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ANALYSIS

UTILITY OF AN OXIDATION REACTION FOR THE SPECTROPHOTOMETRIC DETERMINATION OF ACARBOSE IN CONTROLLED RELEASE TABLETS AT VARIOUS SIMULATED GASTROINTESTINAL MEDIA

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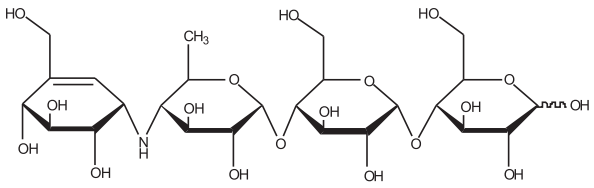
Abstract: A sensitive kinetic method for spectrophotometric determination of acarbose is developed and validated for the determination of the drug in bulk and pharmaceutical formulations. The drug was estimated in simulated gastrointestinal media i.e., 0.1 M HCl (pH 1.2) and phosphate buffer (pH 6.8). The method involves the oxidation of acarbose by treating it with a strong oxidizing agent (potassium permanganate (1×10^{-2} M)) in alkaline media. The reaction kinetics was determined for 20 min at room temperature. The reaction followed first order kinetics and the absorbance of the corresponding manganate ions produced was determined at 610 nm. The absorbance-concentration plot was found to be rectilinear over the concentration range of 2–20 μg/mL. The proposed method was used for estimation of the drug in a novel controlled release dosage form. Thus, the method developed was simple, reproducible and can be successfully applied for the determination of the drug in simulated gastrointestinal fluid.

Keywords: acarbose, alkaline potassium permanganate, oxidation, 0.1 M hydrochloric acid, phosphate buffer, bulk pharmaceutical formulation

Acarbose O-4,6-dideoxy-4-{[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino}-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucose. (Fig. 1) is an oral α-glycosidase inhibitor used in the management of non-insulin-dependent diabetes mellitus (NIDDM). It is obtained by fermentation processes from a microorganism, *Actinoplanes utahensis*. It delays the absorption of glucose in the small intestine by competing with the normal substrate for the binding site of the α-glycosidase enzyme and ceases the enzymatic reaction. This leads to a decrease in the formation and absorption of glucose in the blood stream (1).

Various clinical trials conducted in patients with non-insulin dependent diabetes mellitus indicate that acarbose is a potential drug which reduces postprandial glucose levels significantly along with a marked effect on the hypertriglyceridemia, which is closely linked to carbohydrate and insulin metabolism (2, 3). Acarbose has a short half-life ($t_{1/2}$ = 2 h), which necessitates repetitive administration of the drug i.e., 25–100 mg, 3 times a day. Thus, development of a modified release system of acarbose will obviate the need of multiple dosing of the drug candidate and hence improve patient compliance with reduced gastrointestinal side effects (flatulence, abdominal distension, borborygmus and diar-



(2*R*,3*S*,4*S*,5*S*)-5-((2*R*,3*S*,4*S*,5*S*)-5-((2*R*,3*S*,4*R*,5*S*)-3,4-dihydroxy-6-methyl-5-((1*S*,4*R*,5*R*,6*R*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enylamino)-tetrahydro-2*H*-pyran-2-yloxy)-3,4-dihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-yloxy)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2,3,4-triol

Figure 1. Chemical structure of acarbose

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rhea) associated with repetitive administration of the drug.

Acarbose is an oligosaccharide with molecular weight of 645.6 daltons. The drug moiety lacks a chromophore and hence cannot be estimated directly by spectrophotometry. Till date, various methods have been developed for estimation of the drug in both bulk and plasma samples using high-performance liquid chromatographic separation (HPLC) with tandem mass spectrometric detection (4), HPLC equipped with porous graphitic column (5), gas chromatography-mass spectrophotometry (GC-MS) (6) and capillary zone electrophoresis (CE) (7). All the aforementioned methods available for the analysis of acarbose are expensive, tedious and time consuming. Ibrahim et al., developed a UV-spectrophotometric method for analysis of two α-glucosidase inhibitors viz., acarbose and miglitol using alkaline potassium permanganate in water (8). Kinetic method of analysis is a preferred method as it is more specific, accurate as it aids in determination of the desired molecule individually and the absorbance of the evolved ion is a function of time, as a result there is no possibility of interference with other components or excipients present in the samples (9, 10). In the present work, an attempt is made to develop a sustained release dosage form for acarbose and estimate it in simulated gastrointestinal fluid (pH: 1.2 and 6.8) using a simple, sensitive spectroscopic method.

