melting point of 224-226°C[244]. The ultraviolet spectrum of ND in both methanol and aqueous solution exhibits an absorption maximum at approximately 250nm. Few spectral changes are observed when the compound is stored in aqueous solution under acidic or neutral conditions. In alkaline solution, however, the $E^{1\%}_{1cm}$ at 220nm. is reported to increase whilst at 250nm. a decrease is observed indicative of decomposition[244].

The dissociation constant has been determined, from the solubility of the drug at different pH values as pK_a=6.48 at 25°C. Limited preliminary stability data[244] indicates that the drug is stable under acidic and neutral conditions and in ethanol but undergoes rapid decomposition in methanol and alkaline conditions at

elevated temperatures. No decomposition has been observed when buffered solutions were exposed to light under ambient conditions and at 25°C in a light/dark cycled incubator (12 hours light/12 hours dark). When heated to melting, ND has been reported to produce a single yellow coloured decomposition product.

From the above short term stability data it has been shown that decomposition occurs rapidly at pH values above 7 but more slowly in the pH range 1 to 6. At all pH values the decomposition was found to exhibit a low temperature dependence leading to the assumption of a low activation energy. Although the reaction order has not been clearly established, assuming first order kinetics an over all activation energy of 7.3 $kJmol^{-1}$ was calculated for decomposition at pH 8 (R^2=0.71). Overall it was concluded that the decomposition reaction is complex, particularly in view of the low activation energy and the poor fit of the data to the Arrhenius equation[244].

Although hplc was used as the analytical method to obtain the above stability data, kinetic treatment was limited to the conventional measurement of the parent drug. No information was available regarding the possible decomposition products in aqueous solution although thermal decomposition had been investigated.

In the present work it was intended to examine the decomposition in solid and in solution in more detail. Decomposition was performed under more controlled conditions. Where possible decomposition was allowed to proceed to larger extents than was the case

previously[244]. It was necessary to develop assay methods capable of locating and resolving decomposition products from the parent compound. Where possible the initial rate method was utilised to determine its applicability in the absence of complete identification of the decomposition products.

6.2 Equipment and Materials

Chromotographic measurements were carried out using the apparatus described in Chapter 3. Additional apparatus used included a Waters Systems controller and Data Module and Waters Wisp 710B automated sample injector. Columns used were 100 x 2mm. slurry packed with 5µm ODS-Hypersil (Shandon Laboratories).

Stopped flow measurements were made using a Cecil CE 588 High Performance Micro Computer Scanning Spectrophotometer adapted to take a 10µl flow cell while infrared spectra were obtained on a Perkin-Elmer 681 Infrared Spectrophotometer. Molecular weight analysis in solution was carried out using a Mecrolab Vapour Pressure Osmometer model number 301A.

Nafimidone was supplied by Syntex Research Centre, Edinburgh. Sodium lauryl sulphate (SLS) was obtained from Fisons as was octane sulphonic acid (OSA). Tetraethylammonium (TEA) and tetrabutylammonium (TBA) as the phosphate were obtained from Aldrich Chemical Co. Ltd. Acetonitrile was obtained from Rathburn Chemicals and water was purified using a Millipore MilliQ system. All other chemicals were of AnalaR or equivalent grade.

6.3 Results and Discussion

6.3.1 Preliminary Decomposition Measurements

From the short term stability data available[244], the decomposition of ND was thought to be a complex process. This was indicated in the limited data available by a number of spots in addition to that for ND using thin layer chromatography. These results could not be reproduced during the present work. Hplc using ultraviolet detection was, therefore, investigated as a qualitative means of examining for the presence of decomposition products. ND was subjected to three different methods of decomposition described below.

(A) ND heated to melting, dissolved in methanol and suitably diluted in water. It has been indicated previously that this method of decomposition produced at least one major product[244].

(B) ND dissolved in methanol and made up to volume with 20mM. phosphate buffer pH 6.5. The sample was maintained at 90°C in a water bath for approximately 100 hours.

(C) ND dissolved in methanol and made up to volume with McIlvaine's buffer pH 7.0. The sample was heated as described in (B) above.

Reverse phase chromatography at pH 7 and pH 2 showed that decomposition on melting and in aqueous solution produced different products.

6.3.2 Chromatography at pH 7.0

Sample (A) gave two peaks as shown in Figure 6.1. That for ND exhibited a retention time of 8 minutes while the second peak due to thermal decomposition (TP) showed a considerably longer retention time, in excess of 150 minutes. Figure 6.2 represents a chromatogram of sample (B). Four peaks were observed, three of which, P_1, P_2, P_3, were eluted before the parent compound. No peak coinciding with TP in sample (A) was observed. Sample C also exhibited three peaks in addition to that of ND as can be seen from Figure 6.3. Peaks P_1 and P_3, however could only be detected at very high sensitivity (0.005AU). Peak P_2 appeared to be the major product of decomposition in McIlvaine's buffer, all three peaks being formed more rapidly in phosphate buffer. No peak corresponding to TP in sample (A) was observed.

6.3.3. Chromatography at pH 2.0

Sample (A) yielded two peaks, one being ND with a retention time of 1.5 minutes as is shown in Figure 6.4. The second peak due to thermal decomposition exhibited a longer retention time of 4 minutes. No additional peaks were observed. Injection of sample (B), Figure 6.5, resulted in four peaks one being that for ND. Of the three additional peaks, two showed retention times of less than 1.5 minutes and the third a retention time very similar to that for ND. Figure 6.6 shows a chromatogram for sample (C). A similar pattern as exhibited by sample (B) was observed at very high sensitivity.

From the above it is clear that decomposition under aqueous conditions result in products different from that obtained by thermal decomposition.

Although it is known that ND is a base no information was available regarding the ionic nature of the decomposition products. It is obvious that the thermal product is more lipophilic than ND as evidenced by its longer retention time compared with ND at either pH value. Overall a similar pattern is observed in the different buffers indicating a constant decomposition pattern depending upon the method of decomposition. No other peaks of comparable magnitude were observed. Due to the inadequate resolution between peaks P_1 and P_2 at pH 7 (Figure 6.2) and between P_1, P_2, P_3 and ND at pH 2, these assay methods could not be utilised for stability studies. It has been shown[112,180] that characteristic variation in retention (or capacity factor) is observed depending on the ionic nature of the solutes on addition of pairing ion to the solvent. The above decomposition systems were therefore investigated further as a function of both cationic and anioncic pairing ion concentration.

6.3.4 Chromatographic Separation using Cationic Pairing Ion at pH 7

Stepwise addition of TBA to the solvent resulted in a parabolic variation in capacity factor (k') for P_1, P_2 and P_3 with increasing pairing ion concentration as seen in Figure 6.7. Both TP and ND on the other hand showed in rapid decrease in k' at low TBA concentrations, the

decrease in k' for TP being more rapid consistent with its greater lipophilicity. At high pairing ion concentrations the k' for ND and TP level off. A similar pattern was observed when using a cationic pairing ion of greater (CTAB) and lesser (TEA) hydrophobicity than TBA indicating that P_1, P_2 and P_3 are acidic in nature while TP is either basic or neutral in character. An optimum chromatogram for all five species is shown in Figure 6.8 using 20mM. TBA. It can be seen that P_3 and TP are eluted after ND. While a lower TBA concentration would allow elution of P_3 before ND resolution between P_1 and P_2 would be inadequate for quantitation purposes.

6.3.5 Chromatographic Separation Using Anionic Pairing Ion at pH 2

Stepwise addition of SLS to the solvent resulted in a parabolic variation in k' for both ND and TP as seen in Figure 6.9. The increase in k' for TP with increasing pairing ion concentration confirms that TP is basic in character exhibiting greater lipophilicity than ND. P_1, P_2 and P_3 on the other hand show a marked reduction in their already low k' and were rapidly eluted with the solvent front on addition of SLS. The solvent in this case consisted of 50% acetonitrile, twice that used in the investigation with cationic pairing ion. Current ion pairing[180] ideas indicate that reduction of acetonitrile concentration in the presence of SLS would have the effect of dramatically increasing the retention of ND and TP simultaneously reducing the retention of the acidic

species. This has been demonstrated previously[180] for similar systems. In order to retain the acidic compounds while minimising the increase in ND and TP retention it was necessary to utilise a less hydrophobic pairing ion OSA and a lower acetonitrile concentration. In the absence of OSA, P_1 and P_3 were eluted after the parent ND. TP, however, exhibited a retention time in excess of 120 minutes and was therefore excluded from this study. Figure 6.10 shows the variation of k' for P_1, P_2, P_3 and ND as a function of OSA concentration. As can be seen from this Figure the k' for P_1, P_2 and P_3 is decreased at low OSA concentrations gradually levelling off as the OSA concentration is increased. ND on the other hand shows a parabolic variation in k'. A typical chromatogram obtained using this system at an OSA concentration of 85mM. is shown in Figure 6.11.

For the purpose of stability investigations the solvent system shown in Figure 6.8 comprising water/acetonitrile, 75/25, containing 40mM. phosphate buffer and 20mM. TBA adjusted to pH 7 was chosen even though the solvent system containing OSA allowed elution of all acidic decomposition products before the main reactant peak. This choice was made on the basis that the latter system resulted in poor peak shape for ND as shown in Figure 6.11. Prior to any stability work, reproducibility of analysis was determined by repeated injection of the same partially decomposed aqueous sample (n=5). The relative standard deviation obtained for each compound are as follows; P_1 (1.97%), P_2 (1.51%), ND

(1.64%), P_3 (1.11%). The peak height vs concentration plot for ND was found to be linear (R^2=0.99) over the sensitivity scale used.

6.4 Identification of Decomposition Products

The chromatographic investigations described above indicates that all products of decomposition, whichever method of decomposition is chosen, are ionic in character. Before conducting stability investigations indentification of the decomposition products was attempted.

6.4.1 Identification of the Thermal Decomposition Product TP

ND was heated to its melting point and maintained at this temperature for 30 minutes using an oil bath. On melting, a dark brown glass resulted which on cooling was found to be slightly water soluble. After washing in water, the glass was dissolved in warm methanol and re-precipitated by the slow addition of cold distilled water. The precipitate was filtered, washed with 200 ml. of water and dried under vacuum. The resultant solid was analysed by hplc and found to be approximately 98% pure. Table 6.1 shows a comparison between the ultraviolet spectral characteristics of TP and ND in water. The spectra are shown in Figure 6.12. The spectra are practically indistinguishable other than minimal increased absorption in the 350-400nm. region for TP. Additional minor differences are also observed in the 200-225nm region.

Figures 6.13 and 6.14 show the infrared spectra for ND and TP respectively. As with the ultraviolet spectra the main spectra are similar with differences below $1000cm.^{-1}$.

Vapour pressure osmometry was utilised in an attempt to estimate the molecular weight of TP. The measurements were carried out in methanol and ND was used to calculate the calibration constant. The results are presented in Table 6.2 and 6.3 . Using this technique the molecular weight of TP was calculated as shown below:

$$MW_{TP} = K/(\Delta R/C_{TP})$$

where ΔR is resistance change (ohms)

C_{TP} is the concentration of TP as $\%^{w}/_{v}$

K is the calibration constant given by

$$K = MW_{ND}(\Delta R/C_{ND})$$

The molecular space weight was calculated as 705 suggesting that ND on heating to melting undergoes polymerisation to either a dimer or a trimer. This is consistant with the chromatographic findings regarding TP's greater lipophilicity in contrast to ND. It is likely that mass spectral analysis fragments the polymer so producing results indentical to those for ND.

6.4.2 Identification of P_1, P_2 and P_3

Ultraviolet spectra for these compounds, shown in Figures 6.15 - 6.17 were obtained using the stopped flow technique[245]. Both P_2 and P_3 showed spectra similar to that of ND. Maximal absorption was observed at 248nm. and 285nm., P_1 showing maximal absorption at 270nm.. Of the ultraviolet spectra for the various compounds containing a 2-substituted naphthalene skeleton, the spectrum for 2-naphthoic acid presented as Figure 6.18 showed maximal absorption at 270nm. in the mobile phase. Injection of a sample of 2-naphthoic acid (2-NA) resulted in a retention time similar to that of peak P_1. In order to establish that P_1 was indeed 2-NA, the variation in k' for both P_1 and 2-NA as a function of both cationic and anionic pairing ion concentration was investigated. Three different cationic and an anionic pairing ion were employed. A similar method for the identification of acidic species in decomposed D-glucose solution has been previously demonstrated[245]. Figures 6.19 and 6.20 show the variation in k' for both P_1 and 2-NA using TEA and TBA, and OSA and cetrimide (CTAB) respectively. In the case of CTAB, TBA and TEA both species show a similar parabolic increase in k' with pairing ion concentration while in the presence of OSA the k' are seen to decrease. This confirms both the acid nature and identity of P_1 as 2-naphthoic acid.

In order to identify P_2 and P_3 it was first necessary to separate all the acidic species from the parent ND. This was achieved by extracting with chloroform under

alkaline conditions. The aqueous phase containing P_1, P_2 and P_3 was acidified and extracted into ether which after evaporation yielded P_1, P_2 and P_3. Chromatographic separation followed by further extraction into ether enabled collection of trace amounts of each of the compounds. Mass spectral analysis, however, failed to provide any interpretable results. The spectra of all species contained a large number of contaminent signals thought to be due to plastisisers from the caps of vials containing the sample. Due to the small amount of acids extracted it was not possible to determine the purity of each sample and it is suspected that all three samples were not sufficiently pure to provide conclusive mass spectral evidence as to their nature.

6.5 Stability Studies

It has been shown above that ND undergoes decomposition on melting to produce a single basic product TP. In aqueous solution, however, three acidic products are formed. The absence of TP during decomposition in solution suggests that the mechanisms of decomposition are different depending on the method of decomposition. Decomposition of ND was therefore followed both in the solid state and in aqueous solution.

6.5.1 Decomposition in the Solid State

As the thermal product, with an approximate molecular weight of 705, could be obtained in a relatively pure form it was intended to follow the solid state decomposition by

both the conventional method of measuring ND decrease and the initial rate method by analysing for TP. ND was subjected to decomposition at 130°C for a period of 3 weeks. Samples were initially removed at hourly intervals for the first ten hours and then twice daily. Analysis by hplc was carried out using TBA as the pairing ion. After a period of three weeks no change in ND concentration was observed. Furthermore there was no evidence of TP or any other products being formed. It was concluded that the thermal product formed on melting does not constitute a solid state decomposition product and the kinetics of formation of this product were not pursued further.

6.5.2 Decomposition in Aqueous Solution

Preliminary stability studies have shown that ND decomposed to produce three products in phosphate buffer in substantial amounts whilst in McIlvaines buffer only one major product, P_2, was evident P_1 and P_3 being present in very small amounts. Stability investigations were therefore carried out separately in these two buffers.

(i) Decomposition in phosphate buffer: Decomposition was carried out in 20mM. phosphate buffer at various pH values and temperatures. Because of the inability to identify P_2 and P_3 the initial rate method could not be applied in its fullest sense. Decomposition was therefore followed by determining the decrease in ND concentration and increase in 2-NA concentration as a function of time. P_2 and P_3 were measured by measuring the increase in peak area as a function of time. Monitoring the products

during the experiment showed increases in all decomposition peaks. Figure 6.21 shows the variation in peak area as a function of time for P_1, P_2 and P_3 and the concentration-time data for ND. This typical decomposition pattern permits identification of the route of decomposition. Like the decomposition of TC (Chapter 5) all products are seen to increase with time suggesting that all three products are formed simultaneously, that is, the decomposition follows parallel routes. Unlike the decomposition of TC, where ETC shows a decrease after reaching a maximum (Figure 5.10(B), no such decrease is observed in this instance for any species signifying the absence of a consecutive reaction.

Although P_2 and P_3 remain unidentified the initial rate method was applied to the decomposition of ND to 2-NA (P_1) using Equation (1-16) and the effect of pH determined at 90°C. Decomposition was monitored using this buffer in the pH range 4.5 to 7.5. Figure 6.22 shows the variation in logk-pH profile for both 2-NA and ND. It is seen that the profile for ND is different from that of 2-NA. This is as would be expected as the loss in ND concentration will yield a composite rate constant, while analysing for 2-NA will yield a rate constant purely for the conversion of ND to 2-NA. Table 6.4 shows the first order rate constants for ND decomposition based on ND analysis and that for the ND to 2-NA reaction obtained by the initial rate method analysing for 2-NA. The rate constants for ND decomposed are considerably greater than those for 2-NA formation signifying that 2-NA is a minor product of decomposition.

Although the data for P_2 and P_3 could not be treated in a similar manner with respect to pH, the rate of appearance of each decomposition product P_1, P_2 and P_3 in arbitrary d[Peak Area]/dt units as a function of temperature can be used to calculate the activation energy for the formation of each product in the usual units of $kJmol.^{-1}$. The Arrhenius plots are shown in Figures 6.23 for P_2 and P_3 and 6.24 for 2-NA and ND. The derived activation energies are shown in Table 6.5 together with their relative standard deviations and correlation coefficients. The "students t" test was applied to the slopes obtained from the Arrhenius data for each decomposition product to determine if a significant difference exists between the slopes for the products and that for ND. Using the equation shown below[156]:

$$t = (b_1 - b_2)/sp \times \sqrt{A}$$

where A is

$$A = 1/\Sigma(x_1 - \bar{x}_1)^2 + 1/\Sigma(x_2 - \bar{x}_2)^2$$

and sp is

$$sp = \sqrt{((n_1 - 2)SD_1^2 + (n_2 - 2)SD_2^2)/(n_1 + n_2 - 4)}$$

and

b_1 is the slope of one compound using Arrhenius data.

b_2 is the slope of the second compound using Arrhenius data.

x_1 and x_2 are the reciprocal of absolute temperature.
$\bar{x}_1$ and $\bar{x}_2$ are the mean of the sum of x_1 and x_2 respectively.
sp is a pooled estimator based on both samples.
SD_1 and SD_2 are the respective standard deviations of b_1 and b_2.
n_1 and n_2 are the number of points used to calculate the slope b_1 and b_2 respectively.

No significant difference was observed between the slopes of 2-NA, P_2 and P_3 compared with that of ND (P=0.005). This is taken to indicate that while the ND line represents a composite reaction the activation energies of the various individual reactions are similar.

(ii) Decomposition in McIlvaine's buffer: McIlvaine's buffer was chosen as a means of maintaining a constant ionic strength (μ = 0.5M)[246] during decomposition. In McIlvaine's buffer the rate of decomposition was found to be considerably slower. Also only one decomposition product, that represented by P_2 showed an increase with time. Both P_1 and P_3 though present at the start of the study remained unchanged after 100 hours at 90°C. In addition ND showed only a slight decrease at pH 8.1 representing less than 5% decomposition. Because of the slow nature of the decomposition under these conditions the reaction was followed by analysing for P_2 formation. The concentration of P_2 was determined by equating the loss in ND concentration at PH 8.1 with the peak height of P_2 on the assumption that P_2 is the only product formed

within the time scale of the experiment. The concentration of P_2 was calculated using Equation (6-5) derived below

$$A = P_{ND}/[ND] \qquad (6\text{-}1)$$

$$B = P_P/[P_2] \qquad (6\text{-}2)$$

where

A is the calibration constant for ND in appropriate units
B is the calibration constant for P_2 in appropriate units.
P_{ND} is the peak height of any ND concentration.
P_P is the peak height of any P_2 concentration.

$$[ND]_{LOST} = [P_2]_{GAINED}$$

$$[ND]_{LOST} = ((P_{ND})_0 - (P_{ND})_t/A)$$

$$[P_2]_{GAINED} = ((P_P)_t - (P_P)_0/B)$$

where
$(P_{ND})_0$ and $(P_{ND})_t$ are the ND peak heights at time zero and t respectively.
$(P_P)_0$ and $(P_P)_t$ are the P_2 peak heights at time zero and t respectively.
Therefore

$$((P_{ND})_0 - (P_{ND})_t/A) = ((P_P)_t - (P_P)_0/B)$$

and

$$B = (P_P)_t - (P_P)_0 \times A/(P_{ND})_0 - (P_{ND})_t \qquad (6\text{-}3)$$

rearranging Equation (6-2)

$$[P_2] = P_P/B \qquad (6\text{-}4)$$

substituting Equation (6-3) in Equation (6-4) yields

$$[P_2] = P_P/(((P_P)_t - (P_P)_0) \times A/(P_{ND})_0 - (P_{ND})_t) \qquad (6\text{-}5)$$

Thus the concentration of P_2 can be estimated under these conditions in the absence of the knowledge of its identity. The P_2 concentration-time data so calculated was used to evaluate the initial rate constants for the formation of P_2 at different pH and temperatures. Equation (1-16), shown in Chapter 1, was used to calculate the first order rate constants shown in Table 6.6 together with the derived activation energy. It can be seen from Tables 6.4 and 6.6 that the rate constants determined by following ND decrease in phosphate buffer (Table 6.4) are considerably greater than those calculated by following P_2 increase in McIlvaine's buffer. This signifies that ND is more stable in McIlvaine's buffer than in phosphate buffer. The decomposition in McIlvaine's buffer was however too slow to allow determination of rate constants by conventional methods and no comparison is therefore

possible between the more commonly used method of following the parent compound and the initial rate method. Also the accuracy of the latter results depends largely upon the admittedly crude estimation of P_2 concentration.

The logk-pH profile for the decomposition of ND to P_2 in McIlvaine's buffer is shown in Figure 6.25. Under alkaline conditions conditions the rate of P_2 formation increases with increasing pH. Greater stability is observed at intermediate pH values between pH 4 and 5. Under more acidic conditions the rate is seen to increase slightly between pH 4 and 2.75 before decreasing again. However, as seen from Table 6.6 the overall change in reaction rate below pH 5.9 is small compared with that above pH 6 indicating greater stability under acidic condition.

6.5.3 Determination of Reaction Order

Using the conventional approach of following the decrease in reactant concentration over large extents of reaction, first order kinetics were found to represent the decomposition of ND adequately in phosphate buffer. In McIlvaine's buffer where the decomposition is markedly slower the above method would involve lengthy experimentation. Determination of the order of reaction by the initial rate method was therefore attempted. Using an initial ND concentration ranging from 1.5×10^{-4} to 6.2×10^{-4}M the logk - $\log[ND]_0$ profile was found to be represented by the equation shown below

$$\log (\text{Rate}) = 1.16[ND] - 2.89$$

However, in the instance the error associated with the slope was found to be unduly high (RSD = 32%, R^2 = 0.81) and could not be improved on repetition of the experiment. The only plausible explanation that can be offered for these poor results is the knowledge that ND has been observed to precipitate from solution as the hydrate.

6.6 Conclusion

It has been shown that ND is a stable drug showing no decomposition in the solid state at a temperature well above that experienced under normal storage conditions . In aqueous solution three decomposition products are formed, all of which show an increase in phosphate buffer as ND concentration decrease. A stability indicating assay has been developed capable of simultaneously quantifying all 5 compounds, that is, P_1, P_2, P_3, TP and ND. On the basis of chromatographic evidence one of the decomposition products is identified as 2-NA. Activation energies for the simultaneous formation of 2-NA, P_2 and P_3 in aqueous solution are calculated even in the absence of the knowledge of the identity of two of the decomposition products. The activation energy calculated for ND during the present work was found to be considerably higher than that determined previously.

In McIlvaine's buffer only one product, P_2, is seen to increase with time, little decrease in ND being observed. On the assumption that all products formed are detectable, the loss in ND has been correlated to the gain in P_2 peak height and the effect of temperature and pH

determined.

This particular study demonstrates the limitations of product measurement. While useful kinetic data such as the route of decompostion and the effect of temperature and pH may be determined by measurement of decomposition products, the absence of knowledge regarding product identity does not allow calculation of shelf life for example nor the rate at which each of the products are formed unless considerable assumptions are made concerning the stoichiometry of the reaction.

Table 6.1 Spectral Characteristics for ND and TP in Methanol.

Wavelength(nm)		244	248	288	292	336	341	398
$A^{1\%}_{1cm.}$	ND	1184	1355	290	270	68	68	0
	TP	1047	1375	295	280	80	79	11.6

Table 6.2 Vapour Pressure Osmometry data

Concentration% $^{W}/_{v}$	0.32	0.64
ΔR_1	5.7	9.6
ΔR_2	6.4	8.3
ΔR_3	6.3	7.7
Average ΔR	6.13	8.53
ΔR/Concentration	19.17	13.33

$\Delta R/C$ (C→O)	17.95
Calibration Constant (K)	4882.40

Table 6.3 Vapour Pressure Osmometry data

Concentration% $^{W}/_{v}$	0.32	0.64
ΔR_1	3.3	2.8
ΔR_2	2.0	2.9
ΔR_3	2.8	4.1
Average ΔR	2.7	3.27
ΔR/Concentration	8.44	5.10

$\Delta R/C$ (C → O)	6.93
Mol. Weight K/$\Delta R/C$	705.04

for ND in methanol.

0.96	1.60
13.6	21.7
13.4	21.9
13.0	21.8
13.3	21.8
13.85	13.63

for TP in Methanol.

0.96	1.60
9.0	14.2
7.7	12.9
6.8	11.6
7.83	12.9
8.16	8.06

Table 6.4 Shows the variation in rate constants as obtained by assay for ND decrease and 2-NA increase in phosphate buffer at various pH and temperatures.

Temp	pH	ND Rate Constant ($x10^{2}hr.^{-1}$)	2-NA Rate Constant ($x10^{5}hr.^{-1}$)
90°C	4.75	1.04	0.15
	5.60	1.05	0.50
	6.35	1.15	0.78
	6.59	1.13	1.19
	7.0	2.55	1.28
	7.1	3.19	1.31
	7.4	4.16	1.40
	7.75	4.94	1.99
80°C	7.10	1.50	1.97
65°C	"	0.19	0.22
40°C	"	0.0089	0.007

Table 6.5 Shows the activation energy derived by analysing for the different species in phosphate buffer at pH 7.0.

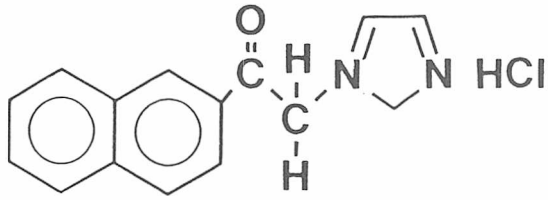
Species followed	ND	2-NA	P_2	P_3 *
E_a kJmol.$^{-1}$	113.6	107.3	130.1	85.49
RSD (%)	4.1	17.70	3.39	10.27
R^2	0.996	0.941	0.998	0.990

* only 3 points used to determine E_a. No P_3 observed at 40^{o}C.

Table 6.6 Shows the variation in rate constant as obtained by asaying for P_2 formation in McIlvaine's buffer at various pH and temperatures.

Temp (^oC)	pH	Rate constant $x10^4 hr.^{-1}$
90	2.0	1.86
	2.7	4.53
	3.95	0.86
	4.35	0.57
	5.35	2.02
	5.65	2.11
	5.90	4.98
	6.50	8.07
	6.73	10.30
	7.59	18.0
	8.10	27.2
80	5.90	2.39
70	"	1.05
60	"	0.44
E_a kJmol.$^{-1}$	79.93	
RSD(%)	2.69	
R^2	0.999	

Scheme 6.1 Showing the formulae for Nafimidone.



1-(-2-Naphthoylmethyl) Imidazole Hydrochloride

Molecular Formula $C_{15}H_{13}ON_2Cl$

Molecular Weight 272·74

Figure 6.1 Chromatogram for sample A. Solvent: acetonitrile : water (25/75) containing 40mM phosphate buffer pH 7.0 using a 100x2mm column. Sample size equals 20ul. Both peaks were measured at a sensitivity of 0.5AU.

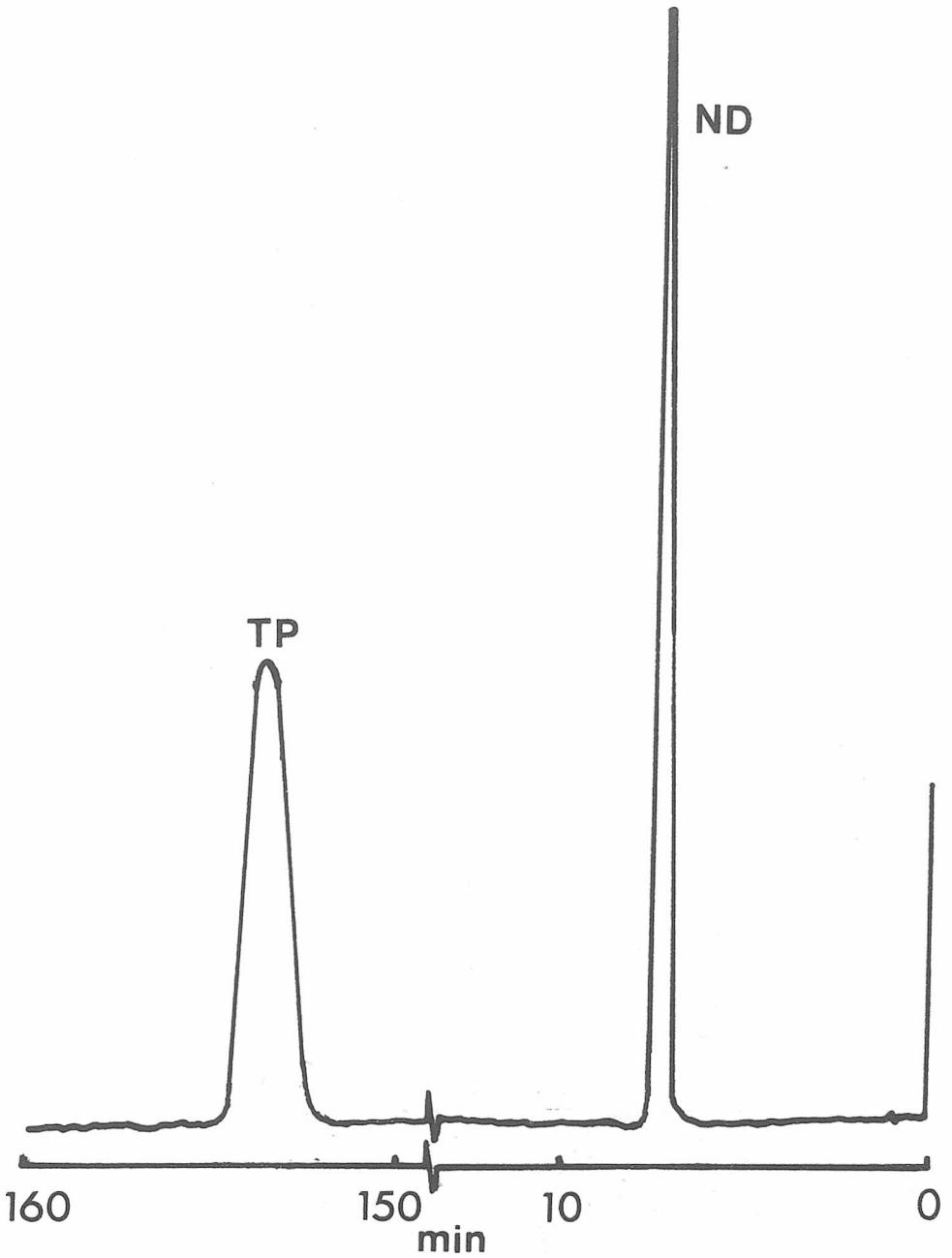


Figure 6.2 Chromatogram for sample B. Solvent: acetonitrile : water (25/75) containing 40mM phosphate buffer at pH 7 using a 100x2mm column. Sample size equals 20ul. All peaks measured at a sensitivity of 0.05AU.

