CHAPTER 4

SPECTROPHOTOMETRIC ANALYSIS OF FORENSIC GOLD JEWELLERY AND PHARMACEUTICAL SAMPLES AND RESULTS COMPLEMENTED WITH ICP AES, EDXRF SPECTRAL ANALYSIS

Introduction:

Gold, Au, is highly malleable, ductile, precious metal and rising living standards shown through ornaments is creating a demand for it [Mike McGlone, 2015]. Dissolving of waste printed circuit boards with aqua regia followed by toluene extraction to recover gold emphasises its value and importance [Young Yun Park and Derek J. Fray, 2009]. Gold is mainly used for jewellery and in investments and is also finding its use in dentistry, electronics, medicine and other fields [Richard. J. Holliday and Christopher. W. Corti, 2010]. Gold is also finding clinical application for the treatment of rheumatoid arthritis [Merchant B, 1998] and in the treatment of many other ailments [Astrid Sigel and Helmut Sigel, 2004]. Gold compounds and nanoparticles are also gaining importance in forensic field [Ahmed A. Mohamed, 2011]. One field of research that involves the use of gold nanoparticles in the detection and treatment of cancer cells in the liver is interesting in its medicinal values [Mohamed Anwar et al., 2011] and fortunately it is possible to prepare such gold nanoparticles not only in high quality, but also in high yield [Xiaohua Huang and Mostafa A El-Sayed, 2010]. Extensive application of gold nanoparticles used in clinical research followed by their electrochemical detection methods are discussed by Pranjal Chandra et al. [Pranjal Chandra et al., 2013]. Upto 20th century, the potential chemistry of gold was left unexplored since, much of the use of gold was concentrated for jewellery and coinage applications [Helgard G. Raubenheimer and Hubert Schmidbaur 2014].
Pledging of gold or gold jewellery is a huge and established market known internationally for getting loans [M.S.Sibi, 2014]. Gold is considered as a sign of prosperity and well-being in society and gold loan has become a fastest growing business in India [Nandakumar V.P, 2015]. Making fake jewellery with gold plating is not a rare issue in the society and attempts are made in pledging such jewellery to banks as a mode of availing loan. It is obvious in such instances to register police complaints in jurisdictional police stations [Times of India, 2013, Times of India, 2015] and in those circumstances it becomes mandatory for the police to check the pledged sample for its genuineness or percentage of gold in it. Since gold is a soft metal, it is being alloyed with many other metals to give strength for making ornaments and it is therefore the determination of gold in the presence of other alloyed metals is of importance in forensic laboratories. It is in all these contexts of gold, an attempt is made in this work to develop a spectrophotometric method involving three systems corresponding to gold(III) oxidizing o-dianisidine to form a dye, and oxidizing catechol which then coupling with that to o-dianisidine or to aniline sulphate and employed those systems for the determination of gold present in some forensic jewellery and pharmaceutical samples and the results so obtained are compared with the results of the same samples determined by ICP-AES method.

