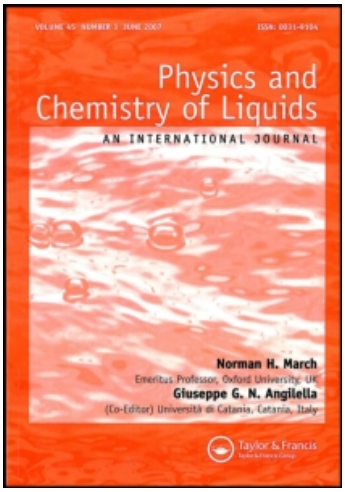

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Micellar catalysis in reactions of some β-lactam antibiotics with *p*-dimethylaminobenzaldehyde

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Kinetic study of the reactions of amoxicillin (I), ampicillin (II) and cephlaxin (III) with *p*-dimethylaminobenzaldehyde (DAB) in weakly acidic $EtOH/H_2O$ solution has been investigated using spectrophotometric method. Relatively slow reversible reactions of first order with respect to the antibiotic have been found. A derived equation for detecting the existence of reversibility from the linearity has been introduced. The effect of anionic surfactants (sodium dodecyl sulfate, SDS) on the kinetic of these reactions in aqueous solution has been studied. The presence of 0.005 M of SDS increases the rate constants by 4.3, 2 and 3.3 times for I, II and III, respectively. The consequence of the rate constants have a similar order in absence and presence of SDS; III > II > I. The rate constants pass through maxima with increasing SDS concentration followed by a gradual but steady decrease in the rate as the surfactant concentration increases further. Multiple linear regression method has been performed to evaluate the binding constants of each drug and DAB with SDS from the resulted kinetic data. The results suggest using multiple linear correlation method for such calculations, which is more accurate, reliable and less time consuming. The calculated binding constants between these drugs with SDS are following the consequence I > II > III which is related to the differences in their substitutions. The kinetic results were employed for spectrophotometric microdetermination of these drugs (I–III) in aqueous solution. The method was based on the reaction of β-lactam with an excess of DAB in presence of SDS and HCl (pH 2) at a wavelength 410 nm. The results indicate that the presented method is simple, precise and accurate. This method is applied to bulk antibiotics and some of their pharmaceutical preparations.

Keywords: β-Lactam; Amoxicillin; Ampicillin; Cephlaxin; *p*-Dimethylaminobenzaldehyde; Micelles

1. Introduction

The use of aqueous micelle solution instead of mixed organic-aqueous solvents has many advantages in biochemistry. Much attention is attracted to the effect of these molecules on the kinetics of reactions due to the fact that many biochemical processes proceed in microheterogeneous systems containing aqueous and lyophilic moiety [1–5]. However, it is well known that there is no certainty in prediction of the effect of micellar

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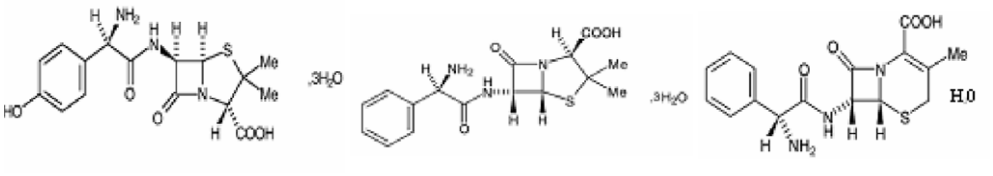

Amoxicillin (I) Ampicillin (II) Cephlaxin (III)

Scheme 1. Chemical structures of the studied β-lactam antibiotics (I–III).

on the kinetic of chemical reactions. This could be attributed to the fact that the observed effects may depend to various factors.

Schiff base formation from the condensation reaction of primary amine with aldehyde carbonyl group is a common synthetic procedure. It seems interesting to investigate the kinetic of the reaction between some medically important β-lactams with *p*-dimethylaminobenzaldehyde (DAB) in the absence and presence of surfactant molecules. No such studies for these antibiotics are found in the literature. The relatively small difference in substitutions of these drugs (scheme 1) may give a good opportunity to investigate their effect upon the rate of reaction and binding constant. The presence of HCl as a catalyst in the reaction medium could certainly make both reactants bear positive charge. Therefore, anionic surfactant such as sodium dodecyl sulfate (SDS) will be suitable to attract those charges of each protonated DAB and ammonium hydrochloride. The latter comes from the interaction of the drugs amino groups with HCl.

