

Reverse Flow Injection–Spectrophotometric Determination of Ethylene Glycol via Oxidative Reaction

Abstract

A reverse flow injection analysis technique (rFIA) was developed for the spectrophotometric determination of EG in antifreeze solutions. The method based on the inhibition effect of EG on the reaction of periodate and OT in the presence of acetic acid. The decrease of blue meriquinone absorbance with EG was used to monitor the reaction spectrophotometrically at 630 nm. Influences of chemical and physical parameters were evaluated. Selectivity of the method and the suitable conditions for sample analysis were investigated. Working on optimized conditions, the detection limit was 10 µg/ml with correlation coefficient of 0.9983. The RSD observed at 40, 100 and 160 µg/ml were 3.27, 1.81 and 0.80% respectively and the analytical throughput was 52 s/h. Possible interferences that related to the determination of EG in antifreeze solutions were studied and the results showed that foreign species caused less than ±5.0% error. Six samples of commercial antifreeze solutions were analyzed according to the developed procedure not presenting any statistical difference in relation to the results obtained by standard method.

Keywords: *Ethylene glycol, reverse flow injection, spectrophotometry, antifreeze*

1. Introduction

The wide applications of EG in both industrial and engine coolants have prompted extensive literature on its determination. Most of the analytical methods employed for the determination of EG are chromatographic methods including gas chromatography (GC) [1, 2], GC-MS [3, 4], high-performance liquid chromatography (HPLC) [5, 6], LC [7], TLC [8]. Also, spectrophotometric methods are among the most analytical methods used for EG determination in antifreeze [9, 10–17]. Other reported methods include amperometry [18], fluorimetry [19, 20] and chemiluminescence [21].

Periodate has been extensively used as an ingredient for the indicators used in catalytic-kinetic analysis involving redox processes. It is of importance for oxidizing many organic and inorganic compounds [2, 3]. Periodic acid and its salts were proposed for cleavage of α-glycol groups (vicinal diols) by Malaprade in 1928 [22–25]. Later it was shown that compounds of other types, in particular, α-hydroxyaldehydes, α-hydroxyketones, and α-aminoalcohols, are also oxidized [22]. The periodate oxidation is used for the determination of the number of adjacent hydroxyl groups, elucidation of the polysaccharide structure [26], and corroboration of the glycoside structure [27]. The α-glycol group is cleaved at the C–C bond with an equimolar amount of periodate; the reaction is applicable for cis- and trans diols and α-aminoalcohols [28].

The mechanism of the periodic acid-induced oxidation of glycols is given in Fig. 1 [28–33]. Depending on the reaction conditions and the structure of the compound oxidized, the limiting stage of the process can be either formation of the cyclic intermediate or its destruction.

As mentioned above, periodate oxidation is used for the quantitation of α-glycol groups, which can be easily done on the basis of periodate consumption or iodate formation [34, 35]. Because of the specificity of the reaction, many materials may be determined in the presence of others by investigating the products of the oxidation [36].

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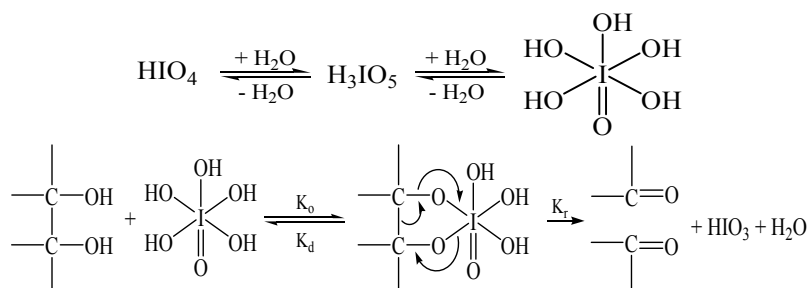


Fig. 1. The mechanism of the periodic acid-induced oxidation of 1,2-glycols.

o-Tolidine (OT) [2-tolidine or 4-(4-Amino-3-methylphenyl)-2-methylaniline] widely used as a reagent or indicator in analytical, clinical and forensic chemistry, such as in the analytical determination of chlorine [37, 38, 39], gold [40], iodine [41], hemoglobin [42], corrogeenon ester sulfate [43], ruthenium [44], manganese [45], bismuth [46], indirect determination of cyanide and thiocyanate [47] and anionic surfactants [48].

Willstatter and Kalb first showed that mild oxidation of benzidine (presence of H instead of methyl group in OT) produced a green substance and that upon oxidation with silver oxide or lead peroxide a deep yellow quinonoid substance was formed [49, 50]. This compound (OT) contained two less hydrogen atoms than benzidine and was *p,p'*-diiminodiphenyl. Such fully oxidized products of diamines were termed holoquinones. In addition to the holoquinones, Willstatter and Piccard isolated colored, crystalline, partial oxidation products of diamines which were called meriquinones [51]. For example, Wurster's red is the meriquinone produced by partial oxidation of *p*-aminodimethylaniline. The structure of meriquinones was shown to be analogous to that of quinhydrone (Fig. 2), but meriquinones are not necessarily composed of equimolecular parts of oxidant and reductant [37].

