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Kinetic study of alkaline induced hydrolysis of the skeletal muscle relaxant chlorzoxazone using ratio spectra first derivative spectrophotometry

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Abstract

2-Amino-4-chlorophenol was found to be the alkaline induced degradation product and the synthetic precursor of chlorzoxazone. The aim of this work is to study different factors affecting the degradation process due to the high toxicity of 2-amino-4-chlorophenol. Chlorzoxazone was found to follow pseudofirst order kinetics. Ratio spectra first derivative spectrophotometry (DR^1) was developed for monitoring the change in chlorzoxazone concentration during the degradation process. Kinetic parameters (rate constant (K) and half-life ($t_{0.5}$)) were calculated at different temperatures (40–120 °C) and different sodium hydroxide concentrations (3–10 M). Activation energy at 3 and 8 M sodium hydroxide concentration and alkaline induced catalysis constant at 60, 70 and 80 °C were also calculated.

Keywords: Ratio spectra first derivative spectrophotometry; Chlorzoxazone; 2-Amino-4-chlorophenol; Stability study; Kinetics; Alkaline hydrolysis

1. Introduction

Investigation of the chemical stability of pharmaceutical products is a matter of growing concern in many analytical laboratories. Systematic kinetic studies of the decomposition of drugs using stability testing techniques is essential for the quality control of such products [1].

Chlorzoxazone, (5-chlorobenzoxazole-2(3H)-one), is a centrally acting skeletal muscle relaxant with sedative properties [2]. It contains a benzoxazolone ring system, which is highly unstable due to presence of both lactam and lactone functional groups in the fused ring system. Both groups are subject to alkaline induced hydrolysis. 2-Amino-4-chlorophenol was found to be the alkaline induced degradate of chlorzoxazone, as shown in Fig. 7 [3]. It is also one of the synthetic precursors of chlorzoxazone as shown in Fig. 8 [4].

2-Amino-4-chlorophenol was found to be toxic and it is classified in Aldrich catalogue as a harmful chemical [5]. It was reported also that high levels of 2-amino-4-chlorophenol could interfere with the ability of blood to carry oxygen; causing headache, dizziness and methemoglobinemia. Higher levels can cause trouble breathing, collapse and even death [6].

Several spectrophotometric methods such as absorbance ratio [7], difference spectrophotometry [8], second derivative [9], zero crossing [10], multi-wave length linear regression [11] and simultaneous equations [12] were tried to resolve chlorzoxazone binary mixture with paracetamol.

Various chromatographic techniques were also applied for the analysis of the same mixture in pharmaceutical dosage forms and in biological fluids such as HPLC [13,14], GC [14,15], TLC densitometry [14,16] and super critical fluid chromatography [17].

The aim of this work is to study different factors affecting chlorzoxazone degradation. It is aimed also to demonstrate the application of ratio spectra first derivative spectrophotometry as a simple, rapid and inexpensive method for determination of chlorzoxazone in presence of 2-amino-4-chlorophenol.

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2. Experimental

2.1. Equipment

- Shimadzu 1601 PC double beam UV–Vis spectrophotometer with fixed slit width (2 nm) connected to an IBM computer loaded with Shimadzu UV PC software was used for all absorbance measurements and data treatment.
- Bruker FT-IR, Infrared spectrophotometer.
- Oakton, pH meter.

2.2. Pure samples

Chlorzoxazone pure substance was obtained from Glaxo Wellcome, Egypt. Its purity was found to be (100.02% ± 0.422) using direct spectrophotometry [18].

2.3. Degraded sample

2.3.1. Preparation of the degradate; 2-amino-4-chlorophenol

Weigh accurately 0.5 g of chlorzoxazone into a 100 ml flat bottom rounded flask, add 50 ml of aqueous 6 M sodium hydroxide and reflux for 2 h. Completion of hydrolysis was checked by recording the absorption spectrum of diluted solution of the reaction mixture against a blank of 0.1 M sodium hydroxide. This process was repeated till disappearance of the absorption band characteristic for chlorzoxazone (at 288 nm) and appearance of that characteristic for 2-amino-4-chlorophenol (at 307 nm). The reaction flask was then cooled and the contents were transferred into a separating funnel. The pH of the solution was then adjusted to pH 7–8 by addition of 6 M hydrochloric acid. Ether was used for extraction using 10 ml portions, the process was repeated five times. The pH was then readjusted to pH 7–8 and the process was repeated till no pH rise was observed after ether extraction. Ethereal extract was collected, filtered and evaporated using rotavap at ambient temperature. The obtained residue was purified by re-dissolving in ether, filtration and re-evaporation till pale brown flakes were obtained. Purity of the prepared 2-amino-4-chlorophenol was confirmed by evaluation of its melting point (m.p.) and comparing it to that found in literature [5].