Recently, Khalil and Al-Khiro [6] studied the effect of SDS surfactant on the kinetic of the reactions of six sulphonamides with DAB. The observed rate constants increase with increasing the amount of SDS except for those of sodium sulphacetamide. The reason for the latter may be attributed to its ionic character and also to the presence of Na which could interact with the polar groups of surfactant molecules.

A characteristic feature of the presented investigations is their applicability for quantitative determination of the studied compounds [6]. According to the literature, there is a relatively remarkable lack of analytical determinations of these compounds, where the use of HPLC technique is the standard procedure [7]. The present study also describes a spectrophotometric method for micro determination of β-lactams (I–III) in presence of surfactant molecules. The method was based on the reaction of the compound to be determined with an excess of DAB in acidic aqueous solution.

2. Materials and methods

All antibiotics (I–III) were obtained in highly pure form from State Drug Industry (SDI), Samarra-Iraq. Their pharmaceutical preparations were obtained from different commercial sources. All other reagents were analytical grade commercial products purified when necessary by standard procedures. Distilled water was used for preparation of all solutions.

All spectral and kinetic measurements were performed on UV-Visible Spectrophotometer Cintra5-GBC (Scientific Equipment) equipped with thermostated cell holder. Absorbance measurements were carried out on Spectrophotometer SP6-350

(Pye Unicam). To control the temperature within ±0.1°C, a water thermostat Hakke Nk22 was used.

The stock solutions were freshly prepared 0.01 M of antibiotics in pure form (I–III) in 0.026 M HCl. Solutions of DAB (0.1 M) were prepared in 99.99% ethanol or in 0.05 M solutions of SDS in water.

Kinetic measurements normally used a solution of 0.6 mM antibiotics and varying concentration of DAB. The solution contained 0.15225 M of KCl only in the absence of SDS to avoid the effect of ionic strength and also for buffering composition (with the desired amount of HCl) of the solution. The use of SDS was at the same content of KCl but the latter causes a negative effect to the former, therefore, KCl is not used in the presence of SDS. Details of the experimental procedure were illustrated in [6]. The observed rate constants were calculated with integral equations from the experimentally obtained profiles absorbance *versus* time. The calculations were performed with a computer using Microsoft Excel program for linear regression analysis. Multiple linear regression analysis was used for determining the binding constants using Minitab program.

The Schiff bases were prepared by reaction of equamolar amounts of the antibiotics (I–III) with stochiometric amounts of DAB. Each antibiotic and DAB were dissolved by the minimum amount of ethanol and then mixed and refluxed for four hours. Then the solution was left for 24 h and the solid product was collected by filtration. The product was redissolved in ethanol for recrystallization to give a colour product of mustard (m.p. = 197–199°C), light orange (m.p. = 210°C decomposed), and yellow (m.p. = 195–197°C) for I, II and III, respectively.

0.6 mM antibiotic solution (I–III) was obtained by dissolving the appropriate weight of the powder of four capsules in distilled water. The solution was filtrated and the filtrate was diluted to 250 mL.

The temperature was maintained at 25°C throughout the experiments.

3. Results and discussion

The reaction of antibiotics (I–III) with the reagent DAB in presence of HCl gives a coloured solution with absorption maximum at 410 nm. This could be attributed to the formation of Schiff bases by the reaction of primary amino group of antibiotics with the carbonyl group of DAB [2,8]. Kinetic study was performed by following the increases of absorption intensity of the aforementioned visible peak with time. It was found that the absorbance of the products at infinite time do not change their positions when the temperature is raised. Also no maximum was observed when following the relation between absorption and time, indicating that the reactions are completely forward and no equilibrium occurs. Such surprising phenomenon was led to extend our exploration through the linearity of following derived equation:

$$\frac{[\beta\text{-lactam}]}{A_{eq}} = \frac{1}{\varepsilon_{sb}[\text{DAB}]K} + \frac{1}{\varepsilon_{sb}} \tag{1}$$

where [β-lactam] and [DAB] are the concentrations of drug and DAB, respectively. A_{eq} and ε_{sb} are the absorbance at infinite time and extinction coefficient of the Schiff base, respectively. K is the concentration equilibrium constant of the reaction. Indeed,

