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Iron complexation of pharmaceutical catechins through selective separation[†]

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Catechins, natural polyphenols from tea leaves are important building blocks in pharmaceutical industries due to their chemoprotective and cardioprotective effects. The concept of metal complexation of catechins is integrated with Liquid Membrane based selective separation technique for recovery of pharmaceutical catechins from natural bio-sources. This is first ever approach to amalgamate the complexation chemistry with engineering approach which is green and energy efficient.

Tea polyphenols, viz. catechin compounds, have been proved to be the efficient scavenger of the biologically damaging oxyradicals such as the superoxide radicals as well as singlet oxygen, due to their ability to act as free radical acceptor.^{1,2} Polyphenols have high reactivity with other phenolics or metal compounds that yields compounds having sensorial properties like color and taste. One of the reasons for many polyphenols lacking the quality to become pharmaceutical drugs is non-adherence to Lipinski's stringent rule of 5.³ However, the quality of the drugs (ligands) depends up on the complementarity of these ligands and corresponding receptors.^{4–6} In their quest for better anti-cancer agents, the researchers have been inspired to design novel polyphenol-based or polyphenol-inspired drugs which contain various functionalized materials that take advantage of the unique physicochemical properties of the phenol functional group.⁷

On the other hand, metal-catechin complexes have strong bioactivities that include even prevention of cancer.^{8,9} Synthesized metal complexes are more effective than their elemental form. The lipophilicity of a drug is increased through the formation of metal chelates and action of the drug is enhanced due to effective permeability of the drug into the site of action. Cisplatin, carboplatin and oxaliplatin are the well-known metal-based drugs widely used in treatment of cancer.¹⁰ Other metal complexes have proven results too, in the treatment of

diseases like diabetes, ulcer, rheumatoid arthritis, inflammatory and cardiovascular diseases.

Catechin molecule has two benzene rings (called the ring-A and ring-B) and a dihydropyran heterocycle (the ring-C) with a hydroxyl group on carbon 3 as shown in Fig.1. The ring-A and ring-B are similar to a resorcinol

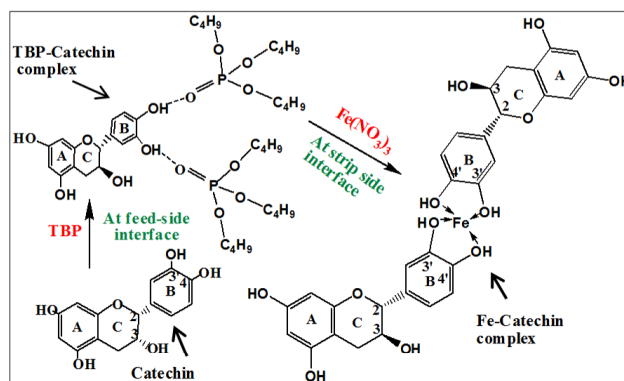


Fig. 1 Schematic of Fe-catechin complex formation from catechin

moiety and catechol moiety respectively. There are two chiral centers on the molecule on carbons 2 and 3. Hence, it has four diastereoisomers. The *trans* and *cis*-configurations are called (+)catechins and (–)epicatechin respectively. The different epimers can be distinguished using chiral column chromatography. Referring to no particular isomer, the molecules can just be called catechin compounds. We have extracted (+)catechins using a neutral extractant, tributyl phosphate (TBP). The possible reaction mechanism between catechin and TBP and the stoichiometry have been discussed elsewhere.¹¹

We have used seven metal ions (Al^{3+} , Cu^{2+} , Ni^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} and Fe^{3+}) as complexing/precipitating agents for catechin in a two-phase stripping set-up. The results (in Table S2 of ESI[†]) indicate the Fe^{3+} as the second best complexing (92%) and stripping agent after Cu^{2+} . Brownish black precipitate was found in the solution mixture in case of iron salt (ferric), that resulted in shifting of equilibrium of the Fe-catechin complexation to

