Photochemical reaction between ascorbic acid and silver (I) in aqueous Triton X-100 and its analytical application

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A new spectrophotometric method for ascorbic acid determination has been described. Colourless silver (I) is photochemically reduced in Triton X-100 (TX-100) medium by ascorbic acid to yellow silver sol having particles with nanometer range size. The particles show a surface plasmon absorption band at 415 nm with molar absorptivity $1.43 \times 10^4$ litre mol$^{-1}$ cm$^{-1}$ and the Sandell sensitivity of $1.23 \times 10^{-2}$ µg cm$^{-2}$. The determination is possible in the range of 0.1-13 ppm of ascorbic acid. The limit of detection is 0.4 ppm. The relative standard deviation is < ±5% for all determinations and the confidence limit for 7.50 ppm of ascorbic acid (10 determinations, 95%) is 7.84±0.32. Statistical parameters, effects of TX-100 concentration, irradiation time and interferents are studied. The method is simple, quick and suitable for vitamin C analysis in pharmaceutical preparations.

The preparation and use of hydrosols has been the subject of research for long and recently added a new dimension due to nanoparticle research. Among all hydrosols, silver sol is most important due to its use in surface enhanced Raman scattering (SERS), photography, catalysis etc. As a consequence stable silver sol was prepared in polymers, ligands, and surfactants in various ways. While chemical reduction using common reducing agents are more common for silver sol preparation, photochemical methods are much less attempted. The photochemical methods reported so far utilized benzoin, alcohol, ethylenediamine tetracetic acid (EDTA), acetone/2-propanol, benzophenone, poly(N-vinylpyrrolidone), etc.

Herein, we report a new photochemical method for silver sol preparation in micellar medium utilising ascorbic acid. This offers a quick and simple method for ascorbic acid determination. Silver (I) is photoreduced by ascorbic acid in aqueous TX-100 medium producing a yellow sol having $\lambda_{\text{max}}$ at 415 nm, the absorbance at which gives a measure of ascorbic acid concentration. The method is suitable for vitamin C determination for pharmaceutical preparations.

The determination of ascorbic acid have been reviewed. They utilize spectrophotometry, redox reactions, derivatization reactions, electrochemical methods, enzymatic methods, chromatographic methods, and fluorimetric methods. But to our knowledge, such a photoreaction has not been used so far for either the preparation of silver sol or ascorbic acid determination. The size of the silver particles are in the nanometer range as observed from transmission electron microscopy (TEM), and this shows the promise for the sol to be used for various other studies.

Materials and Methods

All absorbance measurements were made with a Shimadzu UV-160 digital spectrophotometer. Photochemical reactions were done using germicidal lamp (Sankyo-Denki, Japan; 15-W), in well-stoppered 1-cm quartz cuvettes. The cells were kept at a distance of 3 cm from the light source.

All reagents were of AR grade. Fresh stock solutions of ascorbic acid (1000 ppm) were prepared daily by dissolving ascorbic acid (Aldrich) in cold double distilled water and were standardised by classical titration. Silver nitrate (Aldrich; $1.24 \times 10^{-2}$ mol dm$^{-3}$) and TX-100 solutions were made in double distilled water. The analysed pharmaceutical preparations were purchased locally, and the solutions were made afresh.

Standard procedure for the determination of ascorbic acid

TX-100 ($10^{-2}$ mol dm$^{-3}$) in 2 mL portions were taken in the cuvettes and to each of these were added 60 µL portions of AgNO$_3$ ($1.24 \times 10^{-2}$ mol dm$^{-3}$). To these were then added appropriate volumes of ascorbic acid stock solutions so that the final
concentrations of ascorbic acid ranged from 1-13 ppm. A blank containing no ascorbic acid is also prepared. The solutions were then irradiated for 10 min. Silver sol having a yellow colour was produced in each of them excepting the blank. Absorbance for each of them was measured at 415 nm keeping the reagent blank as the reference. The absorbance was proportional to the ascorbic acid concentration. The colour was stable for >72 hr while kept in the dark.