EXPERIMENTAL

Chemicals

Acarbose (98.23%; Batch No. JA1900) was received as a kind gift sample from CKD Bio Corporation, Korea. Carbopol 71GNF was donated as gift sample by Noveon Inc. (Mumbai, India). All chemicals and solvents used were of analytical reagent grade. Fresh triple distilled water (Sartorius, Göttingen, Germany) was used throughout this study.

Apparatus

Spectrophotometric measurements were carried out using double beam UV-1700, Shimadzu recording spectrophotometer, equipped with kinetic accessory. The reaction kinetics of the drug in various media was studied for a period of 20 min at room temperature at 610 nm, against corresponding blank solution.

Table 1. Composition of samples prepared for estimation of extinction coefficient of acarbose on reaction with $KMnO_4$ in alkaline medium in water.

Concentration (µg/mL)	Vol. of drug stock (100 µg/mL) (mL)	Vol. of 0.5 M NaOH (mL)	Vol. of 0.001 M $KMnO_4$ (mL)	Vol. of water (mL)
2	0.2	1	1	7.8
5	0.5	1	1	7.5
10	1.0	1	1	7
15	1.5	1	1	6.5
20	2.0	1	1	6

Table 2. Composition of samples prepared for estimation of extinction coefficient of acarbose on reaction with $KMnO_4$ in alkaline medium, in 0.1 M HCl (pH: 1.2) and in alkaline medium in phosphate buffer (pH: 6.8).

Concentr. (µg/mL)	Vol. of drug stock (100 µg/mL) (mL)	Vol. of 0.5 M NaOH (mL)	Vol. of 0.01 M $KMnO_4$ (mL)	Vol. Of 0.1 M HCl/phosphate buffer (pH: 6.8) (mL)	Vol. of water (mL)
2	0.2	2	1	4.8	2
5	0.5	2	1	4.5	2
10	1.0	2	1	4.0	2
15	1.5	2	1	3.5	2
20	2.0	2	1	3.0	2

Preparation of stock solution and reagents

Stock solutions of acarbose was prepared by dissolving 10 mg of the drug in 100 mL distilled water, 0.1 M HCl (pH: 1.2) and phosphate buffer (pH: 6.8), respectively. Other concentrations were prepared by further dilution with respective media; so as to get the concentrations in the range of 2–20 mg/mL. 0.001 M $KMnO_4$ and 0.5 M NaOH solutions were prepared in distilled water.

Determination of reaction kinetics

Initially, drug stock solutions of 100 µg/mL were prepared in water (Stock A), 0.1 M HCl (pH 1.2) (Stock B) and phosphate buffer (pH 6.8) (Stock C), respectively. Accurately measured aliquots equal to 20–200 µg acarbose from Stock A were transferred into separate 10 mL volumetric flasks, after that 1 mL of 0.5 M NaOH was added followed by 1 mL of 1×10^{-2} M $KMnO_4$; the mixture was shaken well. The absorbance at 610 nm with time was determined during 20 min at ambient temperature. The rate of the reaction was measured as the tangent to kinetic curve during the first 20 min of reaction using the appropriate graphs. To get the kinetics in 0.1 M HCl and phosphate buffer pH 6.8, the above procedure was carried out with slight modification using respective stock solutions (Stock B and C). To this 2 mL of 0.5 M NaOH was added followed by 2 mL of water and 1 mL of 0.1 M $KMnO_4$ and the reaction mixture was allowed to stand for 20 min. The absorbance of the resulting solution was measured at 610 nm against a blank solution prepared simultaneously in all the cases. Plot of the values of absorbance against the drug concentration in µg/mL was made to obtain the regression equation.

Optimization of reaction conditions

Effect of varying volumes of potassium permanganate

Different amounts of 1×10^{-2} M $KMnO_4$ (0.25, 0.5, 1.0, 1.25, 1.5 and 1.75 mL) were added to the reaction mixtures containing aliquots equivalent to 100 µg of drug and 0.5 M NaOH in all the three media viz., water, 0.1 M HCl and phosphate buffer (pH 6.8), respectively, to study the effect of varying volumes of $KMnO_4$ on the reaction kinetics.

Effect of varying amounts of sodium hydroxide

Different amounts of 0.5 M NaOH (0.5, 1.0, 1.5, 2, 2.5 and 3 mL) was added to reaction mixtures containing aliquots equivalent to 100 µg of drug, 1 mL of 1×10^{-2} M $KMnO_4$ in all the three media viz., water, 0.1 M HCl and phosphate buffer (pH 6.8), respectively.