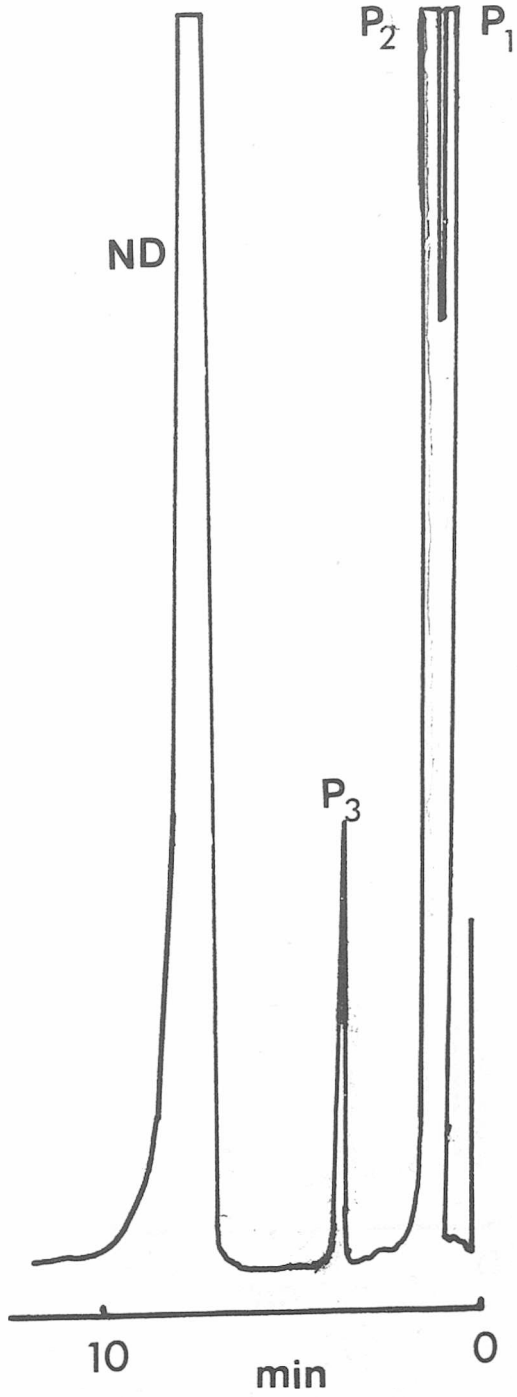


Figure 6.3 Chromatogram for sample (C). Solvent comprises acetonitrile : water (25/75) containing 40mM phosphate buffer at pH 7 using a 100x2mm column. Sample size equals 20ul. All peaks measured at a sensitivity of 0.005AU.

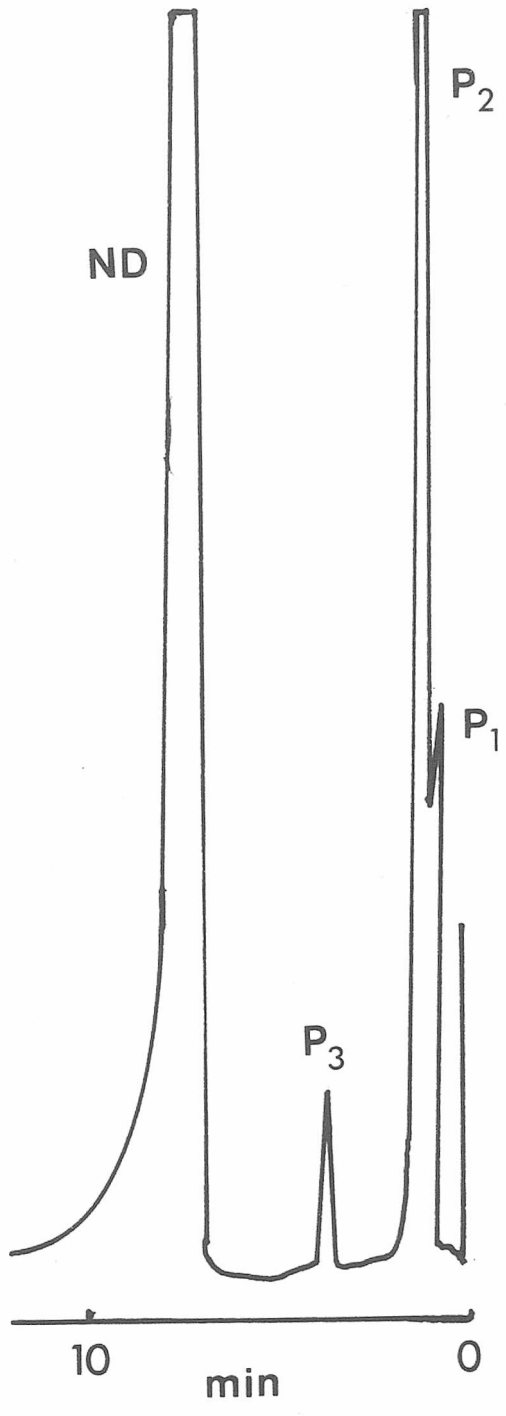


Figure 6.4 Chromatogram for sample A. Solvent: acetonitrile : water (50/50) containing 20mM phosphate buffer pH 2.0 using a 100x2mm column. Sample size equals 20ul. All peaks measured at a sensitivity of 2.0AU.

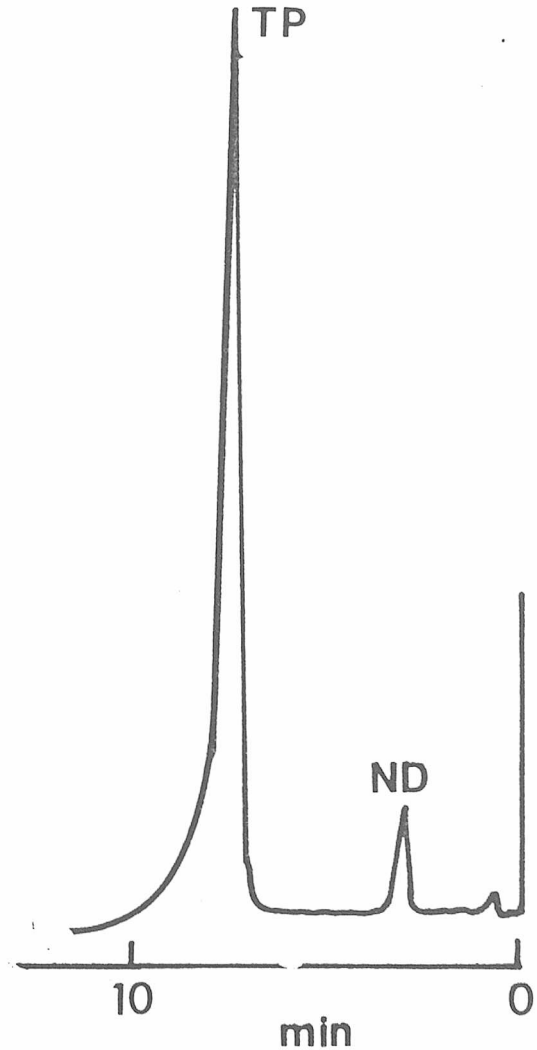


Figure 6.5 Chromatogram for sample (B). Solvent comprises acetonitrile : water (25/75) containing 20mM phosphate buffer at pH 2.0 using a 100x2mm column. Sample size equals 20ul. All peaks measured at a sensitivity of 0.05AU.

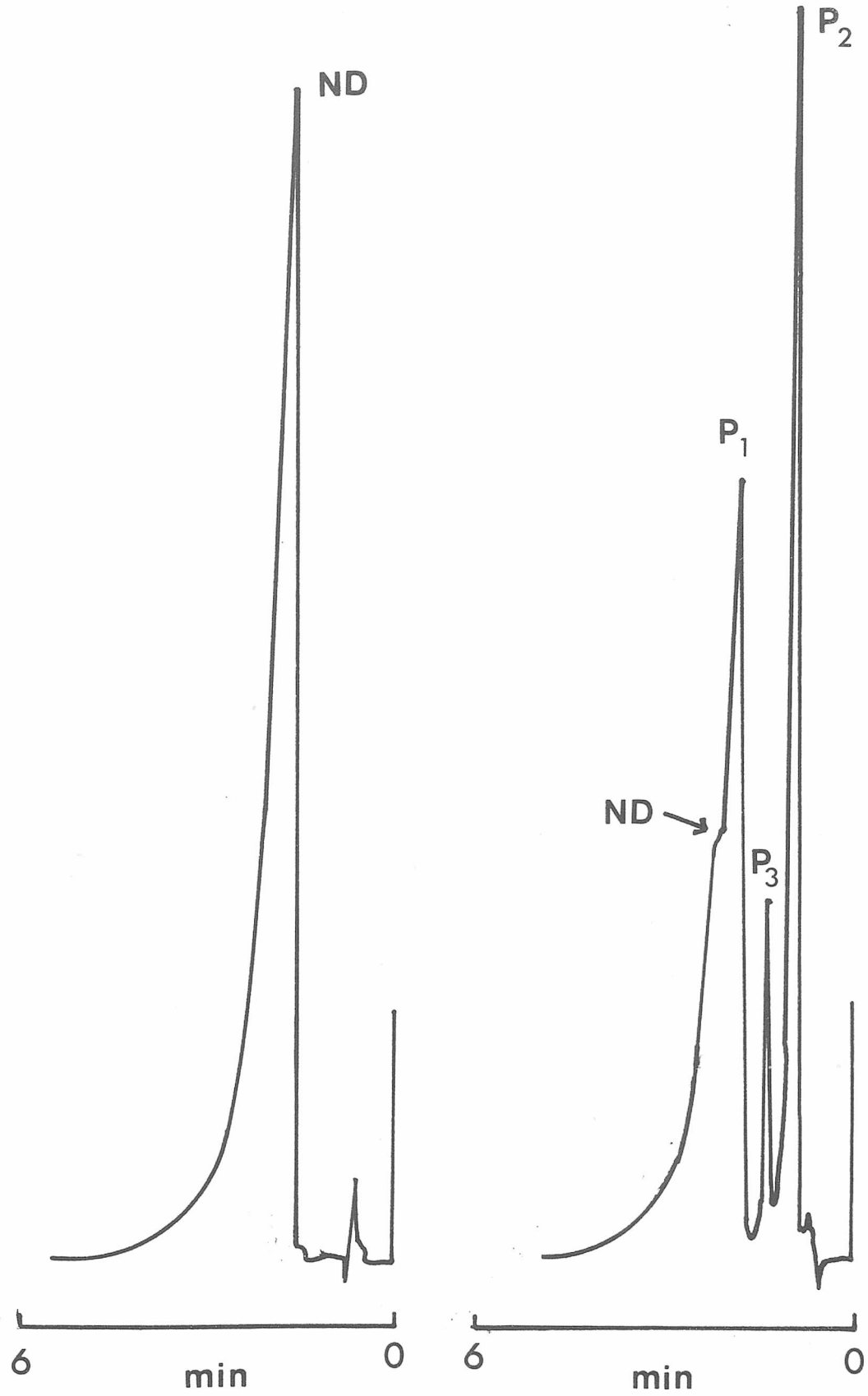


Figure 6.6 Chromatogram for sample C. Solvent comprises acetonitrile : water (25/75) containing 20mM phosphate buffer at pH 2.0. All peaks measured at a sensitivity of 0.005AU.

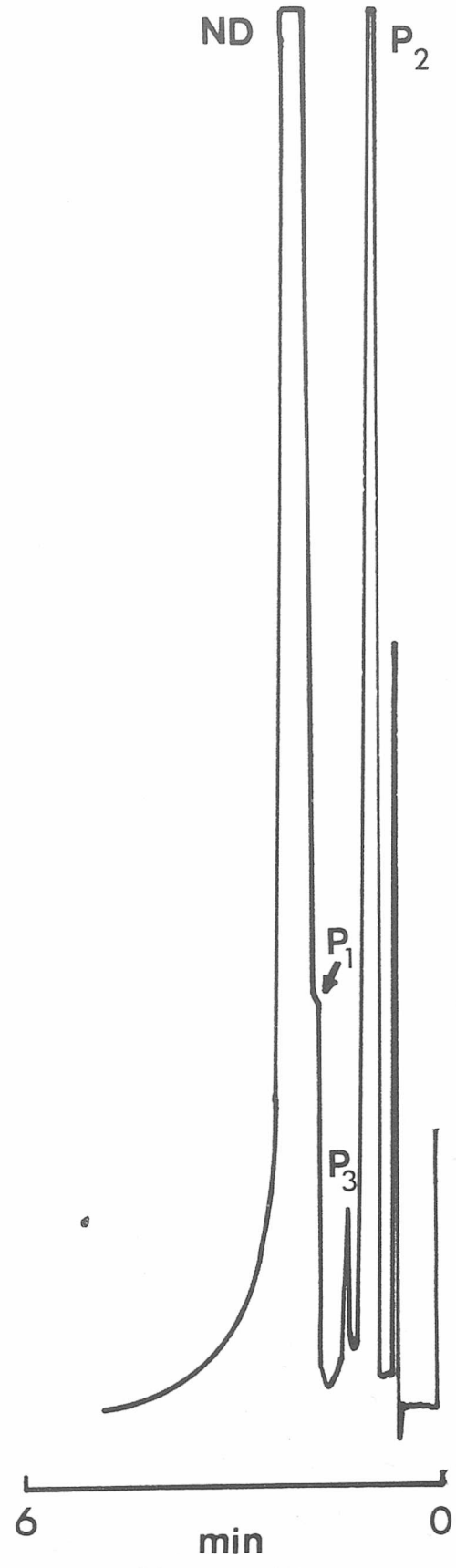


Figure 6.7 Plots showing the variation in capacity factor (k') as a function of cationic pairing ion for ND, TP, P_1, P_2 and P_3. Solvent: acetonitrile : water (25/75) containing 40mM phosphate buffer at pH 7.

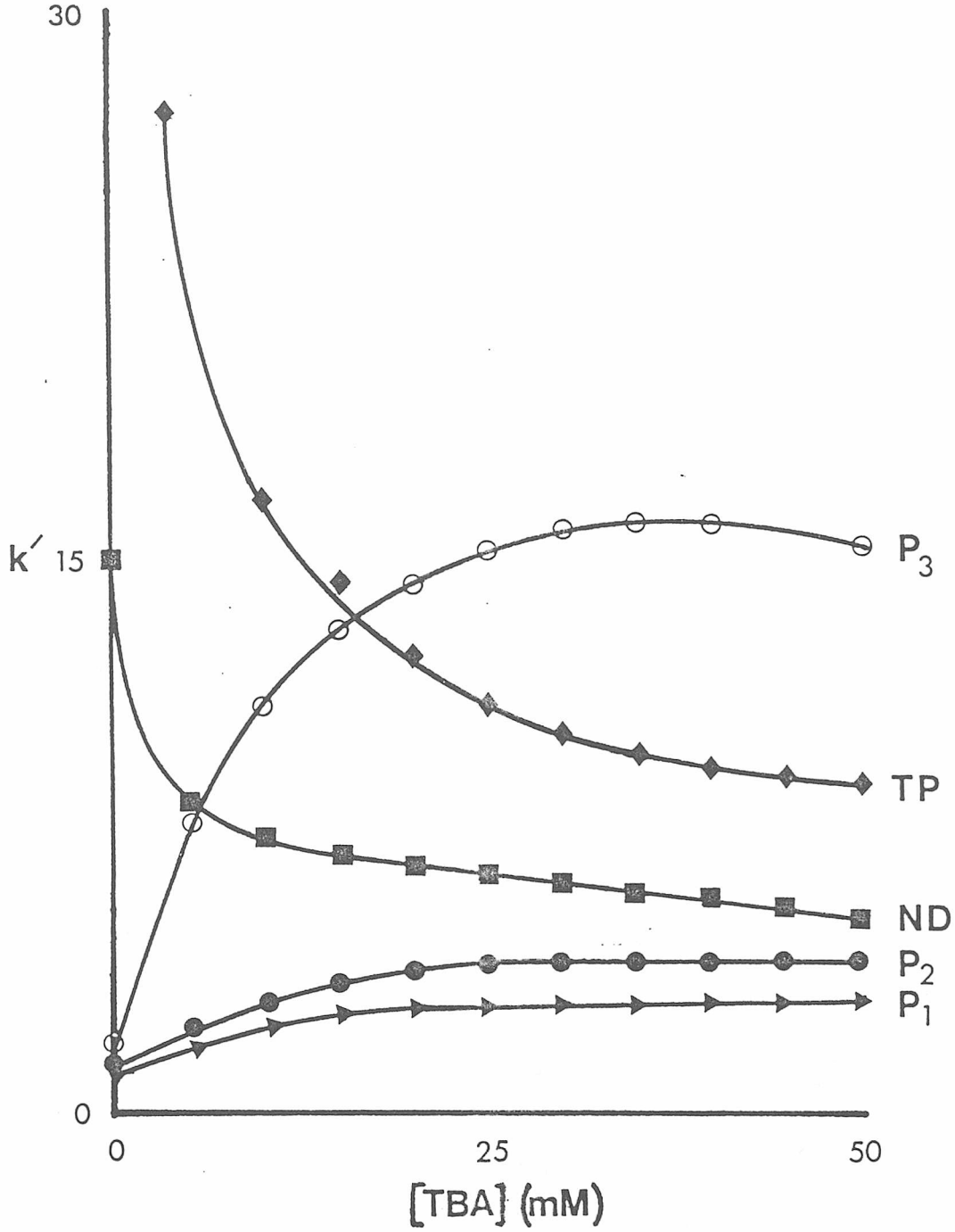


Figure 6.8 Optimum chromatogram showing the separation of P_1, P_2, P_3, ND and TP. Solvent: acetonitrile : water (25/75) containing 40mM phosphate buffer, 20mM TBA at pH 7.0.

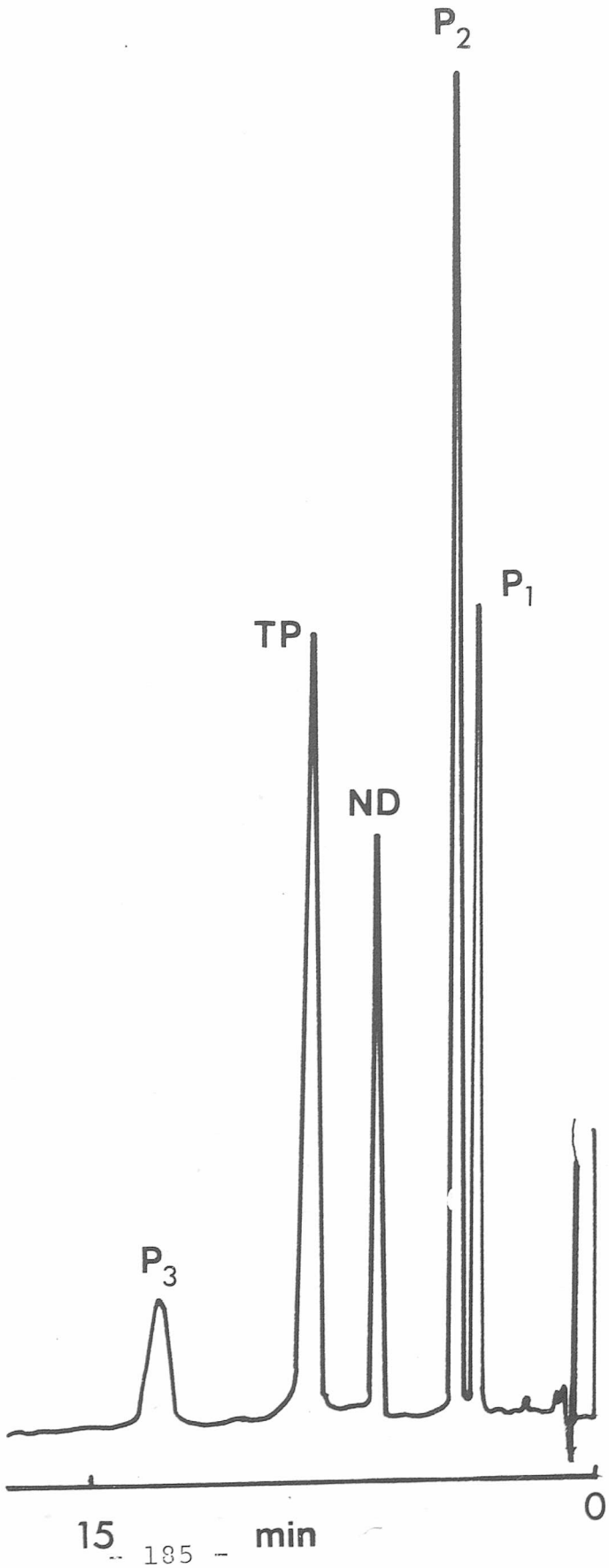


Figure 6.9 Variation in capacity factor (k') for P_1, P_2, P_3, ND and TP as a function of SLS concentration. Solvent: acetonitrile : water (50/50) containing 20mM phosphate buffer at pH 2.0.

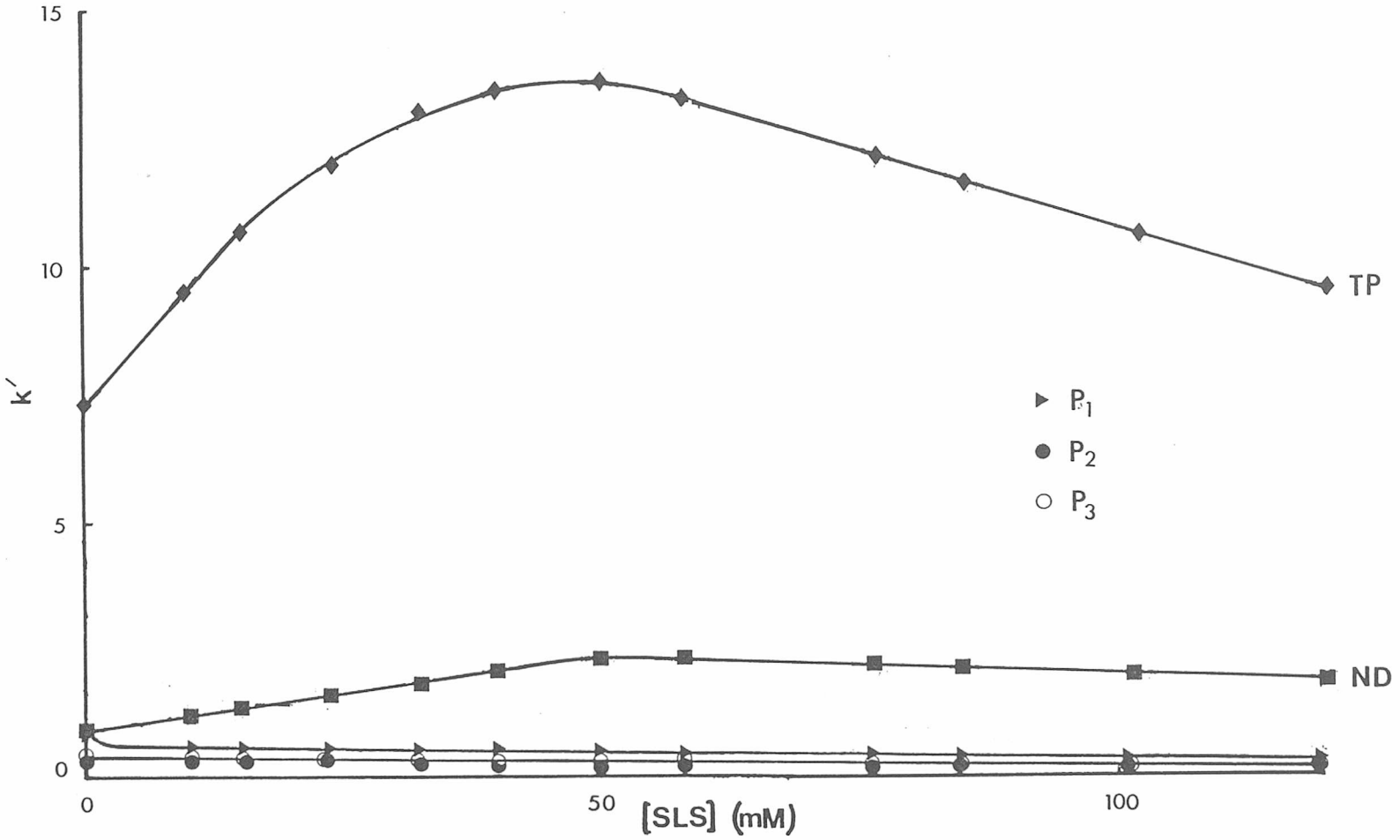


Figure 6.10 Variation in capacity factor (k') for P_1, P_2, P_3 and ND as a function of OSA concentration. Solvent: acetonitrile : water (25/75) containing 40mM phosphate buffer pH 2.0.

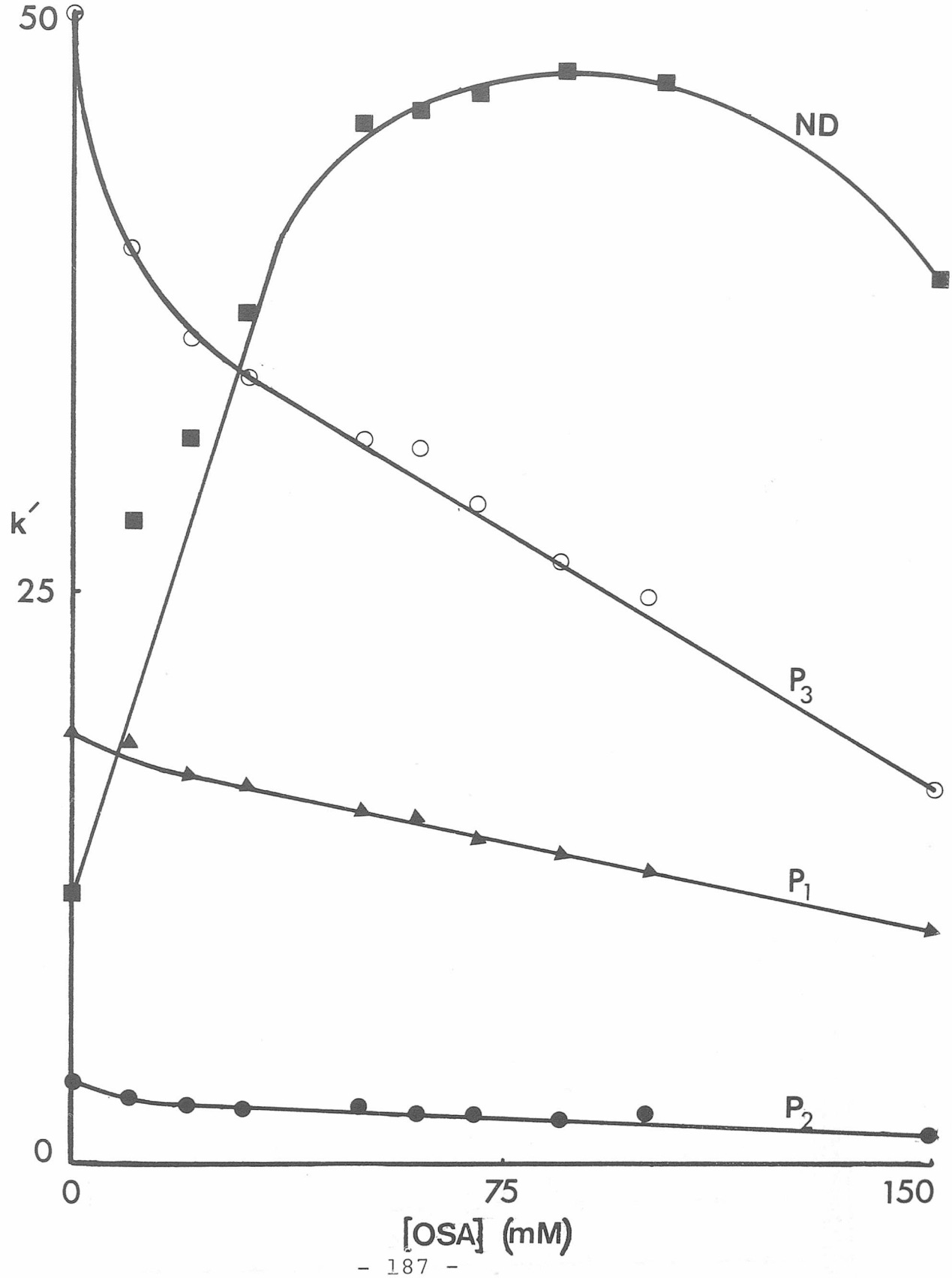


Figure 6.11 Showing the optimum chromatogram for the separation of ND and its decomposition products P_1, P_2 and P_3. Solvent: acetonitrile : water (25/75) containing 40mM phosphate buffer and 85mM OSA at pH 2.

