

COLORIMETRIC ASSAY OF ASPIRIN USING MODIFIED METHOD

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Abstract

In this research, we did this qualitative and quantitative study in order to improve the assay of aspirin colorimetrically using visible spectrophotometer. This method depends on aqueous hydrolysis of aspirin and then treating it with the ferric chloride acidic solution to give violet colored complex with salicylic acid, as a result of aspirin hydrolysis, which has a maximum absorption at 530nm. This procedure was applied to determine the purity of aspirin powder and tablet. The results were approximately comparative so that the linearity was observed in the high value of both correlation coefficient ($R = 0.998$) and Determination Coefficient or Linearity ($R^2 = 0.996$) while the molar absorptivity was $1.3 \times 10^3 \text{ mole}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$. This improvement cancelled the acid or base aided hydrolysis which is widely used during aspirin assay. This method could be also applied to salicylic acid.

Keywords: Aspirin, Ferric chloride, hydrolysis, absorbance.

Introduction

Aspirin, acetyl salicylic acid, was introduced into medicine in 1899⁽¹⁾. It blocks Cox-1 and Cox-2 enzymes that carry out the body's synthesis of prostaglandins⁽²⁾. It is prototype of traditional non steroidal anti-inflammatory drugs (NSAIDs) and it is the most commonly used drug to which all other anti-inflammatory agents are compared. It is a weak organic acid that is unique among other NSAIDs in that irreversibly acetylates (and thus inactivates) cyclooxygenase. The other NSAIDs, including salicylate, are all reversible inhibitors of cyclooxygenase⁽³⁾.

Salicylates, in general, in doses of 500mg inhibit both the renal tubular secretion of uric acid and in large doses of 5-10 gm daily; the tubular reabsorption of uric acid is also inhibited⁽⁴⁾. Several studies indicate that low doses of aspirin reduce the risk of myocardial infarction, stroke, and perhaps colon cancer⁽⁵⁾. Aspirin and other salicylates can be absorbed through the skin and removed by hemodialysis⁽⁶⁾. It is one of the cardiovascular medicines that are available without prescription for primary or secondary prevention of cardiovascular disease⁽⁷⁾.

Aspirin is unionized (lipophilic) in the acid gastric juice and so will be absorbed into the gastric mucosa, once inside the cells it

ionizes and is unable to diffuse out, and so is concentrated there. This property accounts in part for the ulcerogenic property of aspirin and the related drugs⁽⁸⁾.

Aspirin is prepared commercially by the acetylation of salicylic acid (O-hydroxybenzoic acid) with acetic acid in the presence of aqueous sodium hydroxide⁽²⁾. It occurs as white crystals or as a white crystalline powder. It is slightly soluble in water and soluble in alcohol. It slowly hydrolyzes in the presence of moisture giving salicylic acid and acetic acid and salicylic acid can be confirmed by the formation of a violet color on the addition of ferric chloride solution⁽¹⁾.

Aspirin recrystallization is from benzene or various other non aqueous solvents and the alcoholic solution of aspirin is not colored violet with ferric chloride (distinction from salicylic acid)⁽⁵⁾. Aspirin can be identified with various methods such as chemical, spectrophotometric and chromatographic⁽⁹⁾. It is odorless, but in moist air hydrolyzes, it acquires the odor of acetic acid and its aqueous solution is completely one week hydrolyzed⁽¹⁰⁾. In recent study, Li Li and his partners identified that aspirin is unstable to both moisture and heat and they use back-titration to measure the degradation products

of it⁽¹¹⁾. Usually, aspirin is identified with ferric chloride test and assayed after alkaline hydrolysis by titration with 0.5N hydrochloric acid or sulfuric acid (acid-base titration)^(9, 12). Also, UV spectrometric procedure for determination of aspirin in the solution containing 1 % (w/v) of citric acid in some pharmaceutical preparations was presented, at 286 nm; it was found that Beer's law is obeyed from 13.62–68.1 $\mu\text{g ml}^{-1}$ ⁽¹³⁾.