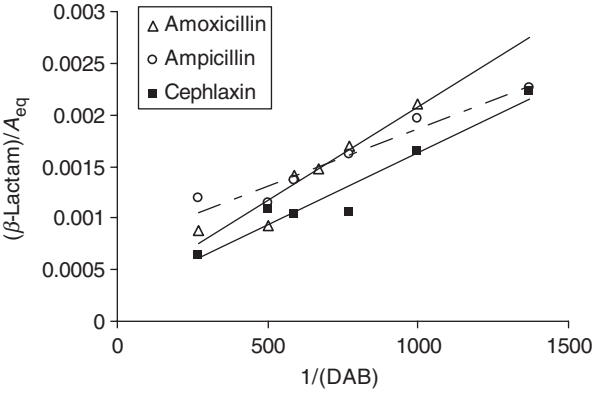

Figure 1. The relation between [β-lactam]/A_{eq} *vs.* 1/[DAB] according to equation (1) for 3.4×10^{-4} M of I, II, and III with various concentrations of DAB in 0.005 M SDS at pH = 2.

Table 1. Calculated values of equilibrium constants K, extinction coefficients ε_{sb}, and other related statistical parameters according to equation (1) for the reaction between 3.4×10^{-4} M of I, II, and III with various concentrations of DAB in 0.005 M SDS at pH = 2.

Antibiotics	Slope	Intercept	r^2	ε_{sb} (L mol^{-1} cm^{-1})	K (M^{-1})
Amoxicillin	1.8153×10^{-6}	2.67×10^{-4}	0.92	3745.3	147
Ampicillin	1.1109×10^{-6}	7.59×10^{-4}	0.94	1317.7	683
Cephlaxin	1.3998×10^{-6}	2.34×10^{-4}	0.94	4273.5	167

this equation was derived through an algebraically rearrangement of the following published relation [8].

$$A_{eq} = \frac{\varepsilon_{sb} K[\text{reactant}][\text{DAB}]}{1 + K[\text{DAB}]} \tag{2}$$

According to equation (1), if the plot of ([β-lactam]/A_{eq}) *versus* 1/[DAB] gives a linear line, the reaction must be reversible. The results show linear lines with correlation coefficients (r) equal to 0.96, 0.97, and 0.97 for I, II, and III, respectively (figure 1). The equilibrium constant (k) can be determined by evaluating ε_{sb} from the intercept of equation (1) which is equal to $1/\varepsilon_{sb}$. Then it can be calculated from the slope by substituting ε_{sb}; slope $= 1/\varepsilon_{sb} K$. Table 1 illustrates the calculated values of equilibrium constants, extinction coefficients and other related statistical parameters through application of equation (1) for I, II, and III. The relatively large values of K may be attributed to the relative stability of the produced Schiff base in comparison to the reverse reaction products [9].

Equal concentrations of both antibiotic and DAB give a first-order reaction when kinetic data were applied to first- and second-order integrated rate equations (table 2). This was confirmed through the application of isolation method (table 3). The results indicate that the reaction is first order with respect to β-lactam and zero order with respect to DAB. Interestingly, the reaction of sulphonamides with DAB obeys first order with respect to DAB and zero order with respect to sulphonamides [6]. The reason for this may be attributed mainly to the steric hindrance since

Table 2. Rate constants (k), order of reaction (n), correlation coefficient (r), and standard error (SE) for the reaction of equal concentration (0.01 M) of β-lactam and DAB at pH = 2.

Antibiotics	k_r	Order of reaction	r
Amoxicillin	$0.00357\,s^{-1}$	First	0.999
	$0.01693\,M^{-1}\,s^{-1}$	Second	0.978
Ampicillin	$0.00663\,s^{-1}$	First	0.999
	$4.35524\,M^{-1}\,s^{-1}$	Second	0.408
Cephlaxin	$0.00737\,s^{-1}$	First	0.999
	$0.38476\,M^{-1}\,s^{-1}$	Second	0.835

Table 3. An application of isolation method to the reaction of 0.001 M β-lactam with 0.1 M DAB, and 0.0053 M Ampicillin* with 0.00026 M DAB in 0.05 M SDS (SDS has been used for the last one* as an accelerator due to the very slow reaction) at pH = 2.

