A solution spectroscopy study of tea polyphenol and cellulose: effect of surfactants†

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Catechin, a bioflavonoid, found in green tea leaves has various applications in the food and pharmaceutical industries. However, little has been studied about its behavior with fabric-type materials especially in the presence of surfactants. Studying this lays the footstone for understanding the cause of tea stains on fabrics, which is a nuisance as they do not wash off easily or completely even after washing with a detergent, especially if the stain is aged. The intriguing question that needs to be addressed is what are the possible molecular interactions occurring between the polyphenols and the cellulosic materials, especially in the presence of a surfactant. To find answers, we studied a model system representing a cotton fabric and investigated the effect of a specific tea polyphenol on it under various conditions like pH, time and the presence of surfactants. Experiments were performed using fluorimetry, UV-Visible spectroscopy, FTIR and 1H-NMR spectroscopic techniques to decipher the molecular interactions. The results showed enhanced oxidation of the polyphenol at elevated pH aggravated by surfactants in the presence of a cellulosic substrate. Furthermore, we showed that adding reducing agents in the medium hinders polyphenol oxidation and prevents staining to considerable extent.

Introduction

Bioflavonoids, found in green tea leaves have various applications in the food and pharmaceutical industries. This is a typical polyphenolic class, a plant secondary metabolite, which may confer a number of benefits to the plant, including the attraction of pollinators, security against predator, and protection from UV damage. Flavonoids include over 4000 compounds, which can be divided into six subclasses and further identified by different substitution patterns.† The flavonoids have generated considerable research interest in recent years because of the significant association between their dietary consumption and protection against diseases.‡ Evidence of the potential health benefits of flavonoids comes from studies on their in vitro activity (such as antioxidant and anti-inflammatory activity) as well as in vivo animal studies.¨–¶ The catechins are naturally occurring flavanols that are found in a variety of foods of plant origin, including fruits, wine, beer, chocolate, and most abundantly in tea (Camellia sinensis).¨–†§ Various catechins found in tea are reported elsewhere.†¶ Tea is the traditional beverage in China, India and many countries in the Indian subcontinent, and it is one of the most widely consumed beverages in the world. The consumption of tea is larger than that of coffee, cocoa drinks, and carbonated drinks and is the second largest consumed beverage worldwide.†¶ There has been a lot of research concerning the health effects of tea; for example, theanine in tea can excite the central nervous system to eliminate fatigue, while tea polyphenols can prevent arteriosclerosis and thrombosis. Unfortunately, these beneficial things have a nuisance value too, as they form stains when spilled on garments, and these are often the hardest to remove. To the best of our knowledge, there is no in-depth scientific study of tea stains on fabrics reported in the literature.

The colour of tea is caused by a variety of compounds. About 60% of the colour is contributed by the presence of catechin derivatives, which are present up to 25% (w/w) in fresh tea leaves.†¶ During black tea processing, which involves withering, fermentation and drying, these catechins are oxidized and polymerized into the theaflavins and thearubigins, which are responsible for the dark coloration in tea. Principal component analysis of black tea has shown that the theaflavins and thearubigins make up 3–6% (w/w) and 12–18% (w/w) of tea beverages, respectively. Other components are: the relatively colourless catechins (3–10% w/w), the light-yellow flavonol glycosides (6–8% w/w) and colorless caffeine, amino acids, proteins, sugars, and minerals.†¶ Thus, the colored compounds in tea consist of three levels of polyphenolic compounds: the catechins, theaflavins and thearubigins. Fresh tea leaf contains four major catechins: epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). The content of normal catechins is lower as compared to their epi-form. Note that catechins are relatively colourless and water-soluble compounds. In contrast, epicatechin is most
resistant against auto-oxidation in comparison with epigallocatechins (EGC and EGCG). Though several studies have been reported on various tea polyphenols, little has been studied about their behavior with fabric-type materials, especially in the presence of surfactants. Studying this lays the footstone for understanding the causes of stains on fabrics caused by the accidental spillage of tea, which does not wash off easily even when a detergent is used, and especially if the stain is aged and the fabric is old. The intriguing question here that needs to be addressed is what are the possible molecular interactions occurring between polyphenols and cellulosic materials, especially in the presence of a surfactant. To find answers, we studied a model system representing a cotton fabric and investigated the effect of a specific tea polyphenol on it under various conditions like pH, time and the presence of surfactants. From time immemorial, cotton has been widely used to make fabrics. Methyl cellulose (MC) was chosen for our study due to its structural resemblance to cellulose, wide availability in its pure form and solubility in cold water, which facilitated the solution phase spectroscopic methods used in this study. (+)-Catechin was chosen as the representative polyphenol. Brij-58 was chosen as the surfactant in order to negate any additional pH effect due to the polymer. If we had chosen a cationic or an anionic polymer, the pH of the solution would have in addition governed the ionic state of the polymers. Therefore, to keep things simple, we chose a non-ionic surfactant. Brij-58 was also chosen because it is commonly used in liquid detergents.