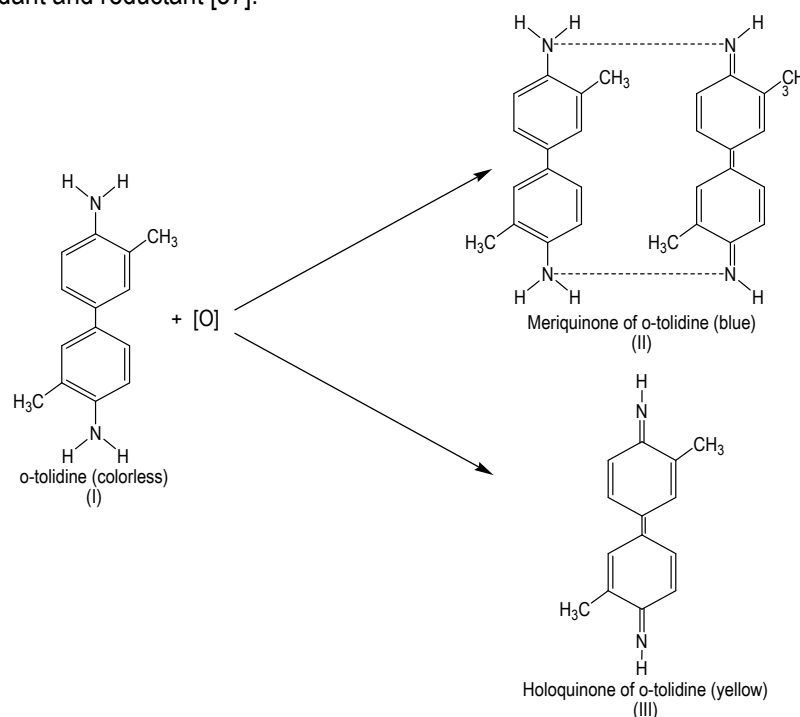


Fig. 2. Oxidation reaction of OT and formation of meriquinone and holoquinone of OT.

Clark, Cohen, and Gibbs applied the above considerations to the OT test and by means of potentiometric and spectrophotometric studies explained the varying color reactions shown by OT. The reactions involved are shown on Fig. 2. From these reactions it became evident that the variations in color experienced in the OT test were due to the superimposing of different degrees

of oxidation upon the effects of variable pH. The use of strong hydrochloric instead of acetic acid favors the development of (III) instead of (II) with resultant greater stability. Likewise, the concentration of OT present is important since low concentration favors the formation of the completely oxidized form or holoquinone [37].

In this work, a simple, sensitive, fast and low-cost rFI – spectrophotometric method based on the oxidation of EG to form aldehyde by excess periodate (Malaprade reaction), the excess of periodate react with OT in acetic acid medium [37, 43, 52, 53, 54]. The quinone diimines formed (II) have very high molar absorption coefficients and thus they can be determined even at very low concentrations, and we have studied the influence of interferences on them.

To minimize the consumption of reagent (OT) and waste production, a rFIA manifold was assembled to determine EG in antifreeze solutions (Fig. 3). The reagent (OT) was injected into a sample stream, and the absorbance obtained was measured. During optimization, all samples were analyzed in triplicate, and absorbance of corresponding blank solution was subtracted.

2. Experimental

2.1. Apparatus

Absorption spectra were measured using UV-Vis spectrophotometer (CECIL CE 3021, 3000 Series) in a 1.0 cm quartz cell and a digital pH meter (WTW, inolab 740 Germany) equipped with a combined glass-calomel electrode was used for pH measurements.

The reversed flow injection system used for the determination of EG in antifreeze samples was shown in Fig. 3. It was equipped with a Desagapl - Heidelberg, England peristaltic pump (6 channels, variable speed) to drive the carrier (D.W.) or sample and the periodate streams and an actuated rotary six-port injection valve (Rheodyne-USA) supplied with variable loops.

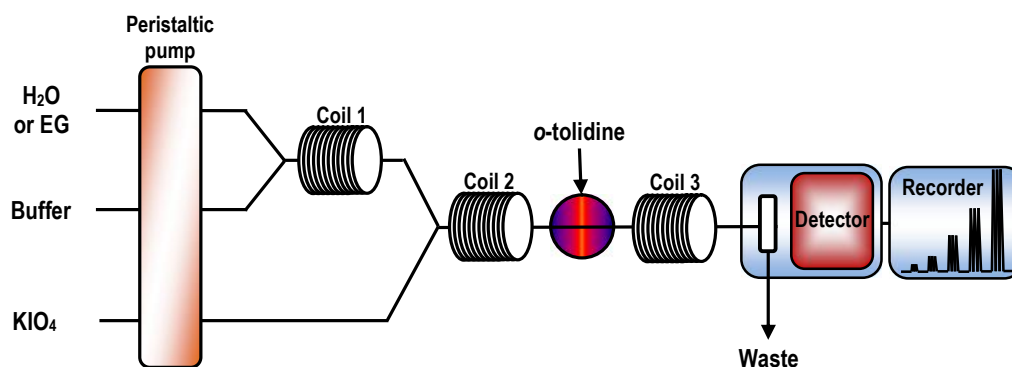


Fig. 3: Reverse flow injection manifold used for the spectrophotometric determination of EG in antifreeze solutions.

