

Cyclic Voltammetric Study of Complexes of Fe (III) with Saponins Isolated from *Cicer aritinum* and *Glycyrrhizin*

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Summary: Cyclic voltammetric study was used to analyze three new saponins (isolated from the seeds of *Cicer aritinum*) along with a known saponin soyasaponin I and β sitosterol glycoside isolated saponins as well as glycyrrhizin. These studies were carried out in aqueous medium at Glassy carbon (GCE) electrode vs. Ag|AgCl reference electrode. Results revealed that the voltammograms of Fe(III) with isolated saponins are irreversible while that of Fe(III)-glycyrrhizin complex is reversible. Even though precise E° values of their Fe(III) complex could not be determined, it is clearly indicated that Fe(III) forms complexes with these saponins. The ability to form strong complexes with Fe(III) therefore reduces the availability of Fe(III) by saponins.

Key words: Cyclic voltammetry, Fe(III), Complexation, saponins, *Cicer aritinum*, Glycyrrhizin.

Introduction

Iron plays a central role in our life process. Lack of iron is by far the most common nutritional deficiency in the world, including Pakistan [1].

The basic requirement of an iron chelating agent is a high and selective affinity to bind iron under physiological conditions. Fe(III) which is preferred oxidation state in aerobic environment has a greater affinity for oxygen donor ligands as compared to sulfur or nitrogen donor ligands. In biological molecules, oxygen donor atoms exist as phenolic hydroxyl, carbonyl or as carboxyl group [2].

Dietary saponins are implicated in the reduction of iron bioavailability [3]. Saponins are plant metabolites consist of carbohydrate moiety (mono or oligo saccharide) bonded with aglycone. Structurally it may be steroidal or triterpenoidal [4]. Saponins occur in wide range of plants, being found in nearly 100 plant families [5]. Their occurrence in food and feed stuffs has been reviewed [6] and their biological activities have been widely observed (like hemolytic, antifungal, antibacterial, antiviral, anti-inflammatory, anti-mitotic, molluscicidal and toxic activity) [7,8].

Southon *et al.* suggested that the saponins may interfere with iron metabolism and argued that reduction of bioavailability of iron in presence of saponins is related to complex formation between iron and saponins [9].

Chickpeas are source of zinc, calcium, phosphorus, iron, magnesium, folate and protein [10]. They are a healthy source of carbohydrates for persons with insulin sensitivity or diabetes due to high content of dietary fiber. From both growth and consumption point of view Chick pea grams are most important pulse in Pakistan and India. The chick pea is an excellent source of dietary-available iron [11].

In hydrolysate of chick pea presence of Soyasapogenol B has been confirmed. TLC and FAB-MASS confirmed presence of soyasaponin I in chick pea [12]. It has also been reported that chick pea contains two saponins [13] and soyasapogenol B as the sole sapogenin. Sterol, possibly originating from sterol glycosides, was also confirmed in chick pea. The second saponin in the chick pea aglycone preparation is yet unknown, but one of the two saponins may be soyasaponin I [13].

Column chromatographic purification of butanolic extract (extracted at room temperature) of flour of gram seeds was performed [14]. This led to the isolation of three new saponins, together with the known saponins, soyasaponin I and β -sitosterol glucoside (Scheme-1). All the saponins possess soyasapogenol B as aglycone structure of the new saponins were elucidated as 3-O- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl]-soyasapogenol B (Compound 1), 3-O- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 2)-

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β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-glucuronopyranosyl]-soyasapogenol B(Compound 2) and 3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl]-(4 \rightarrow 1)- β -D-galactopyranosyl]-soyasapogenol B(Compound 3), respectively (the structures are in Scheme-1 with the help of negative ion FAB-mass spectrum, ^1H and ^{13}C -NMR studies and by chemical reactions [14].

Reduction of bioavailability of iron due to the consumption of saponins rich food has prompted us to investigate their occurrence in gram (*Cicer aritinum*). The subject of present study was whether there was any indication of interaction between iron and isolated saponin as well as ammoniated glycyrrhizin, resulting in reduction of iron bioavailability. For this purpose cyclic voltammetry was used.

Cyclic voltammetry is very widely used for the initial redox characterization of a molecule (i.e. the redox potentials, and the stability of the different oxidation states) and for qualitative investigation of chemical reactions that accompany electron transfer [15].