Study of stoichiometry of the reaction

The limiting logarithmic method (11) was used for the determination of the molar ratio between $KMnO_4$ and acarbose in the reaction. This method depends on measuring the absorbances of different solutions of $KMnO_4$ and acarbose in which the concentrations of the two species are varied in turn at a constant total ionic strength.

Linearity

Standard plot of acarbose was prepared in water, 0.1 M HCl and phosphate buffer (pH 6.8). A stock solution of acarbose (100 mg/mL) was prepared by dissolving 10 mg of accurately weighed drug in 100 mL of respective solvents. The dilutions in concentrations ranging from 2 to 20 µg/mL were prepared as stated in Tables 1, 2. The samples were then analyzed spectrophotometrically at wavelength of 610 nm.

Accuracy and precision

The accuracy and precision of the method was evaluated within the linearity range based on the analysis of acarbose in reference standard samples at 2, 10 and 20 µg/mL in all the respective media. Three independent analyses were performed at each concentration level within one day (intra day precision) as well as for three consecutive days (inter day precision).

Recovery studies

Recovery experiments were carried out by the standard addition method. For this, 2.5 mL of reference acarbose solution (30 µg/mL) was transferred into a 10.0 mL volumetric flask followed by 2.5 mL of sample solution (10, 50 and 90 µg/mL) and 2.5 mL of the respective solutions were then diluted and treated with alkaline $KMnO_4$ in both acidic and alkaline media to obtain the final concentrations of 5, 10 and 15 µg/mL, respectively. The total amount was determined by the proposed procedure and compared with the results obtained on analysis of standard concentrations of 5, 10 and 15 µg/mL, respectively.

Specificity and selectivity

The specificity and selectivity of the proposed method was evaluated by estimating the amount of acarbose in the presence of common excipients such as microcrystalline cellulose, magnesium stearate, starch, lactose and talc.

Preparation and evaluation of pharmaceutical formulations

Another objective of the current study was to design novel controlled release matrices for the drug. Initially, various polymers from synthetic and natural sources were screened for their interference in analysis using the oxidation method. It was observed that Carbopol 71GNF exhibited the least interference in analysis. Hence, five batches containing 100 mg of acarbose with varying proportions

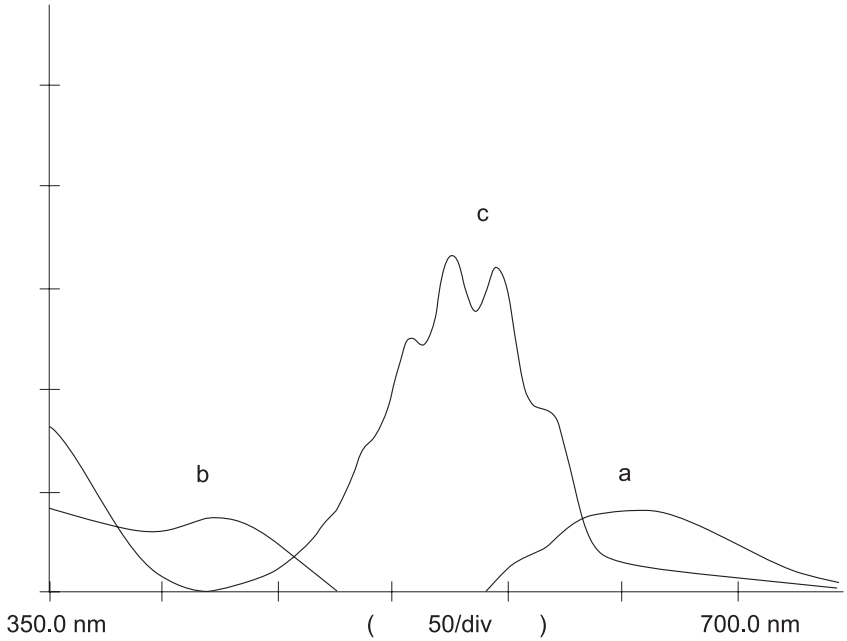

Figure 2. Absorption spectra of acarbose after reaction with alkaline $KMnO_4$. (a) The produced manganate ions after the reaction of alkaline $KMnO_4$ with acarbose (10 µg/mL); (b) Oxidation products of acarbose; (c) $KMnO_4$ (0.01 M)

