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Development of a simple analytical methodology for determination of glucosamine release from modified release matrix tablets

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Abstract

A simple spectrophotometric method for determination of glucosamine release from sustained release (SR) hydrophilic matrix tablet based on reaction with ninhydrin is developed, optimized and validated. The purple color (*Ruhemann* purple) resulted from the reaction was stabilized and measured at 570 nm. The method optimization was essential as many procedural parameters influenced the accuracy of determination including the ninhydrin concentration, reaction time, pH, reaction temperature, purple color stability period, and glucosamine/ninhydrin ratio. Glucosamine tablets (600 mg) with different hydrophilic polymers were formulated and manufactured on a rotary press. Dissolution studies were conducted (USP 26) using deionized water at 37 ± 0.2 °C with paddle rotation of 50 rpm, and samples were removed manually at appropriate time intervals. Under given optimized reaction conditions that appeared to be critical, glucosamine was quantitatively analyzed and the calibration curve in the range of 0.202–2.020 mg ($r = 0.9999$) was constructed. The recovery rate of the developed method was 97.8–101.7% ($n = 6$). Reproducible dissolution profiles were achieved from the dissolution studies performed on different glucosamine tablets. The developed method is easy to use, accurate and highly cost-effective for routine studies relative to HPLC and other techniques.

Keywords: Glucosamine; Ninhydrin; Dissolution; Sustained release (SR) matrix; Glucosamine analysis

1. Introduction

Glucosamine (2-amino-2-deoxyglucose, chitosamine), a component of chitin, muco-polysaccharides like heparin, and other complex polysaccharides, is employed for the treatment of osteoarthritis. It is usually in the acetylated (–NH–$COCH_3$) or sulfated (–NH–SO_3^-) form. However, both glucosamine and its derivatives have no absorbance in ultraviolet and visible wavelength range with conventional dissolution methods and therefore measurement of glucosamine from dissolution medium cannot be determined in a cost-effective way.

Currently, sophisticated methods such as capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) with specific detection (e.g. pulsed amperometric detection (PAD)) or pre-column derivatization are used in the determination of glucosamine [1–10].

Recently, a new method for the glucosamine release study was proposed [11], in which sufficient quantity of standard ninhydrin solution was introduced into the samples of glucosamine solution, removed periodically from the medium, and measurement of the color changes was determined spectrophotometrically. The method, however, was not validated or optimized for accuracy and reproducibility. Ninhydrin has UV absorption, λ_{max} 260 nm, and under optimized conditions, the concentration of ninhydrin–glucosamine reaction product can be correlated.

Ninhydrin (triketohydrindene hydrate) is a white to brownish white crystalline powder, soluble in water and alcohol. It turns red when heated above 100 °C and its

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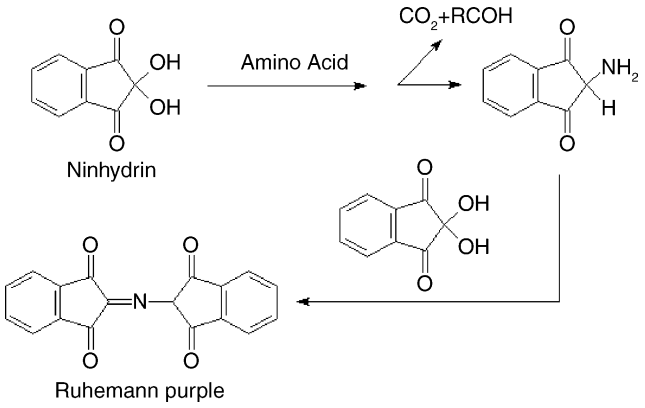

Fig. 1. Mechanism of the ninhydrin–amino acid reaction.

melting range is 240–245 °C with decomposition. Ninhydrin is extensively used in the analytical determination of amino acids and related structures, and potentially can react with a variety of primary and secondary amines producing *Ruhemann* purple color. The mechanism of ninhydrin reaction and color formation has been recently reviewed, and some of the concerns regarding the process outlined [12].