The identity of 2-amino-4-chlorophenol was confirmed by interpretation of the characteristic peaks and comparing the IR spectra of both the drug and the degradate. Figs. 1 and 2 show the IR spectra of chlorzoxazone and the prepared 2-amino-4-chlorophenol, respectively. The peak at 1772 cm^{-1} shown in Fig. 1 is characteristic for the carbonyl group in chlorzoxazone (keto form). This peak has disappeared in the spectrum of the degradate as shown in Fig. 2. This goes with the hydrolysis reaction shown in Fig. 7. Appearance of a doublet peak at 3382 and 3314.3 cm^{-1} in the spectrum of the degradate indicates formation of primary amino group that was absent in chlorzoxazone [19].

2.4. Stock solutions

Stock solutions 400 μg ml^{-1} of chlorzoxazone and of 2-amino-4-chlorophenol were prepared in 0.1 M sodium hydroxide. These solutions were used to prepare the synthetic mixtures shown in Table 1.

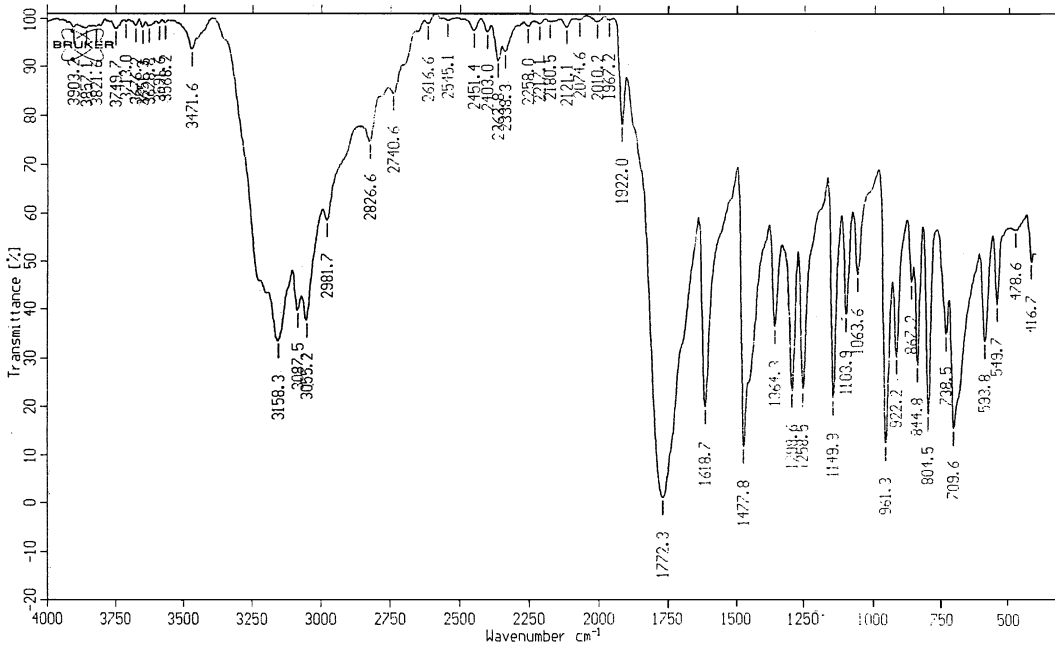

Fig. 1. IR spectrum of chlorzoxazone.

