Research Article

Drug Testing and Analysis

Received: 27 April 2010 Revised: 21 July 2010 Accepted: 24 August 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.208

Kinetic spectrophotometric methods for the determination of artificial sweetener (sucralose) in tablets

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Two simple and sensitive kinetic spectrophotometric methods for the determination of sucralose are described. The first method is based upon a kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time of 30 min. The absorbance of the green coloured manganate ions produced was measured at 610 nm. The second method is based on the reaction of sucralose with cerium (IV) ammonium sulfate in the presence of perchloric acid with the subsequent measurement of the excess unreacted cerium (IV) ammonium sulfate at 320 nm at a fixed time of 30 min in a thermostated water bath at 60 ± 1 °C. This principle is adopted to develop a kinetic method for sucralose determination. The absorbance concentration plots in both methods were rectilinear over the range 4–16 and 10–30 μg ml^{-1}, for the first and second methods, respectively. The different experimental parameters affecting the development and stability of the colours were carefully studied and optimized. The determination of sucralose by rate constant method, fixed concentration method, and fixed-time method was also feasible with calibration equations obtained but the latter method was found to be more applicable. The two methods have been applied successfully to commercial tablets. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: kinetic spectrophotometric methods; sucralose; $KMnO_4$; cerium (IV) ammonium sulfate; tablets

Introduction

Sucralose (trichlorogalactosucrose or 1, 6-dichloro- 1,6-dideoxy-b-D-fructofuranosyl-4-deoxy-a-D-galactopyranoside) is a non-nutritive sweetener used to manufacture diabetic foods and beverages. Sucralose (SUC) is a zero-calorie sugar substitute artificial sweetener. SUC is approximately 600 times as sweet as sucrose (twice as sweet as saccharin, and 3.3 times as sweet as aspartame). Unlike aspartame, it is stable under heat and over a broad range of pH conditions. Therefore, it can be used in baking or in products that require a longer shelf life.

$KMnO_4$ and Ce (IV) were used for the quantitative determination of many drugs in dosage forms and in biological fluids. The oxidation with these reagents was extended to study the reactions kinetically with certain drugs.[1–5] Detection of SUC and other carbohydrates is challenging because they lack a strong chromophore and, therefore, cannot be detected at low concentrations spectrophotometrically or chromatographically with UV detection. Literature survey reveals that there are only a few methods for the determination SUC either in food or in beverages including high performance liquid chromatography (HPLC),[6,7] HPTLC[8] and capillary electrophoresis,[9] but there is no reported method for the determination of SUC in tablet form. The aim of the present work is to develop kinetically based methods for the determination of SUC in tablets by measuring the absorbance at 610 nm after oxidation reaction with alkaline $KMnO_4$ or at 320 nm after reaction with cerium (IV) ammonium sulfate.

Some specific advantages in the application of kinetic methods can be expected:[10]

1- Sensitivities improved due to the measurement of the increase in the absorbance with the time of reaction instead of the measurement of a concrete absorbance value.
2- The coloured excipient components and/or turbidity background of the samples or of other active compounds present in tablets are not interfered providing resistance to the chemical reaction conditions established for the proposed method.

Experimental

Apparatus

A thermo-spectronic UV-Vis dual beam spectrophotometer connected to a Harvest computer system was used. The absorption spectra were measured in 1-cm quartz cells. The absorbance data were processed using Excel software.

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Table 1. Regression and statistical parameters for the determination of SUC using the proposed methods

Parameters	Method A	Method B
Linearity range (μg ml^{-1})	4-16	10-30
LOQ (μg ml^{-1})	4.00	10.00
LOD (μg ml^{-1})	1.14	3.20
Intercept	5.56×10^{-3}	-1.08×10^{-2}
Slope	6.97×10^{-2}	3.48×10^{-2}
Correlation coefficient	0.9993	0.9992
S_a	1.66×10^{-2}	1.67×10^{-2}
S_b	1.54×10^{-3}	7.99×10^{-4}
$S_{y/x}$	1.38×10^{-2}	1.24×10^{-2}
a/S_a*	0.335	0.64
$S_b{}^2$	2.37×10^{-6}	6.39×10^{-7}
S_b%	2.15	2.29
F	2046.37	1890.94
Significance F	2.38×10^{-5}	2.68×10^{-5}

S_a is standard deviation of intercept, S_b is standard deviation of slope, and $S_{y/x}$ is standard deviation of residuals
* Theoretical value of t (a/S_a) = 2.31 at the 95% confidence level

Reagents and materials

The following reagents were used:

- Potassium permanganate (Prolabo, Paris, France): 5×10^{-3} M aqueous solution, freshly prepared.
- Sodium hydroxide (BDH, Poole, UK): 0.5 M aqueous solution.
- Acetonitrile (Aldrich, Germany).
- Ce IV ammonium sulfate (Prolabo, Paris, France) was prepared as 1×10^{-2} M in 0.5 M sulfuric acid, and then step-dilution with 1 M perchloric acid to obtain 1×10^{-3} M of cerium (IV).
- Perchloric acid was of analytical grade (BDH, Poole, UK).
- SUC was obtained from Borg Pharmaceutical Co, Borg El-Arab-Alexandria, Egypt. Standard solution 1 mg ml^{-1} of SUC was prepared in acetonitrile. From this solution intermediate dilution was made with water to obtain 0.4 mg ml^{-1} working standard solution for method A and 0.2 mg ml^{-1} working solution for method B.

Procedures

Recommended general procedures

Method A. Aliquots of 3 ± 0.2 ml of 5×10^{-3} M $KMnO_4$ solution and 1 ml of 0.5 M NaOH solution were placed in a set of 10-ml volumetric flasks. Accurate volumes of working solution covering the concentration range cited in Table 1 were added, and the mixtures were shaken well. Then the solutions were diluted to volume with distilled water. At a fixed time of 30 min, the absorbance was measured directly at 610 nm against the appropriate blank.

The SUC concentration was determined by measuring the rate of the reaction as the tangent to kinetic curve during the first 30 min of reaction and using the appropriate graphs. After the elapse of 30 min, two plots were made: the first one is a plot of log reaction rate versus log [SUC] to get the order of the reaction, and the second one is a plot of the values of the absorbance against the final concentration in mg ml^{-1}. Alternatively, the regression equations were computed.

Method B. Aliquot portions of working standard solution within the range stated in Table 1, were transferred into a set of 10-ml volumetric flasks; then, 4 ml of 1×10^{-3} M Ce (IV) was added to each flask. The contents of each flask were mixed well and allowed to stand at 60 °C ± 1 in a thermostated water bath for 30 min, then completed to the mark with distilled water after cooling. The absorbance of the blank was measured against each solution at 320 nm.

The SUC concentration was determined by measuring the rate of the reaction during the first 30 min of reaction and using the appropriate graphs. After 30 min elapsed, two plots were made: the first one is a plot of log reaction rate versus log [SUC] to get the order of the reaction, and the second one is a plot of the values of the absorbance against the final concentration in mg ml^{-1}. Alternatively, the regression equations were computed.

Procedures for tablets

From the average weight of 20 crushed tablets into fine powder, an accurately weighed quantity of the mixed powder containing an equivalent of 25 mg of SUC was transferred into a 25-ml volumetric flask, diluted to volume with acetonitrile and then filtered with Millipore filter (0.45 μm). An aliquot of this filtrate was diluted with distilled water to prepare the working solution. The procedure was continued as described under general procedures.

Results and Discussion

Optimization of the reactions conditions

Method A:

SUC was found to react with $KMnO_4$ in alkaline medium producing a bluish-green colour due to the production of manganate ion which absorbs maximally at 610 nm (Figure 1). The absorbance of the reaction product remains stable for at least 60 min. The increase in the intensity of the colour by time was used as a basis for a useful kinetic method for the determination of SUC in tablets. The spectrophotometric properties of the coloured product as well as the different experimental parameters affecting the colour development and its stability were studied and optimized. Each factor was changed and investigated individually while the others were kept constant. These factors include the effect of different solvents, the concentration of the reagents ($KMnO_4$ and NaOH), temperature, and time. Acetonitrile was used to dissolve the drug since other solvents could be oxidized by $MnO_4{}^-$ to give green $MnO_4{}^{2-}$ leading to erroneous results. The reaction rate was found to increase by increasing temperature up to 60 °C with the subsequent increase in the slope of the calibration graph. This indicates higher analytical sensitivity, but results in poor linearity. Increasing temperature above 70 °C results in the precipitation of MnO_2. Therefore, room temperature was selected as the optimum temperature. The reaction rate increased by increasing $KMnO_4$ concentration and a volume of 3.0 ± 0.2 ml of 5×10^{-3} M $KMnO_4$ was adequate for maximum absorbance (Figure 2).

The influence of 0.5 M NaOH volume on the reaction rate was investigated. It was found that 1 ml of 0.5 M NaOH was the most suitable volume for maximum absorbance (Figure 2).

