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Rapid Potentiometric Determination of Ascorbic Acid Using Iodate as a Reagent

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What is proposed in is proposed a fast and simple kinetic potentiometric method for the determination of ascorbic acid (AA) in pharmaceuticals. The method was based on the reaction between AA and iodate to form iodide. A combined iodide ion-selective electrode was used in order to monitor the change in iodide concentration. In the process of a univariate optimization study, experimental conditions were determined that allow a linearity between the concentration and the change of potentials, Δ*E*, in the range from $8.0 \cdot 10^{-5}$ to $8.0 \cdot 10^{-4}$ mol L^{-1}. Less than two minutes' time was needed for the proposed method to enable determination of the AA in pharmaceuticals with satisfactory recovery and the usual excipients did not act as interference.

Keywords: ascorbic acid, potentiometric determination, kinetic method, iodide ion selective electrode, iodate, pharmaceuticals

1. INTRODUCTION

The ascorbic acid (AA) is an essential water-soluble vitamin [1-4], its L−chiral form in particular. Due to its reducing and antioxidant properties, AA plays a significant role in human metabolism [1,3-5]. It participates in collagen synthesis, neutralizes the action of free radicals and enhances the immune response [1,2,5,6]. It maintains order in cellular metabolic processes by preventing various chronic diseases and the harmful effects of microorganisms [2,5-7]. The beneficial effect of this vitamin on human health has long been known and discussed; sometimes might it seem as though science has already said its piece on vitamin C. However, scientific research in the field of biomedicine and

chemistry has steadfastly kept its focus on vitamin C. Since the human body requires the intake of ascorbic acid, the content of this vitamin in food, food supplements and pharmaceuticals, but also in cosmetic products, is of extreme importance [1,5-9].

Considering the aforementioned, it is very important to have analytical methods for the reliable determination of ascorbic acid.

Similar to the field of biomedical sciences, in the field of chemical analysis, there is a wealth of information available on a whole host of different methods for analysis, ranging from classical volumetric methods to the application of various instrumental analytical techniques.

The earlier, perhaps most commonly used ones, are direct [10] and trimetric methods [11,12] of analysis that can be applied to samples with a simple matrix that will not interfere. Due to the simplicity of application, spectrophotometric methods of analysis [13,14] or spectrophotometry with the use of flow systems [15] are very often described and developed, and the same can be said of capillary electrophoresis [16] and liquid chromatography[17]. It is quite clear that most of the methods described, especially the electroanalytical ones, are based on the reducing properties of ascorbic acid.

In a review paper from 2015 [18], Skrovankova and co-workers described a number of mainly, mostly electroanalytical ones, but there were other methods important for the determination of ascorbic acid in various samples that were also highlighted.

A Web of Science® search featuring keywords “determination of ascorbic acid” for publications on the topic in the period from 2016. to 2022. yielded about 372 results, mainly scientific papers. On the other hand, a query for “potentiometric determination of ascorbic acid” gave only three results.

In this paper, we want to show that it is possible to offer a reliable method and achieve good results in the determination of ascorbic acid with the use of a very simple potentiometric apparatus and reagent that is well known and available to all laboratories.

To the best of our knowledge, the use of iodate and iodide ion selective electrodes in the determination of ascorbic acid is generally a characteristic of titrimetric methods [11,12] or methods using a flow injection analysis [19]. The advantage of this method is an extremely simple, inexpensive and fast procedure, which allows satisfactory precision by applying three solutions: standard/sample, iodate, and acid. All of the virtues of the method mentioned above will be thoroughly described and discussed in this paper.

2. MATERIALS AND METHODS

2.1. Apparatus

A millivoltmeter (Seven Compact pH/ion meter, Mettler Toledo, Columbus, OH, USA) equipped with combined iodide ion selective electrode (perfectION comb I™ Mettler Toledo, Columbus, OH, USA) and connected to a personal computer was used to measure the change of potentials. The data collected on potentials were then processed and stored with the aid of LabXdirect pH (Mettler Toledo) software. For all the necessary measurements, laboratory glasses identical in volume and size were used for reaction solutions that had been prepared in ultrapure water using the Millipore Simplicity 185

purification system (Millipore, Burlington, MA, USA) with a resistivity of 18.2 MΩ cm^{-1} at 25 °C. In addition, reaction solutions were stirred at a with the constant stirring speed and with Teflon magnetic bars, all of them equal in size.

