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FIA of vitamin C in blood serum in humans at increasing ethanol concentration

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Abstract

The evaluation of the ethanol influence on the changes in vitamin C levels has been done in humans at increasing ethanol concentration. Flow injection analysis method (FIA) with the spectrophotometric detection was used to determine L-ascorbic acid levels. The procedure is based on the oxidation of the analysis with iron (III) and 2,2′-dipyrydyl. Concentrations of vitamin C in the range of 0.4–1.3 ppm have been determined with a relative standard deviation (R.S.D.) 1.2% ($n = 15$). The injection rate was 40 samples h^{-1}. The comparison of L-ascorbic acid levels in humans showed a statistical difference at $P < 0.05$ in the analysed group.

Keywords: Blood serum; Ethyl alcohol; Flow injection analysis; Vitamin C

1. Introduction

Vitamin C (L-ascorbic acid) is one of the most common natural or artificially enriched ingredients in foods and beverages. Our interest in L-ascorbic acid results from the fact that vitamin C has a major biological role as a natural antioxidant [1–3].

L-ascorbic acid is not synthesised by humans, but it is an essential dietary vitamin for the species. Its main sources are citrus, briar-rose and black currant fruits, tomatoes and horseradish root. Clinical deficiency in vitamin C leads to reduced drug metabolism and immmunocompentence and thus affects social and work functions [1,4–7]. Appropriate intake levels of vitamin C for each of its physiological functions have not been fully defined.

About 98% of ethanol is metabolised in the human body. Over 70% of ethanol is converted in the liver and other organs due to activity of ethanol dehydrogenase, about 21% is subject to the activity of the microsomal liver oxidation system, and the remaining part is excreted in an unchanged form by lungs and kidneys (about 1% is subject to catalase activity) [5,10]. Hormones, vitamin B_6 and vitamin C are likely to intensify

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the metabolism of ethanol [5,8,10]. L-ascorbic acid with L-dehydroascorbic acid composes oxidation-reduction system able to transport electrons and hydrogen in reversible processes [10,15–17], thus playing an important role in the tissue respiration and many other metabolic processes. The level of L-ascorbic acid may have an important influence on ethanol alteration [8].

Several methods have been proposed for the determination of L-ascorbic acid. For a broad collection of older method, the reader is referred to the literature cited by Seib [12] and Serbell [13]. Numerous techniques have been used for the determination of L-ascorbic acid based on the reducing property and ability to produce coloured compounds. These methods have been reviewed previously [6,7,12]. Since the introduction of flow-injection analysis [14], many flow systems have been developed for the determination of L-ascorbic acid. Flow injection analysis (FIA) has found wide application in various fields of routine analysis, including pharmaceutical [9]. The versatility and simplicity of the FIA technique allows its adaptation at relatively lower cost to the different requirements of a variety of analytical problems. This type of analysis, in which a large number of samples of very similar composition have to be analysed, is an attractive field for automated FIA.

2. Experimental

2.1. Reagents

A solution of L-ascorbic acid (vitamin C, $C_6H_8O_6$, H_2Asc), from Fluke was prepared by dissolving the requisite amount of sample in distilled water. The solution was prepared fresh every day and kept in the dark and cold to minimise oxidation.

Iron (III) 2.2′-dipyrydyl $(C_5H_4N)_2$ reagent 0.176 g of ammonium salt $Fe(NO_3)(SO_4)_2 \times 12\ H_2O$ was dissolved in 800 cm^3 of water containing 10 cm^3 of 5 M sulphuric acid. Then, 2.5 g of 2.2′-dipyrydyl is added and after dissolving (to boil the solution to 80°C) diluted to 1000 cm^3 with water. A working solution was prepared by addition to 200 cm^3 of iron (III)- 2.2′-dipyrydyl reagent in temperature ca. 80°C a few drops of 2% solution $Ce(SO_4)_2$ in 50% H_2SO_4 (for the oxidation of the trace of iron (II)). The colour of the solution was changed from red to yellow.

All chemicals used were of analytical grade.

2.2. Materials

Research was carried out on subjects at increasing ethanol concentration. Seven groups were indicated. Group I (control) were volunteer blood donors, group II- drivers with blood ethanol level up to 0.5‰, group III- drivers with blood ethanol level up to 1.5‰, group IV, drivers with blood ethanol level up to 3‰, group V, alcoholics (who drink 2–4 weeks), group VI, intoxication alcoholics, and VII, not active alcoholics.

