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DETERMINATION OF ASCORBIC ACID AMOUNT IN GELATIN AQUEOUS SOLUTIONS BY GALVANOSTATIC COULOMETRY USING ELECTROGENERATED BROMINE

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ABSTRACT

The content of ascorbic acid in 0.1-3% aqueous solution of gelatin is determined by galvanostatic coulometry. The value of the concentration of ascorbic acid in the obtained solution is 2-8% lower compared with the entered values. The difference grows with the increasing concentration of gelatin. The proposed operation sequence in the sample preparation procedure allows getting reproducible results. Based on non-linear multiple regression suggested empirical approach to assessing the content of ascorbic of acids in aqueous solutions of gelatin. This is the basis for quantitative determination of ascorbic acid in jelly products from the rest of the food matrix.

Key words: ascorbic acid, gelatin aqueous solution, galvanostatic coulometry, jelly, electrogenerated bromine

INTRODUCTION

High quality, affordable products with a number of useful properties play special value in solving the problem of nutrition of different population groups. These products include jelly products based on natural fruit juice and gelatin, with the addition of vitamin C. Vitamin C (E 300 - ascorbic acid) is one of food additives that are allowed in the production of food products as antioxidant. Number of food additives is regulated by the technological instructions for the production of food. At the same time, ascorbic acid (AA) is a part of the premix along with other vitamins and minerals. If there is clearly defined system of quality control for food additives, there is no such a system for premixes because the absence of standardized requirements for their composition and standardized methods of verification in the world. It is worth noting that the development of standardized methods to quality indicators assessment is practically implemented, but there is no general guidance regarding the implementation of comprehensive research. Therefore, the development of quality requirements to premixes, which include AA, is one of the most actual problems of hygiene. However, this problem is greatly complicated by the fact that quantitative and qualitative composition of the premix largely depends on the type of food that is to be fortified. All the above said is the basis for carrying out the research in the search of perfect methods of AA quantitative determination in food products after fortification.

It is known that vitamin C or ascorbic acid (IUPAC name (5R)-[(1s)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one) is a water-soluble vitamin that is not synthesized in humans and comes only with food. AA belongs to antioxidants with therapeutic properties which play a significant role in activation of the immune protection in preventing the coagulation of blood and other metabolic processes [1-4]. Methods of

identification and quantification of AA content in foodstuffs and food raw materials is a complicated procedure connected with the complexity of sample preparation, characterized by low content of AA in the test object, its instability in the solution through oxidation by atmospheric oxygen, which is catalyzed by metal ions. This question is the subject of many publications including monographs [5-7].

Literature analysis shows that for the determination of AA in food systems various physicochemical methods [8-19] are used. Titrimetric method is the most widely used one, where titrant 2,6-Dichloroindophenol sodium salt hydrate, potassium iodate or potassium bromate. Used spectrophotometric methods are based on the determination of AA in the UV zone, its reaction with iodine, chemiluminescence, and reaction of dehydroascorbic acid with o-phenyl-diamine. Chromatographic methods provide more accurate and sensitive results, especially with the use of HPLC with electrochemical detection [9]. Recently application of such electrochemical methods as different Voltammetry methods, direct potentiometry and coulometry have significantly increased [8]. It worth noting that that most publications concerning the use of the above physical-chemical methods relate to ordinary food systems, such as fruit juices, herbs and extracts. As against the latter, jellied products usually incorporate rather high concentration of hydrocolloids, such as gelatin, agar or pectin. In [20] it is shown that standard method for AA determination by the titration of Voltammetry 2,6-Dichloroindophenol sodium salt hydrate gives poor results relative to the reference method, due to ambiguous mechanism of AA oxidation and the need for high-quality sample preparation. Chromatographic methods [21,22] also point to sufficiently lower results of ascorbic acid content in aqueous solutions of hydrocolloids, compared with the initial amounts of AA. The authors attribute this to the interaction of AA sorption with the molecules of hydrocolloids, including gelatin and agar. Similar results are uncertainty regarding the final result was obtained by titration method [23]. These publications indicate a problem of AA specification in food matrices, which contain hydrocolloids and urgency of the research regarding competent, budgetary and express method that would allow controlling the level of AA in listed above food systems.

