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RESEARCH ARTICLE

# A Modified Version of Generalized Standard Addition Method as Quantitative Determination of Lysineacetyl salicylate-Glycine Complex

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## Abstract

The quantitative determination of lysine (Lys) and glycine ( Gly) in their complexes salts with aspirin was achieved using a modified version of the Generalized standard addition method ( GSAM) that aimed at the validation and standardization of analytical procedures with direct solid sample contain two types of amino acids without previous isolation. It was intended to separate aspirin quantitatively from them before determination by titration. It was intended to use spectrophotometry or titrimetric for determination of lysine and glycine. According to this method, two linear equations were solved to obtain the amounts of (Lys) and (Gly) by using two different wavelengths using ninhydrin as a color developing reagent in the spectrophotometric procedure, as it is the most selective among other coloring agent for spectrophotometric determination for amino acids. The high accuracy and precision of the results indicate that this method is simple, fast, precise and suitable to be used as a quality control procedure for analysis of commercial product (aspegic).

**Keywords**: *Aspirin, glacial acetic acid, glycine (as aspirin amino acid salt), Hydrochloric acid, lysine (as aspirin amino acid salt), ninhydrin, perchloric acid, UV spectrophotometer.*

## Introduction

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution measuring the ratio of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers [1]. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer-Lambert law [1, 2].

Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to concentration. Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law [1]. Numerous methods employed the determination of aspirin could be found in the literature. However, these methods are not suitable for the determination of aspirin when it consist as a salt with lysine in the presence of glycine because both of lysine and glycine interferences the direct titrimetric determination of aspirin; therefore, it was intended to separate aspirin quantitatively from them before determination by the titrimetric method. It was intended to use spectrophotometry and titrimetric for determination of lysine and glycine [3, 9].

The quantitative determination of lysine (Lys) and glycine (Gly) in their complex salt with aspirin was achieved using a modified version of the Generalized standard addition method (GSAM) that aimed at the validation and standardization of analytical procedures with direct solid sample contain two types of amino acids without previous isolation. Many methods were used for the determination of lysine and other amino acid involved previous isolation like the classical analytical

technique uses automated amino acid analyzers [10]. According to this method, two linear equations were solved to obtain the amounts of (Lys) and (Gly) by using two different wavelengths using ninhydrin as a color developing reagent in the spectrophotometric procedure, as it is the most selective among other coloring agent for spectrophotometric determination for amino acids [11]. From the results of these measurements it is possible to use Beer-Lambert's law to construct two linear equations of the form:

$$A_1 = a_{11} M_1 + a_{12} M_2$$
$$A_2 = a_{21} M_1 + a_{22} M_2 \qquad (1)$$

Where; A1 and A2 are the absorbance's at wavelengths $\lambda_1$ and $\lambda_2$ respectively, $M_1$ and $M_2$ are the molar concentrations of lysine and glycine, a11,a12,a21 and a22 are molar absorptivity constant multiplied by the optical path length ( i.e. of the sample).

These two linear equations-spectrophotometric methods seem to be the simplest possible procedure for the determination of the concentrations of a mixture of (n) substances that interfere with each other when measured. Accordingly, precise result from %L and %G when the following two conditions are fulfilled: Firstly there should be a large difference in magnitude between the ratio $a_{11}/a_{12}$ and the ratio $a_{21}/a_{22}$. Secondly, the precision is measuring $L_1$ and $L_2$ should be high. These requirements arise from the fact that the mathematical manipulation magnifies the random error in measurement so that large random error is produced in the calculated concentration of the measured substances (i.e. % L and % G).

The titration was carried out in non- aqueous media using perchloric acid in glacial acetic acid as a titrant. The developed method is accurate, precise, and free from interferences and may provide a useful approach to calibrate in the direct analysis of a solid sample and it is a suitable method to be used as a quality control procedure [12, 13].

Aspirin (acetyl salicylic acid; 2-Acetoxybenzoic acid) as a prototype of traditional non-steroidal anti-inflammatory drugs (NSAIDs) was introduced into medicine in 1899; it blocks Cox-1 and Cox-2 enzymes that carry out the body's synthesis of prostaglandins. It is a weak organic acid that is unique among other NSAIDs in that irreversibly acetylates (and thus inactivates) cyclooxygenase [14, 16]. This method used for the quantitative determination of many chemical compounds and drugs in pharmaceutical forms, providing precise and accurate results, which could be verified by statistical methods [17, 18]. Spectrophotometric methods are widely used for determination of amino acid based on the reaction with coloring agent at different pH forming a colored complex that measured at a specific wavelength [19, 2]. This experimental design of using two different techniques to obtain two linear equation has never been used before in GSAM applications.

It was expected in this work that equations obtained from different techniques are not exactly compatible in mathematical forms to be solved linearly. However, with the aid of some mathematical rearrangement, these two equations could be transformed to linearly solvable forms as could be seen later. It was decided in this work to obtain the first equation using ninhydrin as a color developing agent in the spectrophotometric procedure, as this agent is the most sensitive and selective among other coloring agents for amino acids spectrophotometric determination [20, 21].

It was decided to use titrimetric method to obtain the second equation. The titrimetric method should not be performed in aqueous media since the buffering action of the salt prevents the occurrence of a clear titration end point. This point was evaluated theoretically and observed experimentally. Accordingly, a titrimetric method in non-aqueous media was adopted by using glacial acetic acid as a solvent and a solution of perchloric acid in glacial acetic acid as a standard titrant [22].

