AL-Qadisiya Journal For Science Vol .16 No.4 Year 2011

# Extraction, Identification and Determination of Tannin from Iraqi Pomegranate peels and cores by Aqueous solutions

**Ismail K.Al-Hitti and Sattar Salim Ibrahim**

**Chem.Dept. College of Science, Al-Anbar Univ., Ramadi, IRAQ.**

## Abstract:-

High percentages of tannin were obtained from Iraqi pomegranate peels and cores by aqueous solutions extraction. The extracts were identified by FTIR and UV-Vis spectrophotometry and found to be as tannic acid formed of two units of gallic acid. The proposed combination of extracted tannic acid with copper was in the 1:1 mole ratio as a result of reduction copper (II) to copper (I) by tannic acid and combination with it to form copper tannate. This mole ratio was supported by the volatilization method of copper tannate at $500^0$C in addition to iodimetric titration methods.

## Introduction:

Pomegranate (Punica grantum L.) is one of the oldest fruits and is widely grown in many tropical and subtropical countries. (1)

Pomegranate juice and peel contain substantial amounts of polyphenols such as ellagic tannins, ellagic acid and Gallic acid (2). It has been used in the preparation of tinctures, cosmetic, therapeutic formula and food recipes (3).

In this regard, pomegranate peel is a good source of antioxidants especially tannins which are water soluble phenolic compounds having molecular weights between 500 – 3000 and giving the usual phenolic reactions (4).

In recent years, many attempts have been carried out to study natural antioxidants particularly those of plant origin (5). Great interest has recently been focused on the addition of polyphenols to foods and biological systems due to their well- known abilities to scavenge free radicals as a result of their antioxidant power (5).

Extraction is a key step for obtaining antioxidants tannins with acceptable yield. The extraction yield and economic viability is dependent on the polarity of solvent and the method of extraction.

**Keywords:** **Pomegranate peels and cores, Tannic acid, Extraction, Volatilization, Mole ratio, Stoichiometry.**

The phenolic compounds of pomegranate peel extracted by solvent and ultra sound –assisted with five solvents were compared with the supercritical fluid extraction (SFE) [6]. The overall results showed that acetone with sonication produced the maximum amount of phenolic compounds from pomegranate peel extracts (PPE) [6]. The aim of this research was the aqueous extraction of tannic acid, its identification and determination its amount in Iraqi pomegranate peels and cores.

Experimental:

## Materials and methods:

Pomegranates were obtained from local market. The peels were manually removed and isolated from cores. Both peels and cores were sun – dried or at $120^0$C in an oven until dryness. After complete dryness, the peels and cores were powdered in a grinder to reach 40 - mesh and then packed and stored in a refrigerator at $4^0$C.

All chemicals were of analytical grade and of highest purity obtained from Fluka and BDH.

**Method of extraction:**

The tannins were extracted by addition 75 ml of distilled water to 0.5g of powdered peels and cores separately in boiled water bath for 30 minutes. Four samples were taken for both peels and cores powders. After cooling, the mixture was centrifugated at 2000 rpm for 20 minutes. The supernatant liquid was transferred to 100ml volumetric flask and completed to the mark by distilled water.

**Precipitation method for tannin determination[7]:**

A 20ml of copper acetate (4%) was added to the 100ml of the extract in 400ml beaker and stirred for one hour. The mixture was filtered through whatmann 42 filter paper.

The residue was moved into porcelain crucible and dried in an oven at $110^0$C and then cooled and weighed.

**Percentage of tannin in extracted copper tannate:**

The accurate weight of copper tannate was transferred to a muffle furnace in porcelain crucible and ashed at $500^0$C for two hours. The residue was accurately weighed and the difference in weight before and after ashing represents the weight of volatilized tannin.

**Iodimetric determination of copper after ashing [8]:**

The residue after ashing was accurately weighed and dissolved in 10ml of concentrated nitric acid and heated on water bath to dryness. The residue was cooled and dissolved in distilled water to 100ml.

10ml of the above solution was transferred into conical flask and little amount of solid $Na_2CO_3$ was added to render the solution slightly alkaline. 10ml of 10% KI was added and the liberated $I_2$ (which is equivalent to copper) was titrated with standard sodium thiosulphate solution (0.05N) until the solution became pale yellow. One milliliter of starch (1%) was added and then the titration

was resumed until colorless [9]. The titration was repeated three times more and the average was used in calculation.