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the right (favourable for the complexation) at the stripping side interface. This is in line with the observations reported elsewhere.^{12,13} No precipitate was detected for other metals. As per the chemical coordination mechanisms of catechin, the electron donating catechol 3',4' dihydroxyl groups (at ring-B, catechol moiety) make the coordinate bond with the ferric ion (Fe^{3+}) to form the complex (Fig.1).¹²⁻¹⁴ The coordination of ferric ion occurs with the oxygen atoms of two catechol groups ($-OH$) in ring-B. These two hydroxyl groups are favorable as they are in ortho position and bond length is minimum. The other two hydroxyl groups ($-OH$) in ring-A will not form complex as they are in meta position. The phenoxyl group($-OH$) attached to the ring-C does not form the coordination bond with metal ion. The proposed complexation site between catechin and the ferric ion is shown in Fig.1. The stoichiometry between metal (ferric nitrate) and catechin in complex was determined employing Job Method.^{15,16} The detailed procedure and the results have been discussed in the ESI[†]. Complexation occurred in the strip side interface and the complex diffuses into the bulk strip solution prior to precipitation of so formed complex beyond certain concentration (solubility product). Strip samples are centrifuged prior to measurement of catechin and/or metal-catechin complex. It is assumed that the mass transfer of catechin across the membrane occurs only through diffusion mechanism. The interfacial flux due to the chemical reaction has been neglected, as the chemical reaction is intrinsically very fast, and hence the concentrations at the interfaces will almost be equal to the equilibrium concentrations.

Metal-catechin complex was analyzed by UV-vis spectra and the formation of complex was confirmed by XRD, FT-IR, SEM and EDX analyses. The UV-vis spectral analyses of the retentate (after centrifuge, as described in the experimental section in ESI[†]) are shown in Fig.2. The absorption spectra of retentate containing complexes with Ni^{++} and Cu^{++} were maximum between 386 – 391 nm, whereas the same for Al^{+++} indicates no trace of production of any complex at all. On the other hand, the absorption spectra of retentates involving Fe-catechin complex produced an interesting result. Two separate retentates were studied in this case, one produced in two phase equilibrium study (see ESI[†]) and the other recovered from the stripping phase after a three phase liquid membrane transport study. Both the spectra are nearly same. Hence, lower concentration of catechin in the retentate of the stripping phase indicates the occurrence of complexation reaction, whereas the absence of any UV-vis spectra of Fe-catechin complex in the retentate necessarily indicates conglomeration and precipitation of that complex during centrifuge. Bark et al.¹⁷ reported similar results by UV-vis spectral analyses of the catechin complexes with various metals.

The X-ray diffraction analysis of the precipitated compound with respect to that of pure catechin confirms the formation of

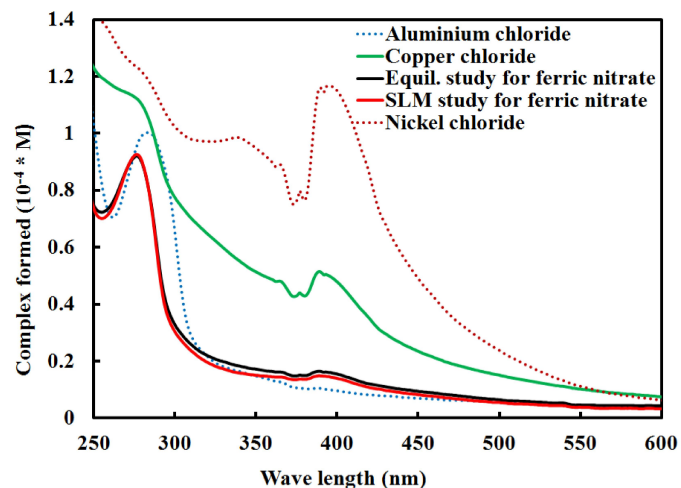


Fig. 2 UV-vis. Spectra of complex (un-precipitated) formed in strip solution ($Fe(NO_3)_3$) and in two phase equilibrium studies

metal-complex. Catechin being a crystalline compound has the maximum XRD peak at $2\theta = 15^\circ$ along with other major peak at $2\theta = 24.84^\circ$. On the other hand, metal-complex has non-crystalline characteristics that shows no peak in XRD analysis at 2θ in the range of $3 - 40^\circ$ (Fig.3). Chen et al.¹⁸ also reported the non-crystalline nature of metal-catechin complex through XRD analyses.