## Mathematical Derivation

The experimental scheme was designed to produce two linear equations in which the ratio $a_{11}$ / $a_{12}$ and $a_{21}$ / $a_{22}$ are very different in magnitude.

A condition required to produce highly precise results. This was expected from the fact that lysine and glycine have nearly the same efficiently to produce colored complexes with ninhydrin reagent in spectrophotometric procedure [23, 24]. While in titrimetric procedures lysine molecule has two titratable amino groups in contrast to the glycine molecule which has one titratable amino group. The first Linear equation obtained from spectrophotometric measurement was derived starting from lambert_ beer, s basic equation for a solution containing a mixture of two substances both capable of absorbing the same wavelength of an incident [25, 26].

$$A = (a_L M_L + a_G M_G) \quad (2)$$

The subscripts L and G stand for lysine and glycine respectively.The second linear equation obtained from the titrimetric procedure was derived from the basic equation of titrating a mixture of two basic substances with standard acid:

$$M_P V_P = (2M_L + M_G) V \quad (3)$$

*Where V is the volume of the solution which has ML and $M_G$ molar concentration concerning lysine and glycine .This volume V is titrated with volume $V_P$ of $M_P$ molar concentration standard solution of perchloric acid to reach the titration end point. The factor 2 in equation (3) is because that lysine contains two titratable amino groups. Equation (2) and (3) were converted to a mathematically compatible linearly solvable set of the equation by the following*

It was assumed that the spectrophotometric procedure starts with preparing a dilute solution of analyzed solid by weighing Wasp (lysine acetylsalicylate-glycine) grams of acetic to prepare $V^0$ ml of stock solution, this stock solution was then serially diluted to prepare the required final concentration that used for spectrophotometric measurement.

Assuming that n step of serial dilution is performed with $F_1$, $F_2$…….$F_n$ dilutions factors to convert the stock solution to the required final concentration .$F_1$ =volume of the volumetric flask used in the first dilution /volume taken from the stock solution. $F_n$ = volume of the volumetric flask used in the nth dilution /volume taken from the stock solution (n-1).

Therefore the final molar concentration, M, of lysine or glycine is given by equation (4):

$$M = \frac{W}{MW} \cdot \frac{1000}{V} \quad (4)$$

*W: the weight in grams of lysine or glycine present in volume v of the titrated solution.*

*M and MW: represent the molar concentration and molar weight of lysine or glycine of the titrated solution:*

$$V = V^{\circ}. F_1. F_2 \ldots\ldots. F_n \quad (5)$$

Where V is the volume of the final solution containing, w grams of lysine or glycine. Also:

$$W = \frac{Wasp}{100} \cdot \% \quad (6)$$

*Where % is the percentage of lysine or glycine in analyzed aspegic.*

*The final equation takes the following form when equations (4), (5), and (6) were substituted in equation (2)*

$$A = \frac{10\ a_L\ w_{asp}}{MW_L\ V^0\ F_1 F_2 \ldots F_n} \%\ L + \frac{10\ a_G\ w_{asp}}{MW_G\ V^0 F_1 F_2 \ldots F_n} \%\ G \quad (7)$$

Equation (7) can be abbreviated in the form:

$$A = a_{11} \%L + a_{12} \%\ G \quad (8)$$

The second linear equation can be derived by taking a volume of V ml of a solution containing lysine and glycine with a molar concentration of $M_L$ and $M_G$ respectively in glacial acetic acid. This V ml is titrated with a standard perchloric acid solution in glacial acetic acid, the molar concentration of perchloric acid in this solution is Mp. Assuming that Vp ml of the perchloric acid solution are consumed to reach the titrations end point, then equation (3) described the material balance, in molar form, at the titrations end points. Assuming that the titrated solution is prepared by dissolving $W_{asp}$ grams of the analyzed aspegic in V ml of glacial acetic acid then, the second linear equation takes the following form when equation (4) and (6) are substituted in equation (3):

$$M_pV_p = \frac{2\ W_{asp} \times 10}{MW_L}\ \%_L + \frac{W_{asp} \times 10}{MW_G}\ \%_G \qquad (9)$$

Equation (9) can be abbreviation to the form:

$$M_pV_p = a_{21}\ \%_L + a_{22}\ \%_G \qquad (10)$$

Equations (8) and (10) are compactable mathematically to be solved linearly. Also the ratio $a_{11}/a_{12}$ and $a_{21}/a_{22}$ has large differences in their magnitudes since, $a_{11}/a_{12}$ ~2 and $a_{12}/a_{22}$~1.

## Materials and Methods

### Materials

- Acetalsalicylate DL-Lysine-glycine (synthesize according to Tagreed N Omar thesis) [27].
- Aspirin (standered); and aspegic.
- Hydrochloric acid (HCl), 36% w/w, Riedel-Deltaen, Germany.
- Glacial acetic acid (99.8 %) was obtained from Sigma.
- Anhydrous sodium carbonate (99.5 %).
- Sodium hydroxide (0.5 N).
- Sodium acetate trihydrate $NaOAc.3H_2O$ (BDH "Analar "99.9).

