Research Article

Determination of Sodium Cromoglycate by a New Kinetic Spectrophotometric Method in Biological Samples

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A new kinetic spectrophotometric method is described for the determination of ultratrace amounts of sodium cromoglycate (SCG). The method based on catalytic action of SCG on the oxidation of amaranth with periodate in acidic and micellar medium. The reaction was monitored spectrophotometrically by measuring the decrease in absorbance of the amaranth at 518 nm, for the first 4 min from initiation of the reaction. Calibration curve was linear in the range of 4.0–36.0 ng mL^{-1} SCG. The limit of detection is 2.7 ng mL^{-1} SCG. The relative standard deviation (RSD) for ten replicate analyses of 12, 20, and 28 ng mL^{-1} SCG was 0.40%, 0.32%, and 0.53%, respectively. The proposed method was used for the determination of SCG in biological samples.

1. Introduction

Sodium cromoglycate, 5,5′-[(2-hydroxy-1,3-propanediyl)bis (oxy)]bis(4-oxo)-4H-1-benzopyran-2-carboxylic acid disodium salt is commonly used in the treatment of both extrinsic and intrinsic bronchial asthma [1]. A number of methods were developed for the determination of cromoglycate, including LC-MS-MS [2] and striping voltammetric [3]. These methods either lack sufficient sensitivity or are time consuming. A number of procedures have already been described for the measurement of SCG in biological samples such as polarography [4], radioimmunoassay [5], enzyme-linked immunosorbent assay [6], and HPLC [7, 8]. These methods require pretreatment for solvent extraction and/or lack sensitivity. The aim of the present study was to develop a sensitive, fast, and economy-analytical method for determination of SCG. Here, we report a kinetic spectrophotometric method for ultratrace determination of SCG, based on its catalytic effect on the oxidation of amaranth by KIO_4 in the acidic and micellar mediums.

2. Experimental

2.1. Reagents and Solutions. All chemicals were of analytical-reagent grade and were provided by Merck and

all the solutions were prepared with double distilled water. Working solutions were prepared daily from stock solutions (1 g/L for sodium cromoglycate, 0.001 M for amaranth, 0.01 M for KIO_4, and 0.01 M for tetrabutyl ammonium bromide (TBAB) solution. The other surfactants tested, namely, Triton X-100, sodiumdodecyl sulphate (SDS), cetyltrimethyl ammonium bromide (CTAB), hexadecylpyridinium bromide (HDPB), and hexadecylpyridinium chloride (HDPC) were prepared in a similar way. Amaranth and SCG have the structure shown in Figure 1.

2.2. Apparatus. Absorption spectrum was recorded with a CECIL model 7500 spectrophotometer with a 1.0 cm quartz cell. A model 2501 CECIL spectrophotometer with 1.0 cm glass cuvette was used to measure the absorbance at a fixed wavelength of at 518 nm. A thermoelectric controller (CE 2024 CECIL) was used to keep the reaction temperature at 25°C. A stopwatch was used for recording the reaction times.

2.3. Sample Preparation. The urine sample was stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for five min at 2000 rpm. The supernatant was filtered through a 0.45 μm filter and then diluted 10 times with water. The solution was transferred into
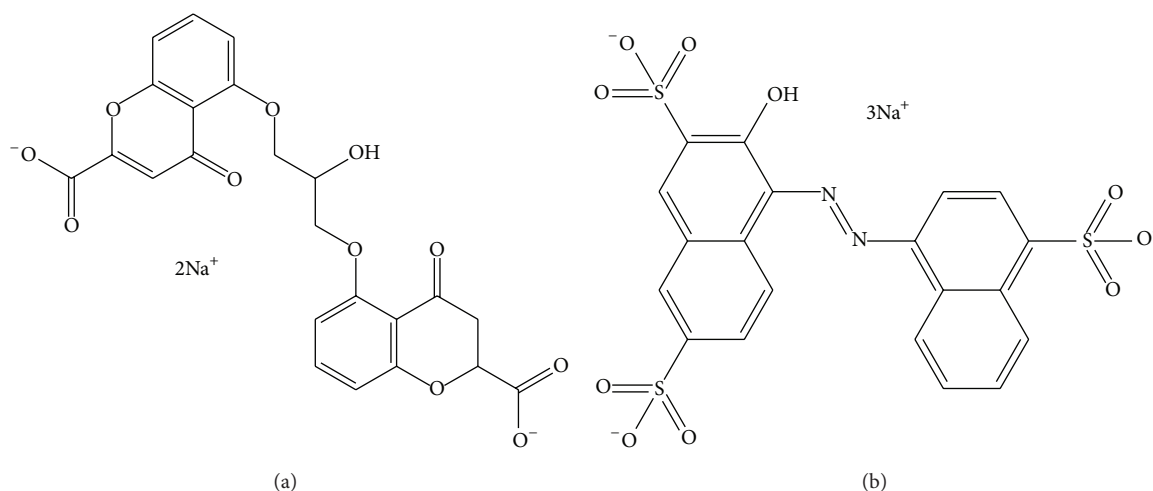
FIGURE 1: Structure of (a) SCG and (b) amaranth.