In this search, we are going to apply ferric chloride test after aqueous hydrolysis to aspirin for assaying it quantitatively by simplified colorimetric procedure via measurement of light absorbance by the violet colored salicylic acid–ferric ion complex.

Experimental Work

1. Materials

- Aspirin (acetyl salicylic acid), Judex Laboratory reagents, England, purity percent 99.8%.
- Aspirin tablets (300mg), SDI.
- Ferric chloride (FeCl_3), Solfine-Chem Limited, India.
- Hydrochloric acid (HCl), 36% w/w, Riedel-Deltaen, Germany.

2. Apparatus

- Volumetric flasks, in volumes 10, 50, 250 and 300 ml.
- Graduated pipettes, in volumes 1 and 2ml.
- UV- Visible Spectrophotometer, double beam, CARY 100 CONC.
- Visible spectrophotometer Optima-Sp-300, Japan.

3. Procedure

The method follows the same principle of the volumetric aspirin assay^(9, 12) which depend on alkaline hydrolysis of aspirin, but, instead, aspirin was aqueously hydrolyzed and the hydrolysate i.e. salicylic acid was treated directly with ferric chloride acidic solution resulting in immediate formation violet colored ferric ion–salicylate complex, as shown in Scheme 1.

1. Preparation of Standard Solutions:

- a. Preparation of 9% w/v ferric chloride (FeCl_3) acidic solution: 4.5 gm of ferric chloride was dissolved in 50 ml volumetric flask using 0.1N HCl solution as solvent.

- b. Preparation and hydrolysis of 1000 ppm aspirin stock solution: this includes heating 250 mg of aspirin in about 50 ml distilled water for 90 min., cooling and then completing the volume to 250 ml using volumetric flask to get a Stock Solution with final concentration of 1000 ppm.
- c. Scanning the violet colored ferric ion-salicylate complex of 100ppm in concentration over a range of 200- 800 nm to obtain, finally, the absorption spectrum using double beam spectrophotometer as shown in Fig.(1); the λ_{max} was 530nm.
- d. Preparation of standards: the (1000 ppm) Stock Solution was then diluted in varying proportions (aliquots) to yield Standards (A, B, C, D and E) of concentrations 50, 70, 90, 110, 130 ppm, this is done by taking 0.5, 0.7, 0.9, 1.1 and 1.3 ml from the stock solution, respectively, and diluted to 10ml, after addition 2 drops of FeCl_3 acidic solution, using volumetric flask, as shown in Table (1). The obtained violet colored standards were read at 530 nm using visible spectrophotometer to get the corresponding absorbances (incremently) that were shown also in Table (1). Once we have determined the concentration and absorbance for these five standards, we plotted the calibration curve, as shown in Fig.(2).
- e. We repeated the experiment of standard D (110 ppm) for three times on a new three different (1000 ppm) stock solutions of the same aspirin used previously and the resultant absorbances were read at 530 nm and this is shown in Table (2).

2. Pharmaceutical Preparations:

- a. Assay and calculate the purity percent of a laboratory synthesized aspirin: we made 1000 ppm solution using 250 mg of this laboratory synthesized aspirin, and then 1 ml of such solution was diluted to 10 ml after adding 2 drops of FeCl_3 solution. The absorbance of this final solution was taken to calculate the corresponding concentration with Beer's law plot or its straight line equation

Fig.(2), from this concentration, we conclude the actual weight of aspirin present and its purity percent.

- b. Assay and calculate the purity percent of (300 mg) aspirin tablet: we dissolved three tablets each in about 50 ml distilled water with heating for 90 min., cooling and then completing the volume using 300 ml volumetric flask to get a solution of 1000 ppm concentration, from which again we diluted 1ml up to 10 ml (after adding FeCl_3 solution) to be ready for obtaining its absorbance which is used to account the concentration, actual weight of aspirin in the tablet and its purity percent that were shown in Table (3).

