Spectrofluorimetric Determination of Dihydric and Trihydric Alcohols by Flow-Injection Analysis

Bin Zhang, H. P. Beck*

Institute of Inorganic and Analytical Chemistry and Radiochemistry, Saarland University, P. O. Box 15 11 50, D-66041, Saarbrücken, Germany

ABSTRACT

A rapid and simple flow-injection spectrofluorimetric method for the indirect determination of dihydric and trihydric alcohols is proposed. The determination mechanism was based on the oxidation of thiamine by periodate at pH 9.0 to form fluorescent thiochrome accelerated by Mn^{2+}, which could be determined by flow-through spectrofluorimetry. The analytes under consideration (ethylene glycol, propylene glycol and glycerol), which can be oxidized by periodate (malaprade reaction), consume the periodate and this results in a decrease of the fluorescence intensity of thiochrome which is proportional to the concentration of the sample analysed. The effects of reagent concentrations, FI system variables and temperature on the determination were studied. Under optimum conditions the working ranges are linear from 2.0×10^{-4} to 5.0×10^{-3} mol L^{-1} for ethylene glycol, 2.0×10^{-4} to 4.0×10^{-3} mol L^{-1} for propylene glycol and 5.0×10^{-5} to 1.0×10^{-3} mol L^{-1} for glycerol with a sampling throughput of 60 samples h^{-1} at room temperature (22°C). The relative standard deviation for ten replicates of analysis of 2.0×10^{-4} mol L^{-1} glycerol is 3.1 %. In addition the result of normal FIA and reversed FIA is compared and the mechanism of sensitivity enhancement in the presence of Mn^{2+} is discussed. The proposed method has been applied for the determination of glycerol spiked in real samples with good results.

Keywords Flow-injection, spectrofluorimetric, dihydric and trihydric alcohols, malaprade reaction

1. Introduction

Dihydric and trihydric alcohols are important chemical reagents in industrial products and the fields of their application are widespread, e.g., ethylene glycol or propylene glycol are used as de-icing or anti-icing reagents [1], glycerol has been reported to have more than 150 applications in foods, pharmaceuticals, solvents, plasticizer industry, etc. [2]. Meanwhile the use of them is known to bring about some environmental impact, e. g., de-icing or anti-icing fluids which contained such reagents could cause contamination of surface drainage [3]. In addition monitoring of the concentration of glycerol is one of the most import parameters in the quality control for some industrial products [4]. Therefore, it is necessary to find a simple and fast method to determine such substances.

Many methods using flow-injection techniques have been reported for the determination of glycerol and ethylene glycol since Betteridge and Ruzicka has proposed the first FIA application for the determination of glycerol based on a refractive index phenomenon [5]. Most of these methods by far are based on enzymatic determination with different detections, e. g., spectrophotometric [6-7], spectrofluorimetric [8-10], ampèrometric [11-14] and chemiluminometric [15-17], etc.. Others included methods with chemiluminescence emission detection [18], schlieren optics [2] and spectrofluorimetric determination with Alizarin Navy Blue [19], etc..

In this paper a simple and fast spectrofluorimetric method for the indirect determination of dihydric and trihydric alcohols with FI technique is based on a reaction proposed for a batch procedure. Periodate and thiamine react at pH 9.0 to produce a fluorescent compound thiochrome (λ_{ex} = 370 nm, λ_{em} = 440 nm). The sample to be determined consumes periodate (malaprade reaction) and this results in decreasing the fluorescence intensity of thiochrome. This decrease is proportional to the concentration of sample injected.

* Corresponding author:
E-mail: hp.beck@mx.uni-saarland.de

2. Experimental

2.1 Reagents

All reagents were of analytical grades and solutions were freshly prepared in ultrapure water (Milli-Q plus system, Millipore, UK). The standard solutions (0.01 mol L^{-1}) of ethylene glycol, propylene glycol and glycerol were prepared by dissolving 0.62 g, 0.76 g and 0.92 g of them (Merck, Germany) respectively in water and diluting to 1000 ml in a volumetric flask. A 0.01 mol L^{-1} thiamine solution was prepared by dissolving 3.3726 g (Merck, Germany) in water and diluting to 1000 ml. 0.01 mol L^{-1} IO_4^- was prepared by dissolving 0.5750 g potassium periodate (Janssen Chimica, Belgium) in 250 ml water. 0.01 mol L^{-1} Mn^{2+} was prepared by dissolving 0.4948 g $MnCl_2 \cdot 4H_2O$ (Merck, Germany) in 250 ml water. The buffer was 0.1 mol L^{-1} borax solution prepared by dissolving 38.1370 g $Na_2B_4O_7 \cdot 10H_2O$ (Fluka, Switzerland) in water and diluting to 1000 ml in a volumetric flask. The cation and anion exchangers were Ionenaustauscher I (Art. 4765) and Ionenaustauscher III (Art. 4767) (Merck, Germany). All solutions used were degassed prior to use in a Sonorex bath (Brandelin, Germany).

