J. Iran. Chem. Soc., Vol. 8, No. 3, September 2011, pp. 662-673.

JOURNAL OF THE
Iranian
Chemical Society

Reverse-FIA with Spectrophotometric Detection Method for Determination of Vitamin C

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(Received 26 May 2010, Accepted 30 August 2010)

Simple reverse flow injection analysis (rFIA) manifold with spectrophotometric detection was developed for an indirect determination of ascorbic acid. Parameters such as stability, accuracy and precision were established for the method and evaluated statistically to assess the applications of the method. Ascorbic acid in this procedure accelerates dediazoniation reaction of formed diazonium ions; hence its quantity can be determined by monitoring the derivatization product from coupling unreacted diazonium ion with phenol to give an azo dye (coupling reaction). The rFIA design was based on the injection of sodium nitrite into an acidic *p*-aminoacetophenon carrier stream in which diazonium ion was formed. This ion was inhibited by ascorbic acid stream before coupling with the phenol-Na_2CO_3 stream. Under optimum conditions, ascorbic acid acts in accordance with the Beer's law at two concentration ranges 0.4-6.5 μg ml^{-1} (R = 0.9995) and 7.0-20.0 μg ml^{-1} (R = 0.9949), with detection limits of 0.25 μg ml^{-1}. The developed method was applied to the determination of vitamin C in pharmaceutical formulations which produced satisfactory results compared with the standard methods reported in the British Pharmacopoeia.

Keywords: Reverse-FIA, Ascorbic acid, Pharmaceutical formulation, Diazonium salt

INTRODUCTION

Ascorbic acid (AA) (also known as L-ascorbic acid, anti-scorbutic vitamin and vitamin C) is a well-known and important water soluble vitamin [1]. Most plants and animals synthesize ascorbic acid for their own requirements. However, humans and apes cannot synthesize ascorbic acid due to lack of gulonolactone oxidase enzyme. Hence, ascorbic acid has to be supplemented mainly through fruits, vegetables and tablets. The major metabolites of AA in the human body are dehydroascorbic acid, 2,3-diketogluconic acid and oxalic acid [2].

Vitamin C is one of the most essential vitamins for both pharmaceutical and food processing industries in view of its nutritional significance, varied uses in food and high daily doses necessary for optimum health [3]. Therefore, it is an important analytical task to determine the content of AA in various products. Accordingly, a large number of methods have been reported for the determination of AA including titrimetry [4,5], voltammetry [6], amperometry [7], conductometry [2], potentiometry [8], fluorometry [9,10], spectrophotometry [3,11,12], chemiluminescence [1,13-15], flow-injection analysis (FIA) [16-19] and chromatography [20,21]. The present investigation involves developing a reverse-FIA method suitable for the determination of ascorbic acid and hence can be used for the routine quality control of pharmaceutical formulations, with advantages of simplicity, reproducibility and accuracy. This method may also be used as an attractice alternative method to the existing time consuming and expensive methods like titrations and HPLC.

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EXPERIMENTAL

Apparatus

The rFIA manifold employed in this study (Fig. 1) consisted of a peristaltic pump (DESAGA Heidelberg-England, with six channels) used to deliver the flow streams of the solutions of sodium carbonate, phenol, AA (standard or sample) and acidic *p*-aminoacetophenon (PAAP). Sodium nitrite solution was injected into the flow system by a six-way injection valve (Rheodyne-USA) *via* variable loop volumes. Eventually, the colored product formed was directed towards the detection device equipped with a flow cell of 100.0 µl capacity (Starna-micro-flow cell) and monitored spectrophotometrically using a JENWAY 6405 UV-Vis spectrophotometer.

Reagents and Sample Solutions

All reagents were analytical grade substances and distilled-deionized water (DDW) was used throughout.

Ascorbic acid. A 1000.0 µg ml^{-1} ascorbic acid (BDH) standard stock solution was prepared daily by dissolving 0.250 g of ascorbic acid in a sufficient amount of DDW, then transferred and diluted to 250.0 ml dark volumetric flask. A series of standard solutions were prepared by diluting the required volumes of the stock solution with DDW.

***p*-Aminoacetophenon (PAAP) solution.** A 0.05% (w/v) stock solution was prepared by dissolving 0.125 g of PAAP (Fluka) in a small amount of 0.05 M acetic acid and made up to 250.0 ml in a volumetric flask with the same solution. Other solutions were prepared by diluting this stock solution with the 0.05 M acetic acid.

Sodium nitrite solution. A stock solution (0.20 M) was prepared by dissolving 1.380 g $NaNO_2$ (Fluka) in a little amount of DDW and then diluted to 100.0 ml in a volumetric flask. This solution was prepared daily and kept in a cold place.

Phenol solution. 0.282% (w/v) solution was prepared by dissolving 1.410 g of phenol (BDH) in a small amount of DDW and then the solution was diluted to 500.0 ml in a volumetric flask.

