The Stepwise Injection Analysis as a New Opportunity for Automation of Chemical Analysis of Liquid, Gaseous and Solid-Phase Samples

Andrey V. Bulatov[*], Aleksey L. Moskvin, Leonid N. Moskvin, Anatoly V. Mozhuhin

St. Petersburg State University, Department of Chemistry, pr. Universitetskij 26, 198504
St. Petersburg, Russia

Abstract

In the article, various methods of flow injection and sequential injection analyses are considered. The relative advantages and disadvantages are investigated. The stepwise injection analysis is proposed as a new universal flow method of analysis which means maximum strict reproduction of all the analytical stages which are typical for automated stationary methods. The opportunities of the new method which can be applied to the sample analysis in any aggregative state are illustrated by the examples of phosphate-ions in urine, anionic surfactants and nitrate- and nitrite-ions in water, hydrogen sulfide in hydrocarbon gases, and phosphates in water-soluble mineral fertilizer.

Keywords Stepwise injection analysis, Sequential injection analysis, Flow injection analysis

1. Introduction

By the present day, flow-injection analysis (FIA) [1] and sequential injection analysis (SIA) [2] have been widely disseminated for problem-solving of chemical analysis automation.

Hydraulic schemes of FIA mean periodical injection of a discrete sample portion into the laminar flow of the carrier. Afterwards the flow of carrier-solution mixes with the flow of the reagent solution in the reaction coil and moves into the flow detector. The detector records concentration peaks the width of which is determined by the sample dispersion during the running through the hydraulic track. So we can define FIA as the flow analysis with "controlled sample dispersion" [3].

In SIA one liquid line is used instead of the "net" as in FIA. Through this line the flow of carrier-solution moves in two opposite directions by turns with the help of bidirectional pump. At the same time the discrete sample portions and reagent solutions are injected serially into this flow by means of the same bidirectional pump through the multiway valve. The mixed solution passes through the flow detector which records the peaks the width of which depends on the sample dispersion as in FIA.

FIA and SIA have got such an irrefutable virtue as an efficient production but they both have one common drawback in comparison with their automated static counterparts of analytical methods the sensitization. It follows on the sample dispersion in the hydraulic traces.

In addition, in FIA and SIA methods it is necessary to assemble individual hydraulic shemes when you change from one analytical task to another one. This necessity complicates the problem of on-line automation too much.

We offer another variant of flow methods – stepwise injection analysis (SWIA) as the most universal solution which has the purpose to remedy a deficiency of FIA and SIA methods. This article deals with experimental corroboration of the opportunity to adapt this method the automation of the sample analyses methods. The samples under investigation are in different aggregative states – liquid, gaseous and solid. In the first case, it is talked about the dedermination of phosphate-ions in urine, anionic surfactants, nitrate- and nitrite-ions in water; in the second case it is the determination of hydrogen sulfide in hydrocarbon gases; in the third case we talk about the determination of phosphates in water-soluble mineral fertilizer.

2. The conception of SWIA

SWIA means strict reproduction of all the analytical stages which are typical for stationary methods. The stages are

*Corresponding author

E-mail: bulatov_andrey@mail.ru

portion sampling; sample pretreatment; the addition of reagent solutions to sample solutions; intermixing of the solutions with the help of gas stream until the equilibrium of the system will be gained; thermostating (if necessary); pauses for attainment of maximum value of analytical signal; analytical signal measuring. Thus, SWIA can be regarded as the return to initial chemical analysis automation scheme which is based on the manual operations reproduction made by analysts by means of automated mechanical devices but with the experience accumulated during the flow analyses methods development.