2.2 Apparatus

Fig. 1 illustrates the FI manifold used. A 12-channel peristaltic pump (ISMATEC, Model IPC-12, Switzerland) was used to generate the flowing streams. A 4-way Teflon rotary valve (Type 50, LATEK, Germany), which was connected with a computer

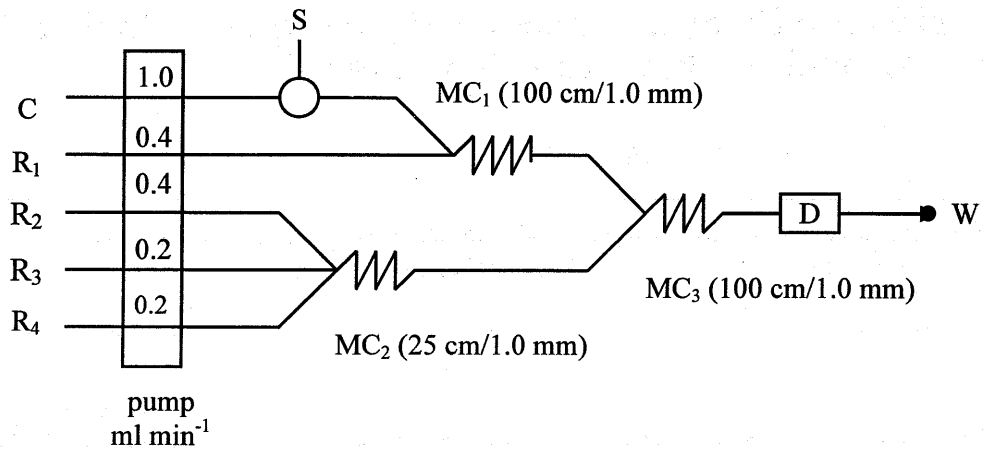

Fig. 1 Schematic diagram of the flow-injection configuration for the spectrofluorimetric determination of dihydric and trihydric alcohols. S – sample injection (200 μl), C - MQ water, R_1 – IO_4^- solution, R_2 – thiamine solution, R_3 – Mn^{2+} solution, R_4 – borax solution, D – spectrofluorimeter with a 8 μl flow-through cell (λex / λem = 370 / 440 nm), MC_{1-3} - mixing coils, W – waste.

controlled motor was used to inject samples automatically. The flow system consisted of 1.0 mm i. d. tubing (Bohlender, Germany) throughout, except that 0.38 mm, 0.64 mm and 1.02 mm i. d. pump tubes (Tygon, England) were used for delivering the aqueous solutions in order to get different flow rates under the same rotation speed of the pump.

The fluorescence intensity was measured by a Model SFM-23 Spectrofluorometer (Kontron, Switzerland) equipped with a flow-through cell (inner volume 8 μl), whose output was recorded by a computer via an A/D converter.

In addition a manifold using a multi-way valve (Bio-Chem Valve Corp, Germany) injection system was developed so that the measurements for different standard solutions and samples could be done automatically under the control of the computer.

2.3 Procedure

The FI system consisted of a carrier stream C and four reagent streams R_1, R_2, R_3 and R_4, which were each propelled via the peristaltic pump working at a speed of 17 r.p.m. (revolutions per minute). Samples were injected directly into the carrier stream C (MQ-water). Then the C-sample stream was merged with 5.0 x 10^{-4} mol L^{-1} IO_4^- and the malaprade reaction took place after flowing through the 100 cm long mixing coil (MC_1). 0.001 mol L^{-1} thiamine (R_2), 0.00075 mol L^{-1} Mn^{2+} (R_3) and 0.04 mol L^{-1} borax solution (R_4) were pre-merged through a 25 cm long mixing coil (MC_2) and then mixed with the stream out of MC_1, which contained the excess of periodate. The detectable fluorescent species thiochrome was formed on passage of the mixture through a 100 cm long mixing coil (MC_3) as the result of a second reaction.