Method B

Ce (IV) has been used for the quantitative determination of some organic compounds.[11,12] Due to its high oxidation potential

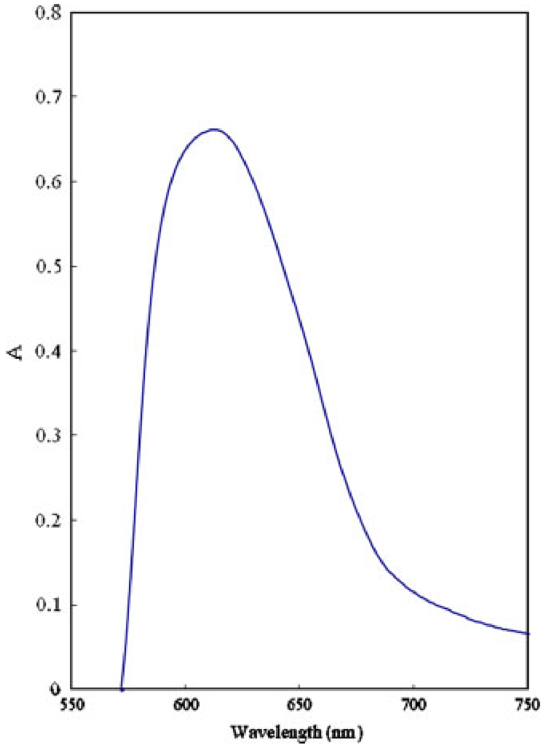

Figure 1. Absorption spectrum of manganate ions resulting from reaction of 9 µg ml^{-1} SUC with 5 × 10^{-3} M $KMnO_4$.

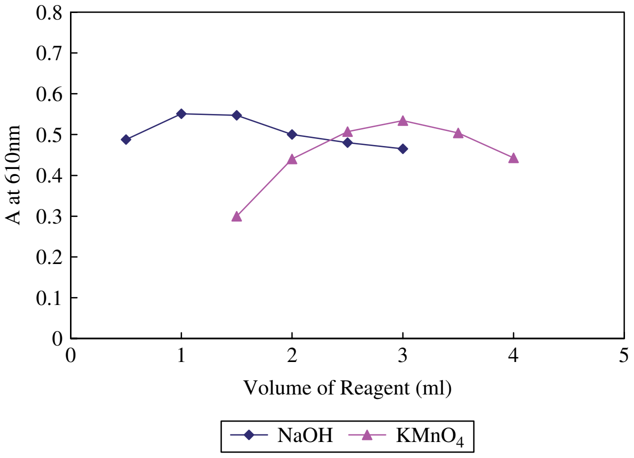

Figure 2. Effect of volume of 0.5M NaOH and 5 × 10^{-3} M $KMnO_4$ on the absorbance intensity of SUC (8 µg ml^{-1}) at 610 nm.

and excellent solution stability, this reagent has been utilized to develop a quantitative method for the analysis of SUC in tablets. The method depends on measuring the absorbance of the consumed Ce (IV) at 320 nm. This was achieved by measuring the absorbance of a blank containing solution of certain concentration of Ce (IV) against the test containing the same concentration of Ce (IV) (Figure 3). Acid medium is needed to prevent precipitation of hydrated cerium (IV) oxide, $CeO_2.xH_2O$. The reaction of the studied compounds with Ce (IV) proceeds quantitatively only in the presence of 1 M perchloric acid. The reaction of Ce (IV) in 3–4 M sulfuric acid ($E^o = 1.44$ V) or nitric acid ($E^o = 1.61$ V) as oxidant for organic compounds proved to be extremely slow

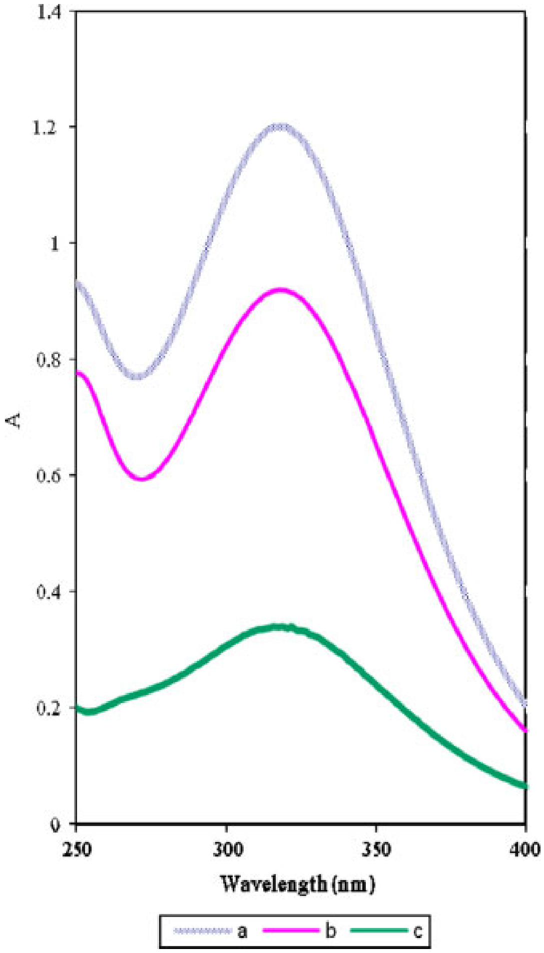

Figure 3. Absorption spectra of 4 × 10^{-4} M Ce IV (a), its reaction product with 10 µg ml^{-1} SUC (b) and of Absorption spectra of 4 × 10^{-4} M Ce IV against its reaction product with 10 µg ml^{-1} SUC (c).

