Microchemical Journal 111 (2013) 97–102

Contents lists available at SciVerse ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Kinetic assay of D-Penicillamine in pure and pharmaceutical formulations based on ligand substitution reaction

Radhey M. Naik [a,*], Surendra Prasad [b,**], Basant Kumar [a], Vimlesh Chand [b]

[a] Department of Chemistry, University of Lucknow, Lucknow-226007, India
[b] School of Biological and Chemical Sciences, Faculty of Science, Technology and Environment, The University of the South Pacific, Private Mail Bag, Suva, Fiji

ARTICLE INFO

Article history:
Received 5 July 2012
Accepted 26 July 2012
Available online 2 August 2012

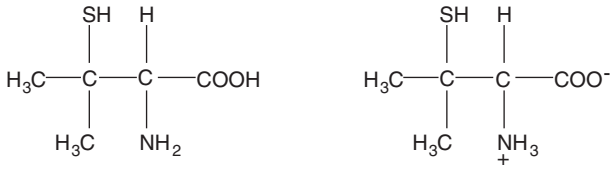
Keywords:
Ligand substitution reaction
Kinetic determination
D-Pencillamine
D-Pencillamine quantification
β,β-Dimethylcysteine determination
Kinetic spectrophotometric determination

ABSTRACT

A kinetic analytical method using uncatalyzed ligand substitution reaction and spectrophotometeric monitoring for the quantification of D-pencillamine (D-PA) *i.e.* 2-amino-3-mercapto-3-methylbutanoic acid or β,β-dimethylcysteine or 3-mercaptovaline or 3-mercapto-D-valine in pure as well as dosage forms has been validated and applied. The method is based on the uncatalyzed ligand substitution reaction between $[Fe(CN)_5(H_2O)]^{3-}$ and D-PA which produces the complex $[Fe(CN)_5(D\text{-}PA)]^{3-}$. The substituted complex $[Fe(CN)_5(D\text{-}PA)]^{3-}$ shows an absorption maximum at 421 nm. The proposed method shows good linear dynamic range of 14.92–149.21 μg mL^{-1}. By measuring the fixed time absorbance as a measure of initial rate of the complex formation during the course of the reaction at this wavelength, D-PA can be determined in the range 14.92–149.21 μg mL^{-1}. The recoveries of D-PA from various samples spiked at the usage level have been quantitative. The limit of quantification (3σ) was determined as 5.16, 3.68 and 3.13 μg mL^{-1} of D-PA corresponding to the fixed time of 2, 3 and 5 min respectively. The common excipients used as additives in pharmaceuticals did not interfere in the proposed method. The method was successfully employed for the analysis of D-PA content in commercial pharmaceutical preparations (tablets) and revealed quantities almost equal to those measured using the standard method, and demonstrated good accuracy and precision. The proposed method is rapid, simple, accurate and precise without the need for authentic analyte standards. It could therefore be used as an alternative to the quantification of D-PA in various samples.

1. Introduction

D-Penicillamine (D-PA) is a sulfur containing amino acid which belongs to aminothiols family where a hydrogen atom in the β-carbon of cysteine is replaced by the methyl group [1]. The thiols are essential for metabolism of cells and indispensable in preventing or healing some diseases [2]. Thus many pharmaceutical preparations contain thiol compounds. D-PA, also called as 3, 3-dimethylcysteine or 2-amino-3-mercapto-3-methylbutanoic acid or β,β-dimethylcysteine or 3-mercaptovaline or 3-mercapto-D-valine, is the major product of the decay of penicillin antibiotics [3,4]. It is the characteristic acid degradation product of β-lactam antibiotics [4]. It exists in two enantiomeric forms D and L. L-Penicillamine has excessive toxicity while D-PA has found many therapeutic applications [1,3,5]. Therefore D-PA, whose structure is shown below, is among the most often used thiols as therapeutic substance [2].



Structure of D-Penicillamine

D-Penicillamine, a well-known heavy metal chelator, is the drug of choice and typically used in the treatment of Wilson's disease, which results from the presence of over and above high concentrations of copper in the body [5–9]. Therefore, D-PA is also useful in the treatment of heavy metal poisoning [5,7,9–11]. It is also effective for the treatment of several disorders including rheumatoid arthritis [1,3,9,11,12], rare inherited disease cystinuria [6,9], primary biliary cirrhosis [7] and progressive systemic sclerosis [7,13]. It is also used as an antifibrotic agent in treatment of scleroderma lung diseases [1,6,7,14]. It may also inhibit the replication of human immune deficiency virus, the cause of acquired

*Corresponding author. Tel.: +91 9450466126; fax: +91 522 2740916.
**Corresponding author. Tel.: +679 3232416; fax: +679 3231512.
E-mail addresses: naik_rm@rediffmail.com (R.M. Naik), prasad_su@usp.ac.fj (S. Prasad).