In the present research work this technique is used to confirm the complexation of Fe(III) with three newly isolated saponins (from *Cicer aritinum*). Complexation was also checked between Fe(III) and Glycyrrhizin. All these analysis were performed at different pH (3 to 8). Complex formation between iron and saponins is implicated in poor iron absorption.

Results and Discussion

Compound 1, 2, 3 and 4 showed no reduction or oxidation peak at pH 6-8 in the potential range from 0.5 to 0.9 Volt (Figs. 1a, 2a, 3a, and 4a). While their complexes showed reduction peak at corresponding peak potentials 0.25, 0.19, 0.12 and 0.06 Volts respectively at pH near about 3 (Table-1 and Figs. 1b, 2b, 3b and 4b) and the reduction peak disappeared at pH 7-9. These results revealed that Fe^{3+} is irreversibly bound to these saponins. The point of attachment of iron to saponin may be carboxyl group of glucuronic acid moiety and oxidation of glucopyranosyl (as shown in the scheme 2 proposed structure of Fe(III)-Soyasaponins complex and Fe(III)-Glycyrrhizin complex) because at low pH carboxylate proton removal is easier than the hydroxyl proton. At the high pH peak is diminished due to the formation of precipitation of complex. The cyclic voltammetric response of Fe(III)-Glucirrhizin

in 1:1 ratio show reversible behavior as compare to its 1:6 complex (Fig. 5).

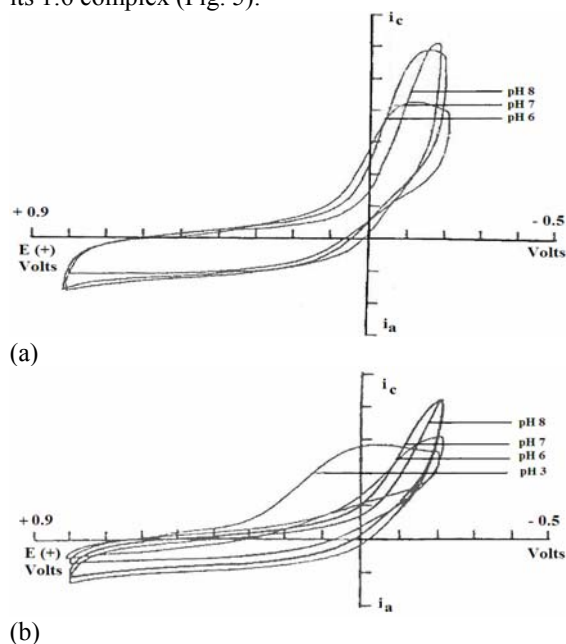


Fig. 1: Cyclic voltammograms showing effect of pH at 100mV/S using Glassy carbon working electrode and Ag|AgCl reference electrode (a) Compound 1 ($0.2 \times 10^{-3}\text{M}$) (b) Fe(III)-compound 1 complex having 1:1 ratio ($0.2 \times 10^{-3}\text{M}$).

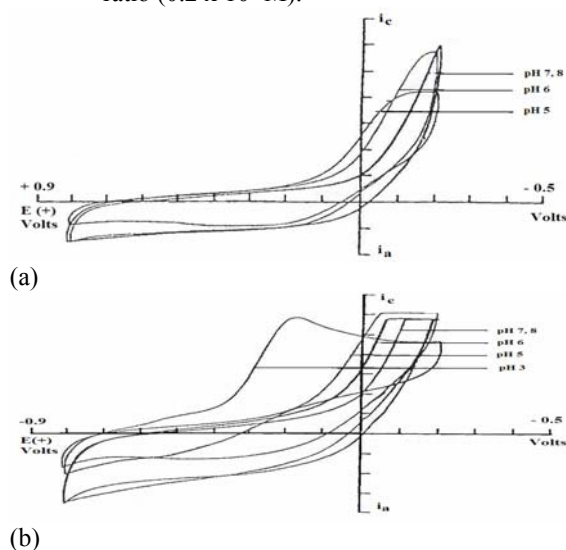
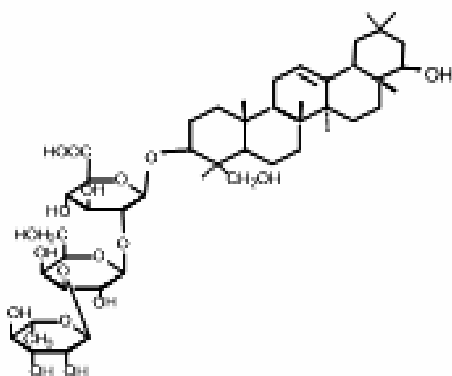
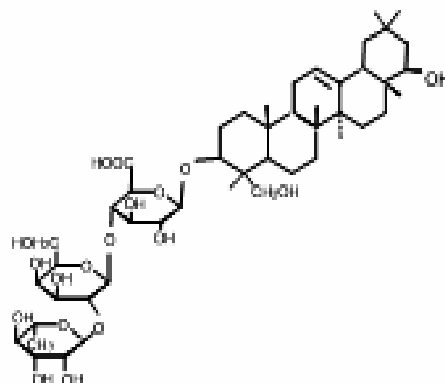


