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Determination of Ascorbic Acid (Vitamin C) by Direct Injection Enthalpimetry (DIE) Technique

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Abstract

The ascorbic acid content of juices of some fruits and pharmaceutical tablets of Vitamin C was determined by a homemade apparatus of DIE technique using a thermocouple as heat sensor. The method is simple, speed, low cost and the different types of turbid, colored samples can be analyzed without any problem. The results were of a valuable accuracy and precision, and the recovery of results was with acceptable values.

Keywords: DIE determination of Vitamin C, Potassium Dichromate.

Introduction

The Vitamin C plays an important role in controlling infections and the body's response to stress. It is also found to be a powerful antioxidant that can neutralize harmful l free radicals; helps make collagen, a tissue needed for health bones, teeth, gums and blood vessels [1, 2, and 3].

The term Vitamin C refers to both ascorbic acid and dehydro-ascorbic acid, since both compounds exhibit anti scorbutic activity [4]. Ascorbic acid, Vitamin C is an important nutrient that is present in many foods. Various reports show that fruits are excellent sources of Vitamin C. Ascorbic acid is synthesized for use as a drug, which occurs as white or slightly yellow crystals or powder or tablets with an acidic taste.

Numerous methods have been reported for determination of ascorbic acid in verity sample of different fruits, vegetables, pharmaceutical preparations, and commercial samples these methods include:

1. Titrimetric Method:

   Depending on reduction of ascorbic acid by iodine [5] or 2,6-Dichlorophenolindophenol [6,7], these methods allowed for determination of 0.01M Ascorbic Acid.

2. Spectrophotmetric Methods

   Many spectrophotometric methods depend on the reduction of Fe (II) to Fe (III) with ascorbic acid, followed by the complexation of reduced Fe (II) with different

reagents [8.11]. Two reagents, nitrosobenzene and P-nitro notrosobenzene have been studied for analysis of ascorbic acid by its hypo chromic effect on reagents [12].

3. Chromatographic methods:

Especially HPLC technique [13], used for determination of ascorbic acid in foods, biological samples, and pharmaceutical preparations the method was used for determination of 10^{-4}M ascorbic acid.

4. Electrochemical methods:

Including potentiometric [14] and amperometric titrations [15] and differential pulse polarograhy [16]

These methods depended also on the oxidation property of ascorbic acid. Amperometric flow-injection methods using immobilized enzyme reactions [15] or photochemical reduction of methylene blue [17] has also been recommended for pH=3.8. The methylene blue method allows the determination of ascorbic acid in the range of 5-90 mg/L.

5. Other Methods like, fluirimetric method [18], photo-oxidation of ascorbic acid [19], and flow-injection method [20], were used the photo-oxidation of ascorbic acid sensitized with thionine blue was investigated by Perez-Ruriz, et.al [19] for its determination ($8*10^{-7}$ – $5*10^{-5}$M) in pharmaceuticals.

DIE technique:

DIE is an analytical method in which a reactant (excess amount) is injected into a colorimetric vessel containing another reagent (usually the analyte). The enthalpy change of the insuring reaction is measured and directly related to the amount of the limiting reagent or analyte.

The main advantages of the DIE method are not necessary to standardize the reagents provided, it is added in sufficient excess to make the reaction at least 99% complete [21]and the speed with which individual determinations may be carried out. The addition of excess reagent favors fast kinetics and a “temperature pulse” may be recorded in less than one minute.

Any rapid process involves a heat of reaction (ΔH) > [1 K.Cal /mol] is suitable to use in direct injection enthalpimetry Fig.-1- (DIE) whether exothermic or endothermic. This technique is successfully applied on different types of reactions. The redox reaction of ascorbic acid with potassium dichromate in acidic medium which is exothermic reaction is the base for this work.

$$3C_6H_8O_6 + Cr_2O_7^{2-} + 8H^+ \rightleftharpoons 2Cr^{3+} + 3C_6H_6O_6 + 7H_2O$$

An acidified solution of ascorbic acid placed in an isolated vessel and excess amount of standard $K_2Cr_2O_7$ injected rapidly to it, then the ΔT was measured, through a 0.5 minute from the injection.

Experimental

Reagents:

All the chemicals used were either of the analytical grade or the highest purity available, unless otherwise stated. Usual distilled water, protected from carbon dioxide was used for all preparations.

- Diluted 5M H_2SO_4 {2ml used for each injection) was prepared from the concentrated acid (98% from Merck).
- Standard potassium dichromate (0.5 M) was prepared from the solid salt $K_2Cr_2O_7$ (BDH).
- A stock solution of ascorbic acid (2M) was prepared from solid reagent (Merck), freshly used for preparation of the calibration curve.
- Tablets of vitamin C from different pharmaceutical factories and juices from different fruits were analyzed.

