The application of kinetic methods in pharmaceutical analysis

M. Ye. Blazheevskiy

National University of pharmacy, 61168, Bluher Street, 4, Kharkov, Ukraine, e-mail: Blazejowski@ukr.net

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The articles are concerning to the application kinetic methods for the determination of drugs and biologically active substances is reviewed. Advantages and limitations using kinetic methods in the pharmaceutical analysis are shown.

БЛАЖЕЄВСЬКИЙ М.Є. ЗАСТОСУВАННЯ КІНЕТИЧНИХ МЕТОДІВ У ФАРМАЦЕВТИЧНОМУ АНАЛІЗІ - Оглянуто праці, які стосуються застосування кінетичних методів аналізу для визначення лікарських та біологічно активних речовин. Показані переваги та обмеження використання кінетичних методів у фармацевтичному аналізі.

Ключові слова: метод кінетики, визначення ліків, фармацевтичний аналіз
Keywords: kinetic method, determination of drug, pharmaceutical analysis

INTRODUCTION

Kinetic methods became of great interest in chemical and pharmaceutical analyses [1]. Automatic kinetic methods are powerful tools for drug analysis as they use modern instrumentation and computers, which are essential for shortening analysis times and enhancing the quality of routine analyses. This paper reviews novel kinetic approaches to the determination of various types of drugs in pharmaceutical materials including chemiluminescent analysis: chemiluminescent analysis with lucigenin (bis-N-methylacridinium nitrate (NMA), 9-cyano-10-methylacridinium (9-CMA); flow-injection analysis (FIA), photo-induced FIA, FIA with electrogenerated reagent, sequential-injection analysis (SIA) and hybrid (FIA/SIA) method, gas-diffusion FIA and the use of micellar catalysis are also discussed.

The literature is still poor in analytical procedure based on kinetics, especially for determination of drug in commercial dosage forms [2]. Experimental and theoretical foundation of the kinetic method analysis was developed by K. B. Yacimirski. [3].

USE OF OXIDATION REACTIONS

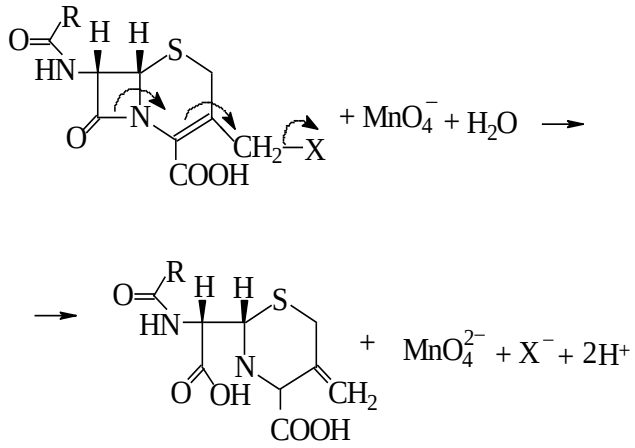
Potassium permanganate as strong oxidizing agent has been used in oxidimetric analytical method for determination of many compounds [4–13]. During the course of the reaction, the valence of manganese changes. The heptavalent manganese ion changes to the green color (Mn VI), while in neutral and acidic medium, the permanganate is further reduced to colorless (Mn II). The behavior of permanganate was the basis for its uses in development of kinetic spectrophotometric method. The absorption spectrum of aqueous potassium permanganate solution in alkaline medium exhibited an absorption band at 530 nm. The additions of any of the studied drugs to this solution produce a new characteristic band at 600–625 nm. This band is due to formation of manganate ion, which resulted from the oxidation of drug by potassium permanganate in alkaline medium. The intensity of the color increases with time; therefore a kinetically based method was developed for determination of drug in their pharmaceutical dosage formulations. The different variables that affect the formation of manganate ion were studied and optimized.

For example, a sensitive kinetic spectrophotometric method for determination of norfloxacin in commercial dosage forms was described, based on the oxidation of norfloxacin with alkaline potassium permanganate. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 603 nm. The initial rate and fixed time (at 3 min) methods are utilized for constructing the calibration graphs to determine the concentration of the drug. The calibration graphs are linear in the concentration ranges 2,0–20 μg/ ml and 1,0–20 μg/ ml using the initial rate and fixed time methods, respectively. The results are validated statistically and through recovery studies. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision [4].

A simple and sensitive kinetic method for the determination of oxamniquine in pharmaceutical preparations and biological fluids was developed. The procedure is based upon a kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time of 20 min. The absorbance of the colored manganate ions was measured at 610 nm. Alternatively, the decrease in the absorbance of potassium permanganate after addition of the drug was measured at 525 nm. The absorbance concentration plots in both procedures were rectilinear over the range 0,5–4 μg/ml. The concentration of oxamniquine is calculated using the corresponding calibration equation for the fixed-

time method. The determination of oxamniquine by fixed-concentration and rate-constant methods was feasible with the calibration equations obtained but the fixed time method had been found to be more applicable. Both procedures were applied to the determination of oxamniquine in formulations. The results obtained were in good agreement with those obtained using the official method. The fixed time method of 20 min was further applied to spiked human urine and plasma, the recoveries (%) were 100,94±0,57 and 98,07±0,88 for urine and plasma, respectively, at 610 nm, and 97,51±1,27 and 95,69±1,23 for urine and plasma, respectively, at 525 nm [5].