**Tannin test [10]:**

The tannins were identified by boiling 0.5g of peels and cores powders separately in 50ml of distilled water. The solution were filtered and cooled. Several drops of 1% of ferric chloride solution were added with stirring.

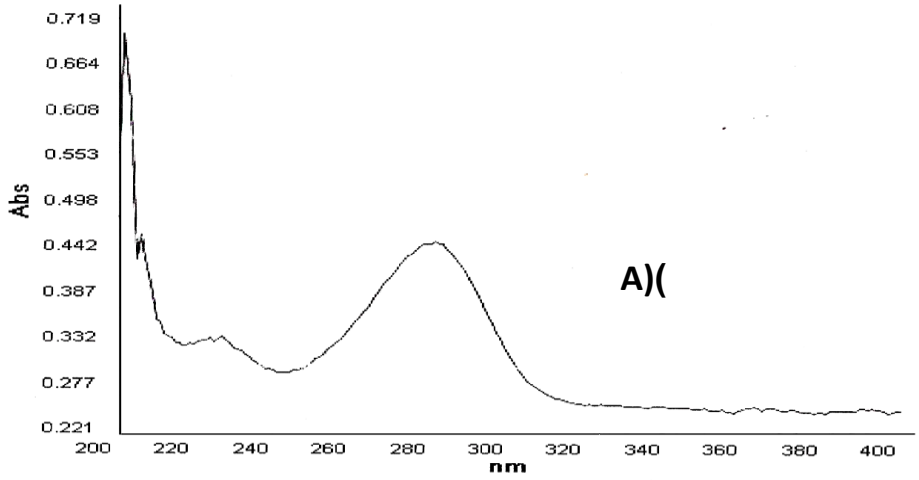
The appearance of greenish blue colour is an indication to tannins evidence [11].

## Results and discussion:

Identification of the extracted tannin:

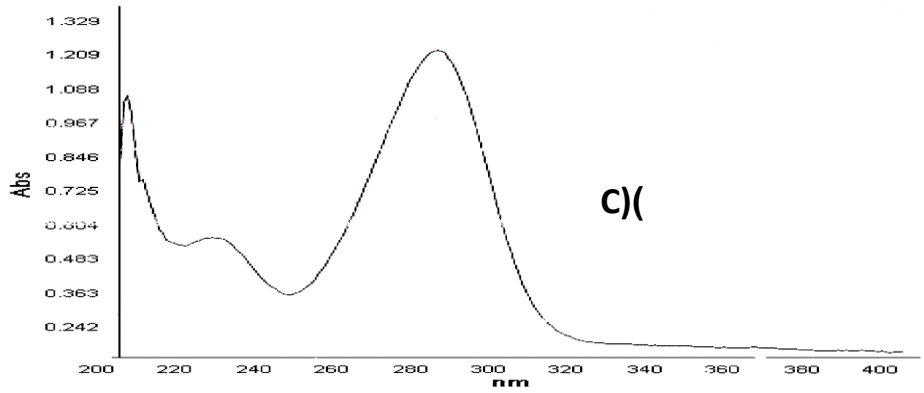
UV-Spectral analysis:

Fig.(1) shows UV spectra of the extracted tannin from peels (A), cores (B), the complex (C) and pure tannic acid (D). The spectra of A and B are similar which means that the extracts of peels and cores of pomegranate are the same.



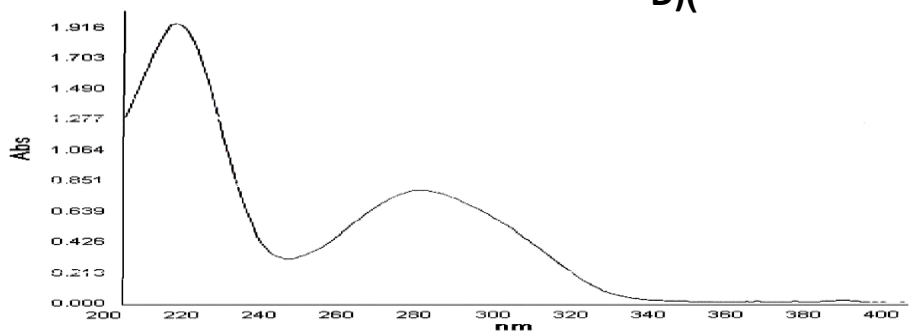

There is small peak in the middle of spectra A and B which may be due to ellagic acid [12].