### Methods

(1gm) of lysine acetylsalicylate-glycine product was weighed and dissolved in (25 ml) of distilled water. The salt was converted to free aspirin by acidification with hydrochloric acid (3 N) until pH (4.5) was reached; then aspirin was extracted by ether by adding 10 ml D.W and 10ml ether to the solution. The solution was transferred to separatory funnel to make extraction for organic layer, after standing few minutes, the solution was separated, the aqueous layer was discard, and the organic layer was evaporated by a rotary evaporator under vacuum. Aspirin was collected to be determined quantitatively by titration and spectrophotometric methods [5, 9]:

### Quantitative Determination of Aspirin in Lysine Acetylsalicylic Acid-glycine Product

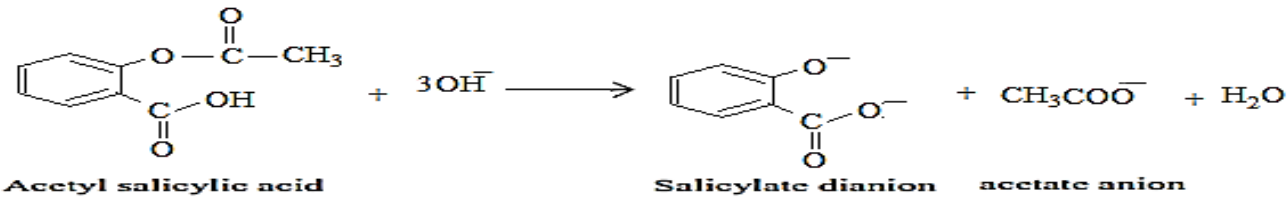
### Titration Method

The obtained aspirin was dissolved in ethanol (95%, 5 ml) and sodium hydroxide (0.5 N, 25ml). The mixture was stoppered and allowed to stand for about 30 minutes to complete the hydrolysis of the ester moiety present in aspirin. Phenolphthalein solution 1-2 drops were added as an indicator and the solution were titrated with hydrochloric acid (0.5 N).

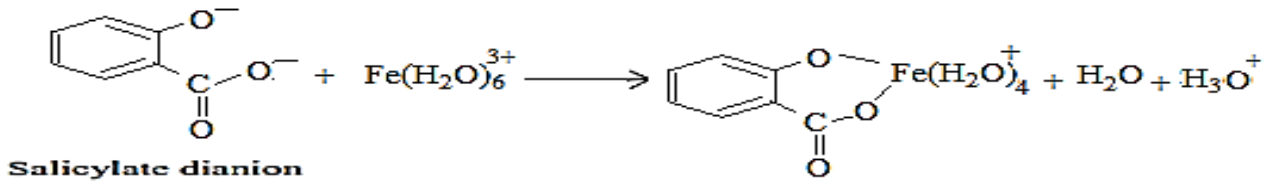
The same procedure were repeated except aspirin was omitted from the solution, to serve as blank .The difference between the volumes of hydrochloric acid used in both titrations represents the required amount of sodium hydroxide (0.5N) to hydrolysis the ester group in aspirin, besides the conversion of COOH group to sodium carboxylate [28]. Each ml of 0.5M sodium hydroxide is equivalent to 45.04 mg of Aspirin.

### Spectrophotometric Method

The acetyl ester group in Aspirin (acetate ester of salicylic acid), is rapidly hydrolyzed to the salicylate anion in a basic medium:




As the de-esterification is complete, and $FeCl_3$ is added to the acidified solution, a violet colored complex ion is formed due to the reaction of salicylic acid with the $Fe^{3+}$ to produce the violet tetraaquosalicylatroiron (III) complex.




**Preparation of Standards and the Aspirin Solutions and our Sample**

- Weigh out 0.500 g (500 mg) of acetylsalicylic acid and place it in a 125 ml Erlenmeyer flask. 10 ml of 1 M NaOH solution was added and heat to boiling. Quantitatively transfer the solution to a 250 ml volumetric flask and dilute with distilled water to the 250 ml mark. This solution will be referred to as the "Standard Aspirin Solution."
- Using a 1 ml graduated pipet, transfer a 0.5 ml sample of this stock solution into a 10.0 ml volumetric flask and dilute this solution to the 10.0 ml mark with 0.02 M iron (III) chloride that is buffered to pH 1.6.
- This solution was placed in a test tube labeled solution A. In a similar fashion, solutions labeled B, C, D, and E were prepared by using 0.40, 0.30, 0.20, and 0.10 ml aliquots of the sodium salicylate solution, diluting to 10.0 ml with iron (III) chloride solution. All of the standard solutions were violet – purple to the human eye.
- The absorbance of the solutions was measured at 530 nm. Iron (III) chloride solution was used for the blank.
- The same steps (1-3) with isolated aspirin from lysine acetylsalicylic acid-glycine product were repeated.
- The absorbance of the solution was measured at 530 nm. Iron (III) chloride solution was used for the blank.

**Quantitative Determination of Each of Lysine and Glycine without Prior Separation by a Modified Version of the Generalized Standard Addition Method (GSAM)**

Lysine and glycine were determined in lysine acetylsalicylic acid-glycine product by applying two methods:

- Ninhydrin as a color developing agent in the spectrophotometric procedure to obtain equation [6].
- A titrimetric method in non-aqueous media was used to obtain equation [8].
- These two simultaneous equations were then solved to calculate the %w/w of lysine and glycine in lysine acetylsalicylic acid-glycine product.