A variable wavelength spectrophotometric (JENWAY, 6300 Spectrophotometer) with a 100 μ L flow cell was used at 630 nm. PTFE (0.8-mm i.d.) tubing was used for knitted reaction coils and all flow tubing. All reagents were pumped at a flow rate of 1.0 ml/min. The signal was recorded by a x-t recorder (Type PM 825A PHILIPS – one line recorder) with various amplification factors and different chart speeds. Peak height was measured for each signal. The analytical signal was calculated as sample output minus blank.

2.2. Reagents and samples

All solutions were prepared using reagent grade substances and distilled water.

Ethylene glycol (EG): A stock standard solution of ethylene glycol (1.0 mol/L) was prepared by diluting 56.3 ml of EG (99 %, BDH) to 1.0 L with distilled water. It was stored in a dark bottle, and protected from daylight. The working standard solutions were prepared by suitable dilution.

119 Potassium periodate (KIO_4) solution: A 0.01 mol/L solution was prepared by dissolving 2.3 g of
120 analytical-reagent grade potassium periodate (solubility 4.20 g/L 20 °C) (RIEDEL-DE HAEN AG
121 SEELZE-Hannover) in distilled water and standardising the solution iodimetrically [36]. The
122 solution was diluted as required, just before use.

123 Buffer solution: A 35.59 g of disodium hydrogen phosphate dehydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) (RIEDEL-
124 DE HAEN AG SEELZE-Hannover) was dissolved in distilled water, the pH is adjusted with 0.10
125 mol/L citric acid (21.01 g in 1.0 L, $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) (HM – G.P.R.) and dilute to 1.0 litre [55].

126 Sample preparation: 1.0 g of antifreeze was dissolved in water, and the resulting solution was
127 diluted to the mark with water in a 100 ml volumetric flask. Then 10 ml of this solution was diluted
128 1000 fold [9]. 5.0 ml of this solution was analyzed for ethylene glycol, as described in preliminary
129 procedure.

130 Acetic acid solution (AA): 0.5 mol/L acetic acid solution was prepared by diluting 2.87 ml acetic
131 acid (99.7%, 1.0485 g/ml – BDH) in DW and diluting to the mark with DW in a 100 ml volumetric
132 flask. Other solutions were prepared by serial dilution.

133 o-Tolidine: 0.004 mol/L OT (solubility in water 1.3 g/L was prepared by dissolving 0.114 g of the
134 reagent (Fluka) in sufficient volume of 0.01 mol/L acetic acid solution the resulted mixture was
135 quantitatively transferred to a 100 ml volumetric flask and diluted with the same acid solution (the
136 pH of this solution was 3.8). It was stored in a dark bottle, and protected from daylight.

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138 2.3. Procedure for rFIA

139 Conditioning of the flow injection system involved pumping 0.01 mol/L periodate through
140 the reactor for auto zero of the detector and was followed by pumping of carrier stream solution
141 (D.W. or EG) and waiting for baseline of detection system at 630 nm to reach a steady state.
142 After conditioning the reagent solution (acidic OT), was injected into the EG – periodate stream.
143 The concentration of EG is proportional to the decreasing in a peak height. When EG solution
144 was inserted into the carrier stream, the magnitude of the signal decreased as a result of the
145 reaction of EG with periodate.

146 Using the experimental setup as shown in Fig. 3, 100 μL of the 0.02 mol/L of OT reagent
147 solution in 0.003 mol/L acetic acid was injected via injection valve into the merged streams of
148 D.W. (or 100 $\mu\text{g/ml}$ EG) with 0.01 mol/L KIO_4 solution (reaction coil 2: 120 cm) flowing with the
149 same flow rate of 1.0 ml/min which was then passed through a glass bead reactor (coil 3: 100 –
150 200 mesh, 5 cm, 2 mm i.d.) where the oxidation reaction (oxidation of OT) was taken place. The
151 resulting blue meriquinone of OT was subsequently monitored at 630 nm.

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153 3. Results and Discussion

154 In this work, we used periodate – OT system for the determination of EG in a reversed
155 flow injection system. The oxidation of EG with a known excess of periodate in Na_2HPO_4 /citric
156 acid buffer (pH 4.5) is indicated by the chemical equation described in Fig. 1. Unreacted
157 periodate react with OT and the formed meriquinone complex is detected spectrophotometrically
158 at 630 nm as a step for the indirect determination of EG.

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160 3.1. Absorption spectra

161 The oxidative reaction between periodate and OT produces a meriquinone of OT having
162 an absorption peak with its maximum value at 630 nm (Fig. 4). Notice that the absorption spectra
163 of the analyte and the blank (OT blank) (curves a and b) have no absorption bands in the region
164 that corresponds to that of the complex periodate – OT. The absorption spectra having their
165 maximum absorbance at 630 nm. Moreover, it is interesting to note that the complex has a higher
166 maximum absorbance value in comparison with that formed in the presence of EG. Hence,
167 subsequent analysis of the colored complex was carried out at 630 nm.