and failed to obey any simple stoichiometric relationship. The greater strength of Ce (IV) in perchloric acid medium ($E^o = 1.75$ V) overcomes both the slowness of the oxidation process and the inexact stoichiometry encountered in sulfuric acid.

All factors affecting the reactions were thoroughly studied, namely Ce (IV) concentration, temperature, and time. The adoption of 4 ml of/1 × 10^{-3} M solution of Ce (IV) in the final solution proved to be adequate for maximum concentration of SUC used in the calibration curve (Figure 4). It was found that 30 min in contact with acidified solution of Ce (IV) at 60 °C ± 1 is sufficient for the complete oxidation of SUC, as indicated by the highest absorbance difference at 320 nm (Figure 5). The maximum absorbance obtained remained stable for at least 30 min. As the intensity of colour increases with time, it was useful to develop a kinetically based method for the assay of SUC.

Kinetic study of the reactions

The rate of the reactions was also found to be [SUC]-dependent. The rates were followed

(1) at room temperature with various concentrations of the drug in the range of 4–16 µg ml^{-1}, keeping $KMnO_4$ and NaOH concentrations constant (Figure 6) applying method Aa; and

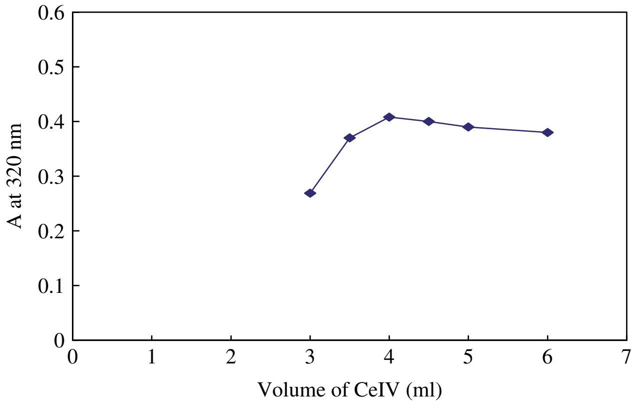

Figure 4. Effect of volume of 10^{-3} M Ce IV on the absorbance intensity of SUC (12 µg ml^{-1}) at 320 nm.

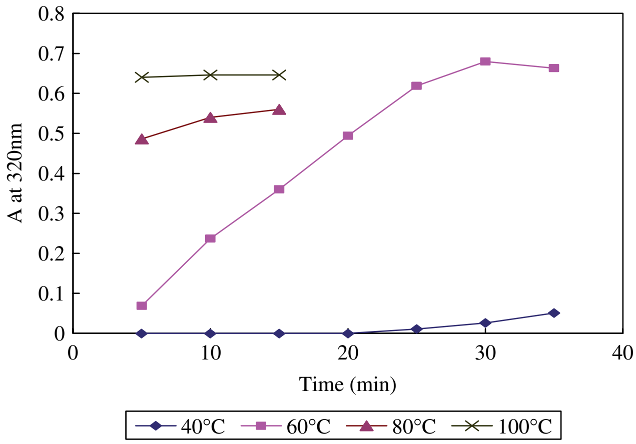

Figure 5. Effect of temperature and heating time on absorbance measurements of reaction product of Ce IV with 20 µg ml^{-1} SUC.

(2) at 60 °C ± 1 with various concentrations of SUC in the range 10–30 µg ml^{-1}, keeping the other reactant Ce (IV) concentration constant (Figure 7) applying method B.