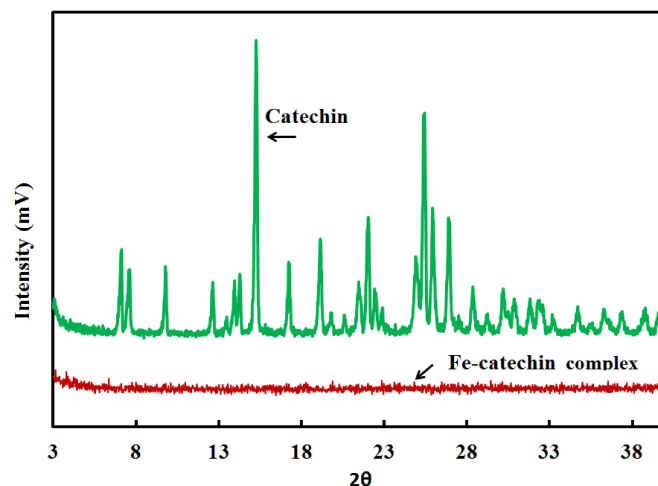


Fig. 3 XRD analysis of complex and pure catechin

The formation of metal-catechin complex was confirmed by FT-IR spectrum analyses too. The wave numbers of FT-IR spectra of pure catechin at 970, 1030, 1143, 1282, 1471, 1519 and 1612 cm^{-1} were assigned to C-H alkenes, $-C-O$ alcohols, $-OH$ aromatic, C-O alcohols, C-H alkanes, C=C aromatic ring and C=C alkenes respectively.¹⁸ Stretching of

O–H was found at 3210 cm^{-1} . The absorption bands at 1030, 1143, 1471 cm^{-1} were absent in the metal-catechin complex. The other wave numbers at 1519 and 1612 cm^{-1} were also shifted to 1504 and 1606 cm^{-1} respectively. A new spectrum was found for Fe–O stretching at 1382 cm^{-1} and the intensity of O–H spectrum at 3210 cm^{-1} became reduced and shifted to 3170 cm^{-1} which might be due to coordination bonding of iron (Fe) with 'O' atom of –OH group¹⁹ (Fig.4).

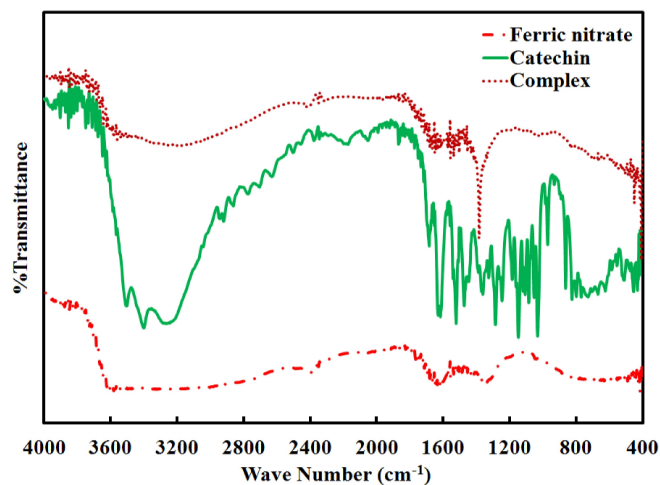


Fig. 4 Comparison of FT-IR spectra of metal-catechin complex and pure catechin with $\text{Fe}(\text{NO}_3)_3$