When ninhydrin reacts with alpha-amino acids, as shown in Fig. 1, carbon dioxide (CO_2), and an aldehyde with one carbon less than the decarboxylated amino acid are produced. On heating the solution, the formed amine reacts with another ninhydrin molecule to form a blue-to-purple color product with a maximum absorbance at 570 nm. It has also been reported that under similar condition, proline and hydroxyproline provide a yellow color with absorbance maxima at 440 nm [13,14]. Hence, the calorimetric reaction makes ninhydrin an important reagent for quantitative analysis of amino acids.

It is interesting to note that under acidic condition, ninhydrin can react with a variety of primary and secondary amines resulting in a so-called *Ruhemann* purple color (diketohydrindamine–diketohydrindylidene) [12]. Comparing this with amino acid reaction, it is significantly slower [12,13]. The published reports [12–17] indicate that for accuracy purposes, the most important factors involved in the quantitative production of *Ruhemann* purple include a high concentration of aqueous ninhydrin solution, a high reaction temperature, and an optimum acidic condition.

In this paper, a new method is developed based on ninhydrin–glucosamine reaction and is used for the determination of glucosamine release from sustained release tablets following USP dissolution method. Furthermore, factors that influence the reaction process were identified and optimized. Controlled release glucosamine tablets were produced using hydrophilic polymers and glucosamine release was determined using the developed colorimetric method.

2. Materials and methods

2.1. Materials

Ninhydrin was purchased from Aldrich, WI; glucosamine sulfate (potassium salt) from USA NutraSource Inc., CA. Monobasic potassium phosphate and sodium hydroxide were purchased from Amend, NJ. Polyethylene oxide (PEO, Polyox WSR 301, MW 4×10^6, and Polyox WSR coagulant), and hydroxypropylmethylcellulose (HPMC, Methocel K100M) from Dow Chemistry, MI; hydroxypropylcellulose (Natrosol, 250M) and hydroxyethylcellulose (Klucel, HXF) from Hercules, VA; microcrystalline cellulose (Avicel PH-200) from FMC, DE, were used as supplied.

2.2. Investigation of UV–vis spectra of the relative compound(s) and mixtures involved in the ninhydrin–glucosamine reaction

Single or mixed solution of glucosamine, ninhydrin and 0.2 M phosphate buffer (pH 6.0) was detected by HP 8452A diode array spectrophotometer (Hewlett-Packard, DE) to find out the best wavelength for the detection with least interference. The solutions included 0.5% glucosamine, 0.2–0.8% ninhydrin, 0.2 M phosphate buffer (pH 6.0), mixture of H_2O–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (4:0.5:0.5, v/v/v), and mixture of 0.3% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (4:0.5:0.5, v/v/v).

2.3. Investigation of factors that may affect ninhydrin–glucosamine reaction

Standard sample, consisted of 2.0 ml of 0.02% glucosamine, 1.0 ml of 0.8% ninhydrin, and 2.0 ml of 0.2 M phosphate buffer (pH 6.0) in a test tube, was placed into a water bath at 100 °C for 10 min. The reaction was then stopped by placing the test tube in a cold-water bath (5 °C) and the produced color was detected at its λ_{max} when samples reached room temperature. In blank sample, glucosamine was replaced with H_2O, ninhydrin with appropriate concentration was added and buffer at appropriate pH value was used. To identify the role of various factors, on the reaction kinetics magnitude of individual factors was altered while keeping others constant. The factors included ninhydrin concentration, pH of the buffer, reaction temperature, reaction time, assay time of reacted sample, and proportions of the reactants.

2.4. Preparation of the calibration curve for glucosamine and recovery test for the method

Four milliliters of glucosamine having different concentrations (i.e. 0.005%, 0.010%, 0.020%, 0.030%, 0.040%, and 0.050%) was supplemented with 0.5 ml of 0.8% ninhydrin and 0.5 ml of 0.2 M phosphate buffer (pH 6.0) as standard samples. For blank sample, glucosamine was replaced with

H_2O. Standards and blank were heated at 100 °C in a water bath for 30 min. Reaction was stopped by placing the test tubes into cold-water bath, and the color changes were detected at 570 nm after they were held at room temperature for 20 min.

