Micelle catalysed methylene blue reduction by ascorbic acid: A procedure for the determination of Vitamin C in pharmaceutical samples

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A new spectrophotometric method for ascorbic acid determination has been described. Methylene blue (MB) being a water-soluble, non-toxic, cationic dye ($\lambda_{\text{max}}$ 660 nm), has been observed to be immobilized successfully in the Stern layer of a cationic micelle (cetyl trimethyl ammonium bromide, CTAB) through hydrophobic interaction. The immobilized dye ($\lambda_{\text{max}}$ 660 nm) is quantitatively reduced by ascorbic acid (AA) in the alkaline medium (pH > 8.5). The progress of the reaction and stepwise bleaching of the blue colour of the dye gives a quantitative measure of ascorbic acid concentration. A single aliquot of the micelle stabilized MB could be used for the determination of ascorbic acid in the range of 50-400 ppm.

The determination of ascorbic acid AA has been reviewed. Very recently photochemical reaction between AA and Ag (I) in aqueous Triton X-100 (chemically known as poly(oxyethylene)iso-octylphenyl ether) has been exploited for the successful determination of AA. An enzyme catalyses a redox reaction in two possible ways: Firstly, by possessing a redox center which is involved in the electron transfer process and thus reducing the activation barrier. Secondly, by increasing the encounter probability through concentrating substrates at its binding sites. Micelles, microemulsions, and organized media have been treated in the light of fractal geometry. The organized assembly is assumed to contain two domains of fractal dimensions, one hydrophobic and other hydrophilic. Thus, reaction kinetics can be studied in terms of fractal dimensions of the reaction medium.

Micelles are well-known membrane mimetic systems. Micelles function in many ways - they lead to the enhancement of the solubility of organic compounds in water, they catalyze many reactions due to the ‘concentration effect’ in the micellar pseudo-phase, and they may also alter reaction pathways. Previous evidence shows that hydrophobic interaction, not charge compensation, plays the deterministic role in dye-surfactant interaction. Hydrophobic effect is the natural tendency of a hydrocarbon like molecule to form aggregates in aqueous solution as to minimize the water-hydrocarbon interfacial area. In the present redox reaction MB has been used to probe the catalytic function of micelle.

Ascorbic acid is a potent reducing agent and its reducing power increases with the increase of pH of a solution. It is used as an analytical reagent and has been reviewed by Erday and Svehla. With these concepts in mind, the study of the reduction of MB by ascorbic acid catalyzed by micelle in alkaline solution is presented here for the spectrophotometric determination of ascorbic acid present in various Vitamin C containing pharmaceutical preparations and anti-oxidant drugs.

Experimental Section

All absorbance measurements were carried out in a Shimadzu UV-160 digital spectrophotometer (Kyoto, Japan) with 1-cm quartz cuvette. Methylene blue (SD Fine Chemicals, India) was purified by repeated crystallization from alcohol. Cetyl trimethylammonium bromide, CTAB (Aldrich), and sodium dodecyl sulfate, SDS (Aldrich) were purified by crystallization. Poly(oxyethylene)iso-octylphenyl ether, TX-100 (Aldrich) and fresh stock solutions of L-ascorbic acid (Fluka) were prepared daily by dissolving the reagent in water. Ascorbic acid was standardized by titration.

Results and Discussion

Micelle – MB interaction

Negatively charged ascorbate anion (AA) is the effective reducing species for the reaction and not the ascorbic acid. This was verified by performing experiments in CTAB medium using same concentration of MB. In one case ascorbic acid (AA) was used as the reducing agent (pH ~ 6) and in other case just making the reaction medium alkaline (pH ~ 10) by adding NaOH. In the former case, there was no decolourization but for the latter rapid reduction was
observed. This indicates that the alkaline condition is essential for the reduction which makes AA a stronger reducing species. Earlier studies have shown that the pKₐ of acids attains a lower value at the surface of CTAB micelle compared to that in bulk water, and this may be responsible for the micellar catalysis of the reduction of MB. Here, the micellar catalysis has been studied at pH ~10. This has been done so that the entire amount of the reductant is present as AA⁻, thus nullifying any effect of pKₐ shift on micellar catalysis.

MB (cationic dye) and AA⁻ are oppositely charged species. The electrostatic attraction between them for an effective reaction in aqueous medium is not observed, presumably because of solvation effect. The extent of solvation is reduced through the use of a suitable micellar environment. The complete reduction of micelle bound MB occurs with AA while the [micelle]: [MB] > 3. This indicates quantitative incorporation of the dye into micelle, which is a prime condition for quantitative bleaching of MB by AA⁻. Otherwise, some amounts of MB remain in the bulk, and for that amount of unincorporated MB micellar catalysis is not observed. The rate of reduction of 0.5 x 10⁻⁴ M MB with 2.8 x 10⁻⁰ M AA⁻ is negligibly small in water, and thus longer time is required before any observation of an appreciable change occurs. The reaction between MB and AA⁻ is thermodynamically favourable but the reaction does not occur appreciably in water due to two possible reasons: (i) due to high energy of activation; (ii) due to low encounter probability because of solvated reactants. The reaction rate does not increase in aqueous SDS or TX-100 micelles. However, the reduction occurs very rapidly, even at 10°C, in aqueous CTAB micelle. More favourable binding of the substrate in micelles leads to a higher encounter probability. Micelle surface bind many organic/inorganic compounds by electrostatic and/or hydrophobic interactions. In this case, MB being a cationic dye binds to SDS by both electrostatic and hydrophobic interactions and to TX-100 and CTAB by hydrophobic forces only. However, the AA⁻ ion binds to CTAB via both forces and to TX-100 and SDS by only a hydrophobic type interaction. This explains, qualitatively, the faster rate of reduction of MB in CTAB micelle where both the substrates are adsorbed onto the micellar surface. When only one of the substrates is adsorbed, then the encounter probability is reduced and so micellar catalysis becomes relatively ineffective. The binding of MB is favoured in all the three micelles through hydrophobic interaction. But for CTAB only, the electrostatic attraction favours the presence of AA⁻ in the Stern layer of the cationic micelle (Figure 1a) for an effective collision with micelle bound MB. For SDS, AA⁻ faces a repulsion which nullify the incorporation of AA⁻ ions in the Stern layer (Figure 1b) of the anionic micelle. In case of neutral micelle, TX-100, some AA⁻ may diffuse into the Stern layer (Figure 1c) and thus the collision between MB and AA⁻ is possible, causing the colour bleaching. Thus, the observed rate of reduction is CTAB > TX-100 > SDS.