The aim is to develop a method for determining vitamin C in aqueous solutions of hydrocolloids by galvanostatic coulometry and assess the possibility of determining the content of AA in jelly products. This includes solving the problems related to the development of methods of quantitative determination of ascorbic acid, which would have simplified the procedure for sample preparation with the ability to determine the content of AA on the background of the rest of the food matrix. To solve the tasks the method of galvanostatic coulometry was chosen. It has significant advantages as an absolute method. It does not require predetermined calibration, has available devices with sufficiently high statistical certainty and sensitivity of the result. Its use for the determination of AA in various pharmaceutical substances of vegetable origin [24,25] and for the analysis of some foods, such as juices, wine, tea and others, proved their effectiveness (e.g. [26]).

MATERIALS AND METHODS

The following reagents with the relevant qualifications: ascorbic acid pharm. grade (China); edible gelatin P-11 (TM "Mriya", Ukraine); potassium bromide, sulfuric acid – all chemical grade. Distilled water were used in the experiment with the following parameters: pH=5.9 and specific electric conductivity 4.8 μSm/sm.

Model gelatin solutions with mass fraction in the range of 0.1-3.0% by weight was prepared as follows: water was added to sample gelatin, then gelatin swelled for 30-40 minutes. Then gelatin was dissolved at a temperature of 40-50°C in a water bath with

temperature control. After cooling the solution of gelatin to a temperature of 30-40° C, ascorbic acid was added as an aqueous solution, AA solution was prepared the same day that model systems were prepared. Then water was added to the sample, which was needed for the solution of a given weight fraction. Then the solution was mixed thoroughly for uniform distribution of AA.

Standard solutions of ascorbic acid were prepared by weight.

Model systems of AA-gelatin-water were prepared by adding appropriate aqueous AA solutions to aqueous solutions of gelatin in proportions corresponding to the final concentration value.

Galvanostatic coulometry titration cell consisted of two separated cells - cathode and anode, volume 10 and 40 ml respectively, connected by a glass membrane. Plate platinum electrode with an area of about 2 cm^2 was used as a generator electrode. Auxiliary electrode was a graphite rod electrode. Pre-treatment of platinum electrode before measurements was carried out in three stages by holding in solution at the operating current of 5 mA: 1) for 5 minutes in a solution of 0.2 M KBr; 2) a solution of concentrated nitric acid (1:1) for 5 minutes; 3) for 15 minutes in a solution of 0.2 M sulfuric acid.

Between measurements, electrodes were stored in a solution of potassium bromide or potassium iodide, depending on the type of research. The measurements were performed at 1-10mA current depending on the concentration of the test solution so that the titration of added weight of the sample was in the range of 150 to 300 seconds. On the one hand, this provides express method, and, on the other, a precision measurement of time. Device T-201M1 (Georgia) were used as a source of stabilized current. The combined device V7-21 with an error less than 0.2% measured the current.

An electromagnetic stirrer mixed the solution in the cell.

Control of the titration end point was carried out by potentiometric method with two electrodes: indicator platinum redox microelectrode EPL-02 and Ag/AgCl reference electrode EVL-1M4 (ZIP plant, Belarus). The potentiometric data of an electrochemical system was measured by 692 pH / ion meter (Metroohm, Switzerland) with an accuracy of 0.1 mV. The above device was used to measure temperature with an accuracy of 0,1º C and 0.002 pH units using the combined glass electrode with temperature sensor Pt1000 (Combined LL pH glass electrode with Pt 1000 temperature sensor, № 6.0238.000 Metroohm, Switzerland).

The specific electric conductivity was measured by conductometer KEL-1M2 (Georgia).

Bromine, obtained from 0.2 M solution of potassium bromide in the 0.1 M solution of sulfuric acid, was as electrogenerated titrant.

Monitoring and experimental data recording (electromotive force-time) was performed electronically with the help of PicoLog Recorder v.5.24 (PicoScope Ltd., UK) program. Timing the end of the titration and statistical processing of results of the research was performed in Excel of Microsoft Office 2010 and Sigma Plot v.11.

RESULTS AND DISCUSSION

To study the possibility of using coulometry titration to quantify the total content of AA in the test solutions validation assessment procedure was performed for measuring the following parameters: specificity, linearity and range, limit of detection (LOD), limit of quantitation (LOQ), accuracy and reproducibility. For this purpose aqueous model solutions AA in the range 0,5-1800 mg/g were prepared. Calculation of AA *m* (mkg/g) with the experimental data was carried out using the following equation (1):

$$m = \frac{ItM}{nFm_s}, \quad (1)$$

where I – current, t – electrolysis time, M – molar mass of the substance, F – Faraday constant, n – the number of electrons in the oxidation half-reaction titrant, m_s – mass of the solution.