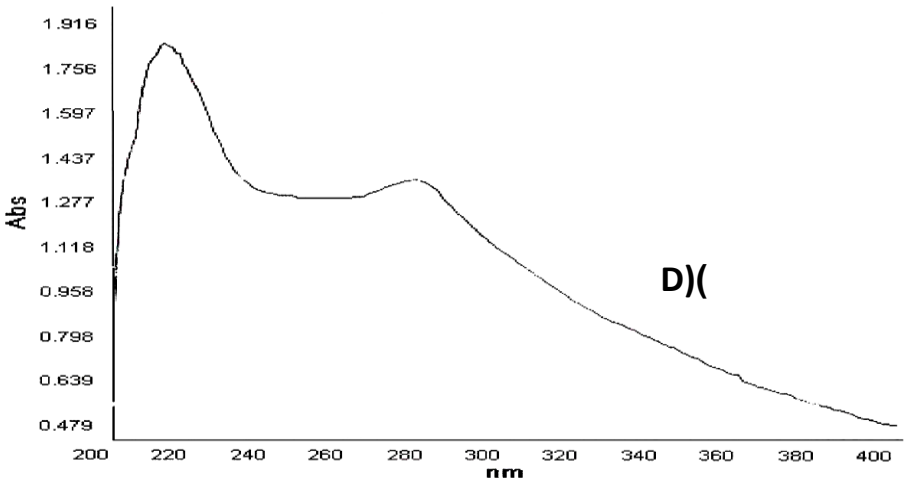
B)(


1.329
1.209
1.088
0.967
0.846
0.725
0.604
0.483
0.363
0.242
Abs
200 220 240 260 280 300 320 340 360 380 400
nm

C)(

D)(


1.916
1.703
1.490
1.277
1.064
0.851
0.639
0.426
0.213
0.000
Abs
200 220 240 260 280 300 320 340 360 380 400
nm


1.916
1.756
1.597
1.437
1.277
1.118
0.958
0.798
0.639
0.479
Abs
200 220 240 260 280 300 320 340 360 380 400
nm

D)(

Fig.(1): UV – Spectra of pomegranate peels tannin (A), cores tannin (B), tannin complex (C) and pure tannic acid (D).

The shifting of spectra to shorter wavelengths in the complex of the extract is an indication to complex formation and preliminary anticipitation that the extracts were tannic acid built of two units of gallic acid (Table 1).

**Table (1): UV- Absorption bands spectra of tannins extracts from pomegranate peels and cores compared with pure tannic acid and tannin complex with copper in ethanol.**

| Component | Absorption bands (nm) |
|---|---|
| Peels extract | 281 227 |
| Cores extract | 281 226 |
| Copper tannate complex | 277 212 |
| Pure tannic acid | 276 214 |

FTIR spectral Analysis:

Fig.(2) depicts the FTIR spectra of peels and cores extracts tannins A and B respectively.

Looking through the spectra, it is obvious that both extracts are identical. These similarities are confirmed from table (2) where the detailed absorptions of the functional groups are compiled. Table (2) shows the absorption peaks of Syrian peels and cores tannin which are also approaching the absorption bands of the pure tannic acid. These spectra assist the combination of two units of gallic acid to form the extracted tannins.