Apparatus:

The homemade isolated system was prepared as shown in Fig. -2- which consists of a block of a Styrofoam and a plastic beaker (50ml) was fixed inside it. A Styrofoam cover was made also and two small holes were made through it, one for passing the sensor and other for passing small tube for injection.

Recommended Procedure:

A- Vitamin C tablets:

A tablet of vitamin C was weighed and dissolved in distilled water in a plastic beaker and acidified with 2ml 5M H_2SO_4, then the volume was completed to about 25ml and the plastic beaker was placed inside the block of Styrofoam in its position, then covered, the thermometric sensor was put inside the solution and also the tube of injection, when the thermal equilibrium was recorded, 2ml of 0.5M $K_2Cr_2O_7$ solution was injected rapidly by a syringe. The output signal (ΔT) was recorded as a peak height (Fig.-2-). The (ΔT) measured by the temperature difference before injection (when a thermal equilibrium reached) and after 30 sec. following the injection. The value of (ΔT) is proportional and to the amount of ascorbic acid present. This process was repeated for five tablets individually and the average value of (ΔT) was reduced.

B- Calibration Curve:

Preparation of the calibration curve was performed by taking a series of (0.5, 1, 2, 3, 4 and 5ml) portion of standard ascorbic acid solution (1000ppm). The same steps performed as above for the tablets to read out the (ΔT) obtained.

The calibration curve (figure -3-) obtained by drawing the (ΔT) obtained against the amount of ascorbic acid in(0.5-5 mg), as shown in (figure -3-). The amount or percentage of the ascorbic acid in the different vitamin C tablets from different pharmaceutical preparations were obtained from the calibration curve. The results were described in (table -1-)

C- Juices of different fruits:

Some fresh ripe orange, lemon and grapefruit were weighed accurately and the fruit were peeled and cut into two transversely and then squeezed to discharge their juice into a pre-washed beaker, sufficient juice was obtained for each kind. A 25ml of a liquate of each juice after acidification with H_2SO_4 solution was put in the plastic beaker and the ascorbic acid content was determined as performed for vitamin C tablets.

Results and Discussion

The sample matrix in pharmaceutical formulation is complex due to presence of a variety of complexs such as active substances and a large number of additives which act as supporting material, binders or stabilizers. Also the fruit juice samples are complex and contain many other constituents. The DIE technique depending only on the (ΔH) obtained from the reaction of the ascorbic acid with the reagent, so the method does not suffer from effect of color or turbidity like in titrimetric or spectroscopic methods or ionic strength of the

sample mixture like in electrochemical methods of analysis, Possible interferences affect on the results of these methods:

Sulfuric Acid Concentration:

Effect of sulfuric acid studied and it was found that the concentration of 5M is suitable for these measurements. Although the process of mixing of sulfuric acid with aqueous solution of the pharmaceutical samples and juice is an exothermic, but during the preparation of sample solution for injection, the reagent was not injected until the thermal equilibrium was obtained as described in the recommended procedure.

$K_2Cr_2O_7$ Concentration:

In DIE technique the main condition is that the amount of the reagent injected in each injection must be in excess, and the addition must be rapid as a pulse so that a maximum (ΔH) obtained in a certain small limited time (0.5 minutes) and it was found that the concentration of (0.5M) is more suitable for this purpose specially for the range of ascorbic acid amount which we work on it. The main advantage of DIE technique as described before is that the standardization of reagents not required, this provides a fast, simple procedure for application.

Determination of Ascorbic Acid % in different fruits and pharmaceutical preparations:

The ascorbic acid content in juices of three different fruits (orange, lemon and grapefruit) were successfully determined by the proposed DIE method, results are shown in table-1- which is the average value of the five samples of each fruit.

The standard deviation of the five measurements for each juice was found between (0.4 – 0.6) which indicate the reproducibility of the results, and also the content of ascorbic acid which obtained for these fruits are agreed with the average contents of ascorbic acid in these fruits.

In order to establish the validity of the new DIE method for determination of ascorbic acid, different types of vitamin C were analyzed, the same type of these tablets were also analyzed by the reference titrimetric method of " British pharmacopeias" [15], results of the two method as shown in table-2- show a good agreement.

Also the ascorbic acid contents of the juices which found are with the acceptable range of these types of the fruits.

The accuracy of the proposed DIE method was also obtained by performing a standard addition method by addition of different amounts (25, 50, 75 and 100 mg) of pure solid ascorbic acid to the solution of vitamin C tablet (250 mg from Alta pharma). The recoveries which obtained as shown in (table-3-) are with acceptable range:

The results obtained from each tablet are the average of four measurements for each tablets, the standard deviations of the four repeated measurements for each tablet was between (0.3 – 0.5), this also indicates the good precision of the results obtained.