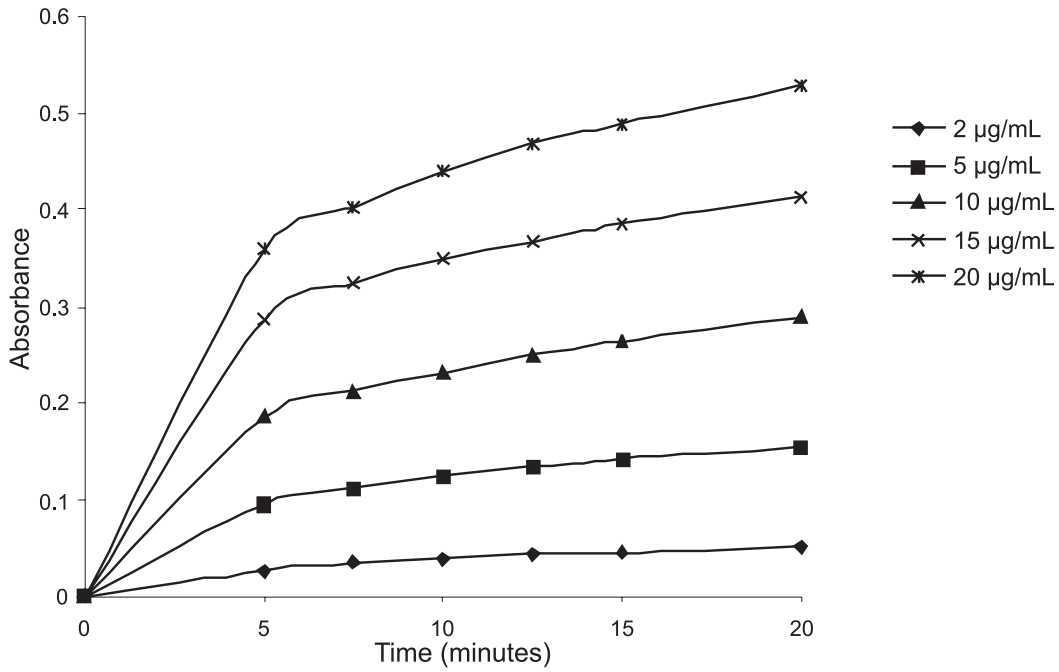

Figure 3. Absorbance *vs*. time graphs for the reaction of acarbose and alkaline $KMnO_4$ showing the dependence of the reaction on acarbose concentration. Concentration of acarbose studied in 0.1 M HCl in insert.

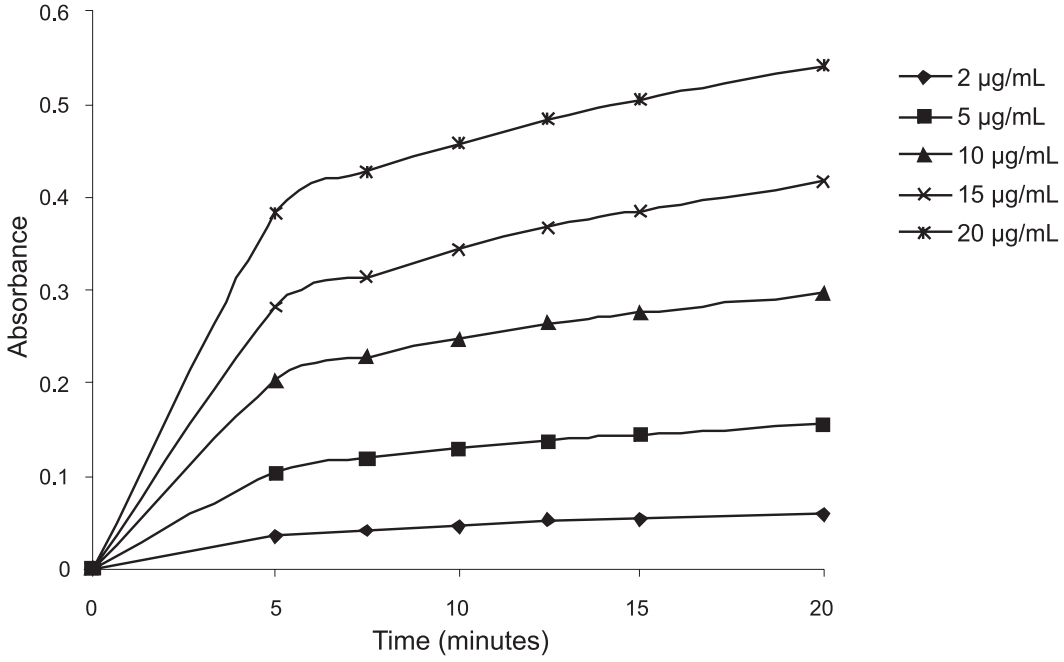

Figure 4. Absorbance vs. time graphs for the reaction of acarbose and alkaline $KMnO_4$ showing the dependence of the reaction on acarbose concentration. Concentration of acarbose studied in phosphate buffer (pH 6.8) in insert.