There are quite a good number of methods available for the determination of gold that are involving electrochemical studies using anodic stripping voltametry for reducing aurate ions in solution to metallic gold [Muammer Kavanoz et al., 2004] and the method needs special glassy carbon electrodes. An ICP–AES method of gold is needing tedious extraction process to remove organic impurities before subjecting gold for its analysis in obtaining reproducible results [MA. Chaudhri and P. Hannaker, 1987]. Other sophisticated
techniques available for its determination include ICP MS analysis of geological samples obtained after nickel sulphide fire assay [Juvonen. R et al., 2002] and Neutron Activation analysis of gold together with other elements present in gold ores [A El-Taher et al., 2003] but such methods are very expensive [Lorenzo Copia et al., 2015, S.S.Ismai, 2010]. Flame Atomic Absorption Spectrophotometric analysis for gold involving organic solvent and its comparison with fire assay and cyanide leach extraction procedures of gold extraction is also available [P.V.Sunder Raju, 2006]. Among all these available instrumental methods for gold determination it appears that its spectrophotometric method is found to be having advantage of procedural simplicity and low cost instrument. Different chromogenic reagents have been used in the spectrophotometric determination of gold, one such methods uses ethopropazine and isothispendyl hydrochlorides [Mahaveer B. Melwanki et al., 2002], and in its solid phase extraction 4-rhodanineazo benzoic acid is used in ethanol medium [Zhang Jie Huang et al., 2009]. There are also visible shortcomings in some of the spectrophotometric methods such as associated with its extraction using of organic solvents dichloromethane, benzene and chloroform considered to be not normal [Patell, K.S and Lieser. K.H, 1986, Tomozo Koh et al, 1986], morin is used in its determination by UV method at 291 nm [Balcerzak. M et al., 2006], there is a lengthy and time consuming procedure of extracton of gold from enriched solid phase cartridge with dimethyl formamide after the formation of red colour gold chelate with 2-carboxyl-1-naphtalthiorholdanine [Chen. Z et al., 2006], and in one method its determination range is very narrow, 0.16-2.24 mg/liter [El-Zawawy et al., 1995]. Phloxine and thiamine as reagents are used for the development of red colour, 570 nm, is highly sensitive but pH of 4.3-4.9 and temperature at 40° C are to be maintained
Solid phase extraction procedures are also available and sensitive but use polymer based cartridges and 5-(2-hydroxy-5-nitrophenylazo)thiorhodanine [Hu. Q et al., 2006], 5-(p-aminobenzylidenc)-thiorhodanine [Zhao, J et al., 2006] and 5-(2-hydroxy-4-sulfo-5-chlorophenolazo)-thiorhodmine [Jingjing Liang et al., 2010]. TritonX-100 is used for staabilizing absorbance at 490 nm for gold(III) with bis(salicylaldehyde) orthophenylenedianmine [Rubina Soomro et al., 2008] and in all these methods the reagents involved are less common and expensive. It is therefore and also knowing the improtance of gold and need for its simple spectrophotometric determination in some real gold samples of forensic and pharmaceutical samples a new spectrophotometric method is developed in this present work for the estimation of gold(III) and the method involves three separate systems each one is either an amine or amine plus catechol based. Specifically, the system 1 is based on the oxidation of o-dianisidine (3,3’-dimethoxy benzidine) by gold(III) ion to form a red coloured dye having \(\lambda_{\text{max}}\) at 446 nm and the reagent was earlier used for developing color with hydrogen peroxide and peroxidase enzyme [Wei Sun et al., 2005, Flavio Luiz Benedito et al., 2003; A.V.Kireyko et al., 2006], the system 2 is involving the oxidation of catechol by gold(III) to produce a nucleophile which will couple with o-dianisidine to produce a dye product with \(\lambda_{\text{max}}\), 540 nm and the system 3 is like system 2 but with catechol and aniline sulphate producing light violet colour dye having \(\lambda_{\text{max}}\) at 505 nm. Unlike o-dianisidine, aniline sulphate alone does not produce colour with gold(III). The concept of using catechol to improve the sensitivity of the colour produced in systems 2 and 3 for gold is borrowed from the earlier methods for the determination of iron(III) [Shyla.B et al., 2012, Mansour S. Abdul Galil et al., 2009], Cr(VI) [Suresha. M.S et al., 2007] and Mn(III)
[Shyla. B and Nagendrappa. G, 2013] that are with catechol and different amines. Conditions of all these three systems constituting this new method are optimized for the effective determination of gold present in the forensic jewellery and pharmaceutical samples. Results obtained from each one of the three systems are compared with the results obtained from Inductively Coupled Plasma Atomic Emission Spectrometric analysis of the same samples and the results from both the methods are found to be accurate and comparable. Among the three systems 1-3 of the spectrophotometric method for gold, the system 2 is found to be having a wider linearity range of gold determination, greater stability of the dye and also better in giving precision results than the other two systems 1 and 3.

**Experimental:**

**Materials and Methods:**

Double Beam UV-Vis Spectrophotometer, model Spectrascan UV 2600 (Chemito, Chennai, India), Inductively Coupled Plasma Atomic Emission Spectrometer, model JY 2000 (Jobin Yvon Horiba, France), God(III) chloride trihydrate from Sigma Aldrich and all other reagents used here were of analytical grade and water used was distilled water.

**Preparation of stock solution of God(III) chloride, 100 µg ml⁻¹:**

An accurately weighed amount of 0.2 g of gold(III) chloride trihydrate (MW=393.84 g mol⁻¹) was dissolved in water and made up to 100 ml to get a stock solution of 1000 µg ml⁻¹ and 10 ml of this solution was diluted to 100 ml to get 100 µg ml⁻¹ gold(III) solution which was used in the experiments.

**o-Dianisidine and aniline sulphate, 1000 µg ml⁻¹:**

An accurately weighed amount, 0.1 g o-dianisidine (MW=244.3 g mol⁻¹) or aniline sulphate (MW=284.33 g mol⁻¹) was weighed and transferred it to a beaker, dissolved
initially in small quantity of alcohol in case of o-dianisidine or in water for aniline sulphate, transferred the solution into a 100 ml volumetric flask and diluted to the mark with water.

**Catechol, 0.005 M:**

An accurately weighed amount, 0.275 g catechol (MW=110.1 g mol⁻¹) was transferred to a beaker. It was dissolved in water and transferred the solution into a 100 ml volumetric flask and diluted the solution to the mark with water.

**Phosphoric acid, 1 M:**

A measured volume, 6.6 ml concentrated orthophosphoric acid (85%, 1.7 g cm⁻³) was diluted to 100 ml with water for obtaining its 1 M solution.