The fluorescence intensity was monitored as the mixture passed through the flow-through cell (λ_{ex} = 370 nm, λ_{em} = 440 nm). The transient signal from the detector was recorded as a negative peak. The signal of the baseline was the relative fluorescence intensity of thiochrome produced by periodate alone without sample injection. The height of the negative peak decreased proportional to the concentration of the sample injected. Three replicate injections per sample were made in all instances.

For the recovery test, all the sample solutions were pre-treated at first by filtering through a No.1 filter paper (Whatman, England) (except drinking water) and then passing through two ion exchangers in turn, which contained a strong acid cation exchanger and a strong basic anion exchanger separately.

3. Results and discussion

Two chemical reactions are used in the proposed FI spectrofluorimetric method for the determination of dihydric and trihydric alcohols. The first one is the malaprade reaction between the sample and periodate.

$$> C(OH)\text{-}C(OH) < + IO_4^- \longrightarrow 2 > C = O + IO_3^- + H_2O$$

The second one is the oxidation reaction of thiamine by periodate to form the fluorescent species thiochrome, which can be detected by a spectrofluorimeter. Without sample injection only the second reaction takes place in the flow system and all the periodate reacts with thiamine. The signal of the baseline is the relative fluorescence intensity of thiochrome, which is formed after all the reagents flowed through MC_3 in Fig. 1. By studying the fluorescence spectrum of the baseline after stopped-flow until the detectable signal reached its maximum, it was found that the maximal excitation and emission wavelengths were 370 and 440 nm respectively (characteristic fluorescence wavelengths of thiochrome). Therefore these two parameters were selected as the suitable wavelengths for detection.

3.1 Effects of reagent concentrations

The effects of reagent concentrations on the sensitivity were studied with fixed manifold parameters at room temperature (22° C): flow rates for the stream of C, R_1, R_2 , R_3 and R_4 were 1.0, 0.4, 0.4, 0.2 and 0.2 ml min^{-1} respectively under the pump rotation speed of 17 r.p.m., glycerol concentration 2.0 x 10^{-4} mol L^{-1}, 0.04 mol L^{-1} borax solution as buffer (to keep the final mixing solution at pH 9.0), 200 μl sample injection volume, the lengths of the mixing coils MC_1, MC_2 and MC_3, were 100, 25 and 100 cm.

The peak height increased by changing the IO_4^- concentration from 1.0×10^{-4} up to 5.0×10^{-4} mol L^{-1}. A greater amount of IO_4^- increased only the signal of the baseline and could cause a decrease of the negative peak height. Thus 5.0×10^{-4} mol L^{-1} IO_4^- was selected for all further experiments.

The effect of varying the thiamine concentration on the peak height was tested from 2.5×10^{-4} to 2.5×10^{-3} mol L^{-1} (5.0×10^{-4} mol L^{-1} IO_4^-). The peak height reached a maximum and remained relatively constant when the concentration was between 0.001-0.002 mol L^{-1}. 0.001 mol L^{-1} was therefore selected for all further experiments.

The effect of the Mn^{2+} concentration on the peak height was studied in the presence of 5×10^{-4} mol L^{-1} IO_4^- and 0.001 mol L^{-1} thiamine (see Fig. 2). The peak height increased by increasing the Mn^{2+} concentration up to 7.5×10^{-4} mol L^{-1}, whereas greater amounts caused a sensitivity decrease. Therefore this concentration was selected. The effect of Mn^{2+} addition on the sensitivity of the redox reaction between IO_4^- and thiamine (signal of baseline without sample injection) will be discussed in the later part of this paper.

The effect of pH on the sensitivity of the redox reaction in the flow system is not discussed in this paper, because the oxidation reaction of thiamine by different oxidants to form fluorescent thiochrome is well known to proceed in the alkaline medium and best results could be obtained between pH 8.0-10.0. Therefore, 0.04 mol L^{-1} borax was used as buffer solution to maintain the final mixing solution at pH 9.0.