Accurate amount of standard sample was added for exact volume and known concentration following the sample analysis procedure to evaluate the recovery rate (%) of the method.

2.5. Tablet manufacturing

The composition of formulations used is shown in Table 1. All ingredients were passed through a No. 20 US standard sieve, all powders except magnesium stearate were blended in a V-blender (Patterson-Kelley, PA) for 10 min, after which time, magnesium stearate was added and mixed for an additional 3 min prior to compression.

Tablets were compressed on a rotary press B2 (Stokes, Pennwalt, PA) at constant pressure using 12 mm diameter deep concave punches.

2.6. Drug release study

The in vitro release studies were conducted in accordance with USP 26 apparatus II procedure (VK 7000, Vankel, NJ) at 37 ± 0.2 °C with paddle rotation of 50 rpm in 900 ml deionized water. At appropriate time intervals, 5 ml of sample was removed manually and replaced with 5 ml of deionized water. Detection of glucosamine was conducted under the given condition as described above (see Section 2.2). When necessary samples were appropriately diluted to match the linear range of the calibration curve. Dissolution profile of glucosamine sustained release tablets was constructed. Dissolution study on all the formulations, from GF-1 to GF-5 was performed in similar manner.

Table 1
Glucosamine formulations

Batch	Code	Ingredient	
1	GF-1	Glucosamine	600 mg
		Polyethylene oxide WSR 301	200 mg
		Avicel PH-200	150 mg
		Magnesium stearate	0.5%
2	GF-2	Glucosamine	600 mg
		Polyethylene oxide WSR 301	100 mg
		HPMC K100M	100 mg
		Avicel PH-200	150 mg
		Magnesium stearate	0.5%
3	GF-3	Glucosamine	600 mg
		Polyethylene oxide WSR coagulant	100 mg
		HPMC K100M	100 mg
		Avicel PH-200	150 mg
		Magnesium stearate	0.5%
4	GF-4	Glucosamine	600 mg
		Natrosol 250M	300 mg
		Klucel HXF	100 mg
		Magnesium stearate	0.5%
5	GF-5	Glucosamine	600 mg
		Klucel HXF	400 mg
		Magnesium stearate	0.5%

3. Results and discussion

3.1. Investigation of UV–vis spectra of the relative compound(s) and mixtures involved in the ninhydrin–glucosamine reaction

Fig. 2 illustrates various spectra. When ninhydrin mixed and reacted with glucosamine under given condition, the reacted mixture turned to purple color and provided two maximum absorbances in visible region at 402 and 570 nm, respectively. The latter one is the same as the wavelength of the blue-to-purple color formed from the reaction of ninhydrin with almost all amino acids [13,14]. Blank where glucosamine was substituted with water shows no absorbance in visible region, especially at 570 nm. According to the spectra, 570 nm is selected as the wavelength of detection with no interferences from the reaction solution.

3.2. Investigation of the factors that may affect ninhydrin–glucosamine reaction

It appears that ninhydrin–glucosamine reaction is affected by many factors which are described in the following sections.

3.2.1. Effect of ninhydrin concentration on the color formation

It is reported that different concentrations of ninhydrin, ranging from 0.05% to 5%, yielded *Ruhemann* purple of different intensity varying with different amines [12]. It is shown that the samples' absorbance increased with increases in ninhydrin concentration from lysine to proline in two extremes

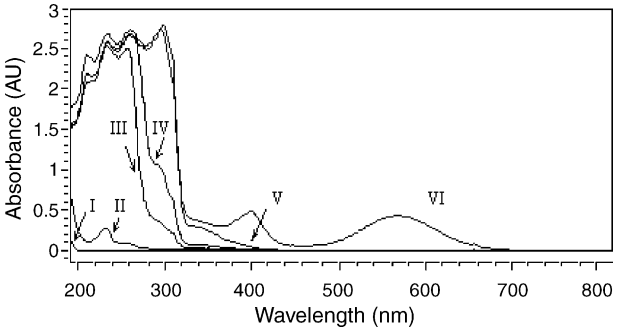

Fig. 2. UV–vis spectra of relative compounds and mixtures: (I) 0.5% glucosamine; (II) 0.2 M phosphate buffer (pH 6.0); (III) mixture of 4.0 ml of H_2O, 0.5 ml of 0.8% ninhydrin and 0.5 ml of 0.2 M phosphate buffer (pH 6.0); (IV) 0.2% ninhydrin; (V) 0.8% ninhydrin; (VI) mixture of 4.0 ml of 0.3% glucosamine, 0.5 ml of 0.8% ninhydrin and 0.5 ml of 0.2 M phosphate buffer (pH 6.0).