Only two variants of hydraulic systems are sufficient enough for the automation of all the variety of chemical analytical methods. In the simple cases, the analytical methods don't include special devices for concentration and analyte conversion such as sorption columns or reducers. The universal hydraulic sheme of SWIA (Fig. 1) includes multiway valve (V), bidirectional peristaltic pump (P), thermostatically controlled reaction vessel (RV), auxiliary vessel (AV) which has cylindrical shape and funnel-shaped at the bottom, and the flow detector (D).
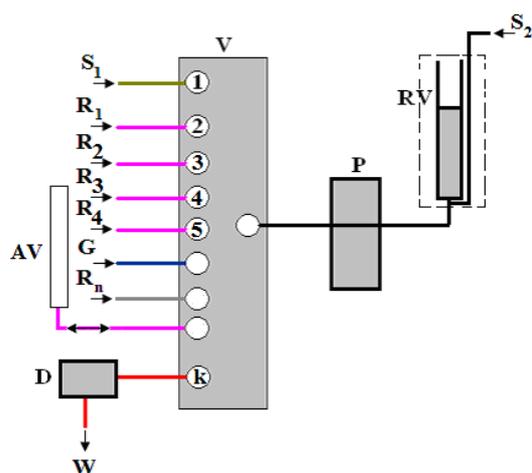
Fig. 1 SWIA scheme: multi-way valve (V); bi-directional pump (P); reaction vessel (RV); detector (D); channel for entering liquid sample (S_1); channel for entering gas sample (S_2); channel for entering 1st reagent solution (R_1); channel for entering n^{th} reagent solution (R_n); channel for entering gas (G); waste (W).

When analyzing liquid samples according to this scheme, optimized volumes of sample (S_1), reagent solutions (R_1, R_2, R_n…) and gas (ambient air or nitrogen) (G) move sequentially into the reaction vessel (RV) through the multiway valve (V) by means of the pump (P) for the intensive intermixing of solutions in the reaction vessel. If there are analytical reactions with delayed analytical form kinetics a pause should be made to complete the reaction of analyte with reagent. Thus, the

analytical signal measuring is assured when the signal comes up to the maximum value for the automated methods of analysis.

The shown scheme allows to automate the methods of solution analysis with analyte extractions and methods of gas analysis without hyphenated flow techniques. When extraction methods are automated the process of analyte extraction proceeds straight in the reaction vessel. Here the necessary volume of extragent (R_2) injected into reaction vessel besides sample portions (S_1) and reagent solution (R_1). After that water and organic phases mixing is assured by means of gas stream (G). After the stop of gas phase flow and a pause which is necessary for phase exfoliation the extract moves into the flow detector (D).

If the methods of gas analysis are automated the gas sample is directed to reaction vessel (RV) through the channel for gas sample (S_2) entering. Such a scheme can be used both for the liquid-absorptive analyte extraction at the gaseous mediums and for the gas extraction at the analysis of the gases which have been dissolved in water or another solvents or of the gases which can be generated in solutions as a result of chemical reactions in the reaction vessel.

If we analyze solid-phase samples the sample portion is placed into the auxiliary vessel (AV). After that the portion of the appropriate solvent (R_1) is placed into it and the gas stream is directed from the reaction vessel or the sample dissolution. The generated solution should be analyzed according the scheme of the liquid sample analysis.

In more complicated cases of analytical methods which include the operations of sample pretreatment on the special devices such as sorption columns, extractor or reducer for conversion of analyte into another chemical form the second unified scheme (Fig. 2) is necessary. In this scheme all the devices for sample pretreatment are called accessorial device (AD).

When you analyze samples according to the scheme illustrated in Fig. 2 in the first stage the sample (S_1) and reagent solution (R_2) get into reaction vessel first. The reagent solution is necessary for favourable conditions creating for following analyte concentration or its conversion, for example, for the necessary pH value generation in the accessorial device. Then the sample and the reagent solution are intermixed by the gas stream and the solution is pumped from the reaction vessel (RV) into the auxiliary vessel (AV) through the accessorial device by switching of the valve. After that the solution returns to the reaction vessel from the auxiliary vessel with the help of the pump. And then two variants are possible. If it is the sorption concentration the solution is discharged out of the reaction vessel, and an eluent (R_3) is sent for analyte desorption into the vacant