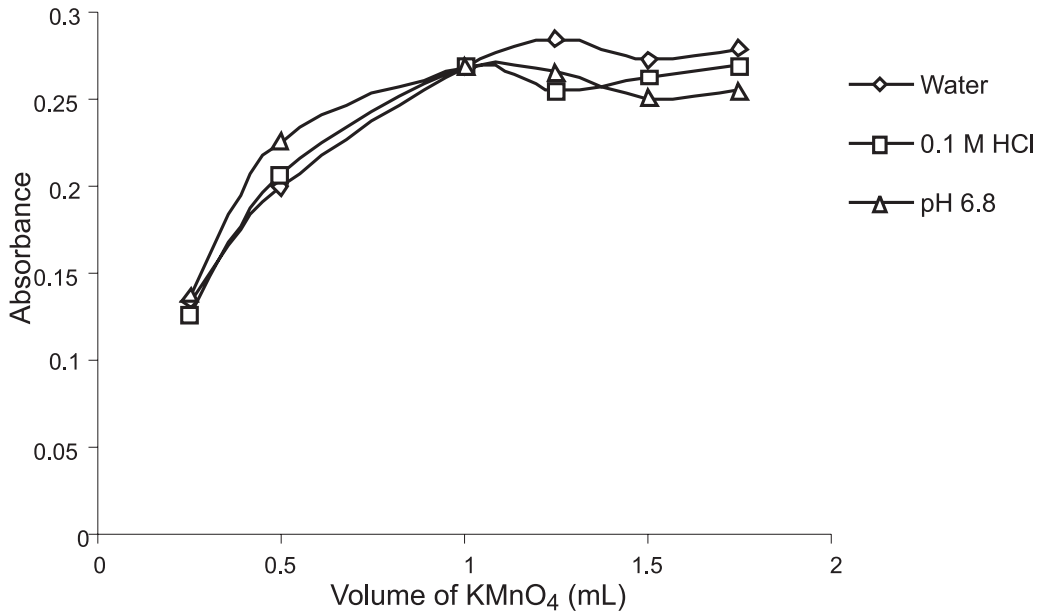

Figure 5. Effect of the concentration of KMnO4 (1×10^{-2} M) on absorbance of acarbose in water, 0.1 M HCl (pH 1.2) and phosphate buffer (pH: 6.8)

(0–60%, i.e., AC0–60) of the release retarding polymer and 2% and 1% w/w talc and magnesium stearate were prepared by direct compression. Microcrystalline cellulose PH 102 was used as the filler in the study, as it is an excellent directly compressible vehicle. All the prepared batches were evaluated for pharmacopoeial parameters viz., friability, hardness, uniformity of weight and drug

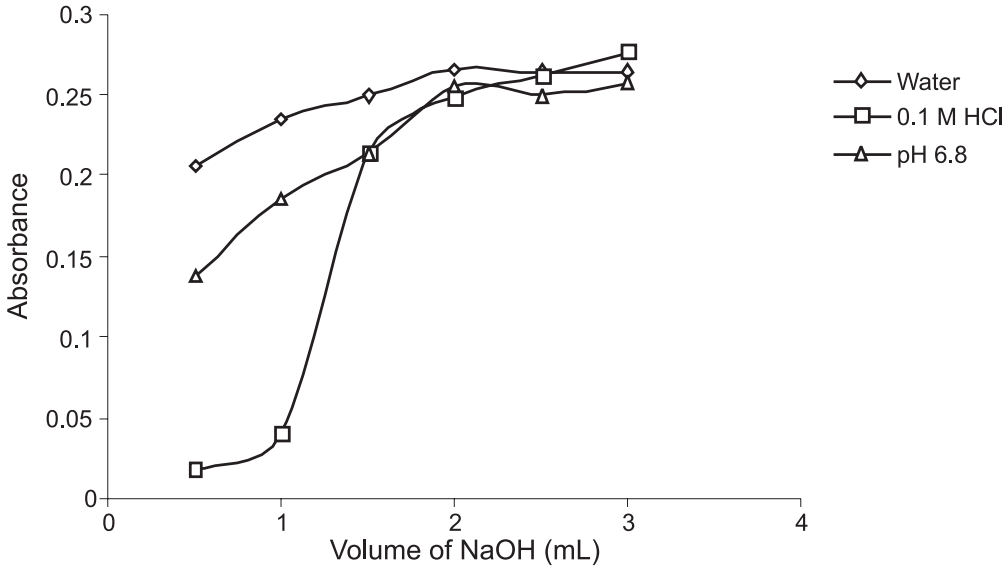

Figure 6. Effect of the volume of NaOH (0.5 M) on absorbance of acarbose in water, 0.1 M HCl (pH 1.2) and phosphate buffer (pH: 6.8)