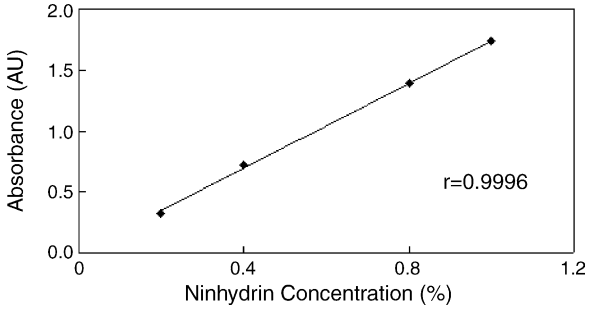

Fig. 3. Effect of the concentration of ninhydrin on the ninhydrin–glucosamine reaction ($n = 3$).

[17]. Others have pointed out that when the ninhydrin concentration was gradually increased from 0.05% to 0.5% at a fixed concentration of asparagines, the color changed from light yellow to brownish purple [12]. In this work, similar result was also obtained. However, there was no wavelength change under the defined experimental condition, all the samples showed purple colors which demonstrated maximum absorbance at 570 nm, and absorbance increased with increases in ninhydrin concentration from 0.2% to 1.0% (Fig. 3). Ninhydrin solution with the concentration of 0.8% was selected for further studies.

3.2.2. *Effect of reaction temperature and exposure time on the reaction rate*

It is known that the reaction temperature is very important. Majority of the reported work on ninhydrin reactions have been performed at elevated temperature. In our study, no color was formed at room temperature in 3 h. Only slight purple color was observed after heating the samples at 37 °C for 1 h. With the increase of reaction temperature, the time for the color formation was significantly reduced. At 100 °C and exposure time of 5 min, dark purple color was rapidly formed as shown in Fig. 4. For this reason, the reaction should be stopped by placing the test tube under tap water immediately after the elapse of specific reaction time. As discussed earlier, the reaction rate between ninhydrin and amines is slower than that with amino acids. Therefore, in order to assure completion of the reaction process, 100 °C for 5 min was selected in this work and absorbance measured within 15–30 min after reaction was quenched in cold water. It should be noted that sample's absorbance increased with the reaction time from 5 to 20 min as shown in Fig. 5.

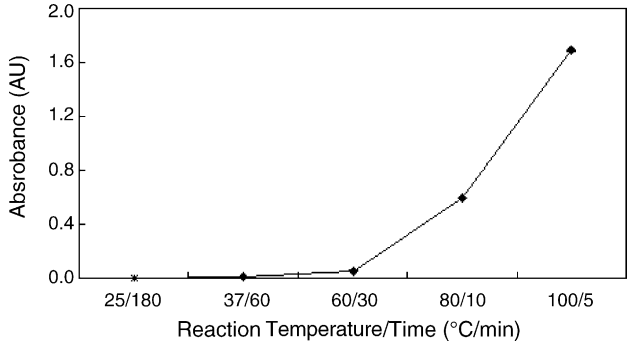

Fig. 4. Effect of reaction temperature on the ninhydrin–glucosamine reaction ($n = 3$).

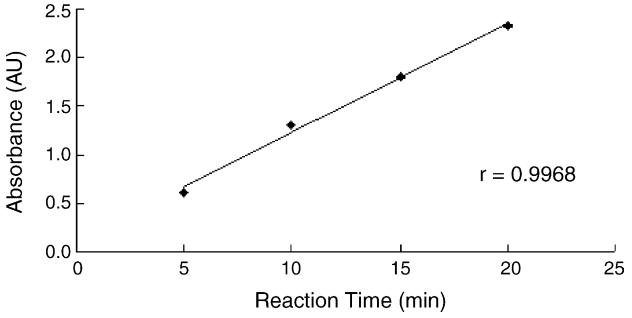

Fig. 5. Effect of reaction time on the ninhydrin–glucosamine reaction ($n = 3$).