Antibiotics	k_r	Order of reaction	r
Amoxicillin	$0.00347\,s^{-1}$	First	0.999
Ampicillin	$0.00561\,s^{-1}$	First	0.999
Cephlaxin	$0.00738\,s^{-1}$	First	0.999
Ampicillin*	$0.00159\,M\,s^{-1}$	Zero	0.994

sulphonamides react with DAB through the para-substituted amino group in contrast to the crowded position of β-lactam amino group. The latter could also be the reason of the substantially slow reaction of β-lactam with DAB in contrast to that of sulphonamides. Thus, the suggested mechanism of presented reactions can be illustrated by the following scheme:

$$\beta\text{-Lactam} + \text{Protonated DAB} \rightleftarrows [\text{Intermediate}] \rightleftarrows \text{Schiff base} + H_2O \qquad (3)$$

where (Intermediate) is the formed hemiaminal through the attack of positively charged carbon atom of protonated aldehyde by β-lactam amino group. It is apparent that the reverse reaction should also be first order due to the high concentration of H_2O (as a solvent). The observed rate constant may be expressed by the relation:

$$k_r = \frac{k_1 k_2}{k_{-1} k_{-2}}.$$

The effect of the addition of SDS on the rate of these reactions is investigated and the results are illustrated in table 4. In this case, an aqueous solution of SDS was used for dissolving DAB instead of ethanol. The plots of observed rate constant and infinite absorbance against SDS concentration are given in figures 2 and 3, respectively. They show a maximum which is usually observed for bimolecular reactions [8] due to the reasons discussed in [1]. It may be concluded that the micelles concentrate the reactants and make them close to each other, and also the anionic surfactant (SDS) stabilizes the positively charged intermediate. On the other hand, the increase in micelle concentration leads to diluted reactants concentration and then decreases the rate of reaction. It should be noted that the minimum concentration of surfactant used in this study (0.005 M) to dissolve DAB is larger than critical micelle concentration (CMC) which is equal to 0.00225 M at pH = 2 [2]. It is apparent that the micelles increase the

Table 4. First-order rate constants for the reaction of 0.01 M of each β-lactam and DAB in presence of SDS at pH = 2.

Antibiotic	Conc. of SDS (M)	k_r (s^{-1})	r
Amoxicillin	0	0.00357	0.9999
	0.005	0.02057	0.9963
	0.0064	0.01264	0.9990
	0.0075	0.01192	0.9989
	0.009	0.01162	0.9976
	0.01	0.01077	0.9996
	0.014	0.00837	0.9988
	0.019	0.00846	0.9985
	0.025	0.00838	0.9970
Ampicillin	0[a]	0.00663	0.9997
	0.005	0.01105	0.9999
	0.0075	0.01093	0.9999
	0.009	0.01001	0.9999
	0.01	0.01024	0.9999
	0.014	0.00721	0.9995
	0.019	0.00639	0.9994
	0.025	0.0056	0.9995
Cephlaxin	0[a]	0.00738	0.9983
	0.005	0.01519	0.9983
	0.0064	0.01418	0.9984
	0.0083	0.01317	0.9992
	0.009	0.01297	0.9997
	0.01	0.01223	0.9990
	0.014	0.00588	0.9999
	0.019	0.00532	0.9997

[a]Those values are excluded from calculations of binding constants to improve the correlation coefficients for both multiple linear regression and old methods.

rate of these reactions by 4.3-, 2-, and 3.3-folds for I, II, and III, respectively. Such magnitudes of increasing are consistent with first order reactions whose order is proportional to the number of rate increase [10]. These numbers may be related to their substitutions of which only the amoxicillin(I) has a hydrophilic OH group, while the cephlaxin (III) contains one hydrophobic methyl group in contrast to I and III which have two methyl groups.

According to the literature, the equation that can calculate the binding constants for these bimolecular reactions from kinetic data is [1]

$$k_r = \frac{k_w + k'_m K_A K_D C}{(1 + K_A C)(1 + K_D C)} \tag{4}$$

where k_r is the observed rate constant, k_w is the rate constant in water, k'_m is equal to k_m/V (where k_m is the rate constant in micelle and V is the molar volume), K_A and K_D are the binding constants of the antibiotic and DAB with micelle, respectively, and C is the surfactant concentration. Since the rate decreases from a maximum as the anionic surfactant concentration increases, k_w can be neglected with respect to the other term in equation (4) [3]. Thus, rearrangement of equation (4) yields

$$\frac{1}{k_r} = \frac{K_A + K_D}{k'_m K_A K_D} + \frac{C}{k'_m} + \frac{1}{k'_m K_A K_D C} \tag{5}$$