Fig. 2: Cyclic voltammograms showing effect of pH at 100mV/S using Glassy carbon working electrode and Ag|AgCl reference electrode (a) Compound 2 ($0.2 \times 10^{-3}\text{M}$) (b) Fe(III)-compound 2 complex having 1:1 ratio ($0.2 \times 10^{-3}\text{M}$).

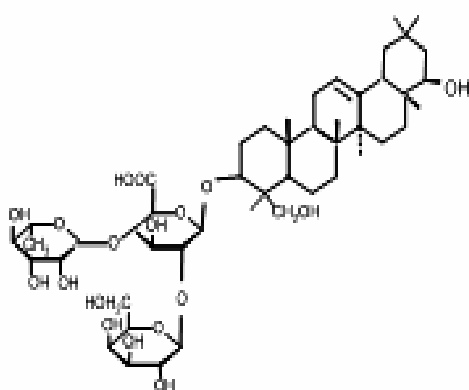
Compound 1



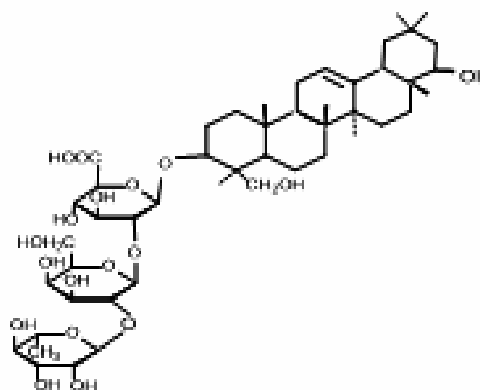
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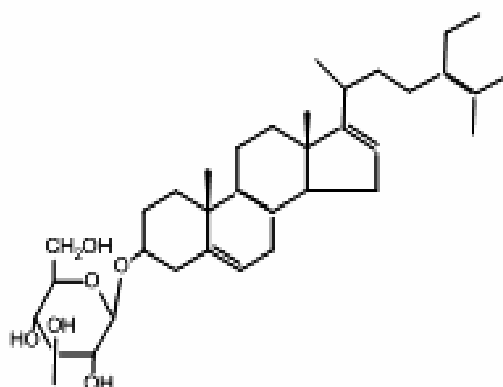
Compound 3



Compound 4



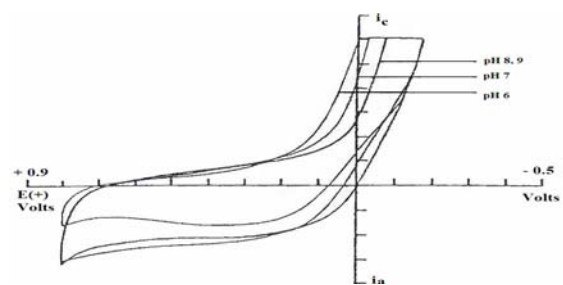
Glycyrrhizin



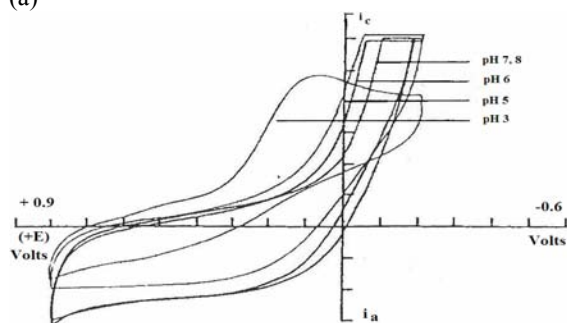
Scheme 1: Structures of the compound 1-4 and Glycyrrhizin.