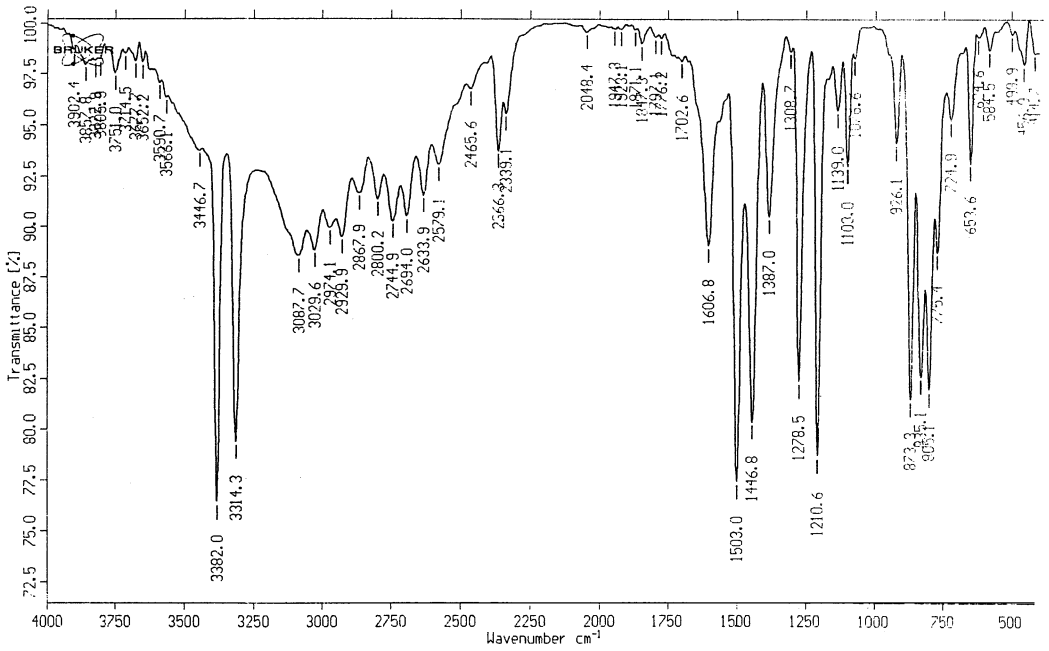

Fig. 2. IR spectrum of 2-amino-4-chlorophenol.

2.5. Laboratory prepared mixtures

Into a series of 25 ml volumetric flasks transfer accurately aliquot portions equivalent to (80–800 μg) chlorzoxazone from its stock solution. Add aliquot portions of 2-amino-4-chlorophenol stock solution equivalent to (80–800 μg) to prepare the binary mixtures shown in Table 1. Complete to volume using aqueous 0.1 M sodium hydroxide.

2.6. Procedures

2.6.1. First derivative of ratio spectra (DR^1) for determination of chlorzoxazone

2.6.1.1. Linearity of DR^1 method. Transfer accurately aliquot portions equivalent to (80–1000 μg) of chlorzoxazone from its aqueous alkali standard solution into a series of 25 ml volumetric flasks. Complete to volume using 0.1 M sodium hydroxide, to obtain chlorzoxazone series (3.2–40 μg ml^{-1}). Transfer accurately aliquot portions equivalent to (160 μg) of 2-amino-4-chlorophenol from its aqueous alkali stock solution into a 25 ml volumetric flask, complete to volume using 0.1 M sodium hydroxide. Record the absorption spectra of chlorzoxazone series and that of the degradate against 0.1 M sodium hydroxide as a blank. Divide the chlorzoxazone series by the prepared degradate divisor (6.4 μg ml^{-1}). Record the first derivative of the ratio spectra obtained for chlorzoxazone series (Fig. 4). Plot the calibration curve using the response obtained at 272.2 nm using instrumental parameters $\Delta\lambda = 4$ and scaling factor $F = 10$. Linear relation between instrumental response (DR^1) and concentration was obtained

Table 1
List of concentrations of synthetic binary mixtures of chlorzoxazone with 2-amino-4-chlorophenol in 0.1 M sodium hydroxide

Mixture number	Chlorzoxazone (μg ml^{-1})	2-Amino-4-chlorophenol (μg ml^{-1})	Percent of degradate (%)	Recovery(%)	Reference method [a]
1	32.00	6.40	16.67	100.19	
2	6.40	3.20	33.33	101.41	
3	6.40	6.40	50.00	99.69	
4	3.20	16.00	83.33	99.69	
5	3.20	32.00	90.91	101.88	
		n		5	5
		Mean recovery (%)		100.34	100.04
		SD		1.068	0.422
		RSD		1.064	0.420
		t (2.776)		0.550	
		F (6.39)		0.16	

Figures in parentheses are the corresponding theoretical t and F values ($P = 0.05$) [21].
[a] Direct spectrophotometry [18].