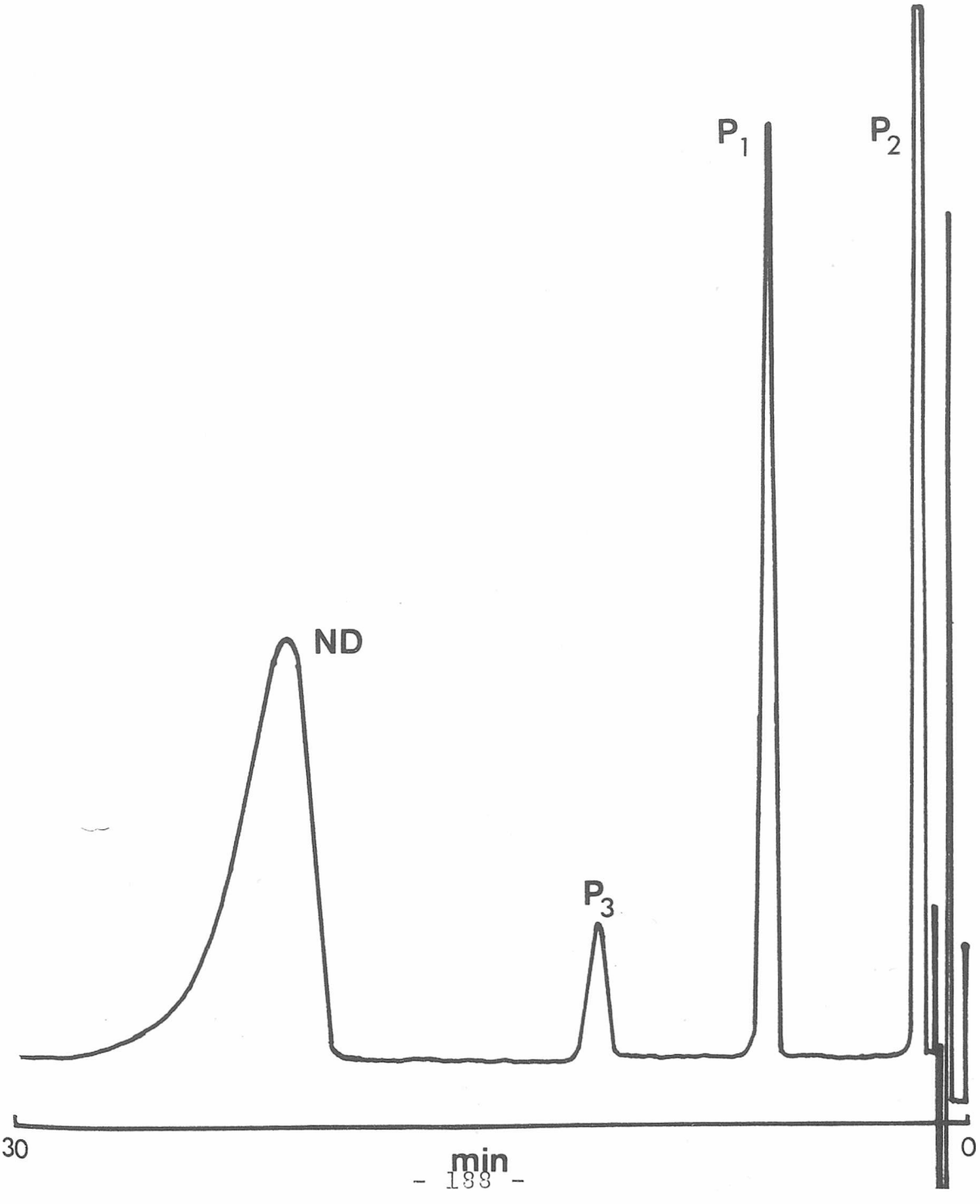


Figure 6.12 Ultraviolet spectra for ND and TP in water.

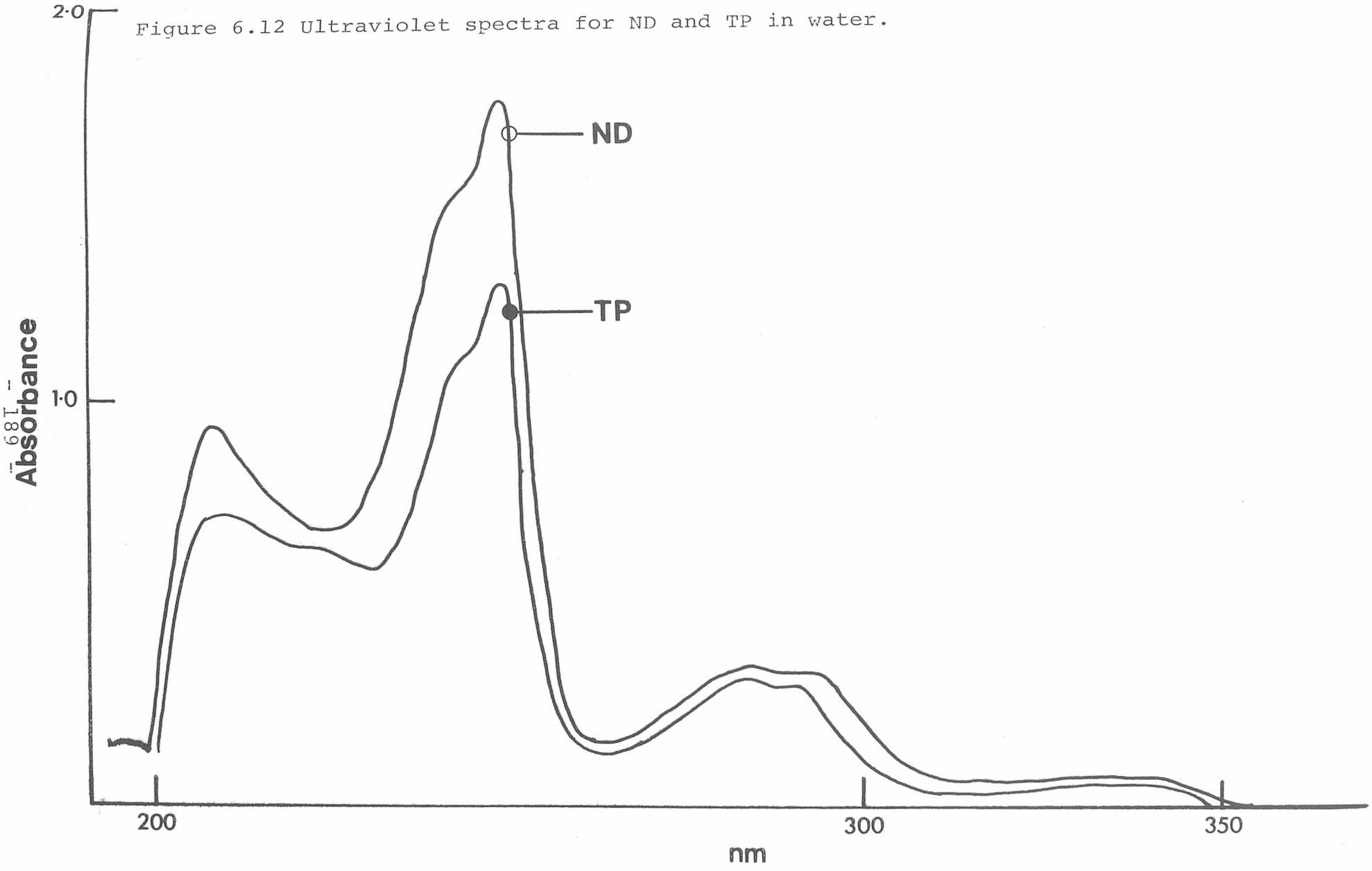


Figure 6.13 Infrared spectrum for ND in Nujol Mull.

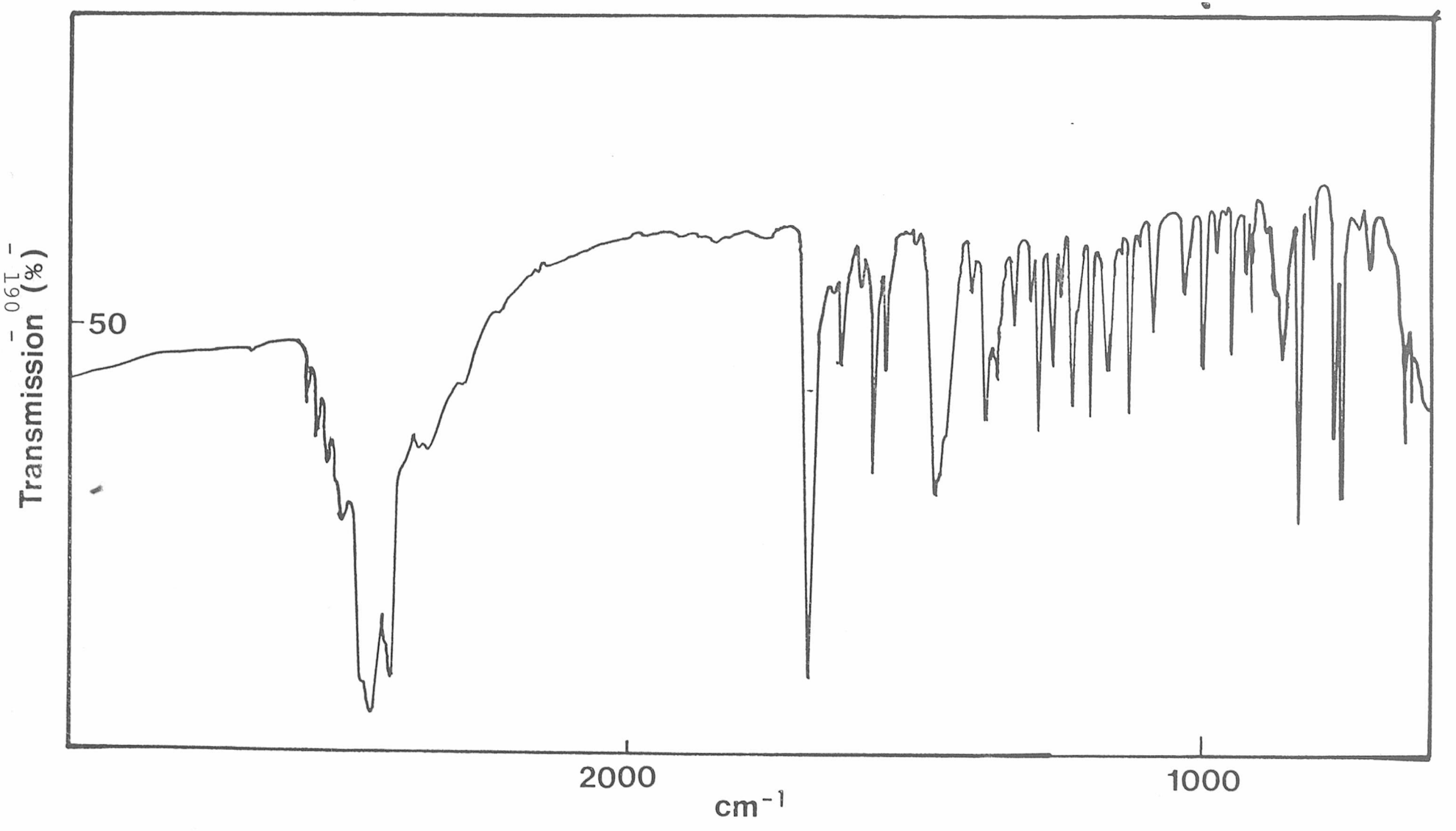


Figure 6.14 Infrared spectrum for TP in Nujol Mull.

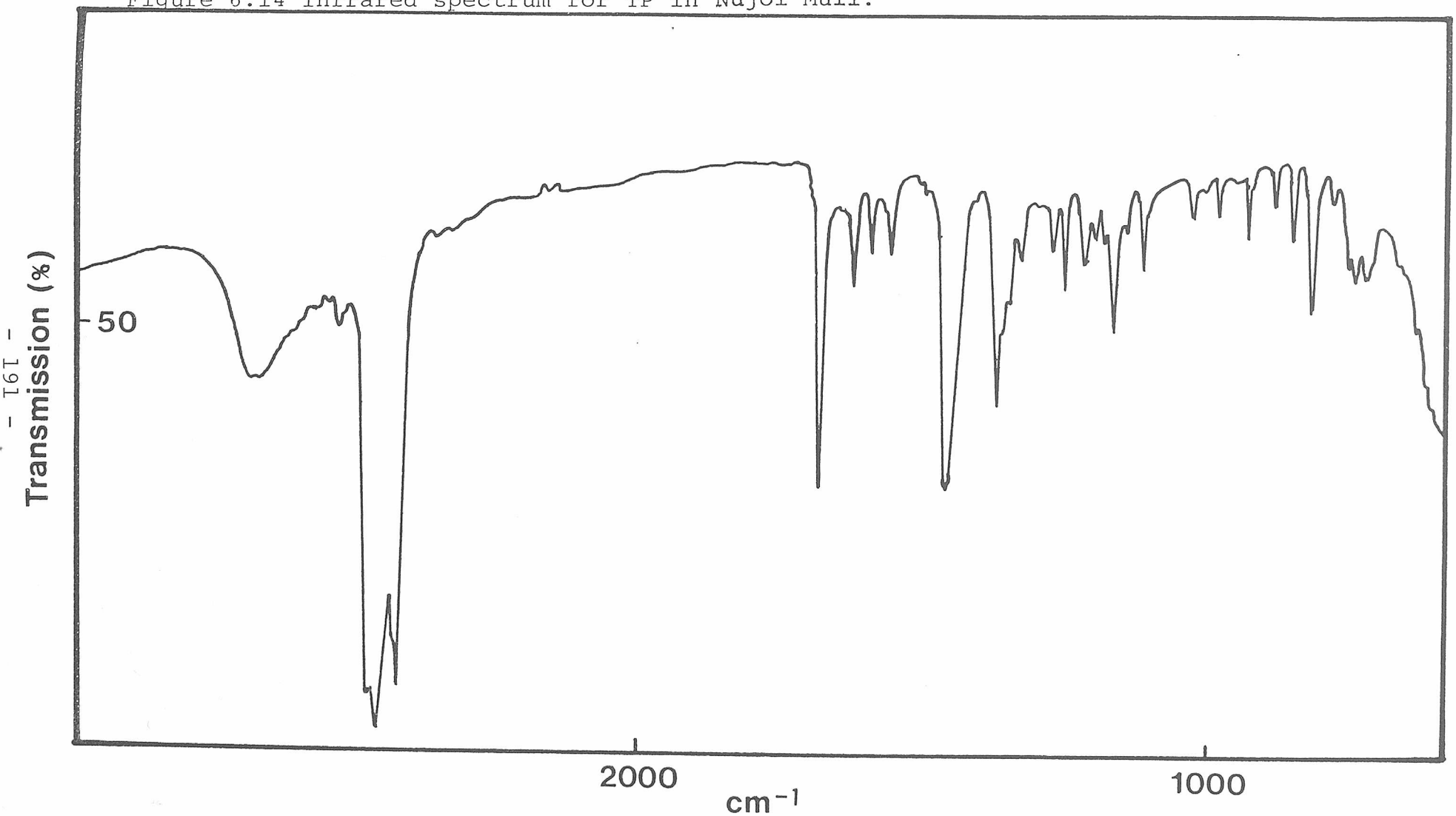


Figure 6.15 Ultraviolet spectrum for P_1 and ND obtained by the stopped flow technique.

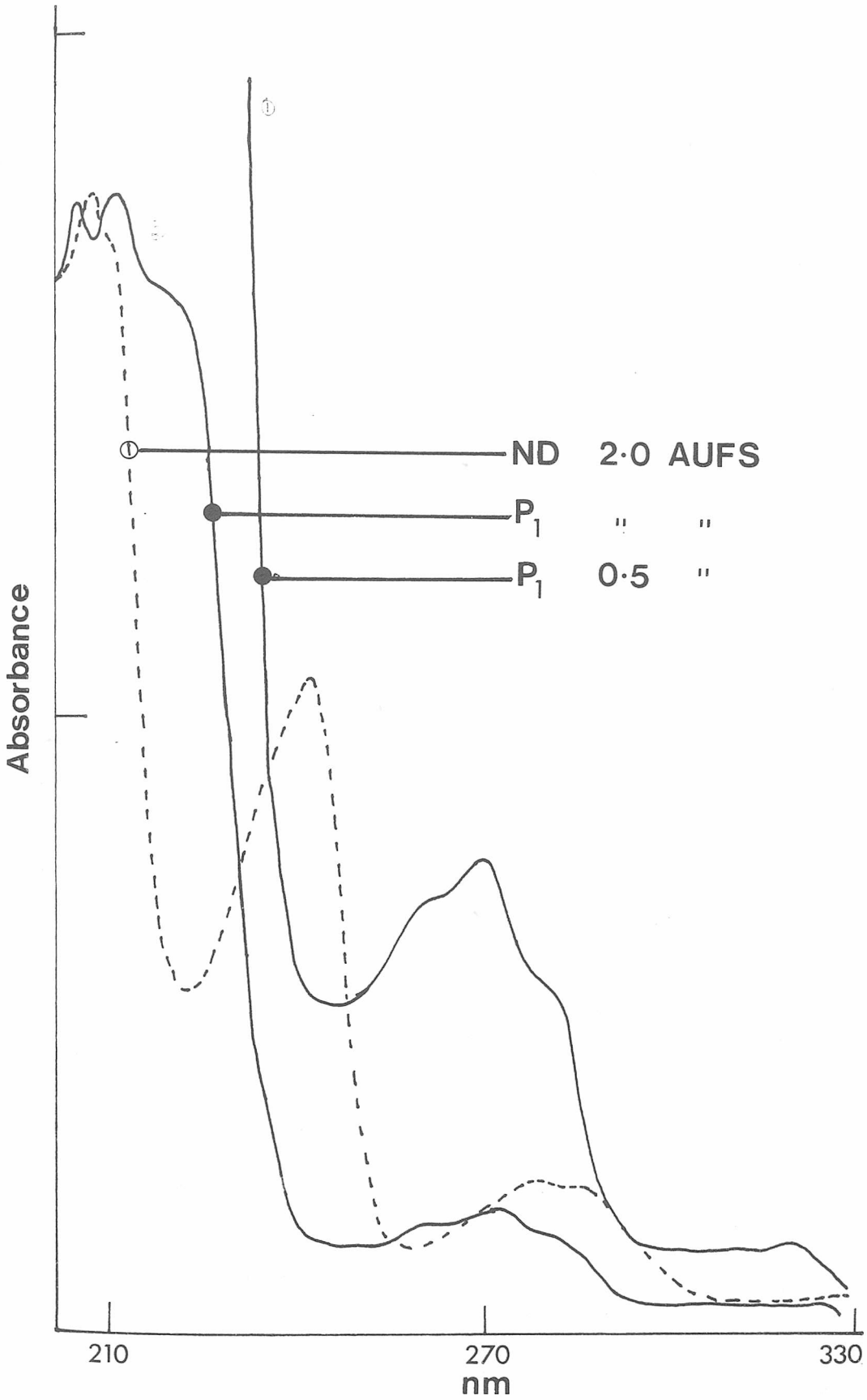


Figure 6.16 Ultraviolet spectrum for P_2 and ND obtained by the stopped flow technique.

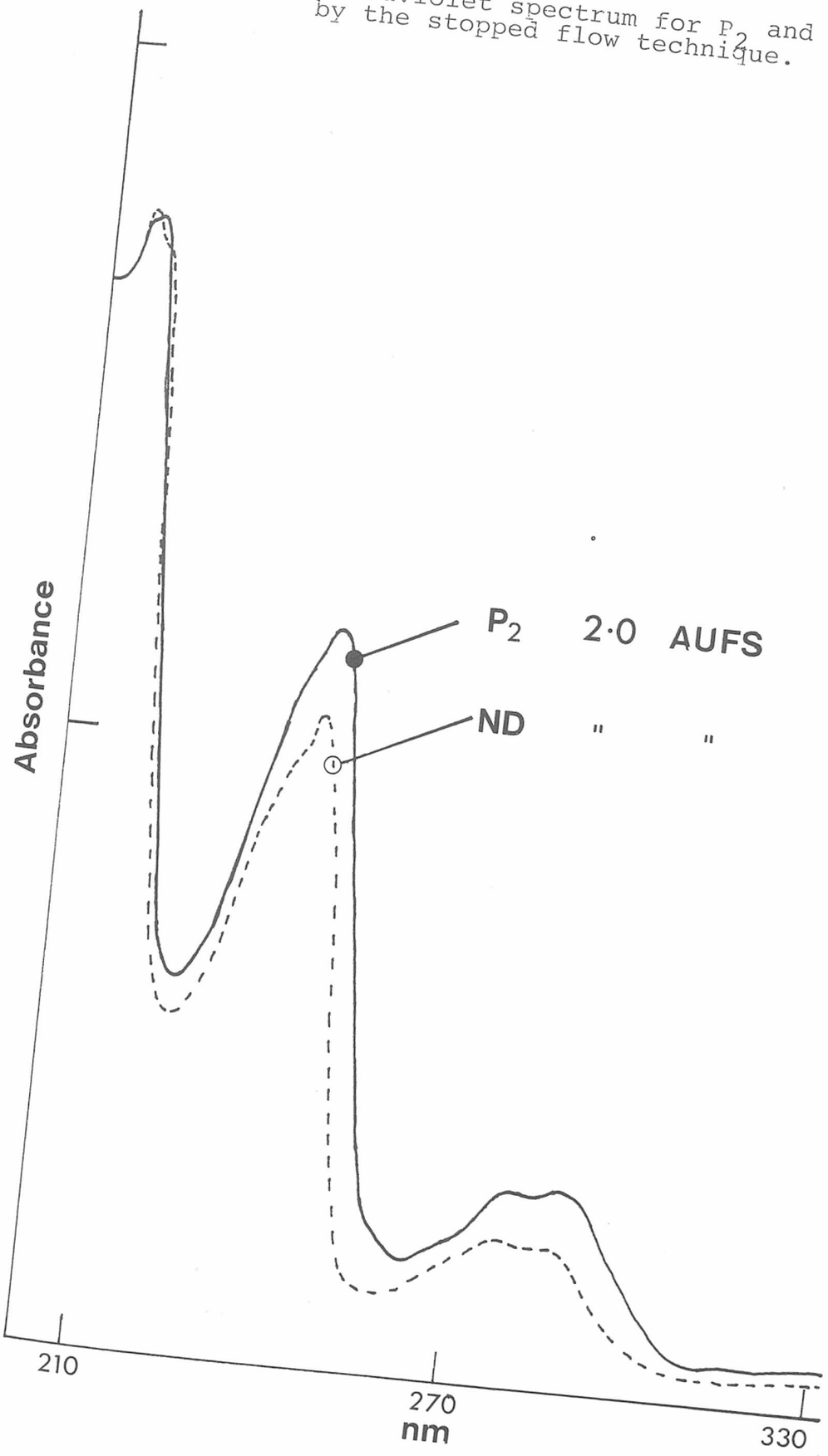


Figure 6.17 Ultraviolet spectrum for P_3 and ND as obtained by the stopped flow technique.

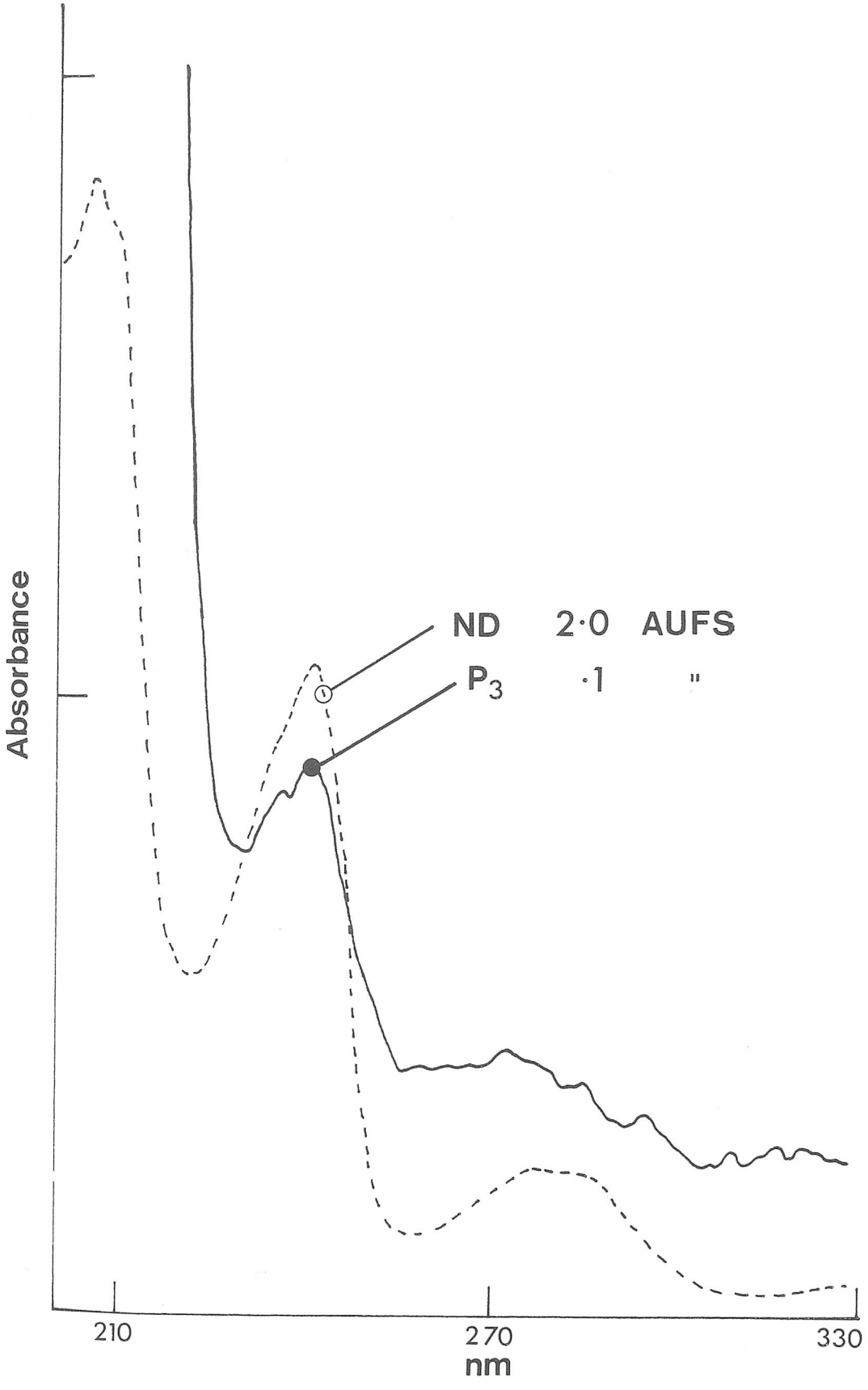


Figure 6.18 Ultraviolet spectra for P_1 and 2-NA in water/ACN (75/25) containing 40mM phosphate buffer and 20mM TBA at pH 7.0.

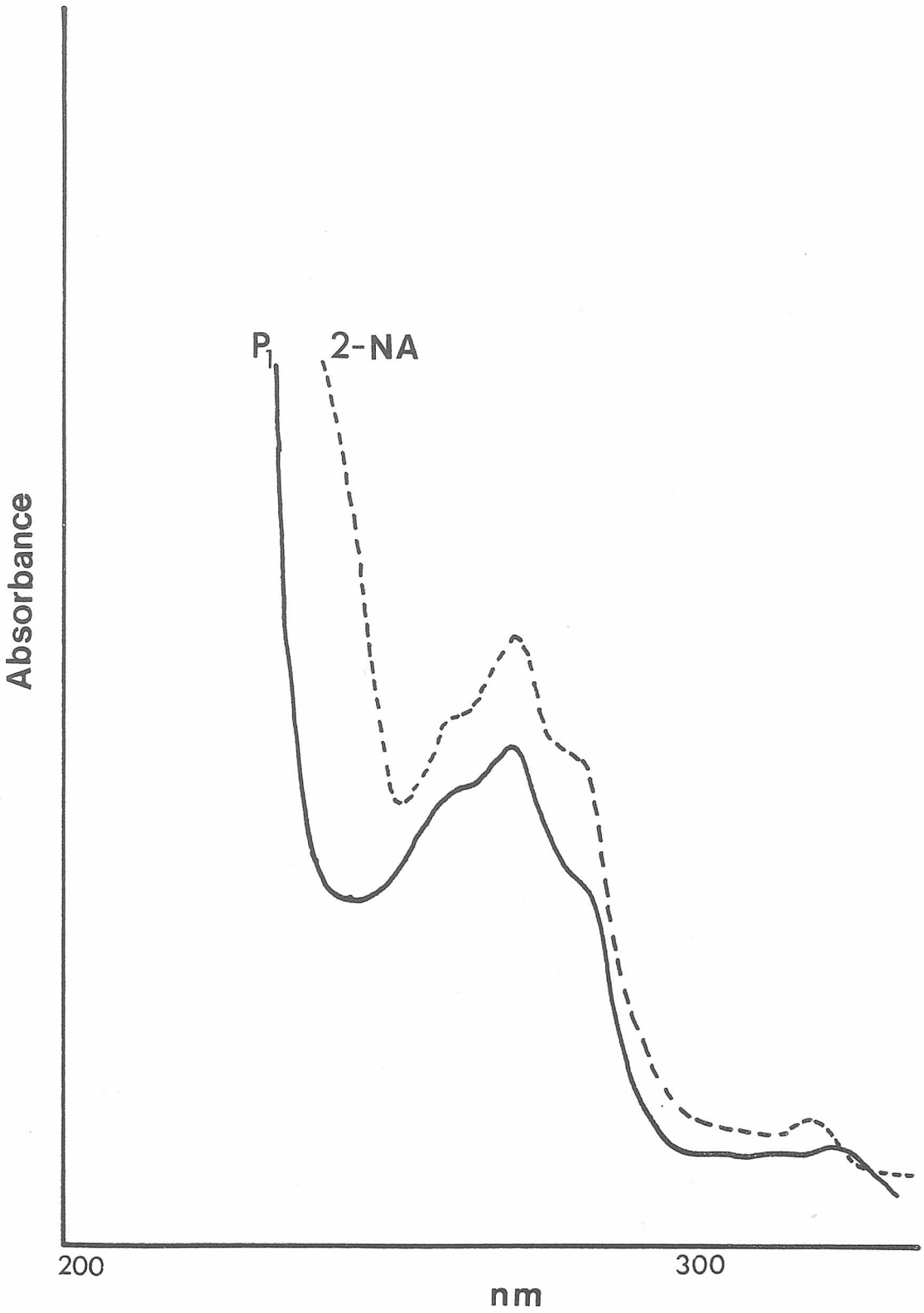


Figure 6.19 Plots showing the variation in capacity factor for 2-NA and P_1 as a function of TEA and TBA concentration.