A simple, reliable, and sensitive kinetic spectrophotometric method was developed for determination of eight cephalosporin antibiotics, namely, Cefotaxime sodium, Cephapirin sodium, Cephradine dihydrate, Cephalexin monohydrate, Ceftazidime pentahydrate, Cefazoline sodium, Ceftriaxone sodium, and Cefuroxime sodium. The method depends on oxidation of each of studied drugs with alkaline potassium permanganate (Scheme 1). The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 610 nm. This band is due to formation of manganate ion, which resulted from the oxidation of cephalosporin by potassium permanganate in alkaline medium. The initial rate and fixed time (at 3 minutes) methods are utilized for construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs are linear in the concentration ranges 5–15 μg/ml and 5–25 μg/ml using the initial rate and fixed time methods, respectively. The results are validated statistically and checked through recovery studies. Statistical comparisons of the results with the reference methods show the excellent agreement and indicate no significant difference in accuracy and precision. The initial rate and fixed time methods can be easily applied for determination of investigated cephalosporins in pure and dosage forms that do not require elaborate treatment and tedious extraction of chromophore produced. The proposed method (initial rate or fixed time) is sensitive enough to enable determination of lower amounts of drug, these advantages encourage the application of proposed method in routine quality control of investigated cephalosporins in industrial laboratories. Finally the developed method provides advantages of improving selectivity, avoiding interference of colored and/or turbidity background of samples because it measures the increase in absorbencies with time against blank treated similarly [6].



Scheme 1. Mechanism oxidation of Cephalosporins is proposed on the basis of the literature.

Two simple and sensitive kinetic methods were developed for the determination of ribavirin in bulk and in its pharmaceutical preparations using alkaline potassium permanganate as an oxidizing agent. The methods are based upon a kinetic investigation of the oxidation reaction of the drug at room temperature for fixed times of 20 and 30 minutes. In the first method, the absorbance of the colored manganate ion was measured at 610 nm, while in second method the reduction in the absorbance of permanganate was measured at 525 nm. The absorbance concentration plots were linear over the range of 3–15 μg/ml with detection limits of 0,028 μg/ml in the first method and 0,229 μg/ml for the second method. The proposed methods were applied successfully for the determination of the drug in its pharmaceutical formulations, the percentage recoveries were 100,15±1,34, 100,06±0.86 in the first method, and 99,60±0,54, 100,43±0,82 in the second method. The results obtained were compared statistically with those obtained by the official method and showed no significant differences regarding accuracy and precision [7].

Two a sensitive kinetic methods for the determination of dothiepin hydrochloride are described. The first method is based on kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time of 25 min. The absorbance of the colored manganate ions is measured at 610 nm. The second method is based on the reaction of dothiepin hydrochloride with 4-chloro-7-nitrobenzofurazan in the presence of 0,1 mol/l sodium bicarbonate. Spectrophotometric measurement was achieved by recording the absorbance at 470 nm for a fixed time of 60 min. All variables affecting the development of the color were investigated and the conditions were optimized. Plots of absorbance against concentration in both procedures were rectilinear over the ranges 4–24 and 50–250 μg/ml, with mean

recoveries recoveries 99,33±0,42 and 99,88±0,53, respectively. The proposed methods were successfully applied for the determination of dothiepin hydrochloride in bulk powder and in capsule dosage form. The results obtained were found to agree statistically with those given by the non-aqueous B.P. method. Furthermore the methods were validated according to USP guidelines and also assessed by applying the standard addition technique. The determination of dothiepin hydrochloride by the fixed concentration method is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable [8].

A new simple and sensitive kinetic spectrophotometric method is described for analysis of nizatidine and ranitidine. The method involves the reaction of the drugs with alkaline potassium permanganate, whereby a green color peaking at 610 nm is produced. The reaction is monitored spectrophotometrically by measuring the rate of change of absorbance of the resulting manganate species at 610 nm. Calibration graphs are linear over the concentration range 0,8–4,0 µg/ml and the precision (% RSD 1,80, 1,53 for nizatidine and ranitidine, respectively) is quite acceptable. The method is satisfactorily applied for direct analysis of pharmaceutical preparations containing nizatidine and ranitidine. A proposal of the reaction pathway is postulated [9].

A new sensitive kinetic spectrophotometric method for the determination of silymarin in pure form and in pharmaceutical formulations is described. The method is based on the oxidation of the drug with potassium permanganate at pH 7,0. The reaction is followed spectrophotometrically by measuring the decrease in the absorbance at 530 nm. The calibration graph is linear in the range of 18–50 µg/ml. The method has been successfully applied to the determination of silymarin in pharmaceutical formulations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision [10].

Four simple and sensitive kinetic spectrophotometric methods (I–IV) for the determination of trimetazidine dihydrochloride have been developed. Method I was based on the oxidation of the drug with alkaline KMnO4 producing green manganate species. Method II was based on the formation of colored condensation product between trimetazidine dihydrochloride and 4-chloro-7-nitrobenzofurazan. Method III wasbased on reaction of trimetazidine dihydrochloride and with 1,2-naphthoquinone-4-sulphonic acid sodium salt forming orange colored product. Method IV was based on the formation of a violet charge-transfer complex between trimetazidine base and p-chloranil. These reactions were followed spectrophotometrically by measuring the rate of color development at 610, 475, 485 and 560 nm for the reactions with KMnO4, 4-chloro-7-nitrobenzofurazan, 1,2-naphthoquinone-4-sulphonic acid sodium salt, and p-chloranil, respectively. The variables affecting the reactions were carefully investigated and the conditions were optimized. The stoichiometries of the reactions were determined, and the reactions pathways were postulated. The initial rate and fixed time methods were utilized for constructing the calibration graphs for the determination of trimetazidine dihydrochloride concentration. The assays limits of detection were 0,2–2,5 mg/l. The analytical performance of the methods, in terms of accuracy and precision, were statistically validated; the results were satisfactory. The methods have been successfully applied to the determination of trimetazidine dihydrochloride in commercial pharmaceutical formulations. Statistical comparison of the results with the reference method showed excellent agreement and proved that no significant difference in the accuracy and precision [11].

A simple and sensitive spectrophotometric kinetic method was developed for the determination of carbocisteine, penicillamine in bulk and in their pharmaceutical preparations using alkaline potassium permanganate as an oxidizing agent. A method involves determination of carbocisteine and penicillamine by kinetic studies of the oxidation reaction of these two drugs at room temperature for a fixed time of 20 minutes. The absorbance of the colored manganate ions was measured at 610 nm. The proposal pathway of the reaction is given as follow:

$$R\text{-}SH + 3MnO_4^- + 6\ OH^- \rightarrow R\text{-}SO_3H + 3MnO_4^{2-} + 3H_2O.$$

2-10 µg/ml of carbocisteine and penicillamine could be determined by the kinetic method with detection limits of 0,14 and 0,21 µg/ml respectively. A method was successfully applied for the determination of these drugs in their dosage forms. The proposed method was simple, accurate, precise, sensitive, rapid, low cost and relating selective compared to the official methods. Furthermore, the proposed method doesn't require elaboration of procedures, which are usually associated with chromatographic methods [12].