To account the influence of impurities in electricity solution, the previous electrolysis time of this solution without AA when determining the time, was taken into account. As stated in [27], AA oxidized to dehydroascorbic acid during interaction with halogens generated in the cell. Thus there is a transfer of two electrons, which corresponds to n = 2 in the eguation (1). Fig. 1 shows the dependence of Q-f (g) solutions for AA at 9 concentrations. As it was expected, this dependence is linear with a correlation coefficient of 0.9987, which proves the linearity condition and the possibility of quantitative determination of AA in this area in the proposed analytical methodology.

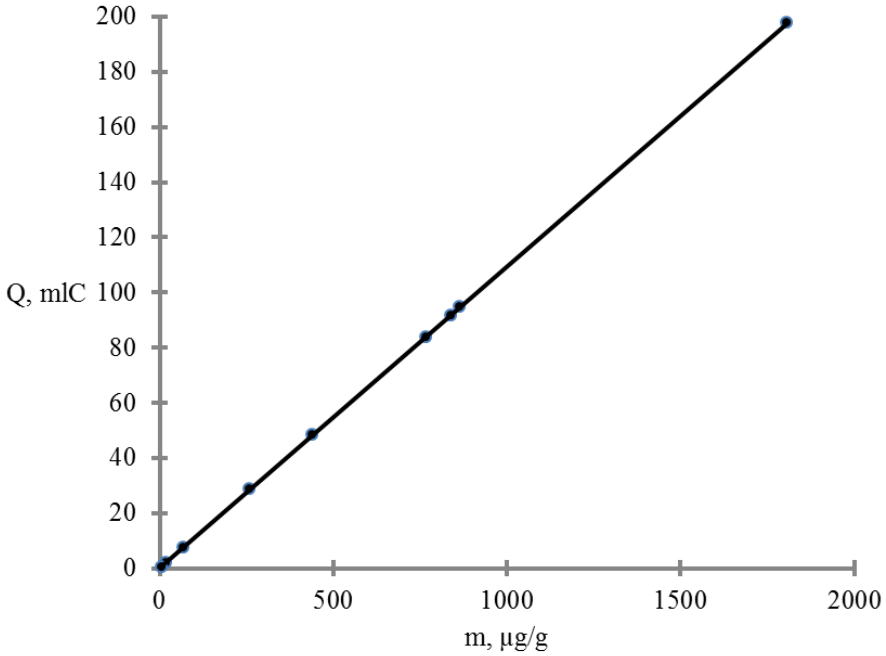


Fig. 1. The dependence between content of AA (m, mg) and the amount of electricity for power generation of titrant bromine (Q, mlC) for model AA aqueous solutions

Residual standard deviation of linear regression σ and S slope of Q-f(m) curve was used to calculate the LOD LOL of the equations:

$$LOD = \frac{3\sigma}{S}, \quad LOQ = \frac{10\sigma}{S} \quad (2)$$

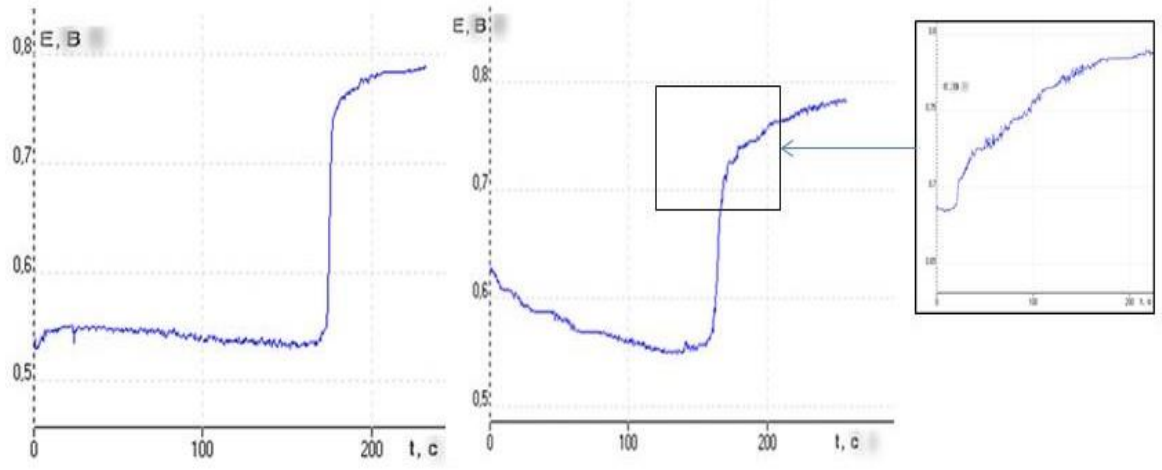
The following results were obtained as the result of calculations by formula (2): LOD=2,7 mkg/g ($1{,}5\cdot10^{-5}$ mole/dm^3) and LOQ=8,1 mkg /g ($4{,}6\cdot10^{-5}$ mole/dm^3).

