# Iron-polyphenol interaction: Its role in catalyzing colour development during sugar manufacturing

**THESIS**

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**2018**

# DECLARATION

I hereby declare that the thesis entitled **"Iron-polyphenol interaction: Its role in catalyzing colour development during sugar manufacturing"** has been prepared by me under the supervision of Prof. Kaman Singh, Head/ Dean, Department of Applied Chemistry, School for Physical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. No part of this thesis has formed the basis for the award of any degree, diploma or fellowship previously. Further, I declare that the material embodied in the present work is based on original research work and the indebtedness to others has been duly acknowledged at relevant places.

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Date: 22 February 2018
Place: Lucknow

## CERTIFICATE

This is to certify that the thesis entitled **"Iron-polyphenol interaction: Its role in catalyzing colour development during sugar manufacturing"** submitted by Mr. **Ajay Kumar, M.Sc., M.Phil.,** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to the Babasaheb Bhimrao Ambedkar University, Lucknow, India satisfies all the requirements as stipulated in *the Doctor of Philosophy (Ph.D.) regulations-1999 as amended in 2018/2010/2013* and it is fit for submission and evaluation for the award of Doctor of Philosophy of the University.

**Date** 23.02.2018

**Supervisor**

**Head of the Department**

# PREFACE

. In the sugar industry, colour isconsider as quality parameter. Since early in the 19th century, the industry has used an estimation of colour in four general ways. First, raw sugars were originally described by their colour and taste. Today, colour is only an incidental factor in the purchase of raw sugars. Second, a comparative measure of colour is used by all manufacturers and refiners as an index to the changes that occur during processing. These values serve for comparison and for record, and from them an operating staff can adjust the variables of the process to obtain an acceptable product. Third, colour is used to evaluate liquid sugar products. This is a contemporary development and makes important demands on visual colour measurement. It has emphasized the need for a universal colour evaluation based on fundamental units.

Increased intensity of colour in raw sugar leads to higher cost of removal of coloured compounds during refining and therefore a potentially lower market value of the raw sugar. Colour is, therefore, a key quality parameter in sugar industry and is defined and measured according to international standards. The types and amounts of pigments in sugarcane juice are dependent on a number of factors such as the variety and maturity of the raw material, climatic conditions, and agricultural practices, time between cutting and processing, quantity of impurities, soil conditions and grinding types.

Sugarcane juice is opaque and viscous liquid which is brownish to dark green in colour that is obtained by pressing the cane stalk. Pigments are naturally occurring compounds present in sugarcane and other plants. Pigments in sugarcane juice are mainly polyphenolic compounds. Polyphenols acids are the most important groups of secondary metabolites and bioactive compounds in plants and good sources of natural antioxidants in human diets. The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing.

A number of polyphenols (Caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarin, 4-hydroxycoumarin etc.), can efficiently chelate metals like Al(III), Fe(II), Fe(III), Cu(II), or Zn(II). Formation of metal complexes play important and

multiple roles in biological systems, and provide sensitive colour stabilization mechanisms. The presence of coloured impurities in sugar process solutions results from chemical reactions taking place during the production process. The compounds responsible for forming coloured impurities are mainly of polyphenols, they exhibit a negative influence both on the quality and quantity of the sugar produced. Iron is main component of the sugar cane juice which plays important role in sugar processing. Chelators in which oxygen atoms serve as the ligand of iron tend to stabilize Fe(III), thus decreasing the reduction potential of the iron. The redox chemistry of iron plays an important role in the oxygen activation and transfer reactions mediated by group of polyphenols.

There is a general update that fast caramalisation catalyzed by iron and relationship between polyphenols and uptake of iron is only hypothesis without experimental data on iron-polyphenols interaction which can complement the above hypothesis that as and when polyphenols comes in contact with iron is accompanied by colour formation during sugar processing. The present study, the caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarine and 4-hydroxycoumarine were chosen as a representative selection of polyphenols and their reaction with iron was investigated with emphasis as colour development. This study has would provided substantial experiment evidences of colour formation by iron-polyphenols interaction during sugar manufacturing. Therefore, their complete removal during clarification must be ensured so that the quality of the end product could be maintained.

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**Ajay Kumar**

*I am indebted to my father for living, but to my teacher to living well.*

*Alexander*

**Department of Applied Chemistry, School far Physical Science, BBAU, Lucknow**

Dedicated
to
My Respected
Teachers
and
Father

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- List of symbol and Abbreviations

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| Nm | Nanometer ($10^{-9}$ m) |
| $e^-$ | Electron |
| $C_o$ | Initial concentration of reactant |
| $C_t$ | Concentration after t time |
| $V$ | Volt |
| C∞ | Concentration at infinite time |
| R | Gas constant = 8.314 J $mol^{-1}K^{-1}$ |
| kcal | Kilo calorie |
| t | time |
| log | Logarithm |
| T | Temperature (Kelvin) |
| $k_1$ | Rate constant |
| T | Temperature |
| K | Kelvin |
| J | Joule |
| kJ | Kilo-joule |
| C | Molar concentration |
| pH | -ve log of $H^+$ ions |
| FT-IR | Fourier transform infrared |
| SEM | Scanning electron microscopy |

# CHAPTER-1

## Introduction and Literature review

There is a general update that fast caramalisation catalyzed by iron and relationship between polyphenols and uptake of iron is only hypothesis without experimental data on iron polyphenols interaction which can complement the above hypothesis that as and when polyphenols comes in contact with iron is accompanied by colour formation during sugar processing. In the present study, the caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarine and 4-hydroxycoumarine were chosen as a representative selection of polyphenols commonly present in cane juice and their reaction with iron was investigated with emphasis on colour development. This study would provide substantial experiment evidences of colour formation by iron-polyphenols which can be extended to systems of significant importance such as role of electron transfer reactions in catalysing colour formation during sugar manufacturing. Therefore, their complete removal during clarification must be ensured so that the quality of the end product could be maintained.

## 1. Sugar industry

### 1.1. Introduction

The understanding of redox reaction of iron in cane juice during manufacturing of sugar is qualitatively understood. Iron in aqueous solution is usually present in Fe(III) form. However, it is reduced to Fe(II) state by various known and unknown species present or deliberately added in the cane juice during pre and post clarification. Reducing action of sulphide being relatively non-permanent, the oxidative enzymes may oxidise hydroxyl group of sugar into a series of a a dark colour compound which are detrimental to sugar quality. The review of the literature [Layrisse, *et.al.,* 2000;] revealed that some work has been reported employing a dilute solution of sugar and its allied products with different salts of iron with an object to understand complex formation. Therefore, it was considered necessary to study the influence of redox behaviour of iron-polyphenol interaction on colour development in sugar manufacturing. Though the polyphenol arises presently in very minute quantities (60-150 ppm) and cannot be removed completely by conventional clarification process. The basic chemistry of iron polyphenol coloured complex formation depends upon the number of phenolic group and their relative positions onto benzene moiety. Iron, the most abundant transition metal, is essential for oxygen transport and many redox reactions. Only slight disturbances to the delicate balance between iron intake and iron loss can push the body into conditions of iron overload or iron deficiency. Phenolic acids are capable of forming Fe(III) complexes and have been generally associated with a negative effect on the absorption of non-haem iron in humans [Layrisse, *et.al.,* 2000; Hurrell *et.al.,*1999]. The caffeic and chlorogenic acids have cis-phenolic groups and are capable of forming chelates with Fe(III). Naringin is also capable of forming a six-membered ring chelate with Fe(III) while quinic, ferulic and

sinapic acids do not appear to form such species. The catechol functional group has been identified as a component of many siderophores [Albrecht-Gary, 1998]. Phenolic acids are also of great interest because their redox properties affect the availability of micronutrients to plants [Deiana *et.al.,* 1997]. The uptake of iron by plants depends considerably on the presence of both complexing and reducing reagents. Chlorogenic acid is one of the major polyphenol compounds found in numerous plant species [ Beate Risch, 1988]. Due to its high sucrose content and low fibre content, sugarcane is one of the important industrial crops of the world. It is principal raw material for the sugar industry as 70% of the world's sugar comes from sugarcane. Besides sugar production, a large number of population in the tropics and subtropics relishes its juice, and consume raw cane. In the Indian system of medicine, chewing raw sugarcane is recommended for sound and healthy body. Both the roots and stems of sugarcane are used in Ayurvedic medicine to treat skin and urinary tract infections, as well as for bronchitis, heart conditions, loss of milk production, cough, anaemia, constipation as well as general debility. Some texts advise its use for jaundice and low blood pressure [Kadam *et.al.,* 2008]. Phenolic compounds in sugarcane juice are partially responsible for its colour [Duarte-Almeida, 2017]. The major flavonoids in sugarcane are flavones, such as naringenin, tricin, apigenin and luteolin derivatives [Williams, 2007; Smith *et.al.,* 1985]. Tricin has recently been reported to interfere with murine gastrointestinal carcinogenesis and may be considered safe enough for clinical development as a cancer chemopreventive agent [Verschoyle *et.al.,* 2006]. A 1996 report [Nakasone *et.al.,* 1996] showed the presence of five antioxidant compounds in a *kokuto* extract, followed by reports [Takara *et.al.,* 2002] on the isolation of several glycosylated phenolic compounds from the same natural extract. Kokuto is a food product similar to Brazilian "rapadura," is highly appreciated as a candy and consisting of a block of raw brown sugar, made from

concentrated sugar cane juice. In addition, found antioxidant activity in different samples of cane brown sugars and proposed various phenolic acids and flavonoids compounds to be at least partially responsible for the observed activity [Payet, 2005]. However, the statistical correlation between phenolics content and antioxidant activity was low. Hence, the authors reported on the possibility of other metabolites to be also involved, possibly, those formed during the sugar production. Thus, although phenolic entities in sugarcane can act as antioxidants, data on its actual polyphenolic composition is scarce and very little is known about its antioxidant activity [Duarte-Almeida, 2006]. Flavonoids are a group of naturally occurring antioxidants and iron chelators, which are widely available in the food we consume, thus their interactions with Fe(III) and possible involvement in the absorption of iron are of interest. Flavonoids have been generally associated with a negative effect on the absorption of non-haeme iron in humans [Layrisse M. *et.al.,* 2000; Hurrell, 1999]. Flavonoids play an important role in the body's antioxidant system. Their ability to reduce the effect of this free radical assault is partly due to their free radical scavenging ability [Acker, 1998] and also due to the fact that they are capable of supplying specific chelators, which are capable of binding any available iron, thus greatly reducing its bioavailability. The antioxidant effect which flavonoids provide arises partly from the specificity of their interaction with Fe(III). Reactions between Fe(III) and polyphenol based ligands are also of interest because of the possible role of plant-derived phenolics in mobilisation of iron in acidic soils.[ Michael J. Hynes *et.al.,* 2001]. The astringency (feeling of extreme dryness in the palate) of certain foods and beverages is due to the interactions between polyphenols and proline-rich proteins [Luck *et.al.,* 1994; Murray *et.al.,* 1994 ]. Epidemiological studies have shown a relationship between high dietary intakes of phenolics and reduced risk of cardiovascular disease and cancer [Paul Ryan, 2007]. Growing evidence suggests that oxidative damage caused by the b-amyloid

peptide in the pathogenesis of Alzheimer's disease may be peroxide-mediated. Many polyphenols possess stronger neuroprotection against hydrogen peroxide than antioxidant vitamins. Recent reports show that a diet rich in fruit and vegetable juices can significantly reduce the incidence of Alzheimer's disease [Dai Q, 2006]. Phenolic compounds, commonly referred to as polyphenols, are naturally occurring chemicals present in all plants and thus are part of our diet, which are largest source of external antioxidants. Over 8000 phenolic structures have been identified that vary structurally from simple molecules to highly polymerized compounds. More than 10 classes of polyphenols have been reported on the basis of chemical structure with the flavonoids being the most common polyphenolic compounds present in plant food [Ryan Paul, 2008]. Polyphenols are plant secondary metabolites consisting of hydrolyzable and condensed forms. Tannic acid, which is part of the first group, has a structure consisting of a central carbohydrate (glucose) and 10 galloyl groups. It occurs in the bark and fruits of many plants. Tannic acid and other polyphenols have antimutagenic, anticarcinogenic and antioxidant activities, but the mechanisms involved in these activities are not completely understood. Polyphenols are -OH radical scavengers because phenolic groups are excellent nucleophiles and are also able to quench lipid peroxidation, acting as chain break antioxidants [George, 1999]. Literature survey suggest that in spite of extensive data on colour complex formation between polyphenols and iron salts, the detailed investigations of deliberately added ferrous, ferric iron in sugar crystal has not been studied so far. Therefore, it was considered necessary to study the influence of iron-polyphenol interaction on colour development in sugar. It is hoped that such reaction will be truly identical to electron transfer reaction. The result is expected to provide relevant mechanistic information regarding common electron transfer reaction, involving solid, moist and solution phase and which can be extended to other systems of significant

importance. The role of electron transfer reaction in catalysing colour formation in sugar processing where polyphenols play important role will be approached which of high technical importance. In the sugar industry, it is generally agreed that colour is of prime importance. Since early in the 19th century, the industry has used an estimation of colour in four general ways. First, raw sugars were originally described by their colour and taste. Today, colour is only an incidental factor in the purchase of raw sugars. Second, a comparative measure of colour is used by all manufacturers and refiners as an index to the changes that occur during processing. These values serve for comparison and for record, and from them an operating staff can adjust the variables of the process to obtain an acceptable product. Third, colour is used to evaluate liquid sugar products. This is a contemporary development and makes important demands on visual colour measurement. It has emphasized the need for a universal colour evaluation based on fundamental units. The fourth important use of colour measurement is for the evaluation of an adsorbent. The first quantitative colour measurement was apparently made in by M. Payen [1822] who reported a procedure to evaluate the capacity of finely divided bone char to remove colour [Victor, 1956]. Sugar industry is the backbone for economic development of the country. India is the second major producer of sugar country in the world after the Brazil. Sugar industry is one of the major agricultural industries in India. Sugar industry has been played a key role in creating social infrastructure in resource mobilization, employment generation, income generation and rural and urban areas. The sugar industry has made social-economic changes in rural areas to provide dairy, poultry, fruit and vegetable processing and educational health and credit facilities in rural India. To improve the competitiveness of the sugar industry as the overall goal work. There has been some new separation techniques and biotechnology sector intention in sugar development, and that the existing agro-business industries to try to apply advance in these areas it is estimated

that this effort for sugar industry is some innovative technology could lead the new methods of applications to remove colour must reduce the cost in the process should and improve quality. This may be effect in the direction of direct production a sugar mill white sugar, traditional system, which is of raw sugar production after sophisticated in a separate Indian refinery. The study of cane sugar to reduce the colour of juice by using new technology to obtained refined sugar. Polyphenols are the non sugars present in sugarcane juice [0.01%] in colourless form but subsequently combining or reacting with other substances form colouring matter. They react particularly with iron depreciated from equipments and atmospheric oxygen to form dark coloured compounds. The iron in raw juice is initially in the ferrous state but changes to ferric state, owing to the simultaneous presence of oxidising enzymes. In the absence of iron, the raw juice becomes brown upon exposure to air but with iron present it turns increasingly green. About 21 polyphenols have been identified in cane plant out of which 10 are carried over up to the stage of raw sugar and four up to even refined sugar [Mahadevaiah, 2009]. Colour arises in raw sugar because of a proportion of the solutes in sugarcane juice being present either as coloured compounds or as precursors of compounds that are coloured [Paton, 1992]. Increased intensity of colour in raw sugar leads to higher cost of removal of coloured compounds during refining and therefore a potentially lower market value of the raw sugar. Colour is therefore a key quality measurement in sugar industries and is defined and measured according to international standards [Chen and Chou, 1993]. The types and amounts of pigments in sugarcane juice are dependent on a number of factors such as the variety and maturity of the raw material, climatic conditions, agricultural practices, time between cutting and processing, quantity of impurities, soil conditions and grinding types [Tariq, 2014]. Food-processing products derived from plants and fruits are

valuable natural sources of bioactive compounds including phenolic compounds [Moure, 2001; Antolovich, 2000].

Numerous studies have been devoted to the bioactive capacity of agricultural byproducts (rice hulls, almond hulls, citrus peels and seed residue, apple peels, olive mill wastes, wine industry byproducts, etc.), in particular, to find some alternatives to synthetic food antioxidants [Balasundram, 2006]. Cane sugar products may contain polyphenols as is the case for other fruits and plants. This assumption arose from reported studies dealing with phenolic acids, polyphenols, and flavonoids in sugarcane [McGhie, 1993; Nutt, 2004; Colombo, 2006]. Phenolic compounds in liquid sugar from cane molasses or in brown sugars [Godshall, 1982; Palla, 1982], and antioxidative phenolic compounds from a non-centrifuged sugar cane [Takara, 2002]. The phenolic compounds were reported in several commercial cane brown sugars and assessed their free radical scavenging activities [Payet, 2005].

### 1.2. Composition of sugar cane juice

Sugarcane juice is an opaque and viscous liquid which is brownish to dark green in colour that is obtained by pressing the cane stalk. Sugarcane juice is an aqueous solution that circulates in the living plants and carrying material required for growth and metabolism. Sugarcane juice is mainly processed in sugar, but the maximum part of the production goes to human consumption as fresh juice or alcohol. Sugarcane juice is an energy-rich food. Sugarcane is an abundant and relatively low-cost agricultural resource, largely produced in tropical and sub-tropical regions of the planet. This raw material contains about 80–85% of water and its dry matter presents an average composition of approximately 30% sucrose and 70% pre-processed lignocellulosic materials [Amalraj, 2008]. Pigments are naturally occurring compounds present in sugarcane and other plants.

During sugarcane crushing, the extracted pigments constitute the juice's non-sugar portion which ultimately impairs the white sugar production process [Diego Matos Favero *et. al.,* 2014]. Flavonoids are a critical group for sugar processing since they cause up to 30% of the raw sugar colour when the sugar is produced at pH 7.0. Anthocyanins, which belong to the flavonoids group, produce a red and blue colour respectively in an acid and alkaline medium [Bourzutschky, 2005]. Sugar cane contains phenolic acids, polyphenols, and flavonoids [Parr, A. J.; Bolwell, G. P, 2000; Fuhrman, B.; Aviram, M. 2002; Manach, C.; Mazur, A.; Scalbert, A.2005; Favier, 2005; Moure, 2001; Antolovich, 2000]. These compounds have also been found in sugar products such as syrup or molasses and in the brown sugars themselves [Balasundram, 2006; McGhie, 1993]. Composition of sugarcane (*Saccharum officinarum*) juice were identified and quantified by analytical high-performance liquid chromatography and photodiode array detection, showing the predominance of flavones (apigenin, luteolin and tricin derivatives), among flavonoids, and of hydroxycinnamic, caffeic and sinapic acids among phenolic acids [Joaquim Maur Icico Duarte –Almeida et, *et.al.* 2006].

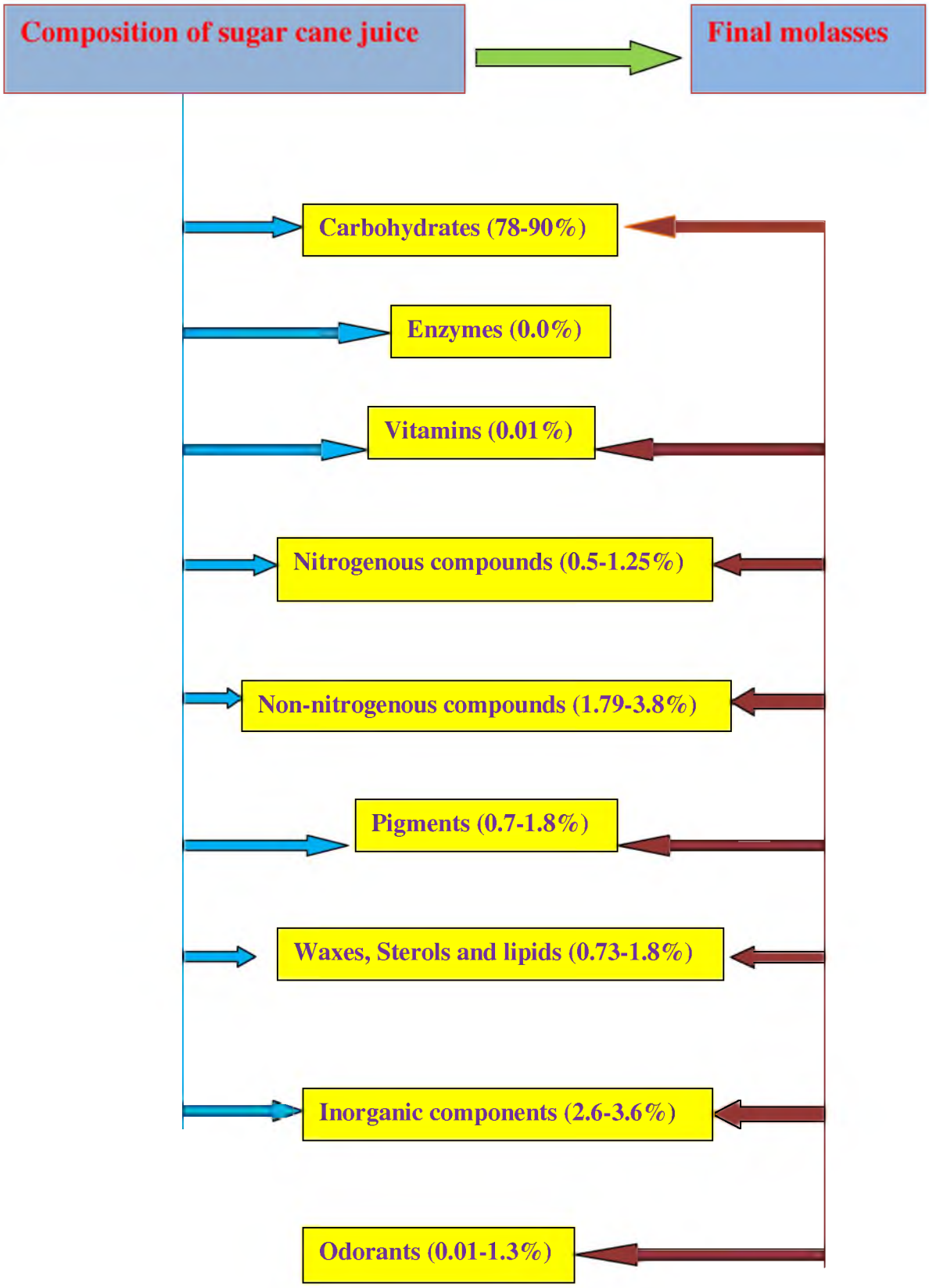



**Fig.1.2. Flowchart showing composition of sugar cane juice**

**Table 1.1. Showing the different chemical components of the sugar cane juices**

| | |
|---|---|
| **Carbohydrates** | Reducing sugar (D-glucose, D-fructose), hemicellulose, soluble gums, pectins etc. |
| **Enzymes** | Colour producing enzymes (Invertase, leccase, peroxidise, tryrosinase etc.) |
| **Vitamins** | A and B group (thiamine, riboflavin, pantothenic acid, niacin, biotin etc.) |
| **Nitrogenous compounds** | amino acids (L-Asparagine, *L(dextro)*-Glutamine, tyrosine, leucine (or isoleucine), valine, -y-aminobutyric acid, alanine, glycine,serine, asparagine, glutamic and aspartic acids, lysine and glutamine etc.) |
| **Non-nitrogenous compounds** | Acids (malic, succinic, glycolic, forinic, and oxalic etc.) |
| **Pigments** | Tannins, anthocyanins (containing phenolic groups), Chlorophyll etc. |
| **Waxes, sterols and lipids** | Wax (free acids, free alcohol, esters, ketones, hydrocarbons), glycerols etc. |
| **Inorganic compounds** | Inorganic salts such sodium chloride |
| **Odourants** | Presence of a substituted benzene structure, of paraffinic methylene and methyl groups, of an acetate group, and of the >C=C< and -C=C-linkages. The presence of a sulfur function is probable. The deionized unfermentable (by yeast) residue from molasses has a raisin-like odour. |

### 1.3. Colouring pigments present in Sugar cane juice

The types and amounts of pigments in sugarcane juice are dependent on a number of factors such as the variety and maturity of the raw material, climatic conditions, agricultural practices, time between cutting and processing, the quantity of impurities, soil conditions and grinding types. Sugarcane contains many colourant compounds that could be extracted from its juice [Rupa T R, 2008]. In 1971, a study on sugarcane juice identified chlorogenic acid, cinnamic and flavones as coloured compounds [Farber *et. al.,* 1971]. Those coloured substances (pigments) are derived from plants and can be found in raw sugar after processing [Bourzutschky, 2005]. The presence of a several colourant compounds, such as chlorophylls [Jangpromma *et.al.,* 2010; Patil *et. al.,* 2014; Qudsieh *et.al.,* 2002], have been described for agricultural and food chemistry aspects, however, the literature has widely described chlorophylls [Streit *et.al.,* 2005]. Other coloured substances described are anthocyanins, flavonoids [Li *et. al.,* 2010; Colombo *et.al.,* 2006; Colombo *et. al.,* 2009; Mabry *et. al.,* 1984] and phenolic acids [Duarte-Almeida et.al., 2006; Duarte-Almeida *et. al.,* 2011; Leal *et. al.,* 1994; Santiago *et. al.,* 2009; Singh *et.al.,* 2015]. Sugarcane pigments are mostly chlorophylls, carotenes, xanthophylls and flavonoids. The first three pigments are insoluble in water are easily separated during cane juices clarification [Farber and Carpenter, 1972].

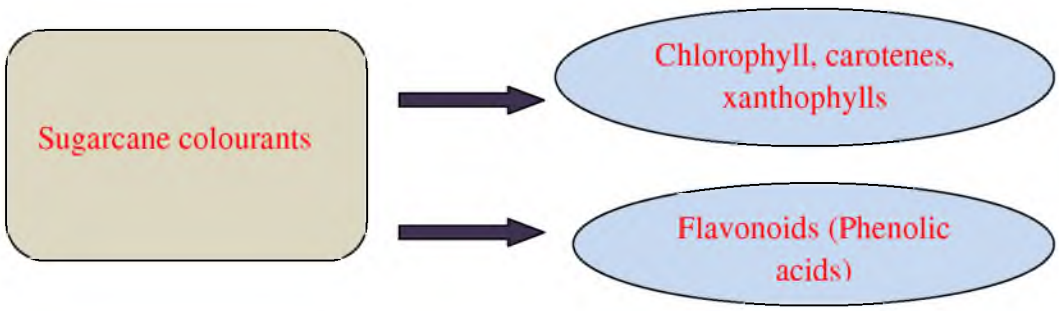



**Fig. 1.2. Classification of colourant present in sugar cane juice**

In the last many decades, there has been an increased interest in identifying antioxidants free radical scavengers or that exhibit positive effects on cardiovascular diseases, cancers, and brain degenerative process due to their significant antioxidant activities [Conte *et.al.,* 2003; Valos *et.al.,* 2005; Gey *et.al.,* 1990; Verzelloni *et.al.,* 2007; Visioli *et.al.,* 2000]. These antioxidants supplied by diets, including vitamin C, vitamin E, carotenoids, and several polyphenolic compounds such as flavonoids can prevent these diseases by scavenging free radicals or interfering with DNA binding [Craig, 1997; Stoner, 1995]. Therefore, antioxidants abundant in foods and plants have been extensively searched and studied because of the important effects on cardiovascular diseases and cancers [Abbas *et.al.,* 2014; Hertog *et.al.,* 1993; Hertog *et.al.,* 1995]. Sugarcane, also known as a noble cane, is one of the important industrial crops in the world due to its high sucrose content and low fibre content. Sugarcane is the principal raw material in the sugar industry and approximately 70% of the world's sugar is made of sugarcane. In addition to sugar production, raw sugarcane and sugarcane juice are commonly consumed by a large number of populations in the tropics and subtropics. Furthermore, chewing raw sugarcane is recommended by some medical systems for keeping sound and healthy status [Kadam *et.al.,* 2008]. Pigments in sugarcane juice are mainly phenolic compounds. Paton [1992] characterized the phenolic composition of sugarcane and its products and found that the main compounds in sugarcane and its products were phenylpropanoids and flavonoids [Harborne *et.al.,* 1964; Smith *et.al.,* 1985]. Nakasone *et. al.,* [1996] isolated five antioxidant compounds from kokuto extracts. Takara *et. al.,* [2002] identified 13 antioxidant compounds, including several glycosylated phenolic compounds in kokuto. Some identified compounds exhibited higher antioxidant activity than α- tocopherol. Payet *et.al.,* [ 2005] reported the antioxidant activity of several samples of brown sugar made of sugarcanes and suggested that a number of phenolic acids and flavonoids

accounted for at least partially the observed antioxidant activity. Phenolic substances in sugarcane juice may have biological activities [Duarte-Almeida *et.al.,* 2011]. For example, an acylated tricin glycoside isolated from sugarcane juice exhibited anti-proliferative activity [Duarte-Almeida *et. al.,* 2006]. While several studies [Duarte-Almeida *et.al.,* 2011; Colombo *et.al.,* 2006; Nuissier *et.al.,* 2008; Nuissier *et.al.,* 2002] have characterized the antioxidant activity of derivative products presented in the by-products such as molasses, filter mud, and bagasse in sugarcane manufacturing industry, there is a lack of systematic study of the phytochemicals and antioxidant activities combined with sugar manufacturing. Characterization of phytochemicals in different parts of sugarcane is useful to understand the potential beneficial effects of sugarcane on human health [Feng *et.al.,* 2014]. The pigment is one of the important indicators affecting the quality of sucrose in sugar production and de-colourization has been a major problem in sugar industry. Numerous studies have been conducted to extract natural antioxidants, however, the raw plant material used for natural antioxidants are expensive and rare. In addition, the plant material was abandoned in the extraction of antioxidant substances, resulting in the waste of the other components. Sugarcane is a rich source for the extraction of natural antioxidants in the tropics and subtropics with relatively low price. The pigment as antioxidant active substance was extracted with no effect on extraction of sucrose, which made full use of the various components of sugarcane. For sugar production, colour is one of the indicators affecting the quality of sucrose and decolourization is the major problems in sugar industry [Fansheng *et.al.,* 2015]. Polyphenols are secondary compounds widely distributed in the plant kingdom. They are divided into several classes, i.e. phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins) stilbenes, and lignans, which are distributed in plants and

food of plant origin [Manach *et al.,* 2004, 2005]. Phenolic acids occurring in sugar cane are usually derived from cinamic acids [Farber Carpenter, 1972]. These phenolic acids are the building blocks of flavonoids [Kennedy and Smith, 1976]. Phenolic acids are very difficult to remove during extraction and refining, reaching the final product. As an example, chlorogenic acid, resulting from the esterification of caffeic acid and quinic acid, was detected in white sugar [Farber and Carpenter, 1972]. This can be a factor for white sugar colour increase at high pH [Riffer, 1988]. Ferulic acid is a pale yellowish compound that can darken by oxidation. Polyphenols can be further broken down into four categories, with additional sub groupings based on the number of phenol rings they contain, and on the basis of structural elements that bind these rings to one another. As a general rule, foods contain complex mixtures of polyphenols, with higher levels found in the outer layers of the plants than the inner parts.

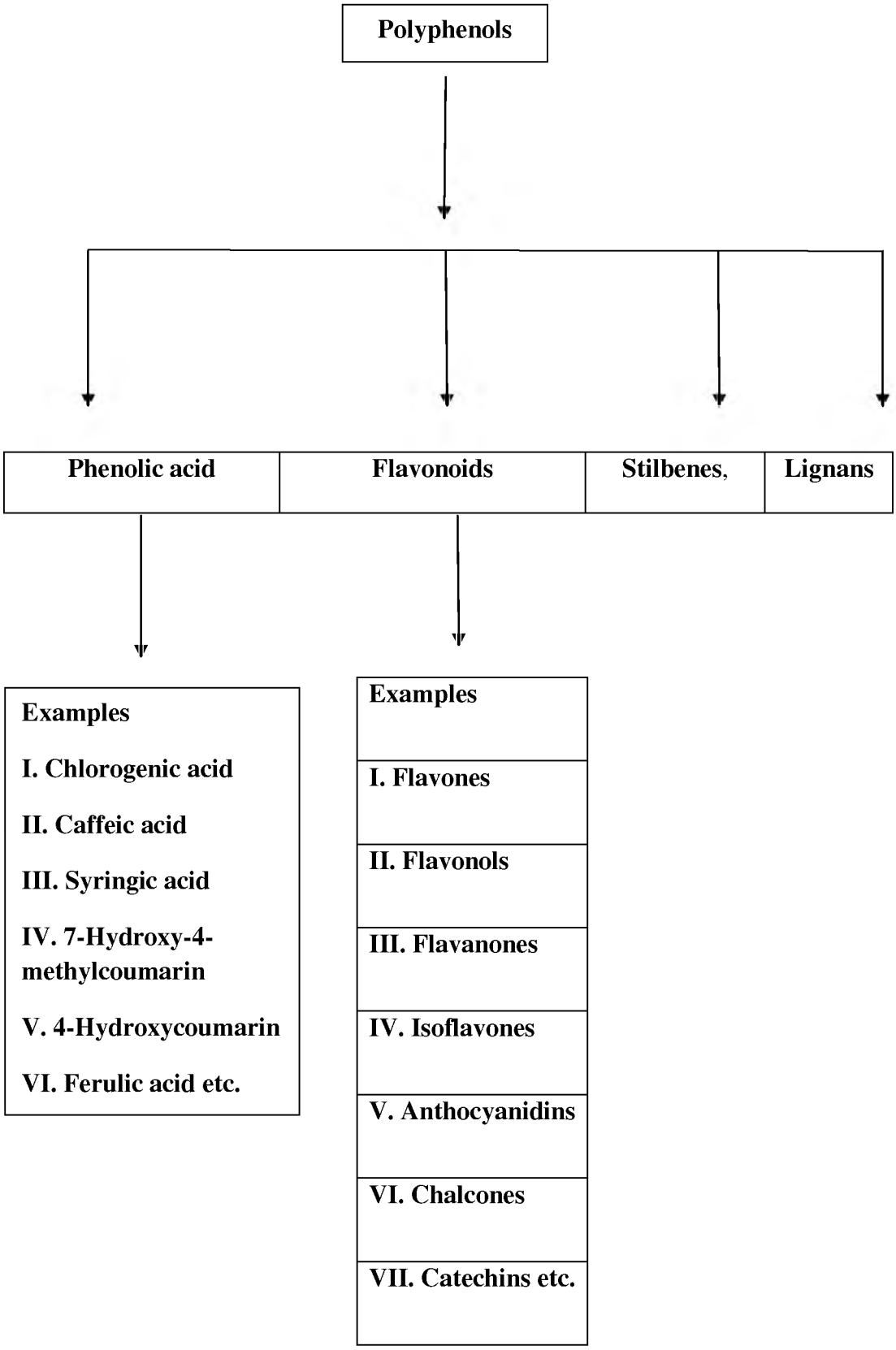



**Fig.1.3. Flow chart showing classification of polyphenols**

The quantity, quality and type of pigments and colourants in juice are dependent upon:

- The variety and maturity of sugar cane
- Climatic condition
- Agricultural practices (use of nitrogenous fertilizers) stored by plant as amino acids.
- Cut to crush delays
- Amount of trashes incorporated into the crushed cane
- Soil condition
- Juice extraction practices.

### 1.4. Properties of phenolic acids

In recent years there is an increase in the areas related to newer developments in prevention of disease especially the role of flavonoids and phenolic acids as antioxidants moreover flavonoids and phenolic acids components play important roles in the control of different human diseases. Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants and good sources of natural antioxidants in human diets [Kim *et. al.,* 2003]. Polyphenols are abundant micronutrients in our diet, and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The health effects of polyphenols depend on the amount consumed and on their bioavailability. Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity [Larson, 1988; Shahidi and Naczk, 1995; Cotelle *et al.,* 1996;

Velioglu *et al.,* 1998; Zheng and Wang, 2001; Cai *et al.,* 2003]. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent [Halliwell, 1994; Mitscher *et al.,* 1996; Owen *et al.,* 2000; Sala *et al.,* 2002]. The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing, but there is still considerable controversy in this area [Hertog *et al.,* 1995; Kuo, 1997; Mclarty, 1997; Yang *et. al.,* 2001; Sun *et. al.,* 2002]. Because of rapid development and employment of modern analytical equipment and technology, natural active components with anticancer activity or anticarcinogenic and antimutagenic effects have been studied and identified from Chinese medicinal plants [Lien and Li, 1985; Ohigashi et al., 1994; Tsai, 2001; Xiao et al., 2000]. They belong to various categories based on their chemical structures. The most commonly found active constituents include terpenes (sesquiterpenes, diterpenes, triterpenes), alkaloids, coumarins, lignans, quinones, flavonoids, tannins, stilbenes, curcuminoids, polysaccharides, etc. Some of them are alleged to have antioxidant activity [Larson, 1988; Ho *et al.,* 1994; Ng *et. al.,* 2000; Xiao *et. al.,* 2000; Mau *et. al.,* 2002]. Antioxidant activity is a fundamental property important for human life. Many of the biological functions, including antimutagenicity, anticarcinogenicity, and antiaging, among others, may originate from this property [Mclarty, 1997; Niki, 1997; Yang *et. al.,* 2001]. Recent reports have been shed light into several biological properties of sugar cane and its derived products. Some investigators [Noa *et.al.,* 2002; Molina *et.al.,* 2005] have consistently proved on *in vivo* models the antioxidant properties of high molecular weight alcohols contained in the wax. Sugar cane also contains phenolic acids, flavonoids and other phenolic compounds [Paton *et.al.,* 1992; McGhie *et.al.,* 1993], which could account for certain antioxidant activity. Phenolic

entities have already been reported as neuroprotectants in models such as MeHgCl intoxication, both *in vitro* and *in vivo* [Magos *et.al.,* 1982; Naganuma *et.al.,* 2000]. Payet *et. al.,* [2005] has been reported that antioxidant activity in different samples of cane brown sugars and proposed various phenolic acids and flavonoids compounds to be at least partially responsible for the observed activity. However, the statistical correlation between phenolics content and antioxidant activity was low. Phenolic compounds, are beneficial for postponing ageing, preventing disorder, reducing disease risk and maintaining health by inhibition of lipid peroxidation [Naganuma *et.al.,* 2000; Dubick, 2001]. Phenolic compounds exist naturally in vegetables, fruits and grains. These compounds possess the ability to reduce oxidative damage because they can act as direct antioxidant by donating a hydrogen atom to free radicals and by chelating metal ions, such as iron or copper, as well as they can act as indirect antioxidants by upregulating antioxidant enzymes [Perumal, 2007; Sun *et.al.,* 2013]. These antioxidant properties of phenolic compounds are directly related to their chemical structure, and particularly to the phenol group [Perumal, 2007]. Phenolic compounds are of interest in pharmaceutical and food industries. Their pharmacological actions are ascribed to the free radical scavenging and metal ion chelating activities, and their effects on pathways of cell-signaling and on gene expression [Van, 1985]. The antioxidant capacities of phenolic compounds are often assessed by the Trolox equivalent antioxidant capacity (TEAC), the ferric reducing antioxidant power (FRAP), the hypochlorite scavenging capacity, the deoxyribose method and the copper-phenanthroline-dependent DNA oxidation assays. Takara isolated several phenolic compounds from Kokuto, a noncentrifuged cane sugar, and showed their antioxidative activity [Takara 2002]. The interest in polyphenols, including flavonoids and phenolic acids, has considerably increased in recent years because of their biological properties, their antioxidant effects, and their possible role in the prevention of several

chronic diseases involving oxidative stress, as well as their protective effect against low density lipoprotein (LDL) oxidation [Dubick *et.al.,* 2001; Fuhrman, 2002]. The ability of phenolic acid to react with metal ions may also render them pro-oxidant: reduce $Fe^{3+}$ to $Fe^{2+}$ and hence allow the formation of initiating radicals. The radical-scavenging antioxidants inhibit the free-radical mediated oxidation of lipids, proteins and DNA, which is involved in diseases. Phenolic compounds act as antioxidants by inhibiting enzymes involved in radical generation [Anouar *et. al.,* 2009; Dewick, 2002; Fukumoto and Mazza, 2000]. Requirement for antioxidants in Indian conditions differ from that of industrialized western countries due to the nutritional differences. There are also a number of dietary supplements rich in antioxidants tested for their efficacy. There are many laboratories from India working on the antioxidant effect of plant compounds, mainly derived from natural sources that are capable of protecting against cell damage and different diseases. Such studies show that compounds with potent antioxidant activity include carotenoids, curcumin from turmeric, flavonoids, phenolic acids, etc. [Atoui *et. al.,* 2005].

### 1.5. Chelation properties of polyphenols

Phenolic acids are aromatic secondary plant metabolites broadly distributed throughout the plant kingdom. Phenolic compounds confer unique taste, flavour, and health-promoting properties found in vegetables and fruits [Tomas-Barberan, 2001]. The term "phenolic acids", in general, designates phenols that possess one carboxylic acid functionality, moreover the reason for including phenolic acids in the family of plant polyphenols lies in the fact that they are bio precursors of polyphenols and, more importantly, they are metabolites of polyphenols. Naturally occurring phenolic acids contain two distinctive carbon frameworks: the hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic acid compounds are produced as simple esters with glucose

or hydroxy carboxylic acids. Plant phenolic compounds are different in molecular structure, and are characterized by hydroxylated aromatic rings [Mandal, 2010]. Quercetin (3, 30, 40, 5, 7-pentahydroxyflavone) is one of the most common flavonols present in nature that has attracted the attention of many researchers because of its biological and pharmaceutical properties [Cornard, Merlin, 2002]. A multitude of substitution patterns in the two benzene rings (A and B) of the basic structure occur in nature and variations in their heterocyclic rings give rise to flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavones. Over 4000 different naturally occurring flavonoids have been described and this list was still growing. Quercetin ($C_{15}H_{10}O_7$) is a flavonoid of the flavonol type that contains five hydroxyl groups in positions 3, 30, 40, 5, 7 and a carbonyl group in the fourth position. Owing to these features, quercetin easily forms complexes with many metals. A great number of flavonoids, especially flavones, can efficiently chelate metals like Al(III), Fe(II), Fe(III), Cu(II), or Zn(II). Formation of metal complexes play important and multiple roles in biological systems and provide sensitive colour stabilisation mechanisms in vivo in higher plants [Kuntic *et. al.,* 1998; Markovic, *et.al.,* 2009; Panhwar *et. al.,* 2010; Sun *et. al.,*2008]. Quercetin complexing capacity, widely used for elucidating the structure of natural flavonoids, can also contribute to the bioactivity of these compounds, by acting as carriers and regulators of metal concentration [Castro, 2004; Marinic *et.al.,* 2006]. Concentrations of cadmium are increasing in the biosphere mainly as a result of its industrial uses. Formation of reactive oxygen species (ROS) involving cadmium suggests that DNA can also be taken into account as a potential target of this metal [Gao *et.al.,* 2001]. Soluble cadmium salt results mainly in acute hepatotoxicity in rodents, which is a well-studied experimental animal toxicological model [Wena, Zhaoa, Bhadauriab, Niralab, 2013]. Metal complexation by organic ligands] changes the speciation of Cd(II) and influences its toxicity [Wena *et. al.,*

2013). Cadmium was absorbed by inhalation and ingestion and has a very long biological half-life. It was classified in group 1 of the International Agency for Research on cancer categories of carcinogens [Blasiak, 2001]. The ability of cadmium to generate oxidative stress has been well documented [Wen *et al.,* 2013]. Redox reactions are also observed through the change of the oxidation state of the metal, jointly with the oxidation of the flavonoids by the loss of hydrogen [Panhwar *et. al.,* 2010]. In this sense, the use of chelated metals in oxidation process became a promising technology. By definition, chelation requires the presence of two or more atoms on the same molecule capable of metal landing. Oxygen, nitrogen, and sulphur atoms of molecules are most commonly the metal ligands. Chelators in which oxygen atoms serve as the ligand of iron tend to stabilize Fe(III), thus decreasing the reduction potential of the iron [Miller *et.al.,* 1990]. As reported [Bucher *et.al.,* 1983], the autoxidation of some iron chelates produces a powerful oxidant, which is stronger than the hydroxyl radical. For instance, the oxidation of methanol using iron chelates does not directly depend on the hydrogen peroxide used, since is believed that ferryl species are formed during oxidation. These species are able to oxidise high reduction potential compounds as methanol. Other authors suggest the possibility of formation of iron-oxo complexes during the process or a Fe(III)-hydroperoxo complex. Therefore, this simple method could be applied to the degradation of refractory organic compounds such as aromatic compounds, like phenol [Bianchi *et. al.,* 2003]. Caffeic acid is a multifunctional naturally available organic acid substance which plays a significant role in binding metal ions from the natural environment, food substances and beverage such as coca cola, mineral water etc. The ligand has two complexing sites in competition: the catechol group (dihydroxybenzene) and the carboxylic function. Several co-workers have reported the complexation of this compound with different metal ions in aqueous solutions, Al(III) [Cornard *et.al.,* 2004;

Cornard *et.al.,* 2006; Khvan *et.al.,*2001], Cu(II), Ni(II), Zn(II) and Co(II) [ Khvan *et.al.,* 2001] and Fe(III)[ Hynes, 2004]. In addition, the complexation of caffeic acid with polyphenol and aromatic compounds were also investigated by spectroscopic and computational methods to design advanced and controllable carriers of drugs and food components [Gornas *et.al.,* 2009; Mate *et.al.,* 2008]. The redox chemistry of iron plays an important role in the oxygen activation and transfer reactions mediated by a group of polyphenols. Polyphenols are widely found in plants and are present in all plant-derived systems [Deshpande *et.al.,* 1984; Singleton, 1981; Bronco, 2006]. Hydroxy-cinnamates, especially caffeic acid and its derivatives, are widely distributed and their presence in fruit juices is due to their easy extractability. Foods containing polyphenols undergo enzymatic and non-enzymatic browning due to autoxidation reactions [Hees *et.al.,* 1985; Singleton, 1987]. Initial oxidation of compounds like caffeic acid results in the formation of their quinone form [Demmin *et.al.,* 1981; Fisher *et.al.,* 1985; Hapiot *et.al.,* 1996], which, being strongly electrophilic, undergoes nucleophilic attack [Cabanes *et.al.,* 1987; Macomber, 1982; Pati, 2006]. The rest of the reaction in which the quinone participates is the same, no matter if the quinone has been produced enzymatically or chemically. The above reaction takes place under acidic conditions and is catalysed in the presence of metals [Hocking, 1985; Speier, 1986; Deiana, 2007]. Food processing that includes treatment in alkaline conditions results in decreased nutritional value due to phenol oxidation reactions with amino acids and proteins through their nitrogen [Hurrell *et.al.,* 1982]. Under alkaline conditions, caffeic acid reacts rapidly with oxygen [Tulyathan *et.al.,* 1983]. Under acidic conditions, the nonenzymatic reaction is slow [Oszmianski, 1985]. Some polyphenols also have the potential to be used as chelators to modulate physiological reactions involving iron and other transition metals [Blache, Durand, Prost, & Loreau, 2002; Elhabiri, Carrer, Marmolle, Traboulsi, 2006; Haslam, 1996]. Under

physiological conditions, iron is constantly bound to maintain its solubility, mainly to proteins (haemoglobin, transferrin, and ferritin), but also to some low molecular weight chelators such as citrate. This non-protein bound iron is called labile iron pool or chelatable iron pool and could be a target to exogenous chelators such as polyphenols [Kakhlon, 2002] but direct experimental evidence for this proposal has been lacking.

### 1.6. Iron-polyphenolic acid complex formation and its role in sugar processing

The entire process of producing refined white sugar, from either sugarcane, is directed toward the removal of extraneous components that adversely affect the final quality of white sugar. Many of these components, while considered extraneous because it is desirable that they are removed, actually are quite normal constituents of the cane or beet plant, examples being soluble cell wall polysaccharides, starch, and other smaller metabolites. Additionally, reactions occur during processing as a result of pH changes, thermal effects and autocatalytic effects, which lead to the formation of polymeric colourant. Extensive research has been performed on the characterisation of sugar colour. Numerous comprehensive reviews have been presented [Riffer, 1988; Clarke *et. al.,* 1985 and Kennedy and Smith, 1976]. There are generally recognised to be four types of colour present in sugar: plant pigments, melanoidins, caramels and alkaline degradation products of fructose (ADF). The last three are factory produced colour pigments. The plant pigments are principally phenolics and flavonoids, which make up about two-thirds of the colour in raw sugar [Smith and Paton, 1985]. The phenolics are generally uncoloured but are oxidised or react with amines or iron to form colourants during processing. Flavonoids are polyphenols that exist in the cane plant and are involved in enzymic browning reactions. Caffeic and chlorogenic acids have cis-phenolic groups and are capable of forming chelates with Fe(III). Naringin is also capable of forming a six-

membered ring chelate with Fe(III) while quinic, ferulic and sinapic acids do not appear to form such species. The catechol functional group has been identified as a component of many siderophores [Albrecht-Gary, 1998]. Phenolic acids are also of great interest because their redox properties affect the availability of micronutrients to plants [Deiana *et.al.,* 1995]. The uptake of iron by plants depends considerably on the presence of both complexing and reducing reagents. Chlorogenic acid is one of the major polyphenol compounds found in numerous plant species [Risch *et.al.,* 1988]. Polyphenols mimic animal iron-binding proteins such as transferrin and protect plants by withholding iron from pathogens. Several studies have shown that beverages containing chlorogenic acid can inhibit the absorption of non-haem iron in man by up to 60% [Hurrell, 1999]. Naringin functions as an antioxidant by chelating iron ions and scavenging peroxyl radicals, whereas it's OH radical scavenging effect is much less important [Deng, 1997]. The widespread interaction of these phenolic components with metal ions and the implications from a biological viewpoint warrants the investigation of their mechanistic interactions with metals. There have been several studies of the kinetics and mechanisms of the interactions of naturally occurring catechols with Fe(III) with the reactions involving a variety of different mechanisms [Mentasti, 1973; Mentasti, 1973; Mentasti, 1976; Linert *et.al.,* 1993; El-Ayaan *et.al.,* 1997; El-Ayaan *et.al.,* 1998]

### 1.7. Removal of colours/pigments in sugar processing

The presence of coloured impurities in sugar process solutions results from chemical reactions taking place during the production process. The compounds responsible for forming coloured impurities are mainly of polyphenols, they exhibit a negative influence both on the quality and quantity of the sugar produced. Since no specific tests exist to determine the different types of colourants, tests for phenolics, pH sensitivity, molecular weight ranges and distribution inside the crystal or in the syrup

layer are the most effective means of determining the fate of colourants in processing. Gel permeation chromatography is an excellent technique for examining the high molecular weight constituents in sugar processing, and diode array detectors and evaporative light scattering detectors are yielding a great deal of information about individual high molecular weight colourants [Bento, 1999]. Adsorption and ionic exchange resins, in either hydrogen or sodium form, are widely employed for softening in the sugar industry. In the chloride form, resins have been used for decolourisation of refined sugar solutions since 1970 [Bento *et.al.,* 1997; Bento *et.al.*, 1998; Bento *et.al.,* 1999]. The resins commonly employed for the decolourisation of refined sugar solutions are anionic, with trimethylammonium groups linked to chloride ions. The resin matrix may be of two types: hydrophobic or acrylic and the colourants retained are different:

- Acrylic resins show a low adsorption selectivity and can be regenerated almost completely with sodium chloride solutions [Fries, 1982; Fries, 1991]. Its use is appropriate for high colour cases [Ramm-Schmidt, 1988].
- Polystyrene resins show higher decolourisation efficiencies but its regeneration is more difficult than acrylic resins.

Pigments are widely distributed in the sugarcane plant cell [Singh *et.al.* 2015]. Authors have reported these pigments with colour in sugarcane juice for white sugar production [Tariq, 2014; Alves *et.al.,* 2013; Prati, 2010; Sartori *et.al.,* 2015; Zerban *et.al.,* 1919]. During the clarification process, plant pigments decompose to form polyphenolic compounds with subsequent enzymatic browning [Tariq, 2014;]. Sugar mills in Brazil remove these pigments, as well as other impurities, for the bleaching action of sulphur dioxide during the sulphitation process [Silva *et. al.,* 2015; Ravnö, 2007]. The clarified sugarcane juice as a matter of sugar production is concentrated up to white crystals [Ravnö, 2007; Aguiar *et.al.,* 2015]. Chromatography is a versatile technique widely used

for the separation of chemical compounds in a suspension or solution [Degani *et.al.,* 1998] and this technique can also be used for separation of pigments from plant extracts [Indriatmoko, 2015]. Chemical compounds can be separated by identification and quantification [Christophoridou et.al., 2005; Ma *et.al.,* 2014]. Adsorption resins have a specific area and pore diameter for rapid diffusion of ions and improved extraction kinetics with an increase in the complexing capacity [Belfer *et.al.,* 1984]. Dowex TM Optipore TM SD-2 has been used in the food industry to remove colour, flavour and odour in sweeteners as it has pore structures to maximize the load, as well as the high mechanical, chemical and thermal capacity [Dow, 2015]. The effect of colour to evaluate the sugar quality without or with different treatments, such as gamma radiation and electron beam has been reported [Aguiar *et.al.,* 2010; Lima, 2015], Fenton-like reaction [Nguyen, 2011], ozone [Aguiar, 2014; Souza-Sartori, 2013] and hydrogen peroxide [Sartori *et. al.,* 2015; Souza-Sartori *et.al.,* 2013; Mandro *et.al.,* 2015]. They evaluated the isolation of pigments in sugarcane juice as potential purification process and isolation system of pigments, which have been associated by antioxidant activities [Aguiar *et. al.,* 2010; Sartori *et. al.,* 2013], by adsorption chromatography column, as described by Lima and Aguiar [Lima and Aguiar, 2015]. During the 1970s, Norman Smith (California and Hawaiian Sugar Co.) tried to determine whether different colourants were harder to remove during refining than others and whether the colourant composition varied between sugars from different sources (Smith, 1972, 1976). He examined the efficiency of colour removal using size exclusion chromatography, attempted to use reverse phase chromatography, followed colourant profiles through the refinery and concluded that colourant material affected the overall colourant removal efficiency through the factory. However, there were a number of problems with this work: the size exclusion columns were inadequate for fractionation of colourants, and the extraction and concentration

methods (methanol extraction and adsorption onto a hydrophobic resin) would have altered the colourant composition during the investigation.

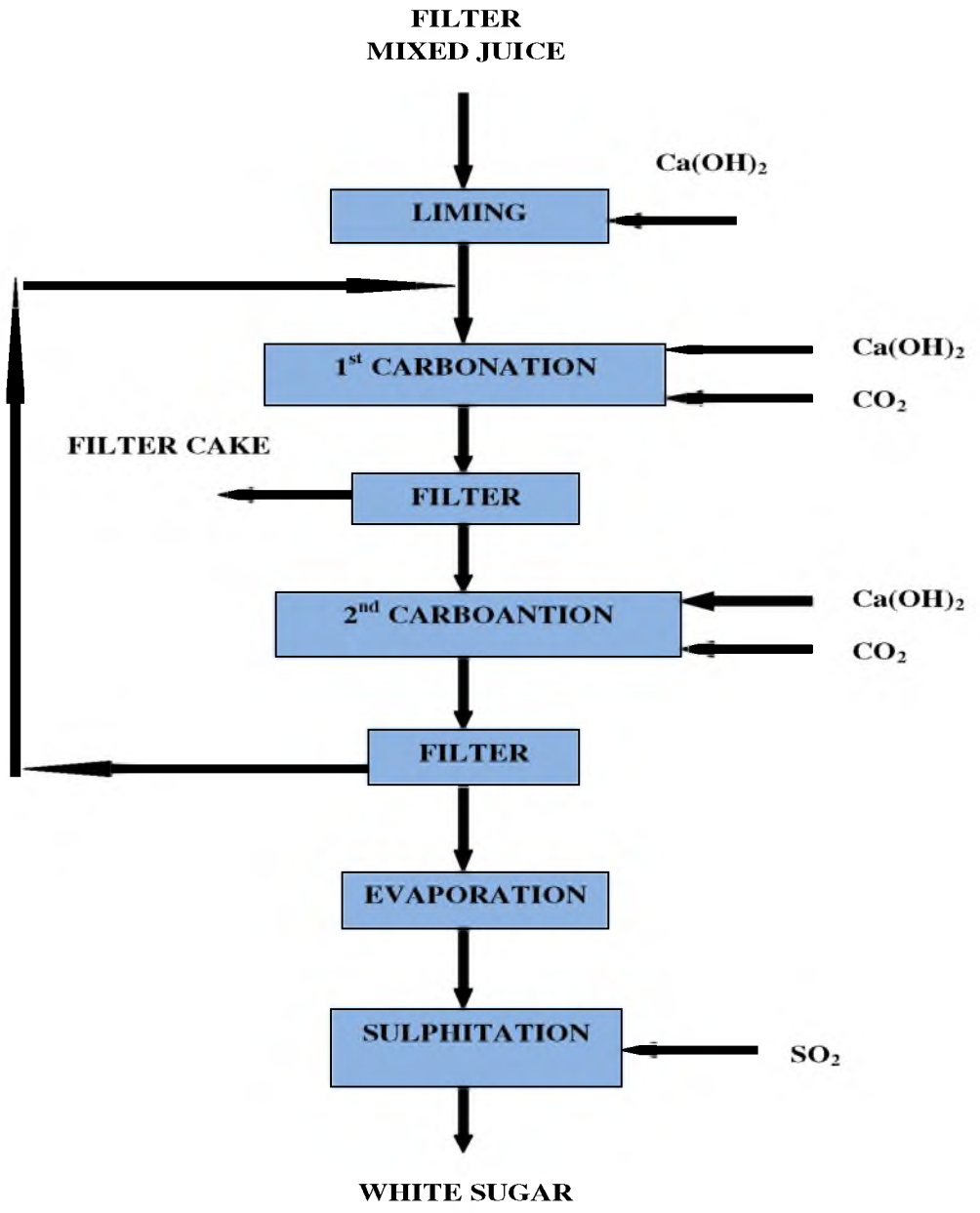



**Fig.1.4. Typical flowchart of sugar manufacturing by carbonation process**

### 1.9. Aim and objective of the research work

In this proposed work, attempt will be made to understand the role of electron transfer (E.T.) reactions in catalysing colour development in sugar processing where iron-polyphenols play an important role as control or elimination of colour formation in sugar processing is of high technical importance. Keeping in view the above problem of the sugar industry following has been proposed:-

1. To determine the kinetics and mechanism of iron-polyphenols interaction taking caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarin, 4-hydroxycoumarin as representative selection of polyphenols commonly present in cane juice.

2. Spectrophotometrical, kinetics and analytical techniques will be used to investigate the kinetics of chelate formation between iron and aforesaid polyphenols.

3. Physiochemical analysis of the iron-polyphenol chelates such as pH stability, solubility, molecular weight, gustatory properties, etc. will be investigated.

4. To determine the various parameters of the isolated complexes such as hardness, electronegativity, softness, total energy, dipole moment and point group symmetry Density functional theory (DFT) was used.

5. To corroborate the experiment findings of the isolated chelates in the pure chemical system by the theoretical study.

This study hopefully appears to generate kinetic data where the application will eventually lead to the introduction of the coating of an inactive material in the surface of iron which remains in contact with cane juice and undergoes various operations and complete removal of polyphenols from cane juice must be ensured. Such preventive measure would also be helpful in minimizing the colour development during storage of sugar as well.

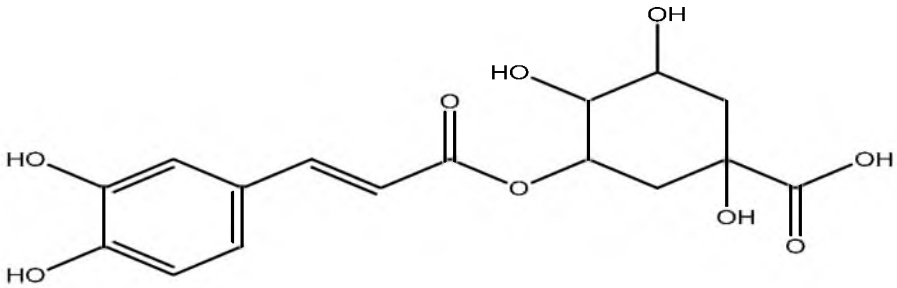
The objective of the present study, therefore, is to substantiate the mechanistic aspects of the interactions between Fe(III) and caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarin, 4-hydroxycoumarin employing experimental and theoretical study.

In the present case, an attempt has been made to corroborate the experimental findings by a theoretical study of the isolated complexes pure chemical system.

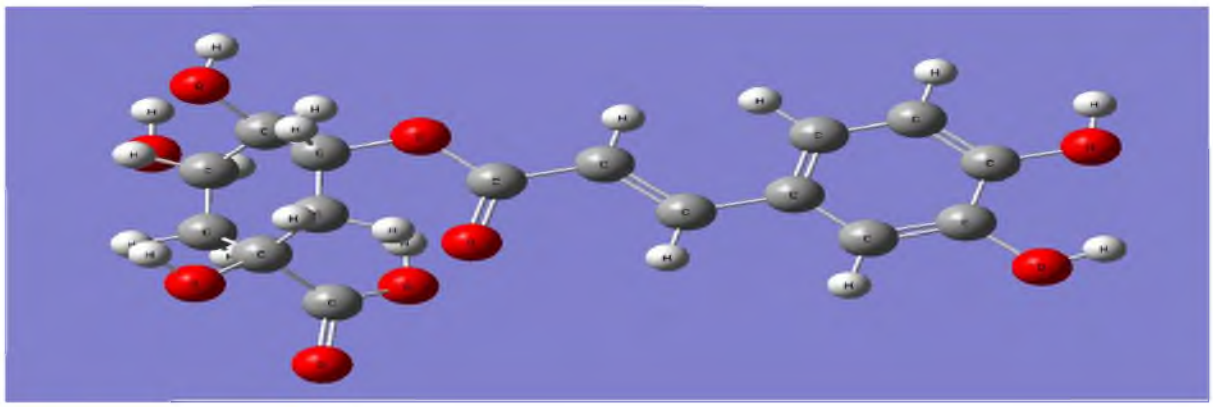
The objective of the present study, therefore, is to substantiate the mechanistic aspects of the interactions between Fe(III) and caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarin, 4-hydroxycoumarin employing experimental and theoretical study.

## 1.10. Structure of representative phenolic acids

### 1.10.1. Chlorogenic acid [3-(3,4-dihydroxycinnamate)]




**(a) 2-D Structure**



**(b) 3-D structure**

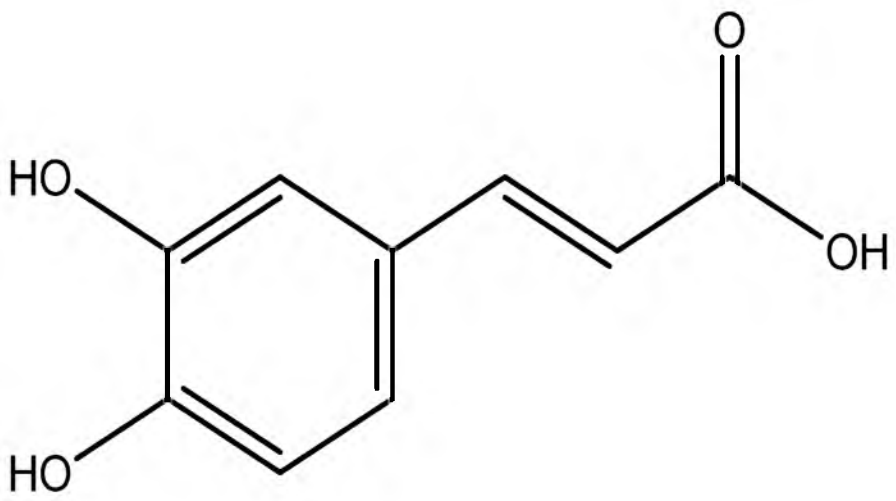
**Fig. 1.6. Showing (a) 2-D and (b) 3-D DFT structure of chlorogenic acid**

**Table 1.2. Properties of chlorogenic acid**

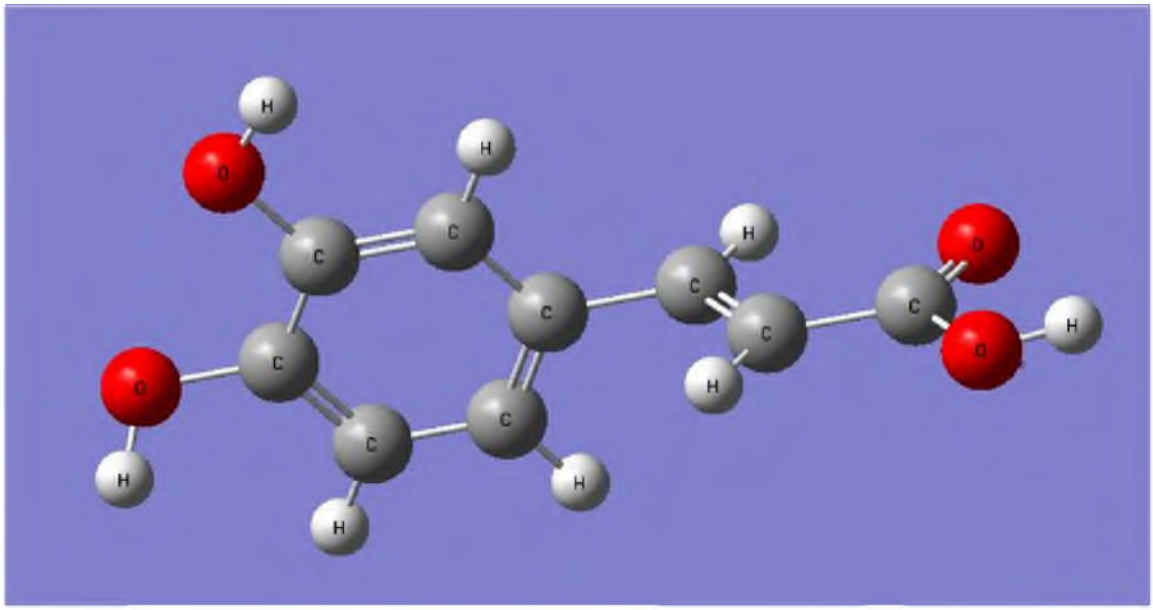
| | |
|---|---|
| Molecular formula | $C_{16}H_{18}O_9$ |
| IUPAC name | 3-Caffeoylquinic acid |
| Melting point | 210 °C |
| Solubility | Soluble in ethanol and acetone |
| Water solubility | Soluble in hot water |
| Colour | Almost white |
| Molecular weight | 354.31 |

(Source: Google chemical book)

### 1.10.2. Caffeic acid (3,4-Dihydroxybenzeneacrylic acid)




**(a) 2-D Structure**



**(b) 3-D structure**

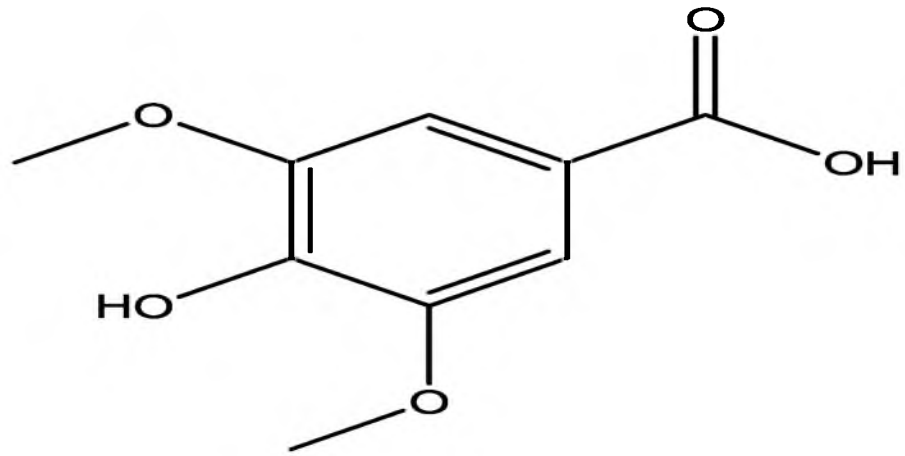
**Fig. 1.7. Showing (a) 2-D and (b) 3-D DFT structure of caffeic acid**

**Table 1.3. Properties of caffeic acid**

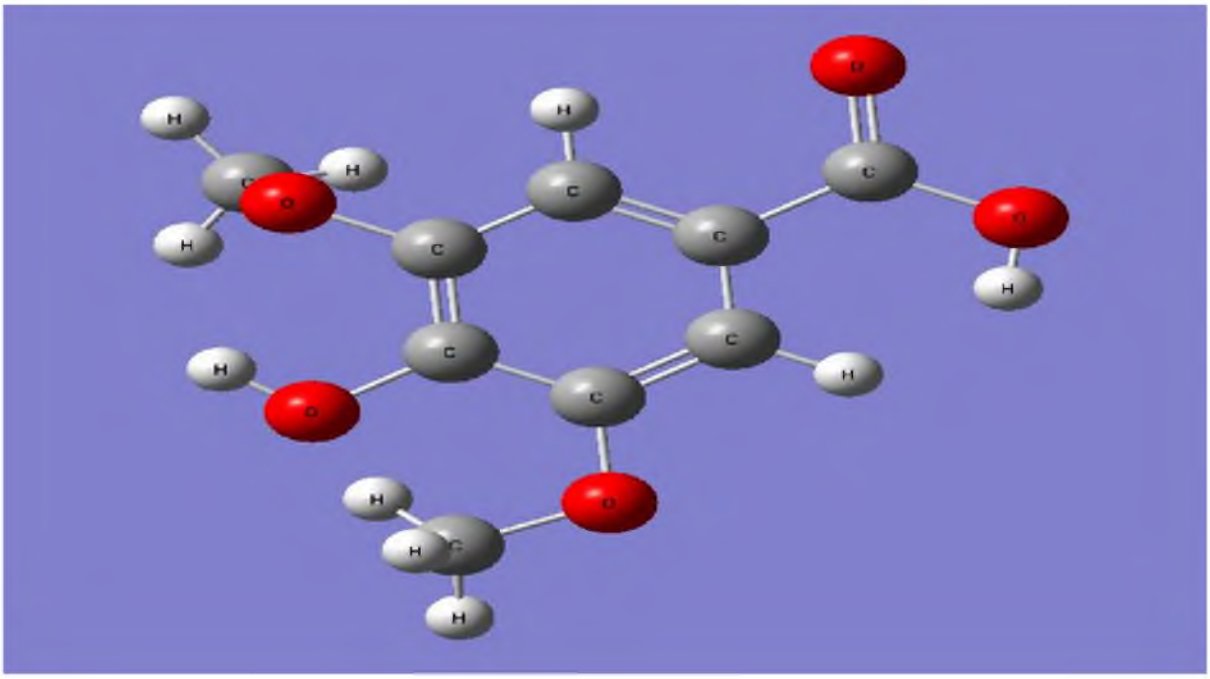
| | |
|---|---|
| Molecular formula | $C_9H_8O_4$ |
| IUPAC name | 3,4-Dihydroxybenzeneacrylic acid |
| Melting point | 211-213 °C |
| Solubility | Ethanol (50 mg/mL) |
| Water solubility | Soluble in hot water |
| Stability | Stable and combustible |
| Molecular weight | 180.16 |
| Colour | Yellow to tan |

(Source: Google chemical book)

### 1.10.3. Syringic acid (3,5-Dimethoxy-4-hydroxybenzoic acid)




**(a) 2-D Structure**



**(b) 3-D structure**

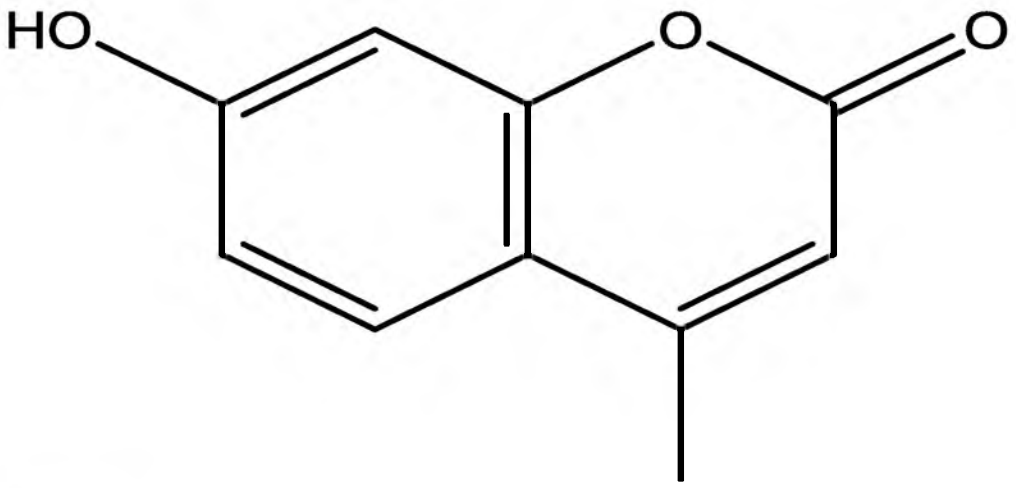
**Fig. 1.8. Showing (a) 2-D and (b) 3-D DFT structure of syringic acid**

**Table 1.4. Properties of caffeic acid**

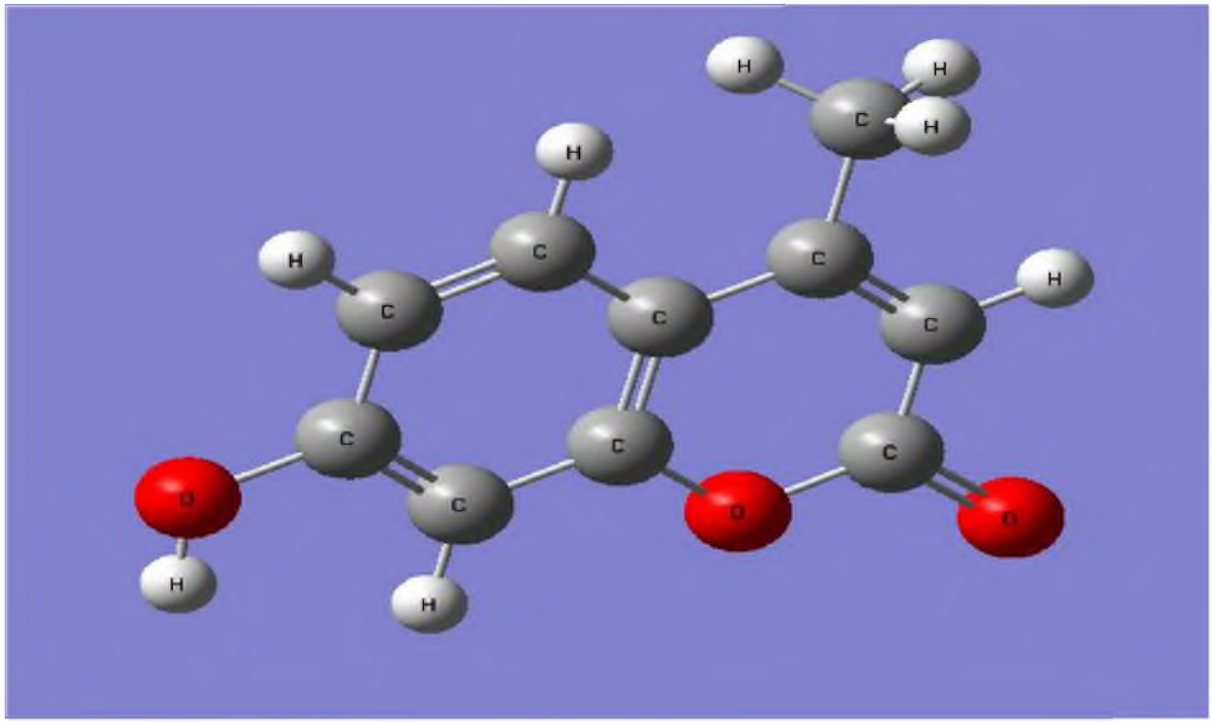
| | |
|---|---|
| Molecular formula | $C_9H_{10}O_5$ |
| IUPAC name | 3,5-Dimethoxy-4-hydroxybenzoic acid |
| Melting point | 205-209 °C |
| Boiling point | 192-193°C 14mm |
| Water solubility | 5780 mg/L (25 ℃) |
| Stability | Stable |
| Molecular weight | 198.17 |
| Colour | Grayish-beige to light brown |

(Source: Google chemical book

### 1.10.4. 7-Hydroxy-4-methylcoumarin




**(a) 2-D structure**



**(b) 3-D structure**

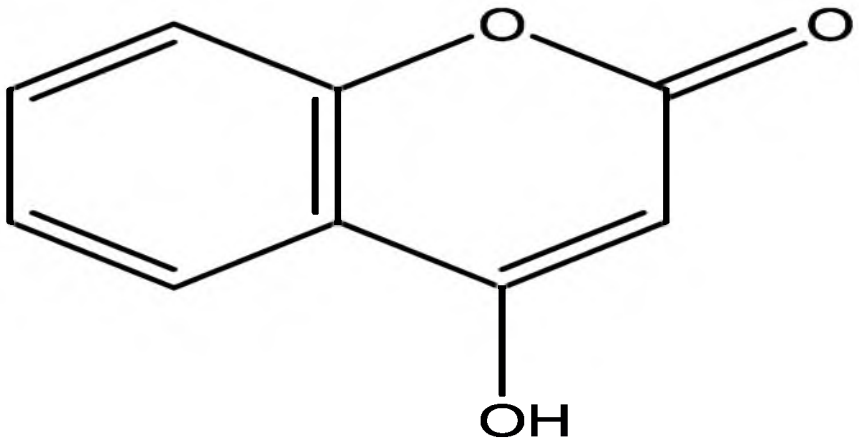
**Fig. 1.9. Showing (a) 2-D and (b) 3-D DFT structure of 7-hydroxy-4-methylcoumarin**

**Table 1.5. Properties of 7-hydroxy-4-methylcoumarin**

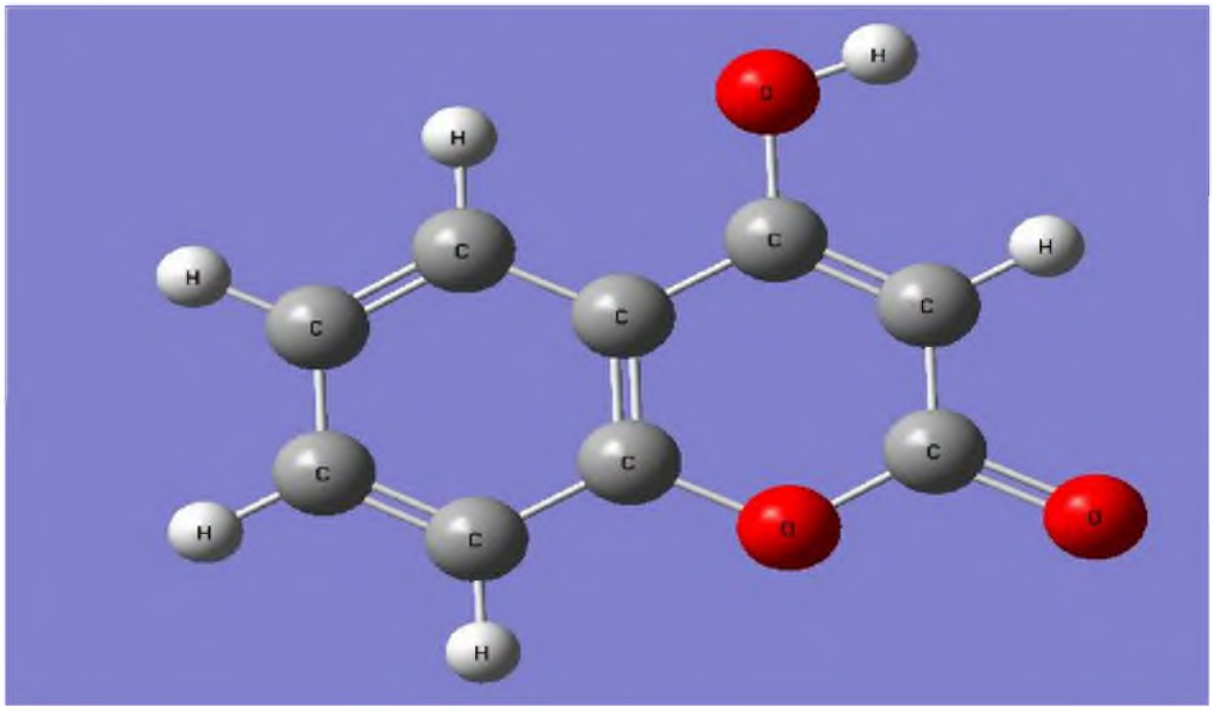
| | |
|---|---|
| Molecular formula | $C_{10}H_8O_3$ |
| IUPAC name | 7-Hydroxy-4-methylcoumarin |
| Melting point | 186-191℃ |
| Water solubility | Insoluble |
| Molecular weight | • 194.18 |

(Source: Google chemical book)

### 1.10.5. 4-Hydroxycoumarin




**(a) 2-D structure**




**(b) 3-D structure**

**Fig. 1.10. Showing (a) 2-D and (b) 3-D DFT structure of 4-hydroxycoumarin**

**Table 1.6. Properties of 4-hydroxycoumarin**

| | |
|---|---|
| Molecular formula | $C_9H_6O_3$ |
| IUPAC name | 4-Hydroxycoumarin |
| Melting point | 211-213 °C |
| Colour | Clear colourless to slightly yellow |
| Water solubility | Insoluble |
| Stability | Stable and combustible |
| Molecular weight | 162.14 |

(Source: Google chemical book)

### 1.11. Scope of the work

Literature survey suggest that in spite extensive data on colour complex formation between polyphenols and iron salts, the detailed investigations of deliberately added ferrous, ferric iron in sugar processing has not been studied so far. Therefore, it was considered necessary to study the influence of iron-polyphenol interaction on colour development in pure chemical system. Furthermore, there is a general update that fast caramalisation catalysed by iron and relationship between polyphenols and uptake of iron is only hypothesis without experimental data on iron polyphenols interaction which can complement the above hypothesis as and when polyphenols comes in contact with iron is accompanied by colour formation during sugar processing. Some polyphenols such as caffeic acid, syringic acid, chlorogenic acid, 7-hydroxy-4-methylcoumarin and 4-hydroxycoumarin were chosen as representative selection of polyphenols and their reaction with iron were investigated.

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# CHAPTER-2

## Experimental methods and characterization techniques

Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible (UV-Vis) spectral techniques were used to characterize complexes of polyphenolic acids with Fe(III). The theoretical studies of the complex have been studied by Density Functional Theory (DFT).

## 2.1.0. Introduction

Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible (UV-Vis) spectral technique were used to characterize complexes of polyphenolic acids with Fe(III). The theoretical study of the complex has been studied by Density Functional Theory (DFT).

## 2.2.0. Experimental

### 2.2.1 Chemicals and reagents

Syringic acid (3, 4-Dihydroxycinnamic acid) chlorogenic acid, caffeic acid (3,4-Dihydroxybenzeneacrylic acid), were purchased from Molekula Ltd., United Kingdom. 7-Hydroxy-4-methylcoumarin and 4-hydroxycoumarin were purchased from Himedia chemicals, India. Source of Fe(III) salt of ferric chloride was obtained from Fisher Scientific India. All the reagents and solvents were of analytical grade and chemically pure and were used as received.

### 2.2.2 Preparation of solutions

All experiment was performed in aqueous solution. Stock solution of Fe(III) was prepared in double distilled water (10 ppm) in 100 ml volumetric flask. Stock solution of syringic acid, caffeic acid and chlorogenic acid were prepared in double distilled water (10 ppm) at pH 9.0 and ethanol (10 ml) was added in their stock solutions for complete dissolution. Similarly stock solution of 7-Hydroxy-4-methylcoumarin and 4-hydroxycoumarin were also prepared in double distilled water at pH 11.0. When equimolar concentration of Fe(III) reacts with different polyphenolic acid, they formed coloured complex after the reaction. After 30 to 45 minutes, the precipitates of different polyphenol complex with iron were formed at room temperature. The precipitates of each complex were filtered and dried separately by rota vapour for further analysis.

**Table2.1. Data showing the polyphenolic acid complex colour formation with Fe(III)**

| S.No. | Fe(III) + Polyphenols complex | Complex colour |
|---|---|---|
| **1.** | Fe(III) + Syringic acid | Pale yellow |
| **2.** | Fe(III) + Caffeic acid | Dark brown |
| **3.** | Fe(III) + Chlorogenic acid | Dark green |
| **4.** | Fe(III) + 4-Hydroxycoumarin | Dark green |
| **5.** | Fe(III) + 7-Hydroxy-4-methylcoumarin | Dark brown |

### 2.3.0. Various characterization techniques

Synthesized complex was characterized by various characterization techniques given below.

### 2.3.1.0. Optical Characterization

### 2.3.1.1. UV-Visible spectroscopy

UV-Vis spectrophotometer uses visible light and ultraviolet to analyze the chemical structure of the substance. A spectrophotometer is a special type of spectrometer, which is used to measure the intensity of light, and the intensity is proportional to the wavelength. When the ultraviolet light project to various organic compounds, these compounds will absorb it. So, you can use UV-Vis spectrophotometer to measure the absorption of a compound by the result and have its molecular structure, as well as the related information.

### 2.3.1.2 Principle of UV-Visible spectroscopy

The spectrophotometer is a much more refined version of a colourimeter. In a colourimeter, filters are used which allow a broad range of wavelengths to pass through, whereas in the spectrophotometer a prism (or) grating is used to split the incident beam into different wavelengths. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can

be as low as 1 to 2 nm. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

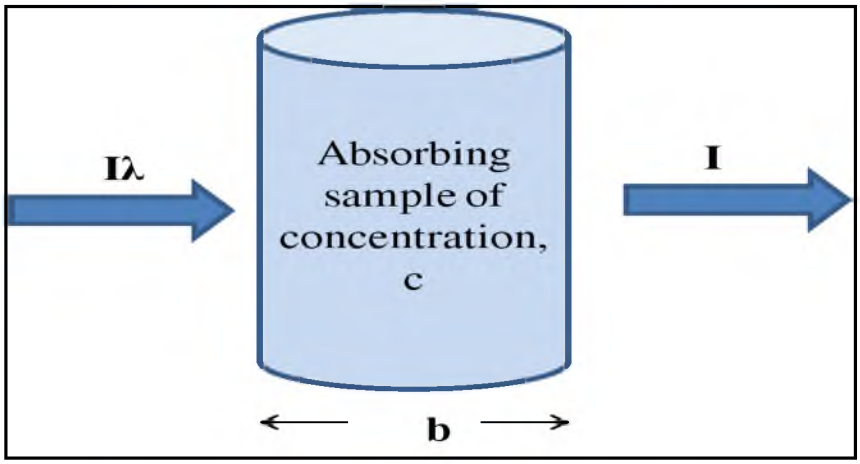


**Fig.2.1. Absorption of light by a sample in UV-Visible spectrophotometer**

### 2.3.1.3 Quantitative relationships for optical spectroscopy

**Beer and Lambert laws**

$$A = \varepsilon bc$$

$$A = -\log T = \log \frac{I_0}{I} = \varepsilon bc$$

$$T = \frac{I}{I_0}$$

(Where A= Absorbance, $I_0$= intensity of incident light, I= Intensity of emitted light, $\boldsymbol{\varepsilon}$ = molar absorptivity coefficient , T= Transmittance, b= path length of sample, c = molar concentration of solute)

From the Beer-Lambert law, it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption.

### 2.3.1.4. Instrumentation and working of UV-Visible spectroscopy

Instrumentation and working of the UV spectrometers can be studied simultaneously. Most of the modern UV spectrometers consist of the following parts-

**Light source**

Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

**Monochromator**

Monochromators generally composed of prisms and slits. The most of the spectrophotometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms. The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

**Sample and reference cells**

One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution. Both sample and reference solution are contained in the cells. These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

**Detector**

Generally two photocells serve the purpose of detector in UV spectroscopy. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

**Amplifier**

The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servometer. Generally, current generated in the photocells is of very low intensity, the main purpose of an amplifier is to amplify the signals many times so we can get clear and recordable signals.

**Recording devices**

Most of the time amplifier is coupled to a pen recorder which is connected to the computer. Computer stores all the data generated and produces the spectrum of the desired compound (Aman Thakur, 2011)

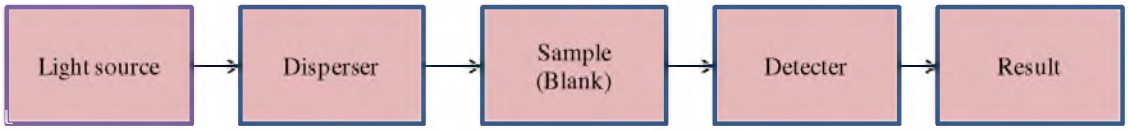


**Fig.2.2. Typical flow diagram of UV-Visible Spectrophotometer working**

With the help of double beam spectrophotometer, the colourimetric study of the various polyphenolic acid complex with Fe(III) has been carried out (Perron N.R., 2010) and such representative data has been given in Table.


**Fig. 2.3. UV-Visible spectrophotometer at DAC, BBA University, Lucknow, U.P.**

**Table 2.2. Data showing the λmax of different iron-polyphenol complexes**

| S.No. | Fe(III) + Polyphenols complex | λ max (nm) |
|---|---|---|
| **1.** | Fe(III) + Syringic acid | 292.5 |
| **2.** | Fe(III) + Caffeic acid | 321 |
| **3.** | Fe(III) + Chlorogenic acid | 323 |
| **4.** | Fe(III) + 4-Hydroxycoumarine | 280 |
| **5.** | Fe(III) + 7-Hydroxy-4-methylcoumarin | 318 |

### 2.3.1.2 Fourier Transform Infrared (FT-IR) spectroscopy

Fourier transform Infrared Spectroscopy (FT-IR) is an extremely useful technique particularly for identifying to different types of chemical bonds in a molecule of unidentified materials [P. Pandey *et. al.,* 2011]. FT-IR spectroscopy is a very powerful method for the identification of functional groups. In general, the goal of FT-IR spectroscopy is to measure how well a sample absorbs or transmits light at each different wavelength. To use the FT-IR, a continuum source of light is used to produce light over a broad range of infrared wavelengths. The principal experimental method in this work is FT-IR, which allows us to detect infrared (IR) absorption and reflection properties over a broad spectral region. IR spectroscopy also known as vibrational spectroscopy, which concerned with the study of absorption of IR radiation by a molecule, causes the molecular bonds to vibrate including vibrational transition in a molecule.

FT-IR is used with the basic goal of determining changes in the intensity of infrared light as it interacts with a material as a function of wavelength. Therefore, infrared spectroscopy can apply as a very powerful tool for qualitative identification of different functional group and chemical bonds in a different environment [B.C. Smith, 1996].

In FT-IR analysis there are three commonly examined pieces of data known as peak position, the peak width and the peak intensity. The peak position is probably the most commonly used for the identification of materials. These peaks are unique since, at characteristic frequencies, certain functional groups will display their own set of peaks. This is because infrared techniques measure the vibrational energies of the molecules. In order for a molecule or functional group to be IR active, the dipole moment of the molecule must change. When the desired sample for testing is opaque, transmission experiments are not

practical. To overcome this problem, reflection experiments in the FT-IR become more appropriate.

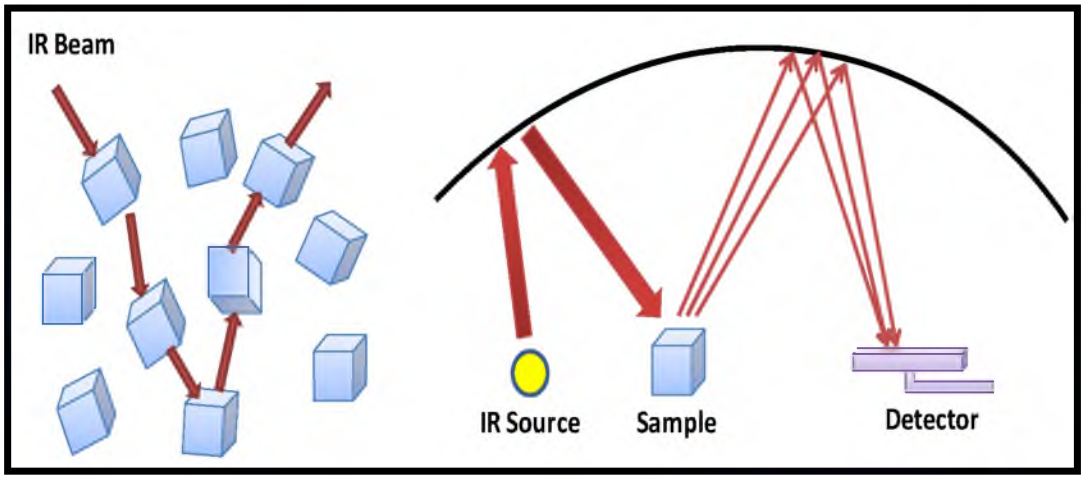


**Fig. 2.4. IR beam interaction in diffuse reflection mode of FT-IR**

When the infra-red beams enter in the sample as shown in Fig. 2.3(a), it can be reflected, transmitted or absorbed. The infra-red energy reflecting off the surface is typically lost. The infra-red beam that passes through a particle can either reflect off the next particle or to be transmitted through the next particle. Scattered infra-red energy is collected by a spherical mirror that is focused onto a detector. These are the basics of how the diffuse reflectance mode of the FT-IR works [Ronald A. Holser, 2012].

Diffuse reflectance mode of the FT-IR (DR-FT-IR) can be successfully used for studying the surface chemistry variation of materials. There have been few studies that have shown that the variation in infra-red absorption of materials can be correlated to their variation in free carrier densities on the material surface.

Absorption by free carriers on the surface in the space charge region may differ that in the bulk and need to be studied when dealing with adsorption of humidity species onto the surface of the material. Beer's law shows that the absorbance can be directly related to the absorption coefficient using the relation given below:

$$I = I_o \exp(-kx) \quad \ldots\ldots(1)$$

In Eqn. 2.6, x is the thickness of measured sample and K represents the absorption coefficient of the sample. This is typically referred to as the broad background of the sample.


**Fig. 2.5. FTIR at USIC, BBA University, Lucknow, U.P., India**

Spectra of the sample prepared during the research period were recorded on KBr pellet using a Thermo Scientific (Nicole 6700) FT-IR spectrometer Fig. 2.3(b), in the wave number region of 400-4000 $cm^{-1}$. For recording the spectra, the sample pellets were prepared by mixing a small amount (2 mg) of the sample with 200 mg of KBr powder and was grinding until a homogenous mixture is formed. The mixtures were then taken in a hydraulic pressure die and pressed under high pressure to form pellets before recording the spectra.

### 2.3.2 Surface morphological characterization

#### 2.3.2.1 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is a special type of electron microscope which produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and give information about the sample's surface topology and composition. The electrons beam is generally scanned in a raster scan pattern and the beam's position is pooled with the detected signal to produce an image. SEM can achieve resolution better than 1 nm. The most common SEM mode for the detection of secondary electrons is emitted by atoms excited by the electron beam. The numbering of secondary electrons depends on the angle at which

beam meets the surface of the specimen, i.e. on specimen topography. By scanning the sample and collection the secondary electrons with the special detector, an image displaying the topography of the surface is created [Antonio da Costa, 2014]. The surface morphological studies of the different polyphenol complex were obtained in mesoporous in nature.

**2.3.3.1.1. Principle and working of SEM**

Different types of signals produced by an SEM include secondary electrons, back-scattered electrons (BSE), characteristic X-rays, cathode luminescence (light), specimen current and transmitted electrons. In all SEM, secondary electron detectors are standard equipment but it is rare that a single machine would have detectors for all possible signals, result from exchanges of the electron beam with atoms at or near the outer part (surface) of the sample. In the standard detection mode, SEM can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. SEM microscopes have a large depth of field yielding a characteristics three-dimensional appearance useful for understanding the surface structure of a sample. A wide range of magnification is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 5,00,000 times, about 250 times the magnification limit of the best light microscopes [C.W. Oatley *et. al.,* 1965].

BSE is the beam electrons that are reflected from the sample by elastic scattering. It is often used in analytical SEM along with the spectra made from the characteristics X-rays because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen. BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can image colloidal gold immune-labels of 5 or 10 nm diameters, which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens [K.C.A. Smith and C.W. Oatley, 1955]. Characteristics X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher-energy electron to fill the shell and release energy and used to identify the composition and quantify the abundance of elements in the sample. The ray diagram of a typical SEM with photograph has been represented by Fig. 2.4(a) and (b).

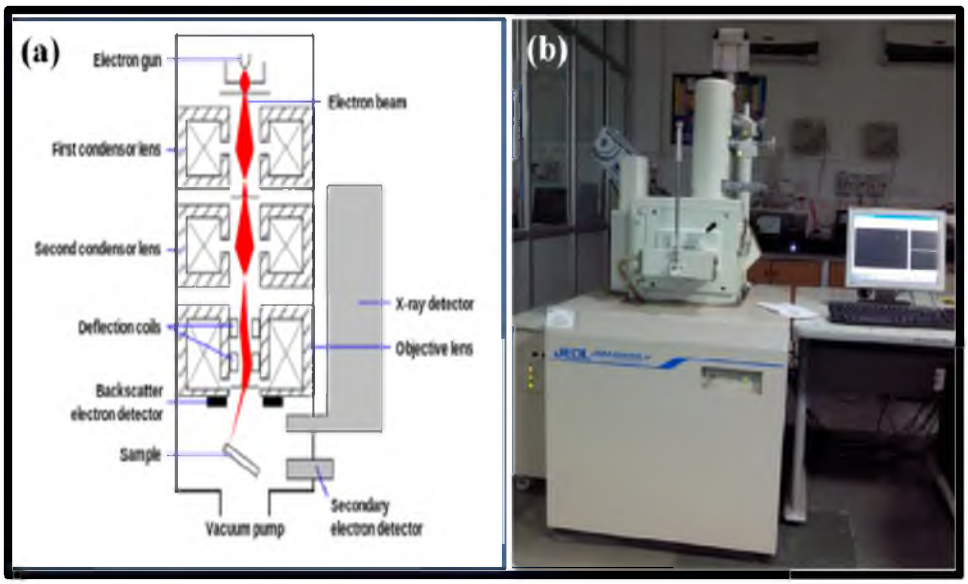


**Fig. 2.6. (a) Schematic diagram of SEM (b) Photograph of SEM at USIC, BBAU, Lucknow, U.P., India**

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# CHAPTER-3

## Kinetics of complex formation of Fe(III) with caffeic acid: Experimental and theoretical study


**Abstract**

Kinetic study on the complexation of caffeic acid with ferric chloride was performed in aqueous solution at pH 9.0. The complex was characterized with IR, UV-Vis spectroscopic and FE-SEM techniques. The complexation reaction was found to be a first-order with rate constants for k (formation) $2.86 \times 10^{-2}$ $min^{-1}$. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 9.0. The apparent activation energy of the complexation reaction was evaluated to be 150 kcal/mol. However, this activation energy is inconsistent with the chemistry of Fe(III) with polyphenols which is supposed to mimic the interaction of Fe(III) with transferrin in biological media. Various parameters of the complex such as hardness, electronegativity, softness, total energy, dipole moment and point group symmetry were calculated employing Density functional theory (DFT) and evaluated as 0.04465, 0.2130, 22.39, $0.5201 \times 10^{-8}$ eV, 15.13 Debye and C1, respectively

### 3.1.0. Introduction

Polyphenols including flavonoids and phenolic acids are products of the secondary metabolism of plants. In plants, polyphenols may act as phytoalexins, antifeedant, and attractants for pollinators, contributors to plant pigmentation, and protective agents UV light [Naczk, 2006]. In addition, polyphenols exhibit many biological properties such as anticarcinogenic, anti-ulcer, antithrombotic, anti-inflammatory, immune modulating, anti-allergic, antimicrobial, vasodilatory, and antioxidant activities [Wollgast, 2000]. Polyphenols have become an intense focus of research interest because of their perceived health-beneficial effects. Phenolic acids, low molecular mass phenolics, are derivatives of benzoic and cinnamic acid. They are widely distributed in sugarcane and are a colour precursor in cane sugar manufacture [Goodacre, 1978; Paton.1992]. Phenolic acids have significant biological and pharmacological properties, some of which were shown to be effective in preventing cancer [McCann, 2007]. They contribute to color formation of juice when sugar cane is crushed and are also involved in the changes that take place during the processing of sugarcane for production of raw sugar [Paton, 1992; Bucheli, 1994]. Several studies have shown that beverages containing chlorogenic acid can inhibit the absorption of non-haem Fe(III) in man by up to 60% [Hurrell *et.al.,* 1999]. Experimental studies demonstrated [Saija *et.al.,* 1995] that it possesses numerous beneficial effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, and antiallergic, antiviral, and anti-inflammatory properties. Many of these effects are correlated to the antioxidant capability that is due to the scavenging of free radicals species and to synergistic effects with enzymes and physiological antioxidants [Miller, 1996]. Metal-chelating compounds remove the metals and can alter their redox potentials rendering them inactive. Another antioxidant mechanism, not exhaustively studied, is based on the ability of some of these compounds to chelate transition metals ions (especially Fe(III) and copper), giving rise to stable complexes that, entrapping metals, prevent these from participating in free radicals generation [Amic *et.al.,* 1995]. Thus, there is emerging interest in the use of naturally occurring antioxidants for the preservation of foods and in the management of a number of pathophysiological conditions, most of which involve free radical damage [Soobrattee *et.al.*, 2005]. This paper

reports the solution structure of the Fe(III) phenolic acids interactions. Because the metal ion coordination resulted in simultaneous deprotonation of the phenolic functions of the aromatic ring, the spectra of the formed metal species resembled those of the anions of the parent molecule, with a bathochromic shift due to the metal ion [Fiallo Marina, 1999]. The kinetics and mechanism of Fe(III) with various polyphenolic compounds have been investigated [Hynes, 2004] and it has been reported that caffeic and chlorogenic acid are generally consistent with the formation of a 1:1 complex that subsequently decays through an electron transfer reaction. However, in the present case, attempt has been made to corroborate the experimental findings by theoretical study of the isolated complex. Objective of the present study, therefore, is to substantiate the mechanistic aspects of the interactions between Fe(III) and caffeic acid employing experimental and theoretical study.

### 3.1.0. Experimental

### 3.1.1. Chemicals

Caffeic acid (3, 4-Dihydroxycinnamic acid) was purchased from Molekula Ltd., United Kingdom. Ferric chloride was obtained from Fisher Scientific India (Fig. 3.1). All the reagents and solvents were of analytical grade and chemically pure and were used as received.

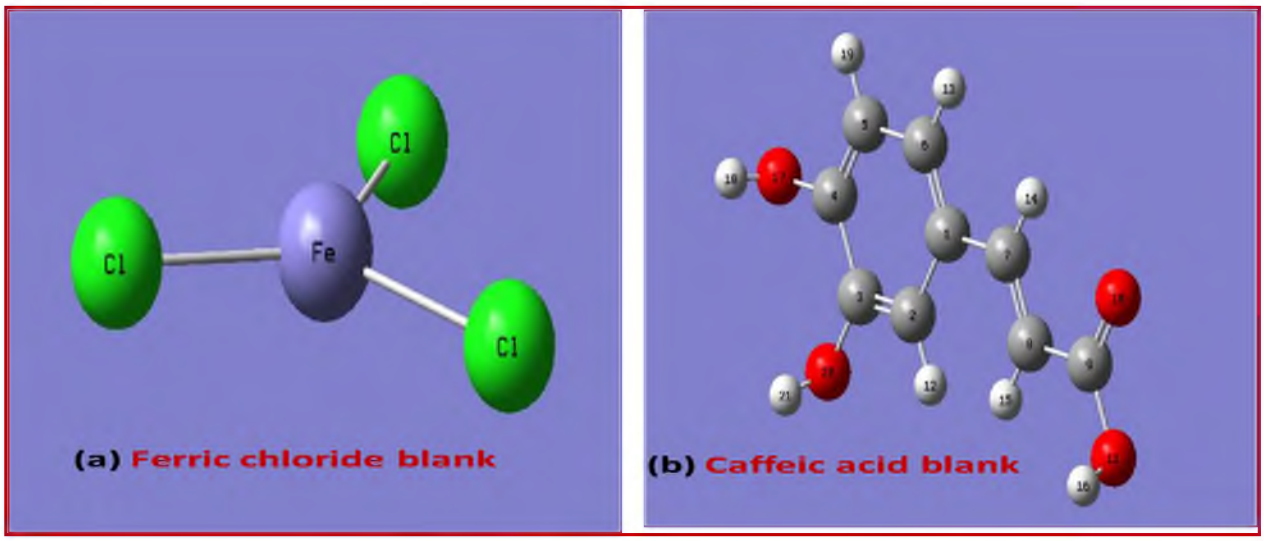


**Fig. 3.1. Molecular structure of (a) Ferric chloride (b) Caffeic acid**

### 3.1.2. Synthesis of Fe(III)–caffeic acid complex

All experiment was performed in aqueous solution at pH 9.0. A stock solution of Fe(III), caffeic were prepared in (10 ppm) in 100 ml volumetric flask. Methanol (10 ml) was added to caffeic and caffeic acid stock solutions for complete dissolution. When the equimolar concentration of Fe(III) reacts with the caffeic acid, the dark green coloured complex was formed. After 30 minutes, the precipitate of caffeic acid was formed at room temperature. The precipitate were filtered and dried for further analysis.

### 3.1.3. Theoretical study

With the help of DFT method, various parameters of Fe(III) and caffeic acid complex were evaluated employing Gaussian09 software.

### 3.1.4. Characterization

The absorbance and maximum wavelength of blank and complexes were recorded using UV–visible spectrophotometer (Carry 100 make). The functional group identification of Fe(III)-caffeic acid complex was determined by Fourier Transform Infra-Red Spectrometer (FTIR) (Thermo-Scientific, Nicole 6700). The morphological analysis of Fe(III)-caffeic acid complex was studied by Field Emission Scanning Electron Microscope (FESEM) by employing JEOL JSM 7610F model.

## 3.2 Results and discussion

### 3.2.1. Fe(III)–caffeic acid complex formation

The theoretical structure of Fe(III) and caffeic acid was obtained from optimizing by DFT method in which Fe(III) attacked carbon 4 due to the availability of pi-electron on the ring. Each valency of Fe(III) will attack carbon 4 of the caffeic acid (Fig. 3.2).

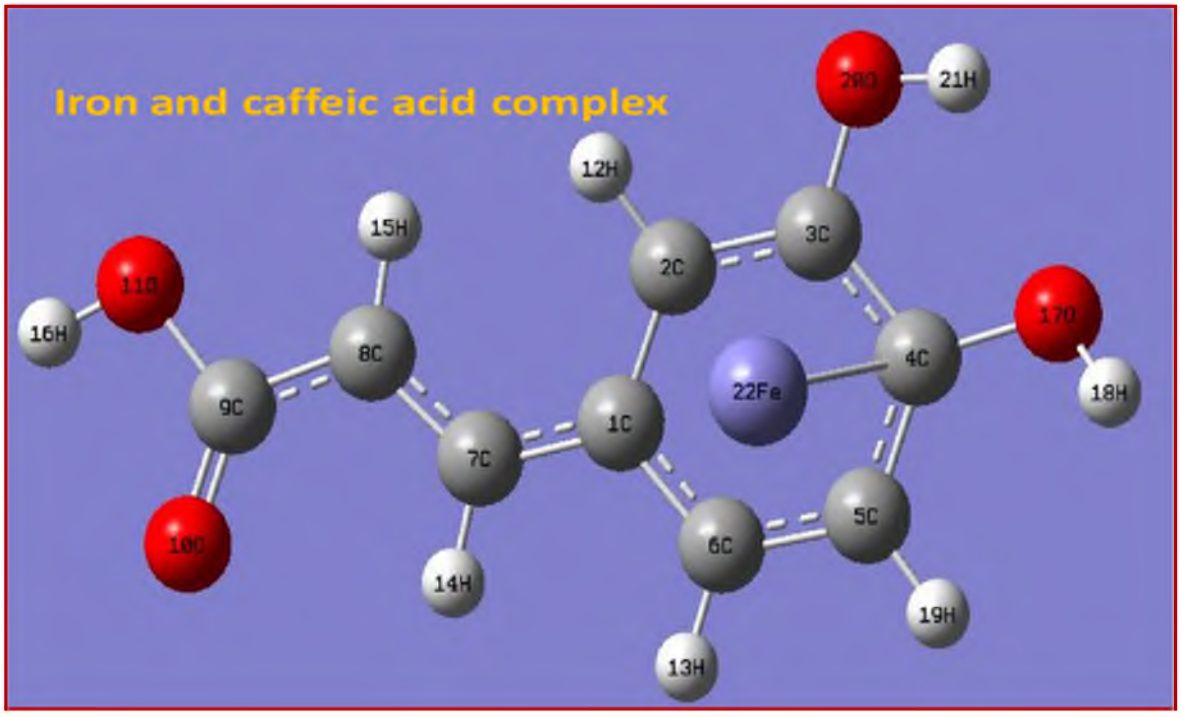


**Fig. 3.2. Complex formation of Fe(III) and caffeic acid by DFT method**

### 3.2.2. FT-IR spectra

The FT-IR spectra of Fe(III)-caffeic acid complex shown in Fig. 3.4 confirmed the complex formation of Fe(III) with phenolic acid. Fig.1 illustrates that peaks were sharp in the complex as compared to caffeic acid (blank). The absorptions at 1617.4 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1520.5 $cm^{-1}$ can be attributed to aromatic C=C stretching vibrations of caffeic acid with Fe(III). The broad peak at 3390 $cm^{-1}$ can result from stretching vibration of -OH groups of caffeic acid. Some peaks of Fe(III)c and caffeic acid disappeared and some of the new peaks appeared, when the complex was formed.

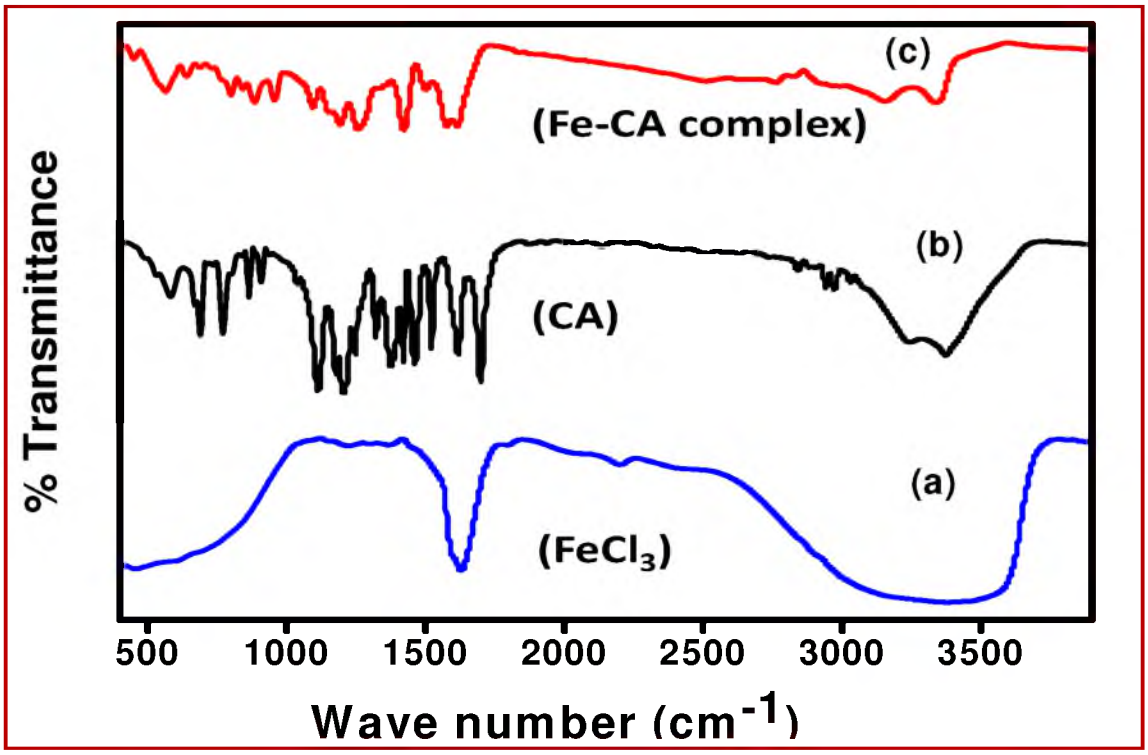


**Fig. 3.3. FT-IR spectra of (a) $FeCl_3$ (b) Caffeic acid (c) Fe(III)-caffeic acid complex**

### 3.2.3. UV-Visible spectra

Since absorbance maxima were not observed in the visible region, therefore, spectra were scanned in 250-450 nm range. The concentration ranges of Fe(III)-caffeic acid complex was maintained at 5, 10, 15, and 20 ppm. The absorbance of Fe(III)-caffeic acid complex (Fig.4) increases with increasing the concentration of caffeic acid. Caffeic acid (blank) solution shows the λmax at 284 nm, whereas Fe(III)-caffeic acid complex show λmax at 321 nm, which confirm the complex formation. Similar results have been reported in the literature surface [Sánchez-Cortés *et.al.*, 2000; Belay, 2012] in the study of adsorption and chemical modification of phenols on a silver.

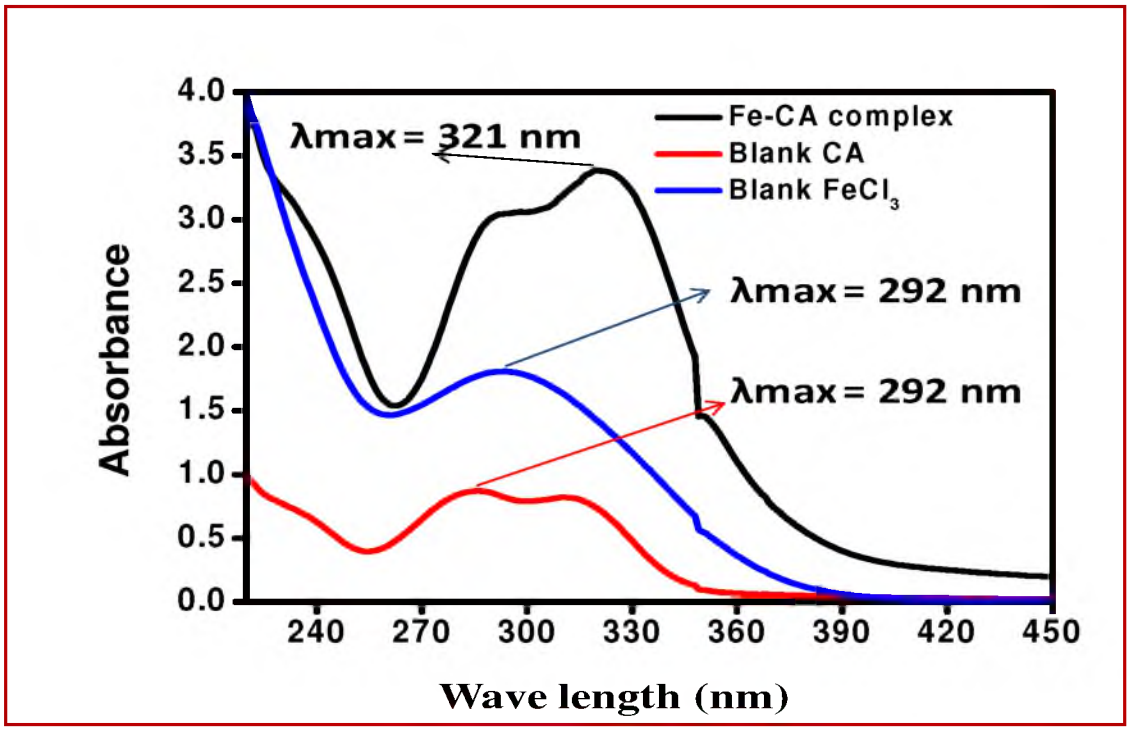


**Fig. 3.4. UV-Visible spectra of ferric chloride, caffeic acid and Fe(III)-caffeic acid complex**

### 3.2.4. FE-SEM spectra

The morphology of Fe(III)-caffeic complex was studied employing the FE-SEM images. The complex of Fe(III) and phenolic acid was prepared at room temperature in double distilled water. These precipitates were dried in rota vapour. Fig. 3.5 shows the surface morphology of complex which reveals that the surface is mesoporous in both cases. The mesoporous nature of complex arises due to the formation of the cavity during the chelation process. The free electron scanning microscopy data indicated that the typical diameter of the Fe(III)-complex powder was in the range of 50–250 nm having mesoporous texture. The morphology of complex did not look in particles form.

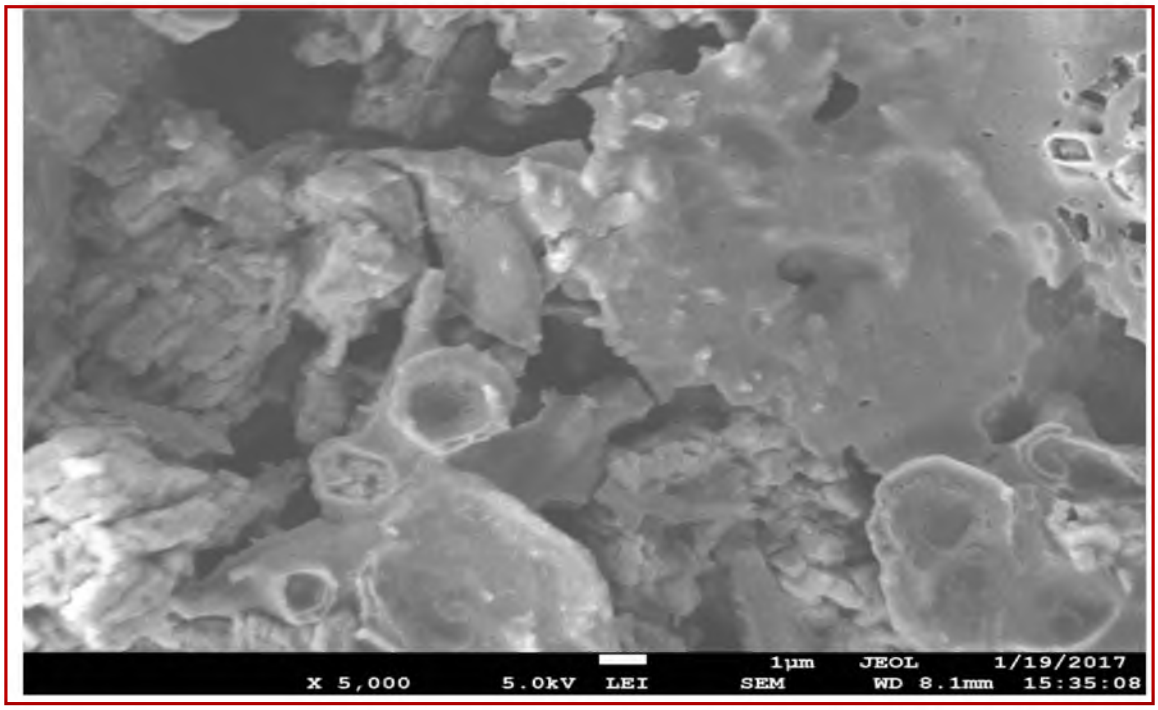


**Fig. 3.5. FE-SEM images of (a) Fe(III)-caffeic acid complex**

### 3.2.5. Gustatory properties

The dried compound was only slightly salty with no metallic taste whatever. It dissolved easily and completely in distilled water to give a dark brown solution at a concentration o.4%, the pH of which was 9.0. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 9.0.

### 3.2.6. pH stability

The stability of the Fe(III)-caffeic acid chelate was investigated at two stages; first, after the chelate had just been formed, i.e. before the precipitation stage, the chelate was found to be stable over the whole pH range (as judged by no formation of a precipitate). Second, the stability of isolated powdered chelate was investigated. A typical ferric hydroxide precipitate formed at about pH 9.0 as the pH was lowered from pH 10.0.

### 3.3.0. Kinetics of complex formation of Fe(III) and caffeic acid

The reaction was followed kinetically by a spectrophotometric method in aqueous solution at pH = 9.0. The initial results of the complex formation showed characteristic absorbance maxima at 321 nm for Fe(III)-caffeic acid complex. The complex formation was, therefore, followed by measuring the colour (absorbance λmax at 321 nm) at different interval of time on double beam spectrophotometer. The data on the development of colour was due to complexation of caffeic acid and Fe(III). To determine effects of each reactant on the observed reaction rate (k), the concentrations of caffeic acid and Fe(III) were independently varied. The increase in absorbance was monitored at the λmax of Fe(III)-caffeic acid complex over time. The slope of the best-fit line through the initial linear sections of the kinetics curves plotted for Fe(III)-caffeic acid complex is shown in Fig. 3.6. The value of the rate constant of Fe(III)-caffeic acid complex varied with a change in concentration over time was evaluated and such representative data are given in Table 3.1. The complexation reaction was found to be a first-order with rate constants for k (formation) $2.86\times10^{-2}$ $min^{-1}$. Hynes and O'Coinceanainn [2004 ] have studied the interaction of Fe(III) with caffeic acid and found a pseudo-first order kinetics with k = 2560 $M^{-1}.s^{-1}$ quite different from the first-order value found in the present work of 2.86 x $10^{-2}$ $min^{-1}$ .

### 3.3.1. Effect of concentration of ligands on rate constant

The data illustrates the dependence of complex formation on changing concentration. The reaction mixture of known concentration of caffeic acids and Fe(III) were used at varying concentrations (viz., 10 ppm 20 ppm & 30 ppm.) at different interval of time, Corresponding results are plotted in Fig. 3.7. The rate constant has been evaluated from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line.

### 3.3.2. Effect of temperature on rate constant

In the previous section, it has been shown that complexation of Fe(III)-caffeic acid obeyed first-order kinetic. In this section, the reported data illustrates the dependence of

complex formation on temperature. The reaction mixture of the known and fixed concentration of caffeic acids and Fe(III) was used at a different temperature, viz., 25°C, 35°C and 45°C and the development of colour at λmax 321 nm for Fe(III)-caffeic acid complex, was recorded at different intervals of time. The corresponding results are plotted in Fig. 3.8. The rate constant has been evaluated from the first order rate of reaction at different temperature suggesting that the equation fits the data in straight line. As shown in Fig. 3.6 the apparent activation energy (Ea) was evaluated employing Arrhenius equation and found to be150kcal/mol for Fe(III)-caffeic acid complex, which is reasonable for a complexation reaction. Similar results have also been reported on Fe(III) flavonoid quercetin complex reaction [Bukhari *et.al*., 2006].

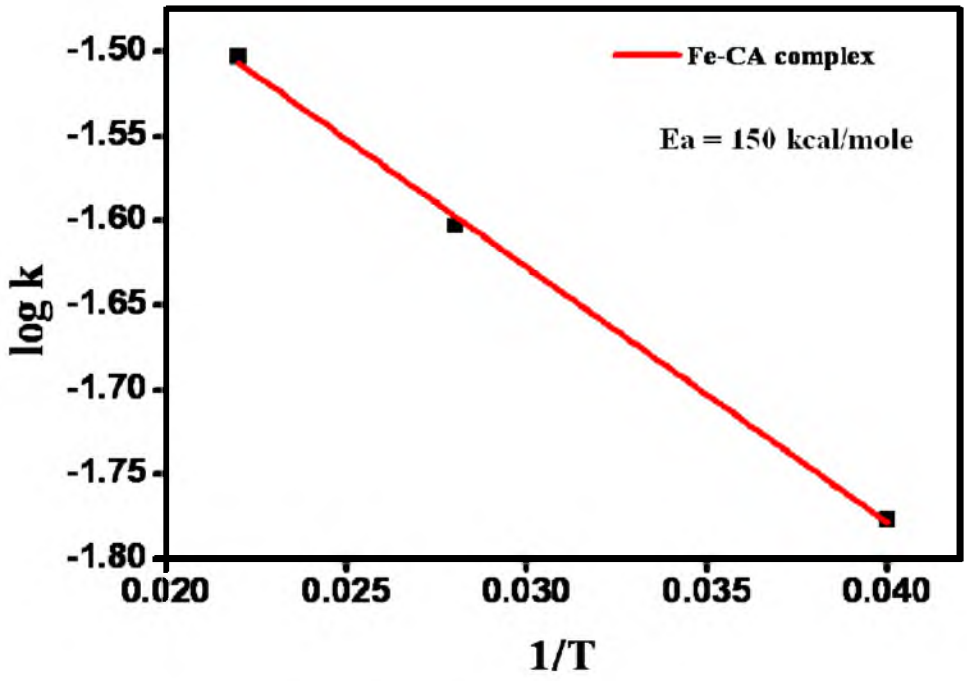


**Fig. 3.6. Arrhenius plots for Fe(III)-Caffeic complex**

### 3.4.0. Effect of various parameters on complex formation

### 3.4.1. Effect of concentration

Influence of increasing concentration of caffeic acid on the fixed concentration of Fe(III) with reference to colour formation was investigated. The absorbance of the complex solution of Fe(III)-caffeic acid was recorded at 321 nm, at different interval of time. When Fe(III) (constant concentration) reacts with variable concentration (5, 10, 15 and 20 ppm) of caffeic acid, its tendency to complex increases with increasing concentration of caffeic acid, the value of absorbance also increases. Such representative results are shown in Fig.3.7. Results obtained in the present case are consistent with earlier studies [Leopoldini *et.al.*, 2006].

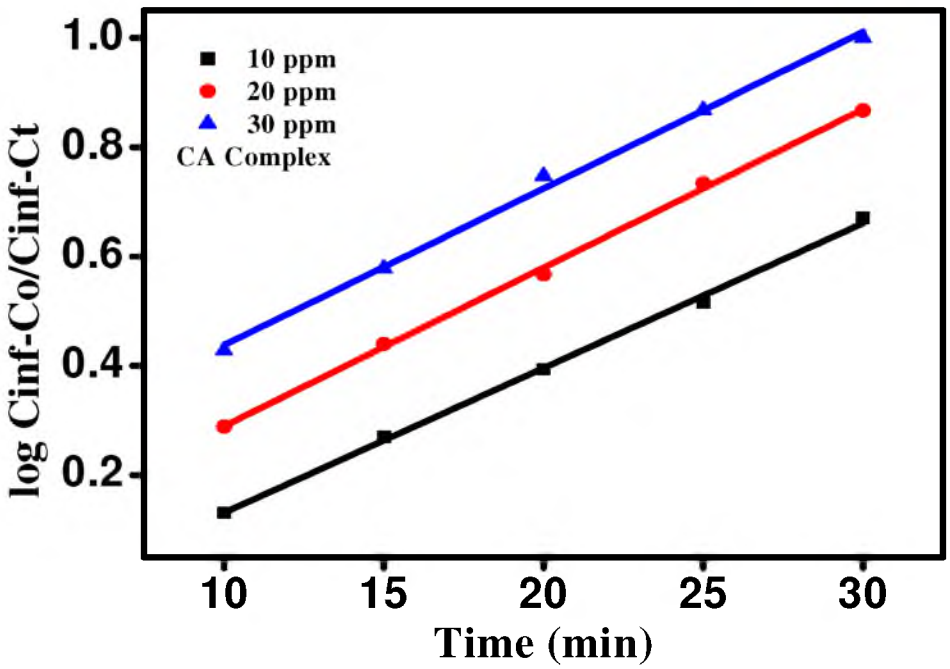


**Fig. 3.7. Plot illustrating the effect of change in concentration with time**

### 3.4.2. Effect of temperature on complexation of Fe(III) and caffeic acid

The development of colour Fe(III)-caffeic acid complex at 321 nm at different temperatures, viz., 25°C, 35°C, and 45°C have been shown in Fig. 3.11. It is observed that amount of colour produced in the reaction mixture, at a fixed temperature increased progressively with time. The colour reached a constant value after a large interval of time. Thus, in a reaction mixture of caffeic acid at different concentration (5, 10, 15 and 20 ppm) temperature (25°C, 35°C, and 45°C), the observation recorded that the curve tended to increases with increasing the temperature. These results increasing absorbance were recorded at the time of attaining the maximum value. It was evident from the above observation that absorbance increased with increasing concentration of caffeic acid. The increases were observed at all interval of time, till a constant value of absorbance at particular concentration was obtained. This trend continued even at the time of attaining a saturation value. Fig. 3.6 indicated the absorbance of complexation with a fixed concentration of Fe(III) and varying concentration of caffeic acid from 5-20 ppm at room temperature. This observation confirmed that absorbance increased with increasing concentration of caffeic acid. The nature of curves was similar to that at 25°C. The increase in absorbance was, however, more pronounced and that the time for achieving the saturation of colour value was also less as compared to that at 25°C. The colour development was, however, sharper at increased concentrations. The time for achieving maximum colour value also decreased successively. Fe(III)-caffeic acid complexation reaction (Fig. 3.11) depends on the temperature of the reaction. The absorbance also increases with increasing the temperature of the reaction which shows that the product formation of the reaction increases with increases the concentration of the reaction.

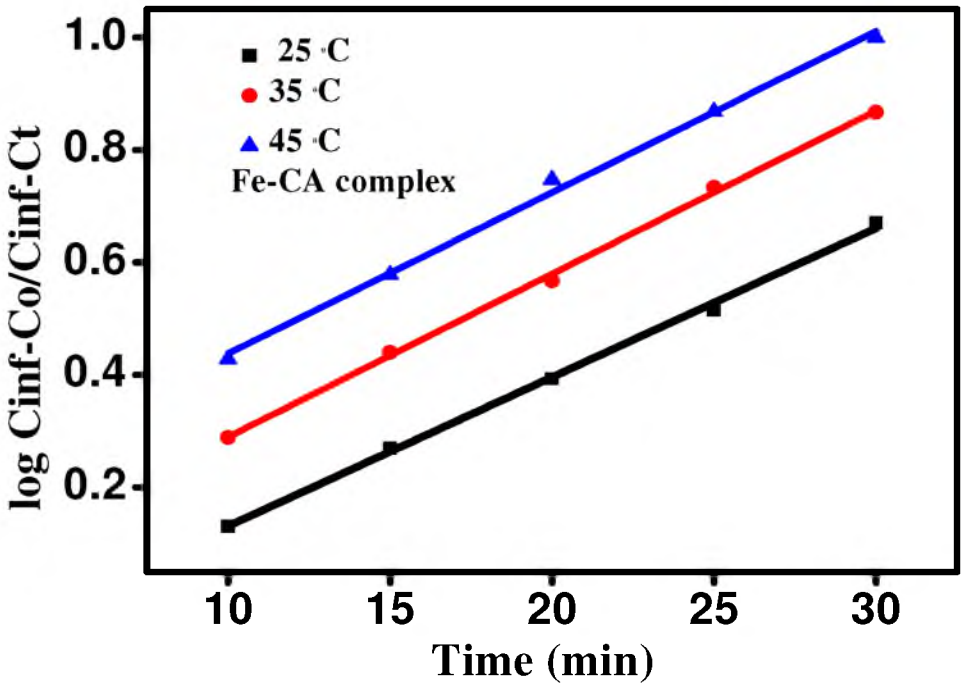


**Fig. 3.8. Plot illustrating the effect of temperatures on Fe(III)-caffeic acid complex formation**

### 3.5.0. DFT study of Fe(III) and caffeic acid complex

With the help of DFT method, various parameters of Fe(III) and caffeic acid complex was evaluated by Gaussian09 software.

### 3.5.1. Hardness

The hardness Fe(III) and caffeic complex was calculated by DFT method on the basis of their HOMO and LUMO and was found to be 0.04465.

### 3.5.2. Electronegativity

The electro negativity of Fe(III) and caffeic acid complex was calculated by DFT method on the basis of their HOMO and LUMO and was found to be 0.2130.

### 3.5.3. Softness

The softness of Fe(III) and caffeic acid complex molecule was calculated by DFT method on the basis of their HOMO and LUMO and was found to be 22.39.

### 3.5.4. Total energy

The total energy of Fe(III) and caffeic acid complex was calculated by DFT method on the basis of their HOMO and LUMO and was found to be $0.5201\times10^{-8}$ eV.

### 3.5.5. Dipole moment and point group symmetry

The dipole moment of Fe(III) and caffeic acid complex was calculated by DFT method and was found to be 15.13 Debye and point group symmetry was C1.

### 3.6.0. Conclusion

In this paper, the kinetic study on the complexation of Fe(III) with caffeic acid was performed in aqueous solution at pH 9.0. The complex formation between $Fe^{+3}$ and caffeic acid has been confirmed by electron spectroscopy. The Fe(III)-caffeic acid complex exhibit maximum absorbance $\lambda$max at 321 nm at which neither of ligands (blank) nor Fe(III) absorbs which give assurance for complex formation between Fe(III) and chosen antioxidant phenolic acid. The FT-IR spectra of Fe(III)-caffeic acid complex showed the alkenyl peaks C=C stretching vibrations at 1622.4 $cm^{-1}$ and at 1519.1 $cm^{-1}$ can be attributed to aromatic C=C stretching vibrations of the caffeic acid complex with Fe(III). The broad peak at 3394.1 $cm^{-1}$ can result from stretching vibration of -OH groups of caffeic acid. The FE-SEM images of Fe(III)-caffeic acid complex with Fe(III) demonstrated the mesoporous nature. Under the experimental conditions, the studied complexation reaction was found to follow first-order kinetics with rate constants for k (formation) $2.86\times10^{-2}$ $min^{-1}$. The extent of colour of the complex was found to increase with increasing the temperature suggesting that complex undergoes dissociation and different products are formed. The apparent activation energy of the complexation reaction was evaluated to be 150 kcal/mol. Various parameters of the complex such as hardness, electronegativity, softness, total energy, dipole moment and point group symmetry were calculated employing Density functional theory (DFT) and found as 0.04465, 0.2130, 22.39, $0.5201\times10^{-8}$ eV, 15.13 Debye and C1, respectively.

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# CHAPTER-4

## Kinetics of complex formation of Fe(III) with syringic acid: Experimental and theoretical study


**Abstract**

Kinetic study on the complexation of syringic acid with Fe(III) was performed in aqueous solution at pH 9.0. The complex was characterized with IR, UV-Vis spectroscopy and FE-SEM techniques. The complexation reaction was found to be a first-order with rate constants for $k_1$ (formation) $3.67\times10^{-2}$ $min^{-1}$. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 9.0. The apparent activation energy of the complexation reaction was evaluated to be 168 kcal/mol. Various parameters of the complex such as hardness, electronegativity, softness, total energy, dipole moment, chemical potential, electrophilicity index and point group symmetry were calculated and found as 0.153, 0.0.484, 6.52, $0.889\times10^{-9}$ eV, 11.03 Debye, -0.484, 0.764 and C1, respectively.

## 4.1. Introduction

Polyphenols are secondary plant metabolites that have been reported to have anti-mutagenic, anti-carcinogenic and antioxidant activities, but the mechanism involved in these activities are not completely understood. Phenolics are described as multifunctional antioxidants with chain-breaking and metal-chelating activities in the same molecule [Afanas'ev et.al.,1989; Harper et.al., 1996; Letan, 1996 ]. There are evidence that some polyphenols act as reactive oxygen species scavengers and as prooxidant metals (e.g. iron and copper) chelators and have thus the potential to modulate physiological reactions involving iron and other transition metals [Haslam, 1996]. Numerous physicochemical studies have dealt with metal complexes formed with polyphenols at equilibrium [Torreggiani et.al., 2005; Mourad Elhabiri *et.al.*, 2007], but very few kinetic studies have been undertaken [Hynes, 200]. Moreover, binding kinetics of Fe(III) to siderophores or biomimetic ligands have been up to now mainly studied under acidic conditions to avoid precipitation of hydroxoferric species [Albrecht-Gary, 1998; Crumbliss, 1999]. The dietary plant polyphenolic compounds were shown to have beneficial effects in preventing oxidative stress, inhibiting the production of free radicals and the formation of lipid peroxidation. Scientific interest in phenolic compounds has been stimulated because of their anti-inflammatory, antimutagenic, and anticarcinogenic properties. They have antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, free radical scavengers, metal chelators, and modulators of enzymatic activity, thereby preventing a lot of diseases, including diabetes mellitus, hypertension, atherosclerosis and cancer [Mattila, 2002]. The reason for the high polyphenols content of green tea is because green tea, unlike other teas, is steamed prior to drying. This inactivates the enzymes present in the tea leaves and inhibits the natural oxidation process, thus preventing the degradation of polyphenols. The neuroprotective effects of both black and green tea have recently been reported [Kumar *et.al.,* 2012]. Several studies of the kinetics and mechanisms of the interactions of naturally occurring catecholates with Fe(III) have been reported, the reactions involving a variety of different mechanisms [Paul Ryan, 2007]. The activity of the phenolic compounds as antioxidants in food systems depends not only on the structure (i.e., number and position of hydroxyl groups bound to the aromatic ring) and chemical reactivity of the phenolics but also on other factors such as their physical location, other food components, and environmental conditions, for example, pH [Ann-Dorit Moltk,

2008]. They reported that phenolic acids generally bind to Fe(III) with 3:1 stoichiometry, but exhibiting different metal affinities for Fe(III) depending on their structure. Moreover, stopped-flow in conjunction with UV/Vis spectrometry was used to determine the reaction kinetics of the phenolic acids with Fe(III) in the presence of citrate under the pseudo-first-order conditions. These thermodynamic and kinetic parameters can provide useful references for understanding the formation of iron-phenolic acids complexes in physiological conditions and food system, which are also beneficial to how to select phenolic acids as chelators for Fe(III) [Senpei Yang, 2014]. Phenolic compounds are predominately polar compounds and are expected to be in the water phase of an emulsion. However, the physical environment can change the partitioning of the phenolic compounds into the emulsifier pseudo phase of an emulsion [Stöckmann, 1999]. Kojic acid contains a specific siderophore structure (hydroxypyranone) which is able to sequester Fe(III) cations by coordinating through the carbonyl and phenolic hydroxyl oxygen atoms, forming a five-member chelate ring with relatively high stability [McBryde, 1961]. This paper reports the solution structure of the Fe(III) phenolic acids interactions. Because the metal ion coordination resulted in simultaneous deprotonation of the phenolic functions of the aromatic ring, the spectra of the formed metal species resembled those of the anions of the parent molecule, with a bathochromic shift due to the metal ion [Fiallo Marina et.al., 1999]. The kinetics and mechanism of Fe(III) with various polyphenolic compounds have been investigated [Hynes, 2004] and it has been reported that caffeic and chlorogenic acid are generally consistent with the formation of a 1:1 complex that subsequently decays through an electron transfer reaction. However, in the present case, an attempt has been made to corroborate the experimental findings by a theoretical study of the isolated complex. The objective of the present study, therefore, is to substantiate the mechanistic aspects of the interactions between Fe(III) and syringic acid employing experimental and theoretical study.

### 4.2.0. Experimental

### 4.2.1. Chemicals

Syringic acid (3, 4-Dihydroxycinnamic acid) was purchased from Molekula Ltd., United Kingdom. Ferric chloride was obtained from Fisher Scientific India (Fig. 4.1). All the reagents and solvents were of analytical grade and chemically pure and were used as received.

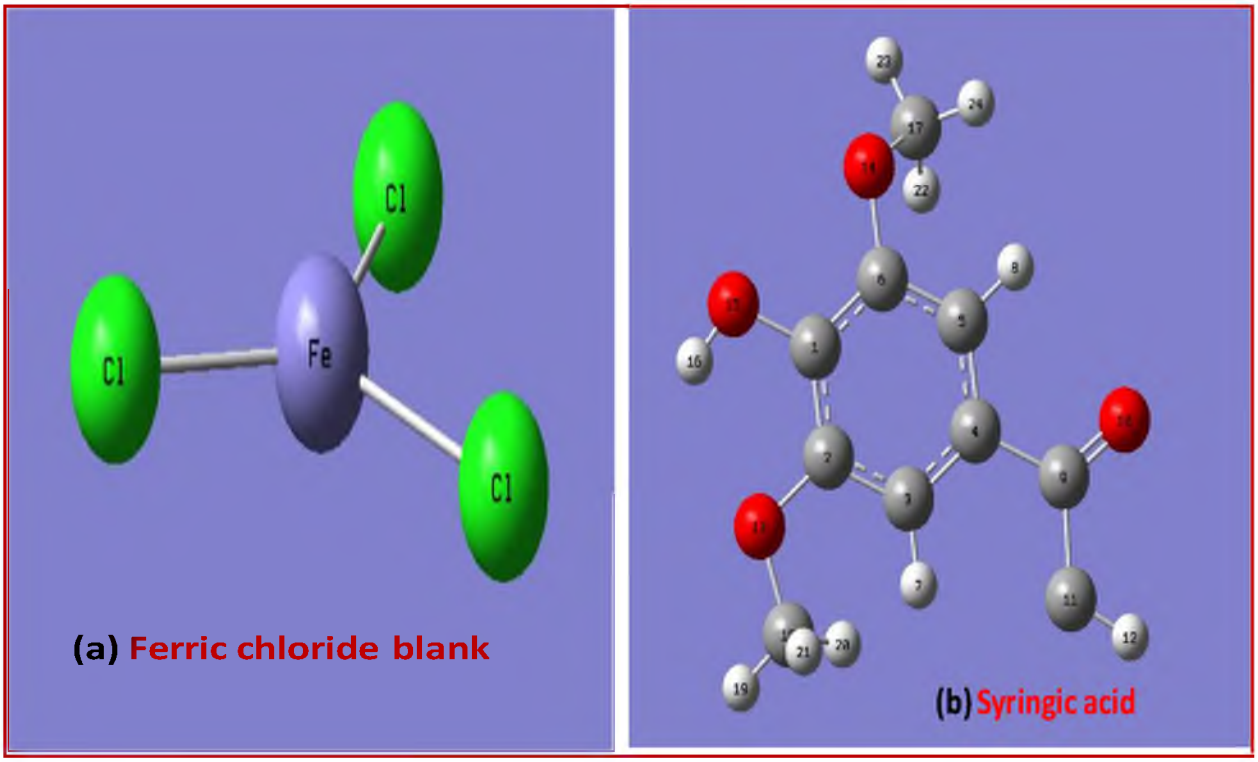


**Fig. 4.1. Molecular structure of (a) Ferric chloride (b) Syringic acid**

### 4.2.2. Synthesis of Fe(III)–syringic acid complex

All experiment was performed in aqueous solution at pH 9.0. A stock solution of Fe(III), syringic acid was prepared in (10 ppm) in 100 ml volumetric flask. Methanol (10 ml) was added to syringic acid stock solutions for complete dissolution. When equimolar concentration of Fe(III) reacts with syringic acid, pale yellow coloured complex was formed. After 30 minutes, the precipitate of syringic acid was formed at room temperature. The precipitates was filtered and dried for further analysis.

### 4.2.3. Analysis of the chelate

The iron content of the chelate and gustatory properties of the dried powder and aqueous solution of the isolated chelate (Fig.4.2) was considering prime importance; therefore analysis of the chelate was investigated.

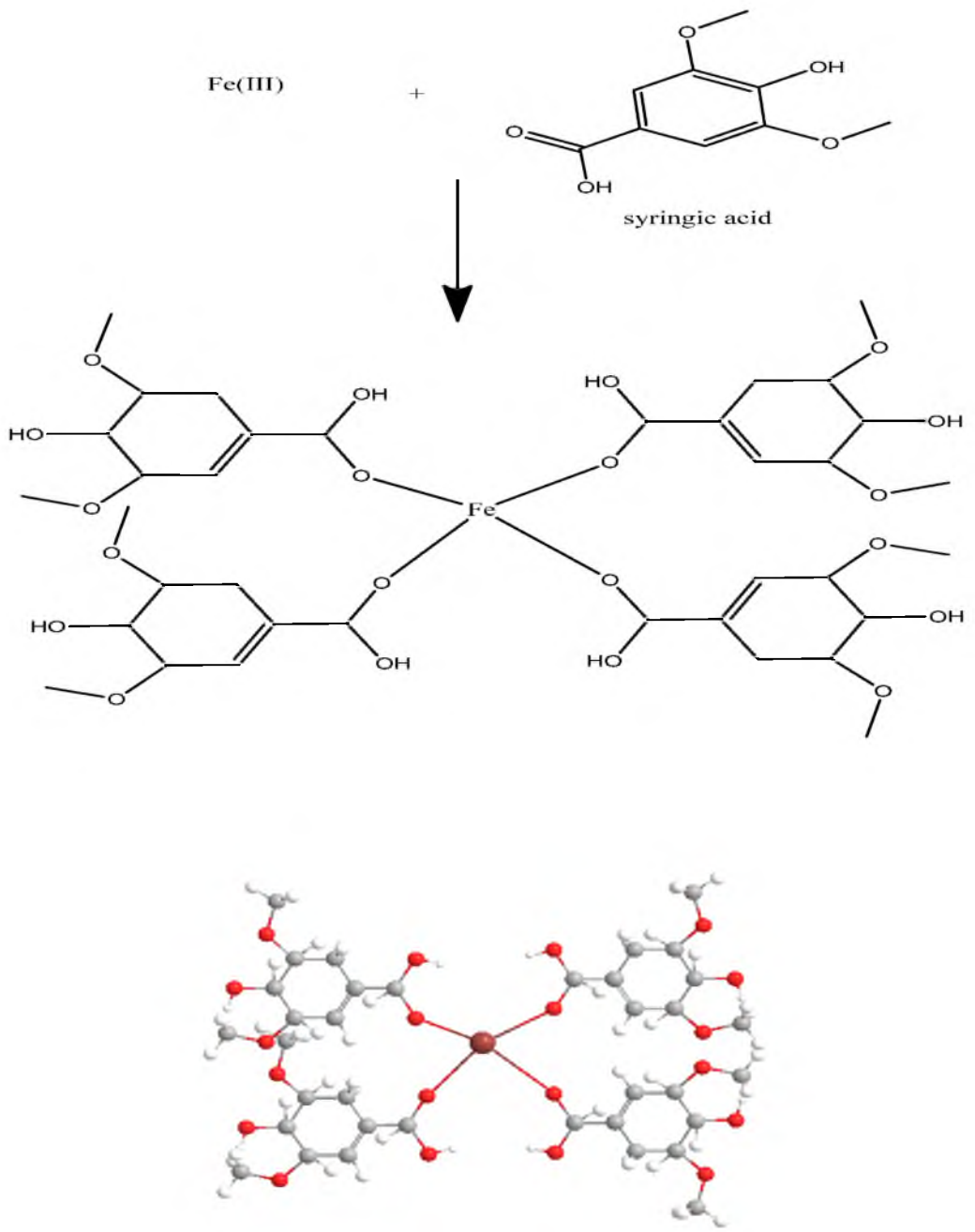


**Fig. 4.2. Images showing the reaction between Fe(III) and syringic acid**

### 4.2.4. Theoretical study

With the help of DFT method, various parameters of Fe(III) and syringic acid complex was evaluated employing Gaussian09 software.

### 4.2.5. Characterization

The absorbance and maximum wavelength of blank and complexes were recorded using UV–visible spectrophotometer (Carry 100 make). The functional group identification of Fe(III)-syringic acid complex was determined by Fourier Transform Infra-Red Spectrometer (FTIR) (Thermo-Scientific, Nicole 6700). The morphological analysis of Fe(III)-syringic acid complex was studied by Field Emission Scanning Electron Microscope (FE-SEM) employing JEOL JSM 7610F model.

### 4.3.0. Results and discussion

### 4.3.1. Fe(III)–syringic acid complex formation

The theoretical structure of Fe(III) and syringic acid was obtained from optimizing by DFT method in which Fe(III) 24 attacked on oxygen 11 atom due to the availability of lone pair. Each valency of Fe(III) 24 will attack to other oxygen 11 of the syringic acid molecule form a chelate product. In this complex, chelation of Fe(III) takes palace where syringic acid act as a ligand (Fig. 4.3).

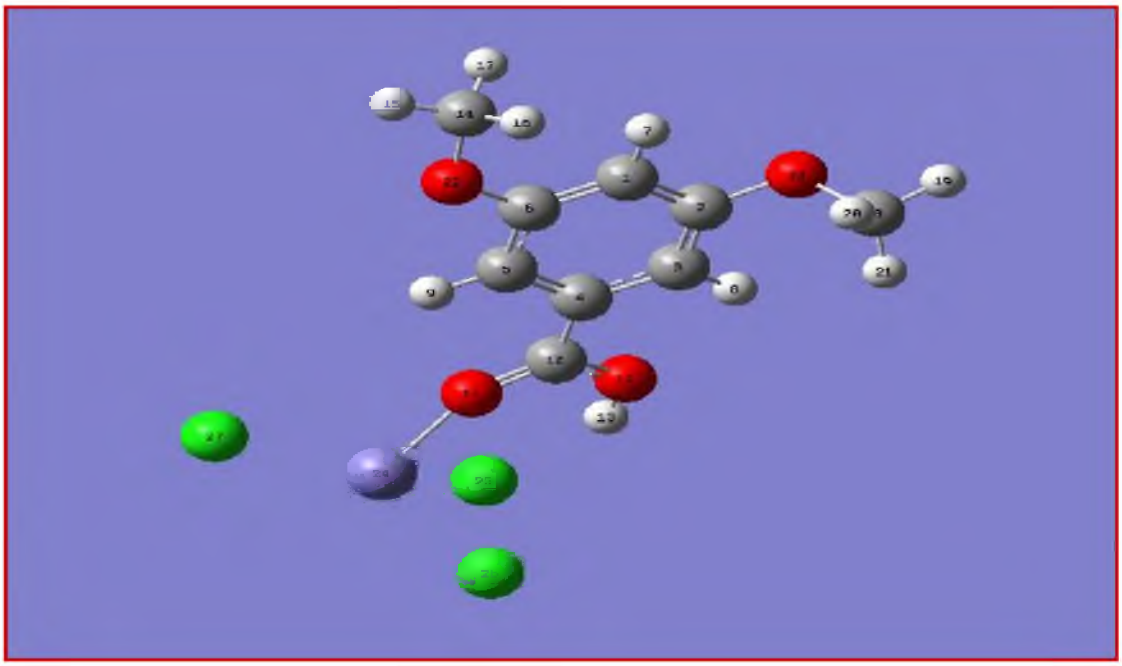

**Fig. 4.3. Image showing complex formation of Fe(III) and syringic acid by DFT method**

### 4.3.2. FT-IR spectra

The FT-IR spectra of Fe(III)-syringic acid complex shown in Fig. 4.4 confirmed the complex formation of Fe(III) with phenolic acid. Fig.1 illustrates that peaks were sharp in the complex as compared to syringic acid (blank). The absorptions at 1622.4 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1519.1 $cm^{-1}$ can be attributed to aromatic C=C stretching vibrations of syringic acid with Fe(III). The broad peak at 3394.1 $cm^{-1}$ can result from stretching vibration of -OH groups of syringic acid. Some peaks of the Fe(III) and syringic acid was disappeared and some of the new peaks of the complex were appeared, when the complex was formed.

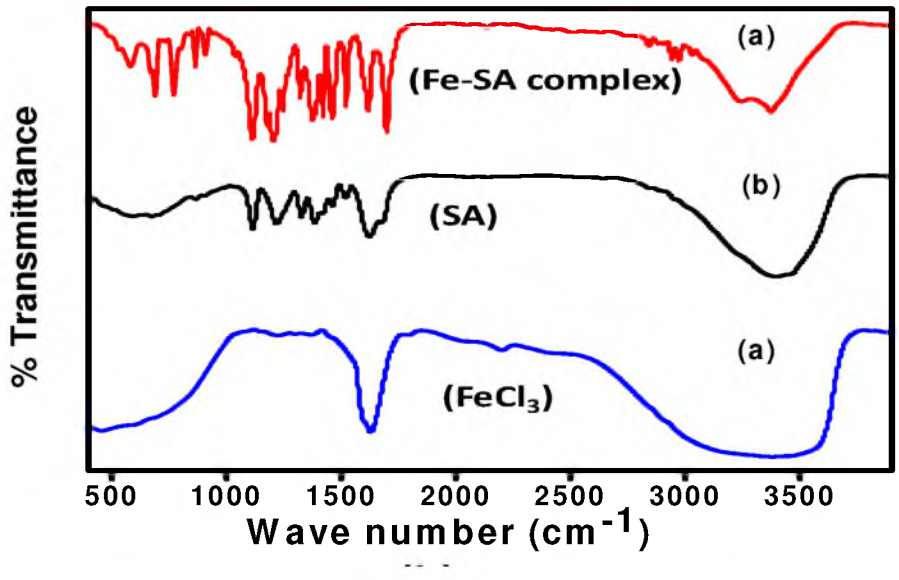


**Fig. 4.4. FT-IR spectra of (a) $FeCl_3$ (b) Syringic acid (c) Fe(III)-syringic acid complex**

### 4.3.3. UV-Visible spectra

Since absorbance maxima were not observed in the visible region, therefore, spectra were scanned in 250-450 nm range. The concentration ranges of Fe(III)-syringic acid complex was maintained at 5, 10, 15, and 20 ppm. The absorbance of Fe(III)-syringic acid complex (Fig.4) increases with increasing the concentration of syringic acid. Syringic acid (blank) shows the λmax at 260 nm, ferric chloride blank solution shows the λmax at 292 nm, whereas Fe(III)-syringic acid complex show λmax at 292.5 nm, which confirm the complex formation.

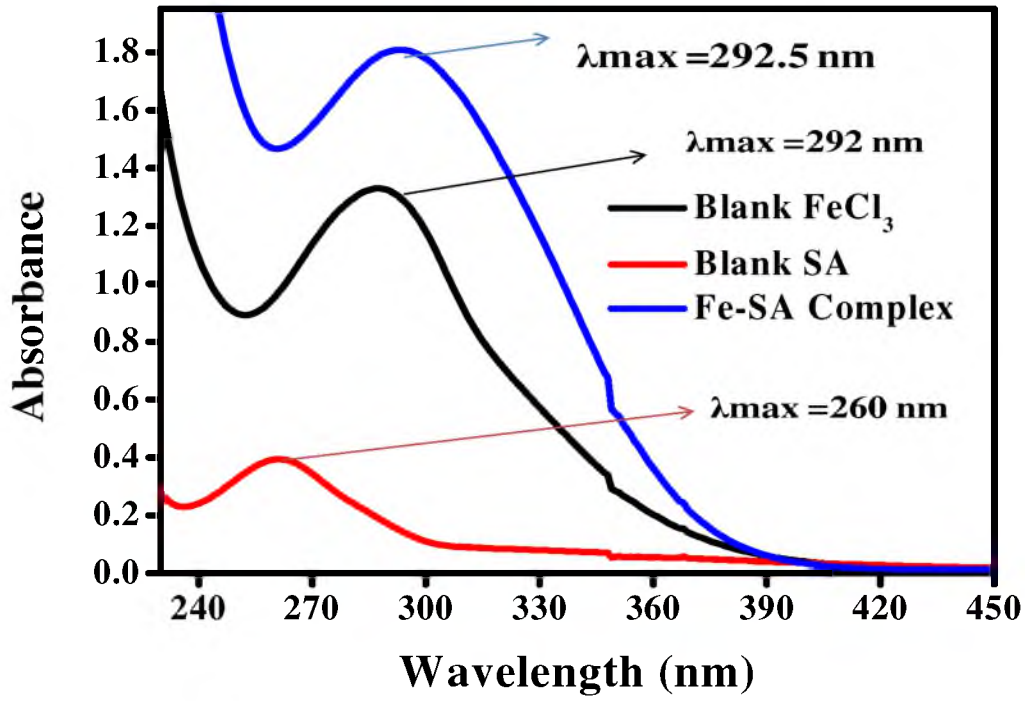


**Fig. 4.5. UV-Visible spectra of ferric chloride, syringic acid and Fe(III)-syringic acid complex**

### 4.3.4. FE-SEM spectra

The morphology of Fe(III)-syringic acid complex was studied employing the FE-SEM images. The complex of Fe(III) and phenolic acid was prepared at room temperature in double distilled water. These precipitates were dried by the rota vapour. Fig. 5 shows the surface morphology of complex which reveals that the surface is mesoporous in both cases. The mesoporous nature of complex arises due to the formation of the cavity during the chelation process. The free electron scanning microscopy data indicated that the typical diameter of the Fe(III)-syringic complex powder was in the range of 50–250 nm having mesoporous texture. The morphology of complex was not looks in particle form.

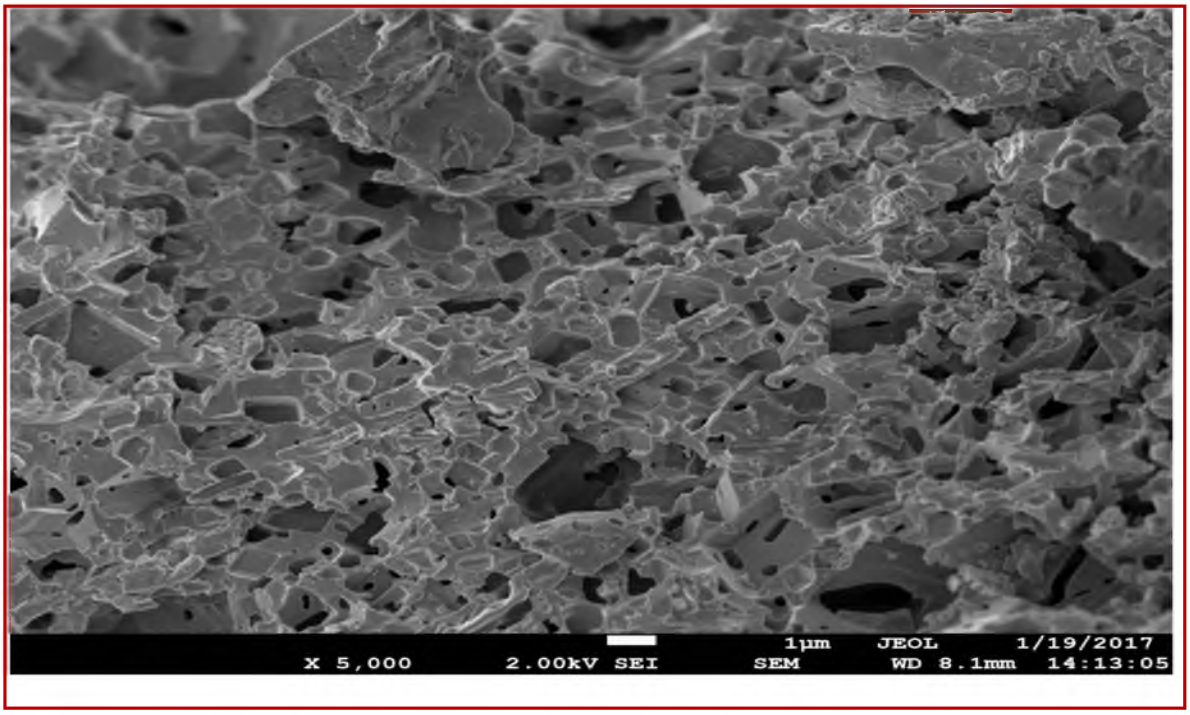


**Fig. 4.6. FESEM images of (a) Fe(III)-syringic acid complex**

### 4.3.5. Gustatory properties

The dried compound was only slightly salty with no metallic taste whatever. It dissolved easily and completely in distilled water to give a pale yellow solution at a concentration o.4%, the pH of which was 9.0. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 9.0.

### 4.3.6. pH stability

The stability of the iron/syringic acid chelate was investigated at two stages; first, after the chelate had just been formed, i.e. before the precipitation stage, the chelate was found to be stable over the whole pH range (as judged by no formation of a precipitate). Second, the stability of isolated powdered chelate was investigated. A typical ferric hydroxide precipitate formed at about pH 7.0 as the pH was lowered from pH 9.0.

### 4.3.7. Geometrical stability

The isolated complex of Fe(III) and syringic acid was formed in a tetrahedral geometry. The chelation by ligand with Fe(III) results chelate complex was stable in tetrahedral geometry due to less interaction in the ligands ring.

### 4.4.0. Kinetics of complex formation of Fe(III) and syringic acid

The reaction was followed kinetically by a spectrophotometric method in aqueous solution at pH 9.0. The initial results of the complex formation showed characteristic absorbance maxima at 292.5 nm for Fe(III)-syringic acid complex. The complex formation was, therefore, followed by measuring the colour (absorbance, λmax at 292.5 nm) at different interval of time on double beam spectrophotometer. The data on the development of colour was due to complexation of syringic acid and Fe(III). To determine effects of each reactant on the observed reaction rate (k), the concentrations of syringic acid and Fe(III) were independently varied. The increase in absorbance was monitored at the λmax of Fe(III)-syringic acid complex over time. The slope of the best-fit line through the initial linear sections of the kinetics curves plotted for Fe(III)-syringic acid complex is shown in Fig. 4.8. The value of rate constant of Fe(III)-syringic acid complex reaction varied with change in concentration over time was evaluated. The complexation reaction was found to be a first-order with rate constants for $k_1$ (formation) $3.67\times10^{-2}$ $min^{-1}$. Hynes and O'Coinceanainn [2004] have studied the interaction of Fe(III) with syringic acid and found a pseudo-first order kinetics with k = 2560 $M^{-1}.s^{-1}$ quite different from the first-order value found in the present work was $3.67 \times 10^{-2}$ $min^{-1}$ .

### 4.4.1. Effect of concentration on rate constant

The data illustrates the dependence of complex formation on changing concentration. The reaction mixture of known concentration of syringic acid and Fe(III) were used at varying concentrations (viz., 10 ppm 20 ppm and 30 ppm) at different interval of time. Corresponding results are plotted in Fig. 4.7. The rate constant has been evaluated from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line.

### 4.4.2. Effect of temperature on rate constant

In the previous section, it has been shown that complexation of Fe(III)-syringic acid obeyed first-order kinetic. In this section, the reported data illustrates the dependence of complex formation on temperature. The reaction mixture of the known and fixed concentration of syringic acid and Fe(III) were used at different temperatures, viz., 25°C, 35°C and 45°C and the development of colour at λmax 292.5 nm for Fe(III)-syringic acid complex, was recorded at different intervals of time. The corresponding results are plotted in Fig. 4.8. The rate constant has been evaluated from the first order rate of reaction at different temperatures suggesting that the equation fits the data in straight line. As shown in Fig. 4.8 the apparent activation energy (Ea) was evaluated employing Arrhenius equation and found to be 168 kcal/mol for Fe(III)-syringic acid complex, which is reasonable for a complexation reaction. Similar results have also been reported on Fe(III) flavonoid quercetin complex reaction [Leopoldini et.al., 2006].

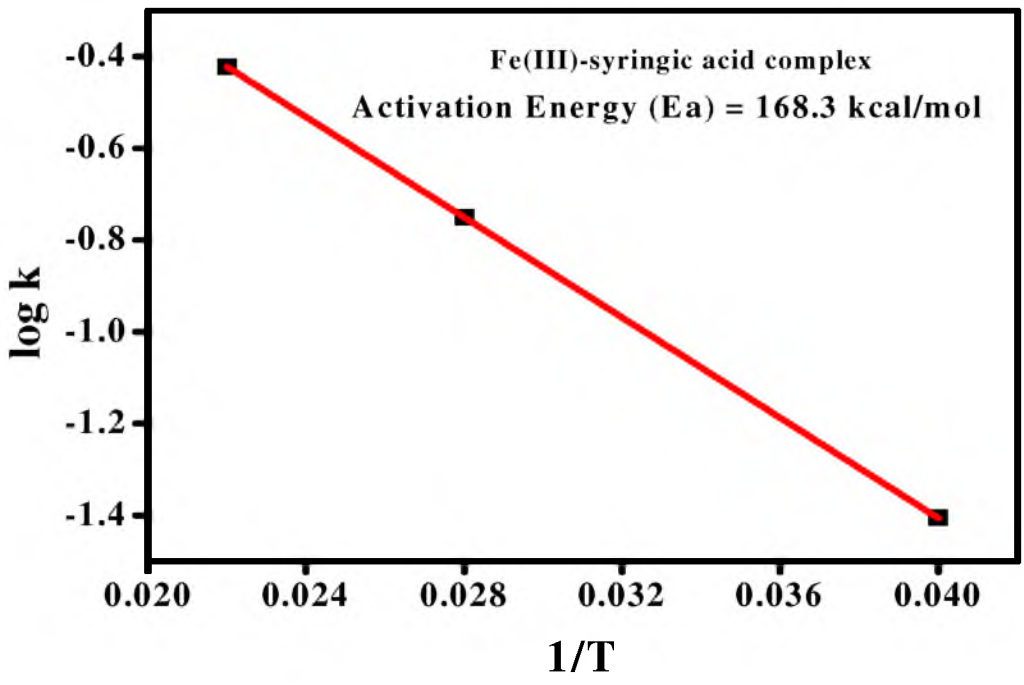


**Fig.4.7. Arrhenius plots for Fe(III)-syringic acid complex**

### 4.5.0. Effect of various parameters on complex formation

### 4.5.1. Effect of concentration

Influence of increasing concentration of syringic acid on the fixed concentration of Fe(III) with reference to colour formation was investigated. The absorbance of the complex solution of Fe(III)-syringic acid was recorded at 292.5 nm, at different interval of time. When Fe(III) (constant concentration) reacts with variable concentration (5, 10, 15 and 20 ppm) of syringic acid, its tendency to complex increases with increasing concentration of syringic acid, the value of absorbance also increases. Such representative results are shown in Fig. 4.8. Results obtained in the present case are consistent with earlier studies [Bukhari et.al., 2006].

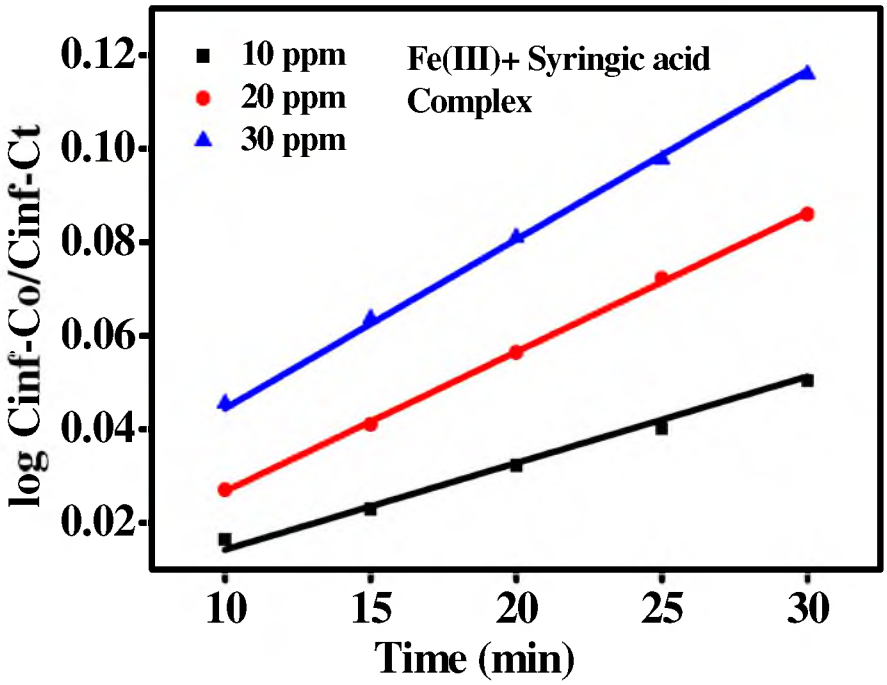


**Fig. 4.8. Plot illustrating effect of concentration of ligands on rate constant**

### 4.5.2. Effect of temperature on complexation of Fe(III) and syringic acid

The development of colour Fe(III)-syringic acid complex at 292.5 nm at different temperatures, viz., 25°C, 35°C and 45°C have been shown in Fig. 4.9. It is observed that amount of colour produced in the reaction mixture, at a fixed temperature

increased progressively with time. The colour reached a constant value after a large interval of time. Thus, in a reaction mixture of syringic acid at different concentration (5, 10, 15 and 20 ppm) and different temperature (25°C, 35°C and 45°C), the observation recorded that the curve tended to increases with increasing the temperature. These results increasing absorbance were recorded at the time of attaining the maximum value. It was evident from the above observation that absorbance increased with increasing concentration of syringic acid. The increases were observed at all interval of time, till a constant value of absorbance at particular concentration was obtained. This trend continued even at the time of attaining a saturation value. This observation confirmed that absorbance increased with increasing concentration of syringic acid. The nature of curves was similar to that at 25°C. The increase in absorbance was, however, more pronounced and that the time for achieving the saturation of colour value was also less as compared to that at 25°C. The colour development was, however, sharper at increased concentrations. Fe(III)-syringic acid complexation reaction (Fig. 4.9) depends on the temperature of the reaction. The absorbance also increases with increasing the temperature of the reaction which shows that the product formation of the reaction increases with increases the concentration of the reaction.

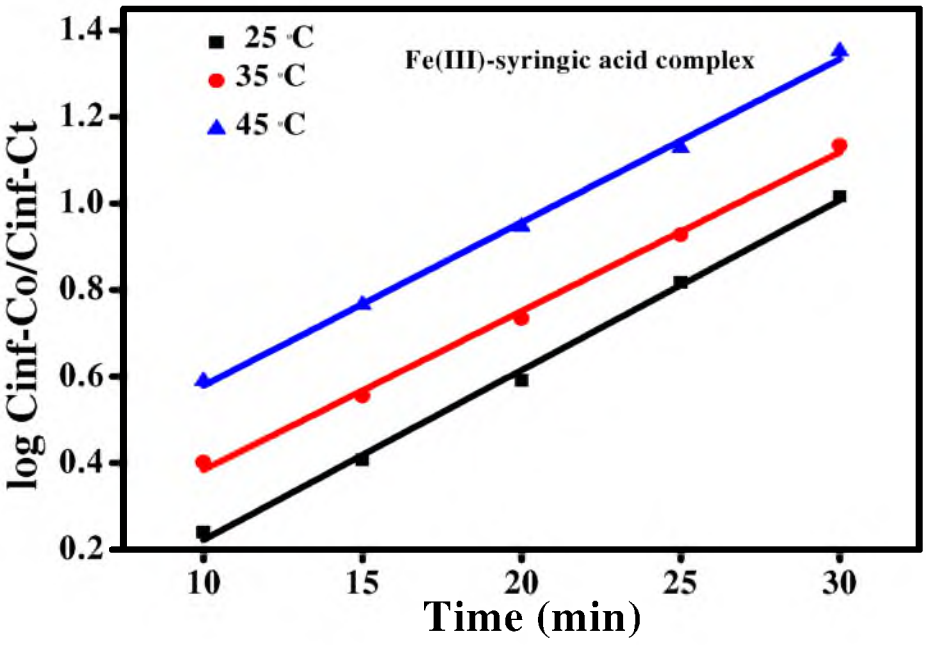


**Fig. 4.9. Plot illustrating the effect of temperature on Fe(III)-syringic acid complex formation**

### 4.6.0. DFT study of Fe(III) and syringic acid complex

With the help of DFT method, various parameters of Fe(III) and syringic acid complex was evaluated by Gaussian09 software in Table 4.1.

**Table 4.3. Showing different parameters of Fe(III)-syringic acid complex**

| S.No. | Name of parameters of complex | DFT values of complex |
|---|---|---|
| 1. | Total energy | $0.889\times10^{-9}$ eV |
| 2. | Dipole moment | 11.03 Debye |
| 3. | Hardness | 0.1532 |
| 4. | Softness | 6.52 |
| 5. | Electronegativity | 0.484 |
| 6. | Chemical potential | -0.484 |
| 7. | Electrophilicity Index | 0.7643 |
| 8. | Point group | C1 |

### 4.7.0. Conclusion

In this paper, the kinetic study on the complexation of Fe(III) with syringic acid was performed in aqueous solution at pH 9.0. The complex formation between Fe(III) and syringic acid has been confirmed by electron spectroscopy. The Fe(III)-syringic acid complex exhibit maximum absorbance ($\lambda$max) at 292.5 nm at which neither of ligand (blank) nor Fe(III) $^{absorbs}$ which give assurance for complex formation between Fe(III) and chosen antioxidant phenolic acid. The FT-IR spectra of Fe(III)-syringic acid complex showed the alkenyl peaks C=C stretching vibrations at 1622.4 $cm^{-1}$ and at 1519.1 $cm^{-1}$ can be attributed to aromatic C=C stretching vibrations of the syringic acid complex with Fe(III). The broad peak at 3394.1 $cm^{-1}$ can result from stretching vibration of -OH groups of syringic acid. The FE-SEM images of Fe(III)-syringic acid complex with Fe(III) demonstrated the mesoporous nature. Under the experimental conditions, the studied complexation reaction was found to follow the first-order kinetics with rate constants for $k_1$ (formation) $3.67\times10^{-2}$ $min^{-1}$. The value of absorbance of phenolic acids complexes was also increased with increasing the temperature suggesting that complex undergoes dissociation and different products are formed. The apparent activation energy of the complexation reaction was evaluated to be 168 kcal/mol. The DFT study of the

complex are shown chelate was stable in a tetrahedral geometry Various parameters of the complex such as hardness, electronegativity, softness, total energy, dipole moment, chemical potential, electrophilicity index and point group symmetry were calculated and found as 0.153, 0.0.484, 6.52, $0.889\times10^{-9}$ eV, 11.03 Debye, -0.484, 0.764 and C1, respectively.

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# CHAPTER-5

## Kinetics of complex formation of Fe(III) with chlorogenic acid: Experimental and theoretical study


**Abstract**

The kinetic study of the complexation of chlorogenic acid with ferric chloride was performed using UV–Vis absorption spectroscopy. The colour complexation reaction was found to be first-order with rate constants for k (formation) $2.4 \times 10^{-2}$ $min^{-1}$ for Fe(III)-chlorogenic acid complex. The gustatory property of the isolated complex was investigated and complex showed no metallic taste. The isolated complex was stable at pH 12.0. The isolated complex was stable at pH 12.0. The apparent activation energy of the complexation reaction was evaluated to be 0.085eV. The DFT study of Fe(III)-chlorogenic acid complex provided hardness (1.0121), electronegativity (0.456), softness (8.26), total energy ($0.106 \times 10^{-9}$ eV), dipole moment (16.62 Debye), chemical potential (-0.456), electrophilicity index (0.859) and point group symmetry (C1).

## 5.1. Introduction

Colour of sugar is the most important commercial feature and sugar mills are spending a lot of resources on the colour of their product to comply with market requirements. When someone says "colourant" means a material that causes colour. There is no colour of pure sucrose, but it appears colourful due to its involvement in lots of complex compounds. Therefore, the colourant is the common word that is used to cover various components which contribute to the colour of sugar. The complexity of sugars is very complex in nature and the volume is not easy to measure. Numerous processes have been developed over the years to achieve efficient and cost-effective removal of colour in order to produce low colour white sugars. Appreciation of the types of colour present in raw sugar is important when choosing an operating refinery processes, as different processes may remove different types of colour bodies. Therefore, combinations of processes are usually required to produce the best quality refined sugar. The colour of the produced sugar is a very important aspect of quality for the industry. The precursors and colourants, which contribute the colour to the sugar, possess two main sources: the generated ones in the sugar cane extracted in the juice (30%) and the coloured compounds formed in the production process (70%) [Lindeman, 2001]. The colour precursors are the substances that by itself contribute significantly to the colour of the juice; they are also substrates for the generation of colour in reactions within the process (enzymatic browning, caramelisation, maillard reactions, the formation of coloured complexes, oxidation). The amount and proportion of colour precursors (Enzymes, phenols, reducing sugars, amino acids, iron, polysaccharides, among others) and natural pigments in the sugar cane is not constant and varies with the variety and maturity of the cane, type and soil moisture, use of fertilizers, the way in which the cane has been harvested and even the use of pesticides and chemical ripeners [Legendre, 1988]. Likewise, the amount of extraneous matter included in the cane affects the colour and the concentration of the colour precursors [Larrahondo *et.al.*, 2009]. It has come to determine that, as part of the extraneous material, the leaves and tops are major contributors of natural colorants and color precursors to the process [Larrahondo, *et.al.*, 2009], an additional 1% of tops or leaves increase the total color of the juice in 4% and 15%, respectively, as which a significant amount of leaves and tops can have a determinant effect on the color, greater than the effect of the variety of the cane [Rein, 2012], as has been mentioned. Several studies have shown that beverages containing chlorogenic acid can inhibit the absorption

of non-haem iron in man by up to 60% [Hurrell, 1999]. Experimental studies demonstrated [Saija *et.al.*, 1995] that it possesses numerous beneficial effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, and antiallergic, antiviral, and anti-inflammatory properties. Many of these effects are correlated to the antioxidant capability that is due to the scavenging of free radicals species and to synergistic effects with enzymes and physiological antioxidants [Miller, 1996]. Metal-chelating compounds remove the metals and can alter their redox potentials rendering them inactive. Another antioxidant mechanism, not exhaustively studied, is based on the ability of some of these compounds to chelate transition metals ions (especially iron and copper), giving rise to stable complexes that, entrapping metals, prevent these from participating in free radicals generation [Amic *et.al.*, 1995]. Thus, there is emerging interest in the use of naturally occurring antioxidants for the preservation of foods and in the management of a number of pathophysiological conditions, most of which involve free radical damage [Soobrattee, 2005]. This paper reports the solution structure of the iron phenolic acids interactions. Because the metal ion coordination resulted in simultaneous deprotonation of the phenolic functions of the aromatic ring, the spectra of the formed metal species resembled those of the anions of the parent molecule, with a bathochromic shift due to the metal ion [Fiallo Marina *et.al.*, 1999]. Colourant may consider as coming from two basic sources: the cane plant and the process. Of the four general types of colourant, the phenolics and flavonoid class comes from the cane plant, where they exist as glycosides attached to sugar residue. Some phenolic are not coloured as they come from the plant, but oxidise or otherwise react (sometimes with amines) to form colourant during process. Other plantst colourants (anthrocanins, chlorophyll, carotenes) go in to cane juice, but are generally remove in factory clarification, although some chlorophyll-type compounds in raw sugars. Plant colourant tends to be charged, more so at high pH, and, if unreacted, are of low to medium molecular weight (the average molecular weight is considered to be about 5000). They also included a test for iron (the batho-phenanthroline spectrophotometric test) because of the importance of iron in colour formation [Margaret *et.al.*, 1986]. There is a general update that fast caramalisation catalysed by ion and relationship between polyphenols and uptake of iron is only hypothesis without experimental data on iron polyphenols interaction which can complement the above hypothesis that as and when polyphenols comes in contact with iron is accompanied by colour formation during sugar processing. In the present study chlorogenic acid was chosen as a representative selection

of polyphenols and its reaction with iron was investigated with emphasis as colour development.

### 5.2.0. Materials and Method

### 5.2.1. Chemicals

Chlorogenic acid (3-(3, 4-Dihydroxycinnamoyl) quinic acid -CA) was purchased from Molekula Ltd., United Kingdom. Ferric chloride was obtained from Fisher Scientific India. All the reagents and solvents were of analytical grade and chemically pure and were used as received.

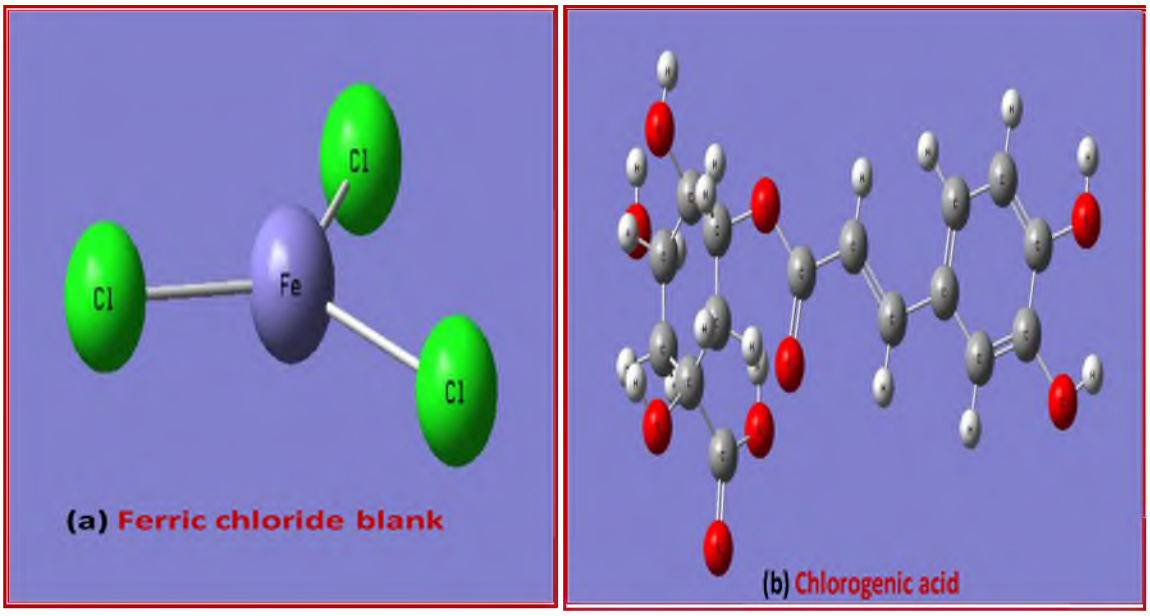


**Fig.5.1. Molecular structure of (a) Ferric chloride (b) Chlorogenic acid**

### 5.2.2. Synthesis of Fe(III)–chlorogenic acid complex

A stock solution of iron and chlorogenic acid were prepared (10 ppm) in 100 ml volumetric flask. Methanol (10 ml) was added to the chlorogenic acid stock solution for complete dissolution. When the equimolar concentration of iron reacts with the chlorogenic acid, dark green coloured complexes were formed (Fig. 5.2). After 30 minutes, the precipitates of chlorogenic acid were formed at room temperature. The precipitates were filtered and dried for further analysis.

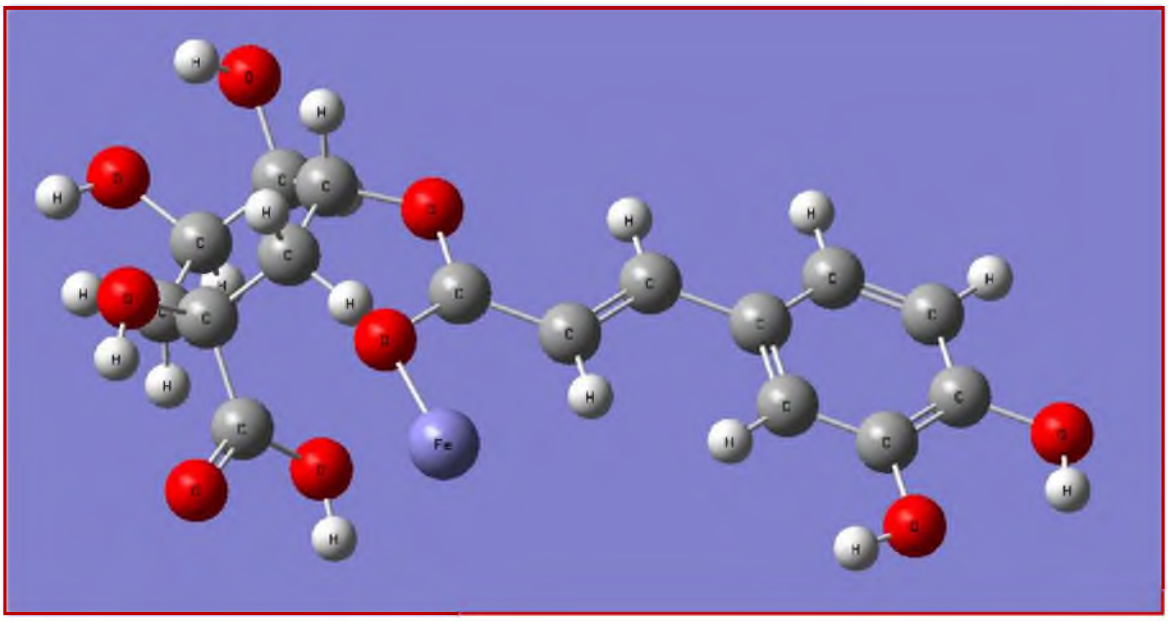


**Fig. 5.2. Showing complex formation between Fe(III) and chlorogenic acid**

### 5.2.3. Characterization

The absorbance and maximum wavelength of blank and complexes were recorded using UV–visible spectrophotometer (Carry 100 make). The functional group identification of iron-syringic/caffeic acid complexes was determined by Fourier Transform Infra-Red Spectrometer (FTIR) (Thermo-Scientific, Nicole 6700). The morphological analysis of iron-syringic/caffeic acid complexes was studied by Scanning Electron Microscope (SEM) by employing JEOL JSM 6490 model.

### 5.3.0. Results and discussion

### 5.3.1. FT-IR spectra

The FT-IR spectra of iron-chlorogenic acid complex shown in Fig.2 confirmed the complex formation of iron with chlorogenic acid. Fig.5.3 illustrates that peaks were sharp in the complex as compared to chlorogenic acid (blank) and iron (blank). The absorptions at 1687.7 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1517.08 $cm^{-1}$ can be attributed to aromatic C=C stretching vibrations of blank chlorogenic acid which were disappeared during complex formation. The broad peak at 3413.19 $cm^{-1}$ can result from stretching vibration of -OH groups of chlorogenic acid. Some peaks of Fe(III) and chlorogenic acid disappeared and some of the new peaks appeared, when the complex was formed.

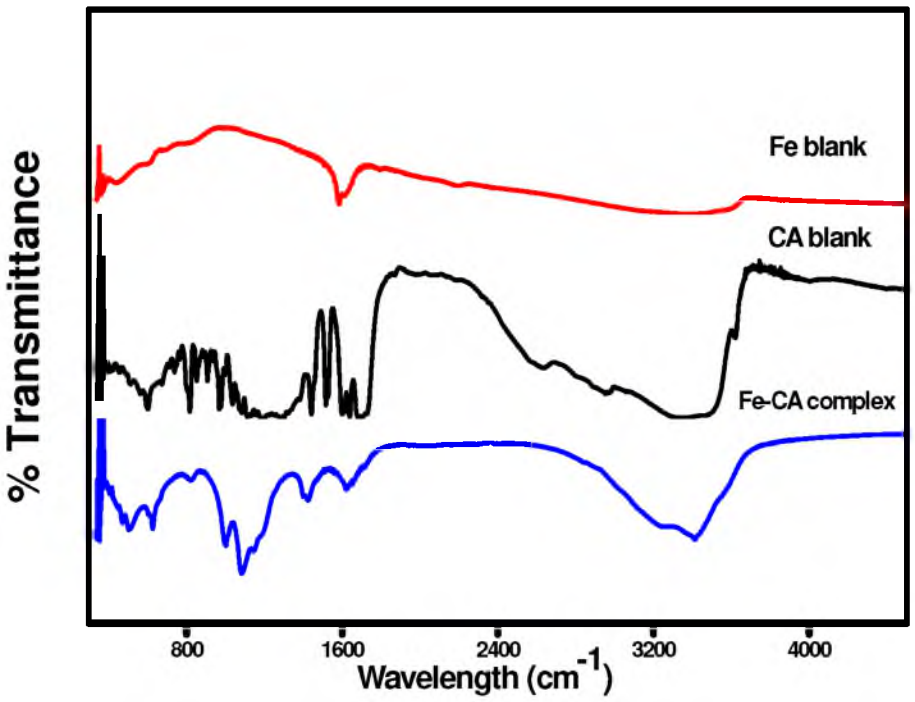


**Fig. 5.3. FT-IR spectra of Fe(III)-chlorogenic acid complex**

### 5.3.2. UV-Visible spectra

Since absorbance maxima were not observed in the visible region, therefore, spectra were scanned with 250-400 nm range. The concentration ranges of Fe(III)-chlorogenic acid complex were maintained at 4, 8, 12, 16 and 20 ppm. The absorbance of Fe(III)-chlorogenic acid complex (Fig. 5.4) increases with increasing the concentration of chlorogenic acid. Fe(III) and Fe(III)-chlorogenic acid (blank) solution shows the λmax at 292 and 323 nm, respectively whereas Fe(III)-chlorogenic acid complexes show λmax at 320 nm, which confirm the complex formation. The absorbance of Fe(III)-chlorogenic acid complex also increases with increasing the concentration of chlorogenic acid. Similar results have been reported in the literature surface [Sánchez-Cortés *et. al.*, 2000; Belay, 2012] in the study of adsorption and chemical modification of phenols on silver.

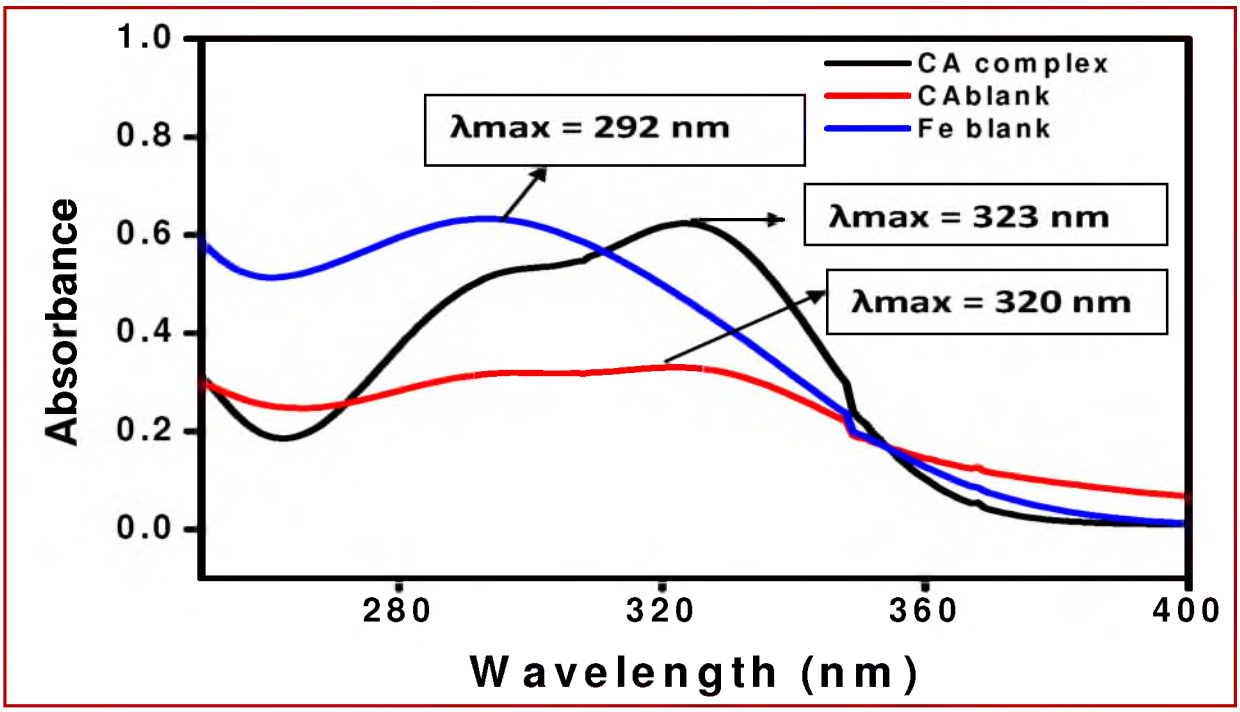


**Fig. 5.4. UV-Visible spectra of Fe(III)-chlorogenic acid complex**

### 5.3.3. SEM spectra

The morphology of Fe(III)-chlorogenic acid complex was studied employing the SEM images. The complex of Fe(III)-chlorogenic acid was prepared at room temperature in double distilled water. These precipitates were dried by the rota vapour. Fig.4 shows the surface morphology of complexes which reveals that the surface is mesoporous in both cases. The micro porous nature of complexes arises due to the formation of the cavity during the chelation process. The free electron scanning microscopy data indicated that the typical diameter of the iron-complex powder was in the range of less than 2 nm has micro porous texture. The morphology of complex did not reveal particles form.

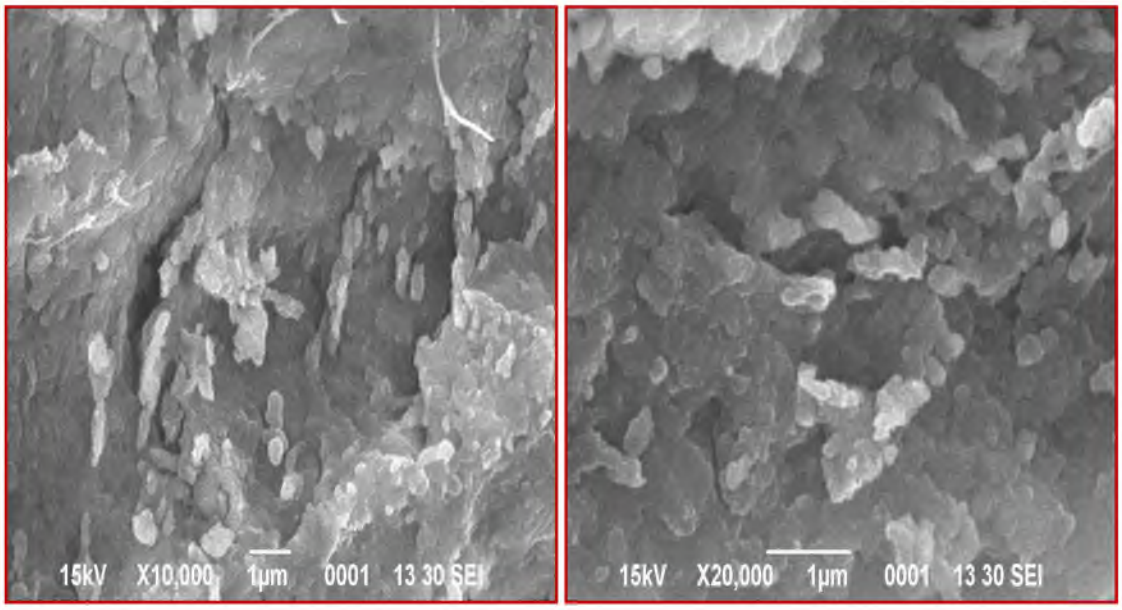


**Fig. 5.5. SEM images of Fe(III)-chlorogenic acid complex**

### 5.3.4. Gustatory properties

The dried compound of Fe(III)-chlorogenic acid was only slightly salty with no metallic taste whatever. It dissolved easily and completely in distilled water to give a dark yellow solution at a concentration o.4%, the pH of which was 12.0. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 12.0.

### 5.3.5. pH stability

The stability of the iron/ chlorogenic acid chelate was investigated at two stages; first, after the chelate had just been formed, i.e. before the precipitation stage, the chelate was found to be stable over the whole pH range (as judged by no formation of a precipitate). Second, the stability of isolated powdered chelate was investigated. A typical ferric hydroxide precipitate formed at about pH 7.0 as the pH was lowered from pH 12.0.

### 5.5.0. Kinetics of complex formation of chlorogenic acid and iron

The reaction was followed kinetically by the spectrophotometric method as described in this paper. The initial results of the complex formation showed characteristic

absorbance maxima at 320 nm for Fe(III)-chlorogenic acid complex. The complex formation was, therefore, followed by measuring the absorbance developed at 320 nm at a different interval of time on double beam spectrophotometer. The data on the development of colour was due to complexation of Fe(III)-chlorogenic acid. To determine effects of each reactant on the observed reaction rate (k), the concentrations of chlorogenic acid and Fe(III) were independently varied. The increases in absorbance were monitored at the $\lambda_{max}$ of Fe(III)-chlorogenic acid complex over time. The slope of the best-fit line through the initial linear sections of the kinetics curves plotted for Fe(III)-chlorogenic acid in Fig. 5.7. The value of the rate constant of Fe(III)-chlorogenic acid complex reaction varied with a change in concentration over time was evaluated and such representative data are given in Table 5.1. The kinetic curve shows that the value of rate constant increases with the change in concentration of phenolic acid with respect to time (Fig. 5.7).

### 5.5.1. Effect of concentration of rate of reaction

The data illustrates the dependence of complex formation on changing concentration. The reaction mixture of known concentration of chlorogenic acids and iron were used at varying concentrations (viz., 10 ppm. 20 ppm and 30 ppm.) at different interval of time. Corresponding results are plotted in Fig.5.7. The rate constant has been evaluated from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line.

### 5.5.2. Effect of temperature on rate of reaction

In the previous section, it has been shown that complexation of Fe(III)-chlorogenic acid obeyed first-order kinetic. In this section, the reported data illustrates the dependence of complex formation on temperature. The reaction mixture of the known and fixed concentration of chlorogenic acid and iron was used at a different temperature, viz., 25°C, 35°C and 45°C and the development of colour at 320 nm for Fe(III)-chlorogenic acid complex were recorded at different intervals of time. The corresponding results are plotted in Fig. 5.8. The rate constant has been evaluated from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line. As shown in Fig. 5.6, the apparent activation energy (Ea) was evaluated employing

Arrhenius equation, and found to 0.085 eV for Fe(III)-chlorogenic acid complexes, respectively which is reasonable for a complexation reaction. Similar results have also been reported on iron flavonoid quercetin complex reaction [Leopoldini *et.al.*, 2006].

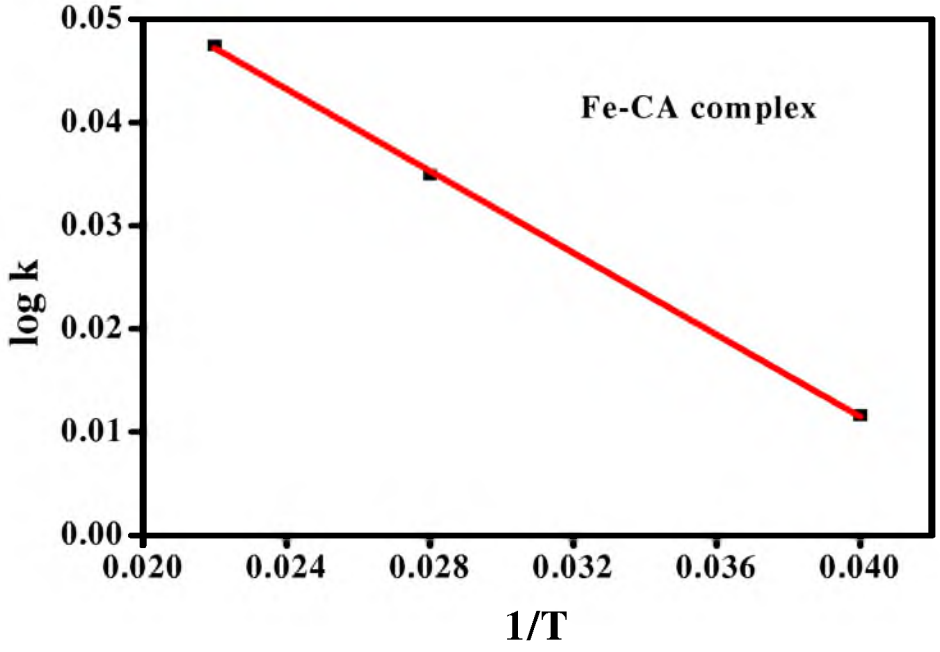


**Fig.5.6. Arrhenius plots for Fe(III)-chlorogenic acid complex**

### 5.4.0. Effect of various parameters on complex formation

### 5.4.1. Effect of concentration

Influence of increasing concentration chlorogenic acid on the fixed concentration of iron with reference to colour formation was investigated. The absorbance of the complex solution of Fe(III)-chlorogenic acid was recorded at 320 nm, at different interval of time. When iron (constant concentration) reacts with variable concentration (4, 8, 12, 16 and 20 ppm) of chlorogenic acid, its tendency to complex increases with increasing concentration of chlorogenic acid, the value of absorbance also increases. Such representative results are shown in Fig. 5.7. If we reverse the condition, as chlorogenic acid reacts with variable concentration of iron, the tendency of complex formation also increases with increasing concentration of iron and absorbance of the complex also increases [Bukhari *et.al.*, 2006].

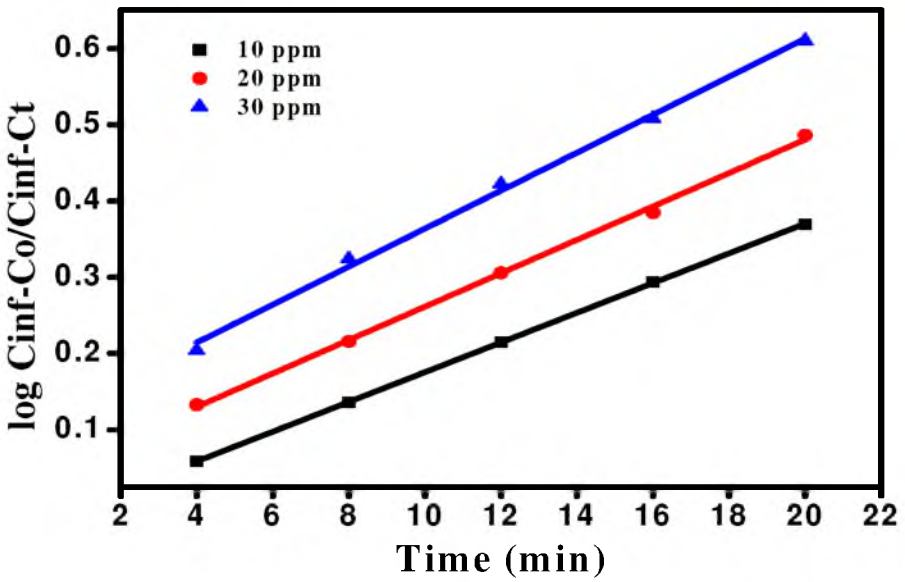


**Fig. 5.7. Plot illustrating the effect of change in concentration with time**

### 5.4.2. Effect of temperature on complexation of chlorogenic acid and iron

The development of colour Fe(III)-chlorogenic acid complex at 320 nm at a different temperature, viz., 25°C, 35°C and 45°C have been shown in Fig. 6. It is observed that amount of colour produced in the reaction mixture, at a fixed temperature increased progressively with time. The colour reached a constant value after a large interval of time. Thus, in a reaction mixture of chlorogenic acid at different concentration (4, 8, 12, 16 and 20 ppm) and temperature (25°C, 35°C and 45°C), the observation recorded that the curve tended to increases with increasing the temperature. These results increasing absorbance were recorded at the time of attaining the maximum value. It was evident from the above observation that absorbance increased with increasing concentration of chlorogenic acid. The increases were observed at all interval of time, till a constant value of absorbance at particular concentration was obtained. This trend continued even at the time of attaining a saturation value. The nature of curves was similar to that at 25°C. The increase in absorbance was, however, more pronounced and that the time for achieving the saturation of colour value was also less as compared to that at 25°C. Fe(III)-chlorogenic acid complexation reaction (Fig. 5.8) depends on the temperature of the reaction. The absorbance also increases with increasing the temperature of the reaction which shows that the product formation of the reaction increases with increases the concentration of the reaction.

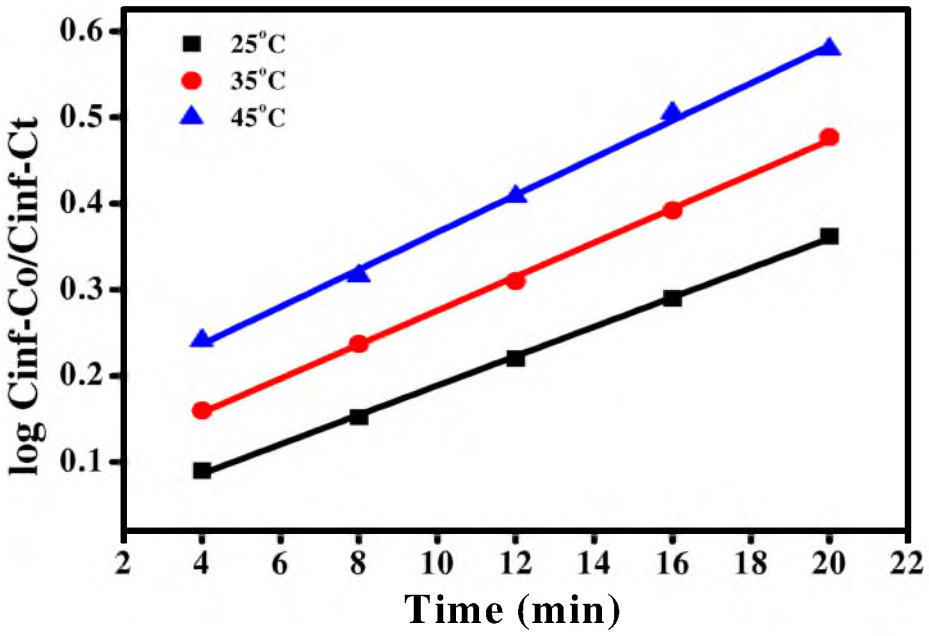


**Fig. 5.8. Plot illustrating the effect of change in temperature with time**

### 6.6.0. DFT study of Fe(III) and 4-Hydroxycoumarin complex

With the help of DFT method, various parameters of Fe(III) and the chlorogenic complex was evaluated by Gaussian09 software in Table 5.3.

**Table 5.3. Showing different parameters of Fe(III)-chlorogenic acid complex**

| S.No. | Name of parameters of complex | DFT values of complex |
|---|---|---|
| 1. | Total energy | $0.106\times10^{-9}$ eV |
| 2. | Dipole moment | 16.63 Debye |
| 3. | Hardness | 0.121 |
| 4. | Softness | 8.26 |
| 5. | Electronegativity | 0.456 |
| 6. | Chemical potential | -0.456 |
| 7. | Electrophilicity Index | 0.859 |
| 8. | Point group | C1 |

### 5.6.0. Conclusion

In this paper, the colour formation and the kinetic study on the complexation of chlorogenic acid with ferric chloride were performed using UV–Vis absorption spectroscopy. The colour complex formation between $Fe^{+3}$ and chlorogenic acid has been confirmed by UV-Visible spectroscopy. Fe(III)-chlorogenic acid complex exhibit maximum absorbance at 320 nm at which neither of ligands (blank) nor Fe(III) absorbs at this wavelength. The FT-IR spectra of Fe(III)-chlorogenic acid complex illustrated that peaks were sharp in complex as compared to chlorogenic acid (blank) and iron (blank). The absorptions at 1687.7 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1517.08 $cm^{-1}$ can be attributed to aromatic C=C stretching vibrations of blank chlorogenic acid which were disappeared during complex formation. The broad peak at 3413.19 $cm^{-1}$ can result from stretching vibration of -OH groups of chlorogenic. Some peaks of chlorogenic acid disappeared when complexes were formed. The SEM images of Fe(III)-chlorogenic acid complex with iron demonstrated the micro porous nature. The value of absorbance also increases with respect to time, after 24-hour saturation has been achieved. Under the experimental conditions, the complexation reaction was found to follow first-order kinetics with rate constants for k (formation) $2.4 \times 10^{-2}$ $min^{-1}$ for Fe(III)-chlorogenic acid complex. . The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 12.0. The isolated complex was stable at pH 12.0. The apparent activation energy of the complexation reaction was evaluated to be 0.085eV. The DFT study of Fe(III)-chlorogenic acid complex provided hardness (1.0121), electronegativity (0.456), softness (8.26), total energy ($0.106 \times 10^{-9}$ eV), dipole moment (16.62 Debye), chemical potential (-0.456), electrophilicity index (0.859) and point group symmetry (C1) were calculated

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# CHAPTER-6

## Kinetics of complex formation of Fe(III) with 4-Hydroxycoumarine: Experimental and theoretical study


**Abstract**

Kinetic study on the complexation of 4-Hydroxycoumarine with Fe(III) was described in aqueous solution at pH 11.0 together with a reappraisal of spectral evidence for chelate formation. The complexation reaction was found to be a first-order with rate constants for $k_1$ (formation) $4.35\times10^{-4}$ $min^{-1}$. Additionally the effect of concentration and temperature on the complexation reaction was investigated. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 11.0. The apparent activation energy of the complexation reaction was evaluated to be 181 kcal/mol. DFT study used for evaluation of various parameters of the studied complex showed hardness (0.153), electronegativity (0.484), softness (6.52), total energy ($0.889\times10^{-9}$ eV), dipole moment (11.03 Debye), chemical potential (-0.484), electrophilicity index (0.764) and point group symmetry (C1).

### 6.1.0. Introduction

The biological importance of coumarin derivatives as anticoagulants, aflatoxins, mycotoxins and antibiotics has led to a considerable amount of synthetic work in the field of coumarin for their pharmacological evaluation. 4-Hydroxycoumarin (4-hydroxy-2H-1- benzopyran-2-one) (HDC) is used in the synthesis of pharmaceuticals especially for anticoagulants. It is used in the manufacturing fluorescent dyes and rodenticides. The 4-hydroxycoumarin derivative, mercamour, is a long oral anticoagulant activity is in the duration of 48-72 hours [Nanndibewoor, 2009]. The hydroxycoumarins are typical phenolic compounds which act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The coumarins display a remarkable array of biochemical and pharmacological actions. Out of some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems. Coumarin (1,2-benzopyrone) [Traykova, 2005] , the parent molecule of coumarin derivatives, is the simplest compound of a large class of naturally occurring phenolic substances made of fused benzene and -pyrone rings. The investigation of coumarin compounds revealed that a wide spectrum of medicinal plant extracts that were in use as early as 1000 A.D., which contained a high content of coumarins. Coumarin derivatives have chelating characteristics have long been observed and the bacteriostatic activity seems to be due to chelation. These metal complexes are found to be interesting due to their biological applications activity. Some chalcones derived from coumarin derivatives, possess significant antimicrobial activity [Vyas, 2009]. The transition metal ions have good capacity to form coordination compounds with O, N donor ligands which are able to donate an electron pair. Some of the coumarins show distinct physiological, photodynamic and bacteriostatic activities and placed for many diverse uses. Their chelating characteristics have long been observed and the bacteriostatic activity seems to be due to chelation. The physicochemical studies of the coumarins with the chelating group at appropriate position and their metal complexes reveal that the ligand can be used as potential analytical reagents [Vyas *et.al.*, 2009]. Iron is an essential element participating in many vital processes. However, in the organism, it has to be firmly stored in proteins and its metabolism has to be meticulously regulated due to possible participation of free iron in reactive oxygen species (ROS) generation [Halliwell, 1999; Mladenka *et.al.*, 2005]. Iron homeostasis is deranged during some cardiovascular pathologies, in particular, there is a substantial drop in pH below 6 with

the release of free iron with known ROS consequences in acute myocardial infarction [Ambrosio *et.al.*, 1987; Chevion *et.al.*, 1983 ]. They contribute to colour formation of juice when sugar cane is crushed and are also involved in the changes that take place during the processing of sugarcane for production of raw sugar [Paton, 1992; Bucheli, 1994]. Several studies have shown that beverages containing chlorogenic acid can inhibit the absorption of non-haem Fe(III) in man by up to 60% [Hurrell *et.al.*, 1999]. Experimental studies demonstrated [Saija *et.al.*, 1995] that it possesses numerous beneficial effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, and antiallergic, antiviral, and anti-inflammatory properties. Many of these effects are correlated to the antioxidant capability that is due to the scavenging of free radicals species and to synergistic effects with enzymes and physiological antioxidants[Miller, 1996 ]. Metal-chelating compounds remove the metals and can alter their redox potentials rendering them inactive. Another antioxidant mechanism, not exhaustively studied, is based on the ability of some of these compounds to chelate transition metals ions (especially Fe(III) and copper), giving rise to stable complexes that, entrapping metals, prevent these from participating in free radicals generation [Amic *et.al.*, 1995]. Thus, there is emerging interest in the use of naturally occurring antioxidants for the preservation of foods and in the management of a number of pathophysiological conditions, most of which involve free radical damage [Soobrattee, 2005]. This paper reports the solution structure of the Fe(III) phenolic acids interactions. Because the metal ion coordination resulted in simultaneous deprotonation of the phenolic functions of the aromatic ring, the spectra of the formed metal species resembled those of the anions of the parent molecule, with a bathochromic shift due to the metal ion [Fiallo *et.al.*, 1999]. The kinetics and mechanism of Fe(III) with various polyphenolic compounds have been investigated [Hynes, 2004] and it has been reported that caffeic and chlorogenic acid are generally consistent with the formation of a 1:1 complex that subsequently decays through an electron transfer reaction. However, in the present case, attempt has been made to corroborate the experimental findings by theoretical study of the isolated complex. Objective of the present study, therefore, is to substantiate the mechanistic aspects of the interactions between Fe(III) and 4-hydroxycoumarin employing experimental and theoretical study.

### 6.2.0. Experimental

### 6.2.1. Chemicals

4-Hydroxycoumarin was purchased from Himidea Ltd., India. Ferric chloride was obtained from Fisher Scientific India (Fig. 6.1). All the reagents and solvents were of analytical grade and chemically pure and were used as received.

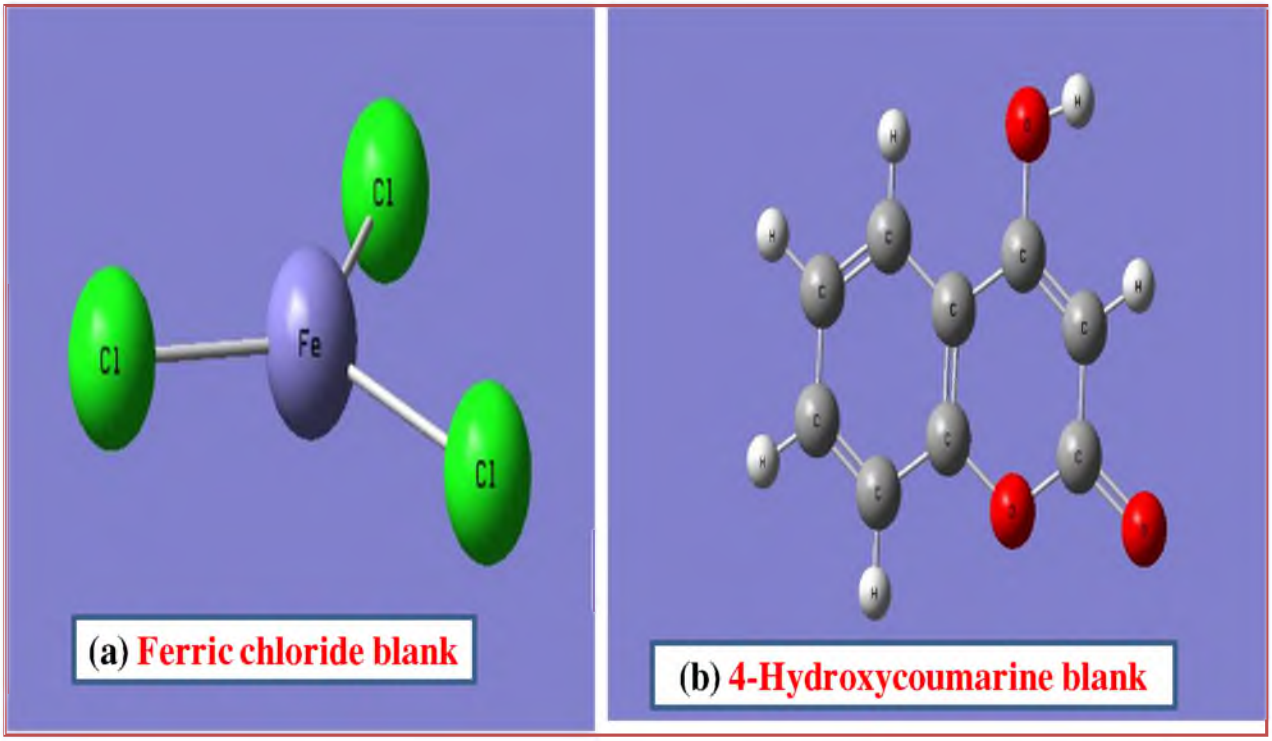


**Fig. 6.1. Molecular structure of (a) Ferric chloride (b) 4-Hydroxycoumarin**

### 6.2.2. Synthesis of Fe(III)–4-hydroxycoumarin complex

All experiment was performed in aqueous solution at pH 11.0. A stock solution of Fe(III), caffeic were prepared in (10 ppm) in 100 ml volumetric flask. Ethanol (10 ml) was added to 4-hydroxycoumarin stock solutions for complete dissolution. When the equimolar concentration of Fe(III) reacts with 4-hydroxycoumarin, the dark yellow coloured complex was formed. After 45 minutes, the precipitate of 4-hydroxycoumarin was formed at room temperature. The precipitates was filtered and dried for further analysis.

### 6.2.3. Analysis of the chelate

The iron content of the chelate and gustatory properties of the dried powder and aqueous solution of the isolated chelate were considering prime importance, therefore analysis of the chelate ( Fig. 6.2) was investigated.

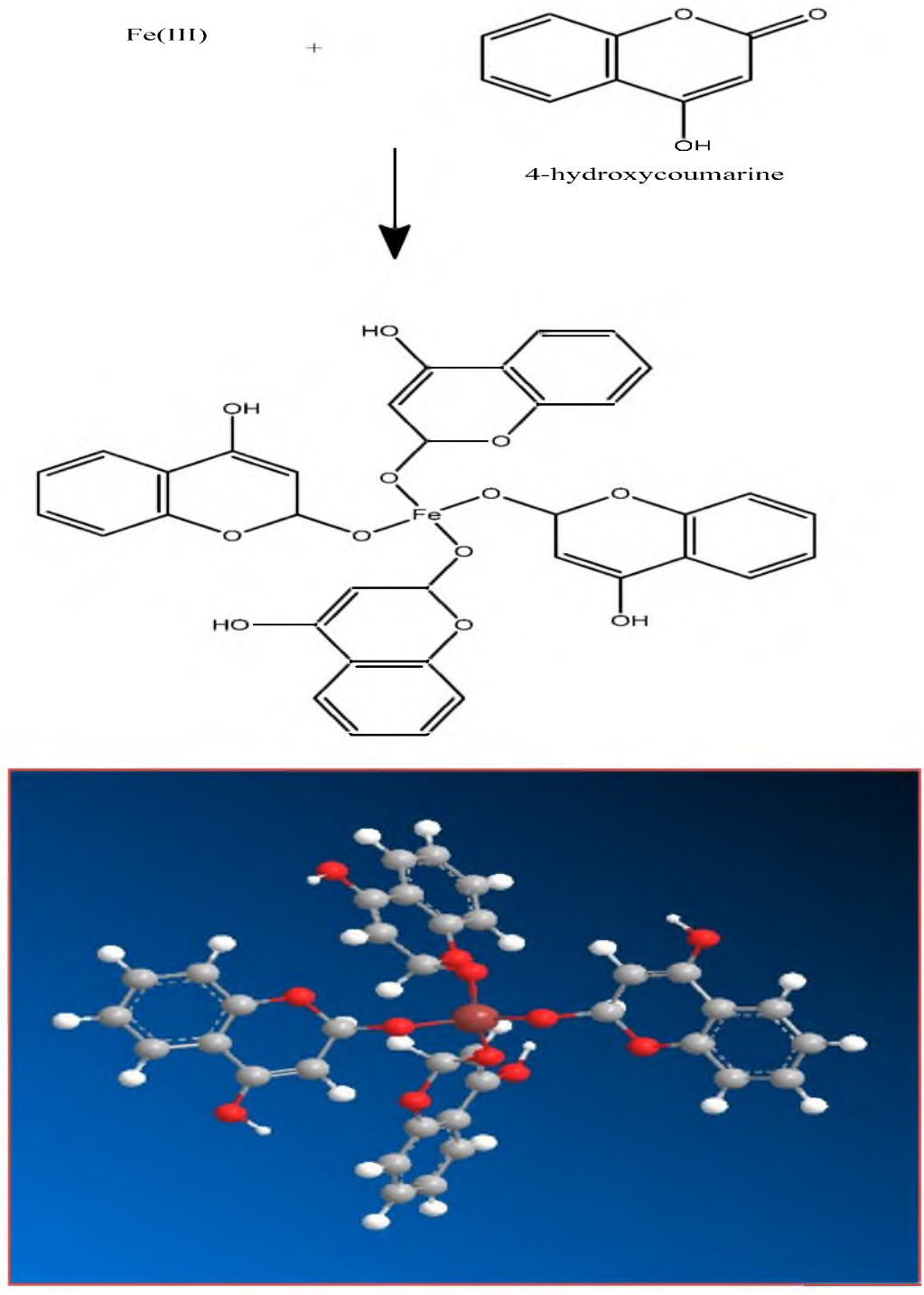


**Fig. 6.2. Showing the reaction between Fe(III) and 4-hydroxycoumarin**

### 6.2.4. Theoretical study

With the help of DFT method, various parameters of Fe(III) and the 4-hydroxycoumarin complex was evaluated employing Gaussian09 software.

### 6.2.5. Characterization

The absorbance and maximum wavelength of blank and complexes were recorded using UV–visible spectrophotometer (Carry 100 make). The functional group identification of Fe(III)-4-hydroxycoumarin complex was determined by Fourier Transform Infra-Red Spectrometer (FTIR) (Thermo-Scientific, Nicole 6700). The morphological analysis of Fe(III)-4-hydroxycoumarin complex was studied by Scanning Electron Microscope (SEM) by employing JEOL JSM 7610F model.

### 6.3.0. Results and discussion

### 6.3.1. Fe(III)–4-hydroxycoumarin complex formation

The theoretical structure of Fe(III) and 4-hydroxycoumarin were obtained from optimizing by DFT method in which Fe(III) attacked on oxygen 16 atom due to the availability of pi-electron. Each valency of Fe(III) will attack to oxygen 16 of the 4-hydroxycoumarin (Fig. 6.3).

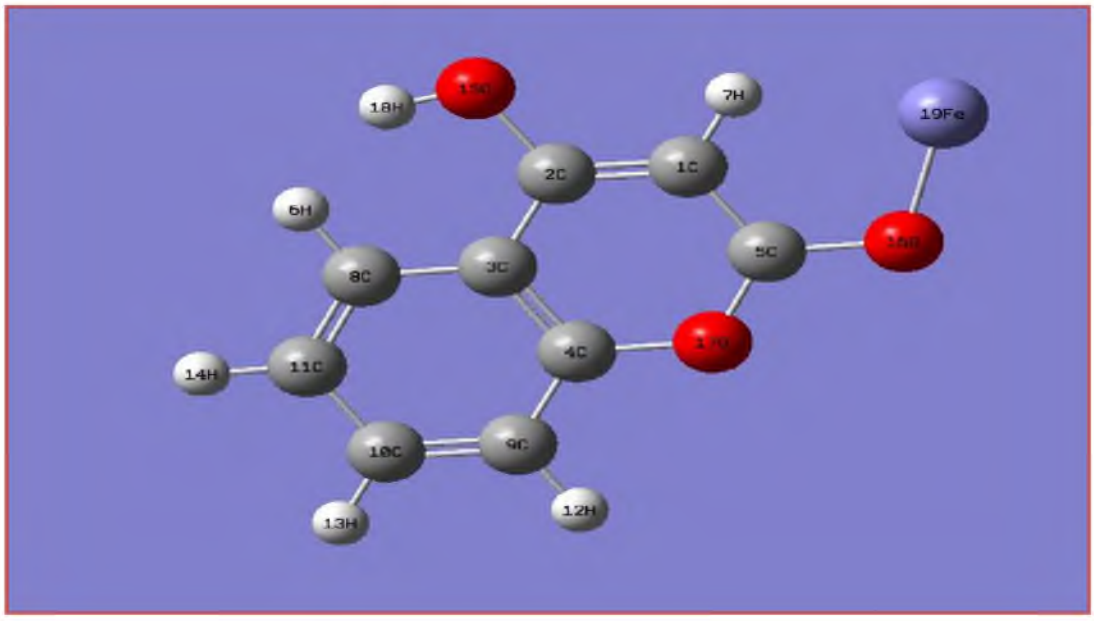


**Fig. 6.3. Complex formation of Fe(III) and 4-hydroxycoumarin by DFT method**

### 6.3.2. FT-IR spectra

The FT-IR spectra of Fe(III)-4-hydroxycoumarin complex shown in Fig. 6.4 confirmed the complex formation of Fe(III) with phenolic acid. Fig.1 illustrates that peaks were sharp in the complex as compared to 4-hydroxycoumarin (blank). The absorptions at 1550.2.4 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1650.4 $cm^{-1}$ can be attributed to aromatic -C=O- stretching vibrations of 4-hydroxycoumarin with Fe(III). The broad peak at 3873.0 $cm^{-1}$ can result from stretching vibration of -OH groups of 4-Hydroxycoumarin. Some peaks of Fe(III) and 4-hydroxycoumarin disappeared and some new peaks appeared, when the complex was formed.