Two simple and sensitive kinetic methods for the determination of tramadol hydrochloride are described. The first method is based upon a kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time at 20 min. The absorbance of the colored manganate ions was measured at 610 nm. The second method is based on the reaction of tramadol hydrochloride with 4-chloro-7-nitrobenzofurazan in presence of 0,1 M sodium bicarbonate. The spectrophotometric measurements were recorded by measuring the absorbance at 467 nm, at fixed time at 25 min on thermostated water bath at 90±1 °C. All variables affecting the development of the colour have been investigated and the conditions were optimised. The absorbance concentration plots in both

methods were rectilinear over the range 5–25 and 50–250 μg/ml, for the first and second methods, respectively. The two methods have been applied successfully to commercial capsule and ampoule dosage form. The results obtained are compared statistically with those given by the reference spectrophotometric method. The determination of tramadol hydrochloride by the fixed concentration and rate constant methods is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable [13].

Two simple and accurate kinetic methods, the fixed time and the fixed concentration methods, for the determination of caffeine were described. The two methods involve the use of 3,20•10-3 M cerium(IV) solution and 8,0•10-2 M sulfuric acid. Reaction rates were followed at 405 nm; absorbance measurements for the fixed time method were taken at 350 s; and the following calibration equation was used for calculating unknown concentrations of caffeine: A = (2,31 – 3,55)•10-3 C. For the fixed concentration method, time was measured at a fixed absorbance of 1,70 and the following calibration equation was used: 1/t = -1,25•10-3+2,48•10-5 C. The two methods were applied to the determination of caffeine in proprietary drugs, interferences were studied, and a statistical comparison with the results obtained by the official BP method was made [14].

A procedure for the simultaneous kinetic spectrophotometric determination of cephalexin and trimethoprim was described. It was based on the different reaction rate of oxidation of these compounds with yellow ammonium cerous (IV) sulfate in acidic medium and colorless cerous (III) sulfate was produced. The overlapped kinetic data was quantitatively resolved by the use of chemometric methods, partial least squares, principal component regression and radial basis function-artificial neural network. The proposed method was also applied to the simultaneous determination of cephalexin and trimethoprim in pharmaceutical preparation and human urine with satisfied results, which compared well with those obtained by HPLC [15].

A simple and sensitive kinetic method has been developed for the determination of ethamsylate in its pharmaceutical preparations. The method is based upon oxidation of ethamsylate with 3-methyl-2-benzothiazolinone hydrazone hydrochloride in presence of cerium (IV) ammonium sulfate at room temperature for 20 min. The absorbance of the reaction product is measured at 514 nm. The absorbance-concentration plot was rectilinear over the range of 4-30 μg /ml (r = 0,999). The lower detection limit was 0,267 μg /ml (9,110•10-6 M) and the lower quantitation limit was 0,808 μg/ml. The different experimental parameters affecting the development and stability of the reaction product were studied and optimized. The proposed method was applied to the determination of ESL in formulations, and the results obtained were in good agreement with those obtained using a reference method. The proposed method was also used for the in vitro detection of ethamsylate in spiked human plasma at its therapeutic concentration level [16].

A procedure for the simultaneous kinetic spectrophotometric determination of aminocarb and carbaryl in vegetable and water samples was described. The method was based on the differential oxidation rate of aminocarb and carbaryl when they were reacted with the oxidant, potassium ferricyanide, in an appropriate alkaline medium. Both species were instantly oxidized, and resulted in a decrease of ferricyanide concentration. This anion has a maximum spectral absorbance at about 420 nm. Under the optimum experimental conditions, the linear ranges were 0,05–0,6 mg/l and 0,1–1,2 mg/l for aminocarb and carbaryl, respectively. The kinetic data collected were processed by chemometrics methods, such as classical least squares, partial least squares, principal components regression, back propagation-artificial neural network, radial basis function-artificial neural network, and principal component-radial basis function-artificial neural network. These methods were applied for the prediction of the two carbamate pesticides. The results showed that the partial least squares and principal component-radial basis function-artificial neural network calibration models gave the lowest prediction errors. The proposed method was successfully applied to the simultaneous determination of aminocarb and carbaryl in vegetable and water samples, and satisfactory results were obtained [17].

Two methods for the simultaneous determination of ascorbic acid and *L*-cysteine from kinetic spectrophotometric data are applied and compared. The fist is the two-rate method, and the second, the differential kinetic method. Present in an excess amount, iron(III) is quantitatively reduced by ascorbic acid or *L*-cysteine to iron(II) that, in turn, interacts with phenanthroline to form a colored product, $Fe(phen)_3^{2+}$ complex possessing a maximum absorbance at λ_{max} = 510 nm. The first method is based on the measurement of reaction rates at two points in the course of successive reactions, and the second method is based on the difference in the kinetics of the ascorbic acid and *L*-cysteine oxidation reactions at different pH values. Both oxidation reactions have been studied by ficting the kinetic curves (absorbance versus time) with suitable reaction rate equations. It is established that the *L*-cysteine + iron(III) reaction obeys a simple fist order kinetics, while the ascorbic acid + iron(III) is successive two-step process. Both ascorbic acid and *L*-cysteine are determined in the 0,5–5,0 ppm rage with satisfactory accuracy. The two methods under consideration have been successfully used for the analysis of model and real samples. The RSE values for the determination of *L*-cysteine and

ascorbic acid by the two-rate method and differential kinetic method were 5,15, 5,12, and 5,18, 11,98 % respectively [18].