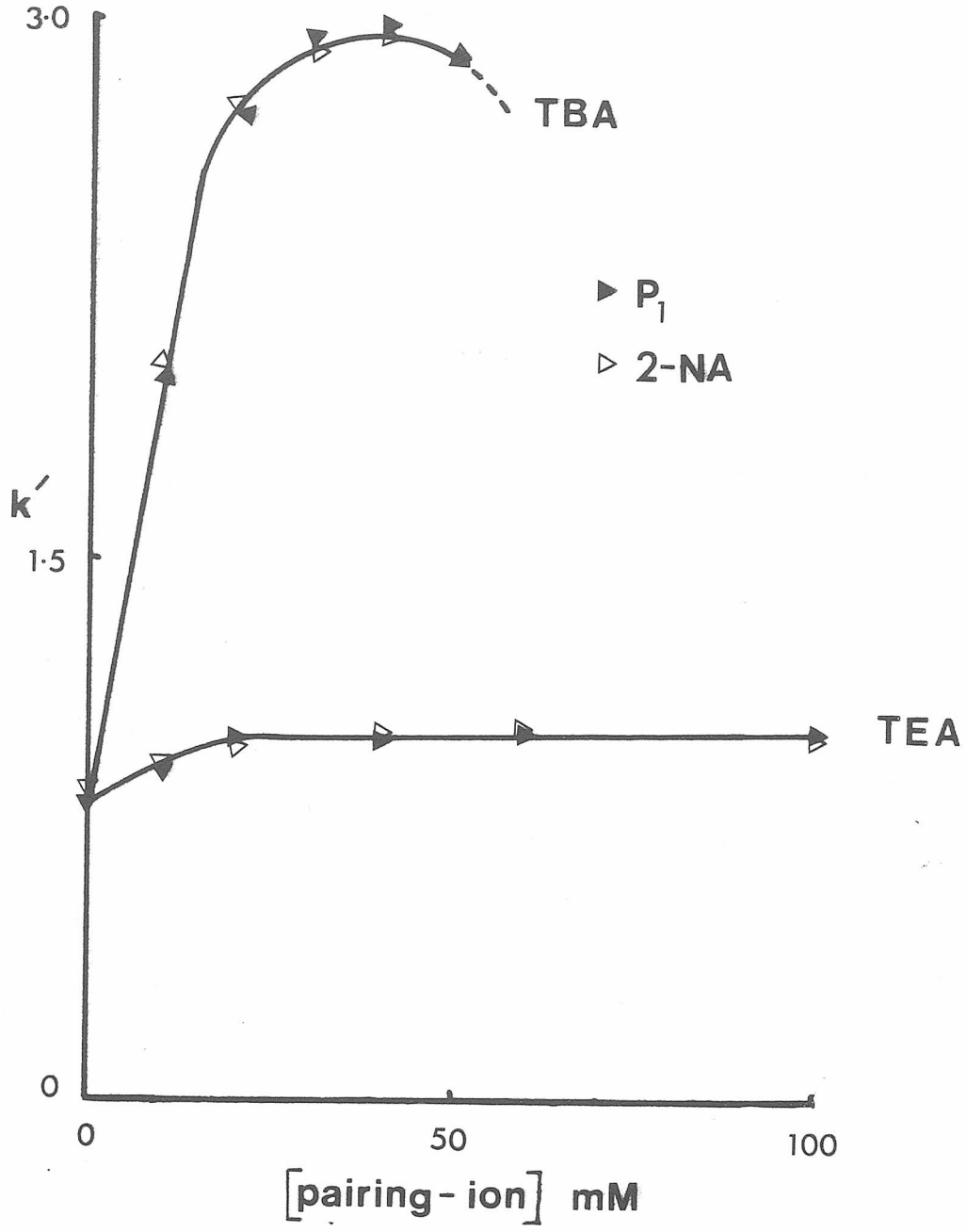


Figure 6.20 Plots showing the variation in capacity factor (k') for P_1 and 2-NA as a function of CTAB and OSA concentration.

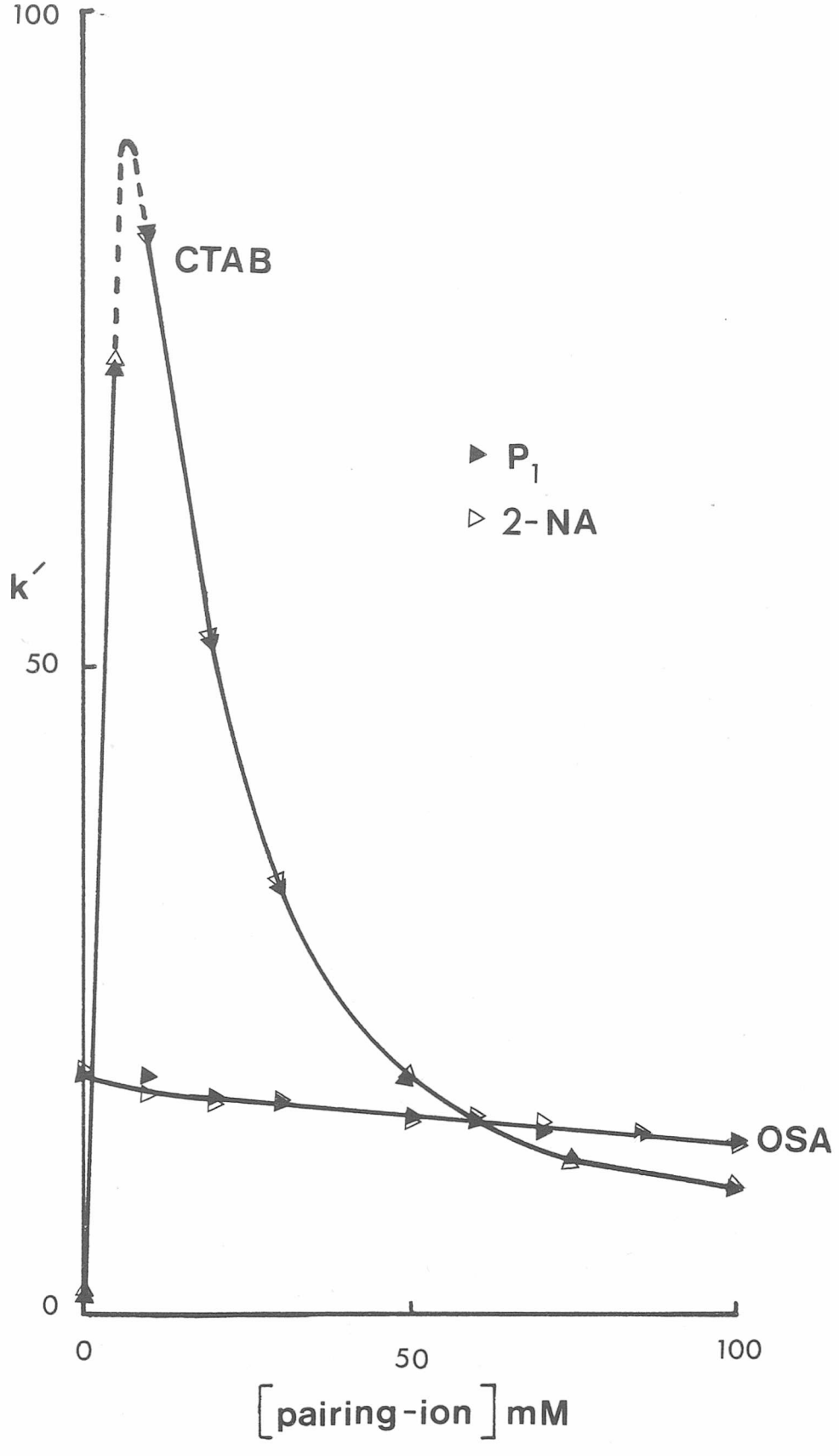


Figure 6.21 Plots showing the variation in peak area as a function of time for P_1, P_2 and P_3 and concentration with time for ND in phosphate buffer at 90°C.

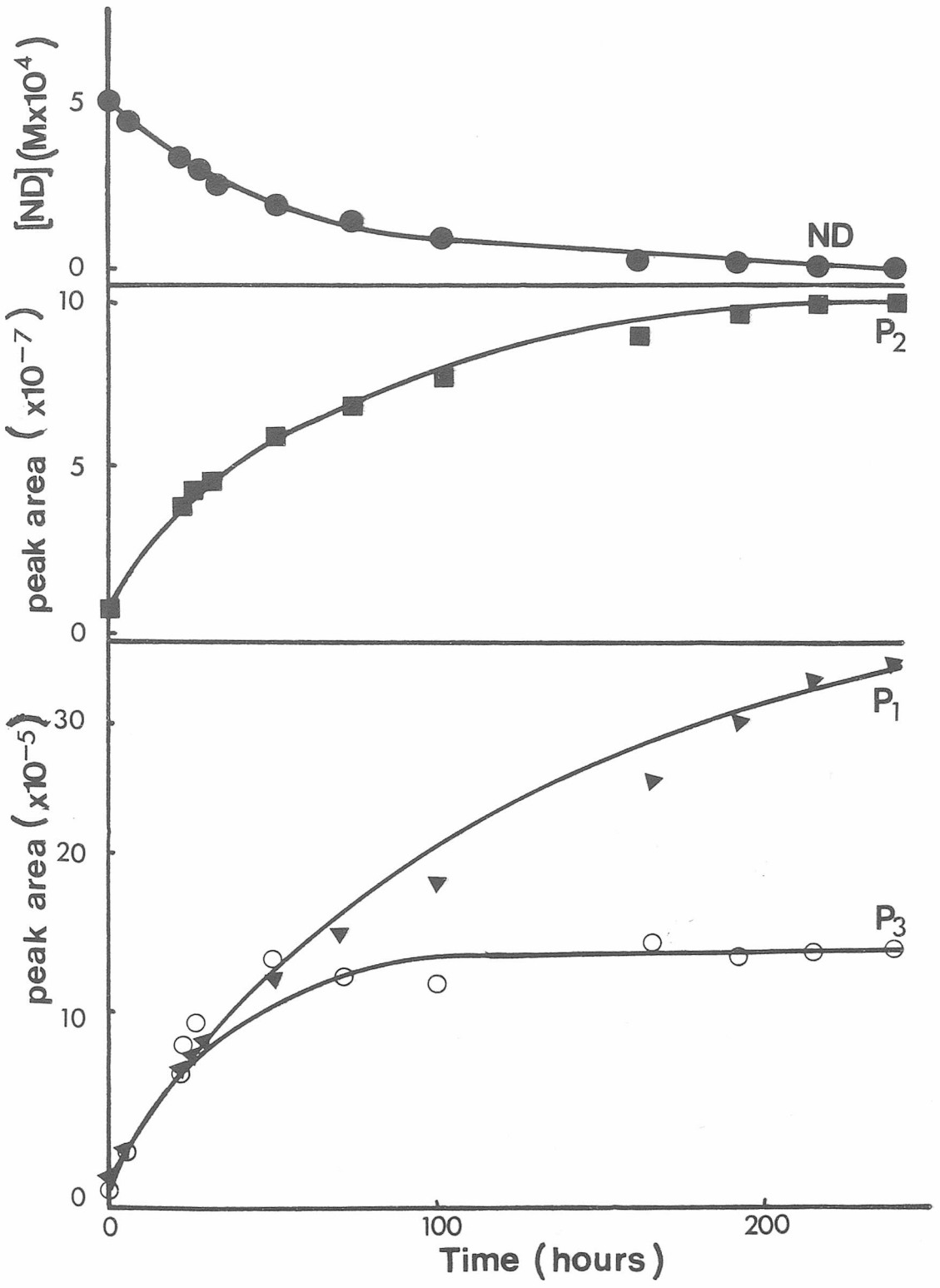
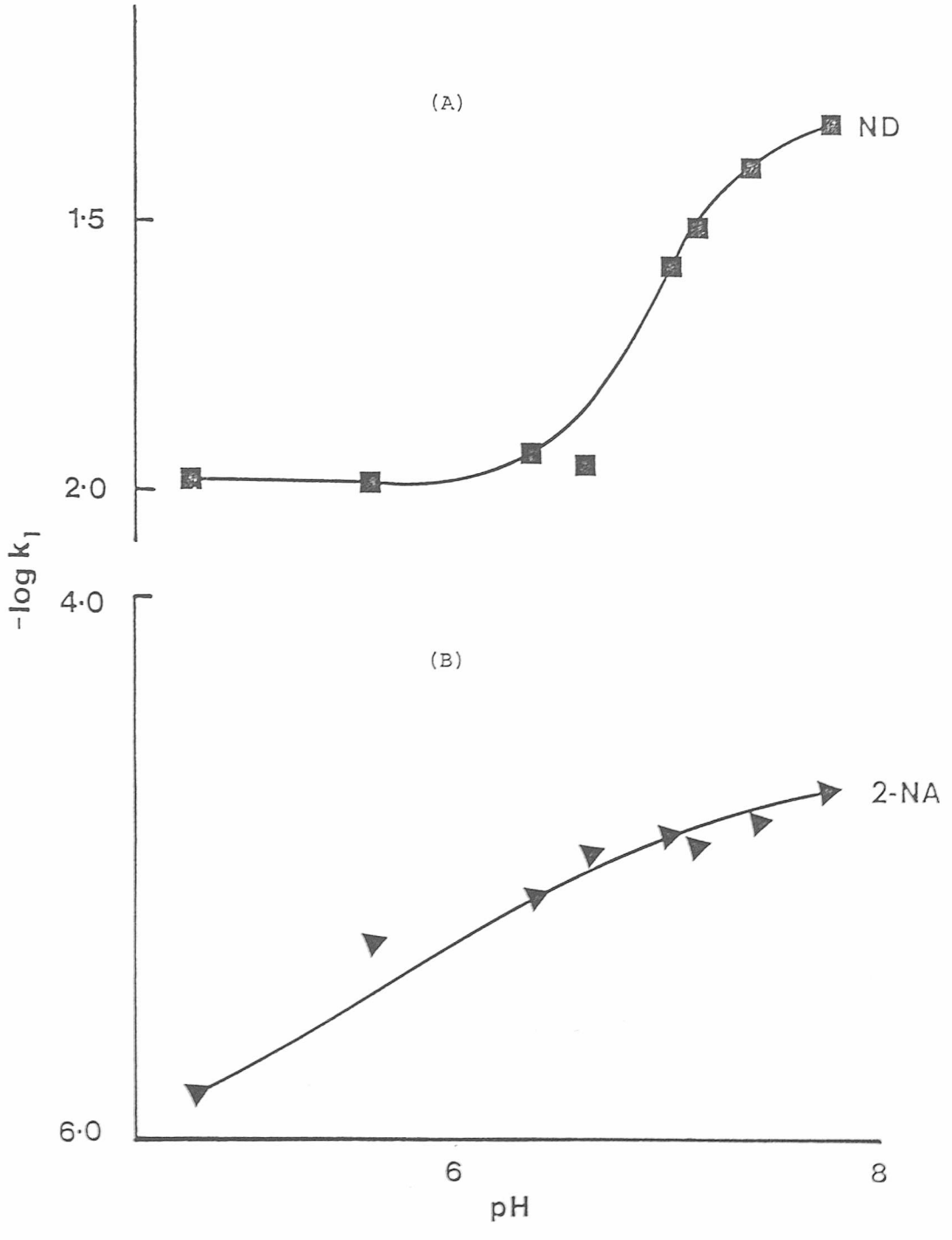


Figure 6.22 Logk_1-pH profile as determined by following (A) decrease in ND and (B) increase in 2-NA in 20mM phosphate buffer at 90°C.



Fiugre 6.23 Arrhenius plot for P_2 and P_3.

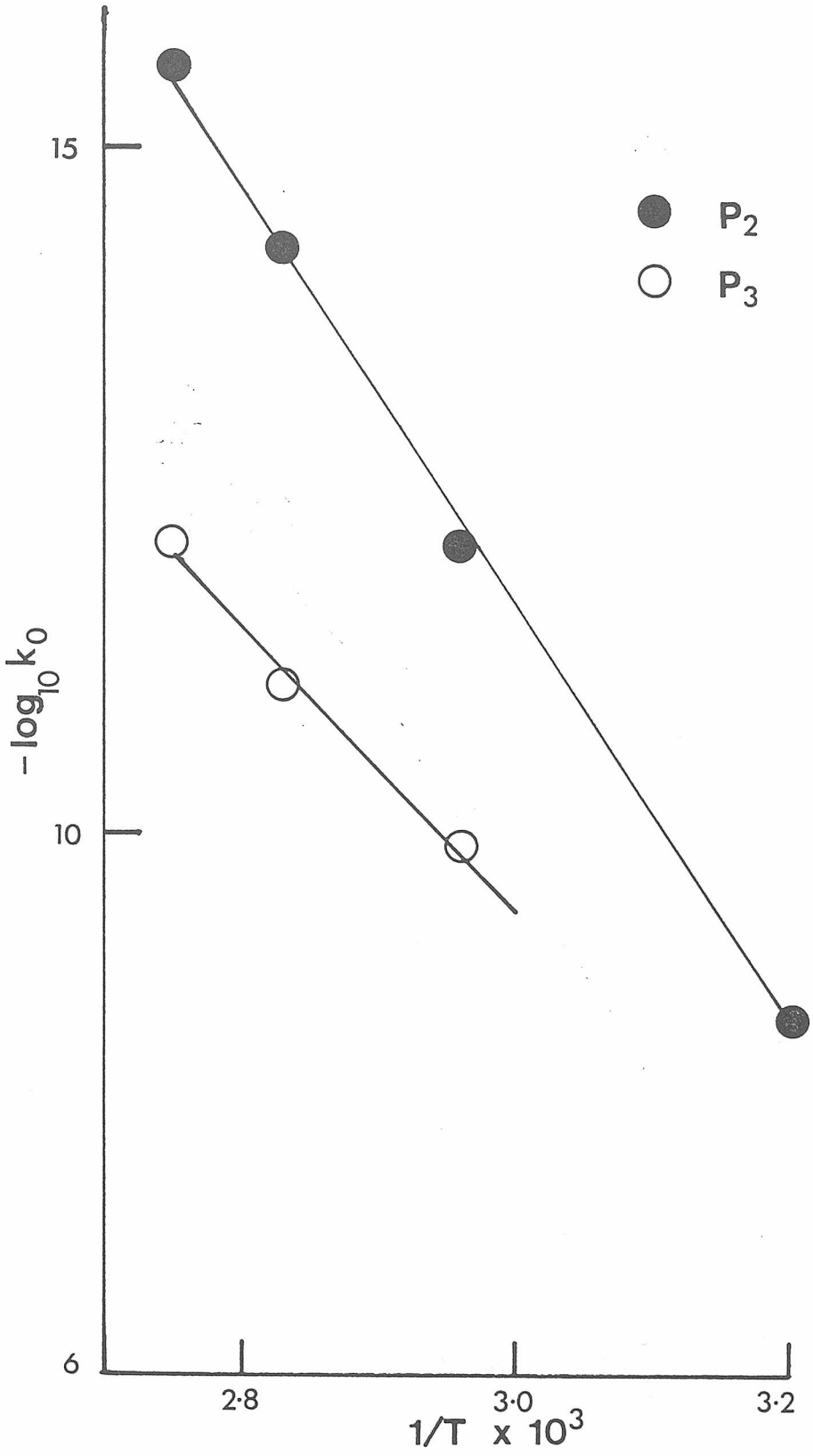


Figure 6.24 Showing the Arrhenius plots for ND decomposition and 2-NA formation during ND decomposition in phosphate buffer.

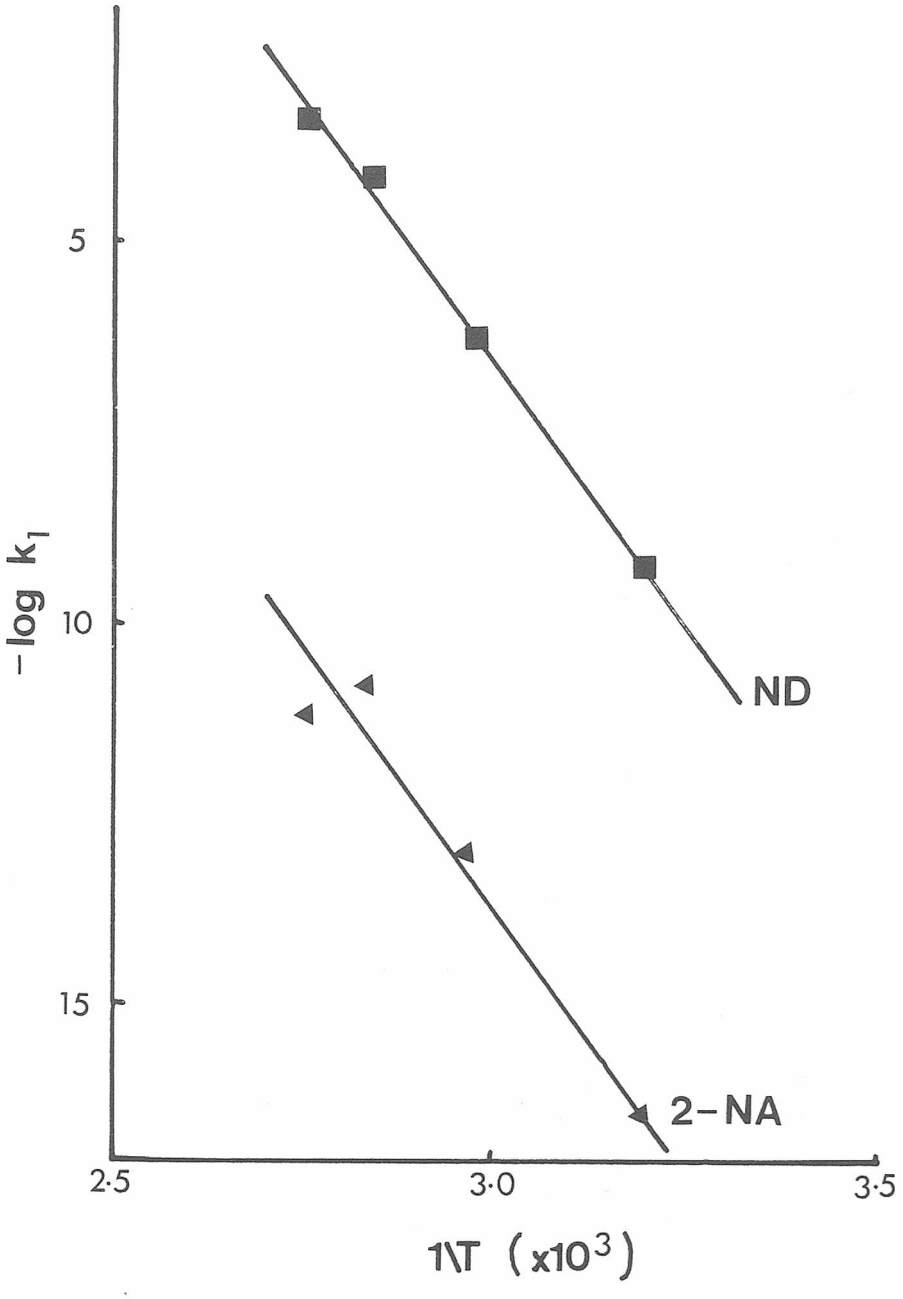
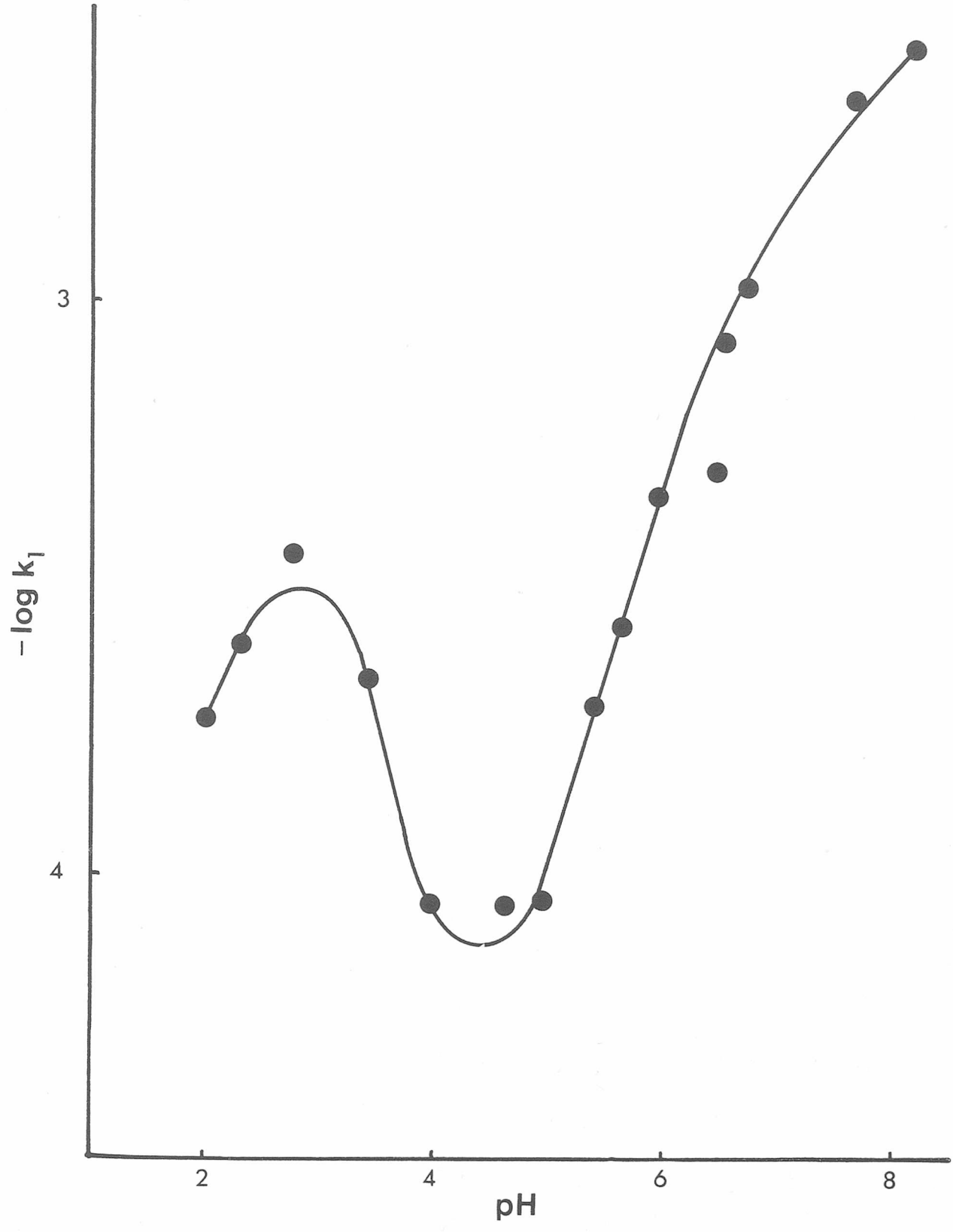


Figure 6.25 Showing the logk-pH profile for ND decomposition in McIlvaine's buffer determined as described in the text.



Chapter 7

7.0 The Decomposition of 5-Hydroxymethylfurfural

7.1 Introduction

It is well established that heating D-glucose and other hexoses under acidic and neutral conditions leads to the formation of 5-hydroxymethylfurfural (5HMF)[247-249]. While it is clear from the previous chapters that potency retained on storage is the main consideration of stability investigations, in the case of D-glucose the literature shows a change in emphasis from this. The retention of potency in this latter case is not as important a consideration as the formation of impurities. The degradation of D-glucose represents an example of a decomposition where the decomposition product has been extensively used[13,250-253]. The fact that 5HMF is observed to be formed from D-glucose during autoclaving has led to the rate of 5HMF formation being taken as an indication of the rate of D-glucose depletion[13,250-253]. This approach has been found attractive and convenient presumably because analysing for 5HMF is easier than analysing for D-glucose. However, such measurements have been made on the assumption that the 5HMF formed reflects the D-glucose lost, that is stoichiometry has been assumed[254].

The decomposition of D-glucose has been the subject of numerous reports[13,247-267]. These investigations can be said to have been conducted for three main reasons;

(i) to establish the mechanism of 5HMF formation from D-glucose

(ii) to establish the cause of increased acidity observed on autoclaving D-glucose

(iii) to establish the rate of 5HMF production.

There is considerable confusion in the literature regarding both (i) and (ii) above. Scheme 7.1 summarises the numerous hypotheses regarding the mechanism of decomposition and the various acids postulated to be the cause of increased acidity. Both the mechanism and cause of acidity still remain unclear.

Ultraviolet spectroscopy and hplc have been used in (iii) above to determine the rate of 5HMF production. Perhaps the only conclusion that can be drawn from these previous investigations is that 5HMF is the main decomposition product formed. Indeed it is on this fact that the pharmacopoeial limit tests for D-glucose infusion fluids is based. This limit test involving direct spectrophotometric measurement at 284nm. after suitable dilution purports to be a measure of 5HMF and its related decomposition products. These products are regarded as being responsible for the increased acidity observed during autoclaving of D-glucose infusion fluids and although they have yet to be unequivocally identified their concentrations are limited in the British Pharmacopoeia by a pH limit and in the European Pharmacopoeia by a simple titration method. In the light of the non-specificity of these limit tests for such a widely used infusion fluid, a new limit test capable of specific and precise

quantitation of 5HMF and its related products (see below) has been proposed[268].

When exposed to the atmosphere under ambient conditions, 5HMF is known to be unstable[245]. It is oxidised to two acidic species which have been identified as 5-hydroxymethylfuroic acid (HMFA) and furan-2, 5-dicarboxylic acid (FDA). However, it is not known if other decomposition products accompany the formation of HMFA and FDA during 5HMF decomposition under these conditions. While it may be argued that 5HMF present in autoclaved D-glucose infusion fluids is unlikely to be exposed to such oxidative decomposition the same argument cannot be applied to the relatively recent use of D-glucose in continuous ambulatory peritoneal dialysis (CAPD) fluids where it is conceivable that invivo oxidation may occur.

Peritoneal dialysis involves the introduction of dialysis fluid into the peritoneal cavity by means of a catheter placed surgically so as to link the abdominal cavity and the exterior of the abdominal wall. In an adult up to two litres may be run into the cavity under gravity over a 15 to 20 minute interval and drained a few hours later. Such use of CAPD fluids produce conditions where a patient may be exposed to two or more daily changes over a number of years. Discomfort and pain have recently been reported in addition to peritonitis from bacterial contamination during change over [269]. A very recent report [270] has evaluated the addition of certain drugs to CAPD fluids. While the stability of drugs in

such an environment is questioned, the stability of 5HMF, which is present in the CAPD fluid has not been considered.