Using fluorescence, UV-Visible spectroscopy, FTIR and 1H-NMR spectroscopic techniques we report a thorough and systematic study to provide insight on the stain forming nature of catechin at
various pH, and explore its molecular interactions in the presence of representative cellulosic molecules and surfactants.

**Experimental section**

(+)-Catechin, polyethylene glycol hexadecyl ether (brij-58) and methyl cellulose (MC) were purchased from Sigma-Aldrich (USA) and used without further purification (Scheme 1). Millipore water obtained from a Milli-Q purification system was used wherever required. NaOH and HCl (AR grade) were purchased from Merck and used to adjust the pH. Freshly prepared catechin stock solutions were used for all experiments. A deaerated aqueous solution of catechin was prepared by bubbling nitrogen gas through distilled water for about 1 hour and then adding catechin to the air-free distilled water under an nitrogen atmosphere followed by homogenization.

UV-Vis absorption spectra were measured on a Perkin-Elmer Lambda-35 spectrophotometer with properly corrected background. Fluorescence studies were performed using a Shimadzu RF-5301PC fluorimeter with both excitation and emission slits set at 5.0 nm. Room-temperature Fourier transform infrared (FTIR) measurements were performed on a Perkin-Elmer FT-IR spectrophotometer, Spectrum-100, using the diffused reflectance mode. The spectra were normalized and separated for the convenience of comparison. \(^1\)H-NMR studies were performed on a Bruker AV-200 NMR spectrometer, operated at 200 MHz and 303 K. The scan number was fixed at 1024 for all samples. Samples were prepared in D\(_2\)O to mimic the actual experimental environment where water was used as solvent. The pH of the sample solutions were adjusted to 8.5 and recorded in Wilmad 535 pp NMR tubes, pre-thermostatted (Lauda K2R thermostat) and equilibrated for 10 minutes before data acquisition. The digital resolution for \(^1\)H NMR spectra was 0.04 Hz per data point. For the experiments, TMS (tetramethylsilane) was used as the reference to calculate the chemical

**Scheme 2** The mechanism of catechin oxidation.

**Fig. 3** Emission spectra of an aqueous solution of catechin (0.2 mM) at pH 7 with an increasing concentration of MC at different time intervals. The concentration of MC is 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM, respectively.

**Fig. 4** Relative fluorescence intensity (for the peak at 316 nm) for the interaction of catechin with MC at different pH.
shifts. The observed chemical shifts (δ) of both aliphatic and aromatic protons were examined.

All experiments were performed in water and spectroscopic tools were exploited to decipher the molecular interactions. (+)-catechin (5,7,3′,4′-tetrahydroxyflavan-3-ol), the major constituent of green tea, is one of the flavanols bearing 5 hydroxyl groups. The antioxidant action of catechins originate from its favorable one-electron donation properties.\textsuperscript{15,16} An aqueous solution of catechin initially formed a colorless solution but developed a pale yellow color after a few hours with the intensity of the color changing with time, indicating a chemical change to the catechin moiety. The new chemical species had a different spectroscopic signature in solution, which was investigated by both absorption and emission spectroscopy.

**Results and discussion**

**Absorption and emission studies**

**Studies on the polyphenol.** The UV-Vis absorption spectrum of a 0.2 mM solution of catechin in deaerated water revealed a very sharp and strong absorption maximum peak at 275 nm (Fig. 1). No appreciable change was observed even when the solution was kept for 5 days at 25 °C. However, in the case using an aerated aqueous solution of catechin, the absorption spectrum changed within 24 hours. In the latter case, the absorption maximum peak at 275 nm shifted to a slightly longer wavelength, and new absorption maximum peaks appeared at 430 nm and 480 nm. This indicated that the presence of dissolved oxygen in water was responsible for affecting a change to the catechin moiety through oxidation. Torreggiani et al. reported oxidation of the catechol moiety on the B-ring of catechin in an aqueous solution.\textsuperscript{17} Catechols are known to be oxidized by a similar mechanism.\textsuperscript{18-20} Catechin contains the catechol moiety and can be converted to its oxidized form in an aqueous solution at neutral pH.