**Inductively Coupled Plasma Atomic Emission Spectrometer parameters:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>1100W</td>
</tr>
<tr>
<td>Plasma gas flow rate</td>
<td>15(PL1) L/min</td>
</tr>
<tr>
<td>Sheath gas flow rate</td>
<td>0.1(G1) L/min</td>
</tr>
<tr>
<td>Sheath gas stability time</td>
<td>3.0 s</td>
</tr>
<tr>
<td>Nebulization flow rate</td>
<td>0.02 ml/min</td>
</tr>
<tr>
<td>Nebulization pressure</td>
<td>1.0 bar</td>
</tr>
<tr>
<td>Sample uptake</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Rinsing time</td>
<td>5.0 s</td>
</tr>
<tr>
<td>Rinsing pump speed</td>
<td>High</td>
</tr>
<tr>
<td>Transfer time</td>
<td>10.0 s</td>
</tr>
<tr>
<td>Stabilization time</td>
<td>0.5 s</td>
</tr>
<tr>
<td>Transfer pump speed</td>
<td>Normal</td>
</tr>
<tr>
<td>Wavelength</td>
<td>242.795 nm</td>
</tr>
</tbody>
</table>

The spectrum of gold solution obtained with ICP AES under the above mentioned conditions [Li-qiang Xu and Peter Schramel, 1992] is as shown in Fig.1(a) and calibration graph produced for the intensities of 0, 4, 8, 12 and 16 ppm solutions is as shown in Fig. 1(b).
Fig. 1: [a] CP-AES spectrum of 8 µg ml\(^{-1}\) gold(III) chloride solution showing the wavelength of maximum absorption at 242.795 nm (top). [b] Calibration graph used in the determination of gold in real samples (bottom).

**Recommended procedure:**

**System 1:** To a series of labeled 10 ml volumetric flasks, 0.5 ml, 1000 µg ml\(^{-1}\) o-dianisidine and 0.5 ml, 1 M phosphoric acid were added. Different aliquots of gold(III) solution was added to each flask (corresponding to 0.05-5 µg ml\(^{-1}\) of gold) and made up to mark with water. Absorbance of each solution was measured at 446 nm.

**System 2:** To a series of labeled 10 ml volumetric flasks, 0.5 ml 1000 µg ml\(^{-1}\) o-dianisidine, 1.0 ml 0.005 M catechol and 0.5 ml, 1 M phosphoric acid were added. Different aliquots of gold(III) solution was added to each flask (corresponding to 0.5-40
µg ml\(^{-1}\) of gold) and made up to mark with water. Absorbance of each solution was measured at 540 nm.

**System 3**: To a series of labeled 10 ml volumetric flasks, 0.5 ml 1000 µg ml\(^{-1}\) aniline sulphate, 1 ml of 0.005 M catechol and 0.5 ml, 1 M phosphoric acid were added. To each flask a known aliquots of gold(III) corresponding to its concentration, 0.8-25 µg ml\(^{-1}\) was added and made up the solution to the volume with water. Absorbance of each solution was measured at 505 nm.

**Results and discussion:**

Selection of wavelengths of the systems 1, 2 and 3: Gold is known to produce colours with number of reagents including o-tolidine, rhodanine and o-dianisidine [Beamish F.E, 1966, Mahadevaiah et al., 2007]. Using o-tolidine the spectrum was recorded for gold(III) solution in acid medium which produces green colour with λ\(_{\text{max}}\) at 628 nm not very stable but with using catechol the colour obtained was pink with λ\(_{\text{max}}\) at 537 nm, Fig. 2 were recorded by scanning between 400 to 700 nm. However, in the present study, reactions of o-tolidine were not considered. The wavelengths of maximum absorption (λ\(_{\text{max}}\)), 446 nm, 540 nm and 505 nm were found for the system 1, 2 and 3 respectively when the respective system gold(III) solution was scanned with the spectrophotometer from 400 to 700 against the reagent blank and the resulting spectra are shown in Fig. 3.
Fig. 2: Spectrum of gold(III) solutions with o-tolidine having λ max at 628 nm (above) and o-tolidine along with catechol with λ max at 537 nm (below).

Fig. 3: Absorption spectra of the solutions A, B and C under their optimized conditions, [A]: 4 µg ml⁻¹ gold(III) with o-dianisidine having λ max, 446 nm, the system 1; [B]: 10 µg ml⁻¹ Au(III) with catechol and o-dianisidine having λ max, 540 nm, the system 2 and [C]: 8 µg ml⁻¹ Au(III) with catechol and aniline sulphate having λ max, 540 nm, the system 3.

After optimizing the conditions of all the three systems, linearity for gold determination was established. The linear range and other analytical parameters obtained are shown Table 1. For the estimation of gold in real samples, calibration graphs were
plotted in the concentration range 1.0–5.0 ppm by recording the absorbances of the solutions at 446 nm for the system 1 and the overlaid spectra are shown in Fig. 4. Similarly, spectra were recorded at 540 nm and 505 nm for the systems 2 and 3 respectively in the gold(III) concentration range 2.0-10.0 ppm and their corresponding spectra are shown in Fig. 5 & 6. Five replicates of these determinations were carried out for each concentration and the average absorbance values obtained were used in the construction of their calibration graphs and are indicated in Fig. 7.