a spectrophotometric cell to be analyzed without any further pretreatment. Mineralization of 2 mL of the human serum samples was carried out for 1 h at 100°C with the addition of 4 mL of concentrated nitric acid [9, 10]. Then samples were analyzed directly after neutralization with sodium hydroxide solution and diluted with double distilled water to a suitable volume. For recovery test, the necessary standards were spiked in samples at this stage.

2.4. Recommended Procedure. All the solutions and distilled water were kept in thermostated water bath at $25 \pm 0.1°C$ for 30 min before starting the experiment. An aliquot of the solution containing $4.0–36.0$ ng mL^{-1} cromoglycate was transferred into a 10 mL volumetric flask, and then 2.6 mL of sulfuric acid solution 9.1 M, 1.0 mL TBAB 0.01 M, and 0.4 mL KIO$_4$ 0.01 M, and 1.4 mL amaranth 1.0×10^{-3} M were added to the flask, and the solution was diluted to the mark with water. The solution was mixed and a portion of the solution was transferred into the spectrophotometric cell. The reaction was followed by measuring the decrease in the absorbance against water for 0.5–4.0 min from initiation of the reaction at 518 nm. The signal was labeled as ΔA_s. The same experiment was repeated without cromoglycate to get the blank signal, and it was labeled as ΔA_b. Time was measured just after addition of the last drop of amaranth. The calibration graph was constructed by plotting of $(\Delta A_s - \Delta A_b)$ versus cromoglycat concentration at a fixed time of 0.5–4.0 min from initiation of the reaction.

3. Results and Discussion

Amaranth undergoes an oxidation reaction with periodate in the acidic medium at a very slow rate. We found that in the presence of tetrabutyl ammonium bromide (TBAB) as a micellar medium, this reaction rate is sharply increased by the addition of trace amounts of SCG. There are many methods, such as fixed time, initial rate, rate constant, and variable time methods for measuring the catalytic species. Among these,
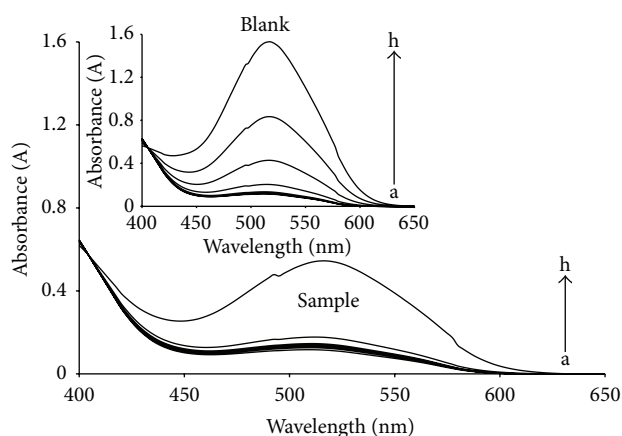

FIGURE 2: Absorption spectrum for the SCG-amaranth-IO$_4^-$ system with time. Conditions: H$_2$SO$_4$, 2.4 mol L^{-1}; SCG; 12 ng mL^{-1}; TBAB, 1.0×10^{-3} M; amaranth, 1.4×10^{-4} M, IO$_4^-$ 4.0×10^{-4} M; temperature, 25°C interval time for each scan, and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 min from initiation of the reaction.

the fixed time method is the most conventional and simplest, involving the measurement of ΔA at 518 nm. Figure 2 shows the relationship between A and reaction time. It was found that the rate of reaction is proportional to the SCG concentration. The reaction rate was monitored spectrophotometrically by measuring the decrease in absorbance of the characteristic band of amaranth at 518 nm. Therefore, by measuring the decrease in absorbance of amaranth at a fixed time of 0.5–4.0 min from initiation of the reaction, the SCG contents in the sample can be measured.

