Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der ChemicaSinica, 2016, 7(1):9-19

Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Determination of the total content of diterpene glycosides in *Stevia rebaudiana* plant by the method of direct potentiometry

Natalia V. Lutsenko, Maria O. Mironyak, Oksana V. Labyak, Olena V. Volnyanska and Volodymyr I. Tkach

Department of Analytical Chemistry and Chemical Technology of Food Additives and Cosmetics, Ukrainian State University of Chemical Technology, Gagarina avenue, Dnipropetrovsk, Ukraine

---

ABSTRACT

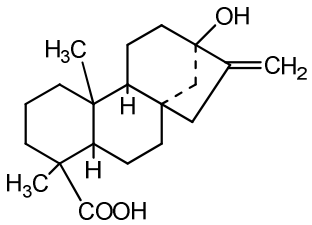
We have investigated the reaction of diterpene steviol glycosides with heteropolyanion of Keggin structure of 12-molybdophosphoric acid by UV and IR spectroscopy. The associative nature of the interaction was confirmed. The resulting slightly soluble associate was used as electrode active substance in plasticized membranes of ion-selective electrodes sensitive to steviol glycosides. The developed new film ion-selective electrodes, being sensitive to stevioside, have satisfactory electrode characteristics. They are proposed for the quantitative determination of steviol glycosides in extracts, food products and cosmetics by direct potentiometry. A method for the quantitative determination of diterpene steviol glycosides in extracts and industrial output by direct potentiometry has been developed. The developed method meets all the requirements of modern analysis - it is simple, express, safe and affordable. It has sufficient selectivity, accuracy, and sensitivity.

Keywords: 12- molybdophosphoric acid, direct potentiometry, electrode active substance, ion-selective electrode, stevia.

INTRODUCTION

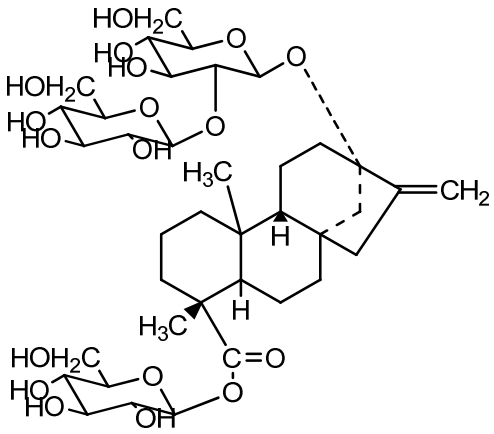
Stevia is a natural non-carbohydrate sweetener which is 300 times sweeter than sugar. It was used for centuries by Guarani Indians as a sweetener and a medicinal plant.

The sweetness of the plant is provided by sweet substances named diterpene glycosides, which are derivatives of steviol and have a collective name - "stevioside". In its turn, diterpene glycosides consist of four major glycosides: stevioside, constituting 8-12% of dry weight of the plant's leaves (ratio of sweetness to sugar – 250 - 300), rebaudioside A - 3-4% (400-450), rebaudioside C - 1-2% (60-80), dulcoside A - 0.4-0.6% (40-50) [1].