0026-265X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.microc.2012.07.015

immune deficiency syndrome (AIDS) [9]. Thus D-PA is widely used in medicine. However, it can also cause severe adverse effects such as loss of appetite, nausea, abdominal pain, loss of the sense of taste, bone narrow suppression and serious kidney disease [8]. Therefore due to the biological and the medicinal importance of D-PA, its determination in pure form and pharmaceutical preparations (dosage forms) is of great public importance.

Literature search assembled a number of different methods, which have been used for detection, determination and analysis of D-PA in pure and pharmaceutical preparations, biological tissue and serum. These are spectrophotometry [3], spectrofluorimetry [4,15], kinetic potentiomentric [2], flow injection analysis [3], chemiluminescence-based detection [6], voltammetry/electrochemical [5,8,9,16–19], liquid chromatography [1,15], high performance liquid chromatography [7,20,21] and NMR spectroscopy [3,9,22]. As the thiol lacks sufficient UV absorption so a pre or post-column derivatization procedure is normally required which is one of the important limitations of liquid chromatography techniques in determination of D-PA [9]. Among all, the electrochemical methods are an alternative for the D-PA determination because they are cheap, simple, fast and sensitive. Various chemically modified electrodes have also been used to determine D-PA [5,8,9,16–19] but their use in flow injection analysis and liquid chromatography detections are limited because of the requirements of particular mechanical and chemical stability towards the flowing solution [9]. Thus many of the reported methods in general suffer from one or other of disadvantages, such as use of sophisticated and time consuming techniques, expensive instruments, reagents that are mostly not available in many of quality control laboratories, and have limitations due to their toxicities [9]. Some of the available methods are discouraged because of low selectivity and higher interference problems. Therefore, we have been interested in developing an analytical method for D-PA determination.

The presence of a thiol, amino and carboxyl groups in D-PA provides a number of ways of interacting with organic [3,23,24] and inorganic [25,26] species or both [27,28], in a variety of complex formation and redox reactions that yield some spectrophotometrically active product. To the best of our knowledge no studies have been reported on the determination in D-PA in pure form and pharmaceutical preparations based on ligand substitution reaction. We have been interested in developing methods for various analytes of environmental, biological and medicinal interest [29–39]. The search for a selective, rapid, accurate and economical method for the determination of D-PA led to an investigation for a highly sensitive method for the quantification of D-PA based on ligand substitution reaction between $[Fe(CN)_5(H_2O)]^{3-}$ and D-PA.

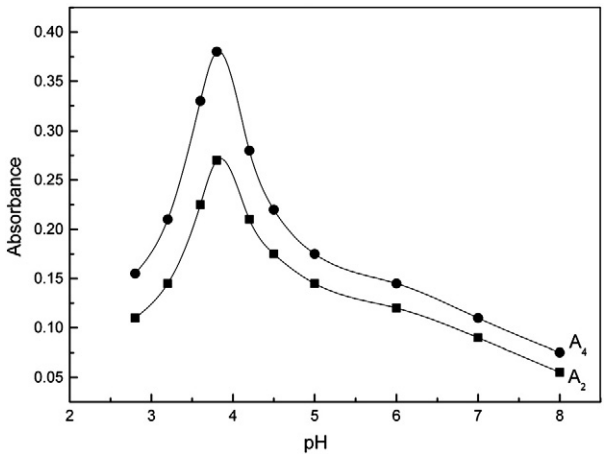


Fig. 1. Effect of pH on the reaction rate under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 8.0\times10^{-5}$ mol L^{-1}, [D-PA] $= 3.2\times10^{-3}$ mol L^{-1}, I = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

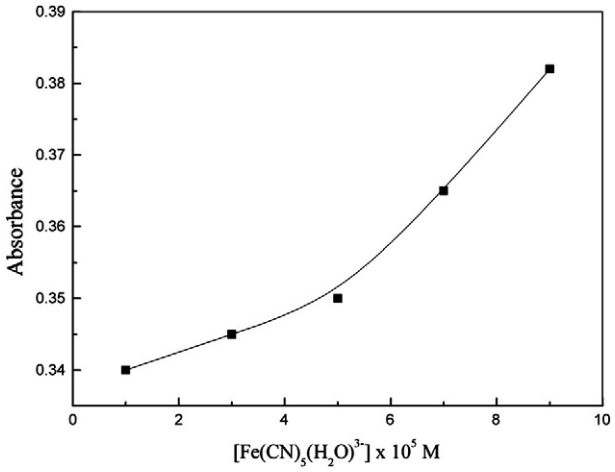


Fig. 2. Effect of $[Fe(CN)_5(H_2O)^{3-}]$ on the reaction rate under the reaction conditions: [D-PA] $= 3.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02, I = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

Kinetics-based analytical methods can be classified broadly into four groups, namely the uncatalyzed reaction rate methods, catalyzed reaction rate methods, differential reaction rate methods, and miscellaneous methods. While catalytic rate methods have drawn greater attention due to the unique selectivity and the low limits of detection attainable [40], uncatalyzed methods provide the theory and scope of kinetic determination of variety of species with simple instrumentation and possess good sensitivity and selectivity [40–43]. Therefore, in continuation of our interest in developing analytical methods based on the catalytic oxidation [31,33,34,37,39] and ligand substitution reactions [29,30,32,35,36,38], in the present work we have considered it worthwhile to report a sensitive, selective, rapid, accurate and economical procedure for the determination of D-PA in pure and pharmaceutical preparations. The methods is based on the uncatalyzed ligand substitution reaction between $[Fe(CN)_5(H_2O)]^{3-}$ and D-PA which leads to the formation of a substituted, highly stable and pharmaceutically important product, $[Fe(CN)_5(D\text{-}PA)]^{3-}$, which absorbs strongly at 421 nm [44].