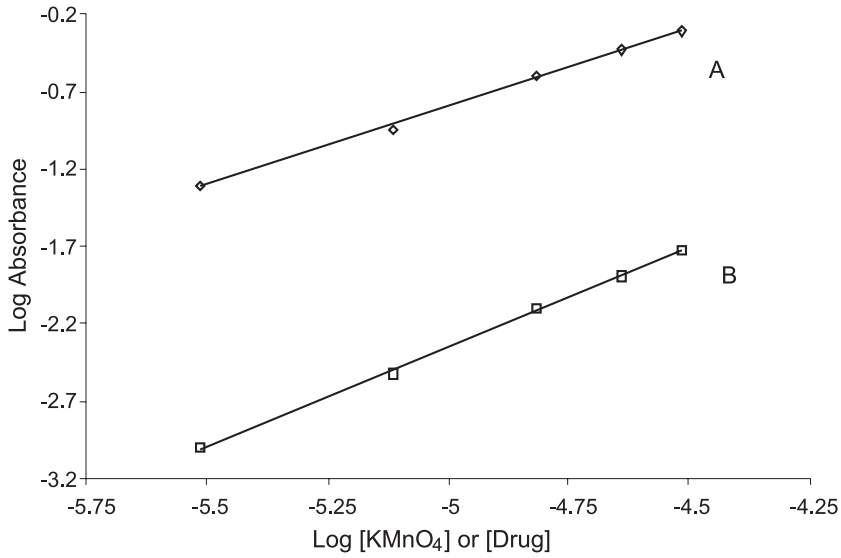

Figure 7. Determination of the molar ratio between alkaline $KMnO_4$/acarbose by limiting logarithmic method. (**A**) Set of solutions with variable $KMnO_4$ concentration and constant acarbose concentration. (**B**) Set of solutions with variable acarbose concentration and constant $KMnO_4$ concentration

content. To study the *in vitro* release profiles the dissolution test was carried out according to USP 27 method for delayed release tablets (Method A) (12). Dissolution study was carried out using USP apparatus type-II i.e., paddle type at 50 rpm and temperature of the dissolution media was maintained at 37 ± 0.5°C. Initial studies were carried out in 750 mL of 0.1 M HCl (pH 1.2) for 2 h, after which 250 mL of a 0.2 M trisodium orthophosphate solution was added into the dissolution media. pH of the media was adjusted to 6.8. Samples were collected at predetermined time intervals till 24 h and analyzed for acarbose content with the oxidation method using a UV-spectrophotometer.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of acarbose were studied in different reaction media in the wavelength range of 350 to 700 nm. The sample solutions were prepared individually as described above. Polysaccharides on reaction with $KMnO_4$ in alkaline conditions yield manganate ions (13). Thus, reaction between the acarbose and alkaline $KMnO_4$ resulted in the formation of various oxidation products and green colored manganate ions, which absorb at 610 nm. The results obtained showed the absorption spectra with maximum absorbance at 610 nm as shown in Figure 2.

The rate of the reaction may be estimated by the variable time method (14). The reaction mixture changed color on standing, indicating the effect of time on the reaction kinetics. Thus, the kinetics of the reaction was studied in all the three media for duration of 20 min against the corresponding blank. At room temperature, the reaction rate was found to

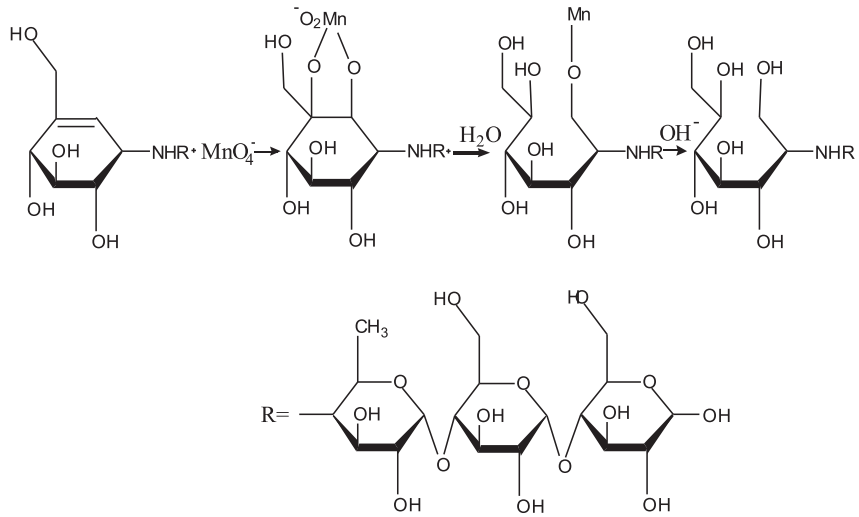

Figure 8. Proposed reactions involved in oxidation of acarbose and generation of manganate ions.