**Colorimetric Method using ninhydrin Reagent [29, 30]**

- Preparation of ninhydrin solution was done by adding 25ml of 2-methoxy ethanol (99.0%) to a mixture of ninhydrin (99.0%) 0.67g and stannous chloride $SnCl_2.2H_2O$ (99.0%) with continuous stirring until completely dissolved.
- Sodium acetate buffer (8.33 ml) was added, and the resulting solution was immediately transferred to dark glass reservoir bottle, and steam of nitrogen gas was bubbled into the reagent solution for approximately 20 minutes.

The ninhydrin reagent was prepared with sodium acetate buffer (4M, pH 5.5) and with pH values of 7 and 9.Solutions of lysine acetylsalicylic acid-glycine product, lysine and glycine were prepared separately as stock solutions by dissolving 36.38 mg, 14.62 mg,

and 7.50 mg respectively in a 50 ml deionized water. Each one of these stock solutions was transferred to 25 ml volumetric flask and diluted to the mark with deionized water. This gives 0.08mM standard solutions of lysine acetylsalicylic acid-glycine product with about 0.08nM lysine and 0.04mM glycine. These solutions were measured at λ 570 nm against a reagent blank. Also, an aqueous solution of lysine acetylsalicylic acid-glycine product of a concentration about 0.08mM and 0.04mM of lysine and glycine respectively were prepared. (1 ml)Of this solution was mixed with ninhydrin reagent (1 ml)prepared at pH(5.5) in a stoppered test tube, shacked and placed in a boiling water bath for (1 5 min). An aliquot (5 ± 0.03 ml) of the diluted reagent (1:1) Ethanol 99%: sodium acetate buffer 4M, pH5.5) was added to the mixture and cooled below 30°.

The sample was shacked thoroughly for 30 seconds and, their absorbance was measured at (λ 570 nm) against a reagent blank using (1 cm). Standard curves for lysine and glycine were constructed using (0.05- 0.2) mM standard solution for each. The values of constants $a_{11}$ and $a_{12}$ in equation (7) were determined from the slopes of the calibration curves. A similar ninhydrine spectrophotometric procedure was applied using (4M) sodium acetate buffer 4M, prepared at different pH values (7 and 9). The absorbance was also measured for ninhydrine solutions prepared at pH values, 5.5,7 and 9 and their absorbances were measured at λ(244,409 and 570) nm against a reagent blank using (1 cm).

### A titrimetric Method in Non-aqueous Media to Obtain Equation [22, 24]

Preparation of standard perchloric acid (0.1 N):

(4.25 ml) Of perchloric acid (71-73%) was added to a mixture of 10 ml glacial acetic acid (99.8%) and 10.5 ml acetic anhydride (98%). The total mixture was completed to 500 ml with glacial acetic acid. The prepared perchloric acid solution was standardized by titration with standard anhydrous sodium carbonate (0.106 g) in glacial acetic acid (50 ml) in a stoppered dry flask containing two drops of crystal violet as an indicator.

The titration end point was determined potentiometrically with potentiometric titration equipped with glass –calomel combined electrode.

The pH was recorded after each addition of perchloric acid delivered from the burette. A plot of pH against the titrant volume was constructed to obtain the titration end point.

Titration of lysine acetylsalicylic acid-glycine product with standard perchloric acid:

A sample of lysine acetylsalicylic acid-glycine product (0.2911) g was dissolved in glacial acetic acid 50ml to be titrated with the standard perchloric acid 0.1N. The same titration procedure was followed as previously described before. Stability studies [31, 32]

- An aqueous solution (0.1 g/ 100 ml) of the complex was made for the commercially available compound (Aspegic) and our prepared sample of lysine acetylsalicylic acid-glycine product.
- Different temperatures (25, 40, 50, 60 & 70)°C incubated in ovens for certain intervals. The decomposition of (aspirin-lysine) glycine complex was indicated by TLC using (n-propanol: 34% ammonia 7:3) as a solvent system.

## Results and Discussion

### Quantitative Determination of Aspirin

The effect of glycine was as a buffer to stabilize (lysine- aspirin) complex at a specific pH [3].

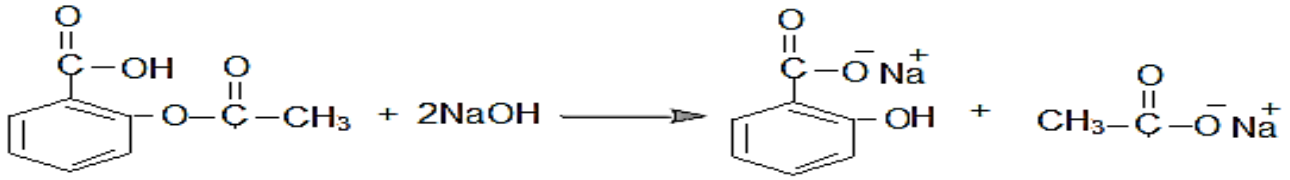
### Titrimetric Methods [5]

Aspirin was extracted from an acidified solution of lysine acetylsalicylic acid-glycine product with three portions of ether; the pH of the acidified aqueous solution was determined by applying the sample complex at different pH values of the extracted sample solution. The result indicates that the optimum pH is (4.5), if pH greater is than (4.5) that leads to incomplete liberation of aspirin from its salt with lysine, so low results were obtained.

The aspirin residue was treated with standard sodium hydroxide solution (0.5 N), the quantity of sodium hydroxide consumed by aspirin was measured by titration with standard hydrochloric acid, The same procedure was repeated but without aspirin (blank solution to reduce any error due to the presence of impurities or to the conditions). The difference between the vol. of HCl used in both titrations represents the required amount of NaOH solution to hydrolyze ester

group of aspirin and the conversion of the COOH group to the corresponding sodium carboxylate.




