

Autooxidation of Some Polyphenols in Various Copper(II) Solutions

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Abstract :The rates of autooxidation of naturally occurring polyphenols, viz., gallic acid, catechin and epicatechin in various aqueous copper(II) solutions – surface active and non surface active types, were measured at 25 °C under a variety of experimental conditions. Compared to gallic acid and the simpler alkyl-substituted diols, such as 4-methylcatechol, and 3,5-di-*tert*-butylcatechol, catechin and epicatechin were found to be relatively inert towards autooxidation. The relative stability of catechins may be related to their tricyclic C₆-C₃-C₆ flavan skeleton in a manner not well understood at the moment.

Key words: polyphenols; autooxidation rate in copper(II) solutions

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Introduction

In nature, particularly in plants, there is abundance of naturally occurring phenols that contain more than one aromatic hydroxyl group, eg polyphenols. These polyphenols are antioxidants the consumption of which are associated with a reduced risk of diseases such as coronary heart disease and cancers [1, 2, 3]. The stability of these polyphenols towards oxygen in aqueous solutions has not been studied systematically. On the other hand, oxidation of catechols catalyzed by transition metal complexes, in particular, copper(II) complexes have been widely studied because of its relevance to biological phenomena involving metalloenzymes [4, 5, 6, 7, 8]. 3,5-di-*tert*-butylcatechol (3,5-DTBC) and catechol have both been commonly used in these investigations.

As part of our group's interest in the catalytic effects of copper(II) complexes, we have initiated a study on the autooxidation of some common natural polyphenols in aqueous solutions. The polyphenols that have been found to be convenient for the study are gallic acid, catechin and epicatechin. See Diagram 1 for the structures of these phenols. Gallic acid is found in gallnuts, tea leaves and fruits in the ester or acid forms and is used as antioxidant in food to prevent rancidity induced by lipid peroxidation and spoilage. Catechins are also found in fruits, tea leaves and cacao beans. They can polymerize to form proanthocyanidins which are responsible for the astringency of fruits [9].

For the purpose of comparison, we have also measured the autooxidation rate of the simpler phenols, namely, catechol, 4-methylcatechol and 3,5-DTBC, under the same conditions as the naturally occurring polyphenols mentioned earlier. The preliminary results are reported in this paper.

Materials and Methods

Catechol (Fisher, certified), 4-methylcatechol (Fluka, 97%), gallic acid (Fluka, >98%), (+)-catechin (Sigma, 98%), (-)-epicatechin (Fluka, >90%) were used as received. 3,5-di-*tert*-butylcatechol (Fluka, >98%) was recrystallized from heptane.

Copper bromide (Fluka, >99%), tetramethylethylenediamine (tmed) (Fluka, >97%), Trizma base (Sigma, >99.9%), Trizma hydrochloride (Sigma, >99%) were used as received.

The two long chain containing ligands, N,N,N'-trimethyl-N'-dodecylethylenediamine (C₁₂-tmed), N,N,N'-trimethyl-N'-hexadecylethylenediamine (C₁₆-tmed) and the surface active copper complexes, [Cu(C₁₂-tmed)Br₂], [Cu(C₁₆-tmed)Br₂] were prepared according to literature procedures [10]. The complexes were purified by recrystallization from a CH₂Cl₂/absolute ethanol mixture. The non-surface active copper complex, [Cu(tmed)Br₂] was not isolated but prepared *in situ* by mixing CuBr₂ and tmed in 1:1 ratio.

Water was freshly distilled from an all-glass apparatus and air-saturated before using it to prepare solutions. In concentration variation studies, stock solutions except phenols were prepared in 1 mM trizma buffer (final pH 6.90 ± 0.05). In measurements involving pH variation, HBr and NaOH were used to adjust pH and no buffer was used. Stock solutions of catechol, 4-methylcatechol, and gallic acid were prepared in pure water, DTBC and catechin in redistilled acetonitrile, and epicatechin in absolute ethanol.

Kinetic studies

All polyphenols used in this work are initially oxidized to their respective quinones which have λ_{max}

at the following values (in the presence of $[\text{Cu}(\text{C}_{12}\text{-tmed})\text{Br}_2]$ at pH 6.9): catechol (431 nm), 4-methylcatechol (469 nm), 3,5-DTBC (400 nm), catechin (448 nm), epicatechin (454 nm) and gallic acid (400 nm). The formation of quinones was monitored at these maxima for the various phenols using a Cintra-5 UV-vis spectrophotometer.

In a typical kinetic run, 3 mL of the copper solutions was introduced into a 1-cm cuvette maintained at 25 °C by a thermostat bath, which circulated water around the cell holder jacket. After 12 min in the sample holder, 5 μL of a stock solution of polyphenol (~ 0.0323 M) was injected, followed by rapid stirring (horizontally and vertically) to start the reaction. The final concentration of the polyphenol was approximately 5.4×10^{-5} M. In the majority of cases the formation of quinone was monitored for at least several half-lives. Under this pseudo-first-order condition where the copper concentration is at least 10 times higher than that of the substrate, good first-order kinetics was always observed.

The data were analyzed by the following equation [11] using a commercial program

$$A_{\text{ob}} - A_{\text{Cu}} = \epsilon_{\text{q}}C_{\text{o}} - \epsilon_{\text{q}}C_{\text{o}}e^{-kl} \cdot e^{-kt}$$

Here A_{ob} and A_{Cu} are the observed absorbance and the absorbance of the pure copper(II) species respectively; ϵ_{q} , the molar absorptivity of quinone; C_{o} , the initial concentration of 3,5-DTBC; l , the time interval between the time when the reactants are mixed and the time when the first absorbance reading is taken; t , the time when absorbance is measured and k is the observed rate constant. For slow kinetics where the expected rate constant is of the order of 10^{-4} s^{-1} or less, the above equation was fitted by assuming a ϵ_{q} value for a particular quinone. This ϵ_{q} value was obtained in a separate experiment by measuring the absorbance at λ_{max} at the time when the polyphenol was completely oxidized to the quinone.

The reported rate constants are the average of three or more independent measurements. The uncertainty is estimated to be less than 20 %.

Results and Discussion

As expected from previous studies [11, 12, 13], the autooxidation rates in the absence of copper(II) species at pH 6.9 and 25 °C were found to be extremely slow compared to those in the presence of copper(II) species. For example, for DTBC and gallic acid, the observed rate constants are 7×10^{-4} s^{-1} and 1.7×10^{-5} s^{-1} respectively, which are many times smaller than those values measured in copper(II) species and reported in Table 1. For catechin and epicatechin the rates were even slower as nearly horizontal lines were observed in the 30-min kinetic curve.

Effects of 1 mM copper(II) solutions:

As observed in Table 1, at pH 6.9, among the substrates, 3,5-DTBC is most susceptible to autooxidation in CuBr_2 , $[\text{Cu}(\text{tmed})\text{Br}_2]$, and second most susceptible in the two surface active copper(II) complexes. The latter result is particularly interesting because micellar copper(II) species have been reported to be a much better rate-enhancing reagent compared to non-micellar copper(II) species [11, 12] at pH 5.7. The apparent contradiction here is most likely due to different pH conditions as our earlier studies have demonstrated that the autooxidation rate is very much dependent on pH of the solution [13]. To confirm the rate-enhancing effect of micellar species at lower pH, we found that at pH 6.0 (in the trizma buffer solution), the observed rate constant in $[\text{Cu}(\text{C}_{16}\text{-tmed})\text{Br}_2]$ and $[\text{Cu}(\text{C}_{12}\text{-tmed})\text{Br}_2]$ micelles were increased to 0.044 and 0.036 s^{-1} whereas in CuBr_2 and $[\text{Cu}(\text{tmed})\text{Br}_2]$, the rate constants were lower, being 0.017 and 0.022 s^{-1} respectively.

In micellar copper(II) solutions gallic acid is most readily oxidized and is second most readily to be oxidized in non-micellar copper(II) solutions. Compared to the simpler diols with alkyl substituent(s) such as 4-methylcatechol and 3,5-DTBC, catechin and epicatechin are relatively unreactive as the rate

Table 1 : Observed rate constants (s^{-1}) of autooxidation of various phenols in 1 mM copper(II) solutions (buffered with 1 mM trizma buffer at pH = 6.9 at 25°C); Error is less than 20% based on five or more independent measurements

| | CuBr_2 | $\text{Cu}(\text{tmed})\text{Br}_2$ | $\text{Cu}(\text{C}_{12}\text{-tmed})\text{Br}_2$ | $\text{Cu}(\text{C}_{16}\text{-tmed})\text{Br}_2$ |
|------------------|----------------------|-------------------------------------|---------------------------------------------------|---------------------------------------------------|
| Catechol | 1.1×10^{-3} | 2.6×10^{-4} | 3×10^{-3} | 2×10^{-3} |
| 4-methylcatechol | 2.7×10^{-4} | 8×10^{-4} | 4.3×10^{-3} | 1.8×10^{-3} |
| 3,5-DTBC | 2.5×10^{-2} | 2.2×10^{-2} | 1.5×10^{-2} | 1.1×10^{-2} |
| Catechin | 2.8×10^{-4} | 1.7×10^{-4} | 1.5×10^{-3} | 1.1×10^{-3} |
| Epicatechin | 5.0×10^{-4} | 2.4×10^{-4} | 1.7×10^{-3} | 1.0×10^{-3} |
| Gallic acid | 1.8×10^{-3} | 1.6×10^{-3} | 2.4×10^{-2} | 2.1×10^{-2} |

constants are of the order of 10^{-3} s^{-1} and less. The unsubstituted diol, catechol is less reactive compared to the alkyl substituted diols and the rate constants are slightly higher than catechin and epicatechin.

For the polyphenols, we have also measured the rates at lower and higher pH conditions and found that the rates do not change significantly between 5.5 to 7.5

for the two micellar copper(II) solutions but drop to 2-3 times slower at pH below 5.5. The observed rate constants vs pH for catechin and epicatechin are shown in Figures 1 & 2 respectively. For the non-micellar copper(II) systems, the observed rates remain nearly constant in the range 5 to 7.4.

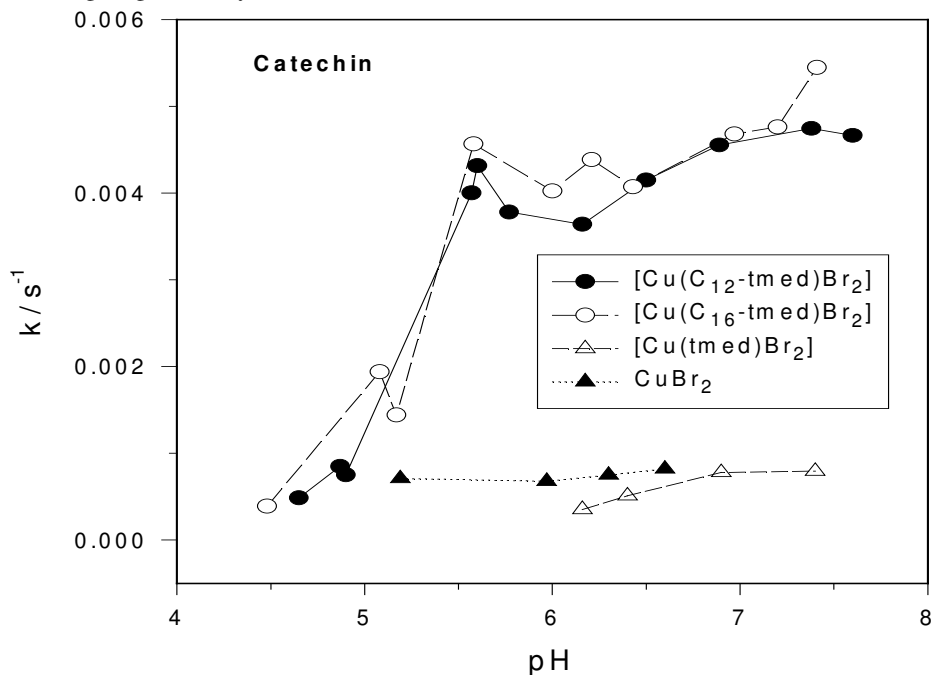


Figure 1: Observed rate constants of autooxidation of catechin as a function of pH in various 1.0 mM copper(II) solutions at 25 °C

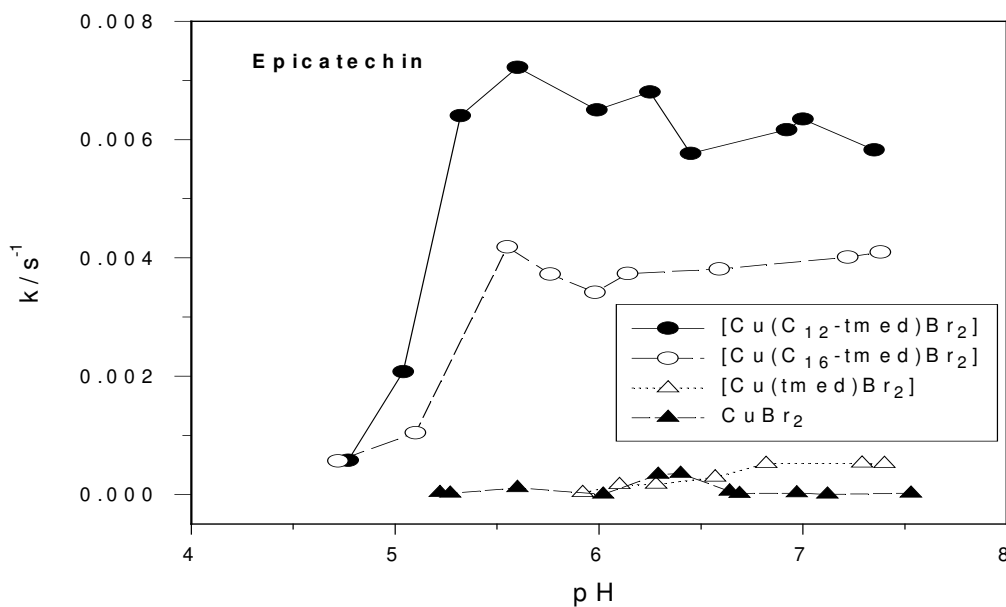
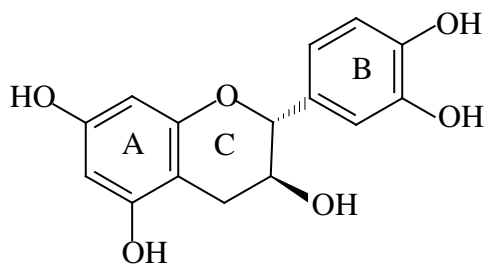


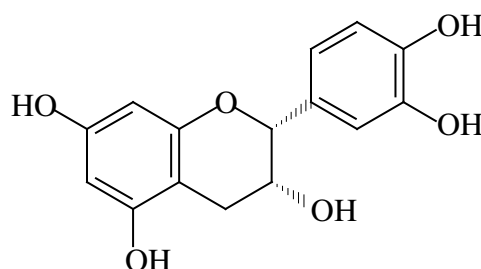
Figure 2 : Observed rate constants of autooxidation of epicatechin as a function of pH in various 1.0 mM copper(II) solutions at 25 °C

The stability of catechins compared to that of the simpler diols and triol may be related to their tricyclic C₆-C₃-C₆ flavan skeleton in a manner not well understood at the moment. The inertness towards dissolved oxygen may explain why hot tea extract still contains substantial amount of catechins and thus retains its nutritional value. In connection with this, an interesting observation is the high reactivity of quercetin, chlorogenic acid, and 3,4-

dihydroxycinnamic acid in the presence of copper(II) micelles. These antioxidants which are found in plants also have two phenolic OH groups in the ortho position (Diagram 1). Quercetin is a flavonol similar to catechin except that the C-ring has ketone and enol groups. They auto-oxidize so fast that the solutions turn brown or give oxidized product in the form of precipitate within seconds.



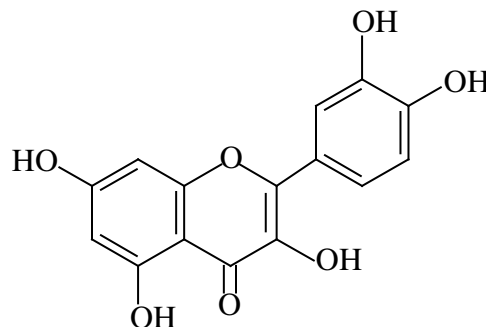
1. Catechin



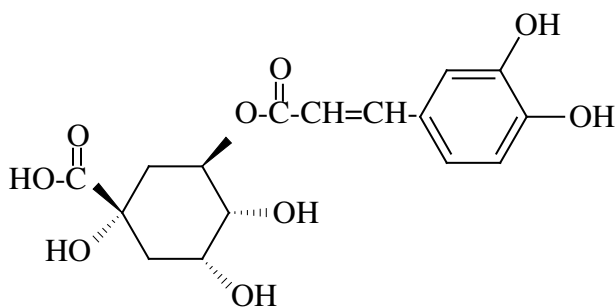
2. Epicatechin



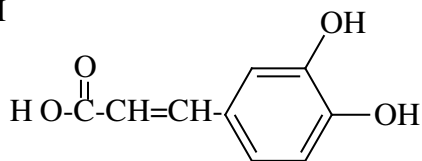
3. Gallic acid



4. Quercetin



5. Chlorogenic acid

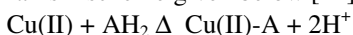


6. 3,4-dihydroxycinnamic acid

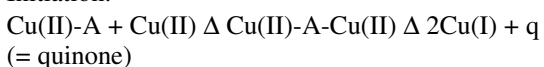
Diagram 1 : Structures of naturally occurring polyphenols

Effects of concentration of micellar copper species

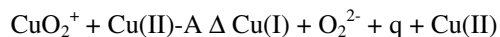
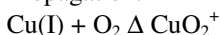
The observed rate constants increase regularly as the concentrations of copper(II) complexes increase from 0.3 to 2.0 mM for all the phenols studied. See Figures 3 & 4 for catechin and epicatechin. The increase has been ascribed to increased concentration effect of micelles as a result of higher concentration of micelles at higher concentration of the surface active copper(II) complexes [11, 12, 13]. Higher copper concentration favours formation of $\{Cu(II)_2A\}$ species (where AH_2 = polyphenol) which is the intermediate species invoked in the initiation step in the reaction mechanism scheme given below [14].



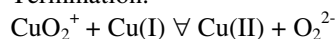
Initiation:



Propagation:



Termination:



One interesting observation is that catechin and epicatechin have different autooxidation rate behaviour in different concentration range of copper(II) micelles. At low concentration range ($\sim 0.5 - 1.0$ mM) both are oxidized at a faster rate in $[Cu(C_{12}\text{-tmed})Br_2]$ micellar solution but at higher concentration range ($\sim 1.5 - 2$ mM) catechin is oxidized faster in $[Cu(C_{16}\text{-tmed})Br_2]$ micellar solution. This stereospecific behaviour warrants further investigation.

Conclusion

This work demonstrates the relative inertness of catechins towards autooxidation when compared to gallic acid, quercetin, chlorogenic acid, 3,4-dihydroxycinnamic acid, and the simpler diols such as 4-methylcatechol, and 3,5-DTBC.

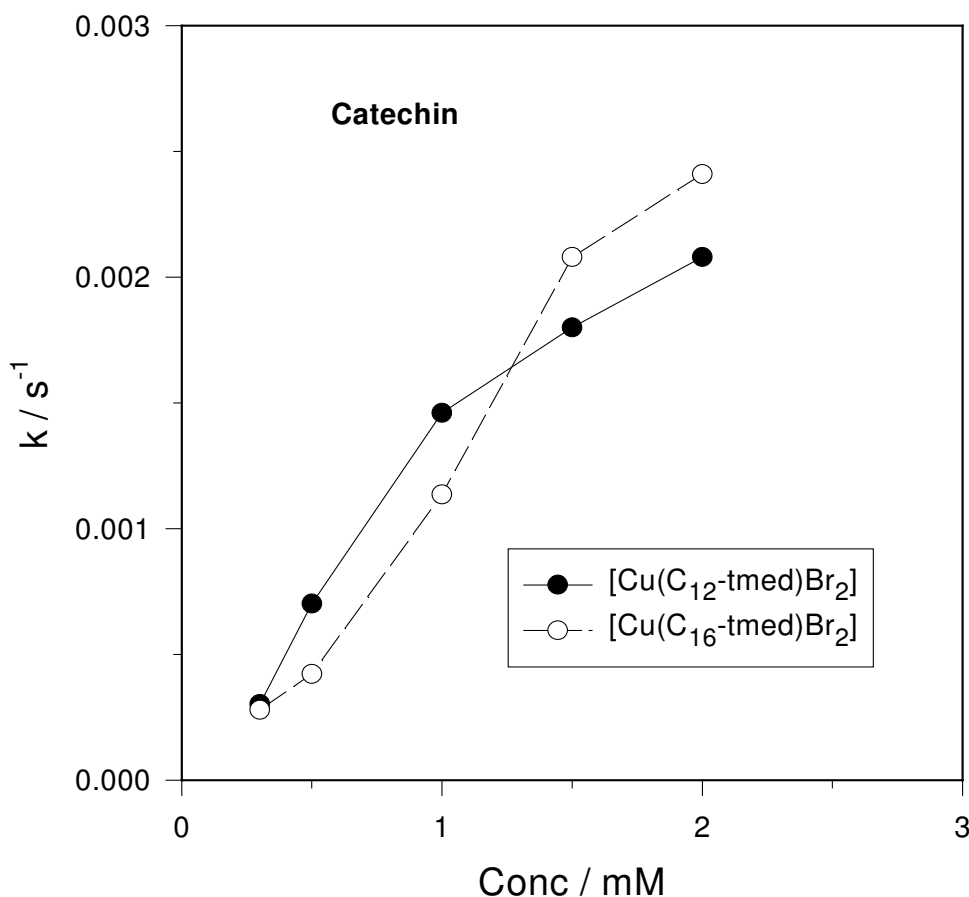


Figure 3 : Observed rate constants of autooxidation of catechin in various concentrations of micellar copper(II) solutions at pH 6.9 at 25 °C

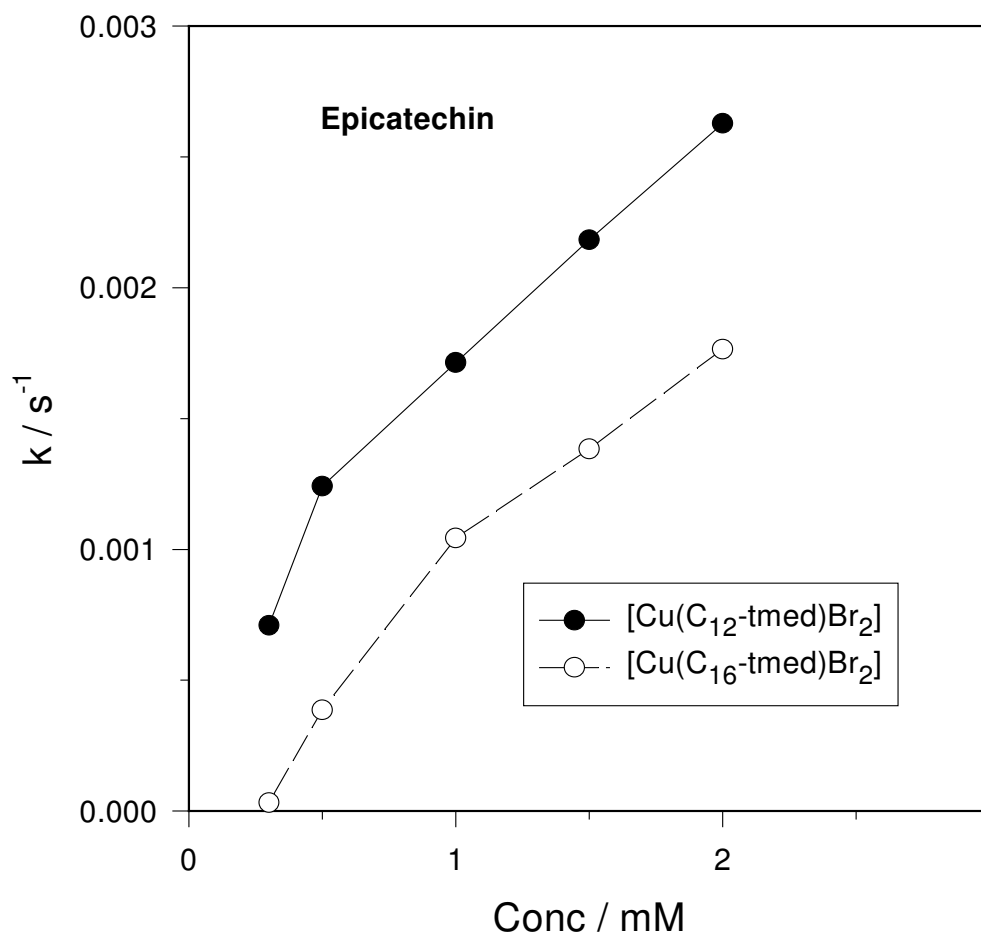


Figure 4 : Observed rate constants of autooxidation of epicatechin in various concentrations of micellar copper(II) solutions at pH 6.9 at 25 °C

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