**Fig. 4:** Overlaid spectra of 1 – 5 ppm Au(III) solutions, each was with fixed volumes of 0.5 ml 1M phosphoric acid and 0.5 ml of 1000 µg ml⁻¹ o-dianisidine, obtained by scanning them from 400 nm to 700 nm, the system 1.

**Fig. 5:** Overlaid spectra of 2 – 10 ppm gold(III) solutions but each solution was having fixed volumes, 0.5 ml 1M phosphoric acid, 1.0 ml of 0.005 M catechol and 0.5 ml of 1000 µg ml⁻¹ o-dianisidine, obtained by scanning the solutions from 400 nm to 700 nm, the system 2.
Fig. 6: Overlaid spectra of 2 – 10 ppm gold(III) solutions but with fixed volumes, 0.5 ml 1M phosphoric acid, 1.0 ml of 0.005 M catechol and 0.5 ml of 1000 µg ml\(^{-1}\) aniline sulphate, obtained by scanning them from 400 nm to 700 nm, the system 3.

Fig. 7: Calibration graphs under optimized experimental conditions obtained from the set of solutions, each one with different volumes of gold(III) solution + 0.5 ml 1M acid and with 0.5 ml of 1000 µg ml\(^{-1}\) o-dianisidine in the system 1 (■), 1.0 ml of 0.005 M catechol + 0.5 ml of 1000 µg ml\(^{-1}\) o-dianisidine in system 2 (x), and 1.0 ml of 0.005 M catechol + 0.5 ml of 1000 µg ml\(^{-1}\) aniline sulphate in system 3 (▲).

**Optimization of conditions:**

a) Effect of different volumes of catechol: Catechol has been used in systems 2 & 3 along with o-dianisidine and aniline sulphate solutions as a coupling agent. To a series of labeled 10.0 ml volumetric flasks, 2.5 ml 100 µg ml\(^{-1}\) gold(III) solution, 0.5 ml of 1 M phosphoric acid and 2.5 ml 1000 µg ml\(^{-1}\) o-dianisidine solution for the system 2 and 2.5
ml 1000 µg ml⁻¹ aniline sulphate solution for the system 3 were added. Then 0.1 – 3.0 ml of 0.005 M catechol was added to each flask, diluted to 10 ml with water and absorbance was measured at 540 nm for the system 2 and at 505 nm for the system 3. The results obtained are indicated in Fig. 8 which shows that 0.5 ml, 0.005 M catechol was required to be used.

Table 1: Analytical parameters for the estimation of gold(III) chloride through the system 1 involving gold(III) with o-dianisidine reagent, the system 2 involving gold(III) with catechol and o-dianisidine reagents and the system 3 involving gold(III) with catechol and aniline sulphate reagents respectively.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Analytical parameters</th>
<th>The system 1</th>
<th>The system 2</th>
<th>The system 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λmax (nm)</td>
<td>446</td>
<td>540</td>
<td>505</td>
</tr>
<tr>
<td>2</td>
<td>Linear range (µg ml⁻¹)</td>
<td>0.01 – 5</td>
<td>0.05 – 40</td>
<td>0.8 – 25</td>
</tr>
<tr>
<td>3</td>
<td>Molar absorptivity (1 mol⁻¹ cm⁻¹)</td>
<td>9.27 x 10⁴</td>
<td>1.97 x 10⁴</td>
<td>1.62 x 10⁴</td>
</tr>
<tr>
<td>4</td>
<td>Sandell’s sensitivity (µg cm⁻²)</td>
<td>0.00212</td>
<td>0.00961</td>
<td>0.0117</td>
</tr>
<tr>
<td>5</td>
<td>Regression equation*</td>
<td>y=0.415x+0.159</td>
<td>y=0.0809x+0.1431</td>
<td>y=0.0657x+0.1262</td>
</tr>
<tr>
<td>6</td>
<td>Correlation coefficient (R²)</td>
<td>0.9978</td>
<td>0.9991</td>
<td>0.9975</td>
</tr>
</tbody>
</table>

*equation of the calibration graph with all the three systems plotted together as in Fig. 5, y=mx+c, where x is the concentration of gold(III) in µg ml⁻¹

However, when the concentration of o-dianisidine was increased, the absorption maximum was found to be shifting towards lower wavelength region and it was producing a positive error in the determination of gold in the system 2. Therefore, it was necessary to always keep the concentration of catechol higher than that of o-dianisidine and in this experiment, 1.0 ml of 0.005 M catechol was used. Similarly, in the system 3, to the standard flasks containing gold(III) solution, 0.5 ml phosphoric acid,
0.1 – 3.0 ml of 0.005 M catechol were added and absorbances the solutions were measured after making them up to the volume. A known volume, 1.0 ml was 0.005 M catechol was required for the system 3 to get the maximum absorbance with 0.5 ml 1000 µg ml\(^{-1}\) amine solutions. Therefore, to maintain uniformity with both systems 2 & 3, 1 ml 0.005 M catechol was used and the graph obtained is represented in Fig. 8.