Sample preparation. Solutions of ascorbic acid of different commercially available pharmaceutical products (tablets, injections and chewable tablets (Table 1)) were prepared as follows:

Tablets and chewable tablets. Ten weighed tablets of vitamin C were placed in a mortar and crushed to a fine powder, from which an accurately representative weighed portion equal to the weight of one tablet was mixed with DDW using an ultrasonic bath, until the powder was completely disintegrated and the resulting solution was allowed to settle. Then, the solution was filtered and diluted to 100.0 ml in a volumetric flask with DDW. Three different

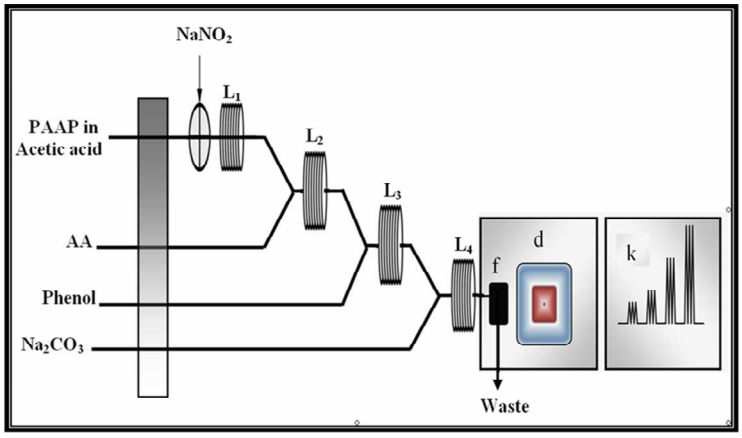

Fig. 1. Schematic diagram of rFIA-spectrophotometric detection; L_1,L_2,L_3 and L_4 are mixing coils, f: flow cell, d: detector, k: recorder; when $NaNO_2$ was injected into the injection valve.

Table 1. Commercially Available Pharmaceutical Products which was Analyzed in the Present Study

Trade name		Ascorbic acid (mg/tablet or injection)
Megavit tablet	Eurostock Ltd.,Bulgaria	500.0
Vitamin C inject	OUBARI pharma-Syria	500.0
Vitamin C tablet	Darou pakhs pharmaceutical Mfg.Co-Iran	250.0
Cetarit tablet	Alshahba HaBs.-Syria	500.0
Vitamin C tablet	Holland Medicines Company-Netherland	200.0
Kana Vitamin C	Kanawati-medical products-Syria	500.0
Vitamin C-chewable tablet	Darou pakhs-Iran	250.0

volumes of the resulting solution were diluted so that the concentration of vitamin C in each case was in the range of the calibration graph, and analyzed by the developed procedure.

Injections. The contents of 10 ampoules were mixed and a volume equal to that of one ampoule was transferred to 100.0 ml volumetric flask and diluted to the mark with DDW. Then, three different volumes of this solution were diluted, so that the concentration of vitamin C was in the range of the calibration graph and determined by the developed procedure.

Solutions of interfering species. Stock solutions of each species that exist in pharmaceutical products under study were prepared in DDW according to Table 2. Other solutions were prepared by serial dilutions of each stock solution.

Preliminary Procedure

In this rFIA system, sodium nitrite solution was injected into a stream of acidic PAAP solution in which diazonium ions were formed. Then, this line was merged with a stream of AA (standards or samples). The resulting mixture of these two streams, containing unreacted diazonium ions, was merged with a stream of phenol solution. Finally, the latter mixture was made into an alkaline by mixing it with sodium carbonate stream to form a red-orange colored azo dye product, which was monitored spectrophotometrically at 460 nm. Accordingly, by measuring the differences in signals obtained in the absence and presence of AA, which appeared as color fading, the quantity of AA was obtained. The possible mechanism of diazotization reaction of PAAP and subsequent coupling with phenol solution is illustrated in Fig. 2.

RESULTS AND DISCUSSION

Absorption Spectrum

The diazonium ion formed from the reaction between nitrite ions and acidic solution of PAAP reacts with phenol solution in alkaline medium to form a red-orange colored azo dye with a maximum absorption peak at 460 nm (Fig. 3).

Optimization of Manifold Design

Depending on the sequence of the reactions and upon keeping all variables constant, it is possible to inject phenol, acidic PAAP, or $NaNO_2$ solutions. Accordingly, three different manifolds were suggested as depicted in Figs. 1 and 4. A comparison was made between these three manifolds depending on the sensitivity, as an absorbance peak height, when three different concentrations of AA were used in each case and also on the differences in the signals obtained between that of the blank and for AA at each concentration. However, depending on the results obtained, manifold design Fig. 4A was not favorable. This may be attributed to the excessive dispersion in the signal due to the instability of the diazonium salt formed as the determination was greatly affected by the degree of unreacted salts. When the designs in Fig. 1 and 4B are compared, better results were obtained with the design in Fig. 1, *i.e.* when $NaNO_2$ was injected into the stream of acidic PAAP; therefore, it was selected for the

Table 2. Interferences Preparation (1000.0 µg ml^{-1})