The specificity was evaluated by "added-found" method (Table 1).

Table 1 - Results of AA coulometric determination by generated bromine (n = 5, P = 0.95) in model aqueous solutions

Substance	Added m_a, mkg/g	Founded m_f, mkg/g	S_r
Ascorbic acid	0.47	0.42±0.15	0.256
	4.60	4.45±0.09	0.017
	17.50	17.46±0.04	0.011
	69.48	68.39±0.09	0.016
	260.0	261.2±0.1	0.009
	440.0	442.0±0.6	0.009
	766.3	766.1±0.3	0.002
	864.7	864.0±0.8	0.004
	1806.5	1805.4±1.9	0.007

Accuracy and reproducibility was assessed by varying the mass of samples at three concentration levels using three masses in each level. Significant results in terms of Criteria Fischer were obtained statistically. In Fig. 2, some examples of experimental titration curve AA in water (a), AA in water-gelatin solutions (b) and gelatin aqueous solution (c) are presented.



a b c

Fig. 2. Listing of experimental curves (dependence of EMF (E, V) on the time (t, s), scan copy from a program PicoLog Recorder):
a - AA solution with a concentration of 76.63 mg/g (I = 9,921 mA, m_s = 2,030 g);
b - AA solution with a concentration of 75.01 mg/g of 3% solution of gelatin (I = 9.923 mA, m_s = 2.145 g);
c – g 3% solution of gelatin (I = 9.919, m_s = 4.140 g)

As we can see from Fig. 2, the obtained curves have a classic look with falling part, the presence of which is characteristic for the titration of AA in redox reactions. Titration of AA aqueous solution (Fig. 2a) gives clearly determined data, with the account of sharp increase in the titration end point, which is 1 to 2 seconds with a change in electromotive force (EMF) of the indicator system by more than 250 mV. Introduction of gelatin makes the titration curve (Fig. 2b) less explicit, and the curve in the vicinity of the end point of the titration becomes less sharp. In addition, the view of the upper part of the titration curve allows conclude that the implementation of the process of gelatin oxidation, as the form of the fragment is identical to the same type of fragments on the titration curves of aqueous solutions of gelatin (Fig. 2b). Given high reactivity of the electro generated bromine, oxidation in aqueous solutions of both components was expected. However, as seen from the titration curve (Fig. 2c) gelatin oxidation occurs within 680-800 mV, while AA is oxidized at lower potentials. Such fact preconditions the implementation of the possibility of determining AA in the presence of gelatin.

It should also be noted that determination of AA content in aqueous solutions of gelatin with the concentration of more than 1.5% is connected with the difficulties conditioned by the formation of gelatinous structures in 30 minutes after cooking solutions. It is a known fact connected with the formation of spatial structure by hydrogen bonds [28]. The attempts to place the sample that has spatial structure of gelatin macromolecules to electrochemical cell and perform the titration did not give adequate and reproducible results. That is why it is recommended for sampling of a structured product, heat it in water bath to a temperature of about 40° C (melt), thereby destroying spatial grid and transferring it to a liquid state, and then use for further determination of AA. As further studies show, the results of AA content, which were determined before the process of jelly formation, and after melting jelly well harmonized regardless of the number of transitions from state to liquid jelly by melting systems studied.

These facts give the basis for the development of methods for determining AA in commercial food products - jelly products based on gelatin, with due regard to their state of aggregation. Table 2 shows the results of determining the content of AA in aqueous solutions of various concentrations of gelatin. Analysis of the data obtained by coulometric titration of AA-gelatin-water suggests systematic reduction of the results of AA content in relation to the amount of AA, which was initially added to the system under study.