![Graph](image)

**Fig. 8:** Variation of absorbances with different volumes of catechol in presence of 5 µg ml\(^{-1}\) gold(III) + 0.5 ml 1 M orthophosphoric acid in system 2 with 0.5 ml 1000 µg ml\(^{-1}\) o-dianisidine (♦) and in system 3 with 0.5 ml of 1000 µg ml\(^{-1}\) aniline sulphate (x)

b) Effect of different volumes of o-dianisidine: o-Dianisidine solution was alone used in the system 1 and along with catechol in the system 2. To check the volumes of o-dianisidine required in systems 1 & 2, to a series of labeled 10 ml volumetric flasks containing 0.5 ml 100 µg ml\(^{-1}\) gold(III) solution, 0.5 ml of 1 M phosphoric acid without catechol solution in system 1 and with 0.005 M, 1.0 ml catechol in the system 2, various volumes of (0.1 to 2.5 ml), 1000 µg ml\(^{-1}\) o-dianisidine was added. Then each flask was diluted to 10 ml with water and absorbances of the solutions were measured at 446 nm for the system 1 and at 540 nm for the system 2. Based on the results obtained after measuring the absorbances, 0.5 ml of o-dianisidine was used in the experiments.
c) Effect of different volumes of aniline sulphate solution: Aniline sulphate solution does not get oxidized to impart any colour with gold(III) solution but only along with catechol it forms colour in the system 3. To a series of labeled 10 ml volumetric flasks containing 0.5 ml 100 µg ml\(^{-1}\) gold(III) solution, 1 ml 0.005 M catechol, 0.5 ml of 1 M phosphoric acid, various volumes of (0.1 to 2.5 ml) 1000 µg ml\(^{-1}\) aniline sulphate solution were added. Then each flask was diluted to 10 ml with water and absorbance was measured at 505 nm for the system 3. Based on the results obtained after measuring the absorbances, 0.5 ml of aniline sulphate was used in the experiments.

d) Choice and effect of different acids: 1 M solutions of hydrochloric, sulphuric acid, nitric acid, acetic acid and orthophosphoric acid were prepared by diluting the required quantity of each acid in water. To a series of 10 ml flasks containing 0.5 ml of 100 µg ml\(^{-1}\) of gold(III) solution, 1 ml catechol and 0.5 ml o-dianisidine / aniline sulphate solutions, 0.5 ml of 1 M acid solutions were added and absorbance values were measured. Phosphoric acid containing solution showing a maximum absorbance value, and phosphoric acid was selected for this new method for gold in addition phosphoric acid was also found to be acting as a masking agent for iron(III) otherwise it was showing interference in the estimation of gold(III). Further, different volumes (0.1 – 2 ml) of 1 M phosphoric acid was added to a labeled set of 10 ml volumetric flasks containing 0.5 µg ml\(^{-1}\) of gold(III) along with 1 ml o-dianisidine in the system 1, 1.0 ml catechol and 0.5 ml o-dianisidine in the system 2 and 0.5 ml catechol and 1 ml of aniline in the system 3 solutions. Absorbance values were measured and plotted against the volume of phosphoric acid added which indicates that 0.5 ml of phosphoric acid was sufficient to
produce maximum colour and further addition beyond this volume, was not increasing the absorbance values and same kind was observed for all the three systems.

e) Order of reagents addition: Experiments were performed by adding same volumes of reagents in all possible sequences. It was found that there was no appreciable change in absorbance values for both the systems 1 and 3. However, in the system 2, to the gold(III) solution, phosphoric acid was added, followed by catechol and then o-dianisidine to get maximum absorbance but following one sequence of addition was found to be not altering the overall results.

f) Colour stability: The colour stability of the reaction system was studied carefully in order to know the reproducibility of the results. Under optimized conditions, absorbances of solutions containing 10 µg ml\(^{-1}\) of gold(III) in systems 1, 2 and 3, were measured after colour development. Colour development was almost instantaneous for all the systems, but the colour formed in the system 2 was staying for a longer time than with systems 1 & 3, and are indicated in Fig. 9.

![Absorbance vs Time Graph](image.png)

**Fig. 9**: Effect of time on the colour stability of solutions containing 2 µg ml\(^{-1}\) gold(III) + orthophosphoric acid in the system 1 (●) with only o-dianisidine, the system 2 (×) with catechol and o-dianisidine and the system 3 (▲) with catechol and aniline sulphate.
**Precision and accuracy:**

Five replicates of 2 µg ml$^{-1}$ of gold(III) for systems 1 to 3 were studied by measuring the absorbances of the solutions prepared following the recommended procedure. The precision of the proposed method expressed as %RSD at selected concentration level was found to be 1.61%, 1.10% and 1.82% at wavelengths of 446 nm, 540 nm and 505 nm in the systems 1, 2 & 3 respectively.