Table-1: Peak Potentials of saponins and their complexes from cyclic voltammograms

	pH	Oxidation Peak Potential (Volts)	Reduction Peak potential (Volts)
Compound 1	6-8	-	-
	3.34	-	+0.25
Complex sol. (1:1)	6-9	-	-
		-	-
Compound 2	5-8	-	-
	3.35	-	+0.19
Complex sol. (1:1)	5-8	-	-
		-	-
Compound 3	6-9	-	-
	3.45	-	+0.12
Complex sol. (1:1)	5-8	-	-
		-	-
Compound 4	6-8	-	-
	3.66	-	+0.06
Complex sol. (1:1)	5-8	-	-
		-	-
Glycyrrhizin	4	-	+0.03
	5	-	-0.01
	6-9	-	-
Complex sol. (1:6)	3	-	+0.145
	4	-	+0.07
	5-9	-	-
Complex sol. (1:1)	3	+0.595	+0.530
			+0.200
	4	+0.560	+0.525
			+0.175
	5-9	-	-

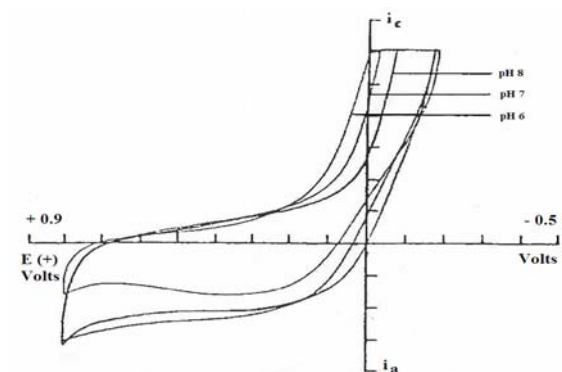


(a)

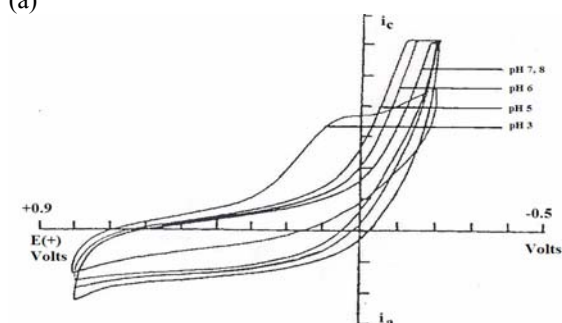


(b)

Fig. 3: Cyclic voltammograms showing effect of pH at 100mV/S using Glassy carbon working electrode and Ag|AgCl reference electrode (a) Compound 3 ($0.2 \times 10^{-3}M$) (b) Fe(III)-compound 3 complex having 1:1 ratio ($0.2 \times 10^{-3}M$)

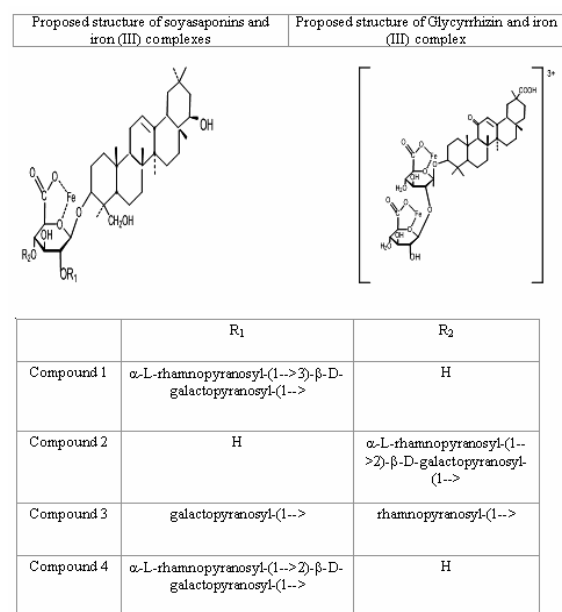


(a)



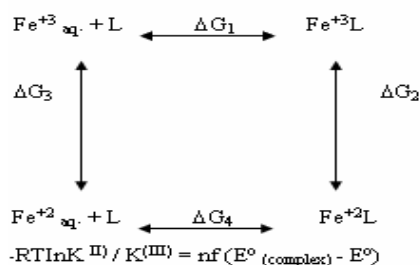
(b)

Fig. 4: Cyclic voltammograms showing effect of pH at 100mV/S using Glassy carbon working electrode and Ag|AgCl reference electrode (a) Compound 4 ($0.2 \times 10^{-3}M$) (b) Fe(III)-compound 4 complex having 1:1 ratio ($0.2 \times 10^{-3}M$).