The graphs shown in Figures 6 and 7, applying methods A and B, respectively, were obtained and from which it is clear that the rate increases as the SUC concentration increases, indicating that the reactions rates obeys the following equation:

$$\text{Rate} = K' [\text{SUC}]^n \qquad (1)$$

where K′ is pseudo-order rate constant and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method measurement[13] as ΔA/Δt, where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations (Table 2), Equation 1 is transformed into:

$$\log(\text{rate}) = \log \Delta A/\Delta t = \log K' + \log[\text{SUC}]. \qquad (2)$$

Regression of log (rate) versus log [SUC] gives the regression equations:

$$\log(\text{rate}) = 1.202 + 1.0026 \log C \ (r = 0.9996) \text{ for method A}$$

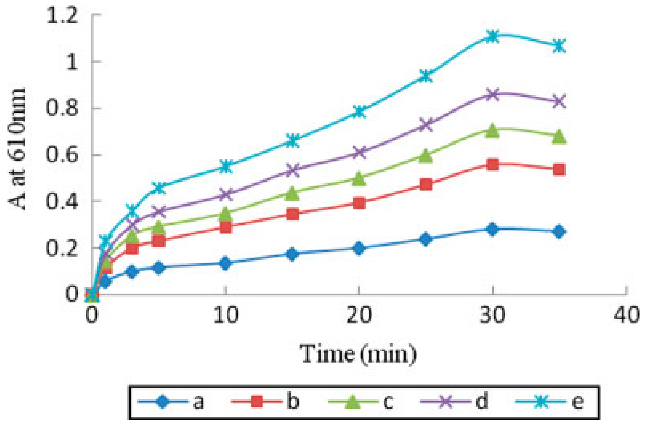

Figure 6. Absorbance at 610 nm versus time graphs for the reaction of SUC and alkaline $KMnO_4$ (Method A) showing the dependence of the reaction on SUC concentration. (a) 1.007×10^{-5} M, (b) 2.013×10^{-5} M, (c) 2.517×10^{-5} M, (d) 3.020×10^{-5} M and (e) 4.027×10^{-5} M.

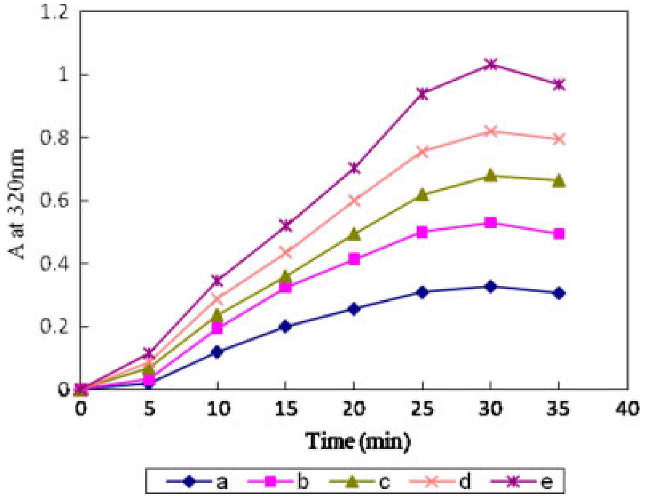

Figure 7. Absorbance at 320 nm versus time graphs for the reaction of SUC and Ce IV (Method B) showing the dependence of the reaction on SUC concentration (a) 2.517×10^{-5} M, (b) 3.775×10^{-5} M, (c) 5.033×10^{-5} M, (d) 6.040×10^{-5} M and (e) 7.550×10^{-5} M.

Table 2. Logarithms of rates for different concentrations of SUC at room temperature applying method (A) & at 60 °C ± 1 applying method (B)

Methods	log ΔA/Δt	log [SUC]
Method (A)	−3.809	−4.997
(λ = 610 nm)	−3.510	−4.696
	−3.406	−4.599
	−3.321	−4.520
	−3.211	−4.395
Method (B)	−3.741	−4.600
(λ = 320 nm)	−3.532	−4.423
	−3.423	−4.298
	−3.341	−4.219
	−3.241	−4.122

Hence $K' = 15.922$ sec^{-1} and the reaction is first-order ($n = 1.0026 \simeq 1$)

$$\log(\text{rate}) = 1.019 + 1.0329 \log C \ (r = 0.9985) \text{ for method B}$$

Hence $K' = 12.35$ sec^{-1} and the reaction is first-order ($n = 1.0329 \simeq 1$) with respect to [SUC].

Evaluation of the kinetic methods

The quantitation of SUC under the optimized experimental conditions outlined above would result in a pseudo-first order reaction with respect to their concentration where

(1) $KMnO_4$ and NaOH concentrations were, respectively, 150 and 5000 times higher than the initial [SUC], applying method A.
(2) Ce (IV) concentration was at least 16 times of the initial concentration of [SUC], applying method B.

However, the rate was directly proportional to [SUC] in pseudo first-order rate equation as follows:

$$\text{Rate} = K' [\text{SUC}] \tag{3}$$

where K' is the pseudo first-order rate constant. Several experiments were then carried out to obtain [SUC] from the rate data according to Equation 3. Rate constant, fixed concentration and fixed-time methods[14,15] were tried and the most suitable analytical method was selected taking into account applicability, sensitivity, intercept and correlation coefficient (r).