Table 2
Assay validation for DR^1 method for determination of chlorzoxazone in presence of 2-amino-4-chlorophenol at (272.2 nm)

Parameter	Value
Range	3.2–40 μg ml^{-1}
Slope	0.4191
SE of slope	0.002
Intercept	0.3367
SE of intercept	0.038
Correlation coefficient (r)	0.9999
RSD ($n=9$) [a]	0.568
RSD ($n=9$) [b]	0.910

[a] RSD is intra-day precision applied on mixtures number 1, 3 and 5 (Table 1) on three different days.
[b] RSD is inter-day precision applied on mixtures number 1, 3 and 5 (Table 1) on three different days.

as described by the following regression equation:

$$Y = 0.4191X + 0.3367$$

where (Y) is the DR^1 response and (X) is concentration in μg ml^{-1}.

2.6.1.2. Validation of DR^1 method. The DR^1, at 272.2 nm and 6.4 μg $ml^{-1}$of 2-amino-4-chlorophenol as a divisor, was applied for determination of chlorzoxazone in presence of 2-amino-4-chlorophenol in their laboratory prepared mixtures. DR^1 was found suitable for chlorzoxazone determination in presence of its degradate up to (90%). Average recovery percent was found to be (100.57 ± 1.008). Statistical analysis of the results obtained as compared with reference method is shown in Table 1. Inter-day and intra-day repeatability was performed by applying the proposed procedures on mixtures number 1, 3 and 5 shown in Table 1. Results are shown in Table 2.

2.6.2. Stability study of chlorzoxazone

Weigh accurately 0.1 g of chlorzoxazone into a 100 ml flat bottom rounded flask. Add 50 ml aqueous sodium hydroxide of the specified molarity and attach a water reflux condenser. The above set was maintained in a thermostated brine water bath with continuous stirring of flask contents using magnetic stirrer. At the specified time intervals, 0.5 ml aliquots were transferred accurately into 25 ml volumetric flasks and completed to volume using aqueous 0.1 M sodium hydroxide to stop the reaction. The rate of degradation was found to be negligible in cold 0.1 M sodium hydroxide. Immediately record the absorption spectrum against aqueous 0.1 M sodium hydroxide as a blank. The obtained spectrum was divided by the chosen 2-amino-4-chlorophenol divisor spectrum. First derivative of the ratio spectrum, obtained using the same experimental conditions described under linearity (Section 2.6.1.1). Chlorzoxazone concentration was calculated from the regression equation of first derivative of the ratio spectra method (DR^1). Data are then manipulated to obtain various kinetic parameters studied. Table 3 shows conditions and the calculated kinetic parameters for each run.The procedure described under Section 2.6.1.2 was used for different temperatures (40–120 °C), where 120 °C is the boiling point of the reaction mixture (reflux temperature) and different alkali concentrations (3–10 M). Reaction rate constant (K) and half-life ($t_{0.5}$) were calculated for each run as tabulated in Table 3.

3. Discussion

Zero order spectra of each of chlorzoxazone and 2-amino-4-chlorophenol show significant overlap that hinders tracing of the remaining amount of chlorzoxazone during degradation process as shown in Fig. 3.

Table 3
List of conditions and calculated kinetic parameters used in the kinetic study of the base induced chlorzoxazone degradation

Temperature (°C)	Alkali strength (M)	Reaction rate constant (K) (min^{-1})	Half life ($t_{0.5}$) (min)
40	10	No visible change	No visible change
60	3	1.399×10^{-3}	495.35
60	6	3.9×10^{-3}	177.69
60	8	5.297×10^{-3}	130.83
70	3	2.390×10^{-3}	289.96
70	6	7.400×10^{-3}	93.65
70	8	0.011	63
75	3	2.994×10^{-3}	231.46
80	6	0.012	57.75
80	8	0.018	38.50
80	10	0.044	15.75
90	3	9.440×10^{-3}	73.41
120	3	0.042	16.5
120	8	0.119	5.82