Schem-2: proposed structures of complexes.

Redox potential of Fe(III)-Saponin complexes would depend upon the relative stability of Fe(III)-Saponin complex. In general the following diagram may be drawn:



Thus if $K^{(III)}$ (the stability constant for the formation of Fe(III)) is larger than $K^{(II)}$, the stability constant for the formation of Fe(II) with the same ligand, then $E^{\circ}_{(\text{complex})}$ (redox potential of complex) would be smaller than the E° (redox potential for the aqueous Complex). Although the cyclic voltammograms for most of the complexes are irreversible because of the extreme lability of the reduced complex with respect to equation and hence we cannot precisely report the E° value.

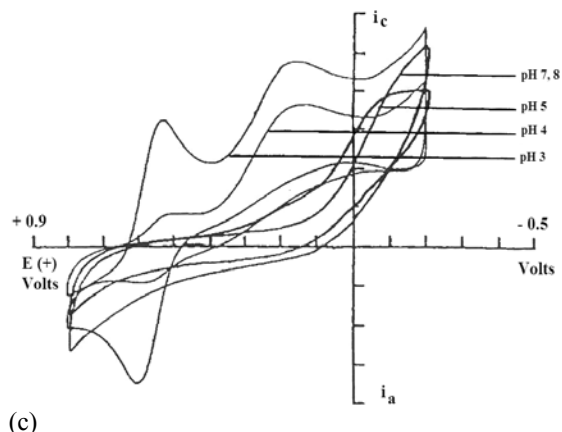
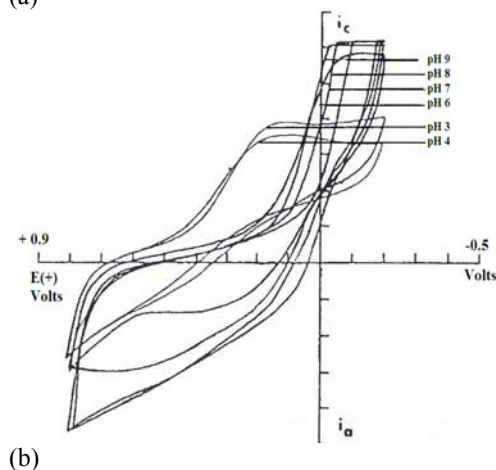
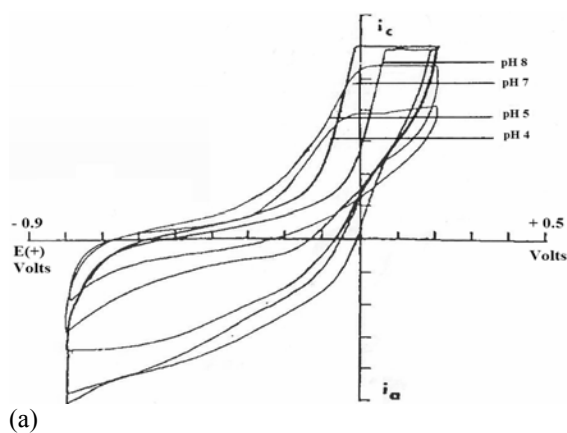


Fig. 5: Cyclic voltammograms showing effect of pH at 100mV/S using Glassy carbon working electrode and Ag|AgCl reference electrode (a) Glycyrrhizin (3.0×10^{-3} M) (b) Fe(III)-Glycyrrhizin complex in 1:6 ratio (0.5×10^{-3} M: 3×10^{-3} M) (c) Fe(III)-Glycyrrhizin complex in 1:1 ratio (3×10^{-3} M).

Experimental

Reagents

All reagents used were of A. R. grade supplied by different sources like Merck, Sigma or Riedel-de-Haen and were employed without further purification. Glass wares were washed with detergent solution and tap water, and then soaked in chromic acid prior to rinsing with deionized water. All solutions were made in double distilled deionized water (deionizer CSW-300) freed from CO_2 from boiling 15 minutes. This water was cooled in an air tight flask.