The 5HMF decomposition system provides a good test system for evaluating the rates of decomposition both by conventional methods and by the product measurement approach since no knowledge is available in the literature. The purpose of the present work is to study the established decomposition of 5HMF to HMFA and FDA[245] in order to determine which products if any are likely to be formed during use. While this work will primarily intend to provide background information on the medicinal use of CAPD fluids the kinetic information obtained will be general to the storage of any preparation containing D-glucose.

7.2 Equipment and Materials

Equipment similar to that used in Chapter 3 were used for this study. Tetraethylammonium and tetrabutylammonium as their bromides were obtained from Aldrich Chemical Co. Ltd. as was 5HMF. HMFA and FDA were synthesised and characterised by the method of Durham et.al.[245].

7.3 Chromatographic Separation and Quantitation

The literature separation[245,268] of 5HMF, HMFA and FDA were found to be adequate. Figure 7.1 shows a specimen chromatogram of all three compounds. The type and concentration of pairing ion was chosen to ensure elution of 5HMF after that of FDA and HMFA. As can be

seen from Figure 7.1 the elution order is FDA, HMFA and 5HMF. This chromatographic system was used to follow the decomposition of both 5HMF and HMFA. Decomposition of FDA was followed using a more hydrophopic pairing ion TBA as shown in Figure 7.2 This choice was made to ensure that if FDA underwent reversible decomposition to 5HMF or HMFA, the latter two would be eluted prior to FDA so allowing for more sensitive detection of these products.

Repeated injection of a partially decomposed sample containing all three species resulted in the following relative standard deviations, 5HMF(1.6%), FDA(1.8%), and HMFA(1.7%) for eight replicate injections.

Using suitable concentrations of standards the peak heights (P) associated with 5HMF, HMFA and FDA at a sensitivity of 2.0AU were established as being proportional to concentration (C). The resultant regression equations are shown below;

(1) 5HMF $P = 2.06 \times 10^5 C + 1.2$ (RSD = 1.63%, $R^2 > 0.99$)

(2) HMFA $P = 1.17 \times 10^5 C + 6.10$ (RSD = 1.84%, $R^2 > 0.99$)

(3) FDA $P = 4.46 \times 10^5 C + 1.0$ (RSD = 1.56%, $R^2 > 0.99$)

This linearity was assumed to hold over the range of sensitivities used during the kinetic experiments (0.01 - 2.0 AU) and peak heights were related to concentration using two freshly prepared standards whose peak heights were similar in magnitude to that of the unknown sample.

7.4 Stability Measurements

While it is known that HMFA and/or FDA are produced from 5HMF[245] the order of appearance has not been reported

in the literature, nor have quantitative measurements been made as to the stoichiometry of the reaction. To obtain the required information 5HMF at several known concentrations was stored open to the atmosphere at 30°C and the rates of disappearance of 5HMF and appearance of decomposition products were measured. Any possibility of a consecutive reaction, that is

$$5HMF \longrightarrow HMFA \longrightarrow FDA$$

was tested by storing HMFA at several known concentrations under similar conditions. These experiments allowed the reaction order to be determined by both the conventional and initial rate methods and provided information on the mass balance.

FDA at a single concentration was also stored under conditions identical with those used above in order to determine if FDA could be regarded as the end product in this scheme.

7.5 Results and Discussion

7.5.1 Decomposition of 5HMF

Two different initial 5HMF concentrations were allowed to decompose in water under atmospheric conditions at 30°C. The reaction was followed to extents in excess of 75% decomposition. Figure 7.3 shows the variation in concentration of all species as a function of time for an initial 5HMF concentration of 4×10^{-4}M. while Figure 7.4 shows the same data for an initial 5HMF concentration of 1.2×10^{-3}M. The results show that

(i) the reaction is relatively slow

(ii) a marked induction period is observed for the appearance of both products and also for the disappearance of 5HMF. Such induction periods are common in radical chain reactions[271] which is the mechanism by which oxidation reactions often occur[44]. It has been suggested[6] that reactions exhibiting an induction period, distinguished from autocatalytic reactions, do so to allow a build-up of a reactive intermediate to some critical concentration when it triggers off the main reaction.

(iii) the total concentration of all compounds at any time is constant for at least 90% decomposition. However, as can be seen from Figure 7.3 above 90% decomposition a mass balance is no longer obtained. It would appear that HMFA and FDA account for the depletion in 5HMF concentration for a large part of the decomposition reaction. No other products were observed.

(iv) the reaction appears to follow a consecutive route. Figure 7.3 indicates that the reaction follows a consecutive pathway with HMFA concentration passing through a maximum. No such maximum is seen in Figure 7.4 presumably as the decomposition was not carried out over a sufficient time scale.

Application of the conventional integrated rate equations to the 5HMF data at different initial concentrations indicated that at the lower 5HMF concentration both zero and first order equations could be applied with similar precision. Table 7.1 shows the results obtained an application of zero, first and second

order integrated equations to both sets of data. At the higher initial concentration of 5HMF the data could be related to both first and second order equations with similar precision (Table 7.1). Thus although the reaction was followed in excess of 50% decomposition, as has been suggested in the literature[12,18,23,64-68], conventional methods did not allow determination of the reaction order. Other instances have been reported where the reaction order could not be determined[57-59].

As the reaction order and rate constants for the decomposition of 5HMF could not be established with any degree of certainty using the conventional integrated approach, the initial rate method was applied to four different initial 5HMF concentrations ranging from 2×10^{-4}M to 1.2×10^{-3}M. The rate of HMFA formation was determined as previously described in Chapter 1. The regression equation so obtained is shown below

$$\log (\text{Rate}) = 1.1[5\text{HMF}] - 1.4 \quad (\text{RSD} = 2.34\%, R^2 > 0.99)$$

indicating that the formation of HMFA from 5HMF follows first order kinetics[6]. While this relationship also allows determination of the numerical value of the first order rate constant, this rate constant will be subject to large errors from extrapolation and the logarithmic scale used. In addition the order of reaction as indicated by the slope of the log-log plot is not exactly unity.

Table 7.2 shows the zero order rate constants for HMFA formation as determined by the initial rate method together with the first order rate constants calculated using Equation(1-16). The mean first order rate constant

for this decomposition was calculated as $1.89 \times 10^{-2} hr^{-1}$. This rate constant must be used in conjunction with the induction period in calculating the concentration of HMFA present at any time. From the initial rate data shown in Figure 7.5 it is apparent that the induction period is variable, typically ranging from 8 to 10 hours. There appears to be no obvious relationship between the induction period and the initial 5HMF concentration. The first order rate constant of $1.89 \times 10^{-2} hr^{-1}$ is found to be comparable with those calculated by measuring HMFA appearance during the extensive 5HMF decomposition at two different initial concentrations and no significant difference (P=0.005) was found between the rate constant above and those determined by the first order integrated equation.

7.5.2 The Decomposition of HMFA

HMFA concentrations ranging from 1×10^{-4} to $1.3 \times 10^{-3} M$ were stored under conditions similar to those described for the decomposition of 5HMF. Both HMFA and FDA concentrations were monitored as a function of time. Figure 7.6 shows a typical concentration-time plot for both compounds. The reaction was found to be slower than that for 5HMF with little detectable change in HMFA concentration even after some 350 hours. The concentration of FDA was however found to increase after a typical induction period of 150 hours. The total concentration at any time was found to be approximately constant indicating that FDA was the only product of

decomposition formed.

Conventional methods of determining the reaction order could not be applied in this instance due to the lack of sufficient change in reactant concentration. Application of the initial rate method to the FDA data, however resulted in the following equation

$$\log (\text{Rate}) = 0.92[\text{HMFA}] - 4.09 \quad (\text{RSD}=2.96\%,\ R^2>0.99)$$

indicating that the reaction followed first order kinetics [6]. Table 7.3 shows the initial rates of FDA formation in moles.$dm^{-3}hr^{-1}$ together with the first order rate constants calculated using Equation (1-16). The average first order rate constant was calculated as $1.48 \times 10^{-4}hr^{-1}$.

7.5.3 Decomposition of FDA

FDA at a single concentration was stored under conditions similar to that for 5HMF and HMFA. As previously described, a different chromatographic system was used to monitor any possibility of a reversible conversion to HMFA or 5HMF in addition to loss of FDA by any other route. No decomposition was observed after some 250 hours either in terms of HMFA or 5HMF appearance or loss of FDA. This was taken to indicate that FDA is the end product of this decomposition reaction.

It would appear, therefore, from the above that the decomposition of 5HMF follows a consecutive decomposition pathway as shown in Scheme 7.2. Using the initial rate method both steps have been shown to obey first order kinetics. Substituting the mean first order rate

constants so calculated into Equations (2-2) to (2-4) shown in Chapter 2, the concentration of each compound has been calculated to allow comparison with experimental data. The concentration at each time interval was calculated as demonstrated in the following example. The concentration at time 100 hours, for example, was calculated using 107.5 hours, that is the induction period was added to the time at which the concentration was to be calculated. The concentration so calculated was regarded as being that at time 107.5 hours. The induction periods used were those obtained from Figure 7.3. Figure 7.7 shows a comparison between experimental and calculated data. There is reasonable agreement for most parts of each curve confirming a consecutive route of decomposition.

7.6 Conclusion

5HMF decomposes by a consecutive route to form FDA, a stable end product. The order of 5HMF decomposition to HMFA could not be unequivocally determined using the conventional approach. The reaction was found to follow first order kinetics on application of the initial rate method and the rate constant for this reaction, under the conditions specified, was calculated as $1.89 \times 10^{-2} hr^{-1}$. No significant difference ($P=0.005$) was found either between the rate constants obtained by following 5HMF decomposition in excess of 75% at two different initial concentrations or between these and those determined by the initial rate method, by following HMFA appearance.

Because of the lack of sufficient decomposition, the

reaction order for the conversion of HMFA, the intermediate, to FDA could not be determined by the traditional methods. Using the initial rate method the reaction was found to follow first order kinetics with a rate constant of $1.48 \times 10^{-4} hr^{-1}$.

The decomposition of HMFA to FDA is approximately a hundred-fold slower than that of 5HMF to HMFA and confirms the simulated findings in Chapter 2, that provided the ratio k_1/k_2 exceeds 2, the intermediate may be analysed to determine the rate constant for the first decomposition step in a consecutive reaction, loss of the intermediate to the end product being small enough to be neglected. This is supported in the present case by the statistical agreement between conventional first order rate constants and those obtained by the initial rate of HMFA formation.

The present work shows that the decomposition of 5HMF under atmospheric conditions is a slow process preceeded by an induction period. It is therefore very unlikely that the 5HMF present in CAPD fluids will undergo any appreciable decomposition to HMFA and/or FDA during use.

Table 7.1 Shows a comparison of the rate constants obtained by application of zero, first and second order integrated rate equations to the 5HMF data used in Figures 7.3 and 7.4.

Initial 5HMF Conc($Mx10^{-4}$)	Order	Rate Constant	RSD (%)	R^2
4.0	Zero	$3.72x10^{-6}$ ($mol.dm.^{-3}hr.^{-1}$)	11.26	0.9725
	First	$2.61x10^{-2}$ ($hr.^{-1}$)	11.44	0.9572
	Second	221 ($mol.dm.^{-3}hr.^{-1}$)	21.54	0.8688
12.0	Zero	$1.57x10^{-6}$ ($mol.dm.^{-3}hr.^{-1}$)	173.0	0.8673
	First	$4.57x10^{-3}$ ($hr.^{-1}$)	11.16	0.9385
	Second	16.25 ($mol.dm.^{-3}hr.^{-1}$)	12.64	0.9223

Table 7.2 Shows the first order rate constants calculated from the initial rate of HMFA formation during decomposition of different initial 5HMF concentrations.

Initial 5HMF Conc($Mx10^{4}$)	Zero Order Rate Constant ($x10^{6}$ $mol.dm.^{-3}hr.^{-1}$)	Calculated First Order Rate Constant ($x10^{2}hr.^{-1}$)
1.96	3.41	1.74
2.29	4.08	1.78
7.89	15.0	1.90
11.47	24.3	2.12

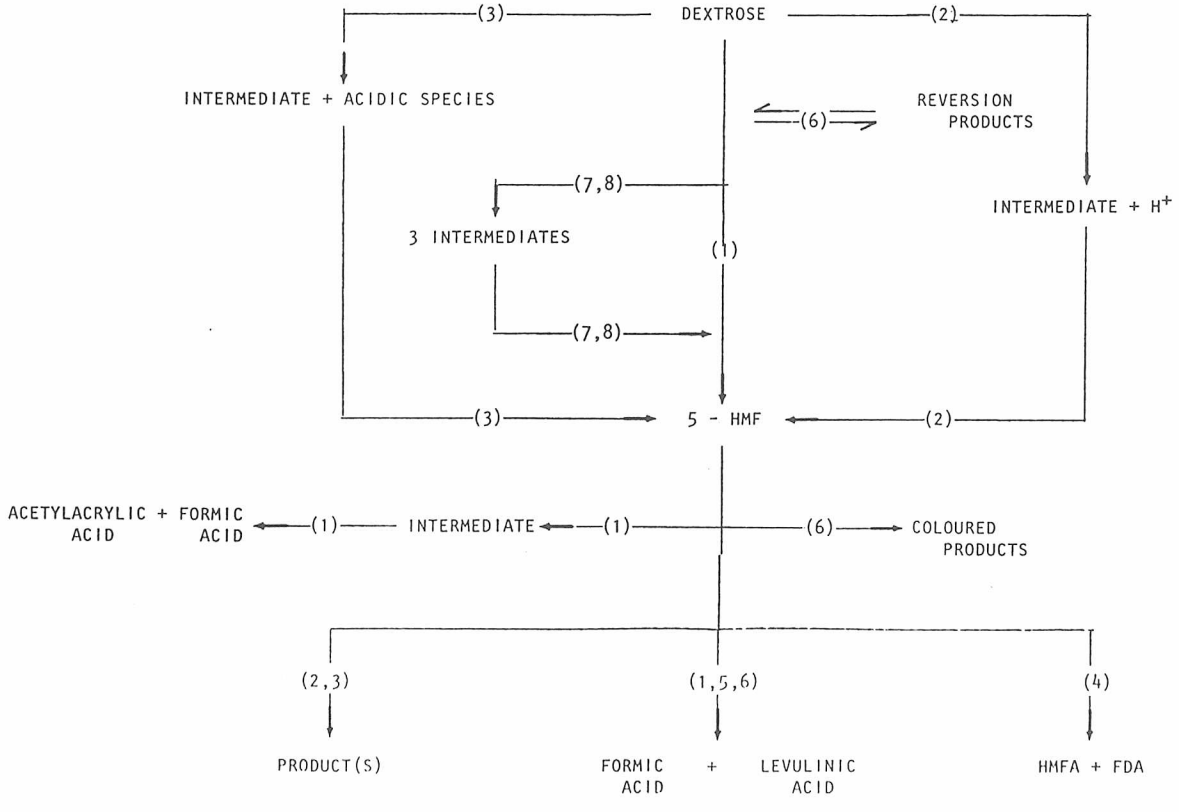
mean $k_1 = 1.89x10^{-2}hr.^{-1}$

Table 7.3 Shows the first order rate constants calculated from the initial rate of FDA formation during the decomposition of different initial HMFA concentrations.

Initial HMFA Conc($M \times 10^{4}$)	Zero Order Rate Constant($\times 10^{8}$ $mol.dm.^{-3}hr.^{-1}$)	Calculated First Order Rate Constant ($\times 10^{4} hr.^{-1}$)
1.26	2.15	1.71
5.04	7.01	1.39
8.82	12.6	1.43
12.8	18.0	1.41

mean $k_1 = 1.48 \times 10^{-4} hr.^{-1}$

Scheme 7.1 A compilation scheme for the Decomposition of Dextrose via 5HMF to various suggested end products.



1. Tahir et al[259]
2. Taylor et al[13]
3. Sturgeon et al[251]
4. Durham et al[245]
5. Tuensenn[261]
6. Heimlich et al[250]
7. Taylor et al[260]
8. Wolfram et al[267]

Scheme 7.2 Showing the route of 5HMF decomposition in water at 30°C.

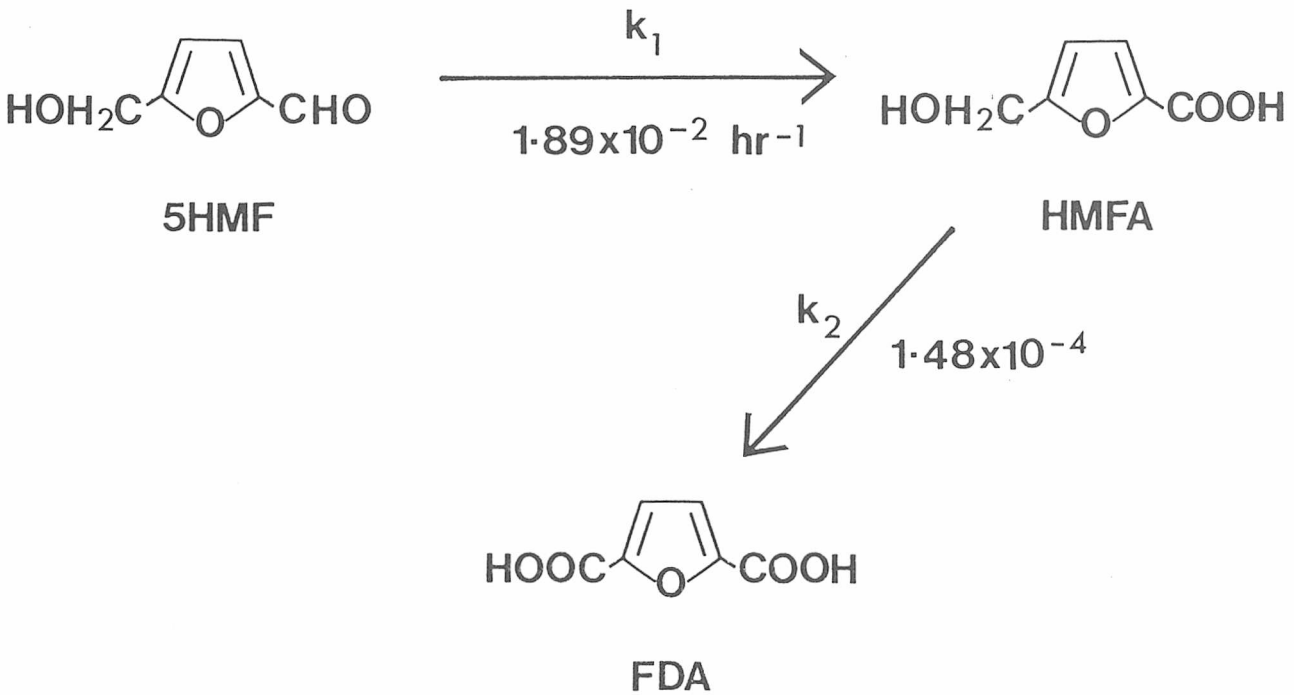


Figure 7.1 Showing an optimum chromatogram for the separation of 5HMF and its decomposition products at 0.24AU. Solvent: water containing 10mM. phosphate and 50mM. TEA at pH 7.

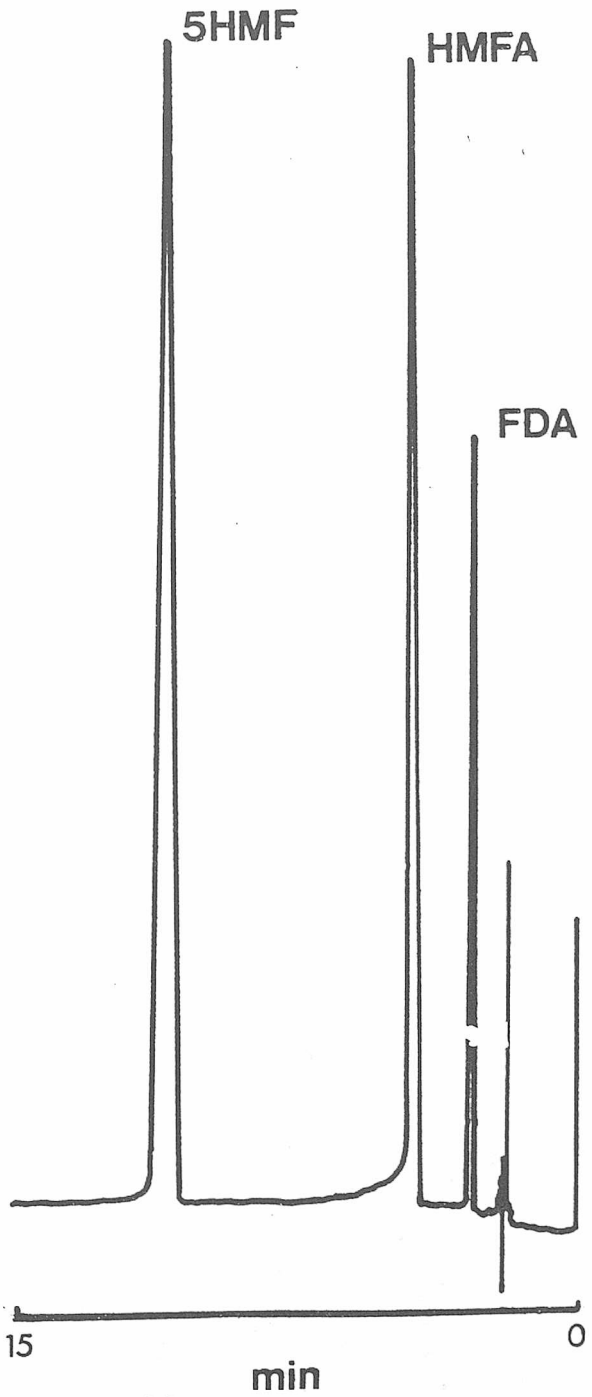


Figure 7.2 Showing the separation of 5HMF, HMFA and FDA at 0.2AU. Solvent: water containing 10mM phosphate buffer and 8mM TBA at pH 7.

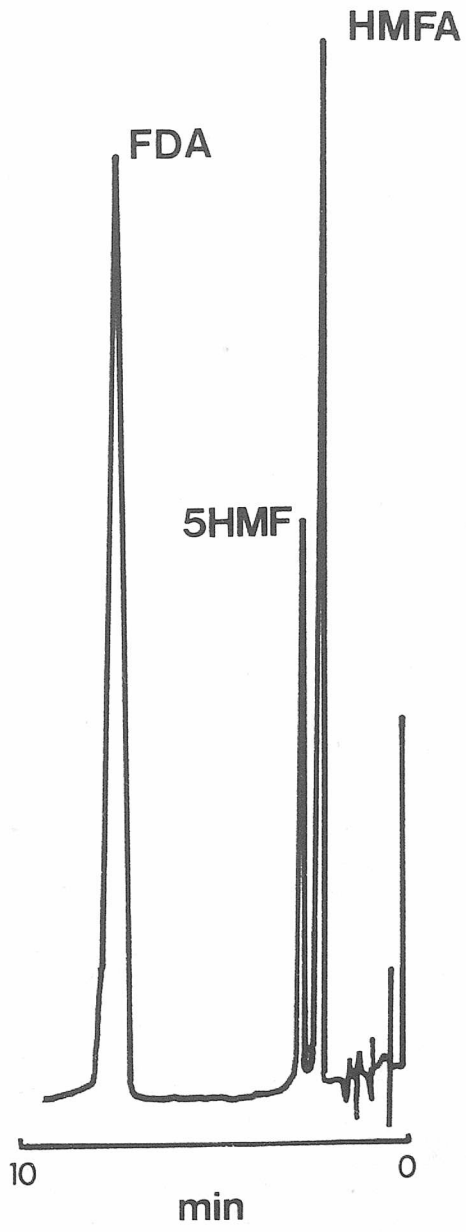


Figure 7.3 Plots showing the variation in 5HMF, HMFA and FDA concentration as a function of time during the decomposition of 5HMF at an initial concentration of 4×10^{-4}M at 30°C in water.

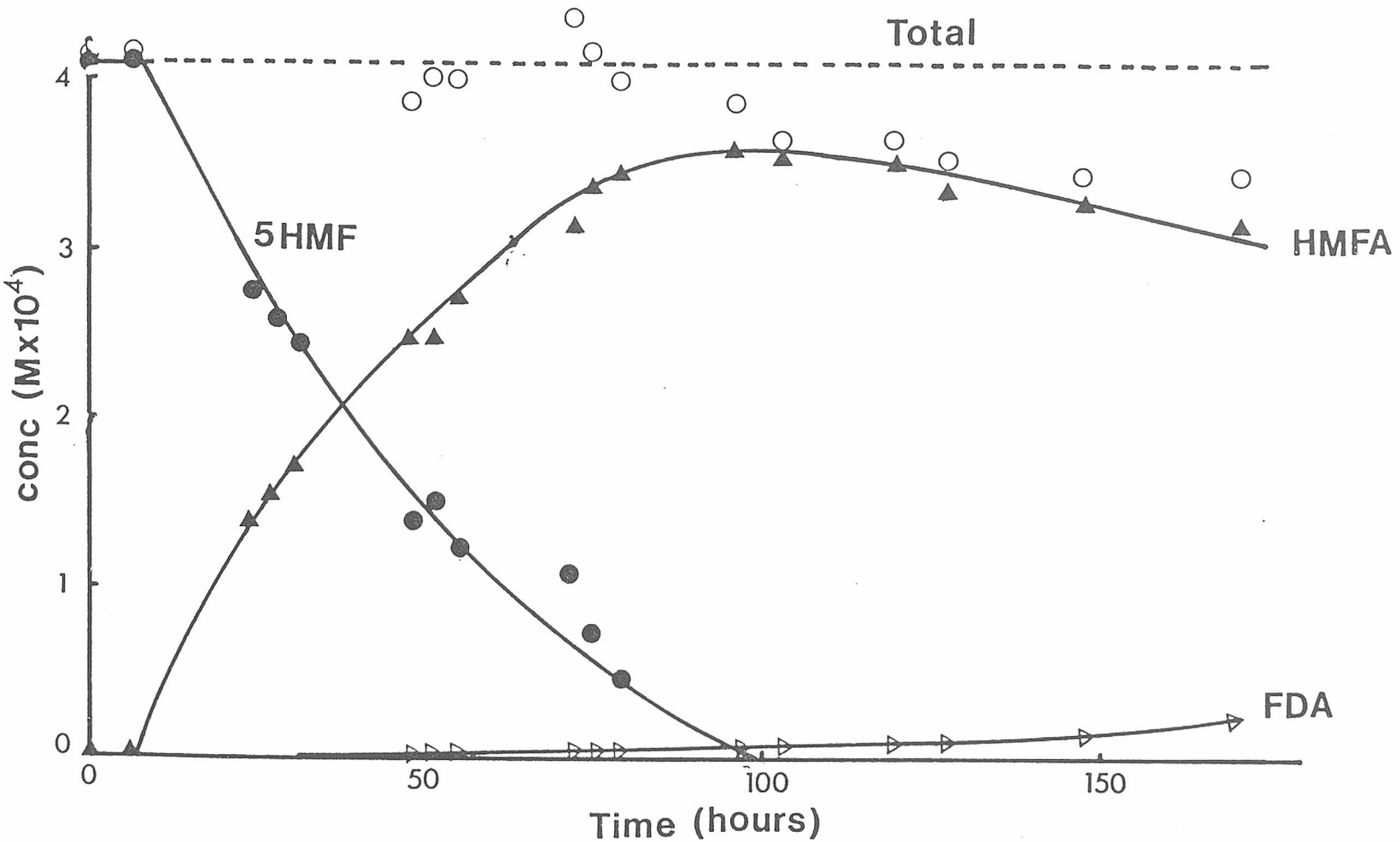


Figure 7.4 Plot showing the variation in 5HMF, HMFA and FDA concentration as a function of time duirng the decomposition of 5HMF at an initial concentration of 1.2×10^{-3}M at 30°C in water.

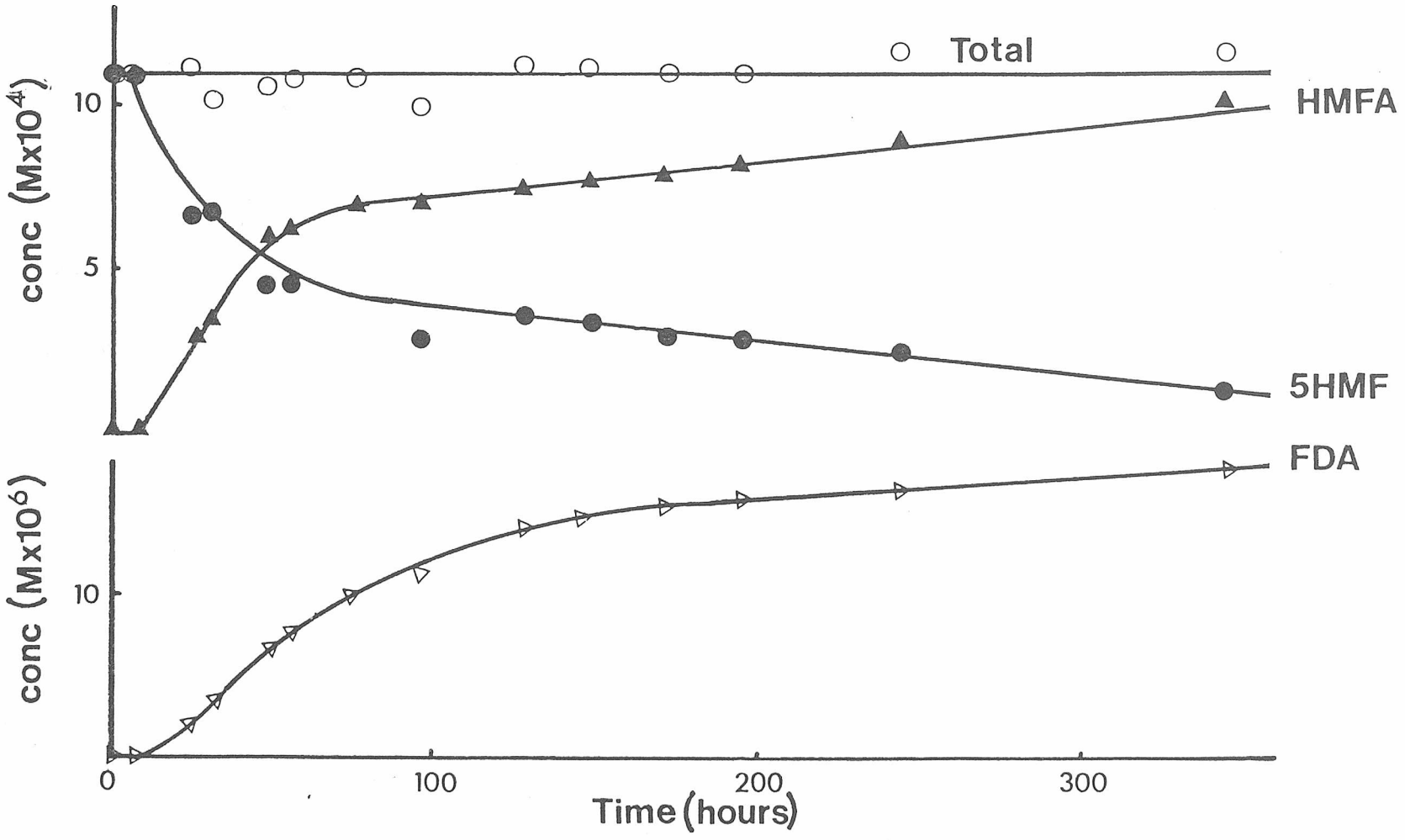


Figure 7.5 Plots showing the initial rate of HMFA formation at various initial concentrations of 5HMF. 5HMF concentrations shown in parenthesis.