Species	Amount weight (mg/100.0 ml)	Supplier
Riboflavine	100.0	Schuchardt münchen
Thiamin	100.0	Fluka
Caffeine	100.0	SDI
Nicotinic acid	100.0	BDH
Nicotinamide	100.0	Merck
Pyridoxine	100.0	SDI
Cysteine	100.0	BDH
Histidine	100.0	BDH
Glucose	100.0	BDH
Fructose	100.0	Fluka
Citric acid	100.0	Fluka
Saccharine	100.0	Fluka
Ethyl glycole	100.0	BDH
Sucrose	100.0	Fluka
Dextrose	100.0	Fluka
Polyethelen glycole	100.0	BDH
Sorbitol	100.0	BDH
Oxalic acid	100.0	Fluka
Tartaric acid	100.0	Fluka
Benzoic acid	100.0	Fluka
Salicylic acid	100.0	SDI
Folic acid	100.0	SDI
Starch	100.0	BDH
NaCl	100.0	Fluka

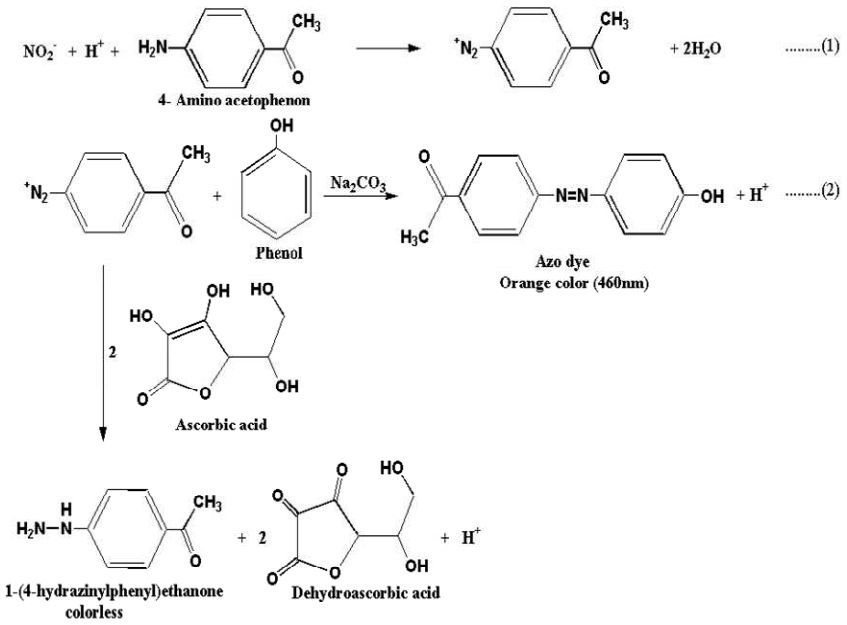

Fig. 2. Mechanism of the diazotization and dediazoniation reaction.

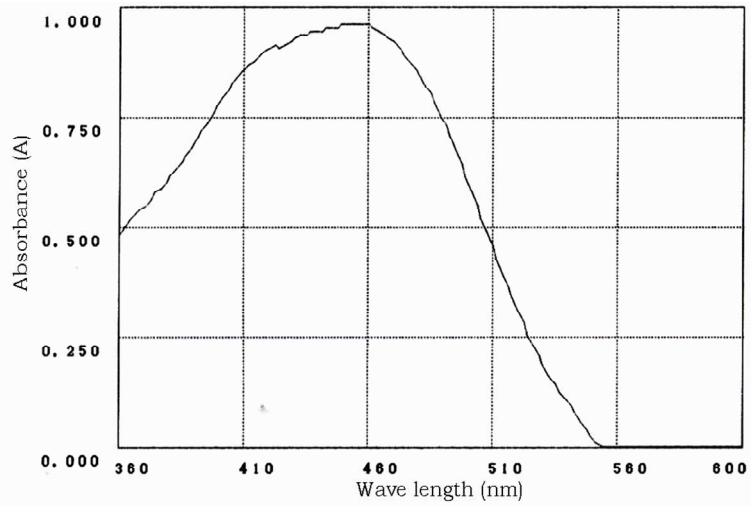

Fig. 3. Absorption spectrum of azo-dye product from acidic PAAP and $NaNO_2$ when mixed with phenol in the presence of Na_2CO_3 base.