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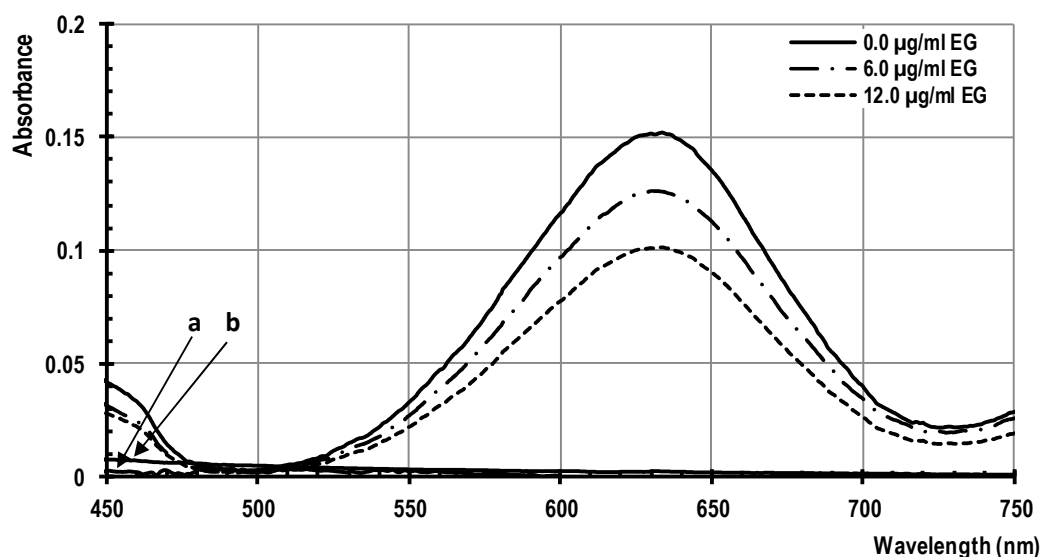


Figure 4: Absorption spectra for the: (a) analyte, (b) blank, and formed meriquinone of OT using (0.0, 6.0 and 12.0 µg/ml) EG.

3.2. Optimization of rFIA method

To optimize the performance of the manifold, the absorbance of the blue meriquinone was measured as a function of buffer pH, periodate concentration, acetic acid concentration, OT concentration, reaction coil length, system flow rate and injection volume of the reagent. To reach the best operational conditions, we used a univariate method, where one variable is varied by maintaining constant the rest. In all optimization experiments, carrier solution was D.W.

3.2.1. Effect of the buffer pH

EG oxidized fastest in a slightly acidic medium. Therefore, the oxidation reaction was studied at various pH values (pH 3.5–5.5). After calculation of the ΔI (blank signal – analyte signal) values which were then plotted against various pH values. Results are shown in Fig. 5. It was evident that the absorption maximum of such a meriquinone studied was increased over the pH range of 3.5–4.5. As can be seen in Fig. 5, at pH values below and above 4.5 the ΔI values decrease significantly, because at higher pH, overoxidation occurs readily and the small fragments produced by the primary oxidation are oxidized at a rate dependent upon the pH of the medium, the concentration, and the molar excess of periodate [17, 56]. Hence, pH 4.5 was chosen as optimum.

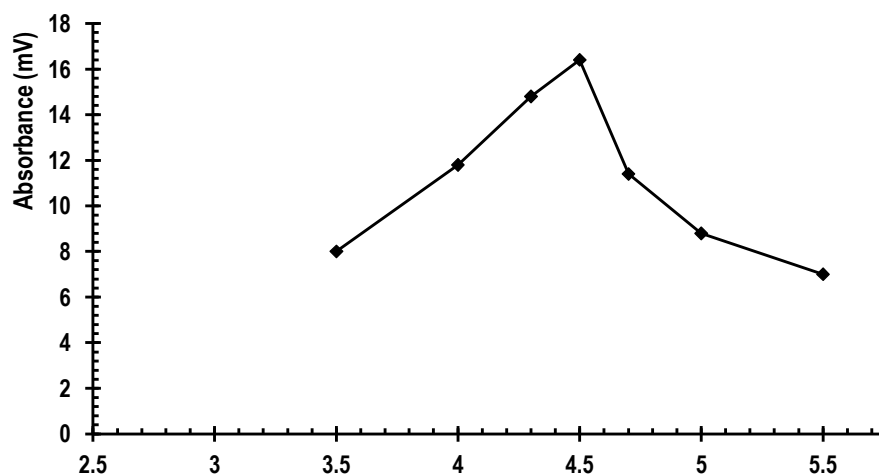


Figure 5: Effect of buffer pH on the determination of 100 µg/ml EG.

3.2.2. Effect of periodate concentration

The effect of periodate concentration on the color development was examined in the range 0.003–0.032 mol/L. Both peak heights of blank and sample remarkably increased with increasing periodate concentration up to 0.015 mol/L (Fig. 6), because at low periodate concentrations the rate of primary oxidation is slow, while at high periodate concentrations nonspecific oxidations may occur [17, 56]. Therefore, the concentration of 0.015 mol/L periodate was selected as optimum because it exhibited maximum ΔI value measured at 630 nm.