2.2. Reagents

The solutions used in this experiment were prepared with ultrapure water using analytical-reagent grade chemicals.

A sulfuric acid solution with the concentration of $1.5 \cdot 10^{-1}$ mol L^{-1} was prepared by diluting concentrated sulfuric acid (Sigma-Aldrich, St. Louis, MO, USA, ACS reagent, purity 98%). This solution was used to achieve the optimal pH of the reaction mixture and for preparing stock solutions of samples and the standard. A stock solution of ascorbic acid (Sigma−Aldrich, St. Louis, MO, USA, ACS reagent, purity >99%), $5.0 \cdot 10^{-2}$ mol L^{-1}, was prepared by dissolving an appropriate amount of available substance in 10.00 mL of $1.5 \cdot 10^{-1}$ mol L^{-1} sulfuric acid solution and then diluting with ultrapure water to final volume of 100.0 mL. Working solutions of lower concentrations were prepared by diluting the stock solution with ultrapure water to the nominal volume. A work solution of potassium iodate (Sigma−Aldrich, St. Louis, MO, USA, ACS reagent, purity >99.5%), $1.0 \cdot 10^{-3}$ mol L^{-1}, was prepared by diluting of $1.0 \cdot 10^{-2}$ mol L^{-1} stock solution with ultrapure water to the nominal volume

2.3. Samples

Ten tablets of pharmaceutical products containing AA, manufactured by different pharmaceutical companies listed in table 2 were weighted and then crushed. The average mass of one tablet was calculated, weighted and transferred quantitatively to a 100.0 mL volumetric flask. After that, this sample was then dissolved by adding 10.0 mL of sulfuric acid ($1.5 \cdot 10^{-1}$ mol L^{-1}) and diluted with ultrapure water to the mark. Work sample solutions were prepared by diluting the stock solution in ultrapure water. The stock sample solutions were filtered before they were diluted, if necessary. Sample solutions and ascorbic acid standards were prepared daily.

2.4. Procedures

10.0 mL of ultrapure distilled water, 2.00 mL 0.15 mol L^{-1} sulfuric acid and 1.00 mL $1.0 \cdot 10^{-3}$ mol L^{-1} potassium iodate. AA reduced iodate to iodide were quantitatively pipetted in the vessel in order to obtain the base reaction solution. The iodide concentrations were monitored during the experiments by a combined iodide ion−selective electrode that was connected to a millivoltmeter (SevenExcellence, Mettler Columbus, OH, USA) and was immersed in the prepared reaction solution and the change of potential was continuously monitored and recorded at a 1.0−second interval. After a short time, no more than 60.0 s, the potential was stabilized (*E*1). At this time, 60 s after beginning measurement, 1.0 mL of standard or sample of AA was pipetted into reaction solution. Potential *E*2 was noted 40 seconds after solution AA was added. The total time for a measurement was 100 seconds. The final volume of reaction

solution in the vessel was 14.0 mL. All measurements were performed under stirring conditions at room temperature.

The calibration graph was constructed by plotting the change in potential ΔE ($E1-E2$) as a function of the negative logarithm of the ascorbic acid concentration, p(AA).

After each experiment, the electrodes were washed with ultrapure water. If it was needed, the electrode was polished between a series of experiments.

3. RESULTS AND DISCUSSION

After the addition of ascorbic acid to the reaction solution containing iodate, there was a redox reaction begun and iodides were formed, which resulted in a decrease in the potential of the cell that was being used.

The amount of iodide that is formed under selected experimental conditions is equivalent to the amount of AA added in the reaction solution. Therefore, the decrease in potential during the measurement will be proportional to the concentration of AA.