2.3. Methods

2.3.1. Flow injection assembly

This is shown in Fig. 1 and included a multichannel peristaltic pump type 304 (Electromedical Coop, Poland) and a laboratory-made rotary injection valve with exchangeable sample loops. Spectrophotometric measurements were made using a spectrophotometer Specol 10 (Carl Zeiss Jena, Germany) equipped with a laboratory-made flow through cuvette and connected to a model TZ 4620 recorder (Laboratorni pristroje, Czechoslovakia). The PVC tubing used (0.7 mm i.d.) was connected with perspex connectors.

2.3.2. Calibration procedure

A 300 μl of sample solution containing 1–10 $mg.dm^{-3}$ of L-ascorbic acid was injected into deionized water carrier and subsequently merged with stream of iron(III)-2.2′-bipyridyl reagent. Product of reaction was carried to the spectrophotometer flow-cell and the absorbance read at 510 nm.

Analytical features of the proposed method were summarised in Table 1.

The values obtained by the presented FIA method were compared with the values obtained by the spectrophotometric Kyaw's method [11]. The comparison of L-ascorbic acid levels showed no statistical differences in the nonparametric Mann–Whitney test.

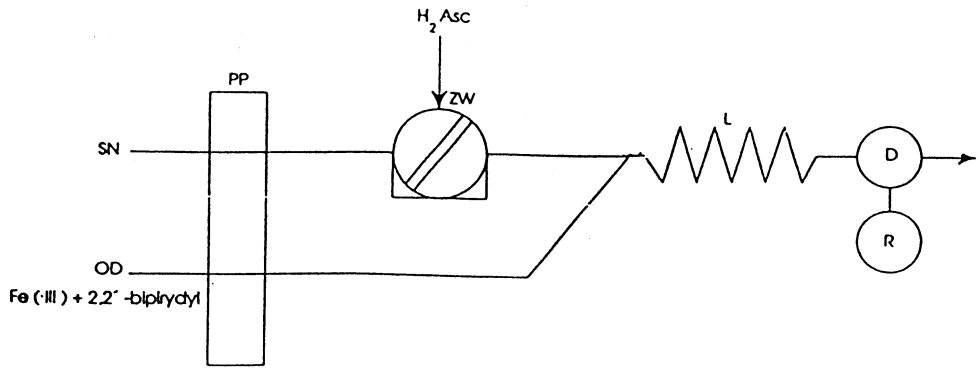

Fig. 1. Schematic diagram of the FI manifold used for the determination of L-ascorbic acid.

2.3.3. Procedure

Blood for the research was collected for 2 days — from the cubits vein of the person. The plasma was collected by 1.34% potassium oxalate and centrifuged at 3000 rpm for 15 min. L-ascorbic acid levels were analysed with the use of FIA method with spectrophotometric detection [9]. Double channel system was applied, with water as a carrier and Fe(III) 2.2′-bipirydyl solution as a reagent (Fig. 1). The supernatants were injected to the carrier stream. In the reaction spiral L-ascorbic acid reduces Fe(III) to Fe(II) and the red coloured complex is created. The concentration of L-ascorbic acid was calculated on the basis of the complex absorbence. A spectrophotometer Specol 10 was used at 510 nm wavelength.

2.3.4. Study of interference

The tolerance level (interfering species/analyte); sacharose, glucose, lactose >500 μg ml^{-1}, glutamic acid 30 μg ml^{-1}, citric acid 5 μg ml^{-1}, acetysalicylic acid 15 μg ml^{-1}.

3. Results and discussion

The L-ascorbic acid levels were compared between volunteer blood donors (group I), ethanol-consuming subjects (group II–IV) and alcoholics (group V–VII). The average L-ascorbic acid level in the blood donors group was 0.99 mg 100 ml^{-1}, in group II 1.02 mg 100 ml^{-1}, in group III 1.06 mg 100 ml^{-1}, in group IV 0.70 mg 100 ml^{-1}, in group V 0.54 mg 100 ml^{-1}, in group VI 0.50 mg 100 ml^{-1}, in group VII 0.73 mg 100 ml^{-1}. Results of the study are presented in Table 2 Table 3.