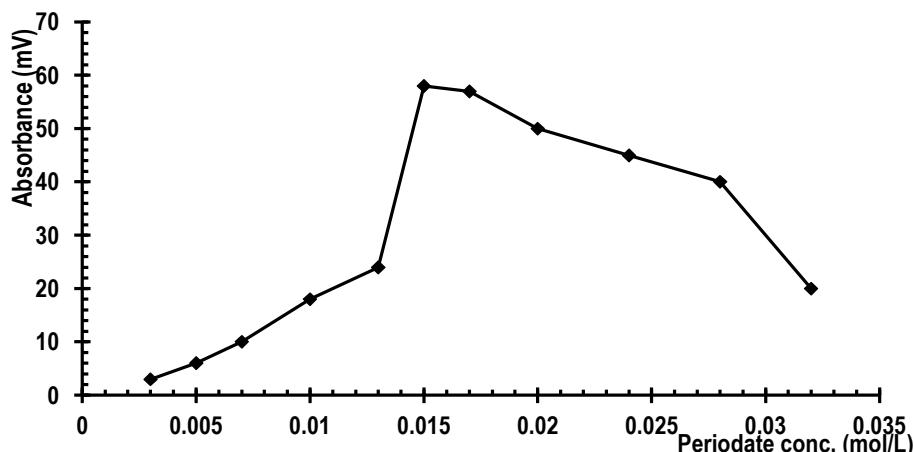


Figure 6: Effect of periodate concentration.

3.2.3. Selection of acid type and concentration

It is known that OT oxidized in acidic media [37, 46]. The following media have been tried in the proposed experiments: sulfuric acid, hydrochloric acid, nitric acid and acetic acid solutions with concentration of 0.01 mol/L. It was found that the value of ΔA is very low in HCl, H_2SO_4 and HNO_3 solutions, because the use of strong acid favors the development of holoquinone instead of meriquinone [37]. Decreasing of absorbance of final product is strong in acetic acid solution and the reproducibility is better. Therefore, acetic acid was selected as the best reaction medium.

In addition to acid type, the effect of acid concentration studied. Different concentrations of acetic acid were tested. The results, graphically presented in Fig. 7, show that the maximum different in absorbance was obtained at 0.005 mol/L. At high concentration of acid the blue compound turned yellow [46]. Accordingly, 0.005 mol/L acetic acid concentration was used throughout the measurements for EG determination.

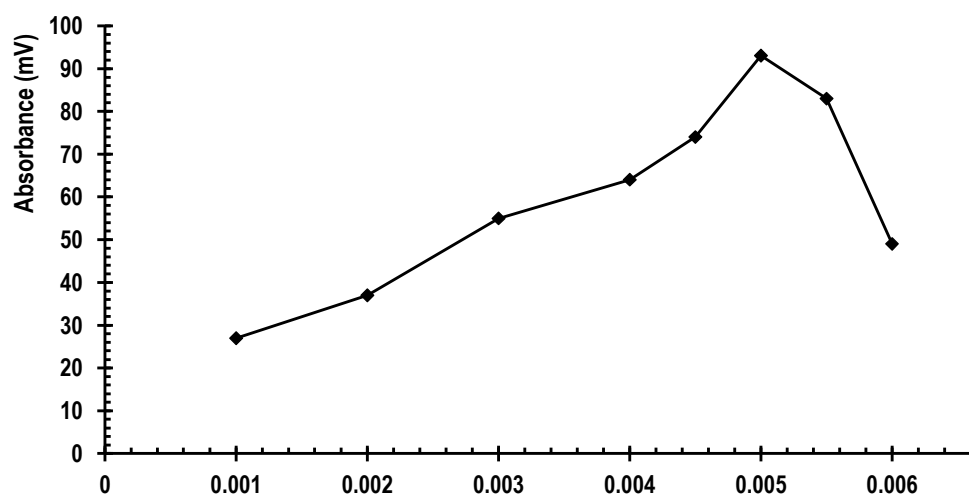


Figure 7: Effect of acetic acid concentration.

3.2.4. Effect of OT concentration

The concentration of OT present is important since low concentration favors the formation of the completely oxidized form or holoquinone (yellow product), while high concentration of OT favors the formation of meriquinone of OT [37, 46]. Therefore, it is necessary to investigate to find out the optimum OT concentration to achieve the best selectivity and sensitivity of the method. Effect of OT concentrations over the range of 0.00–0.03 mol/L were examined for determining 100 µg/ml EG in standard EG solutions.

The sensitivity increased with increasing in OT concentration up to 0.02 mol/L (as shown in Fig. 8) because low concentrations of OT favors the formation of the completely oxidized form or holoquinone, while high concentration of OT favors the formation of meriquinone [37,46]. Further increase in OT concentration gave a stable signal. Therefore, the concentration of 0.02 mol/L was chosen as suitable because it provided reasonable sensitivity.

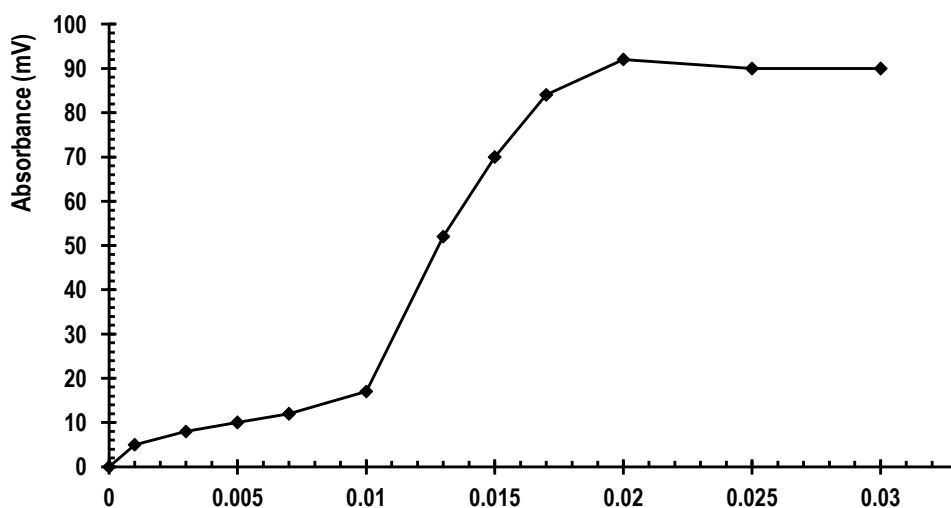


Figure 8: Effect of OT concentration.