Results and Discussion

Photochemical reaction between silver (I) and ascorbic acid

Ascorbic acid absorbs light in the range of ~260 nm both in aqueous and aqueous TX -100 medium. Silver (I) is also photoactive. Although ascorbic acid is known to have the capability to chemically reduce silver (I) to silver metal in alkaline and acid media, the possibility of the same reduction in neutral condition in micellar environment still remains unexplored. The photoreduction, on the other hand, may have the advantage of not being interfered by the presence of other reducing agents such as hydrazine, thiosulfate etc. At the same time, the maintenance of pH of the reaction medium is not necessary. Moreover, photochemical methods have the advantage over chemical methods because they can be applied in milder conditions, the reactions are initiated homogeneously avoiding local concentration gradients of the reactants and can be carried out in a very simple, quick and easy way.

Curve A of Figure 1 shows the surface plasmon absorption band (ref.: reagent blank) of TX-100 stabilized zero valent silver (λ_{max} = 415 nm) obtained from the photoreduction of silver (I) by ascorbic acid. Curve B is the absorption band (ref.: air) for silver (I) after UV irradiation in the absence of ascorbic acid. Without TX -100 no sol could be produced, instead, black precipitate resulted. This indicates that surfactant plays the key role in particle stabilisation. The same photoreduction is possible with sodium ascorbate also. Without light the sol could not be produced even in 24 hr time. Once the sol is produced, it remained stable for >72 hr while kept in the dark, in stoppered condition. The photoreduction is also possible if the dissolved oxygen is purged out by nitrogen. An interesting feature of the reaction is that in the higher concentration range of ascorbic acid (~ 30 ppm) a new band at ~ 650 nm appears in addition to 415-nm band and the colour of the sol appears to be green. This is due to the linear aggregation of silver (O) particles, as observed from transmission electron microscopy. This type of aggregated sol has the potential for being used as a substrate for surface enhanced Raman scattering. Transmission electron microscopy of the sol under description shows that the size of the individual particles are in the range of 10-50 nm. Some elongated clusters, although, are present in the system, but linear aggregation to form string like assembly is not noticed here as observed for the green sol.

The oxidation of ascorbic acid usually occurs via intermediate radicals. The mechanism of the oxidation of ascorbic acid by metal ions has, in several cases, been interpreted in terms of intermediate metal-ascorbate complex. In the photochemical procedure described, however, the oxidation of ascorbic acid by silver (I) possibly follows radical pathway with the concomitant formation of dehydroascorbic acid (M' 174) as evidenced from the GC-MS studies of the ethyl acetate extract of the reaction mixture. The molecular ion then follows Norish type of cleavage leading to mass fragments at m/z 113, 95, 86, 60 and 59. The exact reaction pathway, however, is still unknown and needs more elaborate study.

Effect of TX-100 and irradiation time

The role of TX-100 in the photoreduction of silver (I) to silver (O) is unlike the photoreduction of gold (III) where TX-100 acted dually. There, it caused...
the reduction of gold (III) to gold (0) and, at the same time, being a micelle forming agent it helped in the stabilization of the particles. However, in the present case TX-100 could not bring about the photoreduction of silver (I) to silver (0) but played a key role in particle stabilization. The concentration thus is important because it has a control over the particle size and shape. Studies have been made with TX-100 in the concentration range of $10^{-1}$ to $10^{-4}$ mol dm$^{-3}$ and $10^{-1}$ to $10^{-2}$ mol dm$^{-3}$ worked well. In the range of $10^{-4}$ mol dm$^{-3}$ concentration, TX-100 does not form micelles and so cannot stabilise silver metal particles. In the range of $10^{-1}$ mol dm$^{-3}$ it is too thick to be used. Other surfactants such as cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulphate (SDS) were also tried. In the former case an instantaneous red precipitate was obtained, whereas, the latter interfered in the sol production.