Reagent Solution
To determine the ascorbic acid concentration a stock solution of the reagent was prepared from CTAB (10⁻² mol dm⁻³) and MB (0.5 x 10⁻⁴ mol dm⁻³) solutions maintaining the ratio 3:1. Under this condition all MB remained bound into the micellar Stern layer that is essential for the reduction. Higher [MB] above the prescribed ratio leaves certain amount of MB in the unbound condition in the bulk aqueous medium and that free MB is not reduced by AA⁻. The reagent solution was then stored in an Amber coloured bottle and remained stable for about a month.

Procedure
An aliquot of 2.5 mL of the reagent was taken in a standard quartz cuvette and the dissolved air was purged out from the reagent by nitrogen gas in 5 min time. Then a measured amount of ascorbic acid (0-700 ppm) was introduced into the cuvette and the decrease in absorbance value of the micelle-stabilized dye was noted at 660 nm after 5 min. The bleaching of the dye gave a quantitative measure of the ascorbic acid in solution. The same aliquot of the reagent could be used time and again considering the dilution effect.

Calibration graph
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 0.986. The relative standard deviation (10 determinations) was < ± 3%. The limit of detection was 50 ppm. The 95% confidence limit for 60 ppm of ascorbic acid was 60±0.33 (for 10 replicates).

Determination of ascorbic acid in pharmaceutical preparation
The pharmaceutical samples containing known amount (500 mg) of ascorbic acid was dissolved
separately in double distilled water and the volume was made 100 mL. A measured amount of aqueous solution of the pharmaceutical samples was introduced into a cuvette containing the reagent solution. The decrease in absorbance value was noted at the $\lambda_{max}$ 660 nm of the reagent solution to determine the ascorbic acid concentrations from the calibration curve.

Effect of foreign substances
Organic reducing agents those are frequently present/added in soft drinks and pharmaceutical preparations were added in increasing amount to the reagent solution containing 50 ppm of ascorbic acid. There was no change in the absorbance value due to the addition of foreign organic reducing agents. No serious effect was observed for glucose, maltose and sucrose (~130 ppm), glycine, alanine (~150 ppm), serine (~75 ppm), succinic acid and oxalic acid (~115 ppm). Metal ions such as Fe (III), Zn (II), Cd (II) and Co (III), Pt (IV) (to ~ 60 ppm) and anions like Cl$^-$, SO$_4^{2-}$, ClO$_4^-$, NO$_3^-$, AcO$^-$, tartaric acid and Na$_2$EDTA (~100 ppm) were tolerated (Table I).

Conclusion
Micelle catalyzed colour bleaching procedure has been exploited for a very reliable and reproducible determination of ascorbic acid in the ppm level. The method is very simple and can be used for the analysis of Vitamin C in pharmaceutical preparations in the visible range of spectrophotometer. Using different concentration of MB one can obtain a different calibration graph to deal with the wide variations of ascorbic acid in solutions. Thus a lower detection limit could be achieved. The bleached solution of the dye has been used for the first time to
Table I—Analysis of vitamin C in pharmaceutical preparation.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample</th>
<th>Reported by the manufacturer (mg)</th>
<th>Found* (mg)</th>
<th>% Error</th>
<th>Found by other method* (mg)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Celin (Glaxo)</td>
<td>500</td>
<td>510</td>
<td>+2.0</td>
<td>510</td>
<td>+2.0</td>
</tr>
<tr>
<td>2</td>
<td>Chewcee (Lederle)</td>
<td>500</td>
<td>515</td>
<td>+3.0</td>
<td>515</td>
<td>+3.0</td>
</tr>
<tr>
<td>3</td>
<td>Limcee (Sarabhai)</td>
<td>500</td>
<td>510</td>
<td>+2.0</td>
<td>522</td>
<td>+4.4</td>
</tr>
<tr>
<td>4</td>
<td>Energy (Sofisule Pvt. Ltd)</td>
<td>150</td>
<td>145</td>
<td>−3.3</td>
<td>145</td>
<td>−3.3</td>
</tr>
<tr>
<td>5</td>
<td>Oxy-V (Greenco Biologicals)</td>
<td>120</td>
<td>127</td>
<td>−5.8</td>
<td>126</td>
<td>+5.0</td>
</tr>
<tr>
<td>6</td>
<td>Salace (Universal Medicare Ltd)</td>
<td>100</td>
<td>95</td>
<td>−5.0</td>
<td>95</td>
<td>−5.0</td>
</tr>
</tbody>
</table>

* Average of four determination

Following the method described in reference No. 2

determine as many as ~40 samples of ascorbic acid after repetitive regeneration of MB colour by shaking the same aliquot in air.

References