3.1. Optimization of Variables. The influence of H$_2$SO$_4$ concentration, TBAB concentration, amaranth concentration, periodate concentration, and temperature on the rate of catalyzed and uncatalyzed reactions was studied to find the optimum conditions.
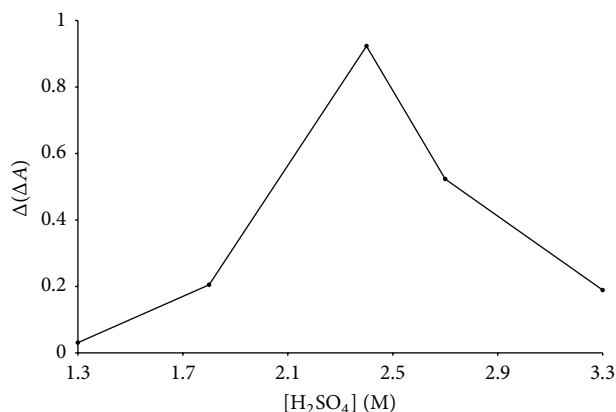
FIGURE 3: Effect of H_2SO_4 on the sensitivity. Conditions: SCG; 12 ng mL^{-1}; TBAB, 1.1×10^{-3} M; amaranth, 1.4×10^{-4} M, IO_4^- 4.0×10^{-4} M; temperature, 25°C.

FIGURE 5: Effect of IO_4^- on the sensitivity. Conditions: H_2SO_4, 2.4 mol L^{-1}; SCG; 12 ng mL^{-1}; TBAB, 1.1×10^{-3} M; amaranth, 1.4×10^{-4} M; temperature, 25°C.
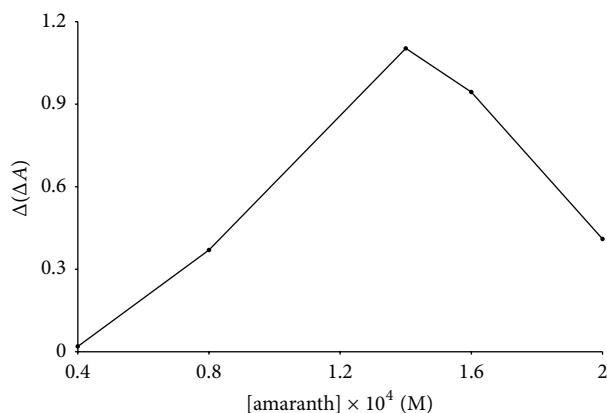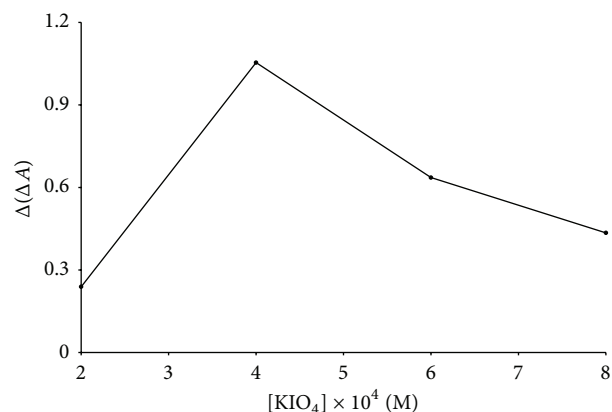

FIGURE 4: Effect of amaranth concentration on the sensitivity. Conditions: H_2SO_4, 2.4 M; SCG; 12 ng mL^{-1}; TBAB, 1.1×10^{-3} M; IO_4^- 4.0×10^{-4} M; temperature, 25°C.

The effect of H_2SO_4 concentration on the sensitivity was studied in the range of 1.3–3.3 M in the presence of 12 ng mL^{-1} SCG, 1.0×10^{-3} M TBAB, 1.4×10^{-4} M amaranth, and 4.0×10^{-4} M IO_4^- at 25°C. Figure 3 shows that by increasing H_2SO_4 values up to 2.4 M, the net reaction rate increases, whereas higher H_2SO_4 values cause decreasing the sensitivity. This phenomenon is due to the fact that in acidic medium, amaranth was protonated. Therefore, a H_2SO_4 concentration of 2.4 M was selected for further study.

Figure 4 shows the influence of amaranth concentration on the sensitivity in the range of $0.4-2 \times 10^{-4}$ M. The results show that by increasing amaranth concentration up to 1.4×10^{-4} M, the net reaction rate increases, whereas greater amounts of the dye decrease the sensitivity. This may be due to the aggregation of the dye in higher concentration. Therefore, an amaranth concentration of 1.4×10^{-4} M was selected for the study.