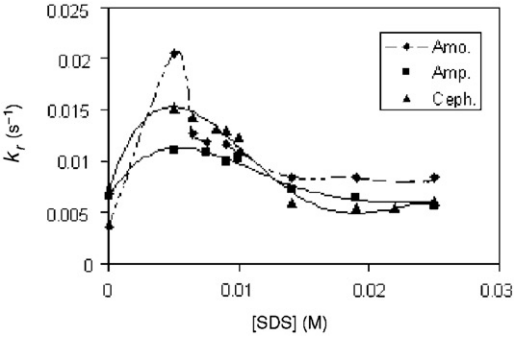

Figure 2. Rate constants of 0.01 M β-lactams (I–III) and DAB *vs.* [SDS] at pH = 2.

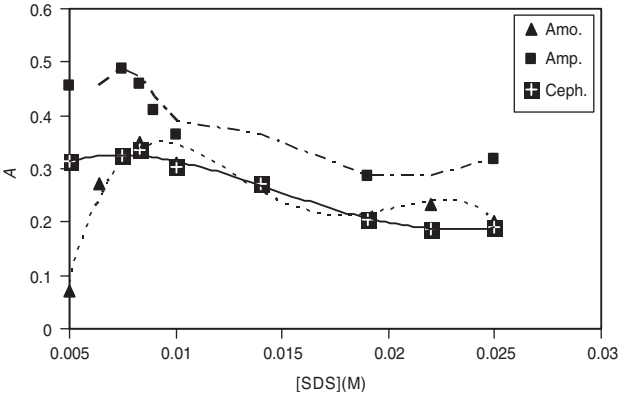

Figure 3. Infinite absorbance (A_∞) of 0.01 M β-lactams (I–III) and DAB *vs.* [SDS] at pH = 2.

Such type of equations are usually solved for this purpose by successive graphical method [1,3], i.e. equation (5) has been represented by Yatsimirskii and his coworkers [1] in the following form

$$\frac{C}{k_r} = \alpha + \beta C + \gamma C^2 \tag{6}$$

where

$$\alpha = \frac{1}{k'_m K_A K_D},$$

$$\beta = \alpha(K_A + K_D),$$

$$\text{and } \gamma = \alpha K_A K_D.$$

k'_m, K_A and K_D can be evaluated through determining α, β, and γ. α represents the intercept of the plot between C/k_r *versus* C, while β and γ are evaluated from intercept and slope of the following relation, respectively [1].

$$\frac{C/(k_r - \alpha)}{C} = \beta + \gamma C \tag{7}$$

Table 5. Multiple linear regression analysis of the results listed in table 4 according to equations (5) and (8).

Compound	Constant $(K_A + K_D/k'_m K_A K_D)$	Coefficient of [SDS] $(1/k'_m)$	Coefficient of 1/[SDS] $(1/k'_m K_A K_D)$	r
Amoxicillin	268.88	4886.3	1.07	0.976
Ampicillin	142.02	1712.5	0.3858	0.912
Cephlaxin	131.0	3123.0	0.517	0.897

Table 6. Binding constants, transfer free energies ($\Delta\mu^\circ$) of β-lactams and DAB and k'_m.

Compound	k'_m s^{-1}	K_A (M^{-1})	K_D (M^{-1})	$-\Delta\mu^{o\,a}_A$ (kJ mol^{-1})	$-\Delta\mu^{o}_D$* (kJ mol^{-1})
Amoxicillin	2.046×10^{-4}	232.04	19.717	23.45	17.34
Ampicillin	3.202×10^{-4}	226.74	26.64	23.40	18.09
Cephlaxin	5.839×10^{-4}	177.818	24.962	22.80	17.93

[a]Calculated from $\Delta\mu^\circ = -RT \ln (55.5\,K)$ [3].

However, equation (5) can be considered as a multilinear equation of the type:

$$Y = a_o + a_1 x_1 + a_2 x_2 \tag{8}$$

where Y is the dependent variable that equals to $1/k_r$, a_o is the constant term which equals to $(K_A + K_D)/(k'_m K_A K_D)$, a_1 and a_2 are the regression coefficients which equal to $1/k'_m$ and $1/k'_m K_A K_D$, respectively, and x_1, x_2 are the independent variables that equal to C and $1/C$, respectively. Thus, using multiple linear regression analysis for different concentrations of surfactant (as mentioned in table 4), one can compute a_0, a_1 and a_2 and then k'_m, K_A and K_D which are given in tables 5 and 6.