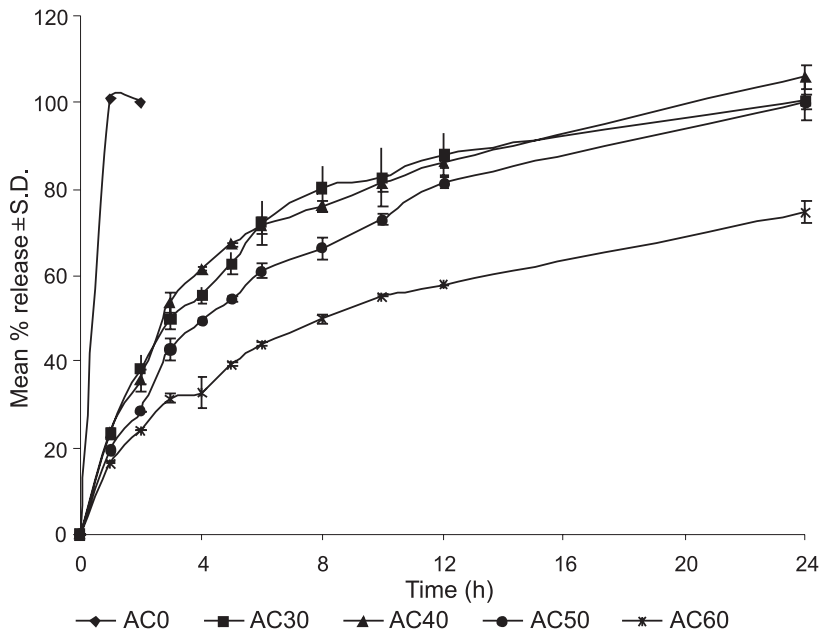

Figure 9. Plot of cumulative percent release *vs*. time for formulations AC0, AC30, AC40, AC50 and AC60 (mean ± S.D.; n = 3)

Table 3. Regression equations of logarithm of the rate for different concentrations of acarbose at room temperature.

Medium	Regression equation	R^2
0.1 M HCl (pH 1.2)	Log rate = 1.104C + 0.786	0.9918
Phosphate buffer (pH 6.8)	Log rate = 1.042C – 0.6713	0.9945

C is a logarithm of the concentration in µg/mL of the final measured solution.

Table 4. Inter-day and intra-day precision.

Parameters evaluated	Amount (µg/mL)		% RSD
	Taken	Found	
0.1 M HCl (pH 2.0)			
Intra-day precision	2	1.92	2.71
	10	10.60	2.78
	20	19.51	1.9
Inter-day precision	2	1.99	1.31
	10	10.63	1.88
	20	19.41	0.27
Phosphate buffer (pH 6.8)			
Intra-day precision	2	2.08	4.99
	10	10.79	1.12
	20	20.82	0.99
Inter-day precision	2	2.16	3.61
	10	10.69	1.12
	20	20.75	0.50

increase substantially with time, as revealed by the intensification of the developed color and corresponding increase in the slope of the calibration graph (Table 3) in both the acidic and alkaline media (Figs. 3 and 4). These data indicate the higher analytical sensitivity of the developed method.

Optimization of reaction conditions:
Effect of volume of $KMnO_4$

The study results obtained indicated that the absorbance of the samples increased significantly on increasing the amount of $KMnO_4$ (0.25–1.75 mL) in the reaction mixture. However, further increase in the amount of the oxidizing agent did not result in rise in the maximum absorbance, hence it was concluded that at least 1.0 mL of 0.01 M $KMnO_4$ (in both acidic and alkaline media) was adequate for the effective development of the colored ions under the given set of reaction conditions (Fig. 5).