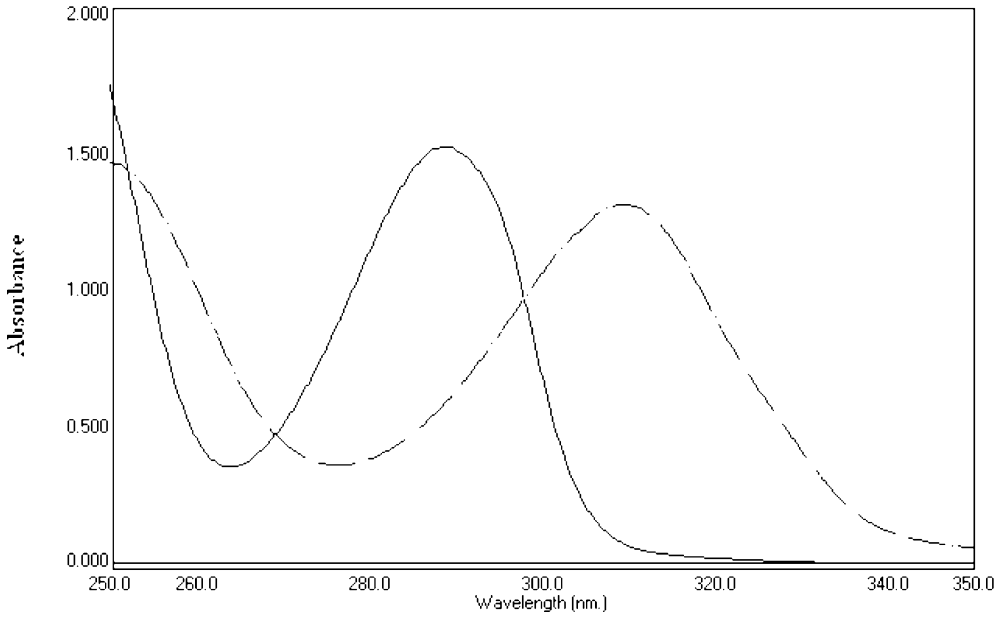

Fig. 3. Spectral characteristics for chlorzoxazone (—) and 2-amino-4-chlorophenol (– –) 40 μg ml^{-1} each, both are dissolved in aqueous 0.1 M NaOH.

First derivative of the ratio spectra (DR^1) at 272.2 nm as shown in Fig. 4 was found suitable for tracing chlorzoxazone remaining during degradation since this method was proved to be able to determine chlorzoxazone in presence of 2-amino-4-chlorophenol up to 90% degradate as presented in Table 1. Different divisor concentrations (6.4, 16, 25.6 μg ml^{-1}) were tried. The first divisor (6.4 μg ml^{-1}) was found the best regarding average recovery percent when the model was used for prediction of chlorzoxazone concentration in its laboratory prepared mixtures. Statistical analysis of the results obtained by the suggested DR^1 method has been carried out. Table 1 shows the results obtained by adopting the proposed procedure as compared with those of the reference direct spectrophotometric method [18]. The calculated t and F values are less than their corresponding tabulated ones, indicating non significant difference between the suggested procedure and the reference one. Inter-day and intra-day precision was also performed according to USP [20] on mixtures number 1, 3 and 5. RSD obtained are shown in Table 2.

Reaction order was determined by plotting the logarithm of chlorzoxazone concentration remaining versus time. Linear relationship suggests first order kinetics as shown in Fig. 5. Alkaline catalyzed hydrolysis is a bimolecular reaction, but one of the reactants is

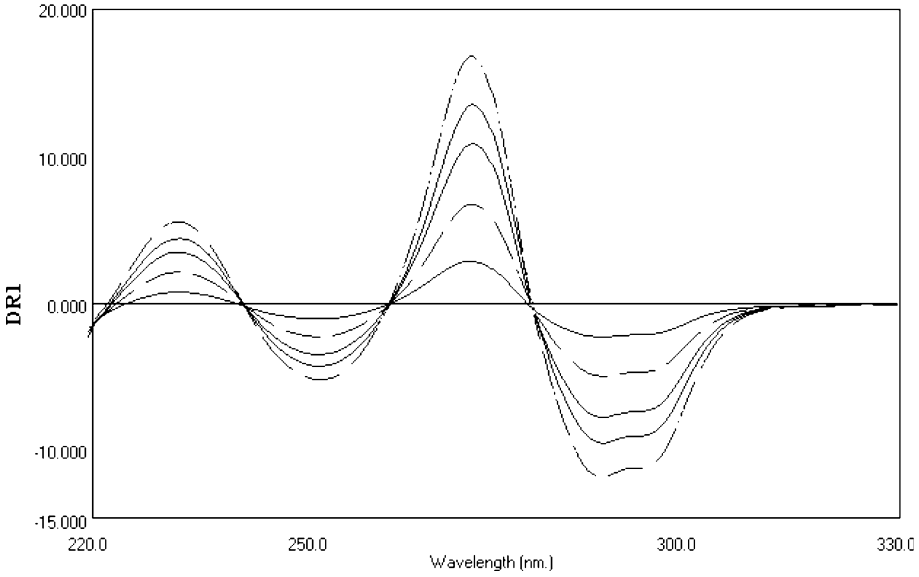

Fig. 4. First derivative of the ratio spectra (DR^1) for chlorzoxazone series (3.2–40 μg ml^{-1}) using (6.4 μg ml^{-1}) of 2-amino-4-chlorophenol as a divisor.