Results and Discussion

- Absorption Spectrum: is the determination of λ_{max} which was at the green light region (530 nm.), meaningly, the violet- colored complex absorbs the green light, as shown in Fig.(1).
- Interacting factors:
 1. Water solubility of aspirin is 1:300⁽⁹⁾ but the optimum stock solution was found to be 1mg/ml which, together with suitable aliquots, gave the proper results and linear relationship. Too high or low concentrations caused deviation and non- linear relationship.
 2. The time interval for complete hydrolysis of aspirin is one week⁽¹⁰⁾ at room temperature, but we accelerated the process with 90 minutes heating of aspirin solution to boiling. This was compared with that obtained after four-week hydrolysis of another 1000 ppm aspirin stock solution giving exactly the same results of complete hydrolysis as shown in Table (4) and Fig.(3). Therefore, we could cancel NaOH – aided hydrolysis step mentioned in all procedures of aspirin assay.
 3. 9% w/v aqueous ferric chloride solution could be used as colorimetric solution for qualitative identification of aspirin, when hydrolyzed, giving a violet color in contrast to the brown color of FeCl_3 solution⁽¹²⁾, but our concept is the applying this identification test for assaying aspirin

using this identification test for assaying aspirin quantitatively with exception of using 9% acidic FeCl_3 solution to form stable violet complex with ferric ion at acidic pH⁽¹⁴⁾.

4. Accordingly, the addition of FeCl_3 solution after releasing all salicylic acid gave violet colored standard solutions of absorbance values that are directly proportional with the concentrations obeying Beer's law⁽¹⁵⁾, with molar absorptivity $1.3 \times 10^3 \text{ mole}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$, and its plot was represented with the following straight line equation Fig.(2):

$$Y = 0.008X - 0.045$$

where:

X : concentration of aspirin solution.

Y : corresponding absorbance for X.

Slope = 0.008

Intercept = - 0.045

$R^2 = 0.996$ and $R = 0.998$.

R^2 : the linearity,

R: the correlation coefficient.

5. So, the highly significant linear correlation of the straight line of absorbance versus concentration is indicated by the high value of (R) and (R^2), which is close to the highest value of perfect correlation (1.0), and this, will ensure the accuracy of the work and the qualification or precision of the spectrophotometric device and the other work circumstances.
 6. The results of repeating standard D was also accepted and nearly the same. This means reproducible values making our procedure and Beer's law plot usable for determining any unknown aspirin concentration. Therefore, this applied for assaying aspirin in powder or tablet form and as a result, calculating its purity percent.
- In respect to assay of synthesized aspirin, the absorbance of 1ml sample was about 0.716 and the corresponding concentration was 93.7 ppm instead of the actual one 100 ppm and the purity percent was 93.7% according to the following equation:

$$\% \text{ Purity} = \frac{\text{Calculated Conc.}}{\text{Actual Conc.}} \times 100\% \dots\dots (1)$$