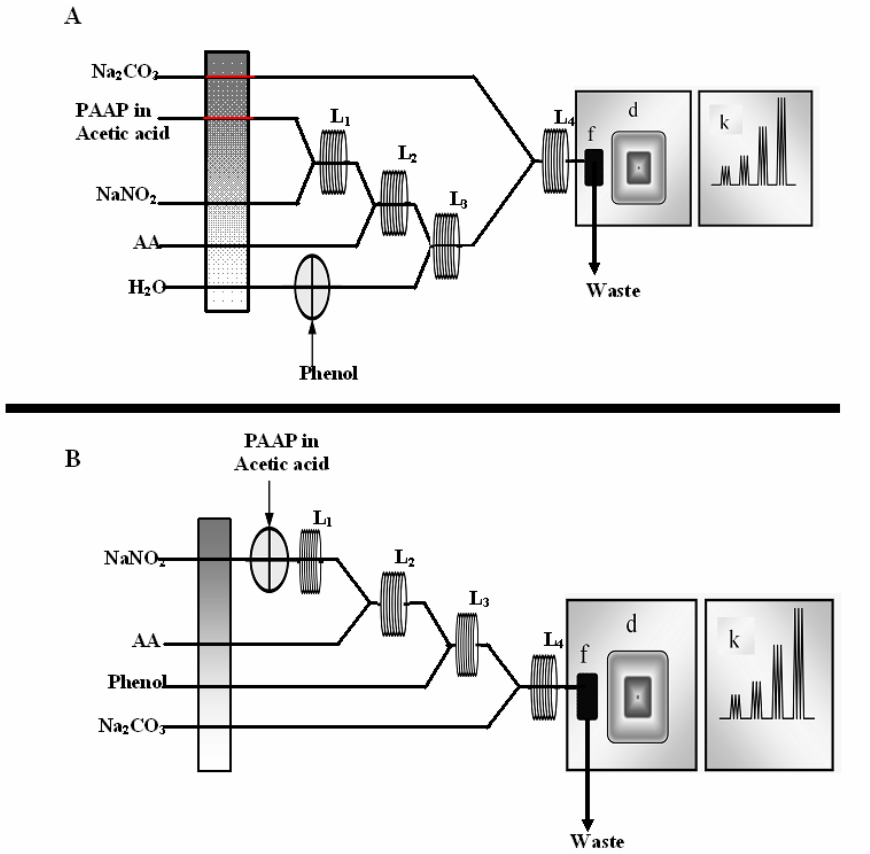

Fig. 4. Manifold design optimization using different reagent injection systems: (A) Phenol injection system. (B) Acidic PAAP injection system.

subsequent work.

Optimization of Chemical Variables

Effect of phenol concentration. Effects of different phenol concentrations in the range of 0.001-0.02% (w/v) as a coupling reagent on the absorption peak height in the absence (blank) and presence of 12.0 μg ml^{-1} AA were investigated using the rFIA design shown in Fig. 1 and fixing all other experimental variables. The results shown in Fig. 5, illustrate that the optimum phenol concentration was 0.008% (w/v) making large differences in the peak height between that of blank and AA. Higher concentration of phenol solution had no effect since the signal remained almost constant, when no further diazonium ion was present.

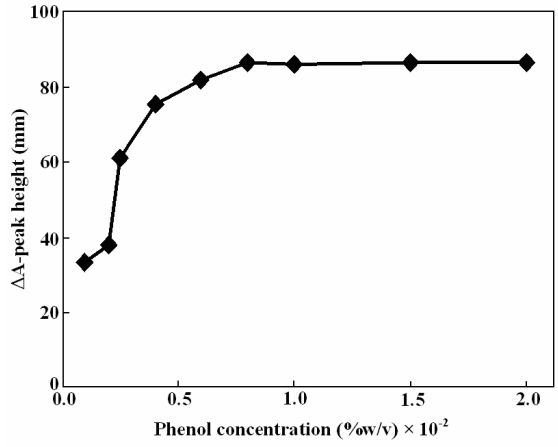

Fig. 5. Effect of phenol concentration.

Effects of type and concentration of the acid. It is of utmost importance that the coupling medium be adjusted to the right degree of pH, because nitrous acid (HONO) (generated by the action of acid on sodium nitrite) is instable. Hence, optimization of type and concentration of the acid used were very important. Accordingly, the effects of different types of acids (namely H_2SO_4, HNO_3, HCl and CH_3COOH) on the absorbance were studied as a peak height (mm). The results illustrated that acetic acid (CH_3COOH) was the best.

Therefore, different concentrations of acetic acid (0.001-0.100 M) were used to find the optimum acid concentration. Results demonstrated in Fig. 6, indicate that increasing acid concentration up to 0.03 M increased the difference in the absorption peak height, beyond which the signals started to decrease. Effect of the acidity on the present system may be attributed to different factors. First of all, the higher the acidity the higher the proportion of amine that lowers the rate of coupling. Furthermore, high acidic medium tends to increase the stability of AA which in turn decreases its ability toward a reduction. In other words, ascorbic acid acts as a reductant of nitrite ions to form nitric oxide under mild acidic conditions [22]. Additionally, phenol is appreciably acidic in aqueous solution and exists in equilibrium with phenoxide ion, which is much more reactive towards coupling reaction. On the other hand, coupling reaction with phenols occurs in mild alkaline solution [23], therefore, great attention must be paid to the optimization of acid concentration. Thus, 0.03 M acid was selected as an optimum concentration.