Table 2 - Results of AA amount in the systems AA-gelatin-water (n = 4 and P = 0.95)

	The added amount of AA m_a, mkg/g									
	90.02		250.1		500.0		750.1		1000	
The concentration of gelatin	The founded concentration of AA m_f and relative difference δm values									
	m_f	δm,%	m_f	δm,%	m_f	δm,%	m_f	δm,%	m_f	δm,%
absent	90.2	0.1	249.7	-0.2	499.2	-0.2	750.5	0.1	1001	0.1
0.1%	89.0	-2.1	245.0	-2.0	489.7	-2.1	688.3	-8.2	*	-
1.0%	86.9	-5.1	235.5	-5.8	465.3	-6.9	695.0	-7.3	*	-
3.0%	85.2	-5.3	234.5	-6.2	466.8	-6.2	702.4	-6.4	970.4	-4.3

* - The study was not performed

This trend is not for aqueous AA solution without gelatin. As we can see from Table 2, relative difference is calculated by the formula (3) of the detected concentration of AA m_f and added amount of AA m_a ranges from - 0.1% to - 8.2%.

$$\delta m = \frac{(m_f - m_a)}{m_a} \quad (3)$$

Fig. 3 shows the deviation value of the content of the added amount of AA from the concentrations of the components gelatin-water. Here, the data is presented for the system without gelatin. For this diagram, the character of linear dependence of the growth of δg value with the increasing concentration of gelatin and a almost linear dependence on the concentration of AA. The non-linear character of dependence of δg value on gelatin concentration correlates with the amount of electricity required for the titration of a unit mass of gelatin. This dependence is well approximated by a parabolic relationship with a correlation coefficient of 0.99.

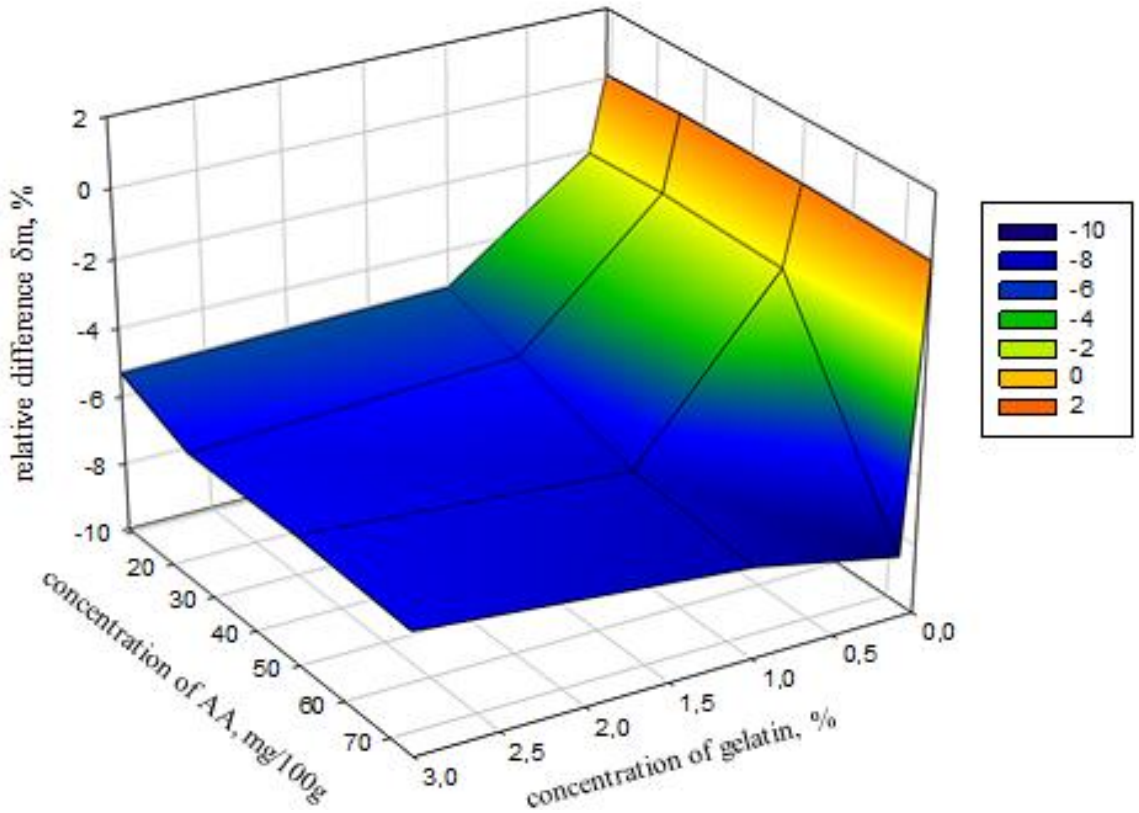


Fig. 3. Dependence δm from the concentrations of AA and gelatin in aqueous solutions