Conclusion

The proposed DIE method is very simple, speed, precise and accurate. The main advantage of this method is that it does not suffer from the effect of interferences which present in the sample matrix (juice or pharmaceutical preparations), the method also can be successfully applied for different samples containing ascorbic acid with acceptable accuracy and precision. The sensitivity of the method can be greatly improved by using a more sensitive heat sensor than thermocouple which used, like a thermistor or any other sensitive heat sensor.

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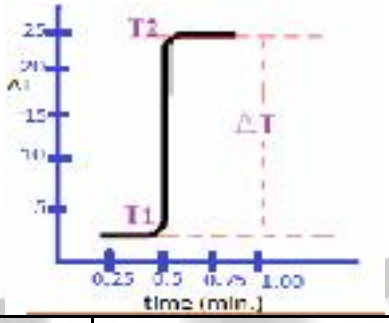
Table(1):Determination of ascorbic acid in different juices

Juice Sample	ppm Ascorbic Acid
Orange	580
Lemon	300
Grupe fruit	450

Table(2): Comparison of the results obtained for determination of ascorbic acid content in different pharmaceutical preparations by DIE method and reference method

Vitamin C	Amount	Amount found by	E%	Amount found by	E%

tablets form	nominal (mg/tablet)	DIE method (mg/tablet)		titrimetric method (mg/tablet)	

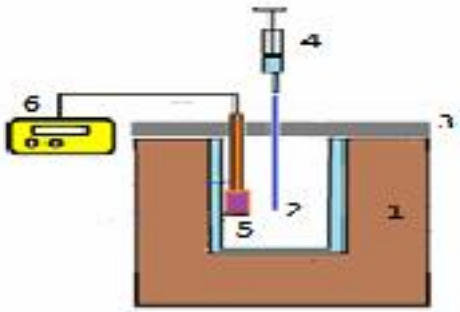


Alta pharma. Germany	250	246.7	-1.32	247.1	-1.16
German Vit. Germany-pol (LTD)	180	184.0	-2.22	183.2	-1.77
Cal-C-Vita "Bayer"	1000	985.6	-1.44	988.1	-1.19
AL SHAHAB labs. "Syria"	500	507.7	-+1.54	506.5	+1.30

Table (3):Recovery experiments for ascorbic acid to Vitamin C tablets

mg Ascorbic Acid in Vitamin C tablets	mg pure A.A. added	mg pure A.A. present	mg pure A.A. found	Recovery %
250	25	275	272.2	98.98
250	50	300	296.4	98.80
250	75	325	330.1	101.50
250	100	350	358.5	102.43

Fig.(1):DIE Thermogram



1. A Styrofoam block
2. Plastic beaker
3. A Styrofoam cover
4. Small syringe for injection
5. Thermocouple sensor
6. Temperature reading

Fig.(2): The arrangement for the Injection Process

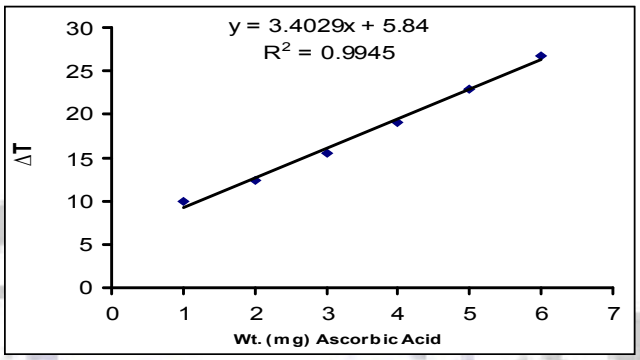


Fig.(3): A Calibration Curve between the (ΔT) obtained and the amount of A.A.

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DIRECT INJECTION ENTHALPIMETRY (DIE)

احمد عبد الله

قسم الكيمياء كلية العلوم جامعة السليمانية

الخلاصة

قدرت نسبة حامض الاسكوربيك (فيتامين c) في عصائر مختلف الفواكه والمستحضرات الصيدلانية (حبوب فيتامين c)بتقنية تقدير المحتوى الحراري عند الحقن المباشر بوساطة جهاز مصنوع محليا في المختبر مع استخدام Thermo couple

الطريقة بسيطة ،وسريعة ،ورخيصة التكلفة وكذلك يمكن تقدير محاليل ملونة وعكرة من دون اية مشكل .

أظهرت النتائج انها ذو دقة وتوافق قيمة وكذلك ذو نسبة استعادة مقبولة ايضا .