Initial rate method

In this method, graphs of the rate (in the beginning of the reactions) versus [SUC] were not easy to obtain, because the first steps of the reactions were too fast to follow. Therefore, this method was abandoned.

Rate constant method

Graphs of log A versus time for [SUC] in the range of $1.007 \times 10^{-5} - 4.027 \times 10^{-5}$ M and $2.517 \times 10^{-5} - 7.550 \times 10^{-5}$ M applying methods A and B, respectively, were plotted. These graphs were shown to be rectilinear. Pseudo first-order rate constants were calculated from the slopes multiplied by −2.303 and are presented in Table 3. Regression of (C) versus K' gave these equations

$$K' = -0.497C - 0.00065 \quad r = 0.6320 \quad \text{for method A}$$
$$K' = 4.050C - 0.00137 \quad r = 0.9001 \quad \text{for method B}$$

The values of (r) indicate poor linearity which is probably due to inconsistency of k'.

Fixed concentration method

Reaction rates were recorded for different [SUC] in the range of $2.013 \times 10^{-5} - 4.027 \times 10^{-5}$ M and $3.775 \times 10^{-5} - 7.550 \times 10^{-5}$ M applying methods A and B, respectively. A pre-selected value of absorbance ($A = 0.4$) for both methods were fixed and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of SUC (Table 4) was plotted and the following equations of calibration graphs were obtained:

Table 3. Values of K' calculated from slopes of log A versus t graphs multiplied by −2.303 for different [SUC] applying both methods

Methods	K' (sec^{-1})	[SUC]
Method **(A)** at ($\lambda = 610$ nm)	-6.659×10^{-4}	1.007×10^{-5}
	-6.559×10^{-4}	2.013×10^{-5}
	-6.601×10^{-4}	2.517×10^{-5}
	-6.654×10^{-4}	3.020×10^{-5}
	-6.794×10^{-4}	4.027×10^{-5}
Method (B) at ($\lambda = 320$ nm)	-1.264×10^{-3}	2.517×10^{-5}
	-1.268×10^{-3}	3.775×10^{-5}
	-1.124×10^{-3}	5.033×10^{-5}
	-1.103×10^{-3}	6.040×10^{-5}
	-1.095×10^{-3}	7.550×10^{-5}

Table 4. Values of reciprocal of time taken at fixed A for different rates of variable [SUC] at constant concentrations 0.5 M NaOH and 5×10^{-3} M $KMnO_4$ at room temperature (method A) and at constant concentration 10^{-3} M Ce IV at 60 °C ± 1 (method B)

Methods	$^1/_t$ Sec^{-1}	[SUC]
Method (A) at ($\lambda = 610$ nm)	7.8×10^{-4}	2.013×10^{-5}
	1.3×10^{-3}	2.517×10^{-5}
	1.9×10^{-3}	3.020×10^{-5}
	4.5×10^{-3}	4.027×10^{-5}
Method (B) at ($\lambda = 320$ nm)	8.3×10^{-4}	3.775×10^{-5}
	1.02×10^{-3}	5.033×10^{-5}
	1.21×10^{-3}	6.040×10^{-5}
	1.47×10^{-3}	7.550×10^{-5}

$$^1/_t = 187.500C - 3.306 \times 10^{-3} \quad r = 0.9766 \quad \text{for method A}$$
$$^1/_t = 17.103C - 1.748 \times 10^{-4} \quad r = 0.9992 \quad \text{for method B}$$

By applying both methods, the range of [SUC] giving the most acceptable calibration graph was so limited that the methods were abandoned.

Fixed-time method

Reaction rates were determined for different [SUC] at a pre-selected fixed time and the absorbance was measured. Calibration graphs of absorbance versus initial concentration of SUC were established at fixed times of 3, 5, 10, 15, 20, 25, 30 and 35 min for method A and 5, 10, 15, 20, 25, 30 and 35 min for method B with regression equations assembled in Table 5. It is clear that the slope increases with time for both methods and the most acceptable values of correlation coefficient and intercept were obtained for a fixed time for 30 min for both methods. Therefore, this period of time was chosen as the most suitable interval for measurements. After optimizing the reaction conditions, the fixed-time method was applied to the determination of SUC in pure form over the ranges of 4–16 µg ml^{-1} for method A and 10–30 µg ml^{-1} for method B.