**Spectrophotometric Method [33, 34]**

The complex ion is formed in two steps. First, the aspirin is reacted with sodium hydroxide to form the salicylate dianion. Then the addition of acidified iron (III) ion

The iron-aspirin solution is violet- red in color; it absorbs yellow-green light (530nm).Which reduces the violet tetraaquosalicylatroiron (III) complex.

Aspirin solution was stored at 4°C to prevent the possible decomposition of ASA (acetyl salicylic acid) to SA (salicylic acid), a solution was freshly prepared before experiments.

500 mg of aspirin was dissolved and diluted to make solution A. Since all of the aspirin solutions were dissolved and diluted the same way as solution A, it is concluded that if the aspirin solution has the same absorbance as solution A, then the ( aspirin-lysine)glycine product must have contained 500 mg of aspirin.

All solutions were compared to solution A because all of the aspirin solutions will be diluted as solution A was diluted. The results are summarized in table-5

**Calculations**

Concentration (in mg/mL) of aspirin in the "standard aspirin solution."

$$\frac{500 \text{ mg aspirin}}{250 \text{ ml}} = \frac{2 \text{ mg}}{\text{ml}}$$

Using the relationship for dilutions:

concentration x volume = concentration x volume

Calculate the concentration (in mg/ml) of aspirin for each of the standard Solutions A- E.

Solution A: (0.5 ml) (2 mg/ml) = X (10 ml) X = 0.10 mg/ml

Solution B: (0.4 ml) (2 mg/ml) = X (10 ml) X = 0.08 mg/ml

Solution C: (0.3 ml) (2mg/ml) = X (10 ml) X = 0.0 6 mg/ml

Solution D: (0.2 ml) (2 mg/ml) = X (10 ml) X = 0.04 mg/ml

Solution E: (0.1 ml) (2 mg/ml) = X (10 ml) X = 0.02 mg/ml

Determination of the concentration(conc.) (mg/ml) ratios of the following solutions.

Conc. B/conc. A: 0.08/0.1 =0 .80, Conc. C /conc. A: 0.06/0.1 =0 .60

Conc. D/conc. A: 0.06/0.1 = 0.40, Conc. E /conc. A: 0.02/0. 1 = 0.20

Calculation of the "mg of acetylsalicylic acid" for each of the standard solutions.

Solution B: 0.80(500 mg) = 400 mg , Solution C: 0.60(500 mg) = 300 mg

Solution D: 0.40(500 mg) = 200 mg , Solution E: 0.20(500 mg) = 100 mg

Draw a graph of concentration (x-axis) vs. absorbance (y-axis).Use the equation from the trend line to find concentrations by entering the absorbance value for Y in the equation, and solving for X.

The results of these experiments were summarized in Table (1) and Figure (1).

**Table 1: The concentrations and absorbance of the standard aspirin solutions are**

| Standard Solution | Concentration (mg) | Absorbance |
|---|---|---|
| A | 500 | 0.744 |
| B | 400 | 0.61 |
| C | 300 | 0.453 |
| D | 200 | 0.301 |
| E | 100 | 0.152 |
| Our sample | x | 0.742 |