The effect of periodate on the reaction rate was studied in the range of $2.0-8.0 \times 10^{-4}$ M. Figure 5 shows that the net reaction rate increases with periodate up to 4.0×10^{-4} M,

whereas the reaction rate decreases with increasing periodate concentration from 4.0×10^{-4} M to greater values. This means that the rate of uncatalyzed reaction increases with periodate concentration ($>4.0 \times 10^{-4}$) to a greater extent than the catalyzed reaction, and the difference between the rates of catalyzed and uncatalyzed reactions ($\Delta A_s-\Delta A_b$) diminishes at higher periodate concentration. Thus, a periodate concentration of 4.0×10^{-4} M was selected for further study.

In many reactions, suitable micelles can affect the rate of reactions [10–15]. A micelle usually can be formed by the aggregation of charged organic molecules. These micelles have the same charge at the outer sphere. For those reactions which have charged species, these micelles can affect the rate of reaction by increasing the effective collisions. In order to choose an appropriate micellar system to enhance the rate of reaction, one should take into account the type of charge of the reactants because the accelerating effect of micelles arises essentially due to electrostatic and hydrophobic interactions between the reaction and micellar surfaces [16]. Cationic (CTAB, HDPB, TBAB, and HDPC), anionic (SDS), and nonionic (Triton X-100) micelles were tested at a concentration greater than the critical micelle concentration (CMC) [13]. The results are shown in Table 1. SCG and amaranth are positively charged, and periodate is negatively charged. Therefore, it seems logical to think that the cationic micelles can enhance the rate of SCG-amaranth-periodate reaction. In fact, CTAB, HDPB, TBAB, and HDPC increased sensitivity, but TBAB increased sensitivity more than HDPB, TBAB, and HDPC; thus, TBAB was chosen for the study (Table 1).

The effect of TBAB concentration on the rate of reaction was studied in the range of $0-2 \times 10^{-3}$ M. The sensitivity increases with increasing TBAB concentration up to 1.0×10^{-3} M and decreases at higher concentrations. This is due to the high aggregation of the surfactant and change in the molar absorptivity of the amaranth in the solution. Therefore, a final concentration of 1.0×10^{-3} M was selected as the optimum concentration of TBAB (Figure 6).

Figure 7 shows the influence of reaction temperature on the sensitivity studied in the range of 10–40°C with the

TABLE 1: Surfactants tested as potential micellar catalysts for the enhanced rate of SCG-amaranth-IO_4^- reaction.

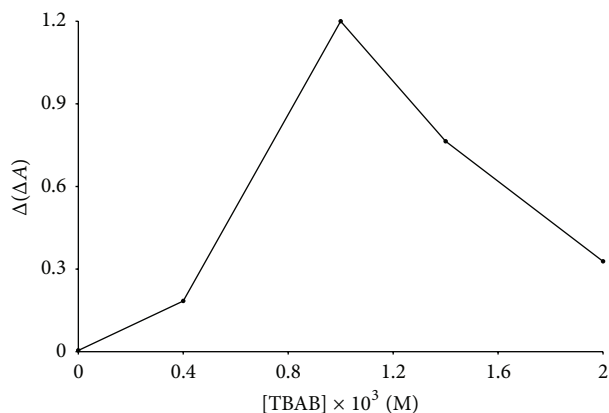| Surfactant	Type	CMC **M**	Micellar catalysis
SDS	Anionic	8.1×10^{-3}	Positive
CTAB	Cationic	1.3×10^{-3}	Positive
HDPB	Cationic	6.5×10^{-4}	Positive
TBAB	Cationic	7.5×10^{-5}	Positive
HDPC	Cationic	2.4×10^{-4}	Negative
Triton X-100	Nonionic	3.0×10^{-4}	Negative

FIGURE 6: Effect of TBAB on the sensitivity. Conditions: H_2SO_4, 2.4 mol L^{-1}; SCG; 12 ng mL^{-1}; amaranth, 1.4×10^{-4} M; IO_4^- 4.0×10^{-4} M; temperature, 25°C.

FIGURE 7: Effect of temperature on the sensitivity. Conditions: H_2SO_4, 2.4 M; SCG; 12 ng mL^{-1}; TBAB, 1.0×10^{-3} M; amaranth, 1.4×10^{-4} M, IO_4^- 4.0×10^{-4} M.

TABLE 2: Effect of foreign ions on the determination of 12 ng mL^{-1} SCG.