Linearity. Under the experimental conditions described, the graphs obtained by plotting A (for method A) and ΔA (for method

Table 5. Regression equations for SUC at different fixed time over the range ($1.007 \times 10^{-5} - 4.027 \times 10^{-5}$ M) at room temperature applying method (A) and over the range ($2.517 \times 10^{-5} - 7.550 \times 10^{-5}$ M) at 60 °C ± 1 applying method (B)

Methods	Time (min)	Regression equations	Correlation coefficient(r)
Method (A)	3	$A = 8761.2C + 2.25 \times 10^{-2}$	0.9912
($\lambda = 610$ nm)	5	$A = 11455.2C + 2.29 \times 10^{-3}$	0.9993
	10	$A = 13754.7C + 4.84 \times 10^{-3}$	0.9983
	15	$A = 16360.3C + 1.86 \times 10^{-2}$	0.9966
	20	$A = 19633.7C + 3.93 \times 10^{-3}$	0.9993
	25	$A = 23483.2C + 4.707 \times 10^{-3}$	0.9993
	30	$\mathbf{A = 27683.5C + 5.549 \times 10^{-3}}$	**0.9993**
	35	$A = 26728.9C + 5.358 \times 10^{-3}$	0.9993
Method (B)	5	$A = 1974C - 0.0338$	0.9906
($\lambda = 320$ nm)	10	$A = 4413C + 0.0168$	0.9965
	15	$A = 6061C + 0.0662$	0.9880
	20	$A = 8752C + 0.0574$	0.9934
	25	$A = 12292C + 0.0116$	0.9982
	30	$\mathbf{A = 13823C - 0.0108}$	**0.9992**
	35	$A = 13159C - 0.0102$	0.9985

B) values versus concentration (in the ranges stated in Table 1) show linear relationships. The slopes, intercepts and correlation coefficients obtained by the linear least squares regression treatment of the results are also given. An important statistical parameter for indicating the random error in the estimated values of y is the standard error of the estimate, or the standard deviation about regression, or the standard deviation of the residuals, $S_{y/x}$. The smaller the standard error of the estimate the closer the points are to the straight line. Standard deviation of intercept (S_a) and of slope (S_b) are also presented for each compound using the proposed methods of measurements. The high values of the correlation coefficients ($r > 0.999$) with negligible intercepts together with the high F-values indicate the good linearity of the calibration graphs.[14,15] The linearity was further evaluated by the calculation of the percentage RSD of the slope (S_b%). Also, the small degree of scatter of the experimental data points around the line of regressions was confirmed by the small values of the variances around the slopes $S_b{}^2$. For more confirmation, the Student's *t*-test was performed to determine whether the experimental intercept (a) of the above-mentioned regression lines was not significantly different from the null hypothesis. The calculated values of t (a/S_a) do not exceed the 95% criterion of $t = 2.31$ for 5 samples. So the intercepts are not significantly different from zero in the proposed methods. Thus, the hypothesis that (a) is of negligible value is confirmed.[16,17]

For equal degrees of freedom, an increase in the variance ratio (F-values) means an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the more steepness of the regression line is. The smaller the mean of squares due to residuals, the less the scatter of the experimental points around the regression line is. Consequently, regression lines with high F-values (low significance F) are much better than those with lower ones. Good regression lines show high values for both (r) and (F) values [17]. Regression equations, correlation coefficients, limit of detection (LOD) and limit of quantitation (LOQ) are given in Table 1.

Table 6. Evaluation of the precision of both methods for the determination of SUC

Methods	Added concentration[(a)]	Recovery ± RSD%[(b)]	E_r%[(c)]
Method(A)	6	99.5 ± 1.00	−0.5
	12	101.3 ± 1.70	1.3
	16	98.5 ± 0.86	−1.5
Mean		99.8 ± 1.18	−0.2
Method(B)	15	96.9 ± 1.00	−3.1
	20	98.8 ± 1.20	−1.2
	30	99.1 ± 1.20	−0.9
Mean		98.3 ± 0.98	−1.7

(a) Final concentration in $\mu g\ ml^{-1}$
(b) Mean recovery ± relative standard deviation for five determinations
(c) Percentage relative error

Accuracy and precision. In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of SUC were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table 6. The low values of relative standard deviation (RSD%) and percentage relative error (E_r%) indicate the high precision and the good accuracy of the proposed methods.

Application

It was found that, when the proposed methods were applied to the determination of SUC in pharmaceutical preparations, the % recovery was around 150%. This might be due to the interaction of the excipients in the formulations (especially lactose monohydrate, croscarmellose Na, aerosil 200 and L-leucine), with both $KMnO_4$ and Ce (IV). Therefore, the tablets had to be extracted with acetonitrile in which only SUC dissolved while the other excipients did not with the exception of aerosil 200 which forms colloidal solution with acetonitrile. Accordingly, millipore filter (0.45 μm) was used to avoid passage of any colloidal solution into the filtrate so that a clear solution of SUC in acetonitrile can be separated