Based on the proposed procedure, described in the previous chapter, it is clear that it is necessary to examine the effect of the following variables on the difference in potential should be examined: time at which the potential is noted and monitored, H_2SO_4 concentration, and iodate concentration.

During preliminary measurements, the slope of the electrode was examined and the time required for a stable initial potential was determined. After a series of measurements, an increase in the time required to establish a stable potential was observed or the potential values were significantly different, and therefore the electrode needed to be polished and conditioned.

Furthermore, the potential ($E2$) sampling time, was chosen because of the sensitivity of the method. Time intervals of 20 s, 40 s and 60 s were tested. The reaction is fast, a linear change of potentials can be observed as quickly as 20 s after AA was added. A time interval of 40 s was chosen as the potential ($E2$) sampling time for further experiments because it enables better method sensitivity. .

During the process of selecting the most suitable experimental conditions, the values of the tested parameter changed while all of the other variables remained the same. $5.0 \cdot 10^{-4}$ mol L^{-1} AA was used for these experiments.

The effect of H_2SO_4 was studied in the concentration range from 0.05 mol L^{-1} to 0.5 mol L^{-1}. The AA concentration selected for this experiment was $5.0 \cdot 10^{-4}$ mol L^{-1}. The analytical signal ($\Delta E = E1-E2$) has the highest value at a H_2SO_4 concentration of 0.15 mol L^{-1} (Figure 1) and this acid concentration was selected as the most suitable for further experiments.

The effect of potassium iodate was studied in the concentration range from $5.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-2}$ mol L^{-1}. The potential change was increased by increasing the iodate concentration until it reached a maximum at a concentration of $1.0 \cdot 10^{-3}$ mol L^{-1},

Figure **2**. At concentrations higher than this one, there was a noticeable decrease in the potential change.. The iodate concentration of $1.0 \cdot 10^{-3}$ mol L^{-1} was selected for further experiments.

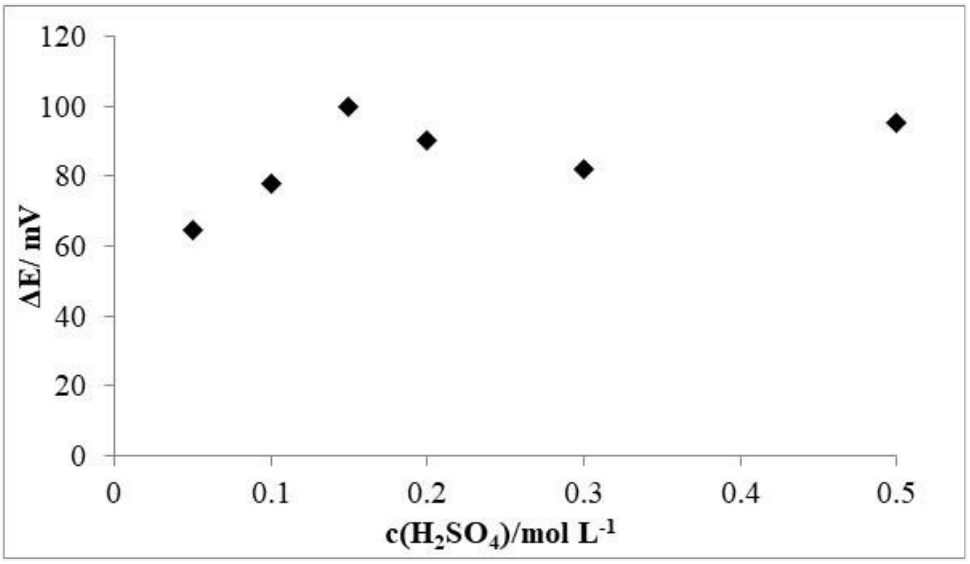

Figure 1. Effect of H_2SO_4 concentration change on potential change. Experimental condition: $c(H_2SO_4)$, mol L-$^{-1}$: 0.05, 0.1, 0.15, 0.2, 0.3, 0.5; c(AA) = $5.0 \cdot 10^{-4}$ mol L^{-1}; c(KIO3) = $1.0 \cdot 10^{-3}$ mol L^{-1}; Potential sampling time is 40 s. *V*total = 14.0 mL.