It was observed that the absorbance of the samples increased significantly on increasing the amount of NaOH (0.5–3 mL) in the reaction mixture. No significant change in the absorbance values were observed on increasing the volume of 0.5 M NaOH beyond 1.0 mL for the samples prepared in water. However, a marked increase in absorbance was observed on increasing the volume of 0.5 M NaOH above 1.0 mL for the samples prepared in 0.1 M HCl and phosphate buffer (pH: 6.8) (Fig. 6). In both these cases the maximum absorbance obtained was found to be relatively constant on addition of at least 2.0 mL or more of NaOH solution to the reaction mixture. Hence, it was concluded that for optimization of the reaction in acidic and alkaline conditions the addition of 2.0 mL of 0.5 M NaOH is adequate to obtain maximum absorbance.

The stoichiometry of the reaction was studied adopting the limiting logarithmic method. The absorbance of the reaction product was alternatively measured in the presence of varying amounts of either $KMnO_4$ or acarbose. The oxidation of polysaccharidic molecules depends on the pH, temperature and ionic strength of the reaction mixture (15). A plot of log absorbance *versus* log [$KMnO_4$] and log [acarbose] gave straight lines; the values of the slopes are 1.001 and 1.287, with high correlation

Table 5. Recovery studies.

Amount taken (µg/mL)	Amount added (µg/mL)	Recovery %	
		0.1 M HCl (pH:2)	Phosphate buffer (pH:6.8)
30	10	98.9	98.7
30	50	102	100.3
30	90	100	101.1

coefficient of 0.9979 and 0.9991, respectively (Fig. 7). Hence, it is concluded that the molar reactivity of the reaction is 1/1, i.e., the reaction proceeds in the ratio of 1 : 1. Based on the obtained molar reactivity, and depending on the structure of the drug, the reaction pathway is proposed to proceed as shown (Fig. 8).

Calibration curves

The calibration graphs and the regression equations were obtained by plotting the absorbance at the specified time *versus* concentration of the drug in µg/mL. The method was found to be linear over a concentration range of 2–20 µg/mL in all the three media. A high coefficient of correlation in all the three media viz., water (r^2 = 0.9942), 0.1 M HCl (r^2 = 0.9948) and phosphate buffer pH 6.8 (r^2 = 0.9977) indicated good linearity of the developed methods.

Accuracy and precision

The results of intra-day and inter-day precision studies are shown in Table 4. They revealed that % RSD values for intra-day studies ranged between 1.08 and 1.63% and for inter-day precision between 0.92 and 1.27%, which are within the permissible limits of 5.0%.

Recovery

The results of the recovery studies in various media are indicated in Table 5. The results obtained clearly indicate that the method is accurate for the estimation of the drug both in bulk form and pharmaceutical dosage form in simulated gastrointestinal media. Values of 0.508, 1.54 and 0.451, 1.366 µg/mL were the LOD and LOQ values for the drug in 0.1 M HCl and buffer, respectively.

Specificity

The developed method is found to be specific for estimation of the drug in the presence of common tablet excipients like microcrystalline cellulose, talc and magnesium stearate. However, sufficient interference was observed in the presence of pharmaceutical excipients like lactose, gums, HPMC as these substances reacted with the $KMnO_4$/NaOH system producing colored products similar to the API.

***In vitro* dissolution studies**

Based on the specificity studies, Carbopol, a polymethacrylate polymer, was chosen for designing of sustained release formulations for acarbose (16). All the prepared batches exhibited excellent hardness (8–10 kg/cm^2), lower friability (< 0.5%) and drug content was found to vary within 97.3–99.67%. *In vitro* release profile indicated that tablets containing 30% and 40% showed a cumulative percentage release of approximately 80% within 8 h, in comparison to batch AC0 containing no polymer, which showed complete release of the drug within 30 min. Batches containing higher proportions of the polymer (AC50 and AC60) showed a controlled release of the drug over a period of 24 h (Fig. 9). However, batch AC60 showed incomplete drug release of 74.61 ± 2.47% even after completion of 24 h. Thus, batches AC30 and AC40 were considered optimal for attaining the desired sustained release of acarbose.

CONCLUSION

The proposed kinetic method is simple, accurate, precise, sensitive, rapid, low cost and selective for the estimation of acarbose both in bulk as well as in pharmaceutical formulations in simulated gastrointestinal fluid. It is advantageous over all the other reported methods as it can be used for drug estimation in different medias., viz. water, 0.1 M HCl and phosphate buffer (pH:6.8), which is the biggest limitation of the other methods proposed till date. The data given above reveal that this method is sensitive enough to detect concentrations as low as 0.5 µg/mL. As a result, the proposed method can be successfully used for the quantitative estimation of

the drug in bulk, immediate release and delayed release pharmaceutical dosage forms.

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