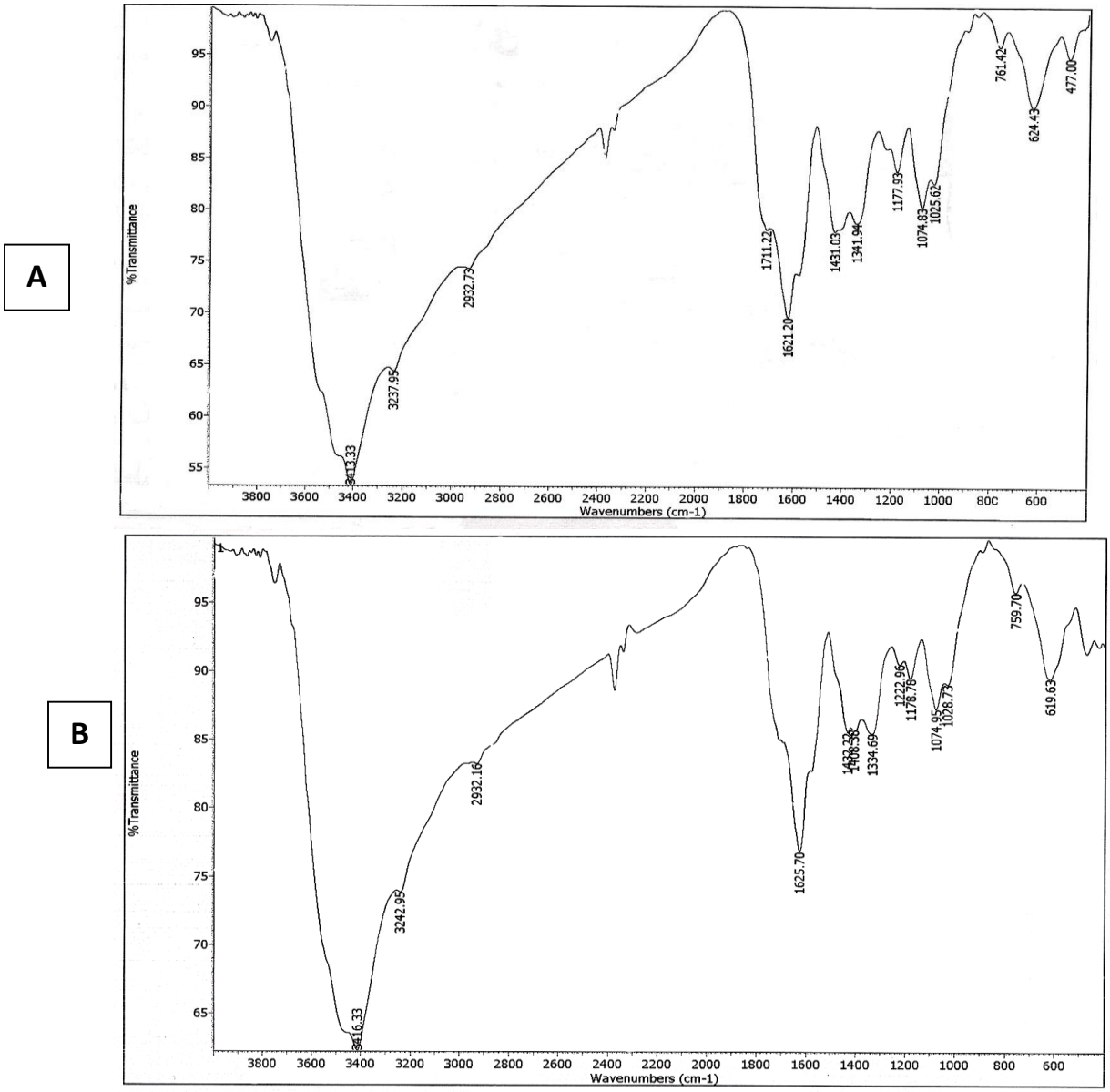



**Fig.(2): The FTIR spectra of peels and cores extracts tannins A and B respectively**

**Table (2): FTIR spectra of pomegranate peels with pure tannic acid.**

| Functional group | Tannic acid pure($cm^{-1}$) | Peels tannins ($cm^{-1}$) | Cores tannins ($cm^{-1}$) | Syrian cores tannins ($cm^{-1}$) | Syrian peels tannins ($cm^{-1}$) |
|---|---|---|---|---|---|
| υ O–H intra H- bond | 3462 | - | - | 3465 | - |
| υ O–H carboxylic acid | 3240 | 3243 | 3238 | 3239 | - |
| υ O–H intra H- bond | 3414 | 3416 | 3413 | 3414 | 3413 |
| υ C–H Aromatic | 2913 | 2932 | 2932 | 2928 | - |
| υ C=O | 1711 | - | 1711 | - | - |
| C=C Aromatic | 1617 | 1625 | 1621 | 1621 | 1624 |
| δ O–H | 1450 | 1432 | 1431 | - | - |
| δ O–H | - | 1408 | - | 1408 | - |
| δ O–H | 1324 | 1335 | 1342 | 1345 | 1343 |
| υ C–O | 1205 | 1223 | - | - | 1263 |
| υ C–O | - | 1179 | 1178 | 1176 | 1178 |
| δ C–O Bending | 1088 | 1075 | 1075 | 1074 | 1074 |
| δ C–O Bending | 1030 | 1029 | 1025 | 1030 | - |
| C–H Aromatic bending | 871 | - | - | - | - |
| C–H Aromatic | 758 | 760 | 761 | 759 | 760 |
| C–H bending | 618 | 620 | 624 | 625 | 619 |

**Percentage of tannin in the extracted peels and cores of pomegranate:**

Three different weights of powdered peels and cores, 0.78g of Iraqi pomegranate peels, 0.80g of Iraqi pomegranate cores and 0.72g of Syrian pomegranate cores were taken to find out the percentage of their tannins containing.

Tannin weights were determined by volatilization of copper tannate at $500^0$C. Table (3) contains the volatilized tannins and their residual weights and the percentages of tannins.

**Table (3): percentages of tannins in three samples of pomegranate peels and cores.**

| Wt. of sample (g) | Wt. of tannins(g) | Wt.of residual(g) | % of tannins |
|---|---|---|---|
| Iraqi peels 0.78 | 0.652 | 0.130 | 83.59 |
| Iraqi cores 0.80 | 0.634 | 0.136 | 79.25 |