The vacuum-dried metal-catechin complex was characterized by scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) analysis in order to characterize the morphology of surface of the complex and to examine global chemical composition of the complex, respectively. The average particle size of complex was $100\ \mu\text{m}$ (Fig.5). The particles were in irregular arrangements indicating the amorphous nature of the complex in accordance with the results of XRD analyses. The presence of iron (Fe) in the complex was detected in EDX spectrum (Fig.6). The pH is an important parameter for extraction of catechin. Source phase of catechin (feed phase) is maintained at pH of 5.5 because aqueous solution of catechin has pH of 5.5 and it is stable in the acidic range of pH. Further, the effect of pH of stripping solution on the transportation of catechin was studied in the range of 1.2 to 2.96 as the complexation reaction at the strip-side interface is pH dependent. As expected, with decrease in pH of stripping solution transportation of catechin increased. The extraction increased from 68.1% to 72%, whereas the recovery increased from 62.1% to 65.6% for the pH reduction from 2.96 to 1.92. The results have been shown in Fig.7.

On further decrease of pH, a sharp increase in the transportation was observed due to the complexation reaction, hence the precipitation of complex was favored in this range

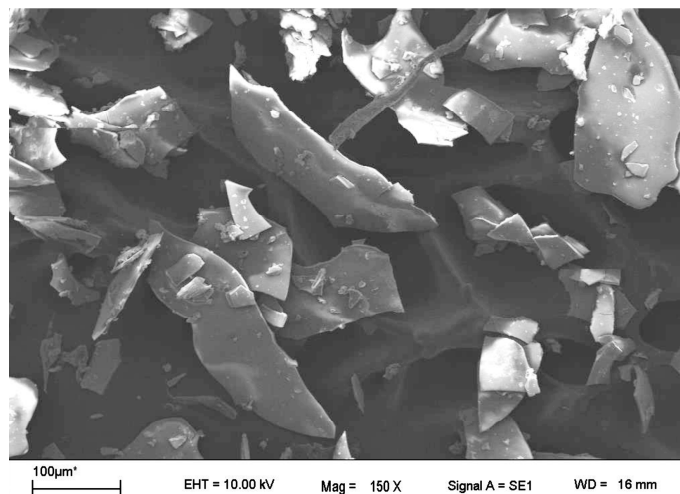


Fig. 5 Vacuum dried Fe-catechin complex powder as observed under Scanning Electron Microscope

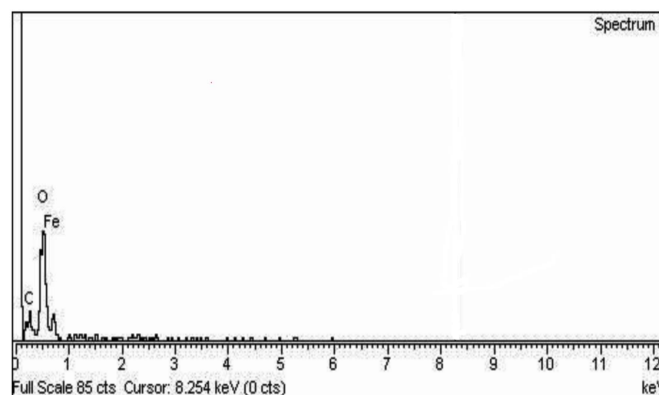


Fig. 6 EDX spectra of vacuum-dried Fe-catechin complex

of pH. Equilibrium of complexation reaction shifted to the right, leading to more catechin transportation from feed solution to strip side interface. Hence, maximum available driving force (concentration gradient of complex across the membrane phase) is available for overall transportation. On further lowering of pH below 1.8, marginal changes were observed in both the extraction and recovery, with a maximum transportation at pH of 1.55.