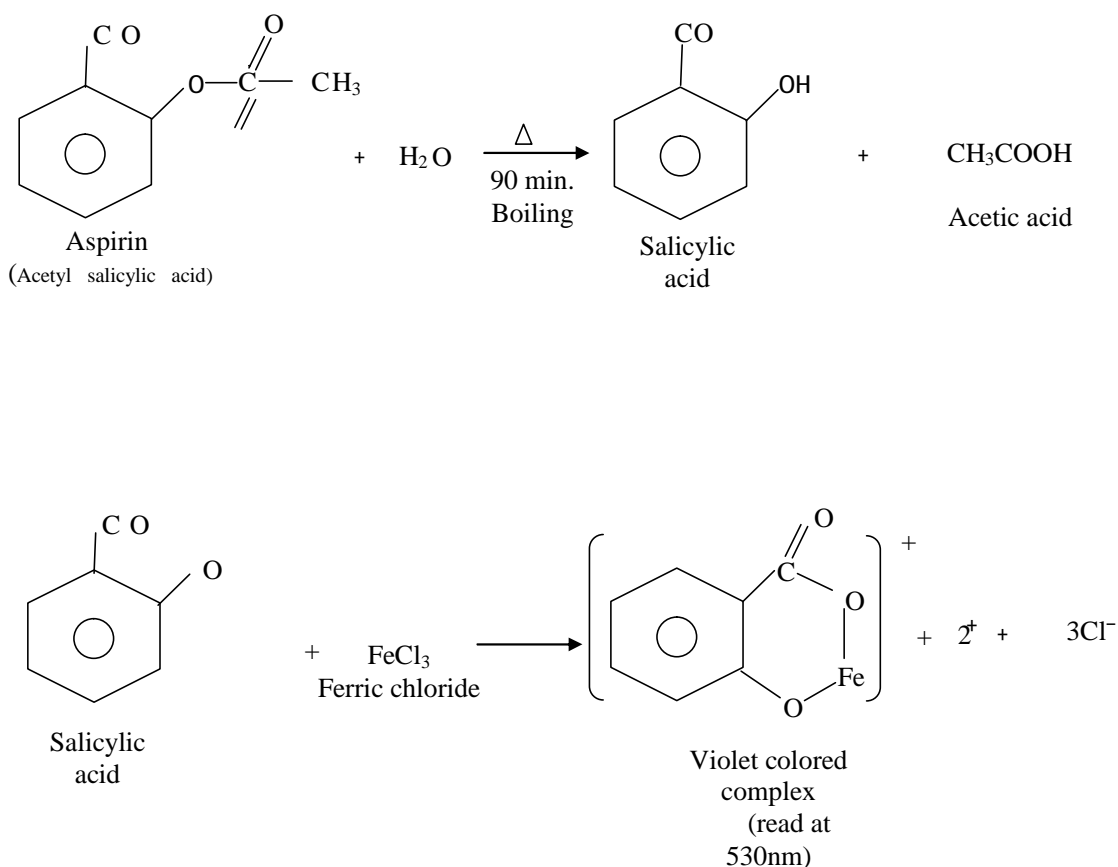
This is not bad value but this laboratory synthesized aspirin needs further purification and recrystallization to be within the allowance of USP or B.P which is not less than 99.5% and not more than 101.0%.

Finally, and as shown on Table (4), the resulted absorbance values of assaying aspirin present in each of three (300 mg)-tablet gave the concentrations of 97.9, 94.2 and 92.2 ppm rather than the actual 100 ppm and ,according to this, the total aspirin weight in each tablet would be 293.7, 282.6 and 276.7 mg respectively. Also the purity percent was calculated for each tablet using equation No. (1).

Therefore, tablet No.1 is more accepted than the two others although they were of the same batch and same strips, this is according to B.P allowance (95- 105%) while USP (90-110%) permits all of these values. This variation between these tablets is owing to the manufacturer itself.

Conclusion

At last, we conclude that we can do the assay of aspirin using this modified method with précised, reliable and reproducible results and without need for alkaline hydrolysis. Also, we can determine the weight of aspirin in tablet packages using this accurate method. This method can be applied in laboratory since it is a simple method.



Scheme (1) : Sequential Equations of Chemical Principle for Assaying Aspirin.

Table (1)
The standards, their concentrations and the corresponding absorbances.

Standard solution No.	Weight of Aspirin (mg) in 1000ppm of stock solution	Final concentration in ppm	Absorbance (unit)
A	0.5	50	0.364
B	0.7	70	0.535
C	0.9	90	0.657
D	1.1	110	0.853
E	1.3	130	1.017

Table (2)
Absorbance values of three different standards D aspirin solutions of concentration 110 ppm.

Standard D of 110ppm concentration	Absorbance (unit)
First experiment	0.849
Second experiment	0.853
Third experiment	0.852

Table (3)
The absorbance values, concentrations and the purity percent of three different 300 mg Aspirin tablets.