2. Experimental

2.1. Reagents

Sodium amminepentacyanoferrate(II) trihydrate, $Na_3[Fe(CN)_5(NH_3)].3H_2O$, was prepared by published methods [45] and characterized by visible absorption spectra and CHN microanalysis. Analytical calculation for $Na_3[Fe(CN)_5(NH_3)].3H_2O$, calculated: %C, 18.42; %H, 2.76; %N, 25.77 and found: %C, 18.36; %H, 2.75; %N, 25.64. The solutions of $Na_3[Fe(CN)_5(H_2O)]$ were obtained by aquation of $Na_3[Fe(CN)_5NH_3].3H_2O$ as required. The solution of $Na_3[Fe(CN)_5(H_2O)]$ was stored in a dark amber colored bottle to avoid its photodecomposition and oxidation. The stock solution of 1.0×10^{-2} mol L^{-1} D-pencillamine (BDH, UK) was prepared by the accurate weighing of its calculated amount in the double distilled or distilled de-ionized water. The pH of the reaction mixture was maintained at 3.80 ± 0.02 using potassium hydrogen phthalate-sodium hydroxide buffer solution [46]. The standard BDH buffers were used to standardize the pH meter before use. Potassium nitrate (Merck) was used to maintain the ionic strength of the reaction mixture.

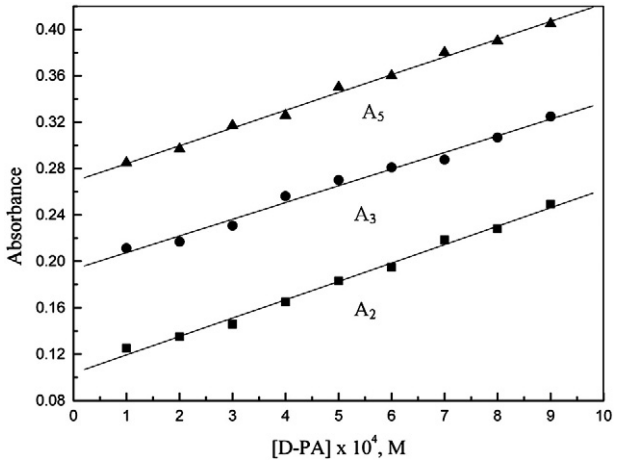


Fig. 3. Linear dependence of D-PA on the reaction rate under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02, I = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

2.2. Apparatus

A shimadzu double beam UV–visible spectrophotometer, model UV-240 attached with a thermostatic cell compartment was used for all absorbance measurements at fixed wavelength of 421 nm, and recording of absorption spectra of reactants and product. The matched quartz cuvettes of 10 mm path length were used in each measurement. All pH measurements were made on a Toshniwal digital pH meter, model CL-46. All the volumetric apparatus used in the present study were of certified 'A' grade, which were steamed regularly before their use.

2.3. Recommended procedure

All the solutions were first equilibrated in a thermostat for 30 min to attain thermal equilibrium at 25.0 ± 0.1 °C. 2.0 mL of each reagent solutions were mixed in a 10 mL Borosil volumetric flask, already placed in thermostatic bath, in the sequence; D-penicillamine, buffer, while the ligand substitution reaction was finally started by adding 2.0 mL of $[Fe(CN)_5(H_2O)]^{3-}$ solution into the mixture. The progress of reaction was monitored by measuring the increase in absorbance of the complex $[Fe(CN)_5(D\text{-}PA)]^{3-}$, formed during the course of reaction at 421 nm, where the product absorbs strongly without any interference from the reactants.