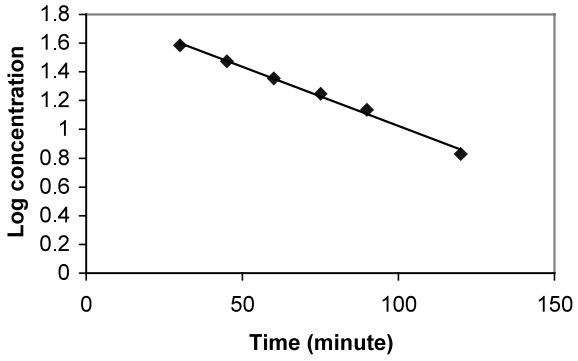

Fig. 5. First order plot for the alkaline degradation of chlorzoxazone (2 mg ml^{-1}) starting concentration, 8 M sodium hydroxide and 80 °C.

water, which is present in large excess, so the change in its concentration is negligible. Such reactions where one of the reactants is present in large excess are considered to follow pseudofirst order kinetics and all equations describing first order can be applied for it [1]. Reaction rate constant (K) and half-life ($t_{0.5}$) for each run are calculated and tabulated in Table 3. Effect of temperature on degradation rate was studied at sodium hydroxide concentration of 3 and 8 M by plotting of the logarithm of the reaction rate constant ($\log K$) versus the reciprocal of the absolute temperature ($1/T$). This plot is called Arrhenius plot and is shown in Fig. 6. Temperature was found to accelerate the reaction as proved by higher reaction rate constants and smaller half-lives as shown in Table 3. Activation energy at 3 and 8 M sodium hydroxide were found to be 62 829.43 and 54 393.2 kcal mol^{-1}, respectively.

Alkaline induced catalysis constant was calculated from the slope of the curve obtained by plotting the reaction rate constant (K) versus alkali molar concentration. Alkaline induced catalysis constant at 60, 70 and 80 °C were found to be 0.0008, 0.0017 and 0.008 min^{-1} mol^{-1}, respectively.

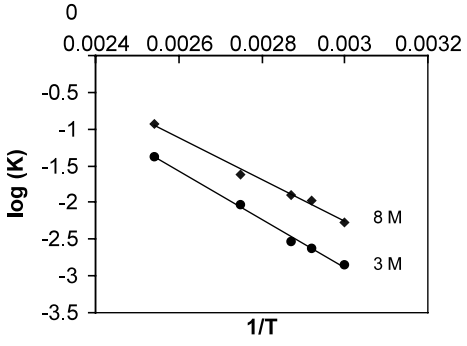

Fig. 6. Arrhenius plot for alkaline degradation of chlorzoxazone at various sodium hydroxide concentrations.

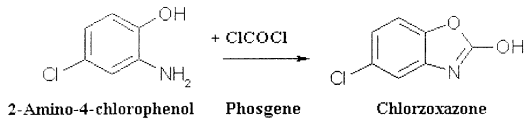

Fig. 8. Preperation of chlorzoxazone by treating 2-amino-4-chlorophenol with phosgene in ethyl acetate.

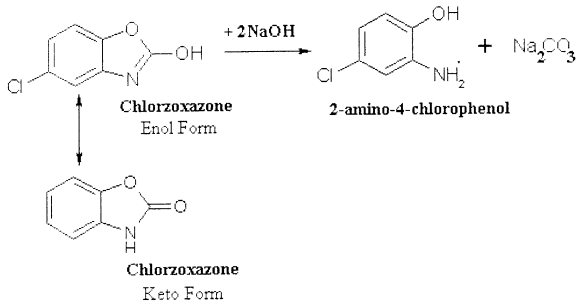

Fig. 7. Hydrolysis of chlorzoxazone to give 2-amino-4-chlorophenol and sodium carbonate.

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