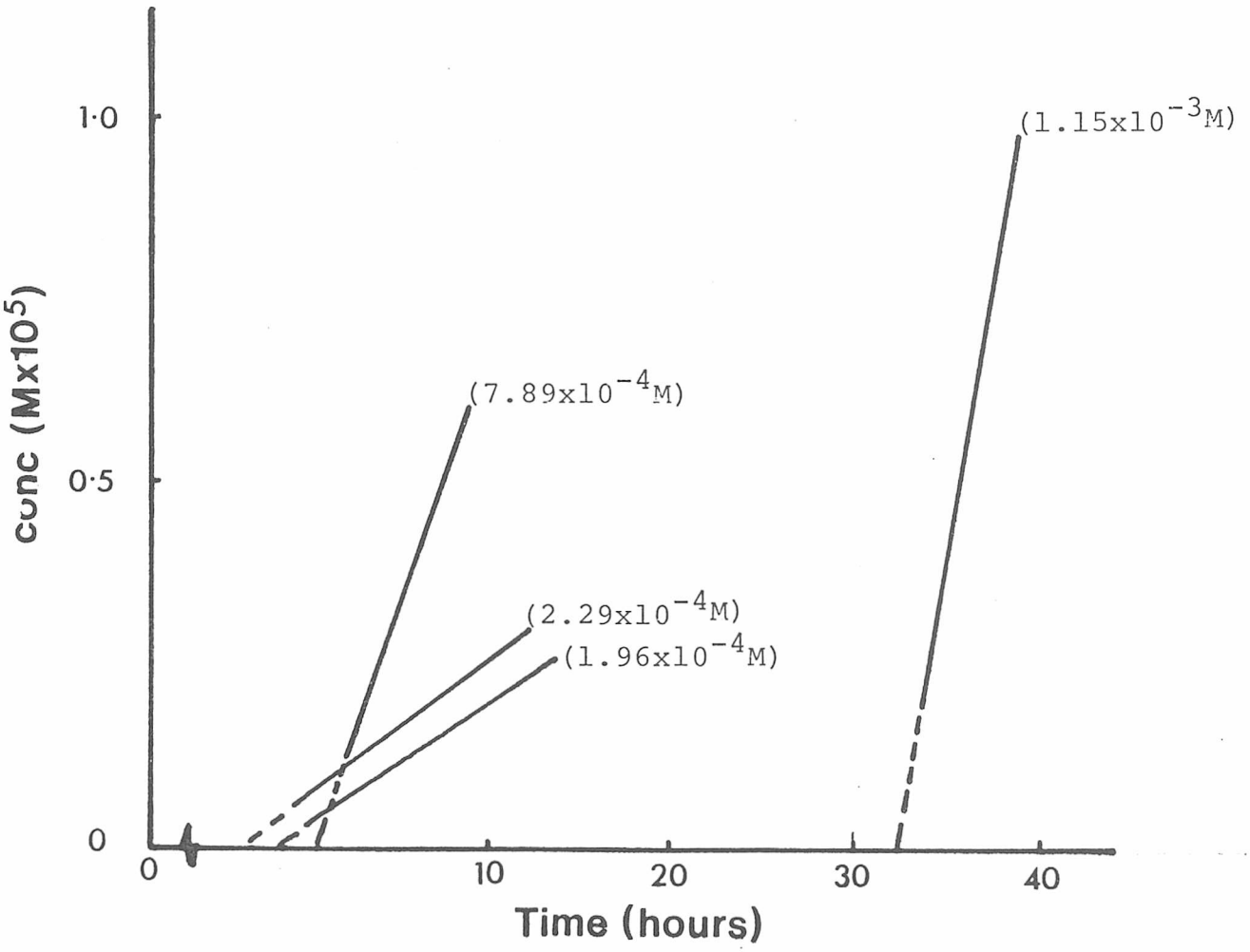


Figure 7.6 Plots showing the typical variation in HMFA and FDA concentration as a function of time during the decomposition of HMFA.

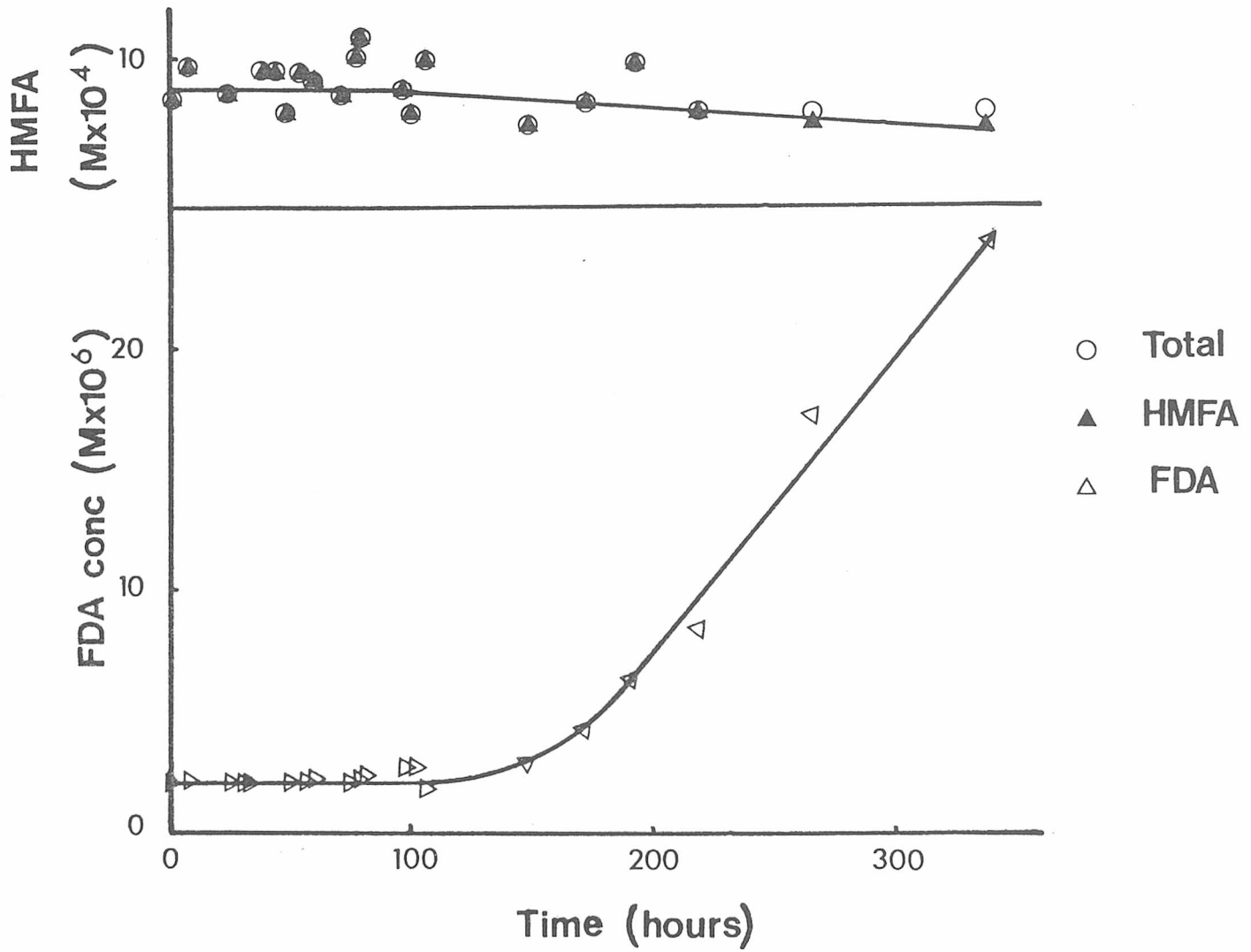
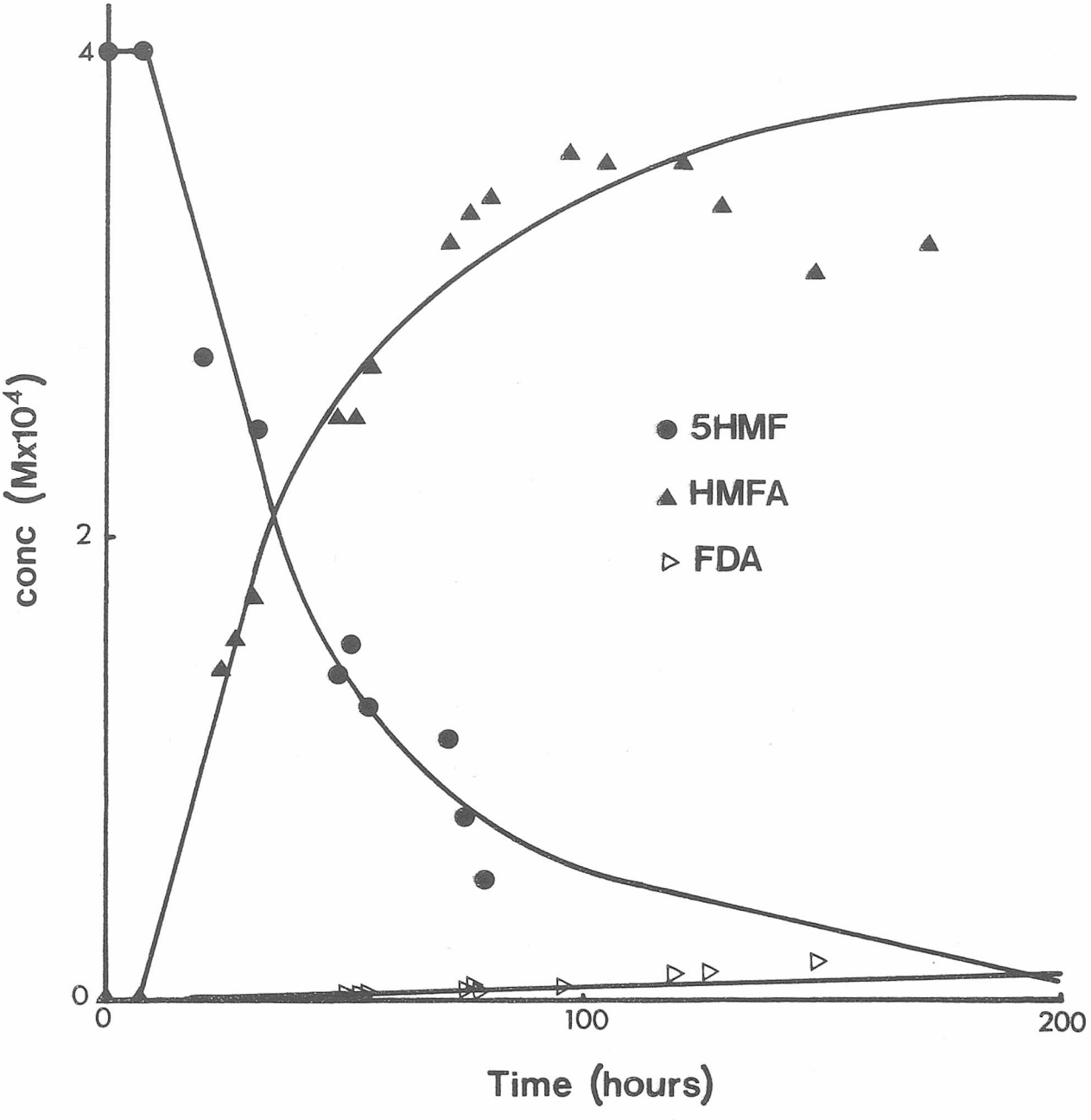


Figure 7.7 Plots showing the comparision between calculated and experimental data for the consecutive decomposition of 5HMF to FDA. Solid lines indicate calculated data.



Chapter 8

8.1 Conclusions

In the Introduction section several disadvantages inherent in conventional treatment of drug stability data were pointed out. Some of these have been discussed in the literature and differing approaches suggested. The initial rate method however has not been seriously proposed as an alternative. It has been suggested in the Introduction that this is because of analytical difficulties.

In this concluding chapter, the advantages of the initial rate method, using decomposition product measurement, will be discussed in the light of conventional treatment of kinetic data. The results of simulated data shown in Chapter 2 allow removal of practical analytical difficulties in consideration of the principles involved. Subsequent results allow evaluation of the initial rate method in practice. The general possibility of designing assay methods with improved stability indicating potential will be described using examples from previous chapters and additional chromatographic results.

A fundamental procedure in pharmaceutical drug stability testing is the determination of order of reaction. This is done to allow application of an integrated rate equation consistent with the data obtained on reactant concentration decrease with time. It carries the implication that the order of reaction is an intrinsic

property of that reaction. This is not so. Order of reaction is an empirical quantity determined by the conditions of an experiment; in this case the monitoring of the decomposition of a drug. That is, if the concentration of a reactant does not change appreciably during an experiment the order of reaction is zero by definition. This corresponds to a situation where the extent of reaction is small which is precisely the relevant situation in shelf life determination.

The accepted need to obtain reaction order results in the requirement that decomposition must be monitored to extents in excess of 25% and, in order to establish adequately precise rate constants, extents of reaction from 50-75% have been suggested as necessary. These facts support the contention that the reaction order is dependent on the extent of reaction but as consequence requires that unrealistic extents of reaction are needed when the accepted criterion of shelf life is 10% decomposition, i.e. t_{90}.

The literature clearly shows that the order of the vast majority of drug decompositions which have been determined, is first order with respect to the drug species when the decomposition is carried out to high extents of reaction. Indeed it has been recommended that reaction order may be assumed to be first in those cases where clear distinction cannot be made. Other workers have suggested zero order assumption to obtain conservative shelf life values.

The determination or assumption of first order

kinetics carries considerable practical advantage in situations where large extents of reaction may be monitored. The units of first order rate constants are those of reciprocal time and are independent of the units of concentration. This allows convenient calculation of t_{90} values independent of the concentration of the drug system under discussion and independent of the units in which such concentrations are expressed. At a more elementary level the adherence of a drug decomposition to first order kinetic behaviour has the advantage that any experimentally determinable property which is linearly related to concentration can be used to determine first order rate constants in the appropriate units of reciprocal time. This has been pointed out by Garrett in a review of stability testing of drugs[19]. Assumption of zero order kinetics on the other hand requires that concentration must be determined from the property measured before rate constants in correct units can be calculated.

As indicated in the Introduction, the order of a drug decomposition can be determined by direct application of the differential equation

$$-d[R]/dt = d[P]/dt = k[R]^n \qquad (8\text{-}1)$$

if the stoichiometry between R and P is known and if the rate of change of product with time can be measured reliably. Thus, if these conditions are satisfied a reaction need not, in principle, be studied to unrealistic extents for the reaction order to be established.

To determine the reaction order as indicated above,

widely different values of $[R]_0$ the initial reactant concentration, are chosen and d[P]/dt is measured over small extents for each initial reactant concentration. At the low extents studied any values of $[R]_0$ chosen will remain constant during the measurement of d[P]/dt. The equation can thus be represented as

$$d[P]/dt = k$$

Thus the gradient of this line is a measure of an apparent zero order rate constant.

The distinction between zero and first order chemical kinetics which has been extensively discussed in the prediction of shelf life is more apparent than real. Consideration of equation (1-16) derived in Chapter 1 which is identical to that previously derived in the pharmaceutical literature shows that for a given reaction the zero, first and second order rate constants are related by the initial reactant concentration, that is,

$$k_0 = k_1[R]_0 = k_2([R]_0)^2 \qquad (1\text{-}16)$$

Thus once the reaction order has been established by the initial rate method first and second order rate constants can be calculated.

It is however questionable whether it is necessary to establish the order of reaction. Firstly, as pointed out above, the order of reaction was required primarily to allow fitting of concentration data covering extensive decomposition. The initial rate method does not suffer from this constraint. Also the use of the correct order of reaction is required in order to calculate concentration of drug present at any time during a

decomposition. The converse is done in the calculation of shelf life. That is the time (t_{90}) is calculated at which 90% of the drug remains.

Although the merits of assuming order of reaction first [46,47,49] or zero [48,60] has been discussed with respect to its effect on shelf life estimation this can readily be quantified.

If the shelf life is defined as t_{90} and a reaction which is in effect first order is treated as zero order with respect to the drug

$$t^1_{90} = 0.105/k_1 \qquad (8\text{-}2)$$

and

$$t^0_{90} = 0.1[R]_0/k_0 \qquad (8\text{-}3)$$

where t^1_{90} and t^0_{90} represent the shelf life on the basis of first and zero order kinetics respectively. Similarly k_1 and k_0 are the respective rate constants and $[R]_0$ is the initial drug concentration. This means that t^1_{90} will be the correct shelf life. From equation (1-16)

$$[R]_0 = k_0/k_1$$

Substituting this in equation (8-3) we obtain

$$t^0_{90} = 0.1/k_1$$

Thus

$$(t^0_{90} - t^1_{90}) = 0.1/k_1 - 0.105/k_1$$

and

$$(t^0_{90} - t^1_{90})/t^1_{90} = - (0.005/0.105)$$

$$= - 0.0476$$

and the percentage error is 4.76%

This error is constant independent of the rate of reaction and the initial reactant concentration.

This means that the shelf life of a drug which decomposes by a first order reaction will be underestimated by some 5% if zero order kinetics are assumed. If then the prime purpose of establishing order is not the determination of rate constants but the determination of shelf life it would appear that in the light of other errors in the determination, the choice of order may be arbitrary.

For a reaction truly second order an assumption of zero order kinetics can be similarly shown to produce a result for t_{90} some 10% lower than the true value.

The concluding section of Chapter 2, 2.4, summerises the comparison made between the initial rate method and conventional kinetic treatment using simulated data free from analytical difficulties. These conclusions in a large part support the ideas presented above.

Because of the procedure of studying reactions to large extents, in order to obtain reliable rate constants, temperature stressing has become an accepted practice in order to obtain sufficient decomposition in a reasonable time. For the prediction of shelf life under storage conditions this requires that extrapolation procedures be used. These also have been criticised in the literature as discussed in Chapter 1.

The initial rate method is advantageous in minimising the time required to obtain rate constants so that the need for severe stressing can be reduced. It has been shown in Chapter 2 using simulated results that for reliable rate constants using integrated equations and reactant measurement some 75% decomposition is required while using the initial rate method 1% decomposition is adequate for calculation of a rate constant of equal precision. A comparison of the relative times required can thus easily be made.

If t_R and t_P represent the times required for the above extents of reaction to be obtained

$t_R = ((\ln(100/25))/k_1)$ and $t_P = (\ln(100/99)/k_1)$

where k_1 is the first order rate constant.

Thus $t_R/t_P = 139$

This potential saving in time can be compared with that obtained by temperature stressing. Although the effect of temperature stressing will be dependent upon the activation energy for a particular reaction a general rule of thumb is a doubling of reaction rate for every 10°C rise in temperature[19]. Thus to obtain approximately the same time saving as indicated above, 90°C must be used as the stressing temperature for a storage temperature of 20°C. This results in a factor of 128. To obtain extrapolation, lower temperatures must also be used with either large increases in time expended or decrease in extent of reaction and consequently precision.

It would appear thus that in principle the initial rate method can obviate the need for temperature

stressing. In practice this may not be the case. Firstly the results in Chapter 2 are based on the assumption that no product is present at the start of the reaction. Any product present will require that a higher extent of reaction be followed to obtain the required reliability. Also the detection capabilities for the product must be adequate. In the limiting case where the product cannot be detected the initial rate method cannot be applied and adequate sensitivity of product detection is a prerequisite.

More realistic comparisons of temperature stressing and the initial rate method in terms of time saving can be obtained by consideration of the results of Chapters 3 - 6 where widely different drug types have been studied by both methods. ASA, DIASA and TC represent examples of drugs which are relatively unstable in solution. In these cases direct comparison may be made of the difference in time required to obtain rate constants at a single temperature by conventional reactant concentration measurement and the initial rate method using product concentration measurement.

The lowest controlled temperature used was in general 30^{o}C to allow adequate thermostating using conventional water baths. This temperature is used for the comparison. For ASA a 106 fold decrease in time was observed. For DIASA a 64 fold advantage was realised and, in the case of TC when ETC was taken as the main decomposition product, the advantage was 122 fold. In the ASA, DIASA cases the wavelength of detection was optimised to give maximum

sensitivity of detection of product and in the TC situation the optimum wavelength of detection was the same for both reactant and product.

The time savings realised in practise by choosing product quoted above can be compared with those obtained by conventional temperature stressing. Comparison of results obtained by conventional methods at 60°C with those of 30°C for ASA, DIASA and TC yielded time savings of 18, 18 and 30 fold respectively and show that fairly high activation energies are associated with these decompositions. The time saving following such a 30°C rise in temperature however is consistently less than that realised by using direct product measurement at the lower temperature. It would thus appear that in practice, for these relatively unstable drugs, the initial rate method as described can eliminate the need for temperature stressing with its associated difficulties and uncertainties of extrapolation.

ND represents a drug which is very stable both in the solid form and in solution. In addition it decomposes into several different products. The results of Chapter 6 indicate the long times required to obtain measurable rate constants by conventional methods even employing temperature stressing to very high temperatures (90°C) in solution. Even under these conditions the suggested 50-75% decomposition could be obtained only after following the reaction for 100 hours. The initial rate method however allowed estimation of initial rates approximately

20 times more rapidly under the same conditions. In such an instance the initial rate method may be required to be used in conjunction with conventional temperature stressing in order to obtain shelf life estimates in acceptable times. The results obtained, however, must be interpreted in terms of the individual rate constants for the different reactions occurring and a composite rate constant calculated before use to predict shelf life.

The reaction studied in Chapter 7 broadly confirms the above results. The oxidation of 5HMF to HMFA can be studied approximately 10 times more rapidly by product measurement but in this case direct comparison is hindered by the existence of a preliminary induction period.

Hplc as a Single General Analytical Method for Stability Studies

The development of temperature stressing methods has arisen, as suggested in the Introduction, for two maian reasons. Firstly, long term storage makes severe demands on the analytical method in terms of long and short term precision. Also such studies are both expensive and time consuming. Although temperature stressing may reduce expense and time, short term precision is still dependent upon the extent of reaction studied. It has been shown in Chapter 2 that at least 30% decomposition must be allowed to occur in order to obtain rate constants of reasonable precision and accuracy when using the conventional integrated method. Most assays for stability studies have been designed to be specific for the reactant, since

conventional kinetic treatments are based on undecomposed drug concentration. An assay method to be useful in applying the initial rate method, however, demands that the product be measured specifically with adequate sensitivity. That is, the assay must be designed to allow sensitive and specific determination of product(s) as well as specific determination of the undecomposed drug. The latter is necessary in order to assess the stoichiometry of the decomposition reaction. These criteria have largely been satisfied for the drug decompositions investigated in Chapters 3-7. Sensitivity of product measurement in the presence of excess undecomposed drug has been achieved by designing the chromatographic system so that the products are eluted before the undecomposed reactant.

To date the criterion for an assay to be termed stability indicating has been accepted as the assay being specific for the reactant in the presence of its decomposition products. It has been implied in the literature that this definition is no longer adequate and any stability indicating assay must be capable of quantifying decomposition products[271]. This suggestion has been made because of the need to quantify toxic decomposition products and has not to date been extended to the application of the initial rate method. To utilise the initial rate method fully the product(s) must be measured. It is suggested as a result of the present work that for an assay to be described stability indicating it should, in addition to being specific for the undecomposed

drug, conform to the following criteria.

1. The assay method should be capable of resolving all decomposition products leading to quantitation if standard compounds are available.

2. The assay method should be designed to elute reaction products before undecomposed drug for maximum sensitivity of product measurement.

3. The assay method should be capable of such resolution and quantitation for different products formed during different decomposition reactions of a drug.

The assays described in Chapters 3 to 7 broadly satisfy these criteria. These assays all depend upon the use of a single analytical method namely hplc which has been widely accepted in the literature as the method of choice for stability indicating assays in terms of specificity. Hplc has the potential of being a universally used assay method for stability investigation purposes. This contention is supported by the appearance of approximately 100 hplc assay procedures reported in 1984 in a single pharmaceutical journal, the Journal of Pharmaceutical Sciences. In addition 80% of these used ultraviolet detection clearly indicating that ultraviolet absorption is a very generally applicable method of quantitation. It has been amply demonstrated that hplc with ultraviolet detection, when applied to product measurement, is capable of comparable relative precision to that reported for reactant concentrations[155]. What has not been demonstrated in the literature other than by the numerous publications is the generality of the hplc method

of analysis in the field of drug stability. Indeed the hplc approach has been utilised only to obtain specificity on a drug by drug basis. The assays demonstrated in Chapters 3 to 7 are based on a rational application of chromatographic principles. In each case it has been possible to arrange the elution order to be that required by the initial rate method. This was achieved largely as a result of considering the relationship between reactant and product in terms of their ionic nature, thus allowing application of the high sensitivity by manipulation of elution order by ion suppression and ion pairing ideas.

In order to demonstrate that such assays can be developed for the vast majority of drug decompositions where ultraviolet detection is possible drug decompositions may be classified in terms of differences in ionogenicity between reactant and product. Such a classification is shown below.

	Ionic Nature of Reactant		Ionic Nature of Product
(i)	acidic	→	acidic
(ii)		→	basic
(iii)		→	neutral
(iv)	basic	→	basic
(v)		→	acidic
(vi)		→	neutral
(vii)	neutral	→	neutral

(viii) → acidic

(ix) → basic

Decomposition type (i) is represented by ASA, DIASA and TC while the decomposition of ND on melting represents type (iv) and in solution type (v). The decomposition of 5HMF is representative of type (viii).

The above classification encompasses all possible drug decomposition types. Stability indicating assays have been developed for ASA, DIASA, TC and ND. To demonstrate that similar assays can be developed for the remaining types of decomposition separations have been developed for decomposition types (ii), (vi) and (ix). The examples chosen are succinylsulphthiazole, diphenhydramine and chloramphenicol respectively.

The decomposition of succinylsulphthiozole to sulphthiazole represents an example of an acidic drug decomposing to produce a basic product. Although a number of hplc methods have been described in the literature these have been concerned with the separation of various sulphonamides[272-276] and not specifically with stability determination.

Reverse phase chromatography can yield directly the correct elution order and resolution for this pair of compounds using a solvent comprising acetonitrile : water (5/95) containing 60mM. phosphate buffer at pH 2. However the capacity factors vary widely and would result in long retention times of succinysulphathiazole (SSPT) or inadequate retention of sulphthiazole (SPT). OSA was used

to decrease the retention of the neutral succinylsulphthiazole and increase retention of basic SPT. The variation in capacity factor (k') for both compounds as a function of OSA concentration is shown in Figure 8.1. As seen from Figure 8.1 addition of OSA results in a decrease in the k' for SSPT which is neutral under these conditions. SPT shows the parabolic variation expected of a base under these conditions. This allows rapid elution of SSPT while maintaining adequate resolution from SPT which is still eluted first at OSA concentrations below 15mM. Above this pairing ion concentration the order is reversed and no longer satisfies the criteria for a stability indicating assay in terms of product measurement. An optimum specimen chromatogram is shown in Figure 8.2 using 5mM. OSA and it is suggested that this could form the basis of an adequate stability indicating assay for this drug decomposition.

The decomposition of a basic drug to neutral decomposition products is represented by diphenhydramine. Although a previous hplc separation of diphenhydramine from some of its metabolites and decomposition products has been reported in the literature[277], addition of salt in the form of sodium chloride to the solvent was necessary for elution of the amines. The use of halogen salts in an hplc system is not ideal.

Diphenhydramine (DPH) undergoes decomposition to benzhydrol (BHL) which in turn may be oxidised to benzophenone (BPN). In this case reverse phase chromatography in the absence of any pairing ion produces

elution of DPH before either of the products at pH 2. Under these conditions DPH will be protonated and can be made to behave as a charged base. A suitable pairing ion was determined to be SLS which was used to increase the retention of DPH. As expected, the effect on the neutral decomposition products was a reduction in retention. The variation in k' for all three compounds as a function of SLS concentration is shown in Figure 8.3. It is seen that above an SLS concentration of 2mM. the elution order is reversed the products being eluted first. DPH shows a rapid increase in k' passing through a maximum before gradually decreasing with increasing SLS concentration. Any base under these conditions would be expected to exhibit this parabolic variation in k'. However while the addition of SLS to the solvent allows reversal of the elution order it also results in the loss of symmetry of the DPH peak. Figure 8.4 shows a sample chromatogram of all three compounds using a solvent of acetonitrile : water (30/70) containing 120mM. phosphate and 10mM. SLS at pH 2. It has been reported that peak symmetry may be restored by the addition of an organic counter ion of the same charge as that on a solute in ion pairing systems[278]. Addition of 10mM. TEA as an organic counter ion to the above solvent showed no improvement in peak symmetry. Addition of 5mM. TBA, however, not only dramatically improved peak symmetry but also reduced the k' for DPH without having any effect on the k' for BHL or BPN. An optimum specimen chromatogram is shown in Figure 8.5.

The above findings are consistent with previously

reported work on the tricylic antidepressant drugs[278] where addition of an organic counter ion altered both peak symmetry and selectivity. In the present work no peak reversal was observed as reported for the antidepressents. This separation obtained by judicious use of appropriate pairing ion and counter ion demonstrates that elution order for this different system can be drastically altered in order to facilitate product measurement.