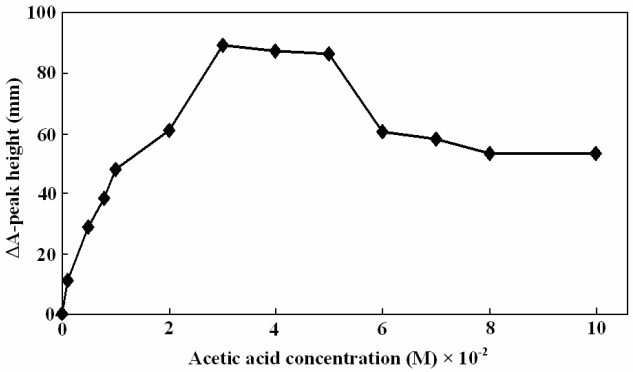

Fig. 6. Effect of acetic acid concentration.

Effect of PAAP concentration. As regards fixed chemical and physical variables, effects of different acidic PAAP concentrations (0.0015-0.12% (w/v) in 0.03 M acetic acid) were studied. Figure 7 shows the results obtained. PAAP concentration 0.02% (w/v) causes the largest differences in absorption signals between blank and AA concentrations.

Effect of concentration and type of the used base. Among different types of bases (NaOH, KOH and Na_2CO_3) that exhibit a rapid coupling reaction and more intense azo dye color Na_2CO_3 is found to be the best. Therefore, effects of Na_2CO_3 concentration, in the range of (0.005 to 0.15 M) on the reaction were investigated. At low base concentration (somewhat acidic), less amounts of phenoxide ion were formed, which led to the low rate of coupling and decrease in the color intensity, whereas, high concentration of the base

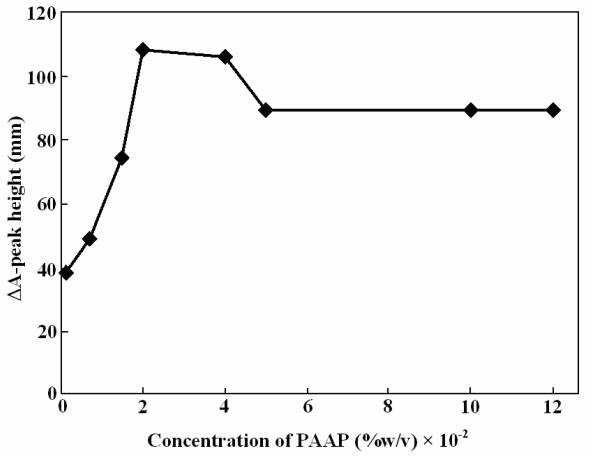

Fig. 7. Effect of acidic PAAP.

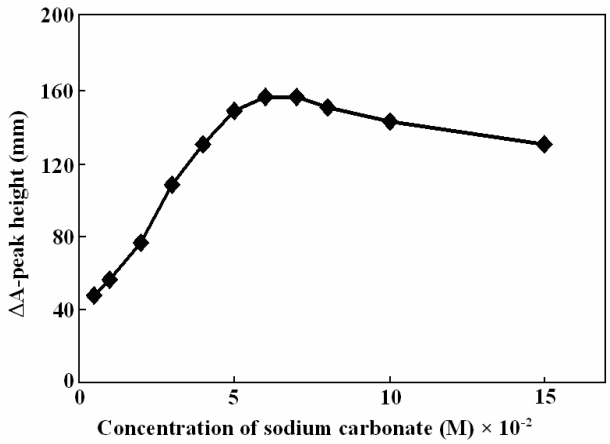

Fig. 8. Effect of different concentrations of the sodium carbonate base.

decreased the degree of diazonium ion formation and hence low signals were obtained. Thus, the results displayed in Fig. 8 demonstrate that optimum absorbance peak height (mm), in the form of the differences between that of the blank and 12.0 μg ml^{-1} AA, was obtained with 0.06 M of Na_2CO_3.

Effect of sodium nitrite. Effect of different concentrations of $NaNO_2$ (1×10^{-3}-1.5×10^{-1} M) on the present system was studied by measuring the extent of the diazonium ion formed as well as the intensity of the formed color. The results obtained are depicted in Fig. 9. Generally, sodium nitrite should not be used in excess since an excess of nitrous acid exerts a very unfavorable influence on the stability of diazo solutions [24]. In addition, it is important to note that ascorbic acid interacts rapidly with nitrite ions to form dehydroascorbic acid [22,25]. Therefore, large excess of the nitrite salts must be avoided. These were in agreement with the results shown in Fig. 9 where the effects of high and low concentrations of the nitrite ions are observed. Hence, 0.5×10^{-2} M is considered as an optimum concentration for the present system.

Optimization of Physical Variables

Effect of flow rate. Effects of different flow rates (0.4-3.0 ml min^{-1}) on the analytical signals as a peak height were investigated. The results obtained are illustrated in Fig. 10. A flow rate of 0.8 ml min^{-1} is found to be the optimum, taking into account shape and value of the obtained signal.

Volume of the injected reagent. Different volumes (50.0-250.0 μl) of sodium nitrite solutions were injected into the system. The results shown in Fig. 11 reveal that an optimum reagent volume in which high absorbance differences are obtained appears with 120.0 μl volume. Increasing the reagent volume leads to the increase in the absorbance signals (as differences in peak height (mm)) due to an increase in the extent of the reaction while the peak signals lose their smoothness. Therefore, a 120.0 μl volume was selected since it led to a reasonable peak height with well-shaped FIA peaks.