reaction vessel through another valve channel. The eluent is directed through the valve into the sorption column which functions as accessorial device here, and than it follows into the auxiliary vessel and again into the reaction vessel. After that into the reaction vessel it is necessary to measure out in doses sequentially the determined volumes of reagent solutions (R_n) required for generation of analytical form of analyte in eluate and the gas stream (G) for mixing. The final stage is pumping of the solution from the reaction vessel into the flow detector with analytical signal measuring according the stopped stream scheme or in the flow conditions. In the second variant which corresponds with the case of analyte conversion in the reducer the returned into the reaction vessel solution acts like a sample with keeping the succession of all the operations according the first of the schemes under consideration.
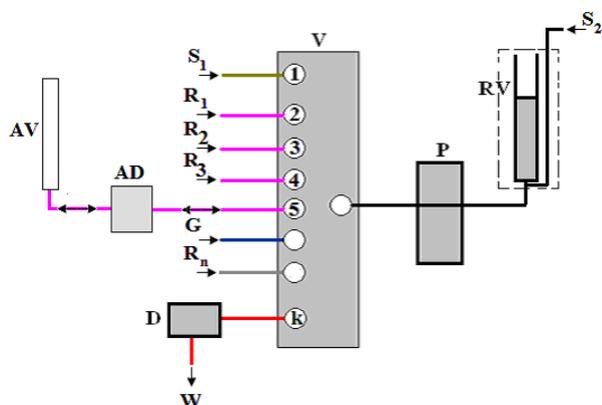
Fig. 2 SWIA scheme, including sample preparation in accessorial device: multi-way valve (V); reverse pump (P); reaction vessel (RV); detector (D); accessorial device (AD); accessorial vessel (AV); channel for entering liquid sample (S_1); channel for entering gas sample (S_2); channel for entering 1st reagent solution (R_1); channel for entering n^{th} reagent solution (R_n); channel for entering gas (G); waste (W).

In SWIA unlike FIA and SIA, the received analytical signal form is the simple difference of registered detector signals which correspond to the sample and the background solution (Fig. 3). In FIA and SIA, the analytical signal is registered as concentration peak even the maximum of which is less than value which it can reach in SWIA (Fig. 3). It is the logical consequence of the fact that in SWIA the sample and reagent solutions are mixed entirely by gas stream. In FIA and SIA, as the sample is moving through the hydraulic circuit lines it is "watered out" party, and as the result, the analytical signal dispersion is observed. The degree of the dispersion depends on such characteristics as sample value, the flow speed, the length and diameter of pipes, the configurations of hydraulic line and

mixing coil, the detector construction [4]. So in SWIA analytical signal measuring is provided in conditions when it comes up to the maximum value for this analytical method.
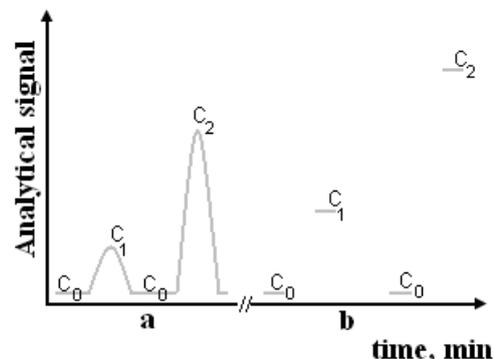
Fig. 3 Registered analytical signals in FIA, SIA (a) and in SWIA (b): for background solution C_0 and sample solutions with concentrations C_1 и C_2.

3. Experimental

3.1. Reagents and solutions

For the preparation of the reagent solution, which is used for the detection of phosphate-ions in urine we mixed 50 mL of 2.5 mol L^{-1} H_2SO_4, 15 mL of 40 g L^{-1} solution of $(NH_4)_6 M_{07}O_{24} \cdot 4H_2O$, 30 mL of 20 g L^{-1} solution of ascorbic acid and 5 mL of 3 g L^{-1} $K(SbO)C_4H_4O_6 \bullet 0.5H_2O$ solution. The solutions were mixed everyday.

The prepared 100 g L^{-1} solution of Greiss reagent was used for the determination of nitrate- and nitrite-ions. To prepare the correcting solution we dissolved 5.4 g of NH_4Cl, 19 g of $Na_2B_4O_7 \cdot 10H_2O$, 0.05 g of $C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$ (EDTA), and 0.01 g of $CuSO_4 \cdot 5H_2O$ in 100 mL of distilled water.

In the capacity of photometrical reagent for the determination of hydrogen sulphide in hydrocarbon gases, the solution of N-dimethyl-p-phenylenediamine was used. The 1.5 g L^{-1} solution of N-dimethyl-p-phenylenediamine was prepared in 4.5 mol L^{-1} HCl and kept in dark glass. To prepare 0.2 g L^{-1} solution of Fe(III) ions which was used for determination of hydrogen sulphide in hydrocarbon gases, the portion of $FeCl_3 \cdot 6H_2O$ was dissolved in 4.5 mol L^{-1} HCl.

To prepare the process solutions of phosphate-ions we took 1 g L^{-1} solution of phosphate-ions which was generated by the dissolving of a portion of KH_2PO_4 in distilled water right before experiment.

To prepare phosphate buffer solution 10 g KH_2PO_4 was dissolved in 1 L of distilled water and 1 mol L^{-1} solution of NaOH was added to get pH=10.

To prepare the 2% solution of zinc acetate 2 g of $Zn(CH_3COO)_2$ was dissolved in 100 mL of 0.1 mol L^{-1} acetic acid.

All the reagents in use ("Reactive", Saint-Petersburg, Russia) have qualification of analytical reagent grade quality.

3.2. Apparatus

The SWIA scheme was brought together on the basic of modular flow analyzer (Rosanalyte, Saint-Petersburg, Russia). In our work we used photometrical detector (the wavelength 670 nm, 10 mm light path), peristaltic pump, which provides the flow direction reversion (the flow speed is 5 mL min^{-1}), seven-way valve (titanium), communication pipelines (polytetrafluoroethylene, the inner diameter is 0.5 mm), reaction vessel. The scheme of reaction vessel is shown in Fig. 4. It is a cell made of polytetrafluoroethylene which has such channels as a channel for solutions and gas injection for their intermixing (1) (its length is 10 mm, inner diameter is 1 mm); a channel for gas samples injection (2) (its length is 10 mm, inner diameter is 2 mm); a channel for mixing of the solutions by gas phase stream (its length is 100 mm, inner diameter is 10 mm). The running of analyzer is automated by a computer.
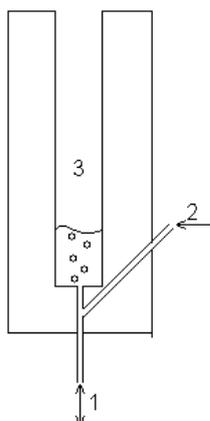
Fig. 4 The scheme of reaction vessel: a channel for solutions and gas injection for their intermixing (1); a channel for gas samples injection (for gas analysis) (2); a channel for mixing of the solutions (3).

4. Results and discussion

For the automation of photometrical methods to determinate phosphate-tons in urine, anionic surface acoustic waves in water, hydrogen sulfide in hydrocarbon gases and phosphates in water-soluble mineral fertilizers, was used the SWIA scheme shown in Fig. 1.

The methodology of the determination of phosphate-ions in urine was developed with the method of standard additions (two additions of phosphate-ions) in the SWIA variant

according the forming reaction of molybdenum blue[5]. The sample portions (S_1), reagent solutions (R_1) and distilled water were injected into the thermostated reaction vessel (RV) (60 $^{\circ}$C) trough the lines of sample and reagent solutions injection (Fig. 1). In the case when samples with additions are analyzed, calculated portions of addition solution (R_3) were injected instead of the distilled water portion (R_2). After the reagent solutions delivery, the stream of ambient air (G) has been let through for 60 s. Then the absorbance value of the sample (A_1) and the sample with additions (A_2 and A_3) was measured in the stopped flow conditions. The volumes of the sample and the solutions added into the sample in every case in the reaction vessel can be found in the Table 1.

In the final stage we made scrubbing of system communications with distilled water and measured background signal (A_3) during the delivery of urine sample and distilled water into the reaction vessel (RV) in ratio1:5, respectively.

The content of analyte in a sample was evaluated on formula

$$\tilde{N}_x = \frac{C_0(A_3 - A_2)A_1 V_\Sigma}{(A_2 - A_1)^2 V_1}$$

where C_x is the concentration of analyte in a sample, C_0 is the concentration of phosphate-ions in solution of phosphate-ions which is used for injection of additions, V_Σ is the total volume of sample solution of phosphate-ions and distilled water (2.5 ml); V_1 is the sample volume (0.5 ml).

Table 1 Volumes of samples, entering in the reaction vessel and volumes of reagents' solutions for phosphate-ions determination in human urine

№	Volume (mL)				Comments
	Sample	Phosphate-ions solution	Distilled water	Reagents solution	
1	0.5	0	2.0	0.5	sample
2	0.5	1.0	1.0	0.5	sample with first addition
3	0.5	2.0	0	0.5	sample with second addition

The methodology is tested at urine samples of different patients (Table 2). The findings coincide with the findings according [5]. The defined methodology ensures the low range

Table 2 The results of phosphate-ions determination in urine samples (n=5, P=0.95)

Sample	Found, mg L^{-1}	
	The developed method	Photometry
1	10.8±0.8	10±1
2	8.4±0.6	8±1
3	11.8±0.8	11±1
4	9.7±0.6	10±1
5	10.5±0.6	10±1

Table 3 The results of determination anionic surfactants in sewage water (n=5, P=0.95)

Purification efficiency	Added, mg L^{-1}	Found, mg L^{-1}
first	0	3.1±0.3
	3.0	5.7±0.4
second	0	1.6±0.3
	1.5	2.8±0.2
third	0	0.11±0.07
	0.10	0.21±0.05

limit of determinate concentrations 2 mg L^{-1}, the analysis duration is 10 min.

The scheme of SWIA with the analyte extraction was realized by the automation of the method of the extraction-photometric determination of anionic surfactants in water according the scheme of their determination in associate formation with methylene blue [6]. In this case according the SWIA scheme (Fig. 1) 10 mL of sample (S_1), 0.5 mL of methylene blue solution (R_1), 1 mL of phosphate buffer solution (pH=10) (R_2), 2 mL of chloroform (R_3) and the stream of ambient air (G) which provides intensive phase intermixing in the reaction vessel were supplied sequentially through the valve into the reaction vessel (RV). After the flow-stop and phase immiscibility the extract was directed into the cell of photometrical detector (D). The extract absorbance measuring was carried out in the stopped-flow conditions. In the conclusion the scrubbing of system communications was made with the solution of isopropyl alcohol (R_4). After that the measuring of background signal at filling of detector cell (D) with chloroform was carried out.

The methodology was tested at sewage water of different purification efficiency. The findings are shown in Table 3. The validity of test results, received from use of the elaborated methodology, was verified by the "added-found" method. As the Table 3 indicates, added and found analyte amounts practically coincide. The methodology provides the low range limit of the determinate concentrations of anionic surfactants at the level of 75 µg L^{-1}, the sample volume is 10 mL and analysis duration is 10 min, i.e. the complication of methodology didn't practically affect the analysis duration.

The scheme of SWIA at the analysis of gaseous samples was tested by example of the automation of the method photometrical determination of hydrogen sulfide in hydrocarbon gases by its reaction with N-dimethyl-p-phenylenediamine [7]. In this case into the reaction vessel (RV) the following reagents were delivered through the different value ways in turn: 1 mL of $Zn(CH_3COO)_2$ solution (R_1) which is used as the absorbent solution for hydrogen sulfide extraction, the stream of hydrocarbon gas sample (S_2) (for 10 min, flow rate 0.3 L min^{-1}), 1 mL of N-dimethyl-p-phenylenediamine (R_2), 1 mL of Fe (III) ions solution, and nitrogen (G) for the solution intermixing in the reaction vessel (RV). After the analytical formation, the absorbance measuring was carried out in the stopped-flow conditions like in the previous cases. In the next stage we carried out the scrubbing of system communications by distilled water (R_4) and background signal measurement at filling of the detector with the solution which was received by mixing of the solution of N-dimethyl-p-phenylenediamine (R_2) and the solution of Fe (III) ions (R_3) in ratio 1:1 in the reaction vessel.

The elaborated method was tested at hydrocarbon gas analysis by "added-found" method. As the Table 4 indicates, the added and found amounts of H_2S practically coincide. The method provides the low range limit of the determinate concentrations in 0.5 mg m^{-3} at the sample volume of 3 L and the duration of one analysis for 15 min.

Table 4 The results of determination hydrogen sulfide in hydrocarbon gas (time of concentration 10 min, n=5, P=0.95)

Added, µg	Found, µg
0	2±0.5
19	21±2
28	30±2
38	39±1

The last evidence of the universality of SWIA scheme shown in Fig. 1 is the solid-phase samples analysis which includes their preliminary dissolving stage.

The verification was carried out by example of the automation of photometrical determination of phosphates in water-soluble mineral fertilizers according the reaction of molybdovanadiumphosphate [8]. In this case into the auxiliary vessel we set the fertilizer sample portion (3-4 mg), fed 6 mL of distilled water (R_1) and the stream of ambient air from the reaction vessel (RV) for 60 s, meanwhile the sample was dissoluting. The sample solution prepared in the auxiliary vessel was analyzed three times. To realize it 2 mL of prepared solution were sampled every time into the reaction vessel, 1 mL mixed reagent solution and the stream of ambient air (G) were added for 30 s, and molybdovanadiumphosphate was generated. In the final stage, we carried out the scrubbing of communications with distilled water and background signal measuring at filling of the detector with background solution, which was received by mixing of 2 mL distilled water (R_1) and 1 mL mixed reagent solution (R_2) in the reaction vessel (RV).

When testing the method, different mineral fertilizers were analyzed according the suggested method and the method as on[8]. The comparison of the results of phosphorus determination in fertilizer, received according the elaborated method and[8] (Table 5), allows to draw a conclusion about their practically total identity.

Table 5 The results of determination water-soluble phosphates in the granulated mineral fertilizers (n=5, P=0.95)

Sample	Found PO_4^{3-}, %	
	The developed method	Photometry
1	28.8±1.0	28.3±1.5
2	26.5±1.3	25.0±1.5
3	24.1±1.6	26.2±1.6
4	7.9±0.5	7.0±0.4
5	6.7±0.3	6.8±0.2

The elaborated method provides the range of determinate concentrations from 6 % up to 35 %, the sample mass is 3 mg and the duration of each analysis is 5 min.

The second of universal SWIA schema which includes sample pretreatment in special devices was tested at the method of separate photometrical determination of nitrate- and nitrite-ions in water [9]. The method is based on the sequential determination of nitrite- and nitrate- ions (after the reduction of the latter ones to nitrite- ions in cadmium reducer) according the of reaction coloured azo dye formation when nitrite-ions interact with Griess reagent at the same time. The method in question illustrates the opportunity to optimize the analytical formations conditions when the reaction kinetics is delayed. Into the reaction vessel (RV) 1 mL of Griess reagent solution (R_1), 1 mL correcting solution (R_2), 2 mL distilled water, and gas stream (G)

are delivered, the mixed solution moves into the flow detector (D), the background signal is measured.

Then 1 mL of Griess reagent solution (R_1), 1 mL correcting solution (R_2), 2 mL sample and gas stream (G) are fed into the reaction vessel (RV), here the analytical form solution generates and is directed into the flow detector (D), and the concentration of nitrite-ions in the sample is measured.

In the next stage 1 mL correcting solution (R_2), 2 mL sample (S_1), and gas stream (G) are delivered into the reaction vessel (RV), after that the mixed solution is directed through the cadmium reducer (AD) into the auxiliary vessel (AV), at this time nitrate-ions reduce to nitrite-ions. From the auxiliary vessel (AV) the solution moves into the reaction vessel (RV), where it mixes with the 1 mL of Griess reagent solution. Afterwards the content of nitrate- and nitrite-ions in the sample is determined. The concentration of nitrate- and nitrite-ions is estimated by the difference of two determinations described above.

The method was tested at samples of natural water. The findings are shown in Table 6. The accuracy of the results which are obtained by using of the elaborated method is proved by "added-found" method. The method provides the low range limit of the determinate nitrate- and nitrite-ions concentration at level of 0.1mg L^{-1}, the analysis duration is 10 min.

Table 6 The results of determination nitrate- and nitrite-ions in waters (n=5, P=0.95)

Sample	Added, mg L^{-1}		Found, mg L^{-1}	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-
Gulf of	-	-	2.3±0.1	43±1
Finland	5	20	7.7±0.2	60±2
Neva river	-	-	<1	29±1
	5	20	4.8±0.2	48±2
Moyka river	-	-	<1	31±1
	5	20	5.4±0.2	49±2
Vontanka	-	-	1.5±0.1	39±1
river	5	20	6.4±0.2	55±2
Olgin pool	-	-	4.4±0.2	57±2
	5	20	9.1±0.2	78±2

5. Conclusions

As follows from the results which were received from the testing of SWIA method opportunities it is possible to keep the sensitivity of automated stationary methods at the expense of sample dispersion elimination and optimization of analytical formation. It can't be attained in FIA and SIA methods. However, SWIA is substantially inferior to know counterparts in

efficiency. The analysis duration according the SWIA scheme which doesn't include the stages of preliminary extraction and concentration or dissolution of samples is principally determined by the analytical formation rate. When the methods which include the stage of preliminary extraction and concentration or dissolution of samples are automated the analysis duration is limited by this stage besides the kinetics of analytical formation.

Thus it is impossible to consider SWIA as the alternative to the known flow analysis methods, but only as one of the possible common methods of approaching to the automation of chemical analysis methods. SWIA can be easily adapted to applied analytical laboratories, because the SWIA methods reproduce static counterparts maximum precisely.

References

[1] J. Ružicka, E.H. Hansen, *Anal. Chim. Acta*, **78**, 145 (1975).

[2] J. Ružicka, G.D. Marshall, *Anal. Chim. Acta*, **237**, 329 (1990).

[3] C. Riley, B.F. Rocks, R.A. Sherwood, *Anal. Chim. Acta*, **179**, 69 (1986).

[4] A. Cerda, V. Cterd. *An introduction to flow analysis*, Palma de Mallorca, 2009.

[5] A.A. Fyodorov, F.V. Chernyahovskaya, A.S. Vernidubov and others. *Analytical chemistry of phosphorous*, Moscow, 1974.

[6] L.N. Moskvin, N.V. Mikhailova, A.L. Moskvin, *Journal of Analytical Chemistry*, **56**, 763 (2001).

[7] Standard of Russia 22387.2-97. *Combustible natural gases. Methods for determination of hydrogen sulphide and sulphur mercaptan.*

[8] Standard of Russia 20851.2-75. *Mineral fertilizers. Methods for determination of phosphates*.

[9] G.F. Wang, M. Satake, K. Horita, *Talanta*, **46**, 671 (1997).