3. Results and discussion

3.1. Indicator reaction

The uncatalyzed ligand substitution reaction between $[Fe(CN)_5(H_2O)]^{3-}$ and D-PA leads to the formation of a yellow $[Fe(CN)_5(D\text{-}PA)]^{3-}$ complex which is quite stable and absorbs at 421 nm assigned due to the metal to ligand charge transfer transitions (MLCT) i.e. Fe(II)dπ → π*(D-PA) [44]. The aquation of $Na_3[Fe(CN)_5(NH_3)].3H_2O$ quantitatively generates aquapentacyanoferrate(II), $[Fe(CN)_5(H_2O)]^{3-}$, which reacts with D-PA to give the final product, $[Fe(CN)_5(D\text{-}PA)]^{3-}$. The overall ligand substitution reaction taking place between D-PA and aquapentacyanoferrate(II) is represented through the following equations.

$$[Fe(CN)_5(NH_3)]^{3-} + H_2O \rightarrow [Fe(CN)_5(H_2O)]^{3-} + NH_3 \quad (1)$$

$$[Fe(CN)_5(H_2O)]^{3-} + D\text{–}PA \rightarrow [Fe(CN)_5(D\text{–}PA)]^{3-} + H_2O \quad (2)$$

In this reaction, while all the reaction variables were held constant, the rate of the ligand substitution reaction was made proportional to the total concentration of D-PA, which formed the basis of development of the kinetic method for its quantification based on the uncatalyzed ligand substitution reaction. The optimization of the reaction variables was carried out sequentially by varying one reaction variable at a time while keeping all others constant.

Table 1
Analytical figures of merit for the determination of [D-PA] at different fixed times under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02, I = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

[D-PA] or linear range (μg mL^{-1})	Time (min)	Linear regression equation	Correlation coefficient (r^2)	Limit of detection (μg mL^{-1}) [a]
14.92–149.21	2	$A_2 = 1.586\times10^2$ [D-PA] + 0.1035	0.9964	5.15
14.92–149.21	3	$A_3 = 1.439\times10^2$ [D-PA] + 0.1932	0.9930	3.68
14.92–149.21	5	$A_5 = 1.532\times10^2$ [D-PA] + 0.2689	0.9973	3.13

[a] $n = 7$.

Table 2
Determination of D-PA at 20.0 μg mL^{-1} level in presence of excipients under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02, ionic strength, I = 0.05 mol L^{-1} (KNO_3), temperature = 25.0 ± 0.1 °C.

Additive species	Tolerance limit [excipient]/[D-PA]	Recovery of drug[a] ± RSD (%)
Starch	90	99.6 ± 0.8
Saccharin	900[b]	102.4 ± 0.4
Glucose	900	101.2 ± 0.6
Sodium phosphate	600	102.8 ± 0.2
Magnesium stearate	25	97.02 ± 0.8
Sucrose	700	101.24 ± 0.8

RSD: Relative standard deviation.
[a] $n = 5$, average recovery based on five replicate determinations per samples.
[b] Maximum ratio tested.

3.2. Effect of pH

While studying the pH dependence, the pH up to 6 was varied using potassium hydrogen phthalate/NaOH or potassium hydrogen phthalate/HCl buffer [46]. However, in the higher pH region, the pH of the working solution was maintained using 5.0 mol L^{-1} NaOH. The reaction is pH dependent. Therefore, the maintenance of pH of the reaction mixture is critical. To obtain a pH corresponding to optimum value, where the product absorbance is the maximum, the influence of pH on the absorbance was studied using fixed time procedure, as a measure of the initial rate, in the pH range 2.5 to 8.0. The variation in absorbance A_t (t = 2 and 4 min of mixing the reagents) at 421 nm as a function of the pH of the media is shown in Fig. 1. It was found that with increase in the pH the absorbance increases and attained the maximum value at pH 3.80 ± 0.02, and then decreases again. Thus pH 3.80 ± 0.02 was chosen for further investigation since it provided the highest initial rate of the reaction.

3.3. Effect of $[Fe(CN)_5(H_2O)^{3-}]$

The influence of $[Fe(CN)_5(H_2O)^{3-}]$ on the rate of product formation i.e. rate of reaction was investigated at optimum pH 3.80 ± 0.02. The changes in the absorbance as a measure of initial rates were evaluated as a function of $[Fe(CN)_5(H_2O)^{3-}]$ by changing its concentration in the range 1.0–10.0 × 10^{-5} mol L^{-1}. Low concentration range was especially studied to prevent any possible dimerization of the $[Fe(CN)_5(H_2O)]^{3-}$ complex [47]. The plot of the absorbance as a measure of initial rate *versus* $[Fe(CN)_5(H_2O)^{3-}]$ is shown in Fig. 2. 8.0×10^{-5} mol L^{-1} $[Fe(CN)_5(H_2O)^{3-}]$ was chosen for further study since it provided the suitable change in the initial rate of the reaction.

Table 3
Accuracy and precision of D-PA determination by the proposed method under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02; ionic strength, I = 0.05 mol L^{-1} (KNO_3), temperature = 25.0 ± 0.1 °C.