Chloramphenicol undergoes both hydrolysis and photochemical decomposition as shown in Scheme 1.1. Below pH 6 hydrolysis results in the formation of 1-(4'-nitrophenyl)-2-amino-1,3-propandiol (AMPD) while above pH 6 1-(4'-nitrophenyl)-2-dihydroxyacetamido-1,3-propandiol (DHPD) has been reported to be formed[279]. Photochemical decomposition of either AMPD or chloromphenicol (CAP) results in the formation of p-nitrobenzaldehyde (PBA) which undergoes further decomposition ultimately yielding p-nitrobenzoic acid (PNA)[280,281]. Of the hplc methods reported only a few involve separation of CAP from some of its decomposition products[281-284]. In no instance are the decomposition products eluted before CAP.

The following chromatographic system has been designed to separate CAP, AMPD, PNA and PBA these compounds being readily available. Attempts to synthesize DHPD by the method of Shih[279-280] proved unsuccessful. Chromatographic separation was carried out at pH 2. Under these conditions AMPD would be expected to behave as a charged base, the remaining three compound being essentially neutral. Although separation in the required

order can be obtained using a solvent of acetonitrile : water (10/90) containing 100mM. phosphate buffer at pH 2 resolution between CAP and PNA was inadequate for quantitation purposes. Addition of a suitable pairing ion, in this case pentane sulphonic acid (PSA) showed the expected variation in k' for all four species. AMPD showed a parabolic variation in k' while CAP, PNA and PBA all showed a reduction in k'. These results are shown in Figure 8.6. Adequate resolution between CAP and PNA, however, could not be achieved at any PSA concentration using a 4.6 x 100mm. column as shown in Figure 8.7. To obtain adequate resolution a longer column, 4.6 x 200 mm, was employed and a specimen chromatogram is shown in Figure 8.8. Unlike the two previous separations where above a certain pairing ion concentration reversal of elution was observed no such reversal was observed in this case at any concentration of PSA. The use of a more hydrophobic pairing ion such as OSA or SLS would have shown more marked changes in k' possibly altering the elution order.

Figures 8.7 and 8.8 were obtained by injecting a sample of CAP exposed to light in solution under ambient conditions for three months. No peaks in addition to those already considered were observed when the chromatograms were run at high sensitivity.

The above results in conjunction with those of Chapters 3 to 7 indicate that the additional criteria for stability indicating assays outlined above can be achieved in practice for eight out of the ten possible

decomposition situations. Decomposition type (iii) represents the reverse of type (viii) and it is suggested that resolution in this case could be obtained as in the case of type (viii) by use of an appropriate cationic pairing ion and choosing a pairing ion type and concentration to elute the neutral species first. This possibility has previously been demonstrated in the literature[269] for the separation of 5HMF, a neutral species from its two acidic decomposition products, and has been used in the present work in Chapter 7. The separation of neutral products from neutral reactant, decomposition type (vii), should not constitute a problem in view of the current ideas on solvent selectivity[285] coupled with the possibility of using normal phase columns which would reverse the elution order albeit at the expense of extraction procedures to allow sample application.

The above discussion has centred upon drug stability for the determination of shelf life. The quotation in Chapter 1 indicates, however, that there are several different interpretations that may be put on the term stability. One of these is undoubtedly the maintainance of potency as quantified by shelf life. Others include mechanistic studies to establish the reaction pathway of decomposition and determination of the amount of decomposition products which may be potentially toxic. This may be regarded as a limit testing situation.

While all of these aspects of drug decomposition are related to stability, they have traditionally been treated

as separate areas of study and while such segregation within an overall stability study may have the advantage of simplifying a given study each has become a separate field. Few attempts have been made to bring together each separate field to describe the overall stability of a drug.

It was suggested above that the availibility of suitable analytical methodology was responsible for the concentration on integrated methods for shelf life determination. It also appears to be the case that separation of stability into the different areas described above is a result of analytical limitations. That is, different analytical methods have been chosen for shelf life determination and for limit testing and this has tended to divorce these areas from one another. While shelf life determination may involve the development of a specific assay method for the undecomposed drug, a different analytical method may be employed in the study of reaction mechanism and yet another may be employed to ensure absence of decomposition product(s) in the preparation. The latter, in the vast majority of cases, is semi-quantitative in nature. Very rarely is the same analytical method used to assess all three areas of stability. Chlorpromazine for example has to be quantified in pharmaceutical preparations by differential ultraviolet absorption (254nm. and 277nm.)[286], spectrofluorimetry[287], GLC[287] and hplc[288]. A recent hplc method reports the separation of chlorpromazine from its two oxidation products[287]. On the other hand both the

British Pharmacopoeia and United States Pharmacopoeia require that the presence of any oxidation products be detected using tlc.

The capabilities of hplc together with the additional criteria for a stability indicating assay when analysing for product(s) now makes it possible to unify these areas of stability on the basis of a single analytical method, that is, the assay method is the common denominator relating these three important aspects of any stability investigation. By designing an assay method specific for both reactant and product and sensitive for the product the assay method not only has the advantages discussed above for the rapid estimation of shelf life by the initial rate method but also has the advantage that it can be applied to investigate the route of decomposition and also be used for limit testing on a quantitative basis. This latter point is of particular relevance in the case of TC where as a result of decomposition a toxic product EATC is formed. The assay method described in Chapter 5 allows all aspects of stability to be investigated. Measuring for ETC allows application of the initial rate method and estimation of shelf life, concentration-time data for each product allows elucidation of the reaction pathway and the toxic EATC is readily quantified for limit testing purposes.

The assay method used to study the decomposition of 5HMF in Chapter 7 was previously reported in the literature[268]. The authors have used this assay method to demonstrate the inadequacies inherent in the present limit

test for 5HMF in D-glucose infusion fluids and have described a quantitative and more sensitive limit test for 5HMF. This same assay procedure has been used in the present work to determine other aspects of 5HMF stability.

The analysis of decomposition products, in addition to enabling shelf life estimation and limit testing, may also enable the route of decomposition to be elucidated. 5HMF has been shown to undergo consecutive decomposition to HMFA and FDA and the knowledge of product identity has enabled determination of rate constants for each step using the initial rate method. The assay method described for ND in Chapter 6 has allowed the suggestion that ND decomposes by a parallel pathway even in the absence of product identity.

The measurement of decomposition product by virtue of its demands on the design of an assay method now makes it possible to determine the overall stability of a compound using the same analytical technique. However the use of hplc coupled with ultraviolet detection demands that the decomposition products be ultraviolet absorbing. In those cases where such detection is impractical other, less sensitive but more general detectors such as refractive index or mass detectors even at the present state of the art, could well provide adequate sensitivity. Measurement of product also demands that the identity of the products be known if the initial rate method is to be utilised completely.

In conclusion it is suggested that while classical methods of shelf life determination provide unequivocal

determination in terms of drug potency, the procedures which have evolved from classical chemical kinetics have many disadvantages mainly in terms of time. They also confine a stability study to discrete areas which further contributes to the isolation of each area since each investigation requires different analytical methods. A change in analytical emphasis to include product analysis in situations where the products have been well characterised offers clear advantages in terms of shelf life determinations, both in the speed of determination and simplification of kinetic treatment, in the study of reaction pathways and in limit testing. Before comparable data on reaction rates can be obtained by product measurement however, the characterisation of products is necessary so that in the case of novel drug compounds traditional methods may well continue with advantage. Once such products are known, however, the initial rate method would appear to be clearly advantageous in determination of the relative stability of different formulations and it would appear that the methodology based on hplc has this analytical capability.

Figure 8.1 Showing the variation in capacity factor as a function of pairing ion concentration for SSPT and SPT using a 4.6x50mm column. Solvent: acetonitrile : water (5:95) containing 60mM phosphate buffer at pH 2.

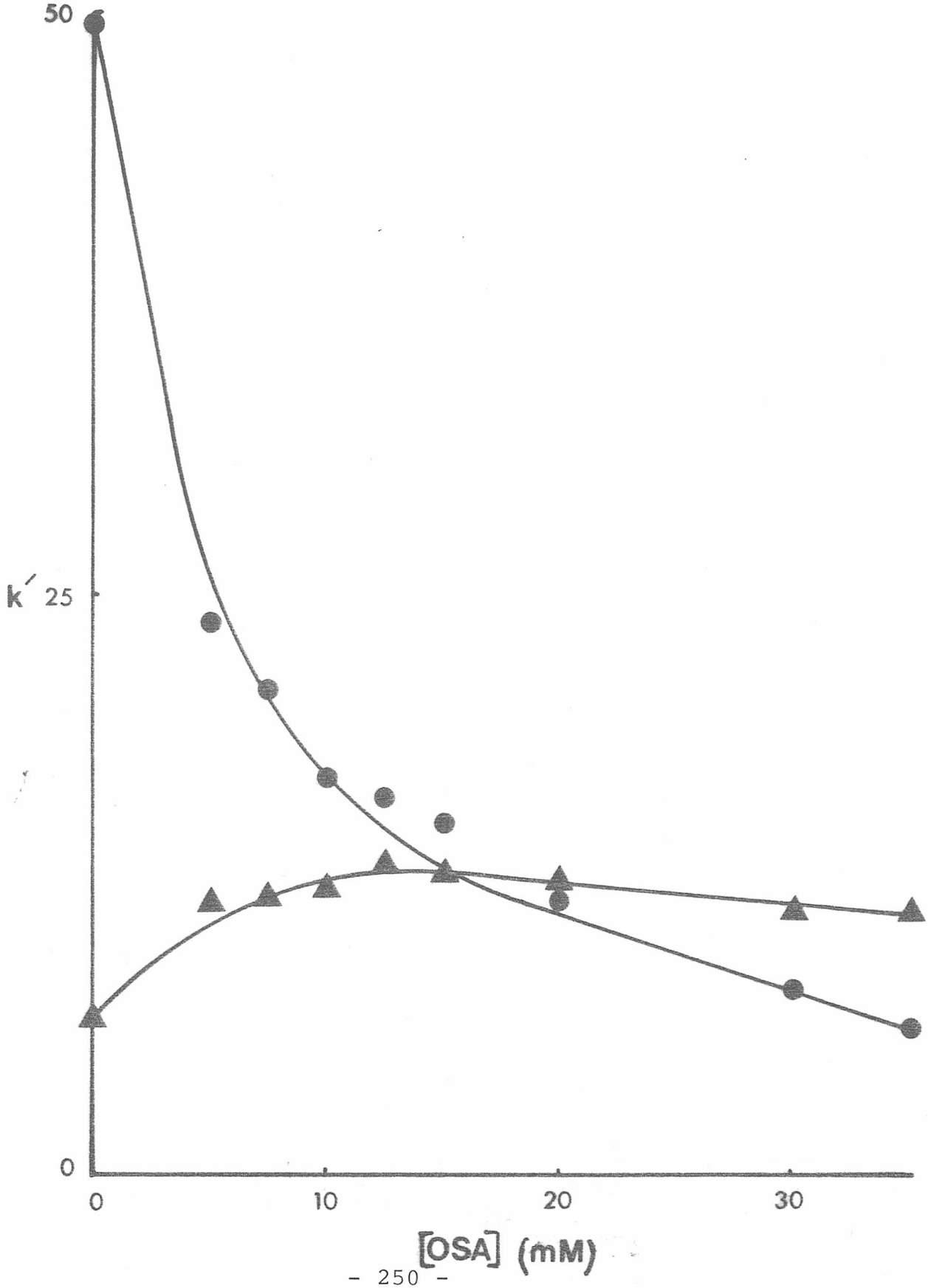


Figure 8.2 Showing an optimum specimen chromatogram for the separation of SSPT and SPT using a 4.6x50mm column at 0.2AU. Solvent: acetonitrile : water (5/95) containing 60mM phosphate buffer and 5mM OSA at pH 2.

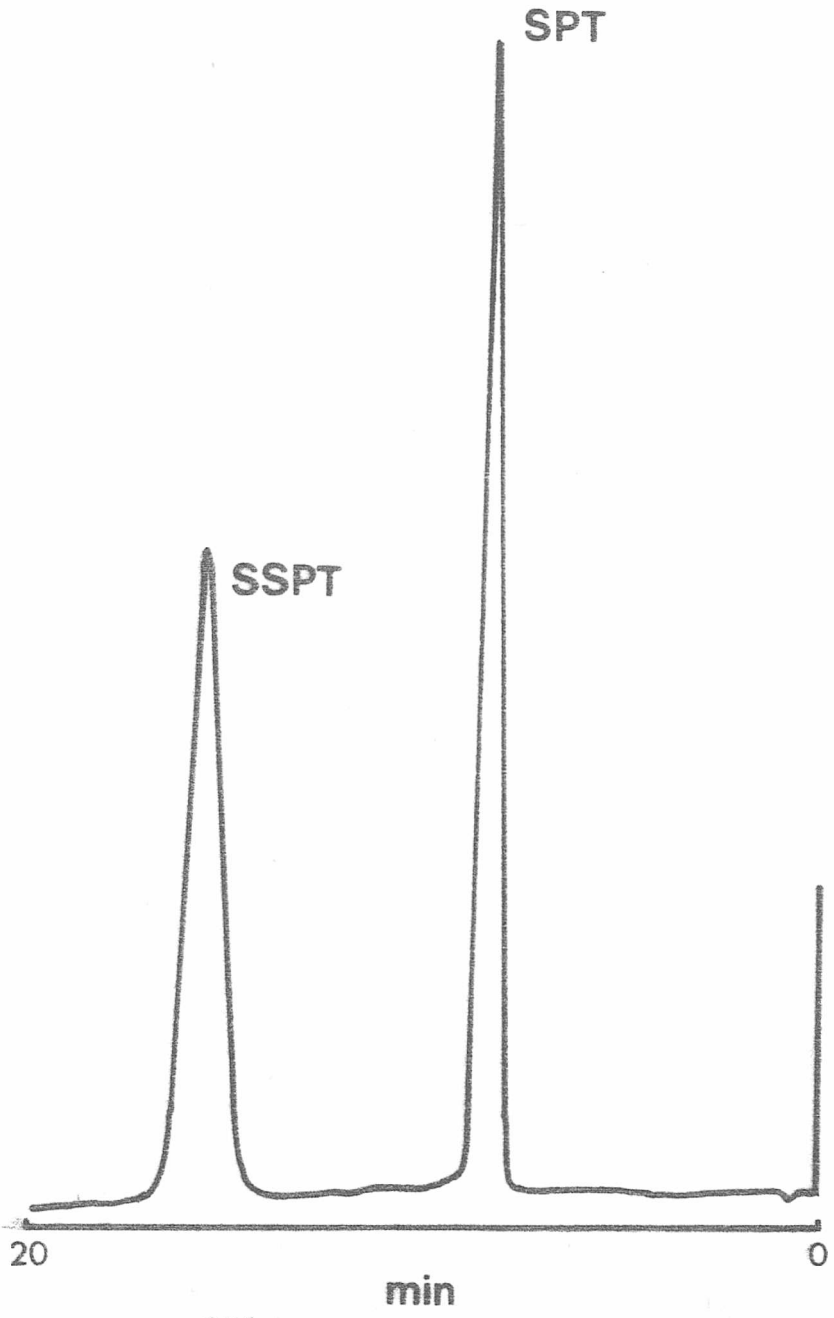


Figure 8.3 Showing the variation in capacity factor (k') as a function of anionic pairing ion concentration for DPH and its decomposition products. Solvent: acetonitrile : water (30/70) containing 120mM phosphate buffer at pH 2.

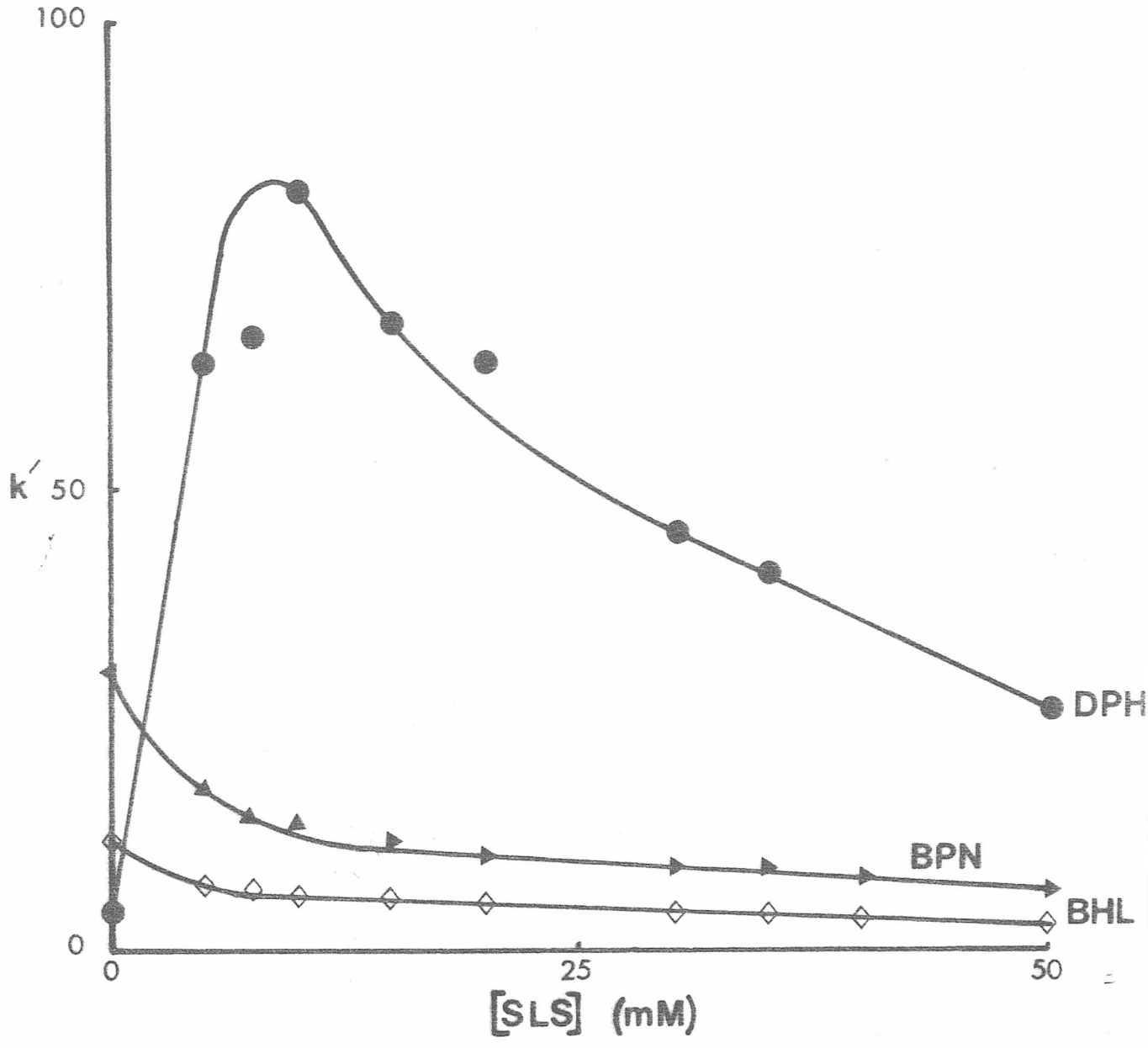


Figure 8.4 Showing a sample chromatogram for the separation of DPH and its decomposition products using a 4.6x50mm column at 0.5AU. Solvent: acetonitrile : water (30/70) containing 120mM phosphate buffer and 10mM SLS at pH 2.

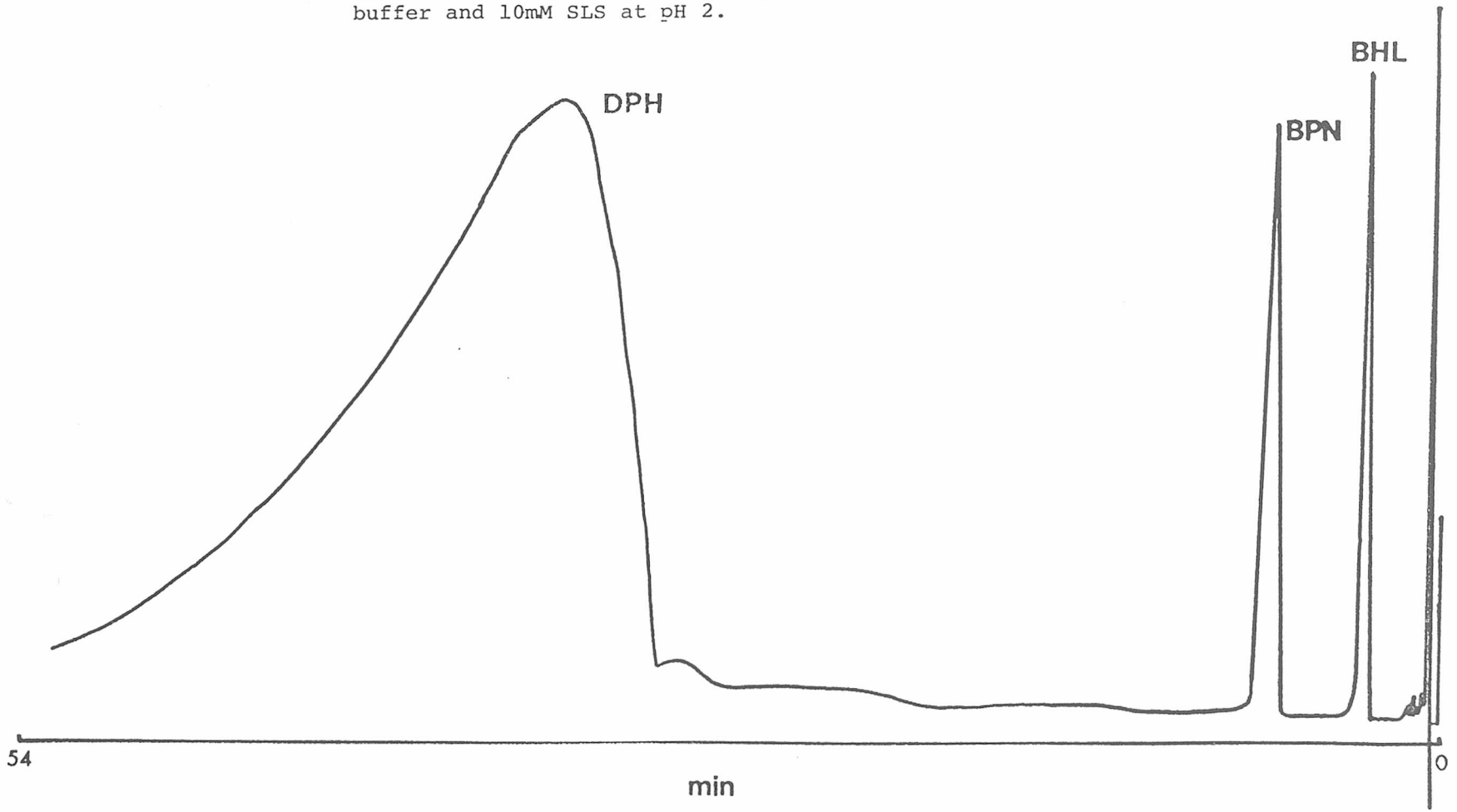


Figure 8.5 Showing the separation of DPH and its decomposition products using a 4.6x50mm column at 0.5AU. Solvent: acetonitrile : water (30/70) containing 120mM phosphate buffer, 10mM SLS and 5mM TBA at pH 2.

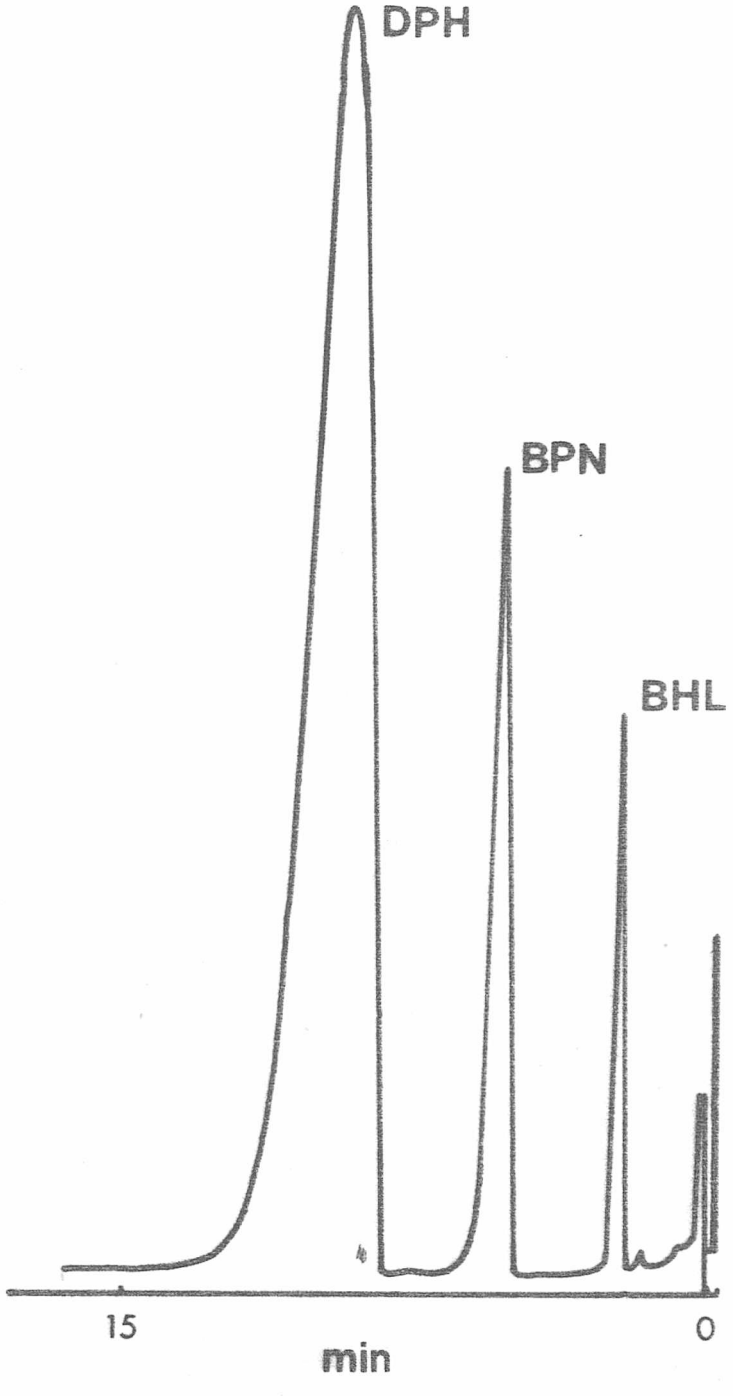


Figure 8.6 Showing the variation in capacity factor (k') as a function of anionic pairing ion concentration using a 4.6x100mm column. Solvent: acetonitrile : water (10/90) containing 100mM phosphate buffer at pH 2.

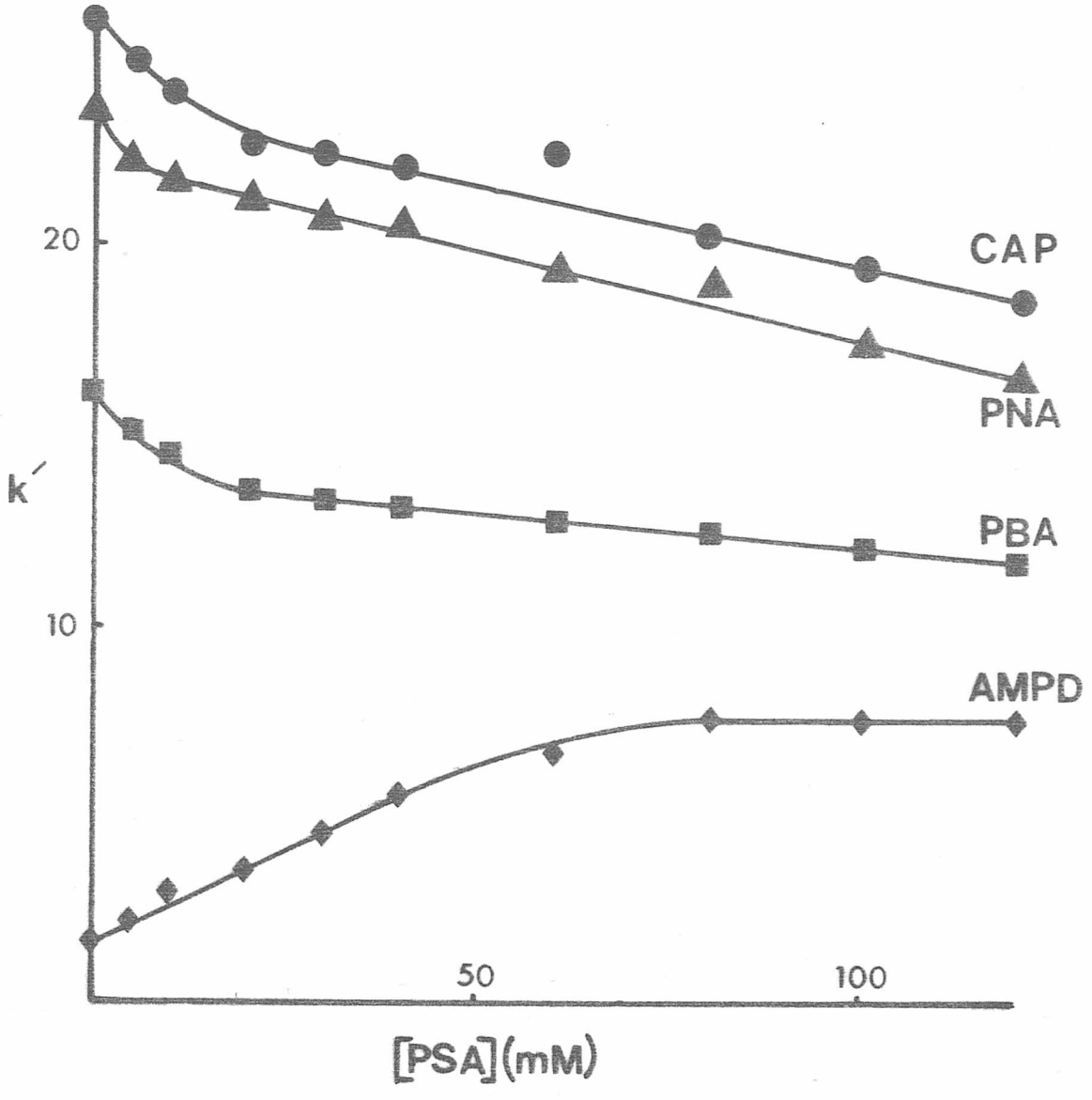


Figure 8.7 Showing a sample chromatogram of the separation of CAP and its decomposition products using a 4.6x100mm column at 1.0AU. Solvent: acetonitrile : water (10/90) containing 100mM phosphate buffer and 20mM PSA at pH 2.