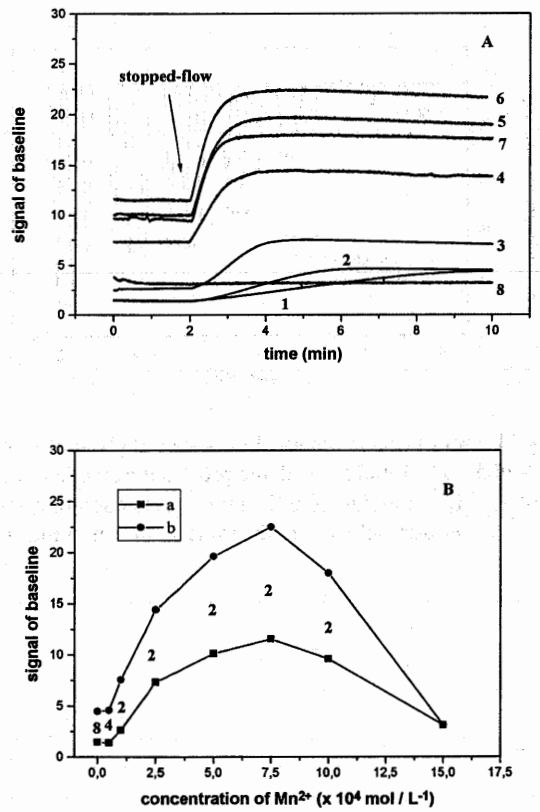

Fig. 2 Effect of Mn^{2+} concentration on the sensitivity of the redox reaction between IO_4^- and thiamine (signal of baseline without sample addition). A - signal of baseline in flow status and in stopped-flow status at different Mn^{2+} concentrations vs. time. Lines 1 to 8 represent 0, 0.00005, 0.0001, 0.00025, 0.0005, 0.00075, 0.001 and 0.0015 mol L^{-1} Mn^{2+} respectively. B - signal of baseline in flow status (a) and maximal value after stopped-flow (b) at different Mn^{2+} concentrations. The digits represent the needed time (minutes) for the signal to reach a maximum after stopped-flow. IO_4^- - 0.0005 mol L^{-1}, thiamine - 0.001 mol L^{-1}, borax solution - 0.04 mol L^{-1}. Other parameters were the same as shown in Fig.1.

3.2 Optimization of the FI system variables

The optimization of the proposed FI system variables was obtained as a compromise between sensitivity, peak width and sampling throughput. The effects of the different factors on the peak height, including pump rotation speed, the volume of sample injected, the lengths of mixing coils, inner diameter and geometric forms of the mixing coils were studied in detail with optimum reagent concentrations. The results are shown in Table 1.

Table 1 Optimization of FI system variables

Variables	Studied Range	Optimum Value
pump rotation speed: r.p.m.	10 -60	17
Injected volume: μl	25 - 500	200
lengths of mixing coil: cm		
MC_1	50 - 200	100
MC_2	25 - 100	25
MC_3	50 - 200	100
Inner diameter of mixing coil: mm	0.5, 0.8 and 1.0	1.0
Geometric forms of mixing coil	coiled and 3-D knitted	3-D knitted

High sensitivity could be obtained when a low flow rate was selected at the cost of a poor sampling throughput. The sensitivity was proportional to the sample injection volume up to 300 μl. Above that the change in height of the signal was insignificant but the peak width was very large. Three different sizes of mixing coils with 0.5 mm, 0.8 mm and 1.0 mm i. d. were tested. The result showed that for the slow redox reaction, high sensitivity could be obtained by using a mixing coil with 1.0 mm i. d. The length of the mixing coil MC_2 did not influence the signal, hence a minimum length of 25 cm was used to mix thiamine, buffer and Mn^{2+} solutions. The peak height increased obviously with increasing lengths of MC_1 and MC_3 up to 100 cm. In addition the result of two geometric forms of mixing coils was compared and higher sensitivity was obtained with a 3-D knitted form.

The contact time (flow rate and length of mixing coil) and reaction space (size and geometric form of mixing coil) were critical factors which affect the sensitivity of the two reactions taking place in the flow system. The sampling throughput for this proposed method was about 60 per hour.

3.3 Effect of Mn^{2+} Addition on the Redox Reaction between IO_4^- and Thiamine

The effect of Mn^{2+} addition on the speed and efficiency of the redox reaction between IO_4^- and thiamine was studied in detail using a stopped-flow method (without sample injection). The result showed that Mn^{2+} played a role as catalyst in the above reaction. It increased the amount and the speed of thiochrome formation (fluorescence intensity of baseline in flow status, as shown in Fig. 2A) and helped to reach the chemical equilibrium after stopped-flow earlier (Fig. 2B). The possible reaction steps are:

$$\text{(1). } IO_4^- + Mn^{2+} + H_2O \xrightarrow{\text{pH 9.0}} IO_3^- + Mn^{4+} + 2OH^-$$

$$\text{(2). } Mn^{4+} + \text{thiamine} \longrightarrow Mn^{2+} + \text{thiochrome}$$