The changes in the UV-Vis absorption spectrum of catechin were aggravated in an alkaline solution using both aerated and de-aerated water. The UV-visible absorption properties of the de-aerated alkaline catechin solution were quite similar to those of the aerated neutral catechin solution, having very close
absorption maxima, indicating similar chemical changes occurring to the catechin moiety, both in the presence of air and in an alkaline solution. However, the yellowish coloration in the alkaline solution appeared faster when compared to the aerated neutral solution of catechin. Moreover, the yellowish color intensified with an increase in pH as well as the storage time of the solution.

The steady-state fluorescence emission spectrum of a 0.2 mM alkaline buffer solution of catechin was measured. Their emission spectra were obtained in the range of 280 nm to 400 nm with an emission maximum peak at 316 nm in the initial state. With passage of time, the fluorescence intensity decreased and a new emission peak appeared in the range of 400 nm to 550 nm with an emission maximum peak at 465 nm, as presented in Fig. 2(a). The emission intensity of this new peak increased with increasing time. This suggested that the changes in the fluorescence spectra depend on the storage time and pH of the solution. Fig. 2(b) shows the change in the fluorescence quantum yield of the emission spectrum in the range of 280 nm to 400 nm. Although the initial concentrations of catechin were the same, their fluorescence quantum yields for the emission spectrum rapidly diminished with an increase in the solution pH. Moreover, the rate of change was promoted by an increase in pH. This finding supports the conclusion that oxidation started with the dissociation of the –OH group in the catechin backbone in an aqueous solution.

In short, catechin displayed a yellowish color in aerated water when the solution was left in the dark for 2 days; consequently, the UV-Vis absorption spectra also changed with time. This phenomenon was aggravated with an increase in the pH of solution. This can be correlated to the oxidation of catechin. The catechol moiety in catechin may be thought to dissociate first, followed by its oxidation in an aqueous solution. The steady-state fluorescence emission spectra of catechin also indicated that the oxidation of catechin was pH dependent. The mechanism for oxidation may be elucidated according to Scheme 2 as reported by Brett et al.\(^{24}\)

**Studies on the interaction of polyphenol with cellulose material.** The effect of methyl cellulose (MC), a cellulose derivative, on the photophysical properties of catechin was studied to ascertain the role of fabric like materials on its oxidation. The effect of pH and time on the interaction of MC with (+)-catechin was studied exhaustively using fluorescence techniques. Experiments were conducted at pH 7, 8.5 and 10 at time intervals of 0, 3, 6, 24 and 72 hours in order to track the changes in the interaction of catechin with MC in an aqueous solution. At pH 7, the fluorescence intensity increased upon the addition of MC to an aqueous solution of catechin at all time intervals (Fig. 3). Similar observations were observed at pH 8.5 (Fig. S1†) and pH 10 (figure not shown due to extremely low intensity) where the fluorescence intensity almost dropped to zero after 72 hours. However, the extent of the increase in intensity for the same concentration of MC was found to be different at different time intervals. Though the absolute fluorescence yield decreased with an increase in time, the relative increase in fluorescence intensity upon addition of MC was found to increase with an increase in time. This is evident from the plot of the relative fluorescence intensity against time at different pH values (Fig. 4).

Therefore, it can be concluded that the interactions between catechin and MC exist at all pH and is enhanced upon increasing pH. However, in this study, a new emission peak at 465 nm was not observed unlike that of an aqueous solution of catechin. Earlier studies have shown that binding polyphenol with cellulose materials can happen for polyesters.\(^{22}\) Through our study, we reiterate that for cellulose substrates too, such interactions are feasible and can lead to stain formation on fabrics. However, this study also indicates that the oxidation of catechin is somehow suppressed in presence of MC. We believe that extensive hydrogen bonding between MC and catechin leads to the suppression of catechin oxidation.

**Studies on the interaction of polyphenol with a surfactant.** The effect of a surfactant on the photo-physical properties of catechin was studied using a representative non-ionic surfactant. For our study, we used polyethylene glycol hexadecyl ether commonly known as brij-58. We studied the effect of pH (7, 8.5, and 10) and time (at an interval of 0, 3, 6, 24 and 72 hours) on the interaction of brij-58 with catechin using fluorometric techniques.

At pH 7, the fluorescence intensity increased upon the addition of brij-58 to an aqueous solution of catechin up to 6 hours (Fig. 5). After 24 hours, a new peak at ~465 nm developed, which can be attributed to the oxidation product of catechin similar to the one elucidated by us from previous studies in an aqueous solution. It was found that the fluorescence intensity of
the original peak decreased after 24 hours and intensity of the new peak increased with an increase in concentration of surfactant and time. At pH 8.5, a new peak developed within 3 hours and the fluorescence intensity increased upon addition of the surfactant (Fig. 6 and S2†). This increased rate in the formation of the new peak confirms that the oxidation of catechin is dependent on the pH of the surfactant solution. Fluorescence intensities were below the detection limit at 0 hours for solutions at pH 10, where the new oxidation peak appeared immediately upon the addition of the surfactant to the solution of catechin. These clearly indicated that the interaction and oxidation of catechin is aggravated by an increase in pH. Moreover, the rate of catechin oxidation is hastened in the presence of surfactant compared to the corresponding aqueous solutions.

Studies on the interaction of polyphenol in the presence of cellulose material and a surfactant. Having studied the interaction between catechin with MC or brij-58, we proceeded to discern the behavior of catechin in presence of both MC and brij-58. The effect of pH (7, 8.5, and 10) and time (at an interval of 0, 3, 6, 24 and 72 hours) are discussed in this section.

As expected, initially, we did not observe any new peaks at pH 7. After 6 hours, an oxidation peak at ~465 nm developed, which was contrary to our previous experiments performed in the presence of a surfactant alone (Inset Fig. 7c). This new peak became more prominent after 24 hours. The fluorescence intensity of the original peak decreased with a progressive increase in the intensity of the new peak and followed a direct correlation with the concentration of brij-58, MC and time.

At pH 8.5, the new peak developed within 3 hours. This result confirmed that the oxidation of catechin is a function of the pH of the surfactant solution and also indicated that this process is aggravated in the presence of both MC and brij-58 at elevated pH (Fig. 8 and 9).

Moreover, a closer look into Fig. 8 (catechin-brij-58 interaction) and Fig. 9 (catechin-MC-brij-58 interaction) revealed that the catechin peak intensity at ~320 nm decreased to a larger extent in the presence of both brij-58 and MC when compared to brij-58 alone at both pH values. This indicates that the extent of interaction with catechin is higher in the MC-brij-58 medium in comparison to brij-58 alone. It is then reasonable to believe why surfactants fail to remove tea stains from fabric surfaces.

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Fig. 7 Emission spectra of an aqueous solution of catechin (0.2 mM) at pH 7 with an increasing concentration of brij-58 and MC (1:1) at different time intervals. The concentrations of brij-58 and MC in all the cases are 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM, respectively.
further validate that catechin oxidation indeed results in stain formation, we performed a series of experiments at pH 8.5 containing a 1:1 mixture of MC and brij-58 in the presence of a few well-known reducing agents, viz., sodium borohydride and sodium disulphite. The concentration of each of these reducing agents in the medium was maintained at 0.2 mM. The stoichiometric concentration of the reducing agents was chosen to ensure that it matches with the concentration of catechin used for the experiments. The prepared solutions were incubated for 24 hours prior to recording their emission spectra. The control solution containing no reducing agents turned brown while no color change was observed in any of the test solutions. Moreover, we did not observe any peak at 465 nm, corresponding to the oxidation of catechin (vide Fig. S3†). Therefore, we can arrive at a reasonable confirmation that tea stains develop from the oxidation of polyphenols such as catechin on the fabric surface, which is hastened by surfactants. We have also demonstrated that use of a reducing agent in the composition can help prevent stains.

FTIR studies

FTIR spectroscopy was performed to study the interaction between catechin, MC and brij-58, and corroborate the results obtained from the fluorometric studies. It is evident from the IR spectra that intermolecular hydrogen bonding occurs between catechin and MC or brij-58 and in the presence of both MC and brij-58 (Fig. 10(a)). A broad absorption band at 2200 cm⁻¹ due to the hydrogen bonded hydroxyl groups, proved the existence of hydrogen bonding between the catechin hydroxyl groups and MC-brij-58. Note that catechin is a polyphenolic compound with five hydroxyl groups. Like other phenolic compounds, catechin acts as proton donor while brij-58 and MC act as proton acceptors.²³ The H-bonding interaction is expected to decrease with an increase in pH of the medium as deprotonation of hydroxyl from the catechin moiety occurs owing to the relative acidity of the aromatic hydroxyl groups. This is evident from the comparative lowering in the absorbance band of 2200 cm⁻¹ with a gradual increase in the pH value.

Fig. 8 Emission spectra of an aqueous solution of catechin (0.2 mM) at pH 8.5 with an increasing concentration of brij-58 and MC (1:1) at different time intervals. The concentrations of brij-58 and MC in all the cases are 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM, respectively.
A broad absorption peak corresponding to the intermolecular H-bonded phenolic –OH and alcoholic –OH groups was also observed between 3550 and 3540 cm\(^{-1}\). There is also a gradual decrease in the peak intensity with time of reaction. This allowed us to conclude that there can be a three way intermolecular complexation between the reactant species. As the reaction progressed, at a particular pH, catechin tends to oxidize resulting in a reduced interaction between the oxidized product of catechin and MC/brij-58. This was possibly due to the formation of keto compounds with lower polarity compared to hydroxyl.

Another characteristic vibrational peak between 2900 and 2900 cm\(^{-1}\) was attributed to the methyl groups of the aliphatic ether. This characteristic peak at 2900 cm\(^{-1}\) corresponding to methyl vibration was expected to be prominent for brij-58. This was retained in all compositions of brij-58 with catechin; however, the interaction reduced with an increase in the pH of the medium.

**\(^{1}\)H-NMR studies**

\(^{1}\)H-NMR experiments were performed to further corroborate the above results. \(^{1}\)H-NMR studies have been used widely to investigate the interactions between organic compounds. The proton chemical shift has been used as a tool to reflect the electron density of the proton. All experiments were performed at pH 8.5 in D\(_2\)O to mimic the actual system. It is noteworthy that the interaction in organic compounds changes with the polarity of solvent; hence, it is desirable to use a water-like polar protic solvent such as D\(_2\)O to maintain similarity in the molecular environment.

For our study, the chemical shifts for both aliphatic and aromatic protons were monitored to derive information about the molecular interactions of catechin with MC/brij-58. The chemical shift for D\(_2\)O was normalized at 4.8 ppm. Complete spectra for all the samples are shown in Fig. S4.† \(^{1}\)H-NMR spectra for all the samples conformed well to the previous reports for the alkyl and aryl protons. Fig. 11(a) and (b) represents the comparative and magnified spectra of the aliphatic protons and aromatic protons for all samples, respectively.

Cooperative interaction between catechin and brij-58 is evident from Fig. 11(i), whereby we observed a downfield shift of the aliphatic protons of brij-58 (spectra (b)) in comparison to the catechin-brij-58 MC mixture (spectra (d)), to an extent of \(\Delta \delta = 0.08\) ppm. This further indicated that interaction of catechin with brij-58 is facilitated by non H-bonded cooperative interaction, especially at elevated pH. It is also evident from Fig. 11(ii) that there is downfield shift of the aromatic protons of catechin.

**Fig. 9** Relative fluorescence intensity (for the peak at 316 nm) for the interaction of catechin and brij-58 and MC (1 : 1) at different pH.

**Fig. 10** FTIR spectra for the interaction of catechin with brij-58 and MC (1 : 1) at (a) 0 and (b) 24 hours. In the figures, curves (i) and (ii) are spectra at pH 7; (iii) and (iv) are spectra at pH 8.5; (v) and (vi) are spectra at pH 10, for 0 and 10 mM concentrations, respectively.
the catechin ring in the presence of a 1 : 1 mixture of brij-58 and MC to an extent of $\Delta \delta = 0.1$ ppm. The shielding of protons is an indication of cooperative interaction of the MC and brij-58 molecules with catechin. This demonstrates that catechin develops a strong interaction with the MC and brij-58, which is promoted with an increase in pH of the medium.

In conclusion, we have studied the effect of methyl cellulose (MC, a cellulose derivative), brij-58 (a representative surfactant) and their combination on the kinetics of conversion of catechin (a potential stain-forming agent in tea) into its oxidized colour-forming polymeric species. The absorption and emission spectral properties of catechin have been investigated in order to obtain insight into the interaction between catechin in an aqueous solution and MC and/or brij-58. Spectral evolution of catechin in different pH media were recorded to quantify the rate of oxidation. The results proved that the kinetics of oxidation were aggravated at higher pH and further enhanced in the presence of brij-58 or a mixture of MC and brij-58 as apparent from the appearance of a new oxidation peak of catechin. The solutions tend to turn a yellowish-brown color, rapidly resulting in enhanced staining for old fabrics that cannot be washed off easily with detergents. Furthermore, we demonstrated that the use of reducing agents in the medium can hinder catechin oxidation and prevent staining.

**Fig. 11** $^1$H-NMR spectra in D$_2$O solvent for (a) catechin, (b) brij-58, (c) MC and (d) a 1 : 1 mixture of brij-58 and MC with catechin. (i) and (ii) are magnified spectra for the aliphatic and aromatic protons, respectively.
References

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