3.2.3. *pH effect on the reaction*

As described earlier, acid environment is one of the most important factors on the ninhydrin–glucosamine reaction. The pH of the mixture of ninhydrin and glucosamine is greater than 4.0, so pH 5.0–6.0 was selected as experimental range. It is reported that pH effect under certain conditions on the ninhydrin reaction may have a fluctuating effect [12]. In our study, samples' absorbances increased with an increase of the pH of the sample solution from 5.0 to 6.0 (Fig. 6), as a result, phosphate buffer with pH 6.0 was selected for further studies.

3.2.4. *Proportion of the reactants*

The quantities and ratios of glucosamine to ninhydrin had to be optimized during construction of the calibration curve. Typical examples showing changes in the ratios and final optimized condition on absorbance is illustrated in Fig. 7. In our specific reaction, under defined conditions, a straight line with absorbance values less than 1.0 was only obtained at a higher ratio of glucosamine to ninhydrin after a dilution procedure as shown in Fig. 8.

Based on the above results, calibration curve for the glucosamine determination from sustained release tablets was successfully obtained under optimized conditions. As shown in Fig. 8, the line did not pass through the origin as color pro-

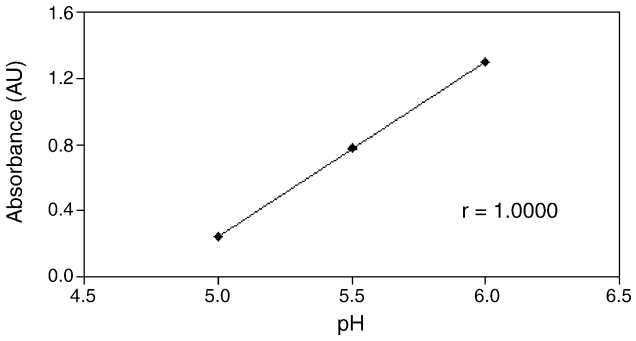

Fig. 6. Effect of pH on the ninhydrin–glucosamine reaction ($n = 3$).

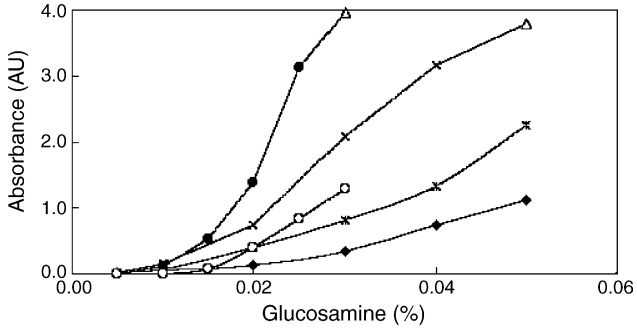

Fig. 7. Effect of the proportion of reactants on the ninhydrin–glucosamine reaction: (♦) 0.02% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (0.5:4:0.5 ml); (●) 0.02% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (2:1:2 ml); (×) 0.02% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (1:3:1 ml); (✱) 0.02% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (0.5:3:1 ml); (◯) 0.02% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (1:2:1 ml); (△) out of dynamic range of the detector.

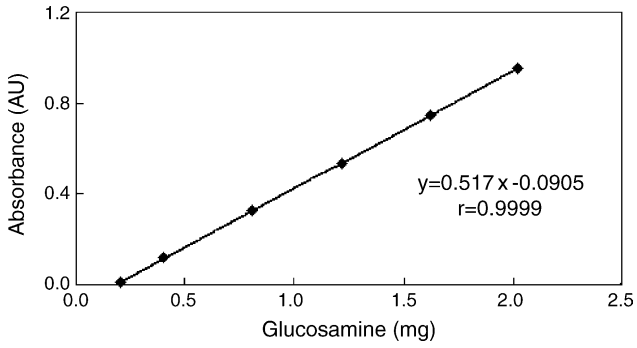

Fig. 8. Calibration curve for glucosamine. 0.02% glucosamine–0.8% ninhydrin–0.2 M phosphate buffer (pH 6.0) (4:0.5:0.5 ml) followed by 10-fold dilution prior to analysis.

duced at a very low level of glucosamine, and ninhydrin was insignificant to be detected. The linear range of the standard curve is 0.202–2.020 mg of glucosamine in sample solution with recovery rates from 97.8% to 101.7% ($n = 6$).