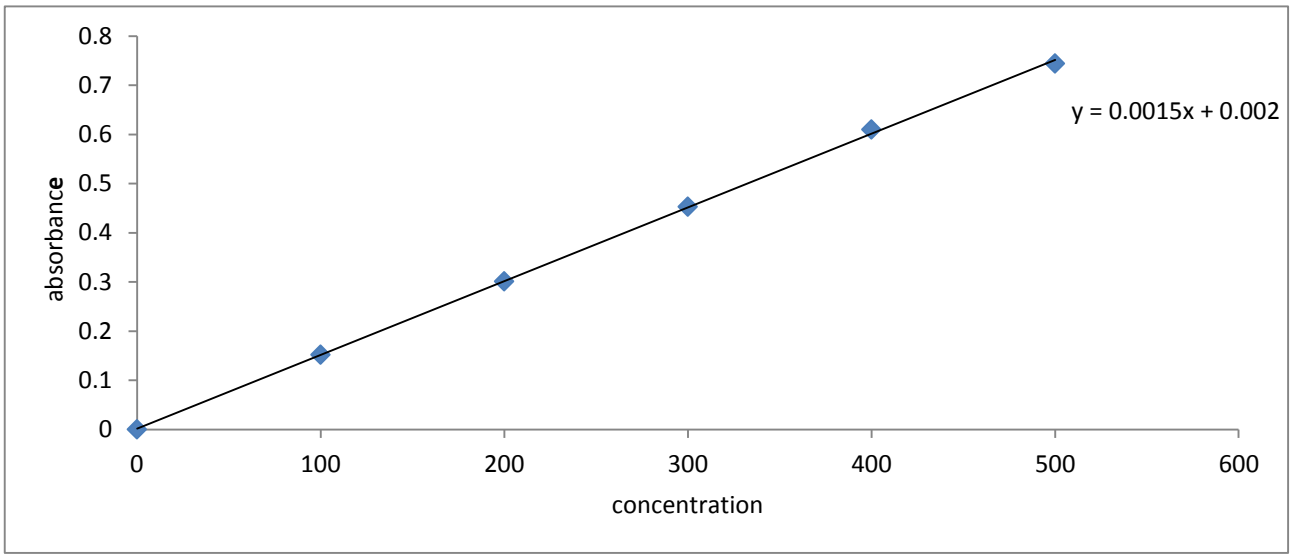



**Figure 1: Calibration curve for standard aspirin and aspirin (aspirin-lysine) glycine product**

## Quantitative Determination of Lysine and Glycine in the Complex: [29]

The quantitative determination of lysine and glycine in the complex using a modified version of the GSAM was achieved by the two linear equations for which the ratio of their constant $a_{11}/a_{12}$ (i.e.: $a_L/a_G$ at the first pH) and $a_{21}/a_{22}$ (i.e.: aL/aG at the second pH) have good difference by a combination of colorimetric method which applied once at different pH media and once by using different wavelengths; (first equation) and non-aqueous titration method (second equation). The results of these experiments were summarized in Table (2) and Figures (2-a-c) and Table (3) and Figures (3-a-c).

**Table 2: Molar absorptivity constant of lysine and glycine colored complex with ninhydrin at different pH values measured at 570nm**

| | pH of ninhydrin solution | aL | aG | aL/aG |
|---|---|---|---|---|
| a | 5.5 | 0.8 | 0.62 | 1.29 |
| b | 7 | 1.04 | 0.78 | 1.33 |
| c | 9 | 1.3 | 0.97 | 1.34 |

**Table 3: Molar absorptivity constant of lysine and glycine colored complex with ninhydrin at different wavelengths λ (244,409 and 570) nm**

| PH of ninhydrin in solutions | λ nm | $a_L$ | $a_G$ | $a_L/a_G$ |
|---|---|---|---|---|
| 5.5 | 244 | 1.17 | 0.93 | 1.27 |
| | 409 | 0.72 | 0.55 | 1.33 |
| | 570 | 0.81 | 0.64 | 1.29 |
| 7 | 244 | 1.26 | 0.98 | 1.3 |
| | 409 | 0.85 | 0.63 | 1.37 |
| | 570 | 1 | 0.76 | 1.33 |
| | 244 | 1.38 | 1.09 | 1.28 |

| | | | | |
|---|---|---|---|---|
| 9 | 409 | 0.9 | 0.68 | 1.34 |
| | 570 | 1.3 | 1.01 | 1.3 |