Concentration of D-PA added (μg mL^{-1})	A_2			A_3			A_5		
	[D-PA] found[a] ± s (μg mL^{-1})	Recovery (%)[b]	Error (%)	[D-PA] found[a] ± s (μg mL^{-1})	Recovery (%)[b]	Error (%)	[D-PA] found[a] ± s (μg mL^{-1})	Recovery (%)[b]	Error (%)
15.00	14.88 ± 0.04	99.20	−0.80	15.22 ± 0.06	101.47	+1.48	15.10 ± 0.08	100.67	+0.67
22.40	22.76 ± 0.06	101.61	+1.61	22.20 ± 0.08	99.11	−0.89	22.62 ± 0.06	100.98	+0.98
30.44	30.88 ± 0.04	101.45	+1.45	30.74 ± 0.06	100.99	+0.99	30.10 ± 0.10	98.88	−1.13
46.16	46.76 ± 0.10	101.30	+1.30	46.32 ± 0.10	100.35	+0.35	46.30 ± 0.20	100.30	+0.30
58.76	58.22 ± 0.12	99.08	−0.92	58.92 ± 0.12	100.27	+0.27	58.98 ± 0.22	100.37	+0.37
72.14	72.72 ± 0.08	100.80	+0.80	72.40 ± 0.14	100.36	+0.36	72.48 ± 0.08	100.47	+0.47
94.32	94.98 ± 0.14	100.70	+0.70	94.10 ± 0.20	99.77	−0.24	94.72 ± 0.07	100.42	+0.42
112.48	112.10 ± 0.09	99.66	−0.34	112.70 ± 0.16	100.20	+0.20	112.30 ± 0.08	99.84	−0.16
130.00	130.76 ± 0.10	100.59	+0.59	130.40 ± 0.21	100.31	+0.31	130.20 ± 0.21	100.15	+0.15

The ± s values represent standard deviation of the mean for three determinations.
[a] Average of five determinations per samples.
[b] Average % recovery of five determinations per samples.

3.4. Effect of temperature and ionic strength, I

The influence of temperature on the reaction rate was studied in the temperature range 20–40 °C. The influence at higher temperature was not tried due to the possibility of decomposition of the reaction product, $[Fe(CN)_5(D\text{-}PA)]^{3-}$. The reaction provided reasonable change in initial rate of the reaction at 25 °C. Hence 25 °C was selected for further study.

The effect of ionic strength, I, on the rate of the reaction was studied in 0.01–0.3 mol L^{-1} range where KNO_3 was used to maintain the ionic strength. The ionic strength 0.05 mol L^{-1} provided the sufficient change in the initial rate of the reaction and was selected for further study.

3.5. Influence of D-PA concentration

To study the influence of D-PA on the reaction rate and also to investigate the linear calibration range for the analytical purpose, the fixed-time procedure was adopted. D-PA concentration was varied in the concentration range 1.0×10^{-6}–3.2×10^{-3} mol L^{-1} under the conditions of 3.2×10^{-3} mol L^{-1} $[Fe(CN)_5(H_2O)^{3-}]$, pH 3.80 ± 0.02, ionic strength 0.05 M and temperature 25.0 ± 0.1 °C. The plot of the initial rate i.e. absorbance at fixed time 2, 3 and 5 min of the initiation of the reaction *versus* [D-PA] was found to be linear in the concentration range 1.0–10.0×10^{-4} mol L^{-1} and is shown in Fig. 3. The linear regression equations relating the A_t to the [D-PA] between 1.0–10.0×10^{-4} mol L^{-1} i.e. 14.92-149.21 μg mL^{-1} are given in Table 1. These linear regression equations were used to calculate the concentration of D-PA in aqueous samples.

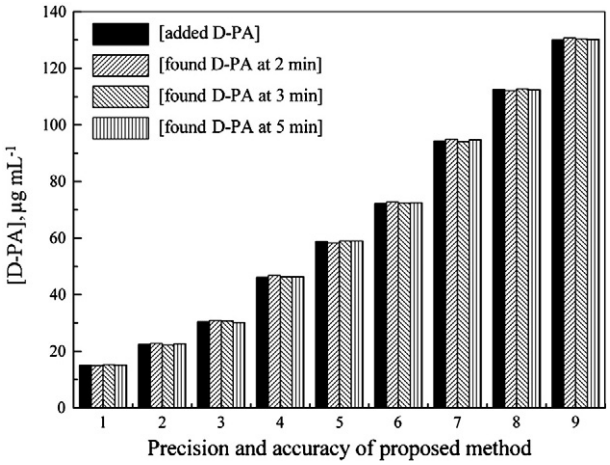


Fig. 4. Precision and accuracy of the proposed method under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02, I = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

3.6. Interference

The extent of interference by some pharmacologically inactive substances, which are generally used as a carrier for the active ingredient D-PA, was ascertained in the aqueous solutions containing 20 μg mL^{-1} of D-PA and various amounts of diverse species. The potential interferents studied included starch, saccharin, glucose, sodium phosphate, magnesium stearate and sucrose present in commercially available penicillamine formulations. It was found that the excipients starch, saccharin, glucose, sodium phosphate and sucrose do not interfere in the present method. However magnesium stearate is tolerable only up to 25 fold excess over D-PA concentration. An error of ±3% was considered tolerable. The typical results of the interference studies are shown in Table 2.