**Stoichiometry of the dye product:**

In order to understand the stoichiometry of the reaction in the system 1, to a series of 10 ml standard flasks containing 1 ml of 0.001 M gold(III) solution, 0.5 ml M orthophosphoric acid and different volumes of 0.001 M o-dianisidine solution were added and absorbances of solutions were measured at 446 nm. Similarly, in the system 2, equimolar mixture of catechol and o-dianisidine were added along with 0.5 ml of 1 M phosphoric acid. Each solution was diluted to mark with water and absorbances of the solutions were measured at 540 nm. The system 3, 2 ml of 0.005 M aniline sulphate, 1 ml 0.005 M catechol along with orthophosphoric acid were added to a series of labeled 10 ml volumetric flasks. The solutions were made up to the mark with water and their absorbances were measured at 505 nm. The results obtained were used for constructing the graph and is shown in Fig. 10 which is accounting for 1:2 stoichiometry between gold(III) solution and o-dianisidine in system 1 and 1:1 stoichiometry between gold(III) and equimolar mixture of catechol along with o-dianisidine in the system 2 and catechol along with aniline sulphate in the system 3 respectively.
After observing the stoichiometry between gold(III) and the reagents, a tentative mechanism Scheme 1 for the formation of the dye has been proposed for the system 1 [Wei Sun et al., 2005]. The dye product is dependent on several factors such as pH and reaction medium and tends to undergo condensation to form an azo compound, bisazodiphenyl product [Wei Sun et al., 2005, Flavio Luiz Benedito et al., 2003, A.V.Kireyko et al, 2006] which is also absorbing at 498 nm as indicated in Fig. 3. In the system 2, gold(III) oxidizes catechol to produce 1,2-benzoquinone, which then undergoes nucleophilic coupling with o-dianisidine to produce a violet colour dye product with \( \lambda_{\text{max}} \) at 540 nm [Shyla.B et al, 2012; Simon P.Fricker, 1996], Scheme 2. Gold(I) which is more stable than gold(III) and it may remain in the solution either as \( \text{Au}_3\text{PO}_4 \) [Gerard Meurant, 1983] or as ion pair having the formula \([\text{Au(I)} \text{ HPO}_4]\) anion associated with further protonated dye cation[Suresha.M.S et al, 2007]. In the system 3 reaction as indicated in Scheme 3 may be similar to the system 2, in which 1,2-benzoquinone, produced from the oxidation of catechol by gold(III) undergoes nucleophilic coupling.
with one molecule of aniline to form 4-anilino-1,2-benzoquinone [Gerard Meurant, 1983].

Scheme 1: o-dianisidine in the system forming quinonediiimine then to bisazodiphenyl product

Scheme 2: Oxidation of catechol followed by coupling with o-dianisidine
**Scheme 3:** Oxidation of catechol followed by coupling with aniline

**Effect of foreign ions:**

For understanding the selectivity of the new method, interferences from the ions as shown in Table 2 and each ion interference was investigated in the determination of 5 µg ml\(^{-1}\) of gold(III) using the three recommended procedures which was producing an effect change in absorbance of more than ±2% from that of the solution containing gold(III) alone. Iron(III) was found to be interfering above 0.25 µg ml\(^{-1}\) concentration if used an acid medium other than the phosphoric acid. Nitrite ion was also interfering in the determination of gold, attempts were made to eliminate its interference by adding urea in the sample preparation step, but its tolerance limit was found to be 40 µg ml\(^{-1}\).

**Table 3:** Interference of foreign ions that have caused in the determination of 5 µg ml\(^{-1}\) of gold (III) for all three systems.

<table>
<thead>
<tr>
<th>Added ion</th>
<th>Tolerance limit (µg ml(^{-1}))</th>
<th>Added ion</th>
<th>Tolerance limit (µg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td>500</td>
<td>Chlorate</td>
<td>100</td>
</tr>
<tr>
<td>Iron (III)</td>
<td>0.25</td>
<td>Sulphate</td>
<td>500</td>
</tr>
<tr>
<td>Iron (III) with phosphoric acid</td>
<td>250</td>
<td>Nitrate</td>
<td>500</td>
</tr>
<tr>
<td>Titanium (IV)</td>
<td>250</td>
<td>Chloride</td>
<td>500</td>
</tr>
<tr>
<td>Silver (I)</td>
<td>500</td>
<td>Nitrite</td>
<td>0.25</td>
</tr>
<tr>
<td>Tin (IV)</td>
<td>250</td>
<td>Nitrite with urea</td>
<td>40</td>
</tr>
<tr>
<td>Copper (II)</td>
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<td>Urea</td>
<td>400</td>
</tr>
<tr>
<td>Palladium (II)</td>
<td>250</td>
<td>Acetate</td>
<td>500</td>
</tr>
<tr>
<td>Zinc (II)</td>
<td>400</td>
<td>Citrate</td>
<td>250</td>
</tr>
<tr>
<td>Sodium (I)</td>
<td>400</td>
<td>Oxalate</td>
<td>250</td>
</tr>
<tr>
<td>Potassium (I)</td>
<td>400</td>
<td>Tartarate</td>
<td>250</td>
</tr>
<tr>
<td>Nickel (II)</td>
<td>1000</td>
<td>Carbonate</td>
<td>500</td>
</tr>
<tr>
<td>Barium (II)</td>
<td>250</td>
<td>Thiocyanate</td>
<td>50</td>
</tr>
</tbody>
</table>
Analytical application:

Determination of gold in forensic jewellery:

Due to the high price of gold, people cheat bankers by pledging gold plated ornaments as genuine in some banks. Those samples were being tested in forensic laboratories for the purity of gold. Part of such samples as received to the laboratory were accurately weighed, dissolved in aqua regia, diluted with water, added urea and boiled the contents to remove nitrite ion interference [Mingming Yang and Hong Wei, 2014], filtered and filtrate collected in 100 ml in volumetric flask was made up to volume with water. This solution used for analyzing gold(III) performing the experiments according to procedures of the systems 1, 2 & 3 of the new method for gold. An aliquot of this solution was also used for the determination of gold using ICP AES method fixing the recommended parameters. The results of gold obtained for each sample from both the methods are tabulated in Table 4 with percentage relative error values of each system given in brackets. Results which are in the table are showing that the new method involving three systems 1-3 could be used successfully for the estimation of gold present in forensic jewellery samples and are also comparable with those results obtained from the same samples determined separately by ICP AES analysis. Energy Dispersive X-Ray Fluorescence spectrometer technique was used for the qualitative determination of the elements present in those jewellery samples by recording their spectra under fixed conditions for medium elements with acquisition live time of 29.9 s, real time of 53.1 s and tube current of 118 µA. The profiles were recorded for each sample separately before and after giving it an aqua regia acid washing and the respective EDX RF profiles are shown in Fig.11-13 and are revealing that they were fake and experimentally clear that
they are gold coated copper-nickel alloys. The analysis with EDX RF spectrometer was carried out under fixed conditions for medium elements with acquisition live time of 29.9 s, real time of 53.1 s and tube current of 118 µA.

**Fig. 11:** EDX RF Spectrometric elemental profiles under fixed conditions for medium elements with acquisition live time of 29.9 s, real time of 53.1 s and tube current of 118 µA of fake gold bangle No. 2, [A] is obtained for aqua regia washed the sample and [B] is that of the same sample before subjecting to aqua regia wash.

**Fig. 12:** EDX RF Spectrometric elemental profiles of fake gold chain, (left) is obtained for aqua regia washed the sample and (right) is that of the same sample before subjecting to aqua regia wash.
**Fig. 13**: EDX RF Spectrophotometric elemental profiles of fake gold chain (left) and bangle No. 6 (right) recorded before giving them acid wash.

**Fig. 14**: Photographs of fake gold chain (left) taken before giving it a acid wash and (right) is that of the same sample taken after its acid wash.

Stereomicroscope photographs of fake gold chain (left) taken before giving it a acid wash and (right) is that of the same sample taken after its acid wash fig 14 and image of transversely cut duplicate gold bangles showing two distinct layers made of inner layer containing copper, zinc and silver with gold covering on the top, a view recoded under stereo microscope Fig.15 are supporting that the pledged forensic samples
were not genuine gold ornaments instead they are gold coated on the samples that are made from other than gold metal and provide a more qualitative support to their EDXRF profiles.

Fig. 15: Image of transversely cut duplicate gold bangles showing two distinct layers made of copper, zinc and silver (white inner layer) with gold covering on the top (outer yellow layer), a view recorded under stereomicroscope for two different bangles.

Table 4: Comparison of results gold obtained for the forensic gold samples determined from three systems of the new method and the results of the same samples but determined from the ICP AES analysis and corresponding %RE are in brackets.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>Amount of gold in mg determined in the sample using</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sys 1*</td>
</tr>
<tr>
<td>1</td>
<td>Fake gold bangle No. 1</td>
<td>1.10 (1.78)</td>
</tr>
<tr>
<td>2</td>
<td>Fake gold bangle No. 2</td>
<td>1.26 (0.78)</td>
</tr>
<tr>
<td>3</td>
<td>Fake gold bangle No. 3</td>
<td>2.25 (0.89)</td>
</tr>
<tr>
<td>4</td>
<td>Fake gold chain</td>
<td>2.31(1.28)</td>
</tr>
<tr>
<td>5</td>
<td>Genuine gold chain 1</td>
<td>105.11 (2.07)</td>
</tr>
<tr>
<td>6</td>
<td>Genuine gold chain 2</td>
<td>97.41 (0.61)</td>
</tr>
<tr>
<td>7</td>
<td>Fake gold bangle No. 4</td>
<td>1.84 (0.54)</td>
</tr>
<tr>
<td>8</td>
<td>Fake gold bangle No. 5</td>
<td>1.41 (0.70)</td>
</tr>
<tr>
<td>9</td>
<td>Fake gold bangle No. 6</td>
<td>2.04 (1.92)</td>
</tr>
</tbody>
</table>

* is an average value of five determinations from the respective system.
2) **Determination of gold in pharmaceutical preparations:**