As the reaction is initiated by light it is important to find out the optimum irradiation time for the system. A kinetic study was performed and it was observed that 4 min exposure was sufficient to get the maximum absorbance. However, irradiation upto 25 min did not cause any change in the absorbance. Thus, 10 min exposure time was selected for our studies. Without light the reaction did not occur even in 24h time.

**Effect of silver (I) concentration**

Since silver (I) is photoactive, high concentration of silver (I) caused higher blank value which is undesirable. To find out the optimum range of silver (I) concentration, a study was made with various concentrations of silver (I). It was found that $6.0\times10^{-3}$ - $2.5\times10^{-4}$ mol dm$^{-3}$ final concentration of silver (I) was optimum in the described working range of ascorbic acid. Lower amount of silver (I) could not bring about the quantitative oxidation of ascorbic acid. So, for the entire study, the final silver (I) concentration was fixed at $3.6\times10^{-4}$ mol dm$^{-3}$.

**Calibration graph and other statistical parameters**

The sample solution containing TX-100 ($10^{-2}$ mol dm$^{-3}$), AgNO$_3$ ($3.6\times10^{-4}$ mol dm$^{-3}$) and ascorbic acid (0-13 ppm) were exposed to UV irradiation for 10 min. A linear calibration graph was obtained in this range of ascorbic acid concentration. Linear regression performed over the linear response region provided a correlation coefficient 0.988 with an intercept of $1.0\times10^{-2}$ and a slope of $7.4\times10^{-4}$ ppm. The molar absorptivity of the sol was $1.43\times10^{4}$ lit mol$^{-1}$ cm$^{-1}$. The relative standard deviation (10 determinations) was < ±5%. The Sandell sensitivity with respect to ascorbic acid was $1.23\times10^{-2}$ g cm$^{-2}$. The limit of detection was 0.4 ppm. The 95% confidence limit for 7.50 ppm of ascorbic acid was 7.84±0.32 (for 10 replicates).

**Effect of foreign substances**

A number of organic acids, sugars and amino acids were added in increasing amounts to 10 ppm of ascorbic acid and no serious effects were observed for glucose, maltose and sucrose (upto 130 ppm), for glycine, alanine (upto 150 ppm) for serine (upto 15 ppm), for succinic acid and oxalic acid (upto 15 ppm) Metal ions such as Fe(III), Zn(II), Cd(II) and Co(III) (upto 60 ppm) and anions like Cl$^-$, SO$_4^{2-}$, ClO$_4^-$, NO$_3^-$, AcO$^-$ and EDTA (upto 60 ppm) were tolerated. Tartaric acid, Mn (II) and Pt (IV) interfered strongly.

**Determination of ascorbic acid in pharmaceutical preparations**

The analysed samples (500 mg of ascorbic acid) were freshly dissolved separately in double distilled water and the volume was made upto 100 mL. Then

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<th>Found* (mg)</th>
<th>% Error</th>
<th>Found by other method* (mg)</th>
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</table>

*Average of three determinations

*Following the method described in reference No.24

**Table I—Analysis of vitamin C in pharmaceutical preparations**

The analysed samples (500 mg of ascorbic acid) were freshly dissolved separately in double distilled water and the volume was made upto 100 mL. Then
these were used to reduce silver (I) following the described procedure. The absorbance values were noted to determine the ascorbic acid concentrations in each case. Results are compared with those obtained using the method described in reference 24 and are shown in Table I.

**Conclusion**

A new photochemical way of silver sol preparation using ascorbic acid in micellar medium is described. This has been used for the spectrophotometric determination of ascorbic acid. The method is simple, quick and reliable. It has been used for the analysis of vitamin C in pharmaceutical preparations.

**Acknowledgement**

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**References**