3.3. Drug release study

The glucosamine dissolution profiles of five batches of tablets are shown in Figs. 9 and 10 with time for 50%, 75% and release kinetics data for batches GF-4 and GF-5 in Table 2.

Table 2
Release kinetic data of five glucosamine formulations

	Formulation				
	GF-1	GF-2	GF-3	GF-4	GF-5
$T_{50\%}$ (h)	1.80	2.68	2.68	2.98	2.02
$T_{75\%}$ (h)	5.17	5.32	6.18	4.40	6.50
k	N/A	N/A	N/A	29.68	34.91
r	N/A	N/A	N/A	0.9964	0.9908

$T_{50\%}$: time for 50% of glucosamine released; $T_{75\%}$: time for 75% of glucosamine released; k and r: the slope and regression coefficient for square root treatment of data.

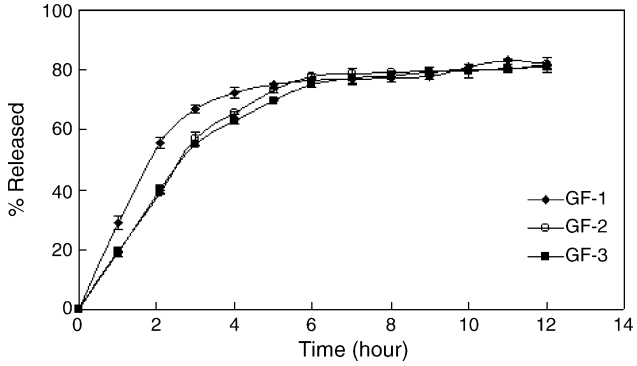

Fig. 9. Glucosamine tablets release profiles ($n = 3$).

It is apparent that formulations GF-1, GF-2 and GF-3 have similar release profiles, an initial linear release during the first 3 h followed by much slower release for the remainder of dissolution time. These formulations were based on PEO, HPMC or their blends as shown in Table 1.

Different types of polymer(s) namely hydroxypropylcellulose (Natrosol) and/or hydroxyethylcellulose (Klucel), used in formulations GF-4 and GF-5 also demonstrated significant variation in dissolution profiles (Fig. 10). The dissolution profiles appear to follow typical square root kinetics. By analyzing the data, a linear relationship existed between amount of drug released (Q) and the square root of time (t), as described by Higuchi [18]. The equation is given as $Q = Kt^{1/2}$, where K is the matrix-controlled constant which incorporates the following:

$$K = [D\varepsilon/\tau(2A - \varepsilon C_s)C_s]^{1/2}$$

where C_s and D are the solubility and diffusion coefficient of the drug in permeating fluid, ε and τ are the porosity and tortuosity of the matrix, and A is the initial concentration of the solid drug in the matrix.

As shown in Fig. 10 and Table 2, the combination of Natrosol 250M and Klucel HXF (GF-4) provided larger $T_{50\%}$ and smaller slope. GF-5 resulted in complete release while only 80% was released from GF-4 in the same time period. The actual operating release mechanisms from the formula-

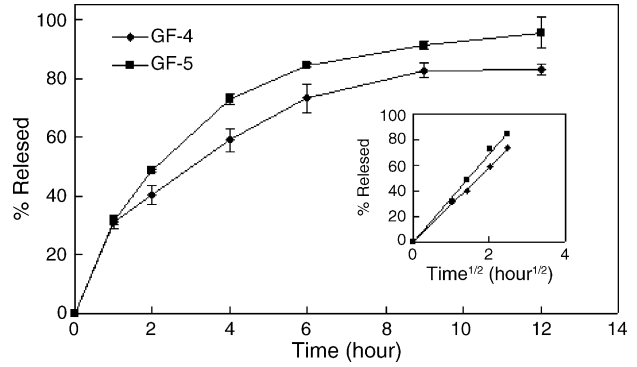

Fig. 10. Glucosamine tablets release profiles ($n = 3$): (♦) GF-4, slope $k = 29.68$ ($r = 0.9964$) in inside plot; (■) GF-5, slope $k = 34.91$ ($r = 0.9908$) in inside plot.