The good values of correlation coefficients (table 5) indicate the success of using this method for determining the binding constants from kinetic data. The results (table 6) show that the binding constants of antibiotics are larger than that of DAB which is attributed to both hydrophobic and hydrophilic binding of β-lactam with micellar phase compared to only hydrophobic binding of DAB. The increase of the values of binding constants takes the following sequence: I > II > III, which can also be related to the difference in their substitution groups discussed above. The change in the standard chemical potential of the antibiotics ($\Delta\mu^o_A$) and DAB ($\Delta\mu^\circ_D$) on passing from water to the micelle have been calculated and the results are listed also in table 6. The somewhat close $\Delta\mu^o$ values of β-lactams and DAB suggest that the reaction occurs inside the core of the micelle. The reasonable values of k'_m and the closeness of K_D and $\Delta\mu^o_D$ values reflect the accuracy of the presented method for regression analysis. Application of the old method represented by equations (6) and (7) to the data of table 4 has been carried out. Poor correlation coefficients of the values of 0.716, 0.291, and 0.187 for I, II, and III, respectively with contradictory binding constants have been observed by applying equation (7). i.e. K_A and K_D for I and II are 396.946, 4.303, 251.062, and 4.854, respectively, while for III fictional or complex values are obtained due to its very bad correlation coefficient. The reason for this may be due to the presence of the independent

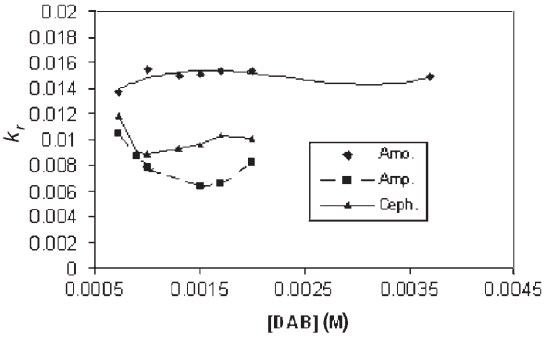

Figure 4. Observed first order rate constants of 3.4×10^{-4} M β-lactams (I–III) at different concentrations of DAB in 5×10^{-3} M SDS and pH = 2.

variable (C) in the left sides of equations (6) and (7) which could disturb the statistical correlation.

The effect of DAB concentration on the rate of reactions and infinite absorption has been investigated and the results are illustrated in figures 4 and 5, respectively. The plot of rate constant *versus* [DAB] (figure 4) confirms the first order reaction which does not depend on the DAB concentration. The increase of infinite absorption of Schiff bases with the increase of DAB concentration (figure 5) also confirms the existence of reversible reaction.

The effect of pH on the rate constants and infinite absorptions has also been investigated and the results are illustrated in figures 6 and 7, respectively. The decrease of the rate constants of β-lactams (I–III) with increasing pH (figure 6) may be attributed to the decrease in [HCl] which acts as a catalyst. While the relation between pH and infinite absorbance (figure 7) exhibits a maximum that indicates the optimum pH for these reactions is approximately equal to 2. The reason for such a maximum could be attributed to the stabilization of β-lactams amino group through forming a chloride salt when pH decreases. The increase of pH leads to decrease of the color of formed Schiff bases, as a result of deprotonation of imine nitrogen that is responsible for the colouring of the products.

On the bases of the aforementioned kinetic results, one can suggest a spectrophotometric method for determining β-lactams (I–III) by their reaction with an excess of dissolved DAB in SDS at pH = 2 and wavelength 410 nm. There are several procedures for determining these compounds in the literature including titration [11–13], spectrophotometric [14–17], chromatographic [18–20], and fluorimetric [21] methods. But, the reaction of the presented compounds with DAB and the use of surfactant have not been observed.