The increased error in the determination of AA gelatin solution (Fig. 3), which change according to the same law as the curve in Fig. 4, may indicate that despite the fact of gelatin interaction with bromine electro-generated at higher potentials than those that characterizing the end point of the titration AA, oxidation gelatin affects the process of quantifying AA. Further study of the mechanism of AA oxidation and its mathematical description of the methodology will improve the quantitative determination of AA in gelatin solution, and thus

in the products based on it. However, with the account of the presented above dependencies, it is possible to consider relative error as a systematic δg error without delving into the chemistry of the phenomenon mechanism and to apply multiple non-linear regression to its approximation depending on the concentrations of the system components based on experimental data.

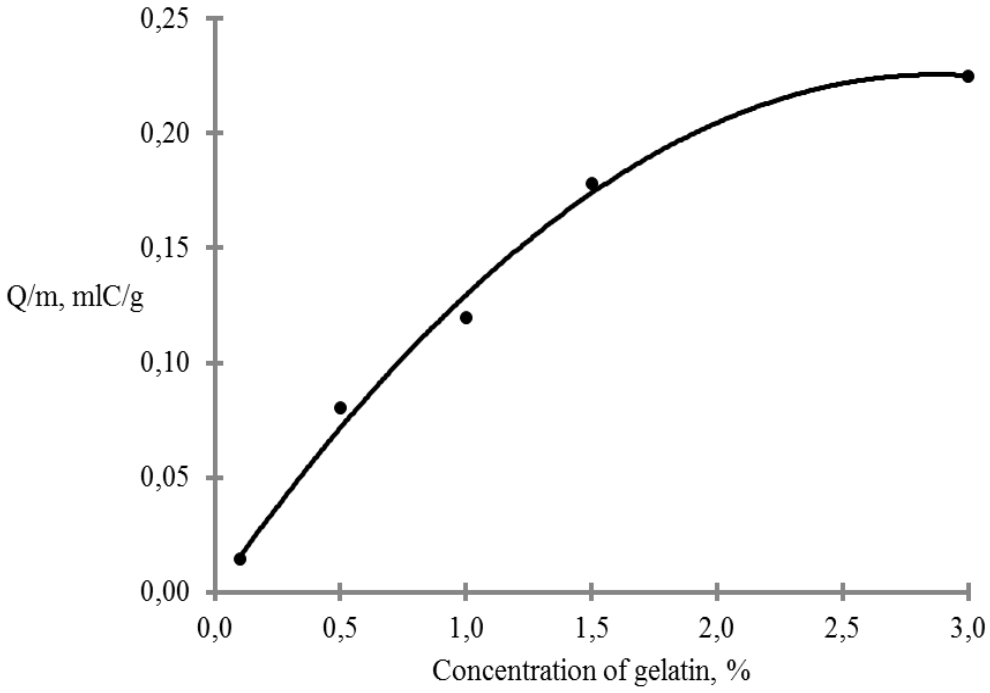


Fig. 4. Dependence of specific quantities of electricity (required for the titration unit mass) of concentration of gelatin

Considering the above dependence we can see relative δg error as systematic, without delving into the chemistry of the reaction. Further, based on experimental data, it is possible to apply multiple non-linear regression to its approximation depending on the concentrations of the system components. Further calculations showed that the most appropriate in terms of standard deviation is the following equation that relates δg with AA g_{AA} and gelatin g_g concentrations considering the corresponding regression coefficients:

$$\delta m = a_0 + a_1 m_{AA} + a_2 m_g + a_3 g_g^2, \quad (4)$$

where regression coefficients are equal to the following values: $a_0 = 0.061$; $a_1 = -0.034$; $a_2 = -7.07$; $a_3 = 1.83$ with standard deviation approximation 1.71. In equation (4) AA concentration is expressed in mg/100 g of solution. To verify the proposed approach, the AA content was determined in 0.5% aqueous gelatin solution, to which AA was added to 9 mg/100 g of solution.

CONCLUSION

The data is purely empirical mathematical approach that does not reveal the chemical mechanism of the phenomenon. However, this approach is promising foundation for further

research on the possibilities of using coulometry adapted from sample preparation to determine the amount of ascorbic acid in jelly products.

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