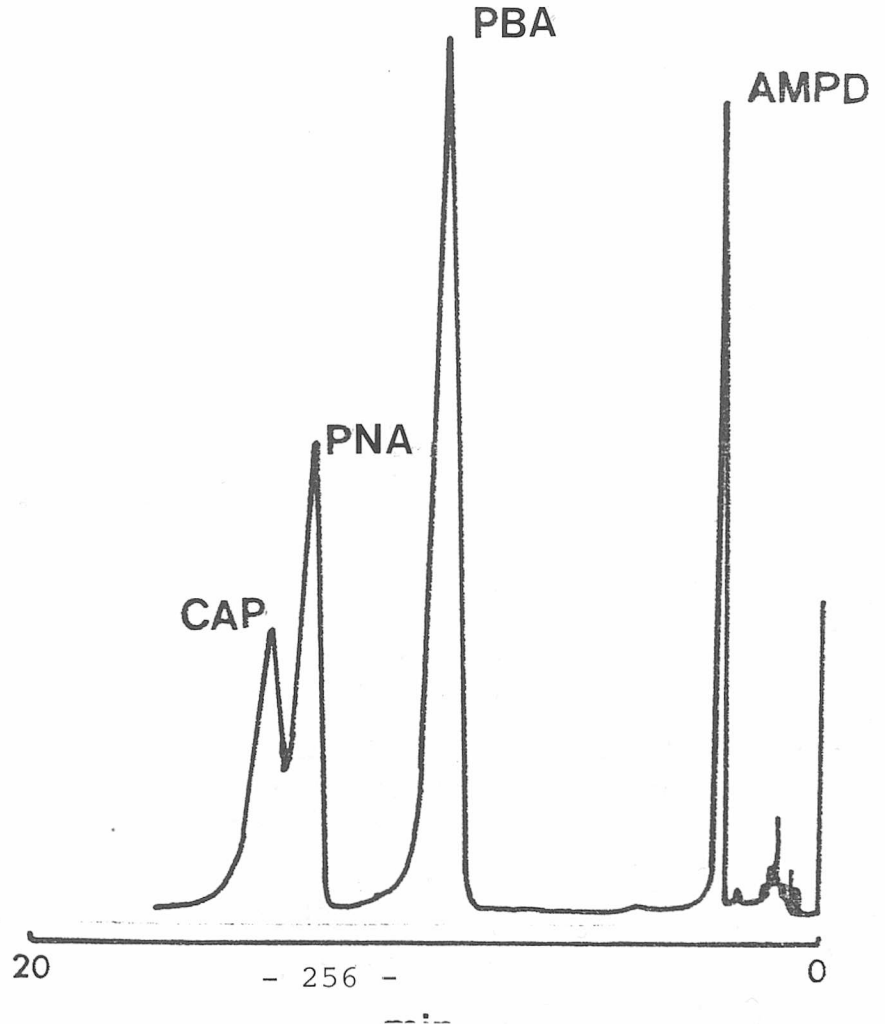
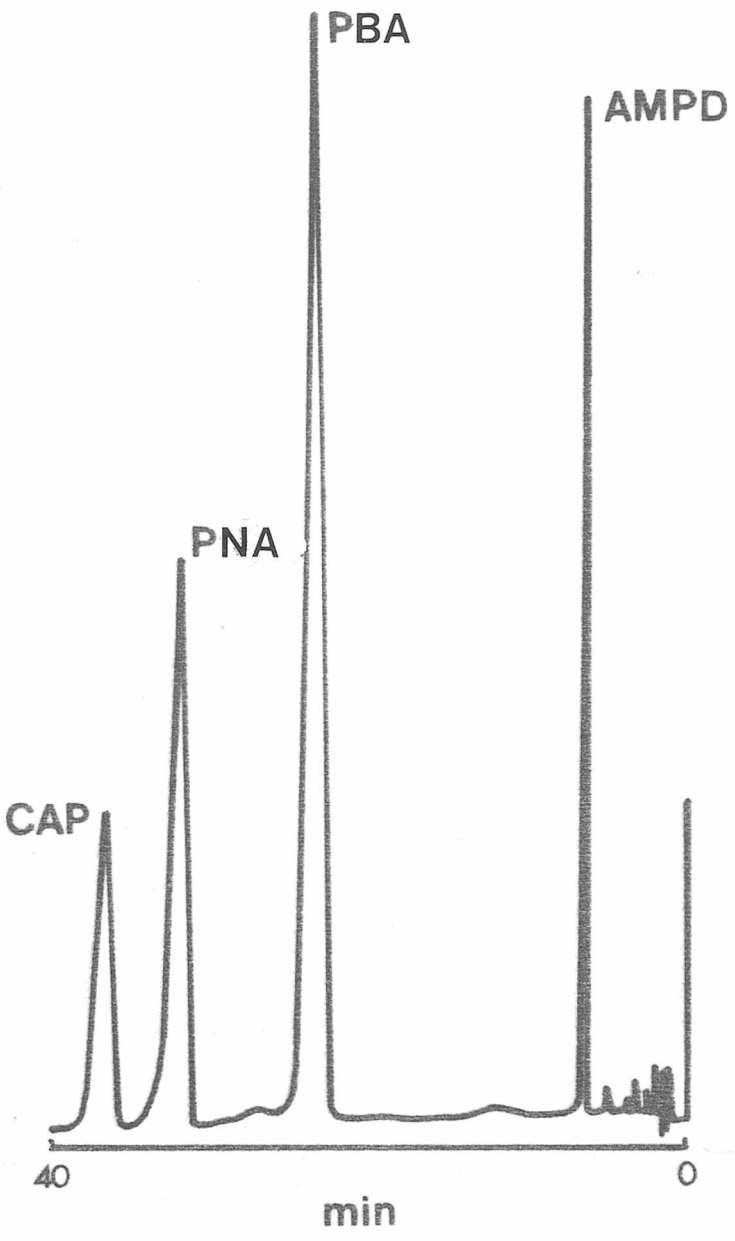


Figure 8.8 Showing an optimum specimen chromatogram for the separation of CAP and its decomposition products using a 4.6x200mm column at 0.2AU.



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APPENDIX I

Postgraduate Courses

The following postgraduate courses and scientific meetings were attended in connection with this program of research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

1. The British Pharmaceutical Conference at the University of London, September 1983.

2. Workshop tutor (D. G. Durham and A. S. H. Shivji) at the Scottish Pharmaceutical Sciences Group meeting on Drug Stability organised by the British Pharmaceutical Society Scottish branch in Edinburgh, April 1984.

3. A residential course in hplc at the University of Sussex, September 1984, sponsored by the Chemical Society of Great Britain.

4. An undergraduate course in statistics for second year students in physical chemistry and third and fourth year students in mathematics in the School of Mathematics, R.G.I.T.

5. Continuous participation in meetings of Aberdeen hplc users group throughout this research program.

6. Continuous personal review of the current literature.

Appendix II

Computer Program for Simulated Kinetics

```
SIMULATED-KINETICS.B20
THURSDAY, JUNE 6, 1985 15:04:19

00010 PRINT "THIS PROGRAM IS CAPABLE OF SIMULATING TIME-CONC."
00020 PRINT "DATA FOLLOWED BY LEAST SQUARE ANALYSIS OF THE   "
00030 PRINT "SIMULATED DATA."
00040 PRINT
00050 PRINT "THE FOLLOWING DATA IS REQUIRED AS INPUT DATA     "
00060 PRINT "(1) THE RATE CONSTANT"
00070 PRINT "(2) LENGTH OF EXPERIMENT"
00080 PRINT "(3) NO. OF DATA POINTS TO BE GENERATED"
00090 PRINT "(4) THE INITIAL CONCENTRATION(S)"
00100 PRINT "(5) THE LEVEL OF DEVIATION (RSD%) TO BE INCORPORATED"
00110 PRINT "    INTO EACH POINT AS EXPERIMENTAL ERROR"
00120 PRINT
00130 PRINT "THE PROGRAM IS CAPABLE OF SIMULATING THE FOLLOWING REACTIONS"
00140 PRINT
00150 PRINT "(1) A ----------> B      SIMPLE 1:1"
00160 PRINT
00170 PRINT "(2) A ----------> B               "
00180 PRINT "      ----------> C      PARALLEL  "
00190 PRINT "      ----------> D               "
00200 PRINT
00210 PRINT "(3) A ----------> B ----------> C    CONSECUTIVE "
00220 PRINT
00230 PRINT "(4) A <==========> B     REVERSIBLE "
00240 PRINT
00250 PRINT "TO SELECT THE DESIRED REACTION ENTER THE NUMBER SHOWN"
00260 INPUT Y
00270 IF Y= 1 THEN GOTO 540
00280 IF Y= 2 THEN GOTO 1260
00290 IF Y= 3 THEN GOTO 2450
00300 IF Y= 4 THEN GOTO 3400
00310 DIM A(500)
00320 DIM A1(500)
00330 DIM A2(500)
00340 DIM A4(500)
00350 DIM A3(500)
00360 DIM B(500)
00370 DIM C(500)
00380 DIM C1(500)
00390 DIM D(500)
00400 DIM E(500)
00410 DIM F(500)
00420 DIM S(500)
00430 DIM T(500)
```

```
00440 DIM Z(500)
00450 DIM D1(500)
00460 DIM D2(500)
00470 DIM D3(500)
00480 DIM D4(500)
00490 DIM A5(500)
00500 DIM A6(500)
00510 DIM A7(500)
00520 DIM A8(500)
00530 DIM A9(500)
00540 PRINT "SIMULATION FOR SIMPLE FIRST ORDER KINETICS"
00550 PRINT "           A ----------> B                "
00560 PRINT
00570 PRINT "ENTER THE RATE CONSTANT FOR THE REACTION"
00580 INPUT K
00590 K1=K
00600 PRINT "ENTER THE NUMBER OF DATA POINTS TO BE GENERATED"
00610 INPUT N
00620 L=N
00630 PRINT "ENTER THE ZERO TIME CONCENTRATION FOR THE REACTANT"
00640 PRINT "                 (MOLES/LITRE)                  "
00650 INPUT C0
00660 PRINT "ENTER THE DEVIATION (%) TO BE INCORPORATED (EACH POINT)"
00670 INPUT P
00680 PRINT "ENTER THE EXTENT OF DECOMPOSITION (%) TO BE STUDIED"
00690 INPUT EX
00700 GOSUB 4460
00710 FOR I=1 TO N
00720 T(1)=TR/N
00730 A(I)=C0*EXP(-K*T(I))
00740 T(I+1)=T(I)+TR/N
00750 Z(I)=A(I)
00760 NEXT I
00770 PRINT
00780 PRINT "REACTANT: ZERO ORDER"
00790 PRINT
00800 GOSUB 4570
00810 GOSUB 4700
00820 GOSUB 4780
00830 FOR I=1 TO N
00840 B(I)=LOG(C(I))
00850 Z(I)=B(I)
00860 C(I)=B(I)
00870 NEXT I
00880 PRINT
00890 PRINT "REACTANT: FIRST ORDER"
00900 PRINT
00910 GOSUB 4780
```

```
00920 FOR I=1 TO N
00930 D(I)=C0-(C0*EXP(-K*T(I)))
00940 Z(I)=D(I)
00950 NEXT I
00960 PRINT
00970 PRINT "PRODUCT: ZERO ORDER"
00980 PRINT
00990 GOSUB 4570
01000 GOSUB 4700
01010 GOSUB 4780
01020 FOR I=1 TO N
01030 E(I)=C0-C(I)
01040 C(I)=E(I)
01050 Z(I)=A(I)
01060 NEXT I
01070 PRINT
01080 PRINT "C0-[PRODUCT]=[REACTANT]: ZERO ORDER"
01090 PRINT
01100 GOSUB 4700
01110 GOSUB 4780
01120 FOR I=1 TO N
01130 F(I)=LOG(C(I))
01140 C(I)=F(I)
01150 Z(I)=F(I)
01160 NEXT I
01170 PRINT
01180 PRINT "C0-[PRODUCT]=[REACTANT]: FIRST ORDER"
01190 PRINT
01200 GOSUB 4780
01210
01220 PRINT "FOR ANOTHER RUN ENTER 1 ELSE ENTER 0"
01230 INPUT R1
01240 IF R1=1 THEN GOTO 680
01250 GOTO 5250
01260 PRINT "SIMULATION FOR FIRST ORDER PARALLEL KINETICS"
01270 PRINT
01280 PRINT " A----------> B"
01290 PRINT "  ----------> C"
01300 PRINT "  ----------> D"
01310 PRINT
01320 L=N
01330 PRINT "THE SIMULATION IS AUTOMATICALLY CARRIED OUT ON"
01340 PRINT "THE ASSUMPTION THAT THREE PRODUCTS ARE FORMED."
01350 PRINT "FOR TWO PRODUCTS ENTER 1 ELSE ENTER 0."
01360 INPUT P2
01370 PRINT
01380 PRINT "ENTER THE RATE CONSTANT FOR A -----> B"
01390 INPUT K2
```

```
Ø1400 PRINT "ENTER THE RATE CONSTANT FOR A -----> C"
Ø1410 INPUT K3
Ø1420 IF P2=1 THEN GOTO 1450
Ø1430 PRINT "ENTER THE RATE CONSTANT FOR A -----> D"
Ø1440 INPUT K4
Ø1450 K1=K2+K3+K4
Ø1460 PRINT "ENTER THE INITIAL CONCENTRATION OF A  "
Ø1470 INPUT CØ
Ø1480 PRINT "ENTER THE INITIAL CONCENTRATION OB B"
Ø1490 INPUT BØ
Ø1500 PRINT "ENTER THE INITIAL CONCENTRATION OF C"
Ø1510 INPUT FØ
Ø1520 IF P2=1 THEN GOTO 1550
Ø1530 PRINT "ENTER THE INITIAL CONCENTRATION OF D"
Ø1540 INPUT DØ
Ø1550 PRINT "ENTER THE NUMBER OF DATA POINTS TO BE GENERATED"
Ø1560 INPUT N
Ø1570 PRINT "ENTER THE DEVIATION (%) TO BE INCORPORATED(EACH POINT)"
Ø1580 INPUT P
Ø1590 PRINT "ENTER THE EXTENT OF DECOMPOSITION (%) TO BE STUDIED"
Ø1600 INPUT EX
Ø1610 GOSUB 4460
Ø1620 FOR I=1 TO N
Ø1630 T(1)=TR/N
Ø1640 A(I)=CØ*EXP(-K1*T(I))
Ø1650 Z(I)=A(I)
Ø1660 T(I+1)=T(I)+TR/N
Ø1670 NEXT I
Ø1680 PRINT
Ø1690 PRINT "REACTANT: ZERO ORDER"
Ø1700 PRINT
Ø1710 GOSUB 4570
Ø1720 GOSUB 4700
Ø1730 PRINT
Ø1740 GOSUB 4780
Ø1750 FOR I=1 TO N
Ø1760 C(I)=LOG(C(I))
Ø1770 NEXT I
Ø1780 PRINT
Ø1790 PRINT "REACTANT: FIRST ORDER"
Ø1800 GOSUB 4780
Ø1810 FOR I=1 TO N
Ø1820 B(I)=BØ+(K2/K1*CØ*(1-EXP(-K1*T(I))))
Ø1830 F(I)=FØ+((K3/K1)*CØ*(1-EXP(-K1*T(I))))
Ø1840 D(I)=DØ+((K4/K1)*CØ*(1-EXP(-K1*T(I))))
Ø1850 Z(I)=B(I)
Ø1860 NEXT I
Ø1870 PRINT
```

```
01880 PRINT "PRODUCT B: ZERO ORDER"
01890 PRINT
01900 GOSUB 4570
01910 GOSUB 4700
01920 PRINT
01930 GOSUB 4780
01940 FOR I=1 TO N
01950 A1(I)=C(I)
01960 Z(I)=0.0000
01970 NEXT I
01980 FOR I=1 TO N
01990 Z(I)=F(I)
02000 NEXT I
02010 PRINT
02020 PRINT "PRODUCT C: ZERO ORDER"
02030 PRINT
02040 GOSUB 4570
02050 GOSUB 4700
02060 PRINT
02070 GOSUB 4780
02080 FOR I=1 TO N
02090 A2(I)=C(I)
02100 Z(I)=D(I)
02110 NEXT I
02120 PRINT
02130 IF P2=1 THEN GOTO 2230
02140 PRINT "PRODUCT D: ZERO ORDER"
02150 PRINT
02160 GOSUB 4570
02170 GOSUB 4700
02180 PRINT
02190 FOR I=1 TO N
02200 A3(I)=C(I)
02210 NEXT I
02220 GOSUB 4780
02230 PRINT
02240 FOR I=1 TO N
02250
02260 A4(I)=A1(I)+A2(I)+A3(I)
02270 C(I)=C0-A4(I)
02280 NEXT I
02290 PRINT
02300 PRINT "C0-[TOTAL PRODUCT]=[REACTANT]: ZERO ORDER"
02310 PRINT
02320 GOSUB 4780
02330 PRINT
02340 FOR I=1 TO N
02350 C(I)=LOG(C(I))
```

```
02360 NEXT I
02370 PRINT "C0-[TOTAL PRODUCT]=[REACTANT]: FIRST ORDER"
02380 GOSUB 4780
02390 PRINT
02400 PRINT
02410 PRINT "FOR ANOTHER RUN ENTER 1 ELSE ENTER 0"
02420 INPUT R2
02430 IF R2=1 THEN GOTO 1590
02440 GOTO 5250
02450 PRINT "SIMULATION FOR FIRST ORDER CONSECUTIVE KINETICS"
02460 PRINT
02470 PRINT "A ---------> B ----------> C"
02480 PRINT
02490 L=N
02500 PRINT "ENTER THE RATE CONSTANT FOR A -----> B"
02510 INPUT K1
02520 PRINT "ENTER THE RATE CONSTANT FOR B -----> C"
02530 INPUT K2
02540 PRINT "ENTER THE INITIAL CONCENTRATION OF A"
02550 INPUT C0
02560 PRINT "ENTER THE NUMBER OF DATA POINTS TO BE GENERATED"
02570 INPUT N
02580 PRINT "ENTER THE DEVIATION (%) TO BE INCORPORATED(EACH POINT)"
02590 INPUT P
02600 PRINT "ENTER THE EXTENT OF DECOMPOSITION (%) TO BE STUDIED"
02610 INPUT EX
02620 GOSUB 4460
02630 FOR I=1 TO N
02640 T(1)=TR/N
02650 A(I)=C0*EXP(-K1*T(I))
02660 Z(I)=A(I)
02670 T(I+1)=T(I)+TR/N
02680 NEXT I
02690 PRINT
02700 PRINT "REACTANT: ZERO ORDER"
02710 PRINT
02720 GOSUB 4570
02730 GOSUB 4700
02740 PRINT
02750 PRINT
02760 GOSUB 4780
02770 FOR I=1 TO N
02780 C(I)=LOG(C(I))
02790 NEXT I
02800 PRINT
02810 PRINT "REACTANT: FIRST ORDER"
02820 PRINT
02830 GOSUB 4780
```

```
02840 FOR I=1 TO N
02850 B(I)=C0*(K1/(K2-K1))*((EXP(-K1*T(I)))-(EXP(-K2*T(I))))
02860 D1(I)=K2*EXP(-K1*T(I))
02870 D2(I)=K1*EXP(-K2*T(I))
02880 D3(I)=(D1(I)-D2(I))/(K1-K2)
02890 D4(I)=1+D3(I)
02900 D(I)=C0-(A(I)+B(I))
02910 Z(I)=B(I)
02920 NEXT I
02930 PRINT
02940 PRINT "PRODUCT B: ZERO ORDER"
02950 PRINT
02960 GOSUB 4570
02970 GOSUB 4700
02980 PRINT
02990 PRINT
03000 GOSUB 4780
03010 FOR I=1 TO N
03020 A1(I)=C(I)
03030 Z(I)=D(I)
03040 NEXT I
03050 PRINT
03060 PRINT "PRODUCT C: ZERO ORDER"
03070 PRINT
03080 GOSUB 4570
03090 GOSUB 4700
03100 PRINT
03110 FOR I=1 TO N
03120 A2(I)=C(I)
03130 NEXT I
03140 PRINT
03150 GOSUB 4780
03160 PRINT
03170 K5=K2/K1
03180 PRINT "THE MAXIMUM CONC OF B IS ";K5^(K5/(1-K5))
03190 PRINT
03200 PRINT "TIME TO MAXIMUM CONC OF B IS ";(1/(K2-K1))*(LOG(K2/K1))
03210 PRINT
03220 PRINT
03230 PRINT "C0-[TOTAL PRODUCT]=[REACTANT]: ZERO ORDER"
03240 PRINT
03250 FOR I=1 TO N
03260 C(I)=C0-(A1(I)+A2(I))
03270 NEXT I
03280 GOSUB 4780
03290 PRINT
03300 FOR I=1 TO N
03310 C(I)=LOG(C(I))
```

```
03320 NEXT I
03330 PRINT "C0-[TOTAL PRODUCT]=[REACTANT]: FIRST ORDER"
03340 GOSUB 4780
03350 PRINT
03360 PRINT "FOR ANOTHER RUN ENTER 1 ELSE ENTER 0"
03370 INPUT R3
03380 IF R3=1 THEN GOTO 2600
03390 GOTO 5250
03400 PRINT "SIMULATION FOR FIRST ORDER REVERSIBLE KINETICS"
03410 PRINT
03420 PRINT " A <==========> B "
03430 PRINT
03440 L=N
03450 PRINT "ENTER THE RATE CONSTANT FOR A -----> B"
03460 INPUT K1
03470 PRINT "ENTER THE RATE CONSTANT FOR B -----> A"
03480 INPUT K2
03490 PRINT "ENTER THE NUMBER OF POINTS TO BE GENERATED"
03500 INPUT N
03510 PRINT "ENTER THE ZERO TIME CONCENTRATION FOR THE REACTANT"
03520 INPUT C0
03530 PRINT "ENTER THE DEVIATION (%) TO BE INCORPORATED (EACH POINT)"
03540 INPUT P
03550 PRINT "ENTER THE EXTENT OF DECOMPOSITION (%) TO BE STUDIED"
03560 INPUT EX
03570 GOSUB 4510
03580 EK=K1/K2
03590 K=K1+K2
03600 FOR I=1 TO N
03610 T(1)=TR/N
03620 A(I)=((K2/K)*C0)+((K1/K)*C0*EXP(-K*T(I)))
03630 Z(I)=A(I)
03640 T(I+1)=T(I)+TR/N
03650 NEXT I
03660 GOSUB 4570
03670 AEK=(K2/K)*C0
03680 FOR I=1 TO N
03690 A1(I)=C(I)
03700 NEXT I
03710 A3=(C0-AEK)
03720 FOR I=1 TO N
03730 A4(I)=(A1(I)-AEK)
03740 A2(I)=A3/A4(I)
03750 C(I)=LOG(A2(I))
03760 NEXT I
03770 PRINT
03780 PRINT "REVERSIBLE REACTION FIRST ORDER"
03790 PRINT
```

```
03800 PRINT "TIME","CALC. REACT.","EXPT. REACT.","GRAPHICAL"
03810 PRINT "(MIN)","    CONC    ","    CONC    ","  VALUE  "
03820 PRINT
03830 FOR I=1 TO N
03840 PRINT T(I),A(I),A1(I),C(I)
03850 NEXT I
03860 PRINT
03870 GOSUB 4780
03880 PRINT
03890 RR=SL/(EK+1)
03900 FR=SL+RR
03910 PRINT "THE RATE OF REVERSE REACTION IS = ";RR
03920 PRINT "THE RATE OF FORWARD REACTION IS = ";FR
03930 PRINT "THE EQUILIBRIUM CONSTANT IS     = ";EK
03940 PRINT "THE CONCENTRATION OF A AT EQUILIBRIUM = ";AEK
03950 TAE=(LOG(C0/AEK))/K1
03960 CAE=C0*EXP(-K1*TAE)
03970 EXA=100-((CAE/C0)*100)
03980 PRINT "EQUILIBRIUM IS ATTAINED AT"; EXA; "% DECOMPOSITION"
03990 PRINT
04000 FOR I=1 TO N
04010 C(I)=LOG(A1(I))
04020 NEXT I
04030 PRINT "REVERSIBLE FIRST ORDER TREATED AS SIMPLE FIRST ORDER"
04040 GOSUB 4780
04050 PRINT
04060 FOR I=1 TO N
04070 D(I)=C0-A(I)
04080 Z(I)=D(I)
04090 NEXT I
04100 GOSUB 4570
04110 PRINT "PRODUCT: ZERO ORDER"
04120 PRINT
04130 GOSUB 4700
04140 GOSUB 4780
04150 PRINT
04160 RRP=(SL/C0)/EK
04170 PRINT "THE RATE OF REVERSE REACTION IS = ";RRP
04180 PRINT "THE RATE OF FORWARD REACTION IS = ";(SL/C0)
04190 PRINT "THE INITIAL REACTANT CONC IS    = ";C0
04200 PRINT
04210 FOR I=1 TO N
04220 A6(I)=C(I)
04230 A5(I)=(C0-C(I))-AEK
04240 A7(I)=A3/A5(I)
04250 A8(I)=LOG(A7(I))
04260 C(I)=A8(I)
04270 NEXT I
```

```
04280 PRINT
04290 PRINT "C0-[PRODUCT]=[REACTANT]: REVERSIBLE FIRST ORDER"
04300 GOSUB 4780
04310 PRINT
04320 PRINT "THE RATE OF REVERSE REACTION IS = ";(SL/(EK+1))
04330 PRINT "THE RATE OF FORWARD REACTION IS = ";SL-(SL/(EK+1))
04340 PRINT
04350 PRINT "C0-[PRODUCT]=[REACTANT]: SIMPLE FIRST ORDER"
04360 FOR I=1 TO N
04370 C(I)=C0-A6(I)
04380 C(I)=LOG(C(I))
04390 NEXT I
04400 GOSUB 4780
04410 PRINT
04420 PRINT "FOR ANOTHER RUN ENTER 1 ELSE 0"
04430 INPUT R5
04440 IF R5=1 THEN GOTO 3550
04450 GOTO 5250
04460 XE=(100-EX)
04470 X=100/XE
04480 XL=LOG(X)
04490 TR=XL/K1
04500 RETURN
04510 XE=100-EX
04520 XE1=(XE*C0)/100
04530 AEK=(K2/(K1+K2))*C0
04540 B1=LOG((C0-AEK)/(XE1-AEK))
04550 TR=B1/(K1+K2)
04560 RETURN
04570 REM RANDOMIZATION/TABULATION/REGRESSION.
04580 RANDOM
04590 FOR I=1 TO N
04600 V=0.0000
04610 S21=0.0000
04620 FOR J=1 TO 6
04630 S21=S21+RND-RND
04640 NEXT J
04650 S(I)=S21
04660 P1=P*Z(N)/100
04670 C(I)=S21*((P*Z(I))/100)+Z(I)
04680 NEXT I
04690 RETURN
04700 GOTO 4710
04710 PRINT "TIME","CALCULATED","EXPERIMENTAL"
04720 PRINT "(MIN)","    CONC    ","    CONC    "
```

```
04730 PRINT
04740 FOR I=1 TO N
04750 PRINT T(I),Z(I),C(I)
04760 NEXT I
04770 RETURN
04780 REM REGRESSION ANALYSIS START**********
04790 PRINT
04800 L=N
04810 S1=0.0000
04820 S2=0.0000
04830 S3=0.0000
04840 S4=0.0000
04850 S5=0.0000
04860 S6=0.0000
04870 FOR I=1 TO L
04880 S1=S1+T(I)
04890 S2=S2+C(I)
04900 NEXT I
04910 T1=S1/L
04920 C1=S2/L
04930 FOR I=1 TO L
04940 S3=S3+(T(I)-T1)*(T(I)-T1)
04950 S4=S4+(C(I)-C1)*(C(I)-C1)
04960 S5=S5+(T(I)-T1)*(C(I)-C1)
04970 NEXT I
04980 PRINT
04990 REM CALCULATION OF REQUIRED PARAMETERS**********
05000 SL=S5/S3
05010 IN=C1-SL*T1
05020 SYX=(S4-(SL*S5))/(L-2)
05030 S6=SQR(S3)
05040 ESE=SYX/S6
05050 STD=((S4-SL*SL*S3)/(L-2)/S3)
05060 SD=SQR(STD)
05070 RSTD=(SD/SL)*100
05080 CC=(S5*S5)/(S3*S4)
05090 FOR I=1 TO L
05100 E(I)=C(I)-(IN)-(SL*T(I))
05110 NEXT I
05120 PRINT
05130 REM REGRESSION ANALYSIS COMPLTET**********
05140 PRINT
05150 REM RESULTS**********
05160 PRINT,," RESULTS "
05170 PRINT,," ------- "
05180 PRINT
05190 PRINT "SLOPE= ";SL,,"INTERCEPT= ";IN
05200 PRINT "STANDARD DEVIATION= ";SD
05210 PRINT "RELATIVE STANDARD DEVIATION= ";RSTD
05220 PRINT "CORRELATION COEFFICIENT= ";CC
05230 PRINT
05240 RETURN
05250 END
```

APPENDIX III

Communications and Publications

1. R. B. Taylor, D. G. Durham, and A. S. H. Shivji

   Comparison of Product and Reactant Concentration Measurement in Stability Investigations. J.Pharm.Pharmacol., 35,101P(1983)

2. R. B. Taylor, A. S. H. Shivji, and D. G. Durham

   Presented as an open paper, entitled Decomposition of 5-Hydroxymethylfural in Aqueous Solution for which A. S. H. Shivji was presented first prize, at the meeting of the Scottish Pharmaceutical Sciences Group, March 1985.

3. R. B. Taylor, D. G. Durham, and A. S. H. Shivji

   A Kinetic Study of Tetracycline Decomposition in Acid Solution Int. J. Pharm., 26,259(1985)

4. R. B. Taylor, D. G. Durham, A. S. H. Shivji and R. Reid

   Development of a Stability Indicating Assay for Nafimidone, 1-(2-Naphthoylmethyl)imidazole hydrochloride, by hplc presented as a poster at the hplc Conference, Edinburgh July 1985 and accepted for publication in the Journal of Chromatography as a paper of the same title. In print.