| Syrian cores 0.72 | 0.576 | 0.104 | 80.00 |

It is clear from table (3) that high percentages of tannins were present in peels and cores of pomegranate in good agreement with the values mentioned elsewhere [9]. It is obvious also from table (3) that tannins in peels are higher than in cores and the Iraqi and Syrian cores are close to each other in tannins percentages.

**Stoichiometric ratio of copper and tannin in copper tannate:**

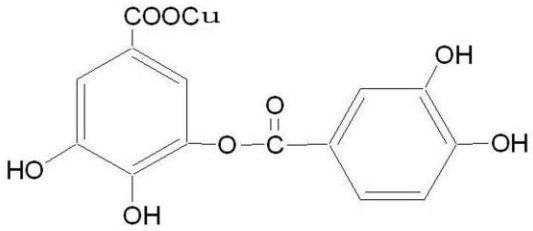
As mentioned in experimental part, the tannin was extracted as copper tannate by addition of 4% copper acetate to the extract.

The precipitate after filtration, washing and drying was ashed at $500^0$C, cooled and weighed. The difference in weight before and after ashing gives the volatilized tannin and the red residue is copper. Table (4) contains the mole ratio of tannin to copper in copper tannate.

**Table(4): Stoichiometric ratio of copper to tannate in copper tannate.**

| Wt. of Copper tannate (g) | Wt. of tannin (g) | Moles of tannins (g) | Wt. of Copper (g) | Moles of copper (g) | Stoichiometric ratio |
|---|---|---|---|---|---|
| 0.782 | 0.652 | 0.002 | 0.13 | 0.002 | 1:1 |
| 0.770 | 0.634 | 0.0027 | 0.136 | 0.0026 | 1:1 |
| 0.680 | 0.576 | 0.00199 | 0.104 | 0.00163 | ~ 1:1 |

It is clear from table(4) that the proposed stoichiometric ratio of copper to tannin in copper tannate is 1:1 this case doesn't occur unless the copper (II) is reduced into copper (I) by tannic acid itself and then the combination with tannic acid to form copper tannate in the following structure:




**Iodimetric determination of copper in copper tannate:**

The average of volumes of sodium thiosulphate solution obtained for iodimetric titration of residual copper were:

8.2 ± 0.09 ml for prepared solution of peels extract.

8.6 ± 0.13 ml for prepared solution of cores extract.

6.6 ± 0.16 ml for prepared solution of Syrian cores extract.