The results of experiments showed that the colour develops immediately and remains stable for more than 24 h. It is obvious from the aforementioned kinetic data that the molar ratio of DAB to β-lactams is 1 : 1. The effect of DAB and SDS concentrations has been investigated, and the results indicate that 3 mL solution of 0.0274 M DAB in 0.0615 M SDS, 0.0282 M DAB in 0.0572 M SDS, and 0.0240 M DAB in 0.0997 M SDS are added with HCl (pH = 2) to I, II, and III, respectively, and then diluted by distilled water to 10 mL. The effect of reaction time at room temperature is investigated. The obtained colour is stable for at least 24 h. The order of addition is determined for each

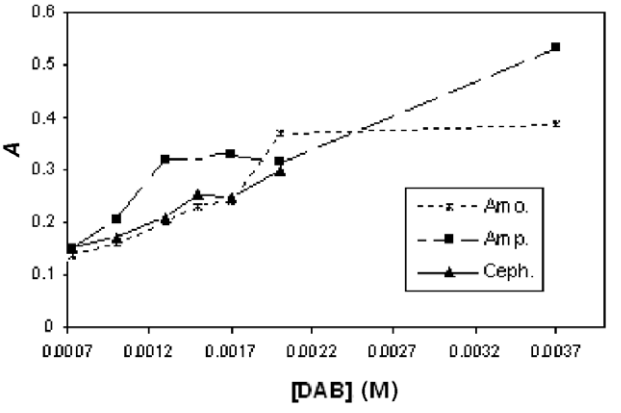

Figure 5. Infinite absorbance *vs*. DAB concentration of 3.4×10^{-4} M β-lactams (I–III) in 5×10^{-3} M SDS and pH = 2.

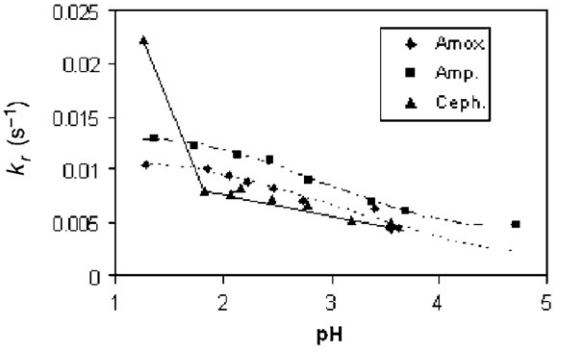

Figure 6. Observed first order rate constant *vs*. pH in the reaction of 3.4×10^{-4} M β-lactams (I–III) with 7.3×10^{-4} M of DAB for II and III, and 1×10^{-3} M DAB for I in the presence of 0.005 M SDS.

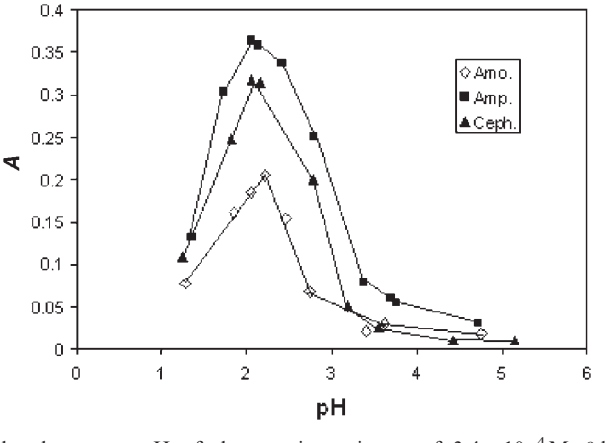

Figure 7. Infinite absorbance *vs*. pH of the reaction mixture of 3.4×10^{-4} M β-lactams (I–III) with 7.3×10^{-4} M of DAB for II and III and 1×10^{-3} M DAB for I in the presence of 0.005 M SDS.

Table 7. Some analytical parameters for the reaction of β-lactams with DAB in SDS.

Antibiotics	λ_{max} (nm)	Limit of det. (M)	r	r^2	Intercept
I	410	2.1×10^{-5} to 6×10^{-4}	0.999	0.997	0.0722
II	410	2.1×10^{-5} to 6×10^{-4}	0.999	0.999	0.0253
III	410	2.1×10^{-5} to 6×10^{-4}	0.999	0.998	0.0380

Table 8. Molar absorpitvities, relative error percentage and relative SD percentage for the presented spectrophotometric method.

Antibiotics	Slope	ε ($L\,mol^{-1}\,cm^{-1}$)	Relative error[a] (%)	Relative standard deviation[a] (%)
I	1575.306	2700	−0.20	±0.06
II	1377.852	1483.333	+0.11	±0.13
III	801.561	1100.0	+0.10	±0.23

[a]Average of five determinations.

compound and the higher absorbances were found as (DAB/SDS + Sample + HCl) for I and II, and (HCl + DAB/SDS + Sample) for III.