3.7. The precision and accuracy of the method

The study of the precision and the accuracy of the method were examined with nine independent experiments using solutions of various concentrations of D-PA under the optimum conditions. Using the three regression equations (*cf.* Table 1) recovery experiments in the range of 15.0–130.0 μg mL^{-1} [D-PA] were performed for the determination of D-PA in various spiked aqueous samples. The corresponding results, where the recoveries of the D-PA concentration were evaluated from the linear regression equations, are presented in Table 3 along with the % recoveries, the % error and the standard deviations (SD). The reproducibility of the method in the range studied is excellent with a standard deviation ±2 for the three fixed times *i.e.* A_2, A_3 and A_5. The results in Table 3 also show that the errors in all three cases of determination are well within acceptable limit where quantitative recovery could be obtained in each case. The recovery results corresponding to the three fixed times *i.e.* A_2, A_3 and A_5 are shown in Fig. 4, which clearly show that the recoveries obtained are in excellent agreement with the spiked values. The limit of detection, defined as three times the standard deviation of the blank (3σ), was determined as 5.16, 3.68 and 3.13 μg mL^{-1} of D-PA corresponding to fixed time of 2, 3 and 5 min respectively.

Table 4
Determination of D-PA in pharmaceutical preparations under the reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.2\times10^{-3}$ mol L^{-1}, pH = 3.80 ± 0.02, ionic strength, I = 0.05 mol L^{-1} (KNO_3), temperature = 25.0 ± 0.1 °C.

Drug	Label claim per tablet (mg)	Amount of drug found in mg, ±SD (n = 5), proposed method	Amount of drug found in mg, ±SD (n = 5), standard method [48]
Penicillamine tablets BP (Generics, UK)	125	124.96 ± 0.8	124.97 ± 0.2
Penicillamine tablets BP (Generics, UK)	250	251.26 ± 0.6	250.98 ± 0.6

3.8. Application of the method to the assay of D-PA in pharmaceutical preparations

The method was successfully applied for the determination of D-PA in pharmaceutical preparations and the results shown in Table 4 agree excellently with the standard method [48]. To study the application of the devised method, twenty tablets were weighed and the average mass per tablet was determined. Accurately weighed 500 mg of the finally grounded tablet material with D-PA was transferred into a 100 mL calibrated flask containing 70 mL de-ionized distilled water and solubilized. The content of the flask was sonicated for 15 min and diluted to 100 mL with de-ionized distilled water. The solution was finally filtered through a 0.45 μm Milli-pore Whatmann filter paper. The concentrations of the drug within the range of the calibration equation were obtained by accurate dilutions with de-ionized distilled water. The solution was directly analyzed, using the calibration equations established according to the proposed procedure (*vide infra*). The result of D-PA analysis by the present recommended procedure is in excellent agreement with those obtained by the standard method [48].

4. Conclusion

This is the first kinetic method proposed for the determination of D-PA using ligand substitution reaction. The proposed present uncatalyzed kinetic method is found to be simple, rapid and economical in comparison to the spectrophotometric methods available in the literature. The proposed kinetic spectrophotometric method is advantageous over many spectrophotometric methods as heating or extraction is not required. The method has a higher selectivity and is sufficiently sensitive to permit determination down to 14.92 μg mL^{-1} with 95% confidence. The method does not make use of any oxidant, organic dye or catalyst, thereby avoiding possible errors in the determination of D-PA. The recovery study data clearly indicate the high reproducibility and accuracy of the method.

Acknowledgments

One of the authors, Basant Kumar, is highly grateful to the Head Department of Chemistry, University of Lucknow, Lucknow, India for providing the necessary facilities for carrying out this research work. S. Prasad is grateful to the University of the South Pacific, Suva, Fiji for the financial support through URC project no. 62002-1321.