The fixed-time method was applied to the determination of SUC tablets. The concentration of SUC was calculated using the corresponding calibration equations in Table 1 at a fixed time of 30 min applying both methods. The results of determination of SUC in tablets obtained from the $KMnO_4$ method were compared with those of the Ce (IV) method. Statistical comparison of the results was performed with regard to accuracy and precision using Student's *t*-test and the variance ratio F-tests at 95% confidence level (Table 7). Since the calculated t- and F- values did not exceed the theoretical ones, this indicated that there was no significant difference between the two methods of analysis

Mechanism of the reaction

Job's method of continuous variation [18] was employed. Equimolar solutions of SUC (1.007×10^{-3} M) with $KMnO_4$ and SUC (5.03×10^{-4} M) with cerium (IV) ammonium sulfate were prepared. A series of 5-ml portions of solutions of drug with the respective reagents were made, comprising different complementary proportions (0 : 10, 0.5 : 9.5, 1 : 9,. . . . . .,8.5 : 0.5, 9 : 1, 10 : 0) in 10-ml volumetric flasks. The absorbance values of the resulting solutions were measured at 610 nm after 30 min at room temperature against reagent blanks for method A and at

Table 7. Application of the proposed methods for the determination of SUC in tablet form using fixed-time method

Pharmaceutical Preparation	Recovery % ± SD[a]	
	Method A	Method B
Blendy® tablet[b]	101.00 ± 1.00	100.50 ± 0.50
t[c]	1.00	
F[c]	4.00	

(a) Average of five determinations
(b) Blendy tablet labeled to contain 5.6 mg SUC per tablet (**Borg Pharmaceutical Co**-the Batch No. is **032003**).
(c) Theoretical value of t and F at P = 0.05 are 2.306 and 6.39, respectively.

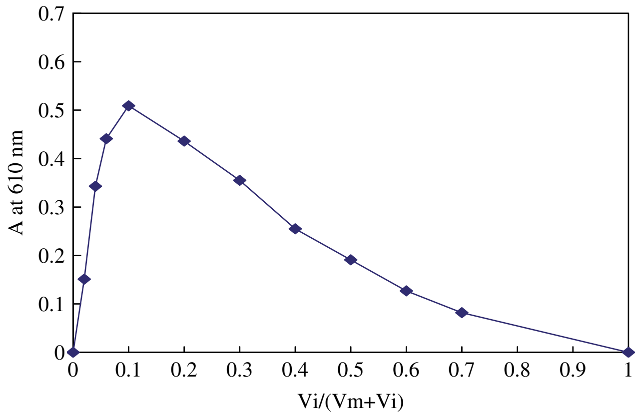

Figure 8. Job method for reaction of SUC with $KMnO_4$.

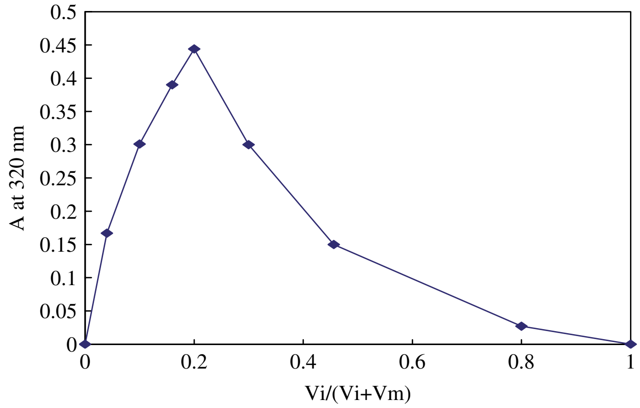

Figure 9. Job method for reaction of SUC with Ce IV.

320 nm at fixed time of 30 min at 60 ± 1 °C for method B. This investigation showed that SUC interacts with $KMnO_4$ in the ratio of 1: 10 (Figure 8) and with cerium (IV) ammonium sulfate in the ratio of 1 : 4 (Figure 9).

Conclusion

Two simple and sensitive kinetic spectrophotometric methods for the determination of SUC have been successfully developed and validated. These methods were based on the oxidation of SUC with alkaline $KMnO_4$ and with Ce (IV). Fixed-time methods for these spectrophotometric methods can be easily applied to the determination of SUC in pure form and in tablets. The proposed methods are the first colorimetric methods used for SUC determination and are sensitive enough to enable the determination of lower amounts of the drug. Furthermore, the proposed methods have the advantage of using the spectrophotometer which is usually available in all quality control laboratories, rather than the previously reported methods that imply more sophisticated and expensive instruments that are usually unavailable.[6–9] These advantages encourage the application of the proposed methods in routine analysis of SUC in quality control laboratories, as alternatives for the existing methods. Taking into account the results obtained for the calibration graphs, method A is more sensitive than method B for the determination of SUC.

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