Coil length. Depending on the progression of the reactions, four reacting coils were proposed for use as described in the manifold shown in Fig. 1. Effects of each coil (2.0 mm i.d.) between 20.0-120.0 cm lengths were studied. The results are shown in Fig. 12.

Coil 1 was used to create diazonium salt from the reaction between acidic PAAP with the injected volume of nitrite. The results clearly show that increasing the length has a positive effect on the absorbance peak height which is due to the sufficient time available to produce the desired salt. Conversely, coil lengths greater than 100.0 cm caused a decrease in the signals probably due to instability of the diazonium salt with time and also due to the excessive dispersion of the product plug. Thus, the peak height would be maximal when the rates of the two processes are equal and this is obtained with 100.0 cm length reaction coil1.

Coil 2 was used to reduce the formed salt in the previous stream quantitatively by ascorbic acid. This reaction proceeded to its great extent only when the reaction coil length

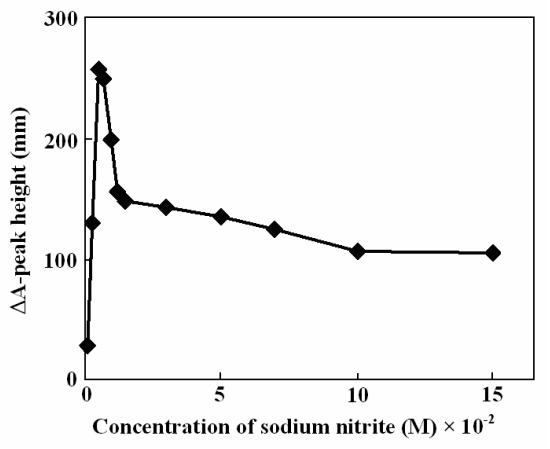

Fig. 9. Effect of sodium nitrite concentration.

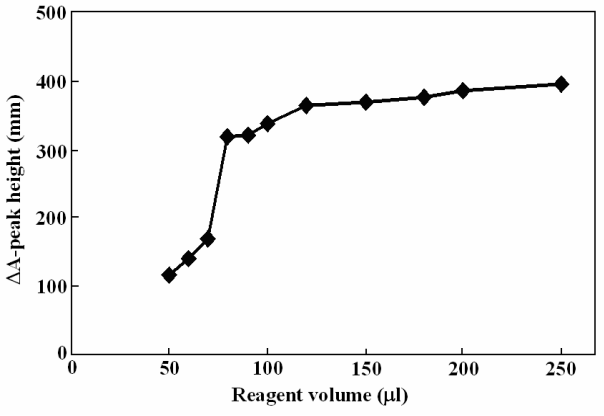

Fig. 11. Influence of injected volume of the reagent.

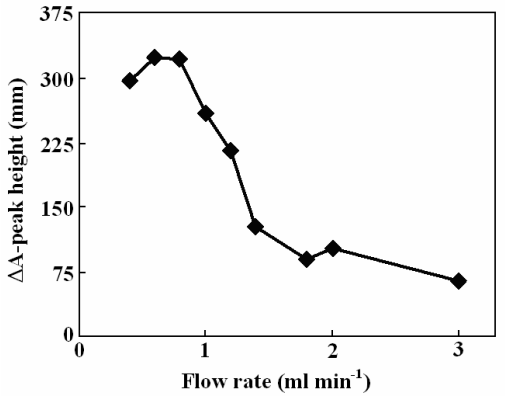

Fig. 10. Influence of flow rate system.

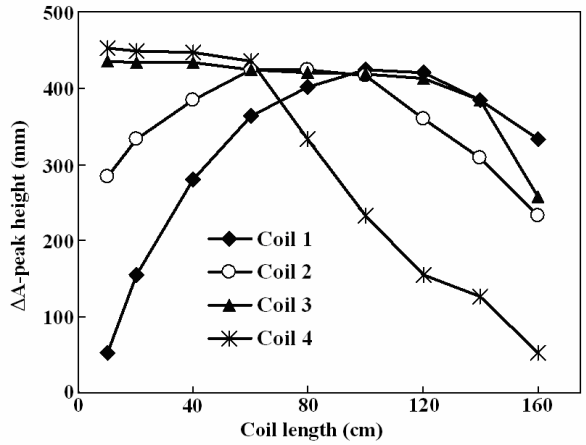

Fig. 12. Effect of different coils in length.

was 60.0 cm. Otherwise, shorter or longer coil 2 has negative effects on the absorption peak.

Coils 3 and 4 were used to produce the coupling reaction between unreacted diazonium salts with a phenol stream in a basic medium stream. This may require only 10.0 cm length of each coil (3 & 4) to reach the optimum value.