In adults, L-ascorbic acid level in blood serum is an individual feature and ranges from 0.53 to 1.20 mg 100 ml^{-1}. In control group of volunteer blood donors (group I), the level averaged 0.99 mg 100 ml^{-1} and was still within the physiological limits reported in the literature [6,7]. Study on humans at increasing ethanol concentration (ethanol level 0.5, 1.5 and 3‰) revealed that there exists correlation among appointed vitamin C, and with level of liquors in the serum of human blood in investigated groups. At low values of liquors in the serum of blood one did not indeed ascertain of statistical differences in levels of L-ascorbic acid. Level of liquor at 3‰ causes enlarged waste of vitamin C, which is significantly different from this group in comparison to the

Table 1
Analytical features of the proposed FIA method

Parameter	
Linear range (mg dm^{-3})	1–10
Regression equation ($y=ax+b$)	$a=12.47\pm0.25$ $b=-2.8\pm1.6$
r^2	0.9995
Detection limit (mg dm^{-3})	0.4
%R.S.D.	1.2
Simple throughput (samples h^{-1})	40

Table 2
L-ascorbic acid levels (mg 100 ml^{-1}) in blood serum in group I–IV

	I Blood donors	II Drivers, ethanol level 0.05–0.5‰	III Drivers, ethanol level 0.5–1.5‰	IV Drivers, ethanol level 1.5–3‰
1	1.02	1.00	1.12	0.65
2	1.12	0.95	1.05	0.74
3	1.05	0.98	0.93	0.70
4	0.53	0.96	0.92	0.80
5	0.90	0.90	1.20	0.92
6	1.20	1.01	1.12	0.90
7	1.12	0.97	1.20	0.59
8	0.96	1.12	1.05	0.61
9	0.70	1.05	1.04	0.87
10	1.14	0.94	1.01	0.82
11	1.00	1.12	0.96	0.59
12	0.98	1.18	0.90	0.81
13	1.20	1.12	1.11	0.55
14	1.06	1.02	1.17	0.59
15	0.94	0.98	1.18	0.50
Mean ± S.E.M.	0.9947 ± 0.04685	1.0232 ± 0.03651	1.064 ± 0.02676	0.7093 ± 0.03542
Significant difference				$P_{1-4} < 0.001$
				$P_{2-4} < 0.001$
				$P_{3-4} < 0.001$

Table 3
L-ascorbic acid levels (mg 100 ml^{-1}) in blood serum in group V–VII

	V Alcoholic	VI Intoxication alcoholics	VII Non active alcoholics
1	0.59	0.51	0.82
2	0.51	0.53	0.90
3	0.57	0.50	0.89
4	0.62	0.42	0.61
5	0.77	0.67	0.87
6	0.56	0.36	0.62
7	0.44	0.52	0.59
8	0.34	0.51	0.81
9	0.37	0.57	0.55
10	0.56	0,49	0.87
11	0.67	0.44	0.72
12	0.58	0.36	0,59
13	0.65	0.37	0.79
14	0.58	0.56	0.81
15	0.42	0.50	0.58
Mean ± S.E.M.	0.5487 ± 0.02998	0.5006 ± 0.03921	0.7346 ± 0.03394
Significant difference	$P_{1-5} < 0.001$	$P_{1-6} < 0.001$	$P_{1-7} < 0.001$
	$P_{2-5} < 0.001$	$P_{2-6} < 0.001$	$P_{2-7} < 0.001$
	$P_{3-5} < 0.001$	$P_{3-6} < 0.001$	$P_{3-7} < 0.001$
	$P_{4-5} < 0.001$	$P_{4-6} < 0.001$	$P_{5-7} < 0.001$

control group. Permanent exposure to liquor causes characteristic statistical difference lowering the level of vitamin C. Intoxication dependent on liquor causes lowering of the level of L-ascorbic acid. A person from prolonged abstinence showed lowering of the level of vitamin C.

The proposed method is superior to other conventional methods in that it is fast and simple. In the described method, the determination of L-ascorbic acid in blood may be a complement to the applied methods. Reagent consumption is minimum, precision and reproducibility of the adopted spectrophotometric systems are good and the values of R.S.D. are low.

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