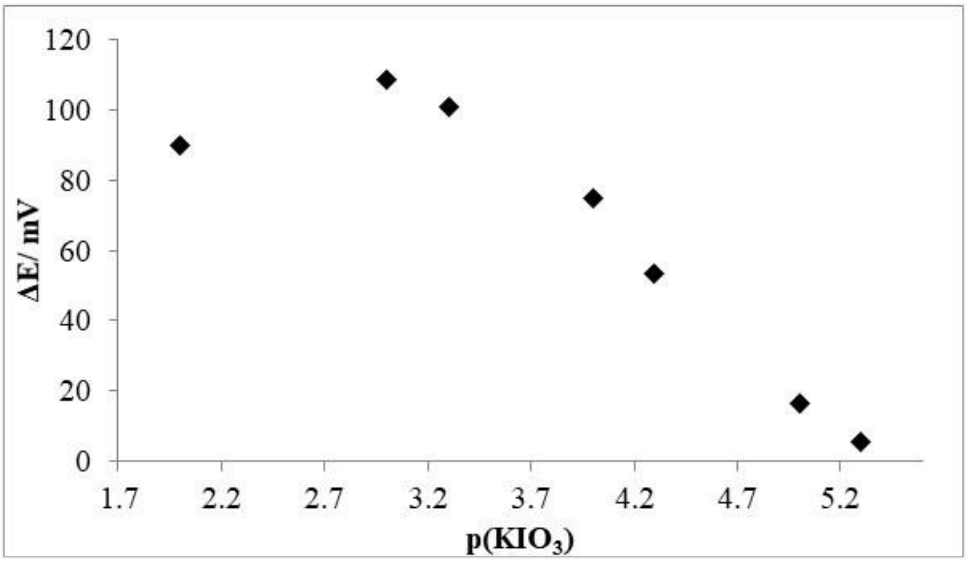

Figure 2. Effect of KIO_3 concentration change on potential change. Experimental condition: $c(KIO_3)$, mol L^{-1}: $5.0 \cdot 10^{-6}$, $1 \cdot 10^{-6}$, $5.0 \cdot 10^{-5}$, $1.0 \cdot 10^{-5}$, $5.0 \cdot 10^{-4}$, $1.0 \cdot 10^{-4}$, $5.0 \cdot 10^{-4}$, $1.0 \cdot 10^{-3}$, $1.0 \cdot 10^{2}$; c(AA) = $5.0 \cdot 10^{-4}$ mol L^{-1}; $c(H_2SO_4)$ = 0.15 mol L^{-1}; Potential sampling time 40 s. V_{total} = 14.0 mL.

3.1. Analytical application of the method

Under the selected experimental conditions, linearity was obtained in the concentration range from $8.0 \cdot 10^{-5}$ to $8.0 \cdot 10^{-4}$ mol L^{-1}. Also, in preliminary experiments linearity between the potential change, at E_2 = 20 s, and AA concentration in the range from $1.0 \cdot 10^{-4}$ to $1 \cdot 10^{-2}$ mol L^{-1} was successfully achieved.

However, potential sampling time at 40 s allows better sensitivity, optimal repeatability and obtained coefficient of regression indicated good linearity.

The potential change for different concentrations of AA is shown in Figure 3.

Details about analytical characteristics of the method, the limit of detection (LOD) determined according to IUPAC recommendations, the same principle was used in references [20,21], linear response range (LRR), regression equation and the coefficient of regression are all shown in

Table **1**.

3.1.1. Interfering species

The proposed method was applied for the determination of ascorbic acid ($1 \cdot 10^{-4}$ mol L^{-1}) in the presence of possible interfering species. The tolerance limit was taken as the amount of added species that caused a relative error less than ±5 % for the determination of AA. Sucrose, glucose, fructose and lactose did not interfere eith the determination of AA even when they were at 500−fold excess. An excess of tested inorganic ions (Ca^{+}, Mg^{2+}, Na^{+}, SO_4^{2-}, NO^{3-}) up to 200-fold did not interfere with the determination of AA and the same proved to be true as was the case with citric and oxalic acid..

Table 1. Analytical characteristics of the proposed method: equation of regression line, the coefficient of regression (R^2), the linear response range (LRR), the limit of detection (LOD), p(AA) = −log*c*(AA).

Equation	R^2	**LRR/mol L^{-1}**	**LOD/mol L^{-1}**
$E = -257.84\text{p(AA)} + 1080.8$	0.997	$8.0 \cdot 10^{-5} - 8.0 \cdot 10^{-4}$	$6.43 \cdot 10^{-5}$

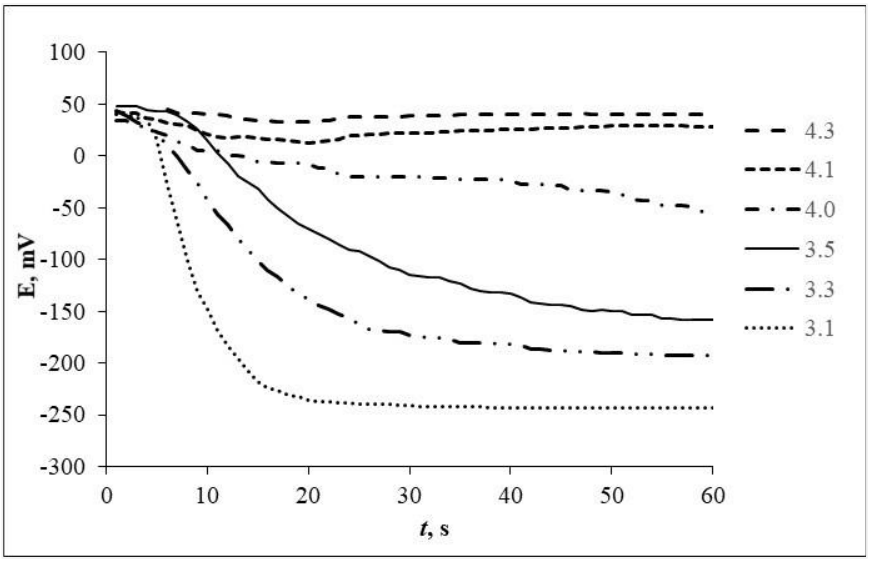

Figure 3. The potential change for different concentrations of AA. Experimental condition: c(AA), mol L^{-1}: $5.0 \cdot 10^{-5}$, $8.0 \cdot 10^{-5}$, $1.0 \cdot 10^{-4}$, $3.0 \cdot 10^{-4}$, $5.0 \cdot 10^{-4}$, $8.0 \cdot 10^{-4}$; $c(KIO_3) = 1.0 \cdot 10^{-3}$ mol L^{-1}; $c(H_2SO_4) = 0.15$ mol L^{-1}; $V_{total} = 14.0$ mL.

The interference effect occurs in the determination of AA with vitamin B in ten−fold excess. It was expected and it was subsequently shown experimentally that other reducing agents such as D−penicillamine, *N*−acetyl−L−cysteine and glutathione were interfered with in the same concentration as AA. It should be pointed out that these thiols are not common ingredients in pharmaceutical products preparations containing AA.

3.1.2. Analysis of pharmaceuticals

Table 2. Results obtained by analysis of different pharmaceuticals

Sample	Amount		
	Labeled/mg	Measured/mg±SD (*n*=3)	Recovery/%
Vitamin C (Alkaloid, Skopje)	500	488.0 ± 0.55	97.6
Vitamin C (Multivita)	250	243.5±0.13	97.4
BIO− C 500 (Dietpharm)	500	491.8 ± 0.15	98.4
Ca−C 1000 Calvive, (Novartis)	1000	1020.9 ± 0.23	102.1
Kalcij + C (Dietpharm)	250	248.9± 0.09	99.6

Table 3. Results obtained by analysis of real samples with known amounts of added standard of ascorbic acid solutions.

Sample	Amount Taken/mg	Added/mg	Measured /mg±SD (*n*=3)	Recovery/%
Vitamin C (Alkaloid,Skopje)	90.0	0.0	87.3 ± 0.30	97.0
		50.0	135.1 ± 0.50	96.5
		120.0	205.8 ± 0.50	98.0
Vitamin C (Multivita)	90.0	0.0	84.8 ± 0.20	94.2
		50.0	132.5 ± 0.98	94.6
		120.0	201.6 ± 1.12	96.0
BIO− C 500 (Dietpharm)	90.0	0.0	86.4 ± 0.25	96.0
		50.0	136.7 ± 1.03	97.6
		120.0	213.7 ± 0.95	101.8
Ca−C 1000 Calvive, Novartis	90.0	0.0	92.6 ± 0.13	102.9
		50.0	141.3 ± 2.06	100.9
		120.0	217.4 ± 1.35	103.5
Kalcij + C Dietpharm	90.0	0.0	87.7 ± 0.20	97.4
		50.0	141.3 ± 0.24	100.9
		120.0	212.8 ± 1.50	101.3

Following a simple procedure, the proposed method was applied for the analysis of pharmaceuticals containing AA, Table 2. In addition, the recovery values were calculated according to experiment results obtained by adding a known amount of AA standard solution to the sample before potentiometric determination,

Table **3**. From the obtained results, it is evident that the similarity between the labelled amount and the determined results are satisfactory. The recoveries were in the range from 94.2 % to 103.5 %.

Recently, only a handful of papers, to be precise only 3, worthy of mention could be found in literature in the last five years. The papers listed in Table 4 describe potentiometric determination of ascorbic acid.

Table 4. Comparison of similar methods with the one proposed in this work for ascorbic acid determination

Reference	**Limit of detection/mol L^{-1}**	**Linear range/mol L^{-1}**	**pH**
This work	$2.4\cdot10^{-5}$	$8\cdot10^{-5} – 8\cdot10^{-4}$	~0.5
[22]	N/A	$8\cdot10^{-6} – 1\cdot10^{-4}$	N/A
[23]	N/A	$1\cdot10^{-3}–1\cdot10^{-1}$	5
[24]	$2\cdot10^{-5}$	$5\cdot10^{-5}–1.5\cdot10^{-3}$	5

N/A – not available

By comparing the data given in Table 4, it could be seen that our proposed method has a very similar limit of detection and linear range, with respect to regard to other methods mentioning them. The main advantage of our method is its possible application in high acid media. Practically, all the literature

references available to us for study suggested potentiometric determination in weak acid media (pH = 5). However, when an experiment is conducted in high acidic media, it is possible to mask those interferences by converting them to their conjugate acids. It is worth pointing out once again this method is very fast since the measurement lasts about 2 minutes and has high reliability. Methods described in literature [22-23] did not give time needed for finishing the analysis nor have they described their reliability. Hence, it is difficult to compare our method with the ones in the table above [22-24] with regard to their time consumption and reliability. Nevertheless, our newly described and newly proposed method can be applied in a flow-injection or a sequential system. By applying it in either a flow-injection or a sequential system, it can be utilized to perform, at the very least, 30 analysis every hour. We hope to apply the method in a flow-injection or a sequential system in our future work.

Finally, according to what was previously stated above, it can be concluded that the advantage of this method is an extremely simple, inexpensive, fast procedure, which allows satisfactory precision by applying only three solutions: standard/sample, iodate, and acid as well as very simple and, relatively affordable apparatus (a millivoltmeter with an iodide ion-selective electrode).

4. CONCLUSION

This paper describes a potentiometric method for the determination of AA, which is based on a redox reaction with iodate as a reagent. The proposed method differs from those previously reported in itssimplicity and shorter analysis time. Furthermore, the low consumption of reagents and solvents, and the low energy consumption with regard to the time of analysis give the possibility to place it in the group of "greener" methods. After selecting the optimal conditions of analysis, this method was successfully applied in the analysis of pharmaceuticals in the concentration range from $8.0 \cdot 10^{-5}$ to $8.0 \cdot 10^{-4}$ mol L^{-1}.

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