Therefore, the weights of residual copper prepared in 100ml were calculated from the following relation:

$$\left(\frac{V \times N}{100}\right)_{thiosulphate} \times Eq.Wt.of\ Cu \times \frac{100}{10}$$

$$\left(\frac{8.2 \times 0.05}{100}\right) \times 31.75 \times \frac{100}{10} = 0.1302g \text{ in peels extract}$$

$$\left(\frac{8.6 \times 0.05}{100}\right) \times 31.75 \times \frac{100}{10} = 0.1365g \text{ in cores extract}$$

$$\left(\frac{6.6 \times 0.05}{100}\right) \times 31.75 \times \frac{100}{10} = 0.1048g \text{ in Syrian cores extract}$$

The weights obtained by iodimetric method were approximate to weights obtained by volatilization method. The little differences were due to experimental errors.

**Conclusion:**

High percentage of tannins were found in pomegranate peels and cores which were considered as a significant source of plant origin.

The proposed stoichiometric ratio of copper to tannin in copper tannate was 1:1 which suggested that tannin is in the form of tannic acid formed of two units of Gallic acid.

**Acknowledgements:**

The authors would like to express their appreciations to Chem. Dept., College of science, Al-Anbar Univ. for supporting this research. The authors also would like to thank Chem. Dept., Education college for girls for their supplying FTIR spectra.

## References

1- Salaheddin, M. E.and Kader, A. A. (1984), Post Harvest Physiology and Storage Behavior of Pomegranate Fruits, Sci. Hort., 24:287-298.
2- Loren, D. J., Seeram, N.P., Schulman, R.N. and Holtzman, D.M (2005),Pediatric Res.,57,858-864.
3- Finkel,T.and Holbrook,N.J.,(2000),Nature,408,239-247.
4- Singh, R. P, Jayaprakasha, G.K.and Sakariah,K.K. (2001), Indian Patent No. 392 Del/01.
5- Zainal,M.K, Abd Hamid, A.,Yusof,S. and Muse, R(2003),Urban. Food Chem., 81,575-581.
6- Yasoubi,P.,Barzegar, M,.Sahari,M.A. and Azizi, M.H.(2007),J. Agric.Sci.Technol.,Vol.9, 35-42.
7- Mohammed, R., Peng, J., Kelly, .M.and Mark, T. (2006), J. Nat. Prod.69 (12), 1739-1744.
8- "Vogel's Textbook of Quantitative Analysis" (1989). Revised by G-H. Jeffery, J. Bassett, J. Merdham and R.C.Denny, 5thed. English Language Book Society, Longmans, Inc., New York, 5th Ed.
9- Dallali, B.K. and Al-Hakeem,S. H (1987),"Food Analysis Dar Al-Kutob for Press and Publication", Mosul Univ.,Iraq,p.479.
10- 10Harris,W.R.,Carrano,C.G,Cooper,S.R and Raymond,K.V. (1979)"Tannins as Metal Io n, 101 Chelatores",J.Am. Chem.Soc.
11- Stafford. A (1990), Flavonid Metabolism, CRC Press, Boca Raton, FL, 65-830

12- Gil, M.I., Tomas- Barberan, F.A., Pierce, B.H., Holcroft, D.M and Kader, AA. (2000), J. Agric Food Chem.,48, 4581-4589.

AL-Qadisiya Journal For Science Vol .16 No.4 Year 2011

# استخلاص ، تشخيص وتقدير التانين من قشور ولب الرمان العراقي بالمحاليل المائية

**إسماعيل خليل إلهيتي و ستار سالم إبراهيم**

**قسم الكيمياء – كلية العلوم- جامعة الانبار**

**الخلاصة**

تم الحصول على نسب عالية من التانين من قشور ولب الرمان العراقي بالاستخلاص بالمحاليل المائية. شخصت المستخلصات بأطياف أشعة FTIR و UV-Vis ووجد بأنها على شكل حامض التانيك متكون من وحدتين من حامض الكاليك.

كانت الصيغة المقترحة لارتباط النحاس بحامض التانيك المستخلص بنسبة 1:1 نسبة مولية مما يدل على إن النحاس (II) اختزل بحامض التانيك إلى النحاس (I) قد ارتبط به مكوناً تانينات النحاس. وقد تم دعم ذلك بعملية حرق تانينات النحاس في فرن حارق في 500 درجة مئوية إضافة إلى طريقة التسحيح الايوديمترية.