1.0M solution of NaCl (E.Merk, MW = 58.5g/mol.) was used as supporting electrolyte. Ferric sulfate (0.001M, $(\text{Fe}_2(\text{SO}_4)_3)$, MW=400g/mol. from E.Merck) was standardized by 1,10-orthophenanthroline method ($\epsilon = 1.1 \times 10^4$ mole $\text{L}^{-1} \text{cm}^{-1}$). Stock solution of glycyrrhizin (0.001M) was prepared by ammoniated glycyrrhizin ($\text{C}_{42}\text{H}_{62}\text{O}_{16}\text{NH}_3$; MW=840g/mol.) in buffer of pH 7.

For pH 4 and 5 sodium acetate, for pH 6 succinate and for pH 7 to 9 Tris/HCl buffer was prepared.

Instrumentation

Cyclic voltammetric studies of saponins were performed on CV-1B Cyclic Voltammetry controller (EF1011-00) unit, Bioanalytical Systems Inc. USA. This apparatus comprise on CV-1B cyclic voltammograph, C-1B cell stand assembly and an XY chart recorder. Conventionally three-electrode cell assembly consists of a GCE (Glassy carbon) working

electrode, Ag|AgCl reference electrode and platinum wire as auxiliary electrode.

GCE surface renewal was needed from time to time, particularly when the analyte/supporting electrolyte was changed. The alumina polishing compound was used for polishing the electrode and rinse with distilled water prior to use. The platinum counter electrode surface was regenerated when it was found fouled while determining base-lines and analyte. It was done by placing platinum counter electrode in dilute nitric acid. Nitrogen gas (99.999%) was used to make inert atmosphere for redox study, which was supplied by Pakistan Oxygen Ltd. The electrode was periodically checked with standard potassium hexacyanoferrate(III) solution.

pH measurements were performed on Orion pH meter (model SA-720). It was calibrated with potassium hydrogen phthalate (0.05M, pH 4.010 at $25^{\circ}\text{C} \pm 1$), along with standard buffer solution made from BDH standard chemicals.

Procedure for Analysis

Residual or background current was run before each cyclic voltammogram. For this purpose base-line was drawn in NaCl(0.1M) as supporting electrolyte at GCE electrode vs. Ag|AgCl reference electrode at $25 \pm 1^{\circ}\text{C}$, which were recorded at 100 mV/s and at $20\mu\text{A/V}$ (current sensitivity).

10mg of compound 1 and 0.56mg of iron(III) was dissolved in 50mL of water that also contained 5mL of 1M NaCl. Voltammogram was recorded after 5min purging at scan rate 100mV/Sec and current sensitivity of $20\mu\text{Amp/Volt}$. This procedure was followed to determine the voltammograms of compounds 2,3,4, and glycyrrhizin and their complexes having the ratio (1:1) as the compound 1.

The voltammetric response of compounds 1,2,3,4 and their complexes were also recorded at pH 3,6,7,8 and 9 in similar fashion. While the voltammograms of glycyrrhizin and its complex was also recorded at pH 3,4,5,6,7,8 and 9. The voltammograms of glycyrrhizin and its complex in ratio of 1:6 were also recorded individually in similar manners.

Conclusion

For complexes of compound 1,2,3 and 4, irreversible behavior was confirmed by the cyclic voltammograms. Although a distinct reduction peak is indicated which is different from the free Fe(III) (shifted towards lower emf value; the standard potential of iron is 0.771 volts). Thus although we can not obtain precise E° value for these complexes, it

is reasonable to infer that complex formation takes place. The irreversibility may be due to lability of the reduced species.

The cyclic voltammogram of the iron (III) and glycyrrhizin in ratio 1:6 shows a irreversible behavior with distinct reduction peak. While the voltammogram of complex of ratio 1:1 shows as reversible behavior at pH 3 and 4. At pH 4 the $E_{\text{red}} - E_{\text{oxide}} = 35\text{ mV}$ which is indicative of $n=1/2$. It may be that two iron (III) are co-ordinated to glycyrrhizin.

Thus it is concluded that reduction of iron absorption in presence of saponin, as reported by Southon *et al.* [9] is caused by formation of insoluble iron-saponins at the pH of the stomach.

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