3.2.5. Effect of coil length

Using a fixed injection volume of 100 µL, the influence of independently varying the mixing coils 1, 2 and 3 (Fig. 9) on final signal response and analysis time was investigated.

Increasing the length of the mixing coil 1 that used for the mixing of buffer solution with D.W or EG, from 5–50 cm, has no significant effect on the absorbance, while with longer coil the absorbance decreased. Therefore, a 5 cm length was selected as an optimum length.

Not surprisingly, increasing the length of coil 2, immediately after mixing of the periodate – EG streams results in an increased difference in a peak response because additional mixing promotes the reaction between EG and the potassium periodate. According to the results illustrated in Fig. 9, optimum coil length was found to be 180 cm that gives a maximum absorbance and higher coil lengths do not enhance the absorbance signal.

The reaction of the excess periodate with OT is rapid, and the obtained results demonstrated that using of mini-column glass bead reactor instead of coil enhanced the sensitivity of the method due to acceleration of the chromogenic reaction. The results show that an increasing the length of mini – column glass bead reactor from 0 to 10 cm increase the peak height, after that, increasing the length had no effect other than to decrease the peak response and increase the peak width. Based on these observations, a length of 5, 180 and 10 cm was chosen for coil 1, 2 and 3 respectively, and this represents a compromise between maximizing the sensitivity and maintaining an acceptable sample throughput.

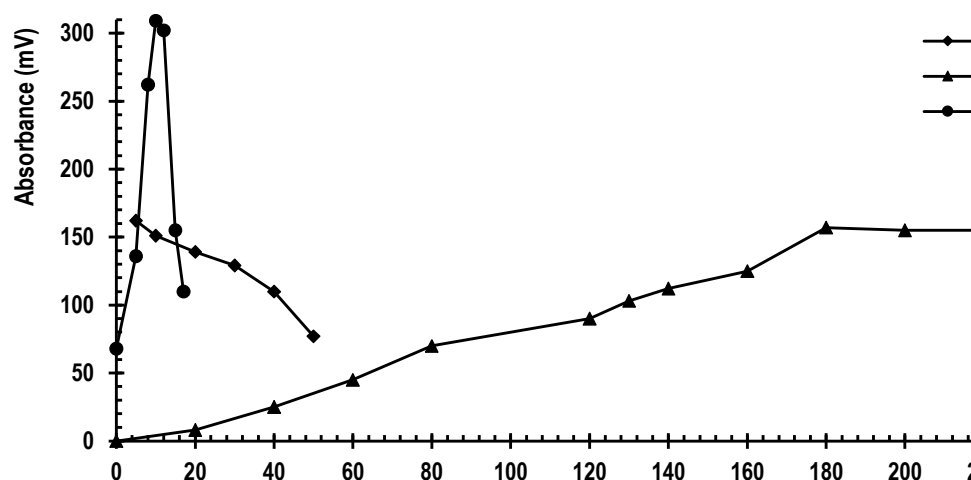


Figure 9: Effect of reaction coil lengths.

3.2.6. Flow rate optimization

The contact time between EG-periodate and periodate-OT solution is very important for the reaction to proceed sufficiently close to the completeness. As this depends on the flow rate of the sample zone through the reactor, a study of the flow rate of the system was conducted. Flow rates between 0.3 and 2.2 ml/min were evaluated. This optimization is shown in Fig. 10.

A high flow rate leads to a shorter time required for each sample passing through the FI system, poor reproducibility and a high rate of sample and carrier reagent consumption. At very low flow rate (0.3 ml/min) the shapes of the obtained signals were not reproducible and the residence time was long giving rise to low sampling rate. Therefore, as a compromise the flow rate of 0.5 ml/min was selected for the subsequent investigations.