A accurate, sensitive and economical procedure for the estimation of amlodipine besylate and nifedipine, both in pure and dosage forms, has been developed. The method is based on the reduction of iron(III) by the studies drugs and subsequent interaction of iron(II) with ferricyanide to form Prussian blue. The reaction develops through a slow kinetics and completes in about 10 min. Both initial slope and fixed time methods were used to derive calibration graphs. The resulted calibration equations were linear in the concentration ranges of 1,0-20,0 μg/ ml and 3,0-19,0 μg/ ml for amlodipine and nifedipine, and the detection limits were 0,10 μg/ ml and 0,19 μg/ ml, respectively. Seven replicate analyses of solution containing three different levels of each drug resulted in very low relative error of prediction (less than 1,6 %) and relative standart deviation (less than 4 %) confirming accuracy and precision of the proposed method. The proposed method was applied to the determination of these drugs in pharmaceutical formulations and excellent recoveries were obtained [19].

A simple and sensitive kinetic spectrophotometric method for determination of captopril has been developed. The method is based on the reduction of Fe(III) with captopril. Fe(II) then reacts with potassium ferricyanide, resulting in the formation of a blue product. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 730 nm. Thus, $1{,}23\cdot10^{-3}$ mol /l $FeCl_3$ and $3{,}04\cdot10^{-4}$ mol/l potassium ferricyanide were used as optimum values for maximum concentration of captopril in the calibration graph. The initial rate is utilized for constructing the calibration graph, which was found to be linear in the range from $4{,}60\cdot10^{-6}$ to $5{,}06\cdot10^{-5}$ mol/l; detection limit is $1{,}99\cdot10^{-7}$ mol /l. The proposed method has been validated; the mean recovery ranges from 99,8 to 101,4% with RSD<2%. Common excipients do not interfere with the determination. The point and interval hypotheses tests have been performed and confirmed that there is no significant difference between the proposed method and the conventional spectrophotometric method. The experimental true bias of all samples is lower than ±2%. The proposed method has been applied to the determination of captopril in bulk and dosage forms [20].

Mixtures of food antioxidants, butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate, were simultaneously analyzed with spectrophotometry, based on their different kinetic properties. These antioxidants react differentially with Fe(III), and the reduced product of which, Fe(II), will be complexed by chromogenic reagent 2,2′-dipyridyl. The differential kinetic spectra were monitored and recorded at 510 nm, and the data obtained from the experiments were processed by chemometric approaches, such as artificial neural network, classical least squares, principal component regression and partial least squares. A set of synthetic mixtures of antioxidants was evaluated and the results obtained by the applications of these chemometric approaches were discussed and compared. It was found that the artificial neural network method afforded better precision relatively than those of classical least squares, principal component regression and partial least squares. The proposed method was also applied satisfactorily to the determination of antioxidants in several commercial food products [21].

The oxidation of *p*-phenetidine with sodium vanadate was proposed for determination of phenothiazine derivatives (aminazine, diprazine, promazine, meterazine, tisercine, triphtazine) by kinetic method. The reaction rate was studied depending on the solution acidity and reagent concentrations, and the optimum determination conditions were selected. The analytical range was 3–100 ng/ml for six phenothiazine alkyl derivatives. The detection limits (3–10 ng/ml) depend on the substituent in the phenothiazine molecule. The relative standard deviations were from 3-20 % [22].

A simple, precise and accurate kinetic spectrophotometric method for determination of cefoperazone sodium, cefazolin sodium and ceftriaxone sodium in bulk and in pharmaceutical formulations has been developed. The method is based upon a kinetic investigation of the reaction of the drug with oxidized quercetin reagent at room temperature for a fixed time of 30 min. The decrease in absorbance after the addition of the drug was measured at 510 nm. The absorbance concentration plot was rectilinear over the range 80–400 μg/ml for all studied drugs. The concentration of the studied drugs was calculated using the corresponding calibration equation for the fixed time method. The determination of the studied drugs by initial rate, variable time and rate-constant methods was feasible with the calibration equations obtained but the fixed time method has been found to be more applicable. The analytical performance of the method, in terms of accuracy and precision, was statistically validated; the results were satisfactory. The method has been successfully applied to the determination of the studied drugs in commercial pharmaceutical formulations. Statistical comparison of the results with a well established reported method showed excellent agreement and proved that there is no significant difference in the accuracy and precision [23].

USE OF HYDROLYSIS REACTIONS

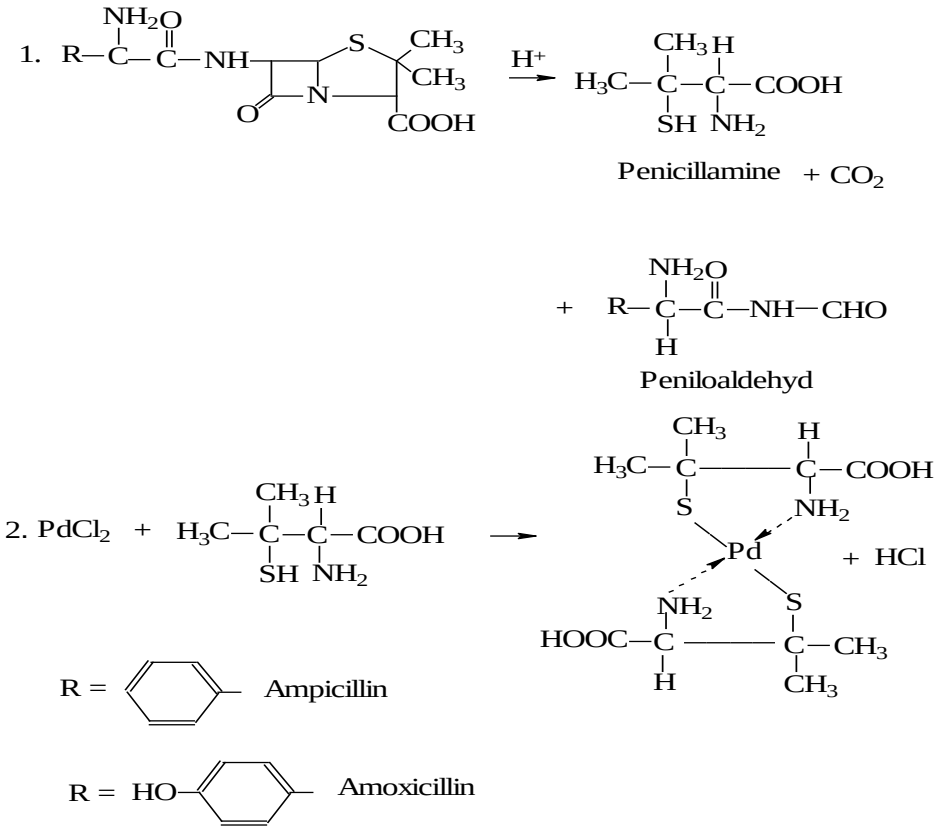
A kinetic spectrophotometric method has been developed for the determination of ampicillin and amoxicillin in commercial dosage forms. The method involves first hydrolysis of the antibiotics

with 1,0 mol/l HCl on a boling water bath for fixed time 1 h, neutralization with 1,0 mol/l NaOH followed by reaction with palladium(II) chloride in Britton-Robinson buffer of pH 6,0 (Scheme 2). The produced yellow complex of penicillamine with palladium(II)chloride is measured at 335 nm.

The proposed method is valid over the concentration range 8–40 μg/ml and 10–40 μg/ml for ampicillin and amoxicillin respectively with minimum detectability of 0,73 μg/ml and 0,76 μg/ml for ampicillin and amoxicillin respectively. The determination of the studied compounds adopting the fixed concentration method is feasible with the calibration equations obtained, but the fixed time method has been found to be more applicable. The proposed method was applied to commercial dosage forms and the results obtained were in good agreement with those given by USP method [24].

A kinetic method for the accurate determination of cephalexin has been described. A solution of cephalexin is reacted with $5 \cdot 10^{-3}$ mol/l cobalt (II) nitrate in $1 \cdot 10^{-3}$ mol/l sodium hydroxide at 60°C for a fixed time of 6 min, after which the absorbance of the reaction product is measured at 310 nm. The concentration of cephalexin is calculated by using the corresponding calibration equation for the fixed-time method. The method has been applied to proprietary drugs and the results were compared statistically with those given by the BP method. The determination of cephalexin by the fixed-concentration and rate-constant methods is feasible with the calibration equations obtained but the fixed-time method has been found to be more applicable [25].

An accurate, reliable, specific and sensitive kinetic spectrofluorimetric method was developed for the determination of seven cephalosporin antibiotics namely cefotaxime sodium, cephapirin sodium, cephradine dihydrate, cephalexin monohydrate, cefazoline sodium, ceftriaxone sodium and cefuroxime sodium.



Scheme 2. Proposal of the reaction pathway between the hydrolysed penicillins and palladium chloride

The method is based on their degradation under an alkaline condition producing fluorescent products. The factors affecting the degradation and the determination were studied and optimized.

The reaction is followed spectrofluorimetrically by measuring the rate of change of fluorescence intensity at specified emission wavelength. The initial rate and fixed time methods were used for the construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs are linear in the concentration ranges 0,2–1,2 μg/ml and 0,2–2,2 μg/mL using the initial rate and fixed time methods, respectively. The results were statistically validated and checked through recovery studies. The method has been successfully applied for the determination of the studied cephalosporins in commercial dosage forms. The high sensitivity of the proposed method allows the determination of investigated cephalosporins in human plasma. The statistical

comparisons of the results with the reference methods show an excellent agreement and indicate no significant difference in accuracy and precision [26].

A new kinetic spectrophotometric method for the determination of acetylsalicylic acid in pharmaceutical formulations was developed. In general, acetylsalicylic acid analysis is not realised directly, and a previous quantitative hydrolysis in a basic medium is necessary, converting acetylsalicylic acid to salicylate ions for its determination. The hydrolysis is carried out by sodium hydroxide solution. The method is based on a ligand-exchange reaction. The reaction was followed spectrophotometrically by monitoring the rate of disappearance of the cobalt (II)-1-nitroso-2-naphthol complex in alkaline medium at 410 nm. The optimum operating conditions regarding reagent concentrations and temperature were established. The initial-rate method is adopted for constructing the calibration curve, which was found to be linear over the concentration range 0,72–9,00 μg/ml. The optimized conditions yielded a theoretical detection limit of 0,35 μg/ml based on the $3{,}3s_o$ criterion. The interference effects of certain ingredients of powdery drugs, foreign ions and amino acids upon the reaction rate were studied in order to assess the selectivity of the method. The results are validated statistically and through recovery studies. The point hypothesis test have been performed which indicate that there is no significant difference between the proposed method and the reference method. The developed procedure was successfully applied to the rapid determination of acetylsalicylic acid in commercial pharmaceutical preparations and human control serum. The unique features of this procedure are that determination can be carried out at room temperature and analysis time is short. The newly developed method is simple, inexpensive and efficient for use in the analysis of a large number of samples [27].

A simple and accurate spectrophotometric kinetic method was developed for the determination of acetylsalicylic acid using strong acidic potassium permanganate as an oxidizing agent. The propose kinetic method doesn't need any preliminary separation procedure or conversion of acetylsalicylic acid to salicylic acid. The reaction was followed spectrophotometric at 520 nm, an appropriate wavelength where permanganate ion exhibits an absorption peak and its disappearance was recorded. The progress of reaction was monitored and kinetic data (absorbance vs. time) were collected and processed by the computer. Each sample was repeated for at least three times for the same set of experimental conditions (hydrogen ion concentration of 0,93 mol/l). Linear calibration graph is obtained representing the pseudo-firs-order rate constant against acetylsalicylic acid (ASA) concentration. The linear regression equation is

$$k_{obs} = (0{,}7 \pm 2{,}8) \cdot 10^{-6} + (4{,}11 \pm 0.06) \cdot 10^{-2} \cdot [\text{ASA}]$$

with r = 0,9989 and $s = 6 \cdot 10^{-5}$ s^{-1}.

The detection limit of the method was calculated as three times of standard deviation of the response (25 determinations) to a blank sample. The value found was 0,02 mg/L. The relative standard deviation was 0,5 % for seven samples containing $3{,}35 \cdot 10^{-2}$g/l ASA. The effect of some organic compounds and heavy metals associated with drugs was studied. The tolerance limit was defined as the concentration of added interference causing less than ±3 % relative error. Most cations and organic substance did not interfere even when present in 200-fold excess relative to vitamins. On the contrary, ascorbic acid, tiamine, pyridoxine, cysteine, and methionine interfere in the determination of ASA [28].

A kinetic method for acetylsalicylic acid determination based on its inhibitory effect upon the catalytic decomposition of hydrogen peroxide was developed. The catalytic reaction of catalase was investigated, by means of a Clark oxygen sensor, in the presence of various concentrations of ASA. Michaelis-Menten kinetic parameters were determined from Lineweaver-Burk plots, obtained in the absence and in the presence of the inhibitor. The inhibition pattern, suggested by the Line-weave-Burk plots, corresponds to a fully mixed inhibition mechanism. Calibration graphs of the reciprocal value of first-order rate costant versus ASA concentration covered the concentration range $(2{,}99–19{,}98) \cdot 10^{-4}$ mol/L, while the detection limit was $4{,}12 \cdot 10^{-5}$ mol/l ASA with a standard deviation of $2{,}1 \cdot 10^{-5}$ mol/l. The method was tested on commercial-available tablets and the recovery of ASA, by the kinetic method, was between 98,4% and 101,4% [29].

The possibility and expedience of using coupled of different types in catalytic methods of pharmauceutical analysis were shown taking as example a rections of alkaline hydrolysis of activated esters and *β*-lactam antibiotics catalized by hydrogen peroxide and/or peroxy acids (reactions of perhydrolysis: with excess hydrogen peroxide and/or or peroxy acids in an alkaline medium) [31-35].

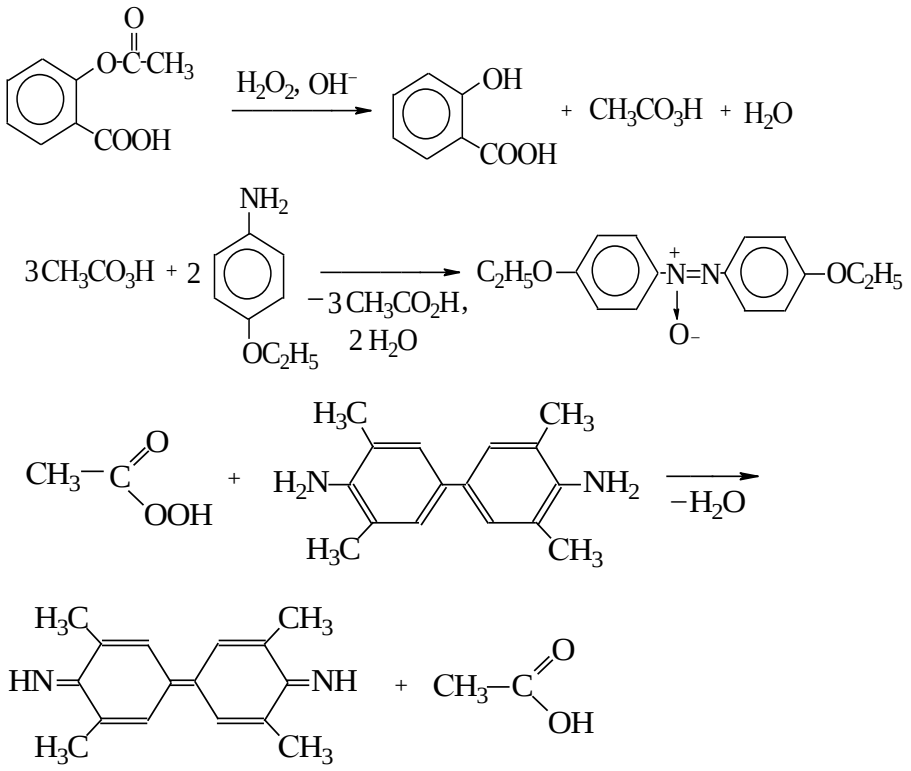
Specrophotometric kinetic methods for determination acetylsalicylic acid [36-37], acetylcholine [38], zopiklone [39-40], ditiline (succinylcholine iodide) [41] in pharmaceutical preparations which based on combination of perhydrolysis reaction with a oxidation reactions of *p*-phenetidine or 3,3',5,5'-tetramethylbenzidine as indicators were developed. Acetylsalicylic acid, acetylcholine, ditiline and zopiklone were hydrolized in the presence of hydrogen peroxide and the peroxy acid generated was detected using a tangent method or fixed time. The reaction

pathways of an alkaline perhydrolysis of acetylsalicylic acid and succinylcholine iodide and peroxyacid oxidation of *p*-phenetidine and 3,3',5,5'-tetramethylbenzidine were proposed (Scheme 3 and 4).

A new simple and sensitive kinetic spectrophotometric method has been developed for the determination of ampicillin and oxacillin in pure forms and in commercial dosage binar preparation «Ampiox» (ampicillin trihydrate 0,125 g and oxacillin potassium 0,125 g). The method involves first oxidation of the antibiotics by peroxy adipic acid for fixed time 1 min to respectively S-oxides followed by the alkaline hydrolysis reaction of *β*-lactam group of the antibiotics at room temperature. The reaction was monitored spectrophotometrically by measuring the increase in absorbance at 305 nm as a function of time. The tangent method was adopted for constructing the calibration curves. Both the calibration curves were linear in the concentration range of 1–50 μg/ ml. The combination of iodometric determination of the summary content penicillines by consumption peroxy acid with the kinetic determination of rapidly reacted component of the tested mixture allows to assay of the both penicillines in binary mixture. The limits of quantitation (LOQ) were 1,0 μg/ml and 2,0 μg/ml for ampicillin and oxacillin respectively. The proposed methods are validated statistically and through recovery studies The relative error of determination is ≤ 3,3 %. The results given by the proposed method are in good agreement with those given by the official UV spectrophotometric method [42].

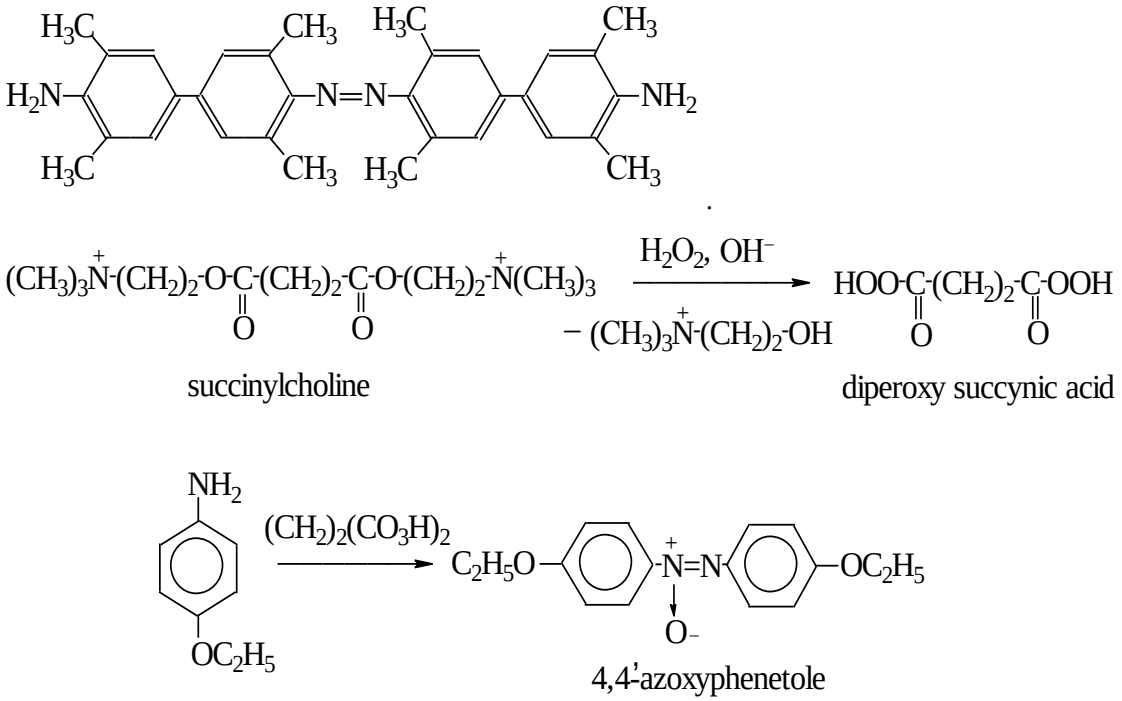
The peroxomonosulphate oxidative alkaline hydrolysis of *β*-lactam antibiotics can be used for their specrophotometric kinetic determination in rage concentration 1–40 μg /ml in drug. The detection limits were 0,3 μg/ml. The proposed methods are validated statistically and through recovery studies (RSD ≤ 2,09 %). The point and interval hypothesis tests have been performed confirming that there is no significant difference between the proposed methods and the reference method (δ= – 0,36%) [43-44].

The reaction pathway of oxidative alkaline hydrolysis (perhydrolysis) of penicillins in the present peroxomonosulphate on an example of ampicillin are described on Scheme 5.

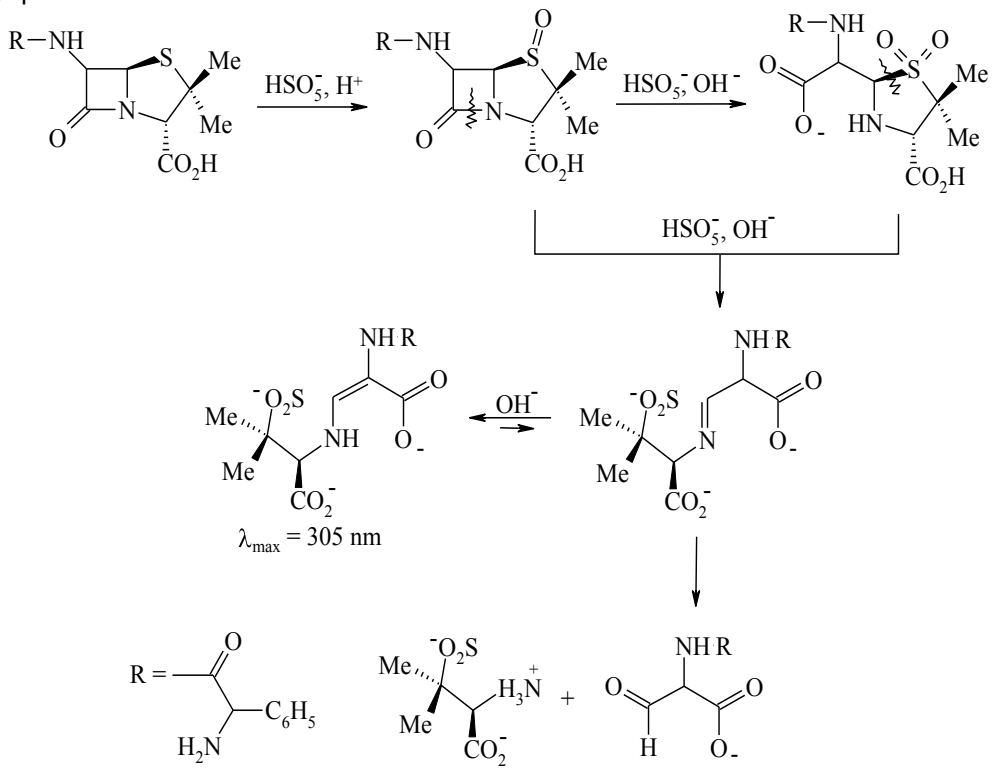


Scheme 3. The proposed reactions of an alkaline perhydrolysis of acetylsalicylic acid and peroxyacid oxidation of *p*-phenetidine and 3,3',5,5'-tetramethylbenzidine

Two molecules of 3,3',5,5'-tetramethyldiphenoquinondiimine are condensated with formation of yellow azodye – *bis*-(2,5,7,10-tetramethyl-6-amino) - azodiphenyl:



Scheme 4. The reaction pathways of alkaline perhydrolysis of succinylcholine iodide and peroxyacid oxidation of *p*-phenetidine



Scheme 5. The reaction pathway of peroxomonosulphate oxidative alkaline hydrolysis (perhydrolysis) of penicillins.