The precipitation of metal-catechin complex begins at its solubility product in the stripping solution. On the other hand, stoichiometry between metal and catechin in their complex depends upon the molar ratio of the reactants (catechin:metal::2:1). The concentration of ferric ion in the stripping solution is important for precipitation and/or recovery due to above two reasons. In order to find the most favorable concentration of the stripping solution, it was varied from

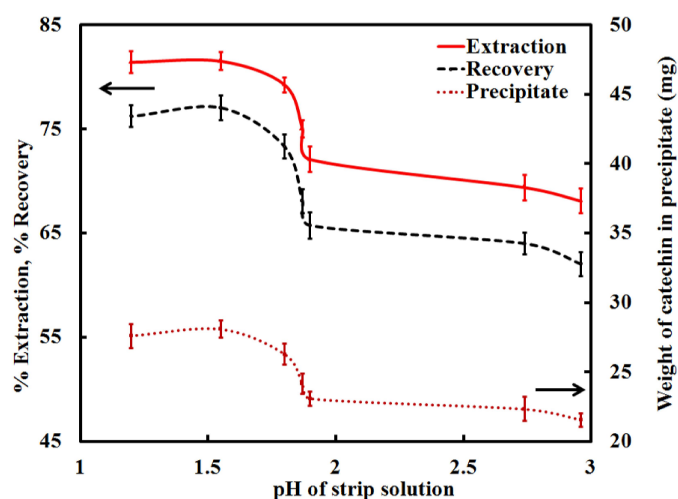


Fig. 7 Role of pH of stripping solution

0.0002M to 0.003 M, while the pH of stripping solution was 1.55 at the beginning of the reaction. Both extraction and recovery increased with the increase in concentration of strip solution up to 0.001M. On further increase in concentration of strip solution, slight decline in extraction and recovery was observed. This is due to the excess Fe^{+3} and nitrate ions in the stripping solution that possibly create extra resistance to diffusion of complex in the bulk of strip phase which eventually reduces the quantity of precipitation. The maximum values of extraction and recovery were 83% and 78.4%, respectively at 0.001 M stripping solution (Fig.8). Recovery includes the catechin contained both in precipitate as well as in the solution. A quantitative analysis (as shown in Table S2 in ESI[†]) indicates that 72% of the transported catechin precipitated in the strip side chamber and 28% remained in the solution.

In summary, owing to the property of precipitation of iron-catechin complex inside stripping section, the pre-concentration and recovery of the catechins become very easy. As a consequence of complex precipitation, equilibrium of stripping reaction shifts towards the right which augments the continuous operation of extraction and recovery of catechin manifold. Facility of easy collection of the recovered product and less downstream processing promises the integrated process to be commercially viable. The skills and methodologies developed in this research can be utilized in a wider context such as purification of other bioactive compounds and their enrichment through metal complexation with various essential metals in a single step.

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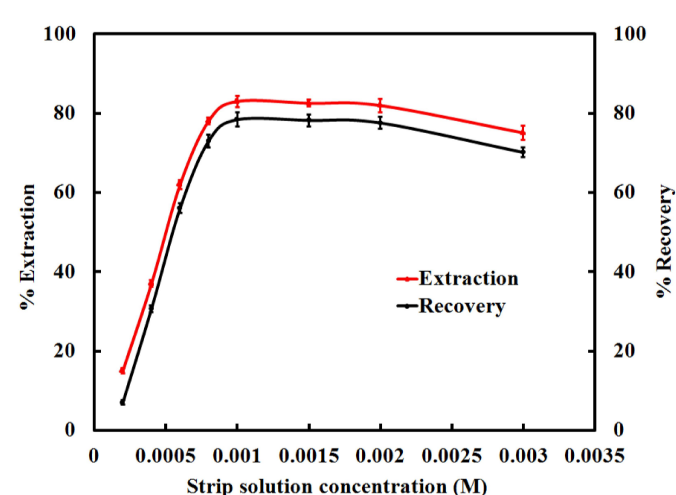


Fig. 8 Role of concentration of metal ion (Fe^{+3}) in the strip solution

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Enrichment of pharmaceutical catechins through iron complexation in their recovery process based on liquid membrane technique