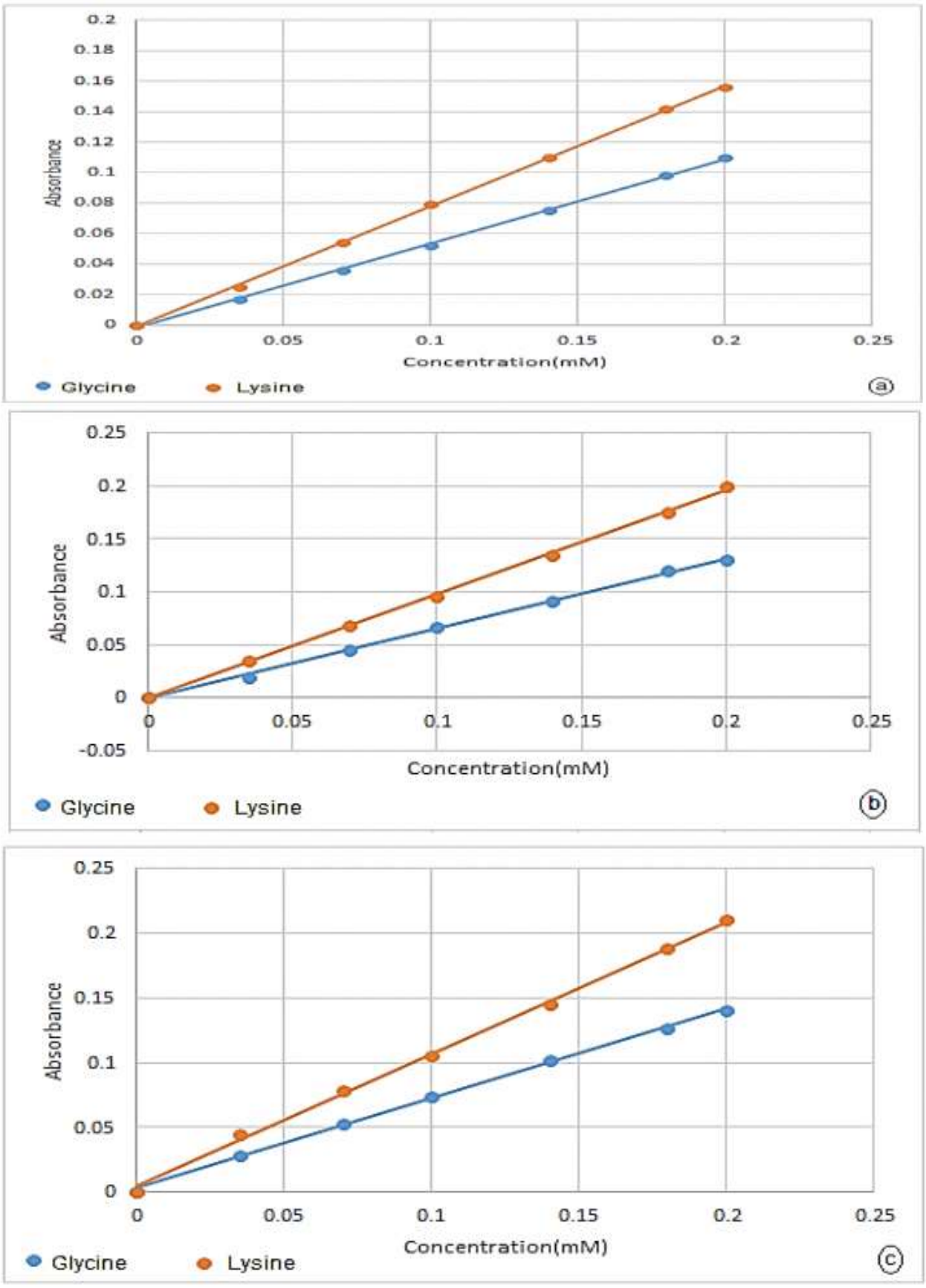



**Figure 2: Standard curve of lysine and glycine colored complexes with ninhydrin at different pH values (a-5.5, b-7and c-9) measuredat 570 nm**

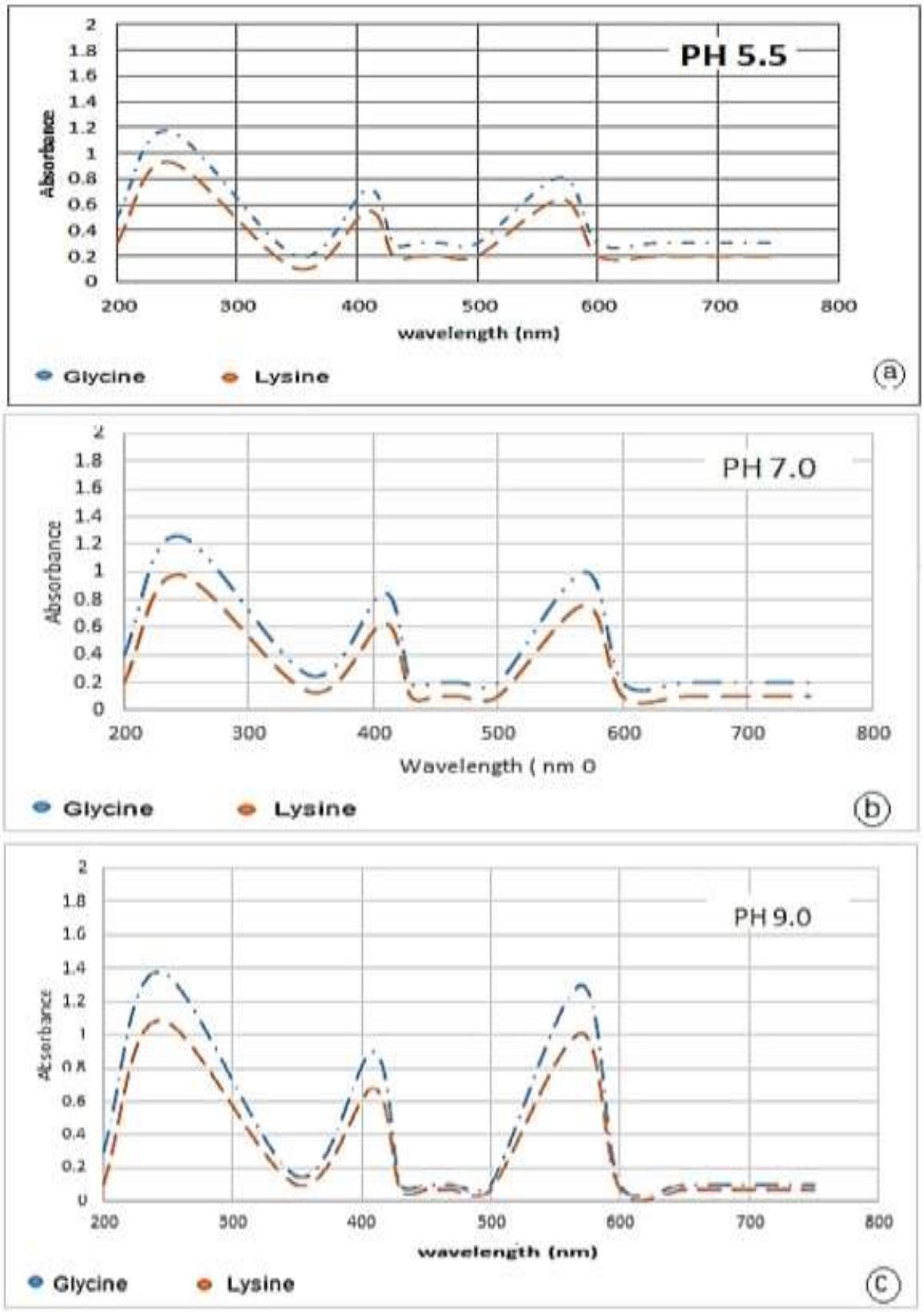



**Figure -3: Uv-visible spectra of lysine and glycine colored c complexes with ninhydrin at different wavelengths**

According to non-aqueous titration, the titration end point of the titration curve between standard perchloric acid with aspirin-lysine glycine product was determined potentiometrically. A typical plot for the titration curve was exhibited in Figure- 4. The result was summarized on Table-5.