The following procedure can be suggested. Three millilitres of 0.02743 M of DAB in 0.03153 M SDS, 0.02823 M of DAB in 0.0572 M SDS, and 0.02402 M of DAB in 0.09967 M SDS for I, II, and III, respectively, plus desired amount of 0.1 M HCl to obtain the desired pH = 2, and a known volume of sample or stock β-lactam solution are placed in 10 mL volumetric flask and diluted with water to 10 mL. The volumetric flasks are placed in water bath at 80°C for 30 min. (It is found experimentally that the heating process increases the extinction coefficients by ratios of 62, 28, and 45% for I, II, and III, respectively.) After cooling of solution the absorbance at 410 nm is measured in 10 mm path-length cell against the blank.

In table 7 typical calibration data are presented for the investigated β-lactams (I–III) obtained from linear regression analysis of absorbance *versus* the concentration of each compound giving limit of detections, slopes, intercepts and correlation coefficients. The linear calibration graphs obtained for the investigated β-lactams can also be used for calculation of the concentration. The apparent molar absorptivities ε of β-lactams are relatively low ranging from 1100 to 2700 $L\,mol^{-1}\,cm^{-1}$ (table 8), while in the absence of SDS the values of molar absorptivities are 59.94, 61.25, and 54.80 for I, II and III, respectively indicating the high increase in sensitivity caused by SDS. The relatively high molar absorpitvities of sulphonamides, ranging from 4.9×10^4 to 8.9×10^4 $L\,mol^{-1}\,cm^{-1}$ [6], can be attributed to the resonance effect resulted from the reaction of directly bonded amino group to benzene ring in contrast to that of aliphatic amino group of the β-lactams. Thus, microgram amounts of these antibiotics can be estimated with an accuracy of better than ±0.4% (relative error) and reproducibility less than ±0.23% (relative SD).

Results for the application of the proposed method for determination of β-lactams (I–III) in pure and dosage forms are shown in tables 9 and 10, respectively.

The effect of interferences on the efficiency in the presented method was investigated by adding 200, 400, and 800 μg of each of glucose, lactose, dextrose, starch, and gum-acacia to the solution of β-lactams. The values of recovery % for each added foreign compound indicate no interference of these compounds on the

Table 9. Assay of three different amounts β-lactams (I–III) in bulk form.

	Recovery (%)[a]		
Antbiotics	6×10^{-5} M	1.2×10^{-4} M	3×10^{-4} M
I	94.01	99.14	113.62
II	98.17	95.47	103.95
III	106.86	98.37	98.04

[a]Average of three determinations.

Table 10. Assay of three different amounts β-lactams (I–III) in dosage form from different commercial sources using DAB/SDS method.

Antibiotics (Company)	Recovery (%)
Amoxicillin (SDI)	113.31
Amoxicillin (Ajanta)	113.31
Ampicillin (SDI)	159.99
Ampicillin (Ajanta)	119.60
Ampicillin (ACAI)	126.94
Cephlaxin (SDI)	111.04
Cephlaxin (ACAI)	109.55

presented method [22]. The effect of addition of other surfactants on the efficiency of the presented method has also been carried out through adding of different amounts (1, 2 and 3 mL) of a solution containing 1×10^{-3} M of cationic (cetyl trimethyl ammonium bromide, CTAB), 1×10^{-3} M of anionic (sodium dodecyl benzene sulphonate, SDBS) and 1.0% of neutral (TritronX-100) surfactants [22]. The results of absorbance also indicate that there is no remarkable effect arisen from the addition of other type of surfactants (in addition to SDS) to the performance of the presented method.

4. Conclusions

On the basis of the results we can conclude the following significant points:

(1) The presented method for detecting the reversibility of chemical reactions through linearity may be considering it as a significant probe for such investigations.
(2) The results of determinations the binding constants strongly suggest uses of the developed multiple linear correlation method for such calculations, which is more accurate, reliable and less time consuming.
(3) The increase in rate constants that is caused by the presence of SDS can be obtained by concentrating the reactants molecules, and stabilizing the positively charged intermediate, by micelle.
(4) The relatively very low extinction coefficients of the products in the absence of SDS may indicate the impossibility of using the presented analytical method without surfactant molecules.

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