Category	Tablet 1	Tablet 2	Tablet 3
Concentration of aspirin solutions in ppm	97.9	94.2	92.2
Absorbance(unit)	0.75	0.72	0.704
Calculated Aspirin weight in tablet	293.7mg	282.6mg	276.7mg
Purity percent	97.9%	94.2%	92.2%

Table (4)
The Standard solutions and their corresponding absorbance values after 4 weeks of hydrolysis process.

Final concentration in ppm	Absorbance (unit)
50	0.34
70	0.513
90	0.671
110	0.848
130	0.995

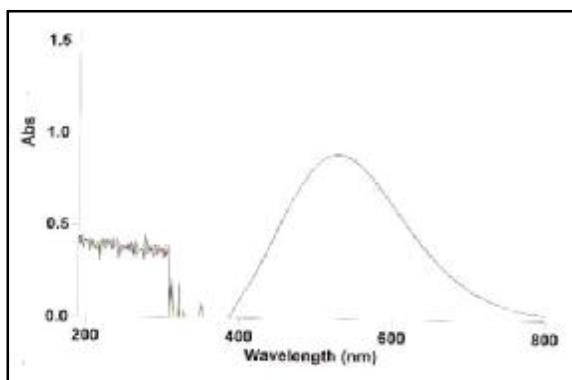


Fig.(1) : UV-Visible Spectrum of the Violet Colored Complex.

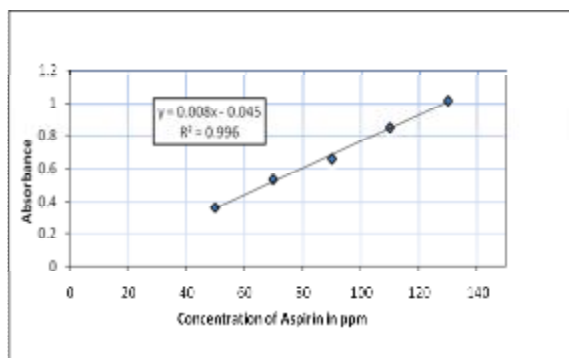


Fig.(2): Calibration Curve shows the relationship between the Concentrations against Absorbance.

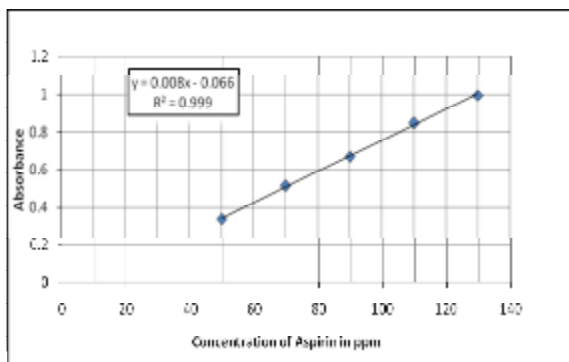


Fig.(3) : Calibration Curve shows the relationship between the Concentrations against Absorbance after four-week hydrolysis.

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الخلاصة

في هذا البحث تم تطبيق دراسة كمية ونوعية لتحسين الطريقة المتبعة لتقييم الأسبرين باستخدام مقياس الضوء الطيفي المرئي. هذه الطريقة تعتمد على التحلل المائي للأسبرين ثم معاملته مع محلول كلوريد الحديدك الحامضي ليعطينا معقداً بنفسجي اللون مع حامض الساليسليك المتكون كنتيجة للتحلل الذي له أعلى أمتصاص في 530 نانومتر. لقد طبقت هذه الطريقة لتحديد نقاوة مسحوق و حبة الأسبرين وكانت النتائج متقاربة حيث ان مقدار الخطية كان ملحوظاً من القيمة العالية لكل من معامل الارتباط ($r = 0,998$) ومعامل التعيين ($r^2 = 0,996$) في حين ان الأمتصاصية (3×10^3 مول⁻¹. لتر. سم⁻¹). ان وجه التحسين في هذه الطريقة هو إلغاء تحلل الأسبرين في الوسط الحامضي أو القاعدي المستخدم في جميع طرق تقييم الأسبرين ويمكن تطبيق هذه الطريقة في تقييم حامض الساليسليك أيضا ."