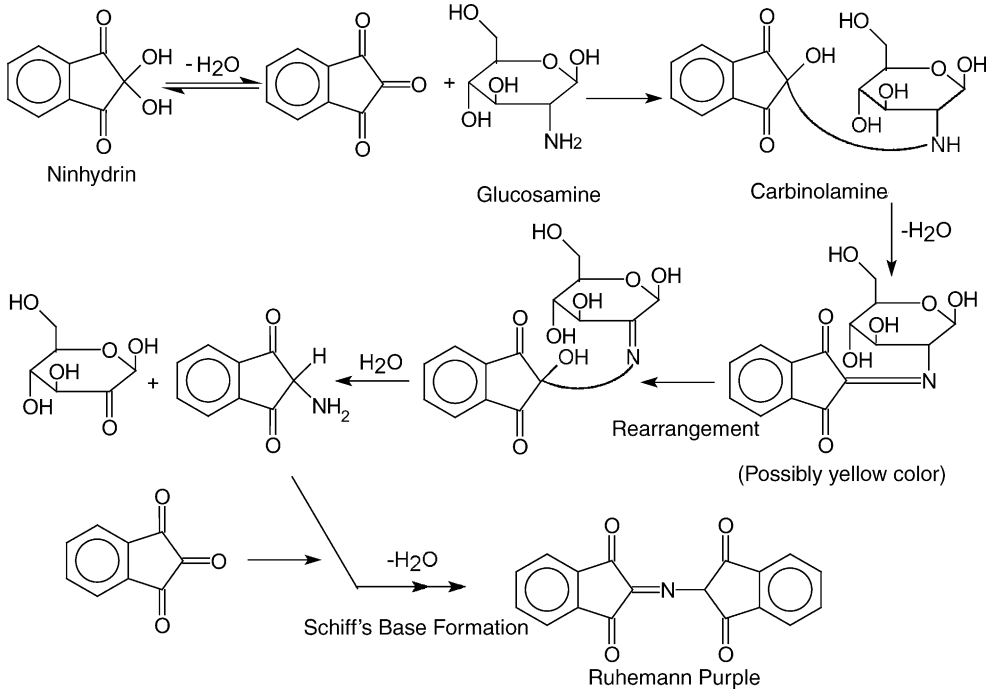

Fig. 11. Proposed mechanism of *Ruhemann* purple from ninhydrin–glucosamine reaction (*Schiff*'s base formation).

tions and associated erosional, diffusional and swelling behavior are the subject matter of another study.

Overall, the consistency in release determination from various batches of tablets indicates that the developed method is reliable with good reproducibility as demonstrated with low standard error of means.

3.4. Proposed mechanism of the ninhydrin–glucosamine reaction

The proposed mechanism of the reaction is illustrated in Fig. 11. This mechanism is based on the *Schiff*'s base formation, as a result of diketohydrindamine–diketohydrindylidene, reaction product which is similar to the ninhydrin–amino acids reaction exhibiting *Ruhemann* purple that can be quantitatively determined at 570 nm.

4. Conclusion

Ninhydrin–glucosamine reaction was affected by the following factors: (1) reagent (ninhydrin) and glucosamine concentrations and ratios; (2) reaction temperature; (3) reaction time; (4) reaction solution pH; and (5) assay time of the reacted solution for maximum stability.

The method for the determination of glucosamine release from tablet dosage form was developed based on the diketohydrindamine–diketohydrindylidene color formation. The reaction conditions were optimized and critical factors identified. Dissolution studies of sustained release glucosamine formulations resulted in reproducible dissolution profiles. The developed method is easy to use, accurate and highly cost-effective for routine studies relative to the other analytical methods using HPLC and CE.

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