Indian traditional system of medicine, Ayurveda makes use of unique metallic-herbal preparations and there are number of such Ayurvedic medicines available in the local market which are containing trace amounts of gold [Simon P.Fricker, 1996; M. Rathore, 2013]. These pharmaceutical preparations were purchased, powdered and 2 g of accurately weighed sample was roasted in a muffle furnace at 650°C [Vysetti Balaram et al, 2013]. After cooling, the sample was digested with 20 ml aqua regia on a hot plate at 200°C, boiled with urea and water [Mingming Yang and Wong Wei An, 2014]. When volume was reduced to 5 ml, the solution was filtered and made up to 100 ml in a volumetric flask. An aliquot of these solutions were analyzed through the recommended procedure using systems 1, 2 & 3 of the new method and the results obtained were cross checked with ICP AES analysis simply by aspirating the samples to the atomizer under conditions which were presented under Materials and Methods, pp 49-50. The results obtained for gold of each sample determined following recommended procedures of all the three systems and are shown in Table 5, with percentage relative error in brackets and these results are comparable with the gold results of the same samples determined separately by ICP AES analysis.

**Table 5:** Determination of gold present in pharmaceutical tablets through procedures of thee systems of new method for gold and also through ICP AES analysis and given %RE in brackets.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>Amount of the gold in mg determined using</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sys 1*</td>
<td>Sys 2*</td>
</tr>
<tr>
<td>1</td>
<td>Tablet No. 1</td>
<td>0.388 (2.51)</td>
<td>0.408 (2.51)</td>
</tr>
<tr>
<td>2</td>
<td>Tablet No. 2</td>
<td>0.437 (2.45)</td>
<td>0.456 (1.78)</td>
</tr>
<tr>
<td>3</td>
<td>Tablet No. 3</td>
<td>1.216 (2.25)</td>
<td>1.274 (2.42)</td>
</tr>
</tbody>
</table>

* is an average value of five determinations from the respective system.
Conclusion:

In the system 1, o-dianisidine forms a red coloured azo dye which absorbs at 446 nm [Wei Sun et al., 2005, Flavio Luiz Benedito et al., 2003, A.V.Kireyko et al., 2006] and the colour formed was less stable and fades away in about 15 minutes after the formation of colour and therefore, measurements were to be made as soon as the formation of the colour. But the sensitivity of this system was very high. The system 3 uses the concept of reaction between catechol and aniline sulphate with gold(III) in acid medium to produce a dye which has absorption maximum at 505 nm. The system 2 uses the concept of catechol oxidation followed by nucleophilic coupling with o-dianisidine [Shyla.B et al., 2012; Mansour S. Abdul Galil et al., 2009] whereby, a stable violet coloured complex was produced which shows maximum absorption at 540 nm and was found to be having more stability than those from systems 1 and 3. The drawbacks associated several spectrophotometric methods for the estimation of gold such as using of organic solvent for its extraction [Patell, K.S and and Lieser. K.H, 1986; Tomozo Koh et al., 1986], determination of gold in UV range [Balcerzak. M et al., 2006], time consuming procedure for gold[Chen. Z et al., 2006], very narrow linear range for its determination [El-Zawawy et al., 1995], its results depending on time and pH [Fujita. Y et al., 1999], its extraction in solid phase [Zhao. J et al., 2006] and surfactant induced sensitivity for it [Rubina Soomro et al., 2008] have all been considered to be effectively overcome in the new method of three systems for gold since it is simple and involving no such difficulties. In addition the method could be practiced for the spectrophotometric determination of gold present in forensic jewellery and pharmaceutical samples either through all of its three systems or through its one convenient system. EDX RF profiles
are indicating presence of more copper and nickler and trace amounts of gold. Therefore, these are effectively complement the results of gold from the new method as well as ICP AES methods. The photographs of the samples do support that the analysed forensic jewellery samples are fake.
Summary:

Three spectrophotometric methods were developed here for the estimation of gold(III) using o-dianisidine, aniline sulphate and catechol. Gold(III) oxidizes o-dianisidine to form a red coloured dye having $\lambda_{\text{max}}$ at 446 nm, the system 1, oxidation of catechol followed by coupling with o-dianisidine to produce a dye product with $\lambda_{\text{max}}$ at 540 nm, the system 2, similarly catechol with aniline sulphate having $\lambda_{\text{max}}$ at 505 nm, the system 3. All the three systems were optimized and analytical parameters were calculated. The molar absorptivity values were found to be $9.27 \times 10^4$, $1.97 \times 10^4$ and $1.62 \times 10^4$ with Sandell’s sensitivities of the systems 1, 2 and 3 were found to be 0.0021, 0.0096 and 0.011 $\mu$g cm$^{-2}$ respectively. The optimized systems were used for the determination of gold present in some forensic jewellery and pharmaceutical samples and the results obtained were compared with the results of the same samples determined by ICP-AES. Compositions of the samples obtained before and after acid washes are in agreement with new method developed for gold and ICP AES analysis.
References:


