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A Solution Spectroscopy Study of Tea Polyphenol and Cellulose: Effect of Surfactants

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Abstract

Catechin, a bioflavonoid, found in green tea leaves has various applications in food and pharmaceutical industries. But little has been studied about its behavior with fabric-type materials especially in presence of surfactants. Studying this lays the footstone for understanding causes of tea stains on fabrics which is a nuisance as it does not wash off easily and completely even after washing with a detergent, especially if the stain is aged. The intriguing question here is: What molecular interactions occur between polyphenol and cellulosic materials and especially in 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 with varied conditions like pH, time and presence of surfactants. Experiments were performed using fluorimetry, UV- Visible, FTIR and ^{1}H-NMR spectroscopic techniques to decipher the molecular interactions. The results showed enhanced oxidation of the polyphenol at elevated pH aggravated by surfactants in 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 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 attraction of pollinators, security against predation, and protection from UV damage. Flavonoids include over 4000 compounds which can be divided into six subclasses and further identified by different substitution patterns.[1] Flavonoids have generated considerable research interest in recent years because of the significant association between their dietary consumption and protection against diseases.[2] Evidence of potential health benefits of flavonoids comes from studies of their in-vitro activity (antioxidant and anti-inflammatory activity, etc.) as well as in-vivo animal studies.[3-5] 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*).[6-10] Various catechins found in tea are reported elsewhere.[11] 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.[12] 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 literature.

The colour of tea is caused by a variety of compounds. About 60% of the colour is contributed by the presence of derivatives of catechins, which are present up to 25% (w/w) in

fresh tea leaf.[13] During black tea processing which involves withering, fermentation and drying, these catechins are oxidized and polymerized to theaflavins and thearubigins which are responsible for thedark coloration in tea. Principal component analysis of black tea has shown that theaflavins and thearubigins make up 3-6% (w/w) and 12-18% (w/w) of tea beverages, respectively. Other components are: the relatively colorless catechins (3-10% w/w), the light-yellow flavonol glycosides (6-8% w/w) and colorless caffeine, amino acids, proteins, sugars, and minerals.[13] Thus, the colored compounds in tea consist of three levels of polyphenolic compounds: 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 the epiform. Catechins are relatively colourless and water-soluble compounds. In contrast, epicatechin is most resistant against auto-oxidation in comparison with epigallocatechins (EGC and EGCG).[14]

Though several studies are reported with various tea polyphenols, however little has been studied about its behavior with fabric-type materials especially in presence of surfactants. Studying this lays the footstone for understanding causes of stains on fabrics caused by accidental spillage of tea is a notorious issue as it does not wash off easily even when a detergent is used, especially if the stain is aged and the fabric is old. The intriguing question here is: What molecular interactions occur between polyphenol with cellulosic materials and especially in 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 with varied conditions like pH, time and 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 pure form and ready solubility in cold water, which facilitated the solution

phase spectroscopic studies. (+)-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. Had we chosen a cationic or an anionic polymer the solution pH would have additionally governed the ionic states of the polymers. Therefore to keep things simple we chose a non-ionic surfactant. Brij-58 was chosen because it is commonly used in liquid detergents.

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

Experimental Section

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

UV-Vis absorption spectra were measured on 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 to 5.0 nm. Room temperature Fourier transform infra-red (FTIR) measurements were performed on a

Perkin-Elmer FT-IR spectrophotometer, Spectrum-100, in the diffused reflectance mode. The spectra were normalized and separated for the convenience of comparison. ^{1}H- NMR studies were done on a Bruker AV-200 NMR spectrometer, operated at 200 MHz and 303 K. The scan number was fixed at 1024 for all the samples. Samples were prepared in D_2O, to mimic the actual experimental environment where water was used as solvent. pH of the sample solutions were adjusted to 8.5 and taken 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 (tetra methyl silane) was used as the reference to calculate the chemical 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 with 5 hydroxyl groups. The antioxidant action of catechins originate from its favorable one-electron donation properties.[15,16] Aqueous solution of catechin initially formed a colorless solution but developed a pale yellow color after few hours, the intensity of the color changed with time indicating a chemical change to the catechin moiety. The new chemical species had different spectroscopic signature in the solution, which was investigated by both absorption and emission spectroscopy.

Results and Discussion

Absorption and Emission Studies

Studies on polyphenol

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

The changes in UV-Vis absorption spectrum of catechin was aggravated in alkaline solution for 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 catechin moiety, both in presence of air or in alkaline solution. However, the yellowish coloration in alkaline solution appeared faster compared to the aerated catechin solution. Besides, the yellowish color intensified with increase in pH as well as with the storage time of the solution.

The steady-state fluorescence emission spectrum of 0.2 mM catechin was measured in alkaline buffer solution. 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 Figure 2 (a). The emission intensity of this new peak increased with increasing time. It is suggested, that changes in the fluorescence spectra depend on the storage time and pH of the solution. Figure 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 same, their fluorescence quantum yields for the emission spectrum rapidly diminished with increase in solution pH. Also, the rate of change was promoted by increase in pH. This finding supports the conclusion that oxidation started with the dissociation of -OH group from the catechin backbone in aqueous solution.

In short, catechin displayed a yellowish color in aerated water when the solution was left in dark for 2 days, consequently the UV-Vis absorption spectra also changed with time. This phenomenon was aggravated with increase in the pH of solution. This can be correlated to oxidation of catechin. The catechol moiety in the catechin molecule may be thought to dissociate first followed by its oxidation in aqueous solution. The steady-state fluorescence emission spectra of catechin also indicated that oxidation of catechin was pH dependent. The mechanism for oxidation may be elucidated according to Scheme 2 by Brett et. al.[21]

Studies on interaction of polyphenol with cellulosic 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 interaction of catechin with MC in aqueous solution. At pH 7, fluorescence intensity increased with addition of MC to an aqueous solution of catechin at all time intervals (Figure 3). Similar observation was evidenced at pH 8.5 (Figure 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 increase in intensity for same concentration of MC was found to be different at different time intervals. Though the absolute fluorescence yield decreased with increase in time, the relative increase in fluorescence intensity on addition of MC was found to increase with increase in time. This is evident from the plot of the relative fluorescence intensity against time at different pH (Figure 4).

Therefore, it can be concluded that interaction between catechin and MC exist at all pH and gets enhanced with increasing pH. However, in this study a new emission peak at 465 nm was not observed unlike that of aqueous catechin solution. Earlier studies have shown that binding of polyphenol with cellulosic materials can happen for polyesters.[22] Through our study, we reiterate that for cellulosic substrates too, such interactions are feasible and can lead to stain formation on fabrics. However, this study additionally indicated that oxidation of catechin somehow gets suppressed in presence of MC. We believe that extensive hydrogen bonding between MC and catechin, led to the suppression of catechin oxidation.

Studies on interaction of polyphenol with surfactant

Effect of 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, fluorescence intensity increased with addition of brij-58 to the aqueous solution of catechin up to 6 hours (Figure 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 aqueous solution. It was found that fluorescence intensity of the original peak decreased after 24 hours and intensity of the new peak increased with increase in concentration of surfactant and time. At pH 8.5 a new peak developed within 3 hours and fluorescence intensity increased with addition of surfactant (Figure 6 and S2). This increased rate in the formation of the new peak confirms that the oxidation of catechin is dependent on pH of the surfactant solution. Fluorescence intensities were below detection limit at 0 hours for pH 10 solutions, where the new oxidation peak appeared immediately with the addition of surfactant to catechin solution. These clearly indicated that the interaction and oxidation of catechin is aggravated with increase in pH. Also, the rate of catechin oxidation is hastened in presence of surfactant compared to the corresponding aqueous solutions.

Studies on interaction of polyphenol in presence of cellulosic material and surfactant

Having studied the interaction between catechin with MC or brij-58 respectively, 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 done in presence of surfactants alone (Inset Figure 7c). This new peak became more prominent after 24 hours. The fluorescence intensity of the original peak decreased with progressive increase in 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 oxidation of catechin is a function of surfactant solution pH and also indicated that this process is aggravated in presence of both MC and brij-58 at elevated pH (Figure 8 and 9).

Also, a closer look into Figure 8 (catechin- brij-58 interaction) and Figure 9 (catechin - MC-brij-58 interaction) revealed catechin peak intensity at ~ 320 nm decreased to a larger extent in presence of both brij-58 and MC compared to brij-58 alone at both pH. 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. To further validate that catechin oxidation indeed results in stain formation; we performed a series of experiments at pH 8.5 containing 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 of the emission spectra. The control solution containing no reducing agents turned brown while no color change was observed in any of the test solutions. Additionally, we did not observe any peak at ~ 465 nm corresponding to the oxidation of catechin (*vide* S3). Therefore, we can arrive at a reasonable confirmation that tea stains develop from oxidation of polyphenols such as catechin on fabric surface, which is hastened by surfactants. We 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 presence of both (Figure 10 (a)).

A broad absorption band at 2200 cm^{-1} due to the hydrogen bonded hydroxyl groups, proved the existence of hydrogen bonding between catechin hydroxyls and MC-brij-58. 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 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 gradual increase in the pH values.

A broad absorption peak corresponding to the inter-molecular H-bonded phenolic -OH and alcoholic -OH was also observed between 3550-3540 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 with MC/ brij-58. This was possibly due to the formation of keto compounds with lower polarity compared to hydroxyl.[24, 25] The three way intermolecular complexation is expected to be most prominent at pH 7 as catechin oxidation is slowest at that pH. Characteristic peaks for cyclic ether occurred at 1100 $cm^{-1.}$ The intensity of the peak increased with time and pH of the medium. This indicated that catechin core moiety (C-ring) did not undergo degradation or oxidative damage with either change in pH or time. However, we got an indication of phenolic –OH oxidation in catechin with increase in pH and time of interaction.

Another characteristic vibrational peak between 2900 and 3000 cm^{-1} was attributed to that of methyl groups of 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 increase in pH of the medium.

^{1}H-NMR studies:

^{1}H NMR experiments were performed to further corroborate the above result. ^{1}H NMR studies have been used widely to investigate the interaction 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_2O to mimic the actual system. It is noteworthy that

interaction in organic compounds changes with polarity of solvent and hence it is desirable to use water like polar protic solvent such as D_2O 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_2O was normalized at 4.8 ppm. Complete spectra for all the samples are shown in Figure S4. ^1H NMR spectra for all the samples conformed well to the previous reports for the alkyl and aryl protons. Figure 11(a) and 11(b) represents the comparative and magnified spectra of the aliphatic protons and aromatic protons for all samples respectively.

Cooperative interaction between the catechin and brij-58 is evident from Figure 11 (i), whereby we observed a downfield shift of the aliphatic protons of brij-58 (spectra (b)) in comparison to 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 Figure 11 (ii) that there is downfield shift of the aromatic protons of catechin ring in presence of 1:1 mixture of brij-58 and MC to the 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 molecule. It demonstrates that catechin develops strong interaction with the MC and brij-58 which is promoted with 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 aqueous solution and MC and/ or brij-58. Spectral evolution of catechin in different pH media was recorded to quantify the rate of its oxidation. The results proved that the kinetics of oxidation aggravated at higher pH and further enhanced in presence of brij-58 or a mixture of MC and brij-58 as apparent from the appearance of new oxidation peak of catechin. The solutions tend to turn yellowish brown color rapidly resulting in enhanced staining for old fabrics that cannot be washed off easily with detergents. Furthermore, we demonstrated that use of reducing agents in the medium can hinder catechin oxidation and prevent staining.

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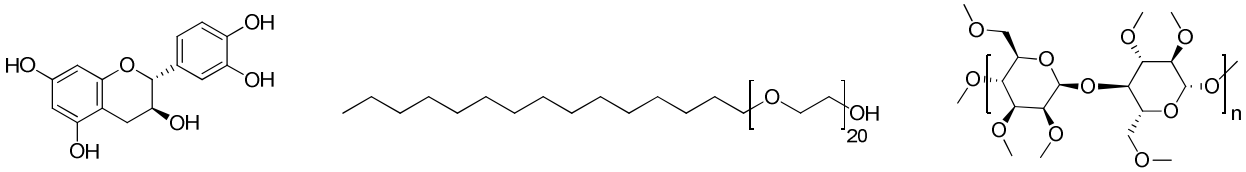
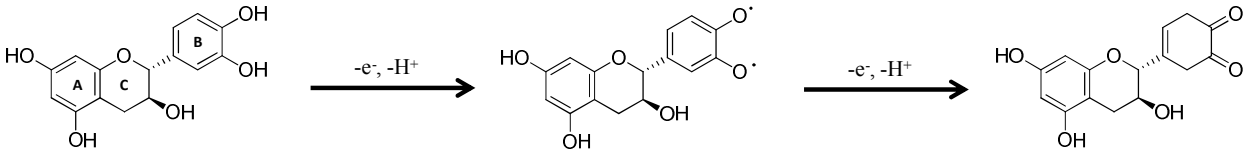


(+)- catechin **Polyethylene glycol hexadecyl ether (Brij 58)** **Methyl cellulose**

Scheme 1: Structure of (+)- catechin, polyethylene glycol hexadecyl ether (brij-58) and methyl cellulose (MC)



(+)- catechin **semiquinone** **quinone**

Scheme 2: Mechanism of catechin oxidation.

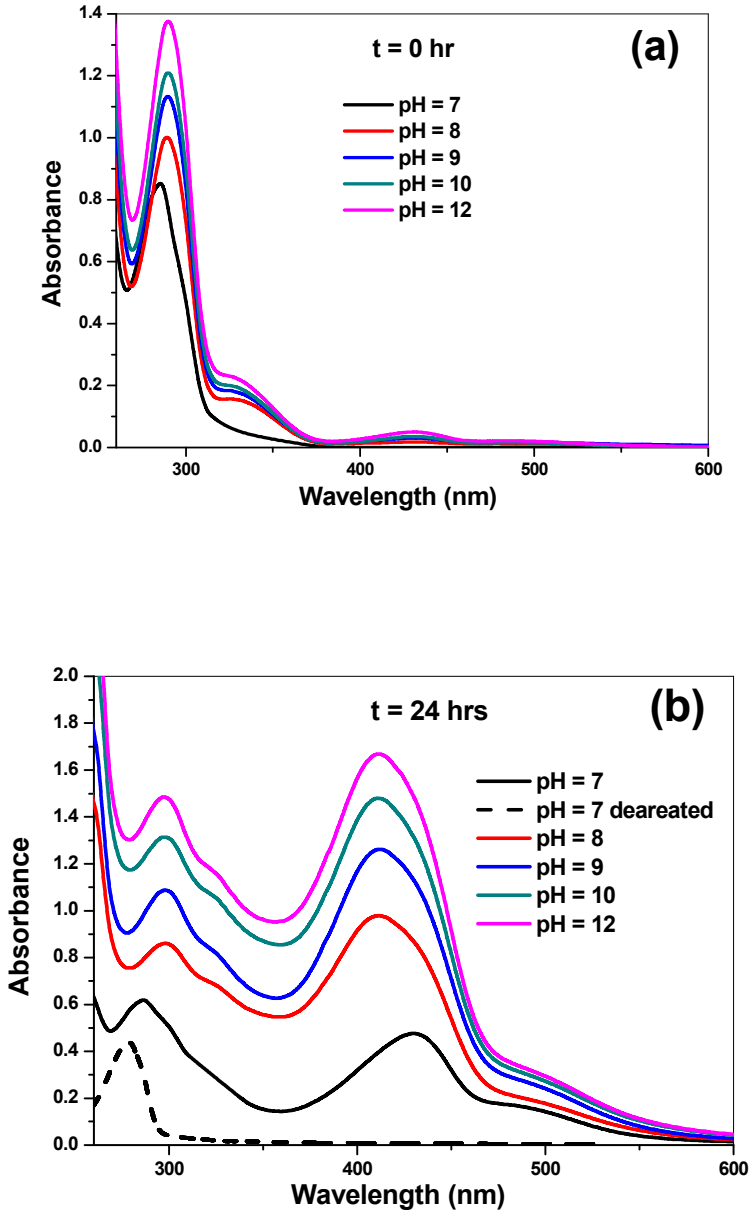


Figure 1: (a) Absorption spectra of 0.2 mM catechin in water at t=0 at different pHs (as shown in the legends). (b) Absorption spectra of 0.2 mM catechin in water at different pHs after 24 hours. The dashed line shows the absorption spectra of a deaerated solution of catechin at pH =7 after 24 hrs.

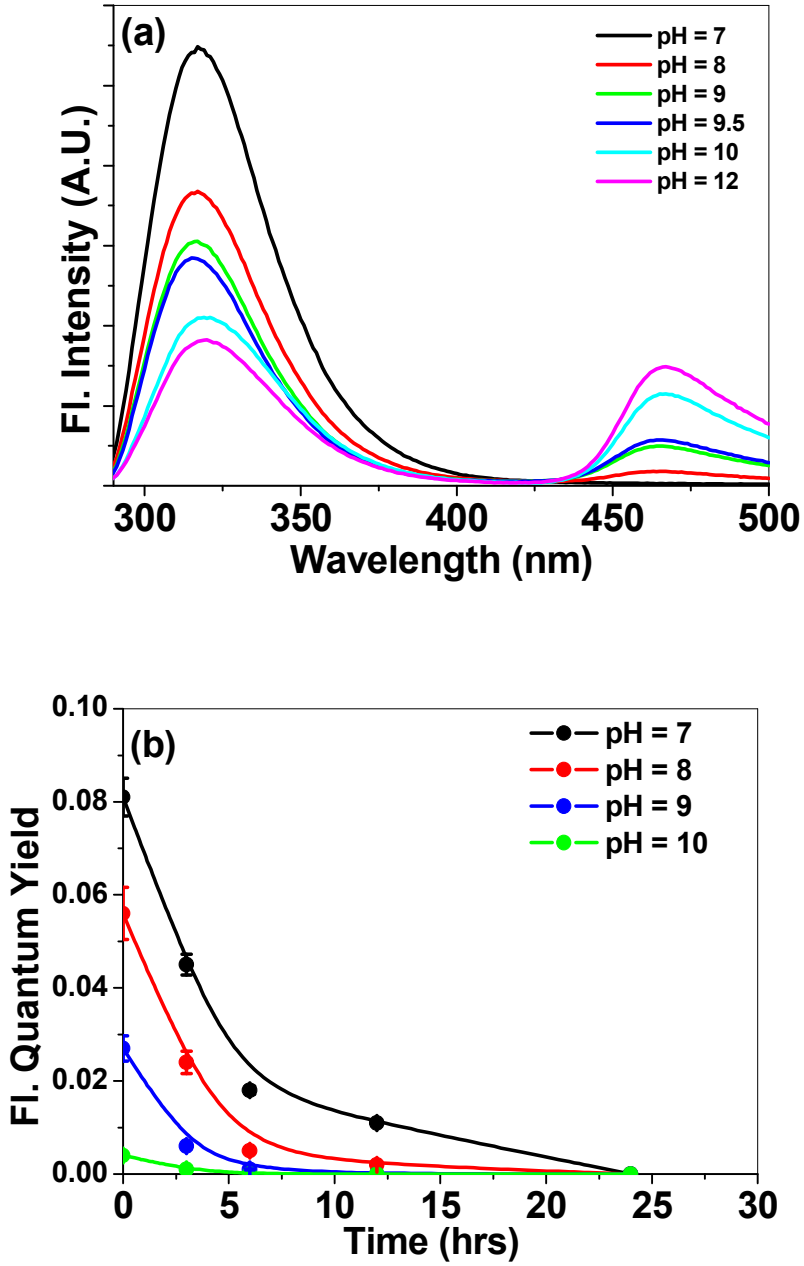


Figure 2: (a) Fluorescence spectra of 0.2 mM catechin taken after 6 hours of adding catechin to different pH solutions. (b) Fluorescence quantum yields as a function of the storage time at various pH.

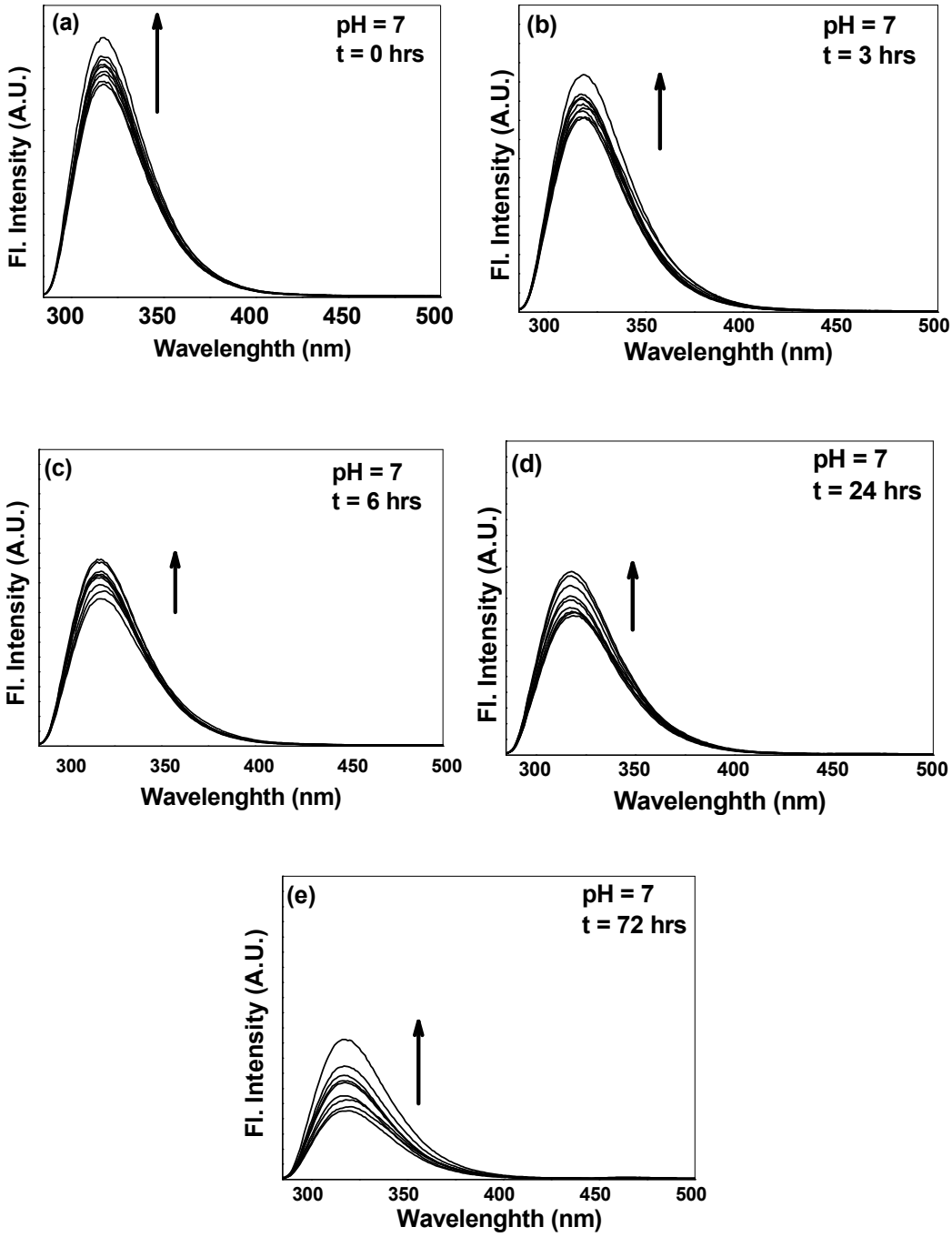


Figure 3: Emission spectra of aqueous solution of catechin (0.2 mM) at pH 7 with increasing concentration of MC at different time intervals. Concentration of MC is 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM respectively.

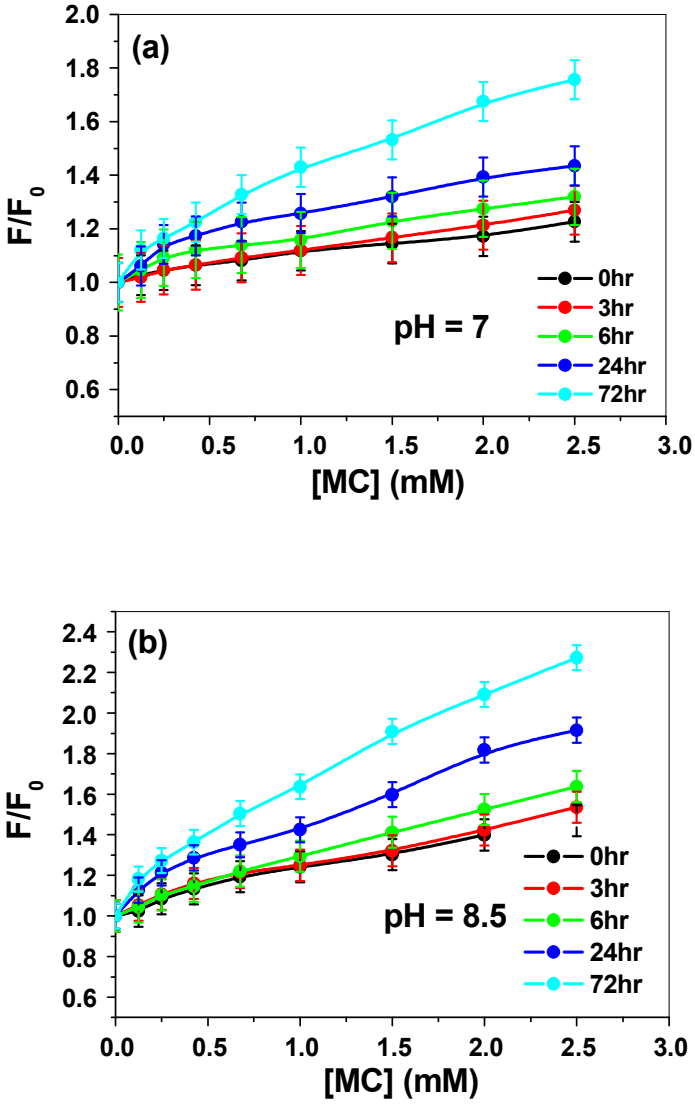


Figure 4: Relative fluorescence intensity (for the peak at 316 nm) for interaction of catechin with MC at different pH.

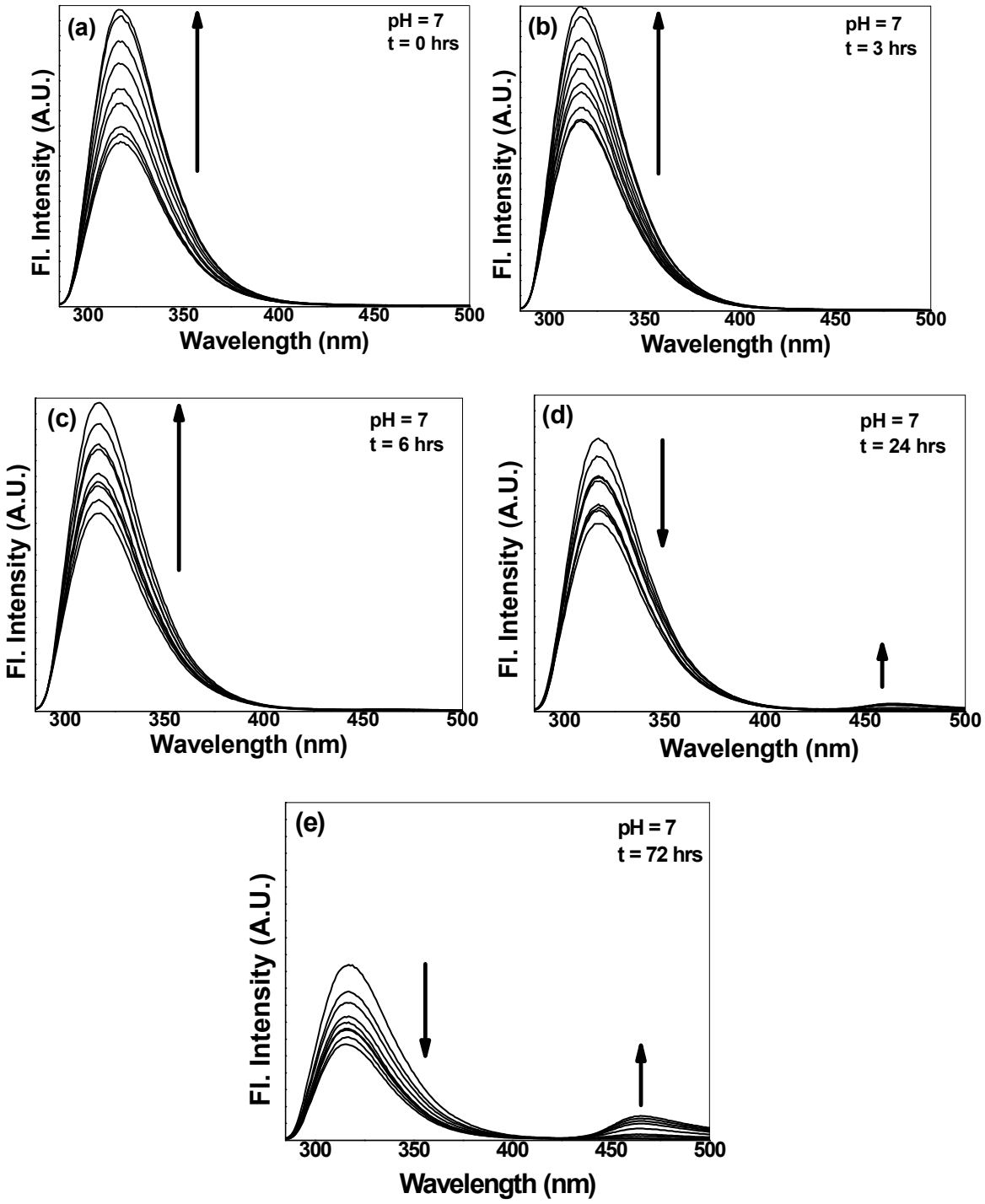


Figure 5: Emission spectra of aqueous solution of catechin (0.2 mM) at pH 7 with increasing concentration of brij-58 at different time intervals. Concentration of brij-58 is 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM respectively.

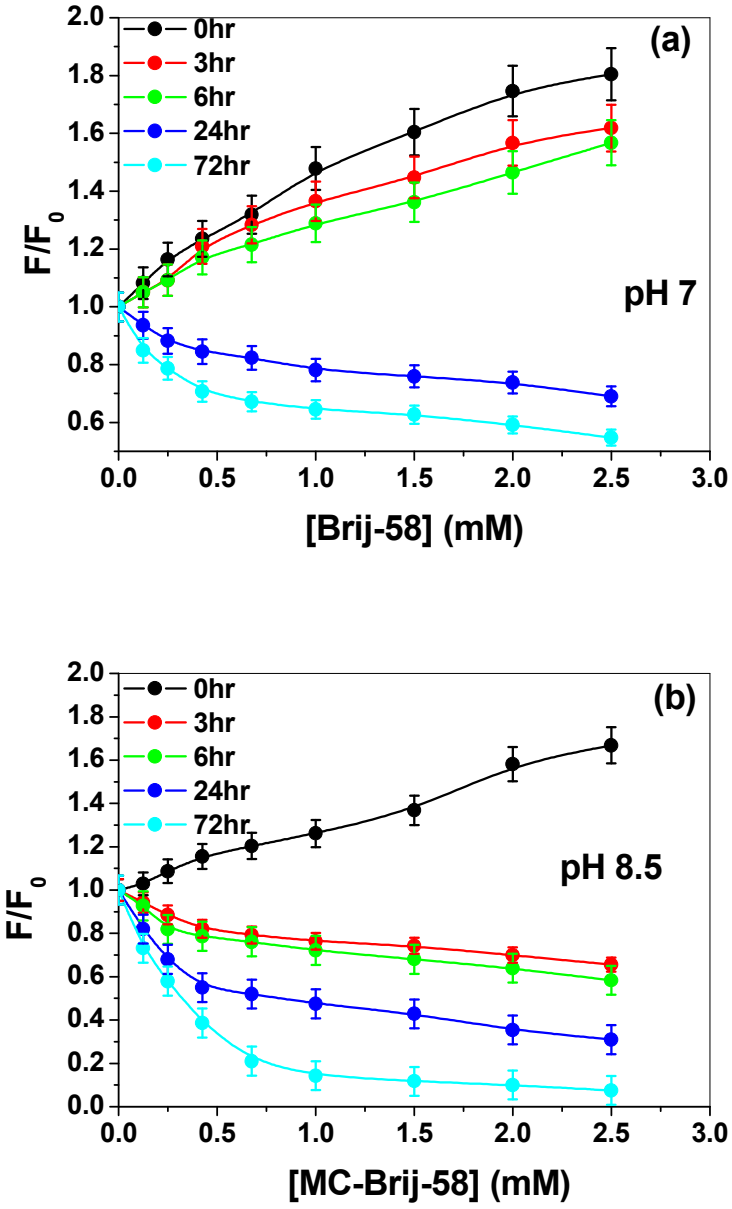


Figure 6: Relative fluorescence intensity (for the peak at 316 nm) for interaction of catechin and brij-58 at different pH.

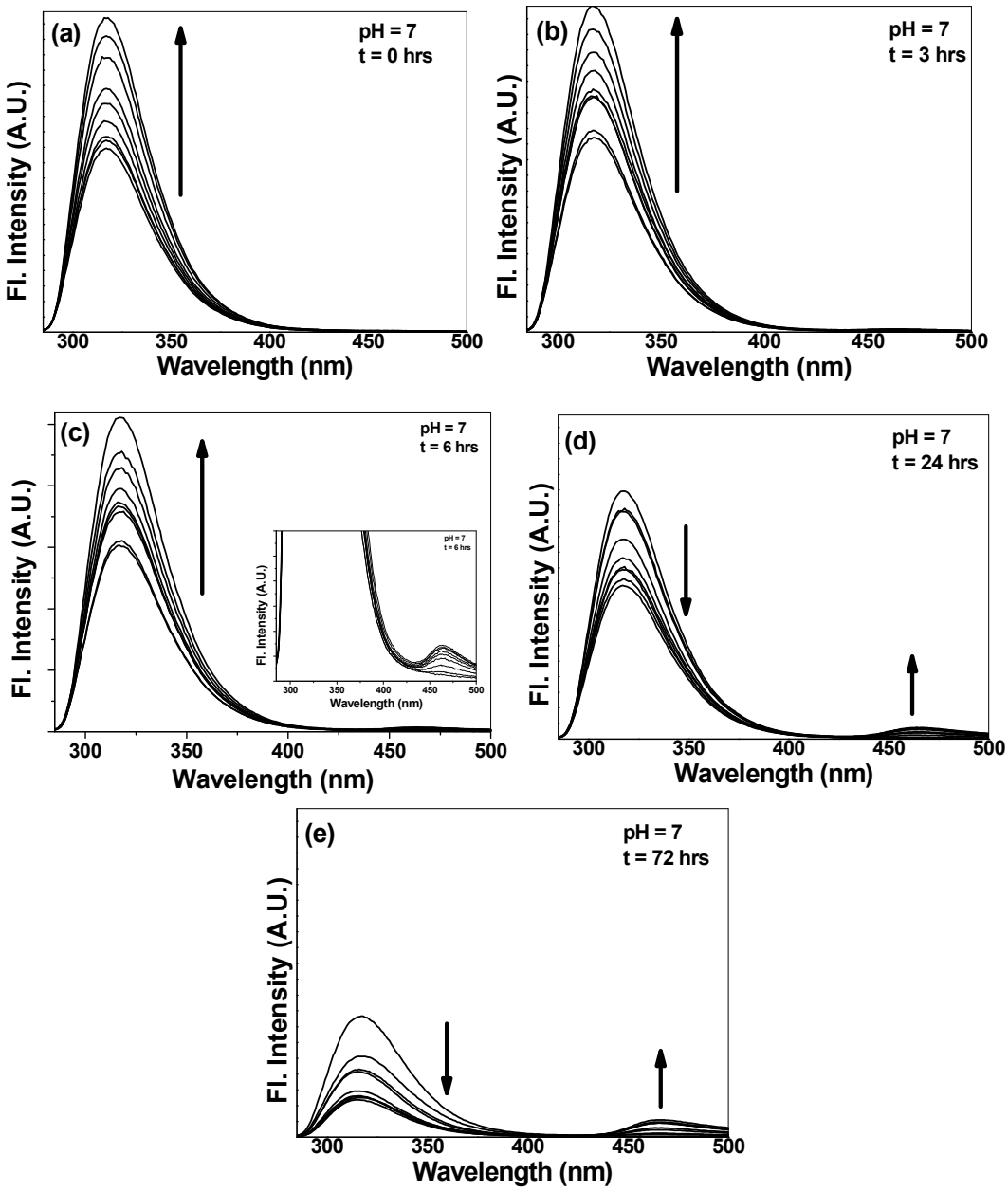


Figure 7: Emission spectra of aqueous solution of catechin (0.2 mM) at pH 7 with increasing concentration of brij-58 and MC (1:1) at different time intervals. Concentrations of brij-58 and MC in all the cases are 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM respectively.

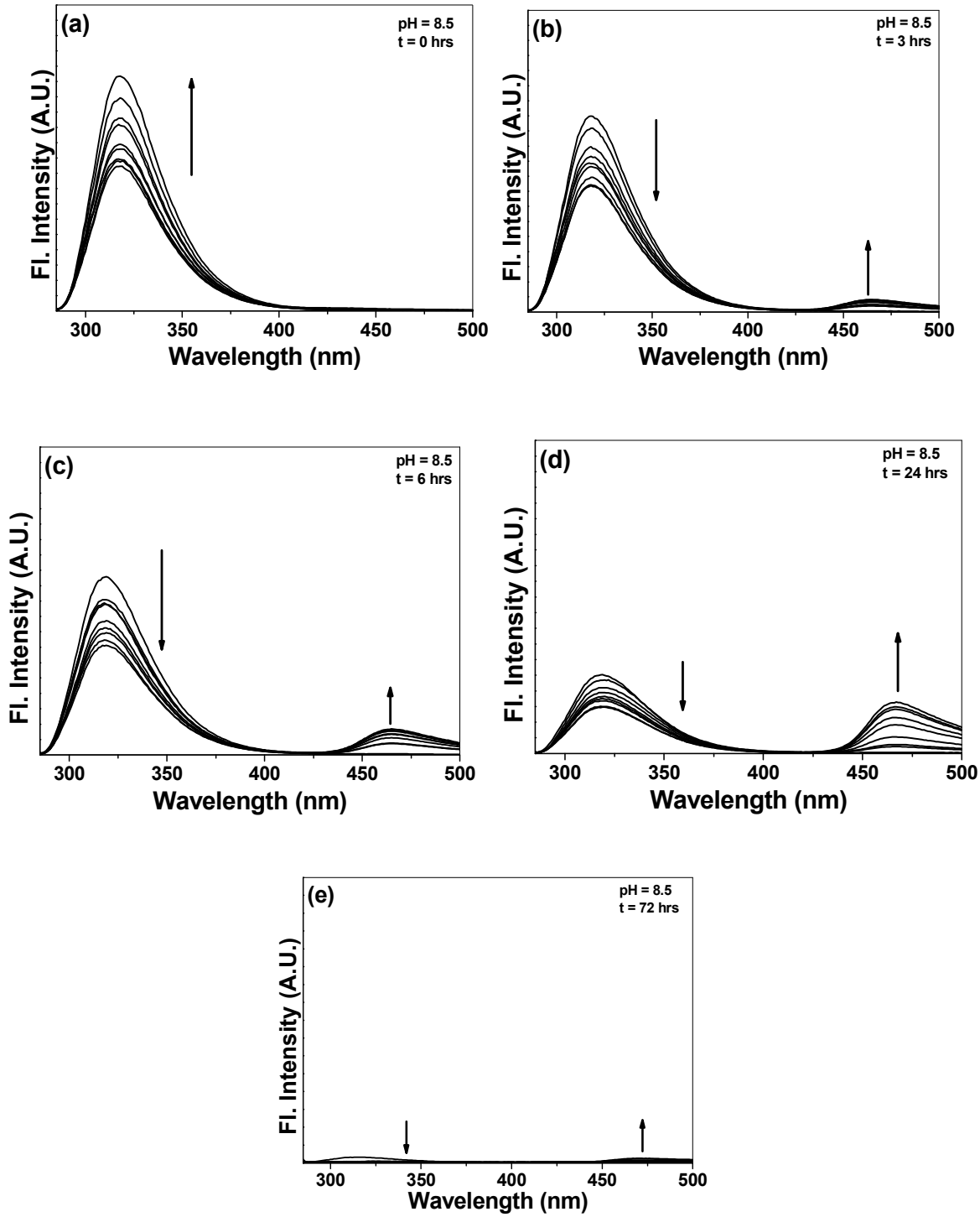


Figure 8: Emission spectra of aqueous solution of catechin (0.2 mM) at pH 8.5 with increasing concentration of brij-58 and MC (1:1) at different time intervals. Concentrations of brij-58 and MC in all the cases are 0, 0.13, 0.25, 0.5, 0.68, 1.0, 1.5, 2.0 and 2.5 mM respectively.

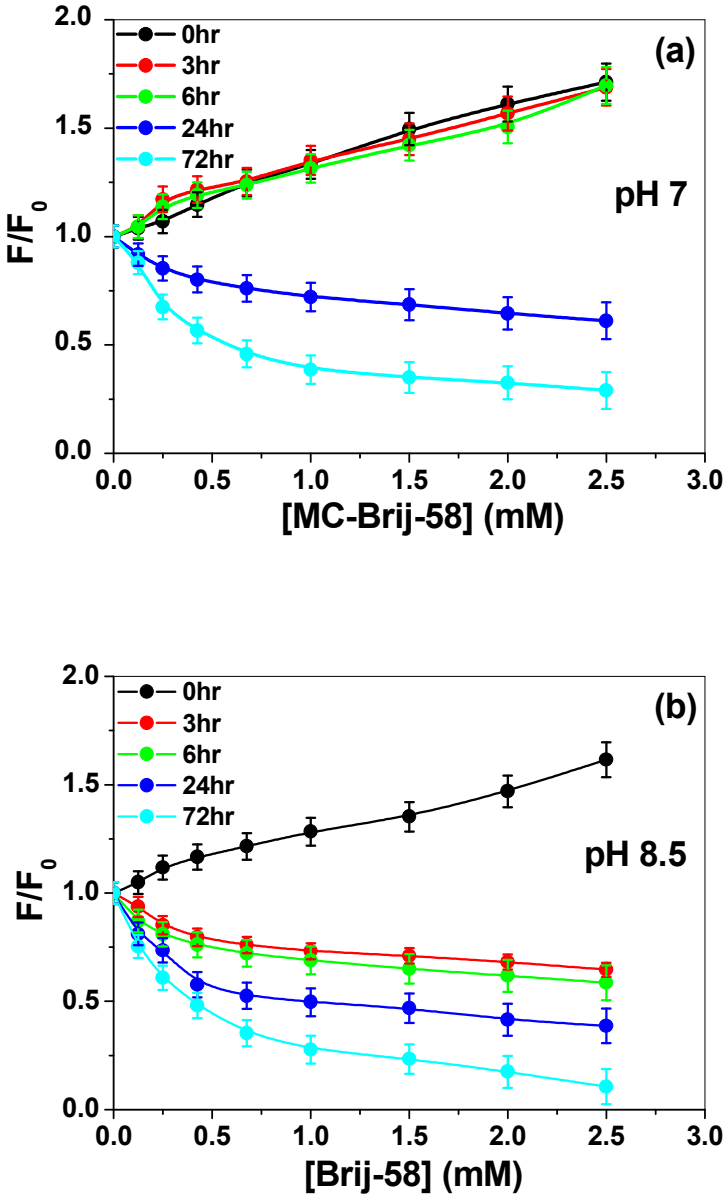


Figure 19: Relative fluorescence intensity (for the peak at 316 nm) for interaction of catechin and brij-58 and MC (1:1) at different pH.

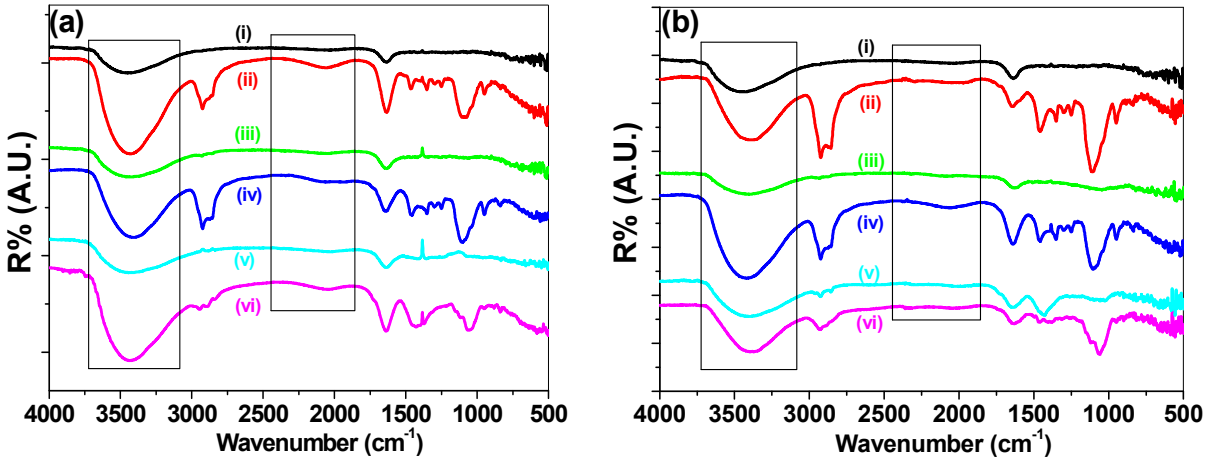
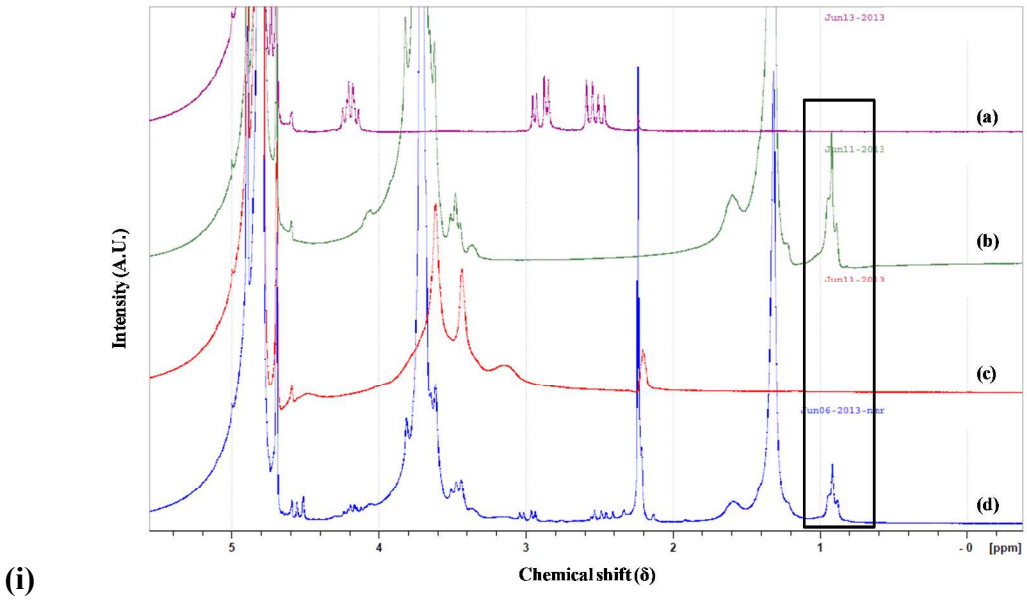
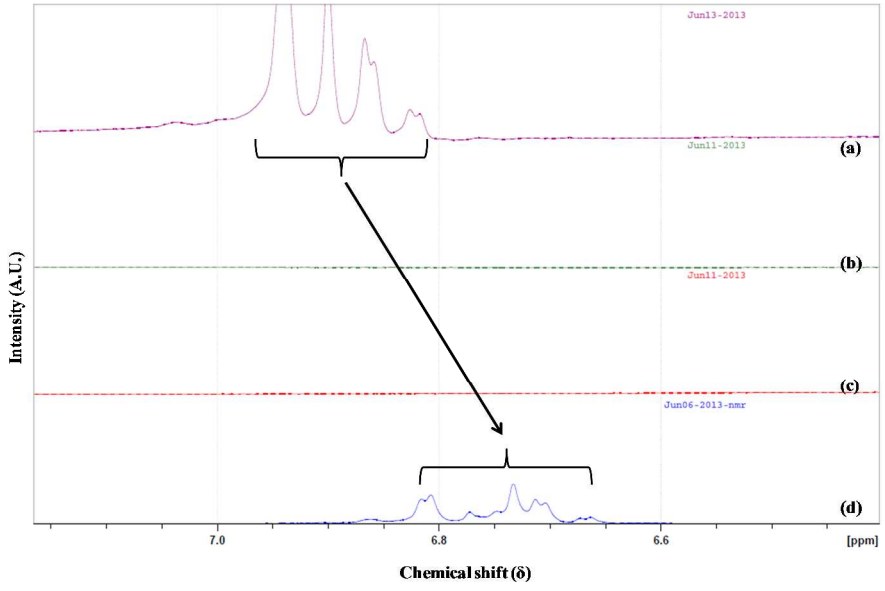


Figure 10: FTIR spectra for the interaction of catechin with brij-58 and MC (1:1) at (a) 0 and (b) 24 hours. In the figures, curves (i) and (ii) are spectra at pH 7; (iii) and (iv) are spectra at pH 8.5; (v) and (vi) are spectra at pH 10, for 0 and 10 mM concentrations respectively.



(i)



(ii)

Figure 11: ^{1}H NMR spectra in D_2O solvent for (a) catechin, (b) brij-58, (c) MC and (d) 1:1 mixture of brij-58 and MC with catechin. (i) and (ii) are magnified spectra for the aliphatic and aromatic protons respectively.