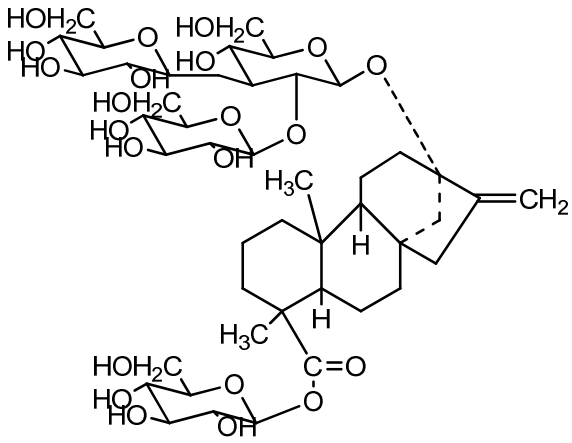


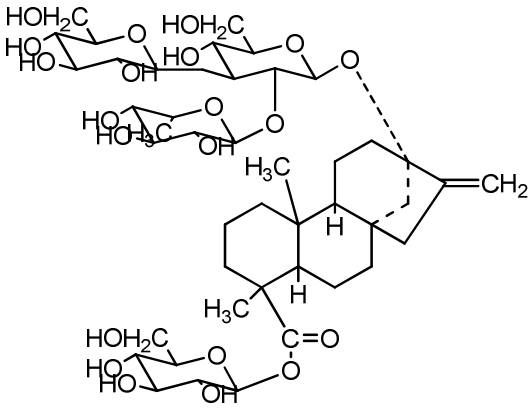
Steviol

Stevioside (molar mass 804)
$C_{38}H_{60}O_{18}$

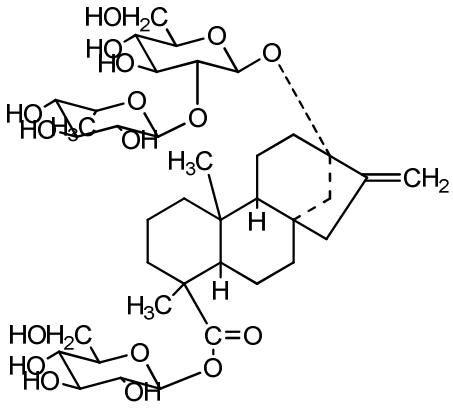


Rebaudioside A (molar mass 967)
$C_{44}H_{70}O_{23}\cdot 3H_2O$





Rebaudioside C (molar mass 950)
$C_{44}H_{70}O_{22}\cdot 3H_2O$



Dulcoside A (molar mass 788)
$C_{38}H_{59}O_{17}$

Stevioside was extracted in 1931 by French chemists M. Bridel and R. Lyavey [2]. It is known from the literature that the amount of glycosides from the plant Stevia Rebaudiana is obtained by water or alcohol extraction from the dry raw material [3-5].

Stevioside is registered in food industry as a food additive E960, a sweetener [6].

The main disadvantage of stevioside is its bitter aftertaste. This is due to the fact that dulcoside A glycosides, rebaudioside C and stevioside least of all have bitter aftertaste. Only rebaudioside A has the best taste most similar to the taste of sugar. The higher is its percentage; the better are the taste characteristics. The content of the latter of less than 60% will ensure a bitter taste [7]. Today, stevia extract of the highest purification degree contains 97% of rebaudioside A.

The production technology of another type of stevioside, having no bitter aftertaste, provides an intermolecular fermentation of the common extract. Application of this technology results to ordinary stevia extract acquiring pure sweet taste without other flavors. [8]

Food additive E960 stevioside is used in the manufacture of chewing gums, juice, soft drinks, alcoholic beverages, dairy products, pastries, marinades, sports nutrition and table sweeteners [9-11]. Stevioside is promising as a sweetener; and, in economic terms, it is three times cheaper than sugar. [12]

Stevioside is safe for use as a food sweetener at doses up to 1500 mg per day for 2 years. It is also recommended for the prevention and treatment of many diseases, among them the problem of excess weight [13-14].

The principal methods of determining the quantitative content of steviosides are capillary electrophoresis, gas and gas-liquid chromatography [15-16], TLC with densitometry [17], high performance liquid chromatography with UV detection [18] and IR spectrometry [19]. There is a modified spectrophotometric method of determination of

diterpene steviol glycosides, its essence being in determining the glucose equivalent to the weight of diterpene glycosides. [20]

Existing methods are difficult to perform and require expensive equipment, pure substance and highly qualified personnel. Therefore, the aim of our work is to use ion-selective electrodes for the quantitative determination of sweet diterpene steviol glycosides.

MATERIALS AND METHODS

UV absorption spectra were measured on a spectrophotometer SF-46 in λ=200-350 nm range in quartz cuvettes (l=1 cm). Distilled water was used as a solvent in all cases. UV spectra of these substances were recorded relatively to the solvent (distilled water). IR spectra were recorded on Nicolet iS10 with Fourier converter, manufactured by Thermo Scientific. Wavelength is given in cm^{-1}, in the direction of the long-wavelength region. All spectra were recorded in the range from 5000 to 400 cm^{-1} with resolution of 4 cm^{-1} and scanning speed of 1 cm/s.

Electrode characteristics of ion-selective electrodes were obtained using electrochemical cell:

Ag	AgCl, KCl	Test solution	Membrane	Solution of the test substance $C=1.0\cdot10^{-4}$ M	KCl, AgCl	Ag

The solution of MPA (12-molybdophosphoric acid $H_3PMo_{12}O_{40}\cdot26H_2O$) concentration $C=1.0\cdot10^{-2}$ mol/l was prepared in the way described hereafter. 2.2940 g of the sample was dissolved in 100.0 ml of distilled water at temperature 70-80 ^{0}C. Further solutions were prepared by diluting the previous one.

The solution of $Ba(NO_3)_2$ (barium nitrate) concentration $C=1.0\cdot10^{-2}$ mol/l was prepared as follows: 2.6100 g of the sample was dissolved in 100.0 ml of distilled water at temperature 20 ^{0}C.

Stevia leaves extract was prepared as follows: a sample of dry Stevia leaves ~ 1.0 g poured with 70-80 ml of water was being water bath extracted at 80-90 ^{0}C for 30 min. The resulting extract was cooled, filtered, poured into the volumetric flask of 100.0 ml and made up the volume with water.

The solution of industrial extract of Stevia leaves, the product of company «Novachem (Wuhan) I&E Co., Ltd. » (China), the content of rebaudioside A - 95% and concentration $C=1.0\cdot10^{-2}$ mol/l, was prepared as follows: a sample of 0.4830 g was dissolved in 50.0 ml of distilled water at room temperature.

The solution of industrial extract of Stevia leaves, the product of company «Novachem (Wuhan) I&E Co., Ltd. » (China), the content of rebaudioside A - 50% and concentration $C=1.0\cdot10^{-2}$ mol/l, was prepared as follows: a sample of 0.4530 g was dissolved in 50.0 ml of distilled water at room temperature.

Dry extract of Stevia leaves for IR spectra registration was prepared as follows: a sample of dry Stevia leaves ~ 5.0g poured with 70-80 ml of water was being water bath extracted at 80-90 ^{0}C for 30 min. The resulting extract was cooled, filtered, transferred to the Petri dish and dried in the oven at 40-50 ^{0}C to remove the moisture completely.

The method of ionometric determination of stevioside in industrial extract of Stevia leaves: a sample of stevioside ~ 2.4 mg was dissolved in distilled water and pH of the solution was adjusted to 3.0. The resulting solution was transferred into the volumetric flask of 25.0 ml, made up the volume with distilled water and quantitatively transferred into the electrochemical cell with a system of electrodes: ion-selective electrode (ISE), sensitive to stevioside, was an indicator, and silver chloride was a reference electrode. Electromotive force of the obtained sample was measured with the ionomer. Quantitative content of stevia glycosides was determined by the calibration graph.

The method of ionometric determination of stevioside in sweets "Chocolate bar with stevia": a sample of sweets ~ 22.42 g poured with 90-100 ml of water was water bath heated at 50-60 ^{0}C to dissolve. The resulting solution was centrifuged, the upper fat layer was removed and the remaining solution was filtered. The resulting solution was poured into the volumetric flask of 100.0 ml and made up the volume with distilled water. Aliquots of 25.0 ml were quantitatively transferred into electrochemical cell, pH was adjusted to 3.0, electromotive force of the obtained sample was measured, and quantitative content of steviol glycosides was determined by the calibration graph.

RESULTS AND DISCUSSION

To study the reaction between diterpene steviol glycosides and MPA by UV spectroscopy after the preliminary formation of cationic complex particles of stevioside-Ba [21-22], the absorption spectra of Stevia extracts (Fig. 1), MPA and their associate (Fig. 2) have been registered at pH=6 [1].

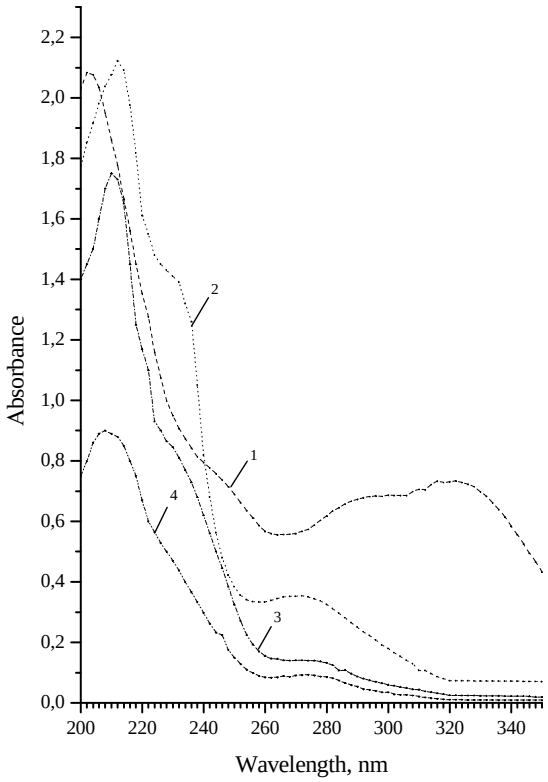


Fig. 1 – UV absorption spectra of Stevia leaves extracts (1 – an extract obtained by the author), 2 – steviol glycoside preparation (contain Rebaudioside A 95%), 3 – steviol glycoside preparation (contain Rebaudioside A 50%), 4 – steviol glycoside preparation (contain Rebaudioside A 40%)

The obtained experimental data show that the absorption bands of steviol glycosides (220-240 nm) are stored in all extracts and correspond to the literature data [17]. Table 1 shows the results of spectrophotometric study of Stevia leaves extracts.

Table 1 - UV absorption spectra of STEVIA REBAUDIANA leaf extracts

	λ_{max}, nm	Characteristic	$\varepsilon_{conditional}$
An extract obtained by the author	206	An intense absorption band	208400
	240	Shoulder (220-230)	55400
	322	Absorption band of medium strength	73400
Steviol glycoside preparation (contain Rebaudioside A 95%)	212	An intense absorption band	212000
	228	Shoulder (220-234)	140800
	272	Absorption band of medium strength	35180
Steviol glycoside preparation (contain Rebaudioside A 50%)	210	An intense absorption band	174500
	228	Shoulder (220-234)	85640
	280	A weak absorption band	14160
Steviol glycoside preparation (contain Rebaudioside A 40%)	208	Absorption band of medium strength	88810
	228	Shoulder (220-234)	50410
	274	A weak absorption band	8890

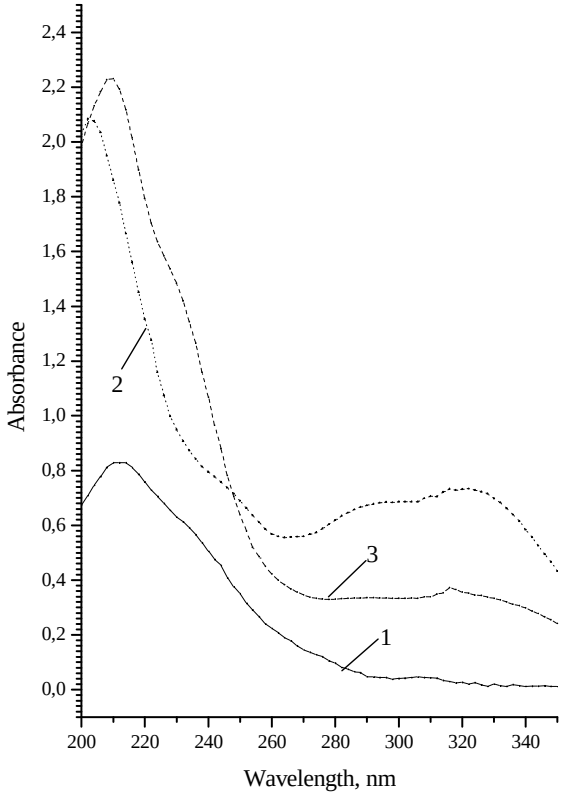


Fig. 2 – UV-absorption spectra 1 – solution MPA, 2 - STEVIA REBAUDIANA leaf extract, 3 – associate St-Ba^{2+}- MPA

Spectral characteristics of the aqueous solution of ionic associate St-Ba^{2+} - MPA retain absorption bands, characteristic for the initial substances (Table. 2). This indicates the invariability of chromophoric system during the reaction, and confirms the associative nature of the interaction.

Table 2 - UV absorption spectra of STEVIA REBAUDIANA leaf extract, MPA and their associate

Compound	λ_{max}, nm	Features UV spectra	$\varepsilon_{conditional}$
$H_3PMo_{12}O_{40}$	210	An intense absorption band	219200
	235	Shoulder (230-235)	113000
	310	A weak absorption band	28000
STEVIA REBAUDIANA leaf extract	202	An intense absorption band	208400
	240	Shoulder (220-230)	55400
	322	Absorption band of medium strength	73400
Associate St-Ba with MPA	212	An intense absorption band	200000
	226	Shoulder (220-230)	158300
	312	A weak absorption band	42600

In Raman spectrum, registered for the dry extract of the crushed leaves of Stevia rebaudina (Fig. 3), the following characteristic bands are observed [23-25]: intense absorption band is clearly shown at 3400 cm^{-1}, characteristic for oscillation of OH groups; the band at 2927 cm^{-1} corresponds to methine CH protons; the band at 1715-1661 cm^{-1} is characteristic for oscillations of C = O group; the band at 1635 cm^{-1} — for oscillations of C = C, and the band at 1100 cm^{-1} — for oscillations of ether C-O-C.

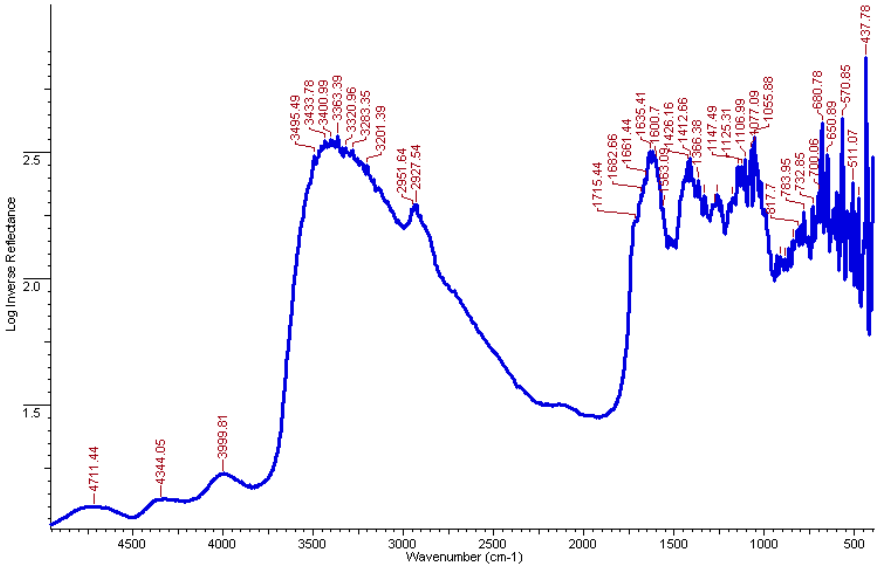


Fig. 3 – Raman spectrum of dry STEVIA REBAUDIANA leaf extract obtained by the author

For comparison, Raman spectra of purified extracts were taken with different content of rebaudioside A manufactured by «Novachem (Wuhan) I&E Co., Ltd.» (China) (Fig. 4).

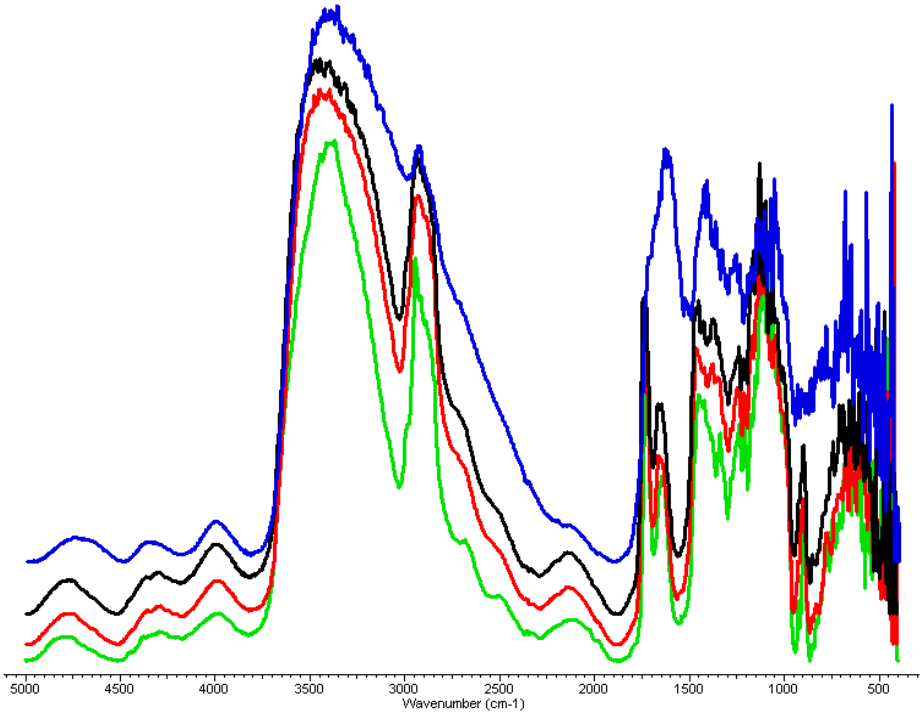


Fig. 4 – Raman spectra of STEVIA REBAUDIANA leaf extracts: a) green, content of Rebaudioside A 95% «Novachem (Wuhan) I&E Co., Ltd.» (China); b) red, content of Rebaudioside A 50% «Novachem (Wuhan) I&E Co., Ltd.» (China); c) black, content of Rebaudioside A 40% «Novachem (Wuhan) I&E Co., Ltd.» (China); d) blue, extract obtained by the author

Also, to study the nature of the interaction between the diterpene steviol glycosides and MPA, and to identify the resulting ion associate, Raman spectra of the associate and MPA were registered.

In the area of “fingerprints”, Raman spectra of the extract and associate were identical (Fig. 5), and the absorption bands 1072, 925, 881, 761, 467 cm^{-1} in the associate spectrum correspond to the MPA absorption bands (Fig. 6) [26-28], indicating the associative nature of the interaction between MPA and glycosides of STEVIA REBAUDINA.

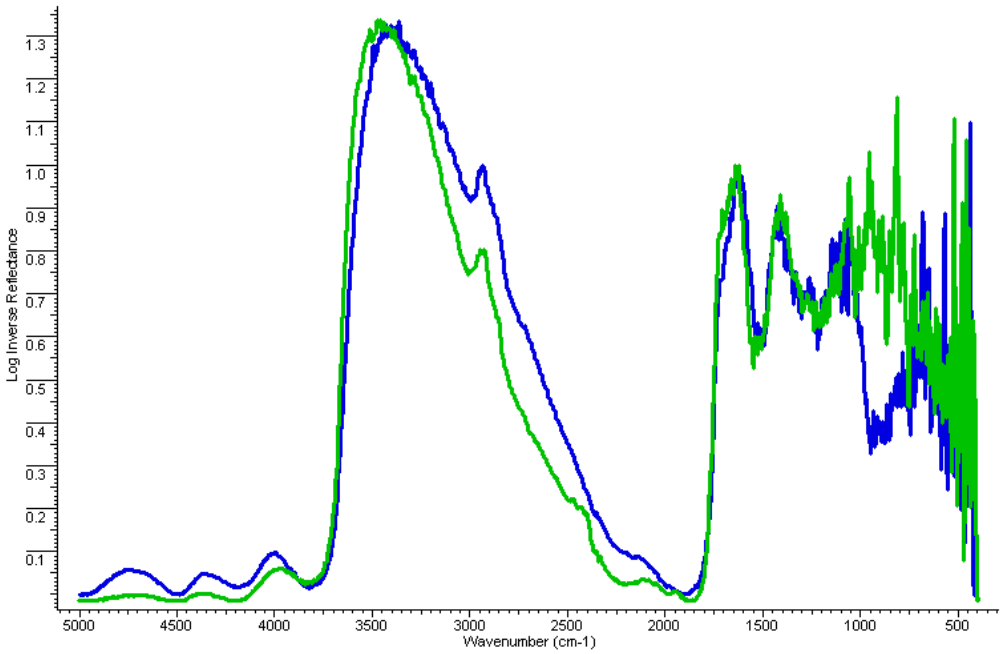


Fig. 5 – Raman spectra of STEVIA REBAUDIANA leaf extract (blue) and associate St-Ba^{2+}-MPA (green)

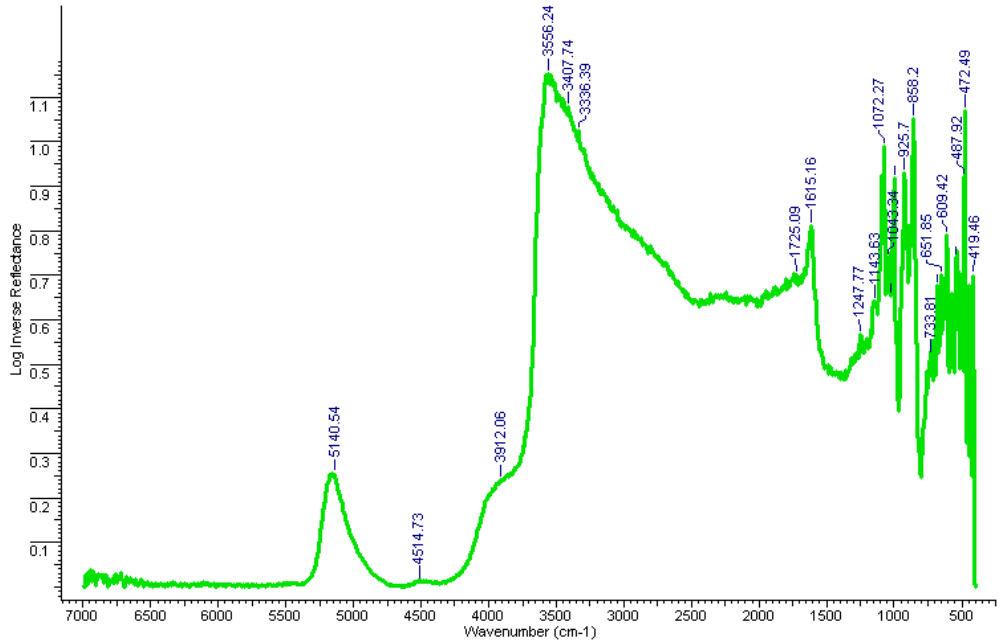


Fig. 6 – Raman spectrum of MPA

Raman, ν_{max} (cm^{-1}): 4711.44; 3444.05; 3999.81; 3495.49, 3433.78, 3400.99, 3363.39, 3320.96, 3283.35, 3021.39 (OH); 2951.64, 2927.54 (CH); 1715.44, 1682.66, 1661.44 (C=O); 1635.41 (C=C); 1428.16; 1412.66; 1366.38; 1147.49; 1106.99; 1077.09, 1055 (C-O-C); 783.95; 680.78; 650.89; 570.85; 511.07; 437.78.

The ratio of reactants in the reaction between the cationic complex particle stevioside-Ba^{2+} and MPA was shown in the previous studies by UV spectroscopy and amperometric titration [29]. This allows us to simulate the following reaction:

$3(St\text{-}Ba)^{2+} + 2PMo_{12}O_{40}^{3-} \rightarrow (St\text{-}Ba)_3(PMo_{12}O_{40})_2\downarrow$.

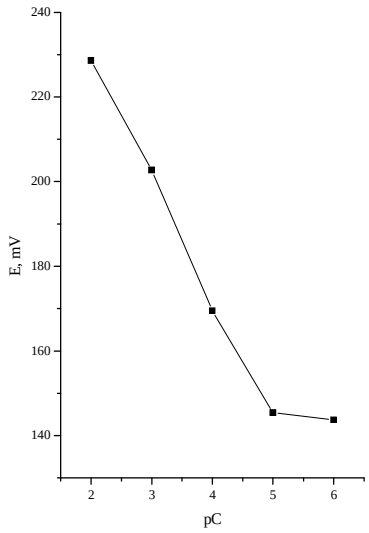
The results of instrumental studies of interaction between heteropolyanion $PMo_{12}O_{40}^{3-}$ and organic cation complex particle stevioside-Ba^{2+} were used in the development of the potentiometric method of quantitative determination of

stevioside. ISEs were designed with plasticized polyvinyl chloride membranes based on membrane solvent-plasticizer - dibutyl phthalate (DBP) and dioctyl phthalate (DOP), wherein ion associate composition $(St\text{-}Ba)_3(PMo_{12}O_{40})_2$ was used as electrode active substance (EAS) [29]. Electrode characteristics of developed ISE, sensitive to cationic particle stevioside-Ba^{2+}, have been studied in different model solutions. The influence of various factors on the characteristics of developed ISE (the slope of the electrode function and the linearity interval of detectable concentrations of stevioside in the solution):

- The pH of the test solution;
- The nature of the membrane solvent-plasticizer;
- Quantitative content of EAS in the membrane.

A series of aqueous solutions of industrial extract of Stevia leaves containing rebaudioside A 98% with concentrations from $1.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-2}$ mol/l was used to make calibration graphs [30].

Graphic dependence of the ion-selective electrode potential on the logarithm of stevioside concentration E = f (pC) is shown in Figure 7.



pH=3.0; m_{EAS}=0.006 g; solvent-plasticizer - DBP

Fig. 7 - Graphical dependence of the ion-selective electrode potential on the logarithm of the concentration of stevioside E=f(pC)

Table 3 - Dependence of electrode characteristics of ISE according to the pH solution

pH	Solvent-plasticizer	S,mVB	Range of linearity, mol/l	C_{min}, mol/l
2	DBP	26.5±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$1.0 \cdot 10^{-5}$
	DOP	25.0±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$
3	DBP	28.3±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$3.0 \cdot 10^{-6}$
	DOP	25.4±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$
4	DBP	22.8±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-5}$
	DOP	20.0±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$
5	DBP	22.0±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-5}$
	DOP	19.0±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-4}$	$5.0 \cdot 10^{-4}$
6	DBP	16.3±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-5}$
	DOP	15.0±1.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$
7	DBP	20.2±2.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-5}$
	DOP	16.0±2.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-3}$	$3.0 \cdot 10^{-3}$
8	DBP	15.8±2.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-5}$	$8.0 \cdot 10^{-5}$
	DOP	15.0±2.0	$1.0 \cdot 10^{-2}$-$1.0 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$

Main electrode characteristics of the developed ISE with membranes sensitive to steviol glycosides, depending on the nature of the membrane solvent-plasticizer and pH, are shown in Table 3.

The experimental data have shown that optimal electrode characteristics are observed at pH=3.0 using the membrane solvent-plasticizer DBP (linearity ~ $C=1.0\cdot10^{-5}$ M, the slope is close to the theoretical for doubly charged cations).

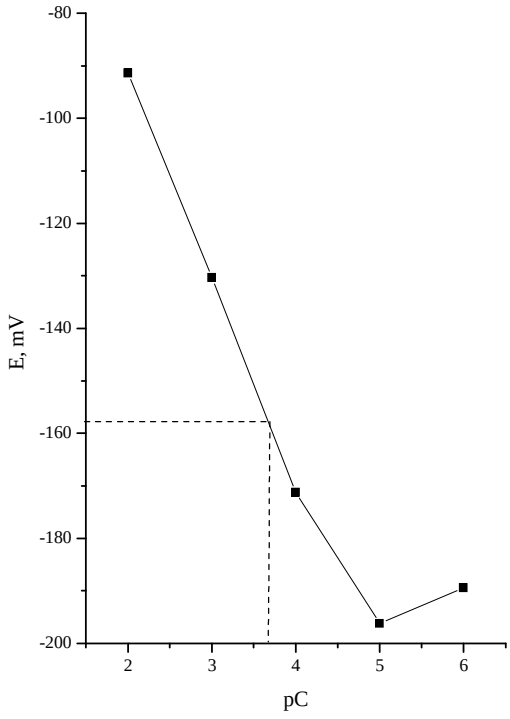
Table 4 shows the results of determining the effect of quantitative content of electrode active substance in the membrane on the characteristics of ISE (EAS-$(Tan\text{-}Ba)_3(PMo_{12}O_{40})_2$).

Table 4 - Dependence of electrode characteristics of ISE according to the quantitative content electrode active substance in the membrane (pH=3.0)

Solvent-plasticizer	Content of EAS in the membrane, g	S, mV	Range of linearity, mol/l	C_{min}, mol/l
DBP	m=0.006	27.9±1.0	$1.0\cdot10^{-2}$–$1.0\cdot10^{-5}$	$3.0\cdot10^{-6}$
	m=0.011	28.3±1.0		
	m=0.050	28.0±1.0		

The experimental data have shown that the quantitative content of EAS in the membrane does not significantly affect the characteristics of ISE.

The developed ISE with optimal electrode characteristics has been used to develop the quantitative method for determination of the total content of diterpene steviol glycosides. Graphic dependence of the ion-selective electrode potential on the logarithm of the stevioside concentration E=f (pC) in the industrial product "Chocolate bar with stevia" manufactured by Zhytomyr confectionary factory "ZS" is shown in Figure 8.



pH=3.0; m_{EAS}=0.006 g; solvent-plasticizer - DBP

Fig. 8 - Graphical dependence of the potential of the ion-selective electrode on the logarithm of the concentration of stevioside E=f (pC) in the industrial product

The determination of steviol glycosides in the industrial extracts of stevia leaves (with different content of diterpene glycosides and rebaudioside A) and in food for diabetics, sweets "Chocolate bar with stevia" manufactured by Zhytomyr confectionary factory "ZS", was carried out to validate the developed method. The results are shown in Table 5.

Table 5 – The results of determination of the amount of diterpene steviol glycosides

Object	Introduction, %	Found, x±δ, %	Sr
An extract containing Total Steviol Glycosides-98%, Rebaudioside A-95% («Vilarus » Ukraine)	95.0	93.0±0.31	0.02
An extract containing Total Steviol Glycosides-90%, Rebaudioside A-50% («Novachem (Wuhan) I&E Co., Ltd.» China)	90.0	88.7±0.26	0.02
Sweets "Chocolate bar with stevia" manufactured in Zhytomyr confectionary factory "ZS"	0.005	0.005±0.0001	0.01
STEVIA REBAUDIANA extract obtained by the author	10.0-20.0	10.95±0.18	0.01

The experimental data confirm the validity of ionometric determination of the amount of diterpene steviol glycosides in industrial products and absence of bias.

CONCLUSION

The method of stevioside determination in the extract of stevia leaves and industrial products was developed using the method of direct potentiometry with the developed ISE. The method was tested on real objects of industrial products. The new technique meets all requirements of modern analysis - it is simple and express, safe and affordable, has sufficient accuracy, sensitivity and selectivity.

REFERENCES

[1] Carakostas M, Curry L, Boileau A, Brusick D, *Food Chem Toxicol*, **2008,** 46(7), 1.
[2] Sadovskiy A, *Himija i Zhizn'* 2005, 4, 28.
[3] Zubcov V, Milorodova E, Jurova E, Rjasenskij S, Osipova L, *Sposob poluchenija steviozida*. Patent RU, 2111969, **1998**.
[4] Shazzo R, Erashova L, Dergunov A, Zhukov A, *Sposob poluchenija jekstrakta iz rastenija Stevia Rebaudiana Bertoni dlja vinodelija*. Patent RU 2167544, **2001**.
[5] Lisicin V, Hakimov H, *Sposob poluchenija jekstrakta iz rastenija Stevia Rebaudiana Bertoni*. Patent RU 2198548, **2003**.
[6] Stevia – a non caloric sweetener of natural origin, *IFST information statement*, **2012**, 12, 1
[7] Morita T, Morita K, Komai K, *Sposob proizvodstva podslastitelja s vysokim soderzhaniem rebaudiozida-A*. Patent RU 2463780, **2012**.
[8] Koyama E, Kitazawa K, Ohori Y, Izawa O, Kakegawa K, Fujino A, Ui M, *Food and Chemical Technology*, **2003**; 41(3), 359.
[9] Lastuhin Ju, *Harchovi dobavki. E- kodi. Budova. Oderzhannja; Vlastivosti*, Centr Evropi, L'viv, **2009**.
[10] Nechaev A, Kochetkova A, Zajcev A, *Pishhevye dobavki*, Kolos, Moscow, **2001**.
[11] Bashura A, Polovko N, Gladuh E, *Tehnologija kosmeticheskih i parfjumernyh sredstv*, Zolotye stranicy, Kharkiv, **2002**.
[12] Korpacheva-Zinych O, Chernaja Ju, *Jekstrakty stevii saharozameniteli s lechebnymi svojstvami*, Steviasjan, Kiev, **2010**.
[13] Malyshev V, *Tihaja revoljucija v pishhevoj promyshlennosti*; Sozvezdie, Samara, **2010**.
[14] Krasina I, Hodus N, *Pishhevaja tehnologija*, **2004**, 5-6, 121.
[15] Pavlovskaja I, Solohina I, Gneusheva I, *Vestnik Oreal GUA*, **2012**, 2, 48.
[16] Krahmalev I, Gubanova L, Hadzhieva Z, *Farmacevticheskie issledovanija*, **2012**, 5, 431.
[17] Kedik S, Jarcev E, Stanishevskaja I, Fedorov S, *Khimija i tehnologija lekarstvennyh preparatov i biologicheski aktivnyh veshhestv*, **2008**, 3, 79.
[18] Vanidze M, Kalandija A, Chanukvadze H, *Khimija Rastitel'nogo Syr'ja***, 2009**, 4, 155.
[19] Kedik S, Fedorov S, Janul' N, Prohorova L, Smirnova E, *Pharmaceutical Chemistry Journal*, **2003**, 10(37), 19.
[20] Tolstikov G, Gorjaev M, *Glicirretovaja kislota*, Nauka, Alma-Ata, **1966**.

[21] Golovej O, *Analitichne vikoristannja reakcij nitrogenovmistnih fosfolipidiv ta polimernih spoluk z 12-molibdofosfatom*, UDHTU, Dnipropetrovs'k, **2005**.
[22] Golovej O, Kuprin V, Tkach V, *Voprosy khimii i khimicheskoi technologii*, **2003**, 3, 27.
[23] Chaturvedule V.S.P, Mubarak Ch, Prakash I, *Food Nutrition Sci*, **2012**, 3, 1467.
[24] Kaur G, Pandhair V, Cheena G, *J. Med. Plants Stad*, **2014**, 5(2), 41.
[25] Upreti M, Strassburger K, Chen Y, Sh. Wu, Prakash I, *Int. J. Mol. Sci*, **2011**, 12, 7529.
[26] Tkach V, *Geteropolianioni jak analitichni reagenti na azotvmishhujuchi organichni rechovini*, DDU, Dnipropetrovs'k, **1995**.
[27] Tkach V, Karandєєva N, Ciganok L, Vishnikin A, *Vikoristannja geteropolianioniv strukturi Keggina v analizi organichnih spoluk*, UDHTU, Dnipropetrovs'k, **2002**.
[28] Tkach V, Maga I, Bolotin O, *Elektrohimichnij analiz nitrogenovmisnih organichnih likars'kih ta bioaktivnih rechovin z vikoristannjam geteropolianioniv strukturi Keggina jak analitichnih reagentiv*, Padjaka, Uzhgorod, **2012**.
[29] Lucenko N, Mironjak M, Tkach V, *Voprosy khimii i khimicheskoi technologii*. **2014**, 5-6, 22.
[30] Nikol'skij B, Materova E, *Ionoselektivnye jelektrody*, Himija, Lviv, **1980**.