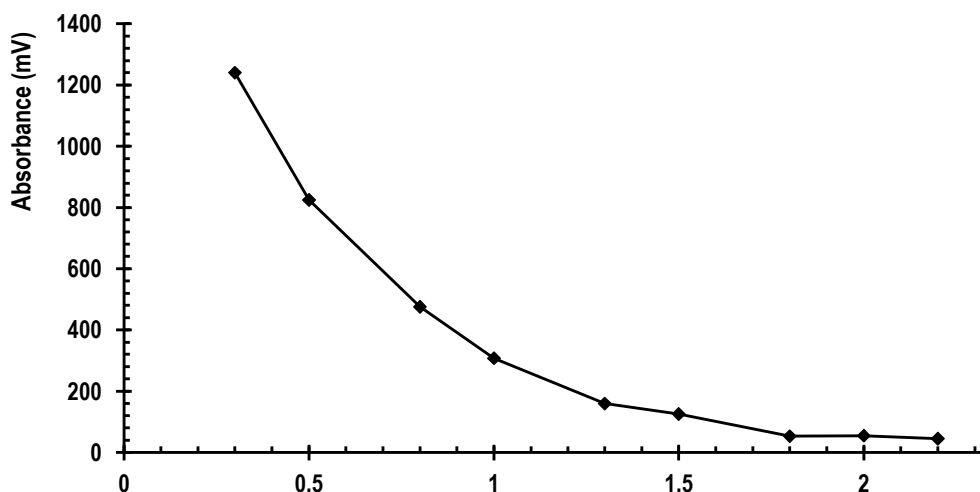


Figure 10: Effect of system flow rate on the determination of 100 µg/ml EG.

3.2.7. Effect of injection volume

The injected reagent should be sufficient for complete color development in rFIA. Therefore, it is very important to optimize the injection volume. The injected volume of the reagent was varied from 50 to 225 µL. The difference in absorbance increased with increasing reagent volume up to 100 µL, after this volume the blank signal remain constant while the analyte signal increased that's lead to decreasing of ΔI (Fig. 11). Therefore, 100 µL 0.02 mol/L OT was chosen for further work.

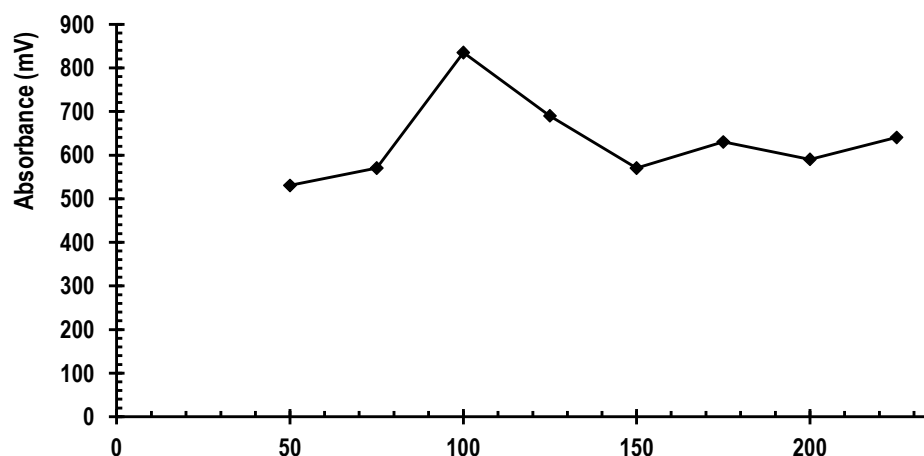


Figure 11: Effect of reagent volume (OT volume).

The best performance of the rFI spectrophotometric system used for the determination of EG was achieved in the conditions summarized in Table 1.

Table 1: Optimum conditions for the determination of EG by proposed method.

Parameter	Condition
pH of Buffer Solution	4.5
KIO ₄	0.015 mol/L
Acetic acid	0.005 mol/L
OT	0.02 mol/L
Coil 1 length	5 cm
Coil 2 length	180 cm
Coil 3 length	10 cm
System flow rate	0.5 ml/min
Injection volume	100 µL
Sample frequency	52 s/h
Wavelength	630 nm

4. Calibration graph

Using the proposed rFI spectrophotometric manifold for EG determination (Fig. 3) under the optimum conditions, the linear calibration graph over the range of 15 – 200 µg/ml EG was established (Fig. 12) which can be expressed by the regression equation $y = 7.4539x + 97.87$ ($R = 0.9983$) where y represents the peak height (absorbance) in mV and x is EG concentration in µg/ml after subtraction of blank.

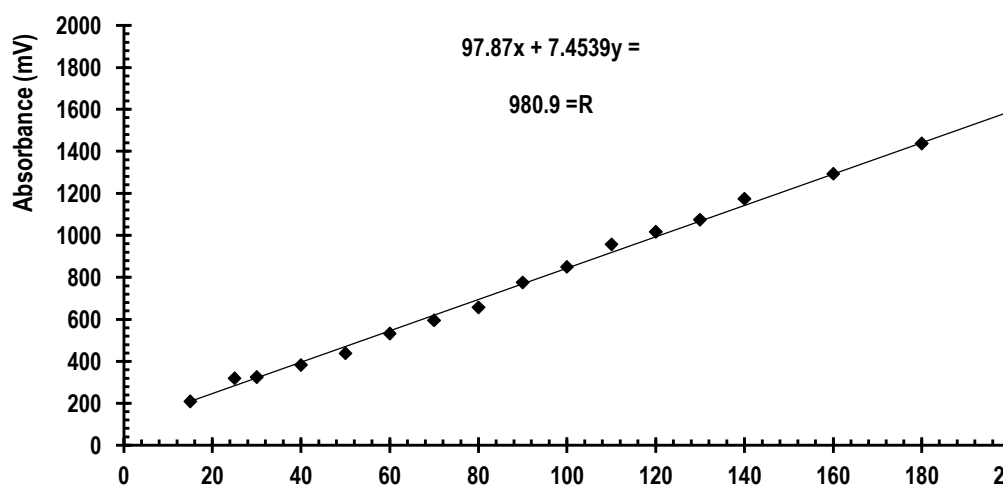


Figure 12: Calibration graph for the determination of EG.