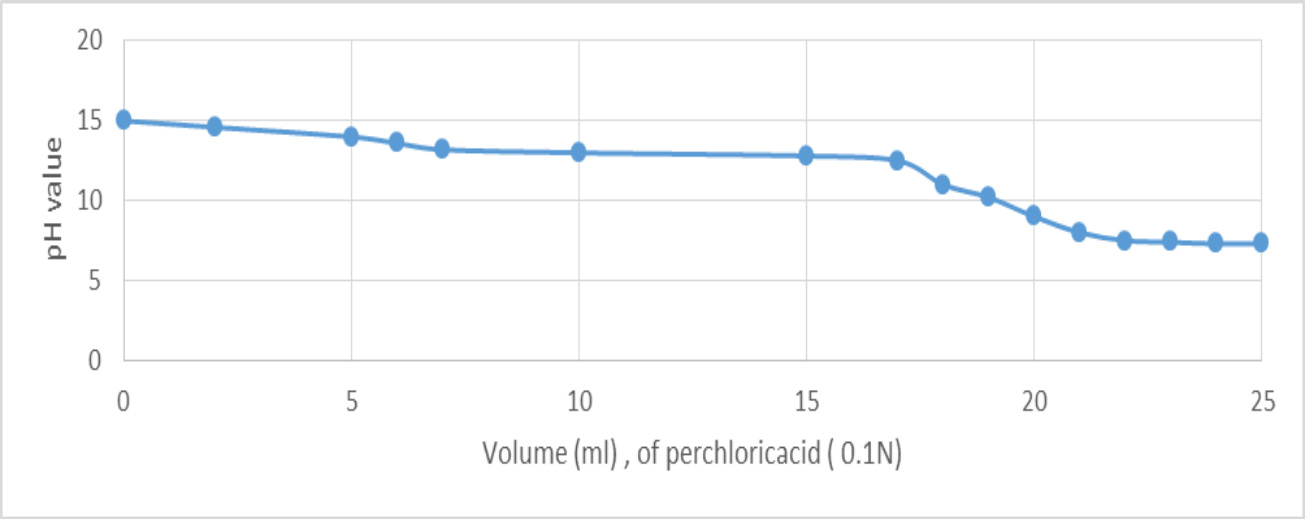



**Figure 4: titration curve for standard perchloric acid (0.1N) and aspirin-lysine glycine product**

## Stability Studies

The decomposition of (aspirin-lysine) glycine complex was indicated by the appearance of three spots on TLC-plates using (n-propanol: 34 % ammonia 7: 3) as a solvent system, which means the releasing of aspirin, lysine and when the samples were incubated at (50, 60 & 70) °C while at (25 and 40) ˚C only one spot for lysine acetylsalicylic acid-glycine product is appeared. The results are summarized in Table (4).

**Table 4: *$R_f$ values of the lysineacetylsalicylate – glycine at different temperatures.**

| °C | Initial | 1 st. | 2 nd. | 3 rd. | 4 th. |
|---|---|---|---|---|---|
| 25 | 0.42 | 0.42 | 0.42 | 0.41 | 0.41 |
| 40 | 0.42 | 0.42 | 0.42 | 0.41 | 0.41 |
| 50 | 0.42 | 0.43 | 0.46 | 0.52 | 0.55 |
| 60 | 0.42 | 0.44 | 0.45 | 0.56 | 0.57 |
| 70 | 0.42 | 0.45 | 0.55 | 0.54 | 0.56 |

* The solvent system is (n-propanol: 34% ammonia 7:3)

**Table 5: the result of aspirin, lysine, and glycine in lysineacetylsalicylate – glycine complex**

| Item | | Expected W/W % | Calculated ( w/w ) % ± SD | C.V % |
|---|---|---|---|---|
| Commercial aspegic | Aspirin | 50 | 49.7 ± 0.2 * , 49.6 ± 0.2 ** | 0.4 , 0.6 |
| | Lysine | 40 | 40.2 ± 0.6 | |
| | glycine | 10 | 9.8 ± 0.2 | |
| Synthesize lysineacetylsalicylate – glycine complex | Aspirin | 50 | 49.6 ± 0.2 * , 49.5 ± 0.3 ** | |
| | Lysine | 40 | 40.3± 0.5 | |
| | glycine | 10 | 9.7 ± 0.2 | |

The result expressed as the mean ± SD of three measurements. SD: standard deviation.; C.V.: Coefficient of variation. * : titration method, **: Spectrophotometric method

## Conclusion

In this work, the complex was acidified to liberate aspirin before extraction by ether, the optimum pH was determined to be pH 2.7 which is the optimum one higher that leads to incomplete liberation of aspirin from its salt with lysine and to get a low yield. The extraction step was followed by two applied methods for the determination of extracted aspirin lead to a good yield. The modified version of GSAM for the determination of both lysine and glycine, it is less time consuming and more convenient to obtain the two equations.

The close similarity in structure between lysine, and glycine makes it difficult to find two wavelength at which the absorbance of lysine and glycine colored complexes with ninhydrin are sufficiently different and this makes the ratios of $a_{11}/a_{12}$ and $a_{21}/a_{22}$ not differ in magnitude to an extant to obtain a highly precise results, but the colored – developed procedure for the ninhydrin method is a pH sensitive and at different pH values revealed the differences between the ratios $a_{11}/a_{12}$ and $a_{21}/a_{22}$.The high accuracy and precision of the results shown in the table (5) indicate that this method is simple, fast, precise and suitable to be used as a quality

control procedure for analysis of commercial product(aspegic).

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