Optimization of Different AA Stabilizers

Due to the instability of ascorbic acid, special precautions are required in the preparation of its solutions. Therefore, effects of many stabilizers were studied as shown in Table 3. Deionized-distilled water (DDW) was found to be the best one which causes larger differences in absorbance values (ΔA-peak height (mm)).

The summary of the optimum values of the physical and chemical variables of the developed rFIA-spectrophotometric method used for the determination of AA are illustrated in Table 4.

Calibration Graph

A series of standard solutions of vitamin C was prepared and analyzed using the present rFIA system (Fig. 1) under the optimum operating conditions mentioned in Table 4 with a sample frequency of 56.0 sample/h. The calibration equation was obtained from a plot of the difference in the absorbance of the formed azo dye in the absence (A_1) and presence (A_2) of AA analyte (*i.e.*, $A_1 - A_2 = \Delta A$) as peak heights (mm) versus different concentrations of AA (µg ml^{-1}). The results show that

Table 3. Demonstrate Different Stabilizers Medium Used in the Literatures

Medium	Sample	Ref.
0.1 g EDTA and 4.0 g l^{-1} formic acid	Fruit, jam and vitamin preparation	[26]
0.05 M $HClO_4$	Tablets	[27]
0.1 M H_2SO_4	Pharmaceuticals	[4]
10% Acetic acid containing 5% Metphosphoric acid	Urine	[28]
10% Trichloroacetic acid	Blood, fruits and urine	[29]
Phosphate buffer (pH 6.6)	Pharmaceuticals	[30]
0.014 M HNO_3	Pharmaceuticals	[31]
0.5% Oxalic acid	Pharmaceuticals and fruit juices	[32]
Citric acid-NaH_2PO_4 buffer	Pharmaceuticals	[33]
Water (DDW)	Pharmaceuticals and foods	[3,34,35-38]

Table 4. Optimum Conditions Used for the Determination of AA by rFIA-Spectrophotometric Method

Parameters	Optimum values
Sodium nitrite concentration	5×10^{-3} M
Acetic acid concentration	3×10^{-2} M
PAAP concentration	0.02% (w/v)
Phenol concentration	0.008% (w/v)
Sodium carbonate concentration	0.06 M
System flow rate	0.8 ml min^{-1}
Coil 1 length	100.0 cm
Coil 2 length	60.0 cm
Coil 3 length	10.0 cm
Coil 4 length	10.0 cm
Reagent volume injection	120.0 µl
Sampling frequency	56.0 sample/h

Table 5. Statistical Parameters Obtained from Calibration Graph

Analyte	Linear range (µg ml^{-1})	Detection limit (µg ml^{-1})	Correlation coefficient (R)
Ascorbic acid	0.4-6.5 7.0-20.0	0.25	0.9995 0.9949

there are two ranges in which the graph shows good linearity (Fig. 13). The first linear range is from 0.4 to 6.5 µg ml^{-1} with a regression equation of (Y = 62.157X + 2.6679) and a correlation coefficient of 0.9995 while the second one is between 7.0 to 25.0 µg ml^{-1} with a regression equation of (Y = 7.3074X + 364.47) and correlation coefficient of 0.9949. Table 5 illustrates other statistical variables.

Accuracy and precision of the current system were checked through the applications of two different concentrations of vitamins for each linear range with five

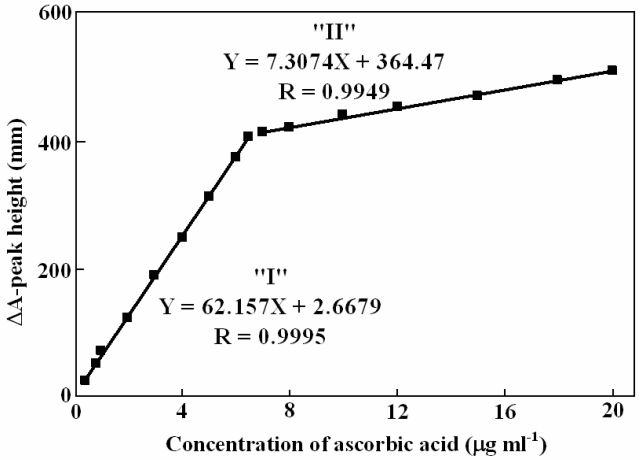

Fig. 13. Linear calibration curve for AA determination; (I) at low concentrations (0.4-6.5 μg ml^{-1}). (II) At high concentrations (7.0-25.0 μg ml^{-1}).

replications as shown in Table 6.

Effect of Interferences

Effects of foreign contaminated species on the determination of AA in real samples were studied through analyzing synthetic sample solutions containing 5.0 μg ml^{-1} AA and excess amounts of the common excipients and tablet fillers used in pharmaceutical preparations that include sugars, vitamins and a number of organic acids. This study was carried out by comparing the signal obtained when AA present alone and in the presence of different concentrations of the interferences. The study showed that the method was not affected by any significant interference, when a substance was considered not to interfere if the variation in the peak height of AA was equal to or less than ±5.0%. The results obtained are tabulated in Table 7. Positive interference effects from some existing vitamins (folic acid and riboflavin) could be attributed to their colors.