Foreign species	Tolerated ratio $W_{species}/W_{SCG}$
NO_3^-, SO_3^{2-}, I^-, CH_3COO^-, $S_2O_8^{2-}$, K^+, Ba^{2+}, Pb^{2+}, Ni^{2+}, Na^+, Mg^{2+}, Cd^{2+}, Al^{3+}, Zn^{2+}, Se^{4+}, Cr^{3+}, and Co^{2+}	1000
Hg^{2+}	800
Ag^+	500
Cl^-	100

TABLE 3: Determination of SCG in synthetic samples.

Sample	SCG added ng mL^{-1}	SCG found ng mL^{-1}	Recovery %
Human serum	6	6.3	105
	7	7.2	102
	8	7.9	99
Urine	6	5.5	95
	7	7.4	105
	8	8.3	103

optimum other reagent concentrations. The results showed that by increasing temperature up to 25°C, the net reaction rate increases, whereas higher temperature values cause decreasing the sensitivity ($\Delta A_s - \Delta A_b$).

3.2. Calibration Graph, Precision, and Limit of Detection. The calibration graph was linear for SCG concentration in the range of 4–36 ng mL^{-1} with the regression equation of ΔA = 0.017 C − 1.39 with ($r = 0.996$ $n = 10$), where ΔA is the change in absorbance for the sample reaction for 0.5–4.0 min from initiation of the reaction (catalytic reaction) and C is the SCG concentration in ng mL^{-1}. The limit of detection (defined as $C_L = 3S_b/m$, where C_L, S_b, and m are limits of detection, standard deviation of the blank signal, and slope of the calibration graph, resp.) is equal to 2.7 ng mL^{-1} SCG. The relative standard deviation (RSD) for ten replicate determination of 12, 20, and 28 ng mL^{-1} SCG is 0.40%, 0.32%, and 0.53%, respectively.

3.3. Interference Study. In order to assess the application of the proposed method to synthetic samples, the effect of various ions on the determination of 4 ng mL^{-1} SCG was studied. The tolerance limit was defined as the concentration of added ions causing a relative error less than 3%, the results are summarized in Table 2. The results show that this method is relatively selective for SCG determination.
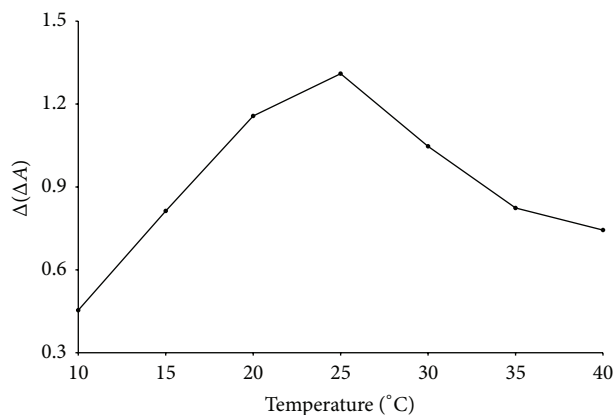
3.4. Sample Analysis. In order to evaluate the applicability of the proposed method, urine and human serum samples were analyzed to determine SCG contents. The results are presented in Table 3. Good recoveries with precise results show good reproducibility and accuracy of the method.

4. Conclusion

The kinetic spectrophotometric method developed for SCG determination is inexpensive and readily available, allows rapid determination at low operating costs, and shows simplicity and adequate selectivity. The detection limit of the proposed method was 2.7 ng mL^{-1}. The method was found to be of very good precision and accuracy, in relation to the
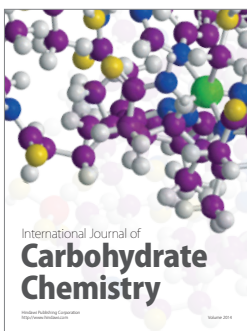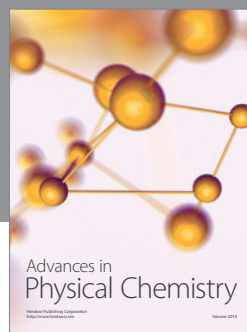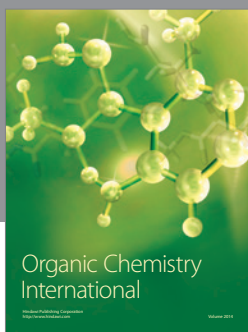
other kinetic procedures. Therefore, the method could be proposed for biological samples.

Acknowledgment

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