The detection limit for EG was 8.0 µg/ml and the sampling rate was found to be 52 s/h. The analytical parameters related to the calibration graph summarized in Table 2.

Table 2: Illustrates the statistical and optical treatment of calibration graph of EG

Analyte	Linear range (µg/ml)	Detection limit (µg/ml)	Intercept	Correlation coefficient (R)
Ethylene glycol	15 – 200	10	97.87	0.9983

The precision test was carried out by five measurements of the working standard solution with the EG concentration of 40, 100 and 160 µg/ml by calculating the relative standard deviation (RSD). The accuracy expressed in term of percentage error (E%) was studied and the results summarized in Table 3.

Table 3: Accuracy and precision of the proposed method.

Analyte	Analyte concentration (µg/ml)		E %	RSD %
	Standard solution	Calculation from proposed method		
EG	40	40.24	0.6	3.27
	100	102.84	2.84	1.81
	160	162.64	1.65	0.80

5. Effect of Interferences

In order to assess the possible analytical application of the proposed method to antifreeze analysis, the effect of some common chemicals used as additives was studied by adding the chemicals to solutions containing 160 µg/ml EG.

Table 4 shows maximum tolerable concentrations of the various compounds. The tolerable concentration ratios with respect to 160 µg/ml EG for interference at 5.0% level were over 12 for borate, phosphate and silicate, 20 for benzoic acid and 0.20 for sodium fluorescein. The same amount of sodium fluorescein produced positively interference because sodium fluorescein could increase the absorbance due to its color. When compared with other interferences studied, however, the concentration of sodium fluorescein in real antifreeze sample is very low (0.01–to 0.50 g/L of the antifreeze solution [17]). Therefore, the concentration of sodium fluorescein is so low in real samples that they do not interfere.

Table 4: The effect of interferences on the proposed method.

Interfering species	Maximum Allowable Concentrations (µg/ml)	Ethylene glycol (µg/ml)		Error %	Tolerable Concentration Ratio ^b
		Added	Found ^a		
Borate (BO ₃) ³⁻	1920	160	160.60	0.38	12
Phosphate (PO ₄) ³⁻	1920	160	157.92	- 1.30	12
Silicate (SiO ₄) ⁴⁻	1920	160	155.91	- 2.56	12
Sodium fluorescein	32	160	161.95	1.22	0.20
Benzoic acid	3200	160	161.28	0.80	20
Mixture of all interfering species		160	161.55	0.97	

^a Mean of three replicate analyses

^b Tolerable Concentration Ratio with no interference [Conc. Interferent (µg/ml) / Conc. EG (µg/ml)]

6. Applications

The proposed method has been applied to the determination of EG in antifreeze samples collected from oil changer markets in Erbil city. Results were listed in Table 5. It was seen that results obtained by the recommended method were in good agreement with those obtained by standard method (iodometric) [9, 57] verified by t -test. t-Test at confidence level 95% ($t_{\text{calculated}} =$

0.80 < $t_{\text{table}} = 2.57$) indicate that there is no significant difference among the achieved results using these two methods. From the values of F-calculated $2.38 < F\text{-table } 5.05$ [58, 59] with a confidence limit of 95% for five degrees of freedom 5, the results indicated that there is no significant difference between the precision of two methods.

Table 5: Application of the developed method in the analyses of EG in antifreeze solutions.

Trade name	Manufacture	Labeled amount (%)	Detectable amount (%)		E %
			Proposed method *	Standard method **	
Prista Ready Antifreeze	Turkey	42 %	42.3	42.13	0.40
Fuchs Antifreeze (Summer Coolant)	Germany	50 %	52.94	52.28	1.26
Deepen Cam Suyu Antifirizi	Turkey	36 %	37.88	36.25	4.50
Sea Horse Antifreeze	Turkey	50 %	51.71	51.63	0.15
Dynagel Plyn Do Chlodnic 2000	Poland	50 %	47.32	49.75	-4.88
National Antifreeze	U.A.E.	50 %	50.01	48.15	3.86

* Average of three replication (n=3).

** Standard method (Iodometric titration).

7. Conclusions

The present method in simple, has the advantages of high sensitivity (detection limit = 10 µg/mL EG). On the other hand, the proposed method is low cost, selective, accurate and precise as indicated by the good results of the antifreeze solutions.

The sensitivity of this method is not less than that obtained for determination EG using spectrophotometric [14, 60, 61] or GC [62] methods that gives linear ranges between 40-200, 15-125, 27-281 and 5.0-10,000 µg/ml respectively. The proposed method is less time consuming (about 52 samples/hour) comparing with some other spectrophotometric methods [61, 63] that need about 30 and 20 minutes respectively for total analysis. In addition, some of them like GC-MS [3, 4] and HPLC [5, 6] higher cost from the proposed method as an additional disadvantage of the proposed method.

The obtained results demonstrated that using of mini-column glass bead reactor instead of coil enhanced the sensitivity of the method due to acceleration of the chromogenic reaction. The proposed method could be applied successfully for the determination of EG in pure form, as well as antifreeze solutions, with no interferences from antifreeze samples.

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