Analysis of Real Samples

Table 8 illustrates a perfect application of the developed method for the determination of AA in pharmaceutical formulations. The results obtained by using the present method are compared with those of a reference method showing good agreement. The comparison was based on the student t-test and variance ratio F-test [39]. The student t-value at the 95% confidence level and 6 degrees of freedom did not exceed the theoretical values for the results obtained (t_{method} = 1.3456 < t_{table} = 2.4500), indicating that there was no significant difference between the developed method and the standard method. It was also noticed that the variance ratio F-values calculated for the recommended procedure did not exceed the theoretical values, (F_{method} = 1.0243 < F_{table} = 4.2800) indicating that there was no recognized difference between the precision of the proposed method and that of the official methods.

CONCLUSIONS

This investigation involved innovation of rFIA with spectrophotometric detection and its application for the determination of vitamin C in some pharmaceutical products available in the local market. The method showed good sensitivity through the modification of the FIA technique. The modification included injecting small volumes of reagent into the flowing sample stream, rather than the conventional injection of the sample into a flowing reagent (or inert carrier) stream. This was carried out to minimize the reagent consumption and to eliminate background absorption from a sample matrix or even from a continuously generated signal.

The method is well-suited for pharmaceutical analysis. Sample preparation is minimal, no pretreatments are required and the analyses are simple, accurate and much precise. The cost of all chemical standards and reagents is low (since no

Table 6. Precision and Accuracy Measurement of the Developed Method

Analyte	Linear range (μg ml^{-1})	Analyte concentration (μg ml^{-1})		RSD%	E%
		Stream line	From the peak height		
Ascorbic acid	0.4-6.5	0.6	0.62	3.00	3.33
		4.0	4.10	2.90	2.50
	7.0-25.0	10.0	9.90	1.20	-1.00
		15.0	15.05	1.25	0.33

Table 7. Influences of Different Interference Species

Substances	MAC ($\mu g\ ml^{-1}$)[a]	Ascorbic acid ($\mu g\ ml^{-1}$)		Recovery (%)	TCR[c]
		Added	Found[b]		
NaCl	100.0	5.0	5.10	102.00	20.0
Caffeine	100.0	5.0	5.03	100.60	20.0
Nicotinic acid	100.0	5.0	5.14	102.80	20.0
Starch	100.0	5.0	5.09	101.80	20.0
Saccharine	100.0	5.0	4.99	99.80	20.0
Ethyl glycol	100.0	5.0	5.12	102.40	20.0
Sucrose	100.0	5.0	4.98	99.60	20.0
Dextrose	100.0	5.0	4.99	99.80	20.0
Polyethelen glycol	100.0	5.0	5.13	102.60	20.0
Sorbitol	100.0	5.0	5.11	102.20	20.0
Glucose	100.0	5.0	4.98	99.60	20.0
Fructose	100.0	5.0	4.99	99.80	20.0
Benzoic acid	50.0	5.0	4.89	97.80	10.0
Acetylsalicylic acid	50.0	5.0	5.10	102.00	10.0
Citric acid	50.0	5.0	5.02	100.40	10.0
Tartaric acid	50.0	5.0	4.99	99.80	10.0
Pyridoxine	10.0	5.0	4.99	99.80	2.0
Thiamin	10.0	5.0	5.12	102.40	2.0
Nicotinamide	5.0	5.0	5.12	102.40	1.0
Cysteine	5.0	5.0	5.09	101.80	1.0
Histidine	5.0	5.0	5.08	101.60	1.0
Oxalic acid	5.0	5.0	5.19	103.80	1.0
Riboflavin	5.0	5.0	5.24	104.80	1.0
Folic acid	5.0	5.0	5.19	103.80	1.0

[a]Maximum Allowable Concentration. [b]Mean of three replicate analyses. [c]TCR: Tolerable Concentration Ratio with no interferences (Conc. Interferent ($\mu g\ ml^{-1}$)/Conc. AA ($\mu g\ ml^{-1}$)).

Table 8. Application of the Two Developed Methods for the Analyses of AA in Pharmaceutical Products

Trade name	Ascorbic acid			E%
	Labeled amount (mg/tablet or injection)	Detectable amount [a] (mg/tablet or injection)		
		1[b]	2[c]	
Megavit tablet	500.0	510.0	502.6	1.47
Vitamin C Injection	500.0	508.9	512.1	-0.62
Vitamin C Tablet	250.0	251.4	249.6	0.72
Cetarit tablet	500.0	506.3	504.3	0.40
Vitamin C Tablet	200.0	201.3	203.5	-1.08
KANA Vitamin C	500.0	508.1	502.7	1.07
Vitamin C- Chwable tablet	250.0	241.5	240.4	0.46

[a]Average of five replication (n = 5). [b]Method 1 = rFIA-spectrophotometric method. Method 2 = standard method.

Large amounts of expensive reagents were consumed when the latter was injected into a small amount, which was one of the rFIA utilities), and it is economic in terms of capital, fast and running costs.

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