USE OF REACTIONS WITH BROMINE AND IODINE

A kinetic method, based on bromination reaction with bromine, was described for the assay of albendazole in bulk drug and in tablets. Proposed method depends on the linear relationship between the concentration of the drug (μg/ml) and time (s) for bromination, as indicated by bleaching of methyl orange acid colour. Kinetic method is applicable in the concentration range of 5 to 25 μg/ ml drug, and albendazole in bulk drug and in tablets can be determined with a fair degree of accuracy and precision. Tablet excipients do not interfere in either method. Recoveries of drug added to

commercial formulations were good. As indicated by *t*- and *F*-values, the methods are as accurate and precise as the reference method [45].

The objective of this research was to develop a kinetic spectrophotometric method for determination of ramipril in pure form and pharmaceutical formulations. The method was based on the reaction of carboxylic acid group of the drug with a mixture of potassium iodate and potassium iodide in aqueous medium at room temperature. The reaction is followed spectrophotometrically by measuring the increase in absorbance at 352 nm as a function of time. The initial-rate and fixed-time methods were adopted for constructing the calibration curves. Both the calibration curves were linear in the concentration range of 10,0–70,0 μg/ ml. The detection limits were 0,02 μg/ml and 0,15 μg/ml for initial rate and fixed time methods, respectively. The proposed methods are validated statistically and through recovery studies. The point and interval hypothesis tests have been performed confirming that there is no significant difference between the proposed methods and the reference method. The experimental true bias of all samples is less than ± 2%. The methods have been successfully applied to the determination of ramipril in tablets and capsules [46].

Al-Momani [47] described a flow-injection spectrophotometric method for determination of amoxcillin, cephalexin, ampicillin, and cephradine in pharmaceutical formulations based on the reaction of drug hydrolysis products with a iodine in acid medium. The reaction is followed spectrophotometrically by measuring the dicrease in absorbance at 460 nm as a function of time.

The implementation of a differential kinetic spectrophotometric method for the determination of angiotensin-converting-enzyme inhibitors in pharmaceutical formulations is described. The determination method was based on the monitoring (350 nm) of the reaction between captopril and iodate, in the presence of iodide, versus time and was fully automated by exploiting the multi-pumping flow concept. The developed multi-pumping flow system included four discretely actuated solenoid micro-pumps as unique flow manifold active components. The automatic control of the solenoid micro-pumps, under time-based and pulse-counting routines, allowed the implementation of a reliable and versatile analytical determination, with the additional advantage of permitting a runtime access to important analytical parameters, such as flow rate, sample insertion and reagent addition synchronisation, facilitating this way the establishment of an approach for kinetic measurements, directly due to the efficient solution handling and accurate timing control. A linear range of determination was verified for captopril concentrations between 10,0 and 60,0 μg /ml with a sample throughput of about 100 determinations per hour. The results were in agreement with those obtained by the reference procedure with relative deviations between 1,81 and 4,48% [48].

Novel selective and simple kinetic spectrophotometric method was developed and validated for the determination of norfloxacin in its pharmaceutical formulations. The method was based on the reaction of N-vinylpiprazine formed from the interaction of the mono-substituted piprazinyl group in NOR and acetaldehyde with 2,3,5,6-tetrachloro-1,4-benzoquinone to give colored N-vinylpiprazino-substituted benzoquinone derivative. The formation of the colored product was monitored spectrophotometrically by measuring the absorbance at 625 nm. The initial rate and fixed time (at 5 min) methods were utilized for constructing the calibration graphs. The graphs were linear in concentration ranges of 20–150 and 10–180 μg/ ml with limits of detection of 8,4 and 3,2 μg/ ml for the initial rate and fixed time methods, respectively. The analytical performance of both methods was fully validated, and the results were satisfactory. No interferences were observed from the excipients that are commonly present in the pharmaceutical formulations, as well as from tinidazole that is co-formulated with NOR in some of its formulations. The proposed methods were successfully applied to the determination of norfloxacin in its commercial pharmaceutical formulations. The label claim percentages were 98,4–100,4 ± 0,52–1,04%. Statistical comparison of the results with those of the official method showed excellent agreement and proved that there was no significant difference in the accuracy and precision between the official and the proposed methods [49].

Three-way partial least squares was applied to kinetic-spectrophotometric data. The coupling reaction of diazotized sulfanilamide with *o*-, *m*- and *p*-amino benzoic acid, and with orciprenaline, to give azodyes was monitored. Three binary mixtures of substrates, i.e., *o*-benzoic acid / orciprenaline, *m*-benzoic acid /*p*-benzoic acid and *o*-benzoic acid /*m*-benzoic acid, with different values of the rate constant ratio and spectra which overlapped seriously were studied. The spectra of the mixtures were scanned with a 2 nm resolution every 30 s during ca. 15 min. The data sets contained from 30×36 to 30×48 time-wavelength data. Nine mixtures of each binary combination of substrates were used for calibration, thus the three-way calibration data sets contained from 9×30×36 to 9×30×48 concentration-time-wavelength data. The two-way partial least squares modelling was constructed on the basis of single wavelength kinetic curves, and the three-way PLS modelling was applied to series of three-way data arrays consisting of a number of selected wavelengths each (up to the whole spectra). The results based on three-way data arrays were better than that of ordinary partial least squares, particularly with

mixtures having both a low rate constant ratio and small spectral differences [50].

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