There was no evidence to assume that Mn^{2+} has associated with thiochrome to form a stronger fluorescence species as reported in [20]. Fig. 2 shows that the fluorescence intensity even decreases at concentrations of Mn^{2+} above 7.5×10^{-4} mol L^{-1} (B, line b). It is possible that under the selected reaction conditions (5.0×10^{-4} mol L^{-1} IO_4^-) the amount of IO_4^- was not enough to react with more and more Mn^{2+}. Therefore the excess Mn^{2+} in the flow system could have a quenching effect. This has been testified by varying the concentration of IO_4^-. When increasing the concentration of IO_4^-, the concentration of Mn^{2+} which corresponded to the maximal signal and above which the signal began to decrease, shifted to higher values.

3.4 Effect of temperature

The effect of temperature on the efficiency (peak height) of both reactions is shown in Fig. 3. The two reactions have been investigated separately. When increasing the temperature from 20°C up to 50°C, there was almost no effect on the malaprade reaction (curve a). An increase in temperature for the reaction between IO_4^- and thiamine had a significant effect on the peak height, as shown in curve b. Therefore the sensitivity could be increased by working at higher temperatures. However, heating could bring about bubbles in the flow system, which seriously interfere with the measurement. Our experiments were therefore done at room temperature (22°C).

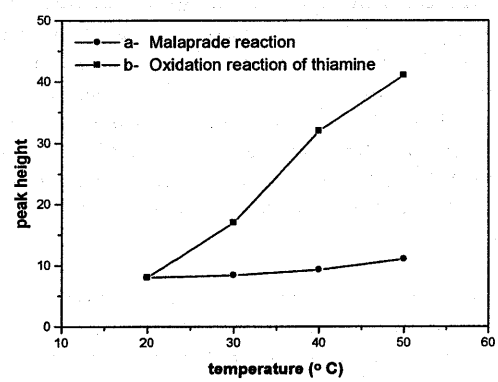

Fig. 3 Effect of temperature on the sensitivity (peak height) (Two reactions were studied separately). Curve a: effect of heating only on the malaprade reaction (MC_1 in Fig. 1). Curve b: effect of heating only on the oxidation reaction of thiamine (MC_3 in Fig. 1). Glycerol - 0.0002 mol L^{-1}, IO_4^- - 0.0005 mol L^{-1}, thiamine - 0.001 mol L^{-1}, borax solution - 0.04 mol L^{-1}. Other parameters were the same as shown in Fig. 1.

3.5 Performance of normal FIA (nFIA) in comparison with reversed FIA (rFIA)

A reversed FIA method was used in order to compare its performance with the normal FIA method. The difference between nFIA and rFIA was to exchange the position of sample and IO_4^- as shown in Fig. 1. In rFIA the reagent IO_4^- was injected into the stream C while the sample solution flowed continually through the system. Compared with nFIA the sensitivity of both methods was almost the same. But the stability of the baseline signal and reproducibility of results in rFIA were better that in nFIA. However the sampling throughput was less.

3.6 Analytical performance characteristics

Under selected optimum conditions, the peak height (relative fluorescence intensity) was a linear function of the sample concentration. Fig. 4 gives the FI response curve for standard glycerol solutions of different concentrations and Table 2 shows calculated results in detail for ethylene glycol, propylene glycol and glycerol. The precision of this proposed flow-injection spectrofluorimetric method, evaluated as the relative standard deviation (R.S.D.) of ten replicates of 2.0×10^{-4} mol L^{-1} of glycerol was 3.1 %. It is possible to determine even lower concentrations if a thermostat system is used so that measurements can be performed at higher temperatures.

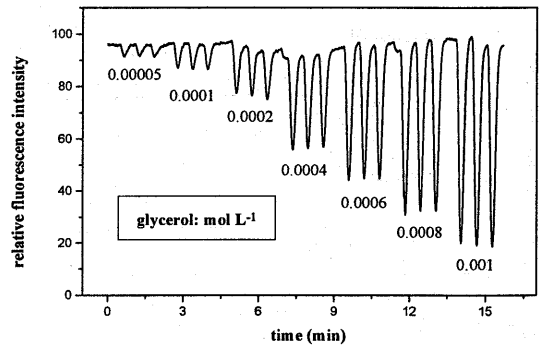

Fig. 4 FI response curve recorded for standard glycerol solutions of 0.00005 - 0.001 mol L^{-1} with the proposed FI method. IO_4^- - 0.0005 mol L^{-1}, thiamine - 0.001 mol L^{-1}, borax solution - 0.04 mol L^{-1}. Other parameters were the same as shown in Fig. 1.