References

[1] K. Kuśmierek, E. Bald, Simultaneous determination of tiopronin and d-penicillamine in human urine by liquid chromatography with ultraviolet detection, Anal. Chim. Acta 590 (2007) 132–137.
[2] A. Martinović, N. Radić, Kinetic potentiometric determination of penicillamine and *N*-acetyl-L-cysteine based on reaction with iodate, Acta Chim. Slov. 56 (2009) 503–506.
[3] A.A. Al-Majed, Spectrophotometric estimation of D-penicillamine in bulk and dosage forms using 2,6-dichloroquinone-4-chlorimide (DCQ), J. Pharm. Biomed. Anal. 21 (1999) 827–833 (and references cited therein).
[4] A.A. Al-Majed, Specific spectrofluorometric quantification of d-penicillamine in bulk and dosage forms after derivatization with4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole, Anal. Chim. Acta 408 (2000) 169–175 (and references cited therein).
[5] J-Bakhsh Raoof, R. Ojani, F. Chekin, Voltammetric sensor for D-penicillamine determination based on its electrocatalytic oxidation at the surface of ferrocenes modified carbon paste electrodes, J. Chem. Sci. 121 (2009) 1083–1091.
[6] L. Ma, M. Fan, X. Xu, W. Kang, H. Shi, Utilization of a novel Ag(III)-luminol chemiluminescence system for determination of d-penicillamine in human urine samples, J. Braz. Chem. Soc. 22 (2011) 1463–1469.
[7] M. Yusof, R. Neal, N. Aykin, N. Ercal, High performance liquid chromatography analysis of D-penicillamine by derivatization with N-(1-pyrenyl)maleimide (NPM), Biomed. Chromatogr. 14 (2000) 535–540.
[8] M. Mazloum-Ardakani1, H. Beitollahi, B. Ganjipour, H. Naeimi, Novel carbon nanotube paste electrode for simultaneous determination of norepinephrine, uric acid and D-penicillamine, Int. J. Electrochem. Sci. 5 (2010) 531–546.
[9] N. Wangfuengkanagul, O. Chailapakul, Electrochemical analysis of D-penicillamine using a borondoped diamond thin film electrode applied to flow injection system, Talanta 58 (2002) 1213–1219 (and references cited therein).
[10] P. Davis, Penicillamine metal chelates and their possible importance in rheumatoid arthritis — a brief review, Clin. Invest. Med. 7 (1984) 41–44.
[11] I.L. Bonta, M.J. Parnham, J.E. Vincent, P.C. Bragt, 5 Anti-rheumatic drugs: present deadlock and new vista, Prog. Med. Chem. 17 (1980) 185–273.
[12] A.O. Muijsers, R.J. Van De Stadt, A.M.A. Henrichs, H.J.W. Ament, J.K. Van Der Korst, D-penicillamine in patients with rheumatoid arthritis serum levels, pharmacokinetic aspects, and correlation with clinical course and side effects, Arthritis Rheum. 27 (1984) 1362–1369.
[13] B. Kang, C. Veres-Thorner, R. Heredia, E. Cha, S. Bose, M. Schwartz, Successful treatment of far-advanced progressive systemic sclerosis by D-penicillamine, J. Allergy Clin. Immunol. 69 (1982) 297–305.
[14] R. Chapela, G. Zuniga, M. Selman, D-penicillamine in the therapy of fibrotic lung diseases, Int. J. Clin. Pharmacol. Ther. Toxicol. 24 (1986) 16–27.
[15] E.P. Lankmayr, K.W. Budna, K. Müller, F. Nachtmann, F. Rainer, Determination of D-penicillamine in serum by fluorescence derivatization and liquid column chromatography, J. Chromatogr. 222 (1981) 249–255.
[16] J-Bakhsh Raoof, R. Ojani, F. Chekin, Electrochemical analysis of D-penicillamine using a carbon paste electrode modified with ferrocene carboxylic acid, Electroanalysis 19 (2007) 1883–1889.
[17] J-Bakhsh Raoof, R. Ojani, M. Majidian, F. Chekin, Homogeneous electrocatalytic oxidation of D-penicillamine with ferrocyanide at a carbon paste electrode: application to voltammetric determination, J. Appl. Electrochem. 39 (2009) 799–805.
[18] J-Bakhsh Raoof, R. Ojani, M. Majidian, F. Chekin, Voltammetric determination of D-penicillamine based on its homogeneous electrocatalytic oxidation with potassium iodide at the surface of glassy carbon electrode, Russ. J. Electrochem. 46 (2010) 1395–1401.
[19] M. Mazloum-Ardakani, H. Beitollahi, Z. Taleat, H. Naeimi, N. Taghavinia, Selective voltammetric determination of D-penicillamine in the presence of tryptophan at a modified carbon paste electrode incorporating TiO_2 nanoparticles and quinizarine, J. Electroanal. Chem. 644 (2010) 1–6.
[20] F. Kreuzig, J. Frank, Rapid automated determination of D-penicillamine in plasma and urine by ion-exchange high-performance liquid chromatography with electrochemical detection using a gold electrode, J. Chromatogr. 218 (1981) 615–620.
[21] M.A. Abounassif, T.M. Jefferies, The determination of D-penicillamine and its disulphide in plasma by reversed-phase ion-pair high-performance liquid chromatography, J. Pharm. Biomed. Anal. 1 (1983) 65–72.
[22] S.E. Ibrahim, A.A. Al-Badr, Application of PMR spectrometry in quantitative analysis of penicillamine, Spectrosc. Lett. 13 (1980) 471–478.
[23] Y. Fujita, I. Mori, T. Yamaguchi, Spectrophotometric determination of biologically active thiols with eosin, silver(I) and adenine, Anal. Sci. 18 (2002) 981–985.
[24] V. Amarnath, K. Amarnath, Specific determination of cysteine and penicillamine through cyclization to 2-thioxothiazolidine-4-carboxylic acids, Talanta 56 (2002) 745–751.
[25] F.E.O. Suliman, H.A.J. Al-Lawati, S.M.Z. Al-Kindy, I.E.M. Nour, S.B. Salama, A sequential injection spectrophotometric method for the determination of penicillamine in pharmaceutical products by complexation with iron(III) in acidic media, Talanta 61 (2003) 221–231.
[26] B. Stypinski-Mis, G. Anderegg, The stability of palladium(II) complexes with sulphur-containing ligands, Anal. Chim. Acta 406 (2000) 325–332.
[27] V.K. Gupta, I. Ali, Determination of stability constants of Cu(II), Co(II) and Fe(II)-penicillamine-NTA ternary complexes, Talanta 46 (1998) 197–201.
[28] P.B. Issopoulos, S.E. Salta, High-sensitive spectrophotometric determination of micromolar concentrations of D-penicillamine by means of a coupled redox–complexation reaction, Farmaco 52 (1997) 113–118.
[29] R.M. Naik, A. Agarwal, S. Prasad, Ligand substitution reaction of hexacyanoruthenate(II) as a tool for mercury(II) estimation at micro level, Spectrochim. Acta A Mol. Biomol. Spectrosc. 74 (2009) 887–891.
[30] R.M. Naik, A. Agarwal, S. Prasad, A.K. Verma, Trace determination of thiosulphate and thioglycolic acid using novel inhibitory kinetic spectrophotometric method, Microchem. J. 93 (2009) 43–48.
[31] V. Chand, S. Prasad, Trace determination and chemical speciation of selenium in environmental water samples using catalytic kinetic spectrophotometric method, J. Hazard. Mater. 165 (2009) 780–788.
[32] R.M. Naik, J. Sarkar, S. Prasad, Kinetic determination of cysteine and thiosulphate by inhibition of Hg(II) catalysed ligand substitution reaction, Microchem. J. 88 (2008) 45–51.
[33] S. Prasad, R.M. Naik, A. Srivastava, Application of ruthenium catalyzed oxidation of [tris(2-aminoethyl)amine] in trace determination of ruthenium in environmental samples, Spectrochim. Acta A Mol. Biomol. Spectrosc. 70 (2008) 958–965.
[34] R.M. Naik, A. Srivastava, S. Prasad, Highly sensitive catalytic spectrophotometric determination of ruthenium, Spectrochim. Acta A Mol. Biomol. Spectrosc. 69 (2008) 193–197.
[35] S. Prasad, Kinetic determination of organosulphur ligands by inhibition: trace determination of cysteine and maleonitrilodithiolate [MNDT], Microchem. J. 85 (2007) 214–221.
[36] S. Prasad, Kinetic determination of mercury(II) at trace level from its catalytic effect on a ligand substitution process, J. Anal. Chem. 60 (2005) 581–588.
[37] S. Prasad, Kinetic method for determination of nanogram amounts of copper(II) by its catalytic effect on hexacynoferrate(III)-citric acid indicator reaction, Anal. Chim. Acta 540 (2005) 173–180.
[38] S. Prasad, Catalytic abstraction of cyanide in hexacyanoferrate(II) by Hg^{2+} in the presence of α-nitroso β-naphthol and trace determination of Hg(II) by kinetic method, Anal. Lett. 37 (2004) 2851–2867.
[39] S. Prasad, T. Halafihi, Development and validation of catalytic kinetic spectrophotometric method for determination of copper(II), Microchim. Acta 142 (2003) 237–244.

[40] S.B. Jonnalagadda, B. Pare, Recent rrends in kinetics in analytical chemistry and kinetic method for determination of ruthenium(III) using aniline blue-acidic chlorite reaction, Anal. Lett. 44 (2011) 1868–1878.
[41] L. Ka´rpa´ti, B. Penke, E´. Katona, I. Balogh, G. Va´mosi, L. Muszbek, A modified, optimized kinetic photometric assay for the determination of blood coagulation factor XIII activity in plasma, Clin. Chem. 46 (2000) 1946–1955.
[42] G. Lo´pez-Cueto, M. Ostra, C. Ubide, J. Zuriarrain, Fenton's reagent for kinetic determination, Anal. Chim. Acta 515 (2004) 109–116.
[43] I.A. Darwish, M.A. Sultan, H.A. Al-Arfaj, Novel selective kinetic spectrophotometric method for determination of norfloxacin in its pharmaceutical formulations, Talanta 78 (2009) 1383–1388.
[44] D.H. Macartney, A. McAuley, Kinetics of the formation and dissociation of pentacyanoferrate(II) complexes of cysteine, penicillamine, glutathione, and 2-mercaptoethylamine, J. Chem. Soc. Dalton Trans. (1981) 1780–1787.
[45] G. Brauer, Handbook of Preparative Inorganic Chemistry, in: 2nd ed., Academic Press, New York, USA, 1965, p. 1511.
[46] R.C. West, in: C.R.C. Hand Book of Chemistry and Physics, vol. 49, Chemical Rubber Co., Ohio, 1969, p. D79.
[47] A.R. Garafalo, G. Davis, Kinetics and mechanism of the thiocyanate-catalyzed dissociation of bis(pentacyanoferrate)(aq)$^{6-}$ in aqueous sodium perchlorate solution, Inorg. Chem. 15 (1976) 1787–1790.
[48] United States Pharmacopoeia (USP), in: XXIII Official Monographs, 1995, p. 1163.