Table 2 Features of the calibration graphs

Sample	Ethylene Glycol	Propylene Glycol	Glycerol
Linear Range: mol L^{-1}	0.0002 - 0.005	0.0002 - 0.004	0.00005 - 0.001
Equation	$y^a = 0.8 + 1.3 \times 10^4 C^b$	$y = 1.5 + 2.1 \times 10^4 C$	$y = 2.6 + 7.7 \times 10^4 C$
Correlation coefficient	R = 0.998 (n=11)	R = 0.998 (n=8)	R = 0.998 (n=7)
Standard deviation	1.2	1.8	1.9

a - peak height, b - concentration of sample: mol L^{-1}.

3.7 Interferences

In order to assess the possible application of this proposed method to real specimens, the interference effects of some cations and anions on the determination of glycerol, which could be present in natural waters, were studied under the otherwise optimum conditions. The tolerance limits shown in Table 3 were obtained by considering that the added ion had no interference if its effect was less than 10 % of the peak height of 2.0×10^{-4} mol L^{-1} glycerol. The result demonstrated that most of the studied ions interfered seriously in the determination of glycerol. Therefore the real sample should be pre-treated by passing through suitable cation and anion exchangers before being analysed in order to remove them and eliminate the interference.

Table 3 Tolerance of different Ions in the Determination of 0.00025 mol L^{-1} (23 mg L^{-1}) glycerol

Interferences	Tolerance Limits (mg L^{-1})	Interferences	Tolerance Limits (mg L^{-1})
K^+	100	NO_2^-	50
Na^+	100	NO_3^-	50
NH_4^+	100	CO_3^{2-}	50
Ca^{2+}	20	SO_4^{2-}	100
Mg^{2+}	20	SiO_3^{2-}	20
Zn^{2+}	20	PO_4^{3-}	50
Cu^{2+}	20	Cl^-	20
Fe^{2+}	20	F^-	20
Fe^{3+}	20	Br^-	20

3.8 Recovery test

As a second procedure to evaluate the application of this proposed method, a standard addition method was used for the determination of glycerol spiked in local drinking water, rain water and river water. All the samples except drinking water were first pre-treated according to the description given above. The result of the recovery test is shown in Table 4.

Table 4 Recovery Test
Determination of glycerol spiked in real samples by the standard addition method

Sample	Glycerol spiked (mol L^{-1})	Found (mol L^{-1})	Recovery %
Drinking water	1.0×10^{-4}	1.03×10^{-4}	103
	2.0×10^{-4}	2.20×10^{-4}	110
Rain water	1.5×10^{-4}	1.55×10^{-4}	103.3
	2.4×10^{-4}	2.42×10^{-4}	100.8
Saar river	2.0×10^{-4}	1.86×10^{-4}	93
	3.0×10^{-4}	3.08×10^{-4}	102.7

4. Conclusion

A flow-injection spectrofluorimetric method based on the malaprade reaction for the indirect determination of dihydric and trihydric alcohols has been developed. Compared with other enzymatic methods the procedure of this automated FI system is simple and the sampling throughput is good. For ethylene glycol, propylene glycol and glycerol linear calibration curves were obtained in the range 2.0×10^{-4} - 5.0×10^{-3} mol L^{-1}, 2.0×10^{-4} - 4.0×10^{-3} mol L^{-1} and 5.0×10^{-5} - 1.0×10^{-3} mol L^{-1} respectively at room temperature. This method was applied for the determination of glycerol spiked in real samples with good results after the samples were pre-treated by passing through suitable ion-exchangers.

Although it is a method with fluorescence detection, the analytes have no direct relations with the detectable fluorophor. Therefore, the results seem to be not so sensitive as far as the fluorescence detection is concerned. Compared with results of the cited methods, this method has an advantage in terms of the simplicity of a FIA system and the high sampling rate, but with a loss of sensitivity as a compromise. It is well suitable for use in typical problems of environmental monitoring, e. g. for the control of runoffs from airports, where glycol is used as anti-icing agent and may be found in concentrations of 0.004 – 0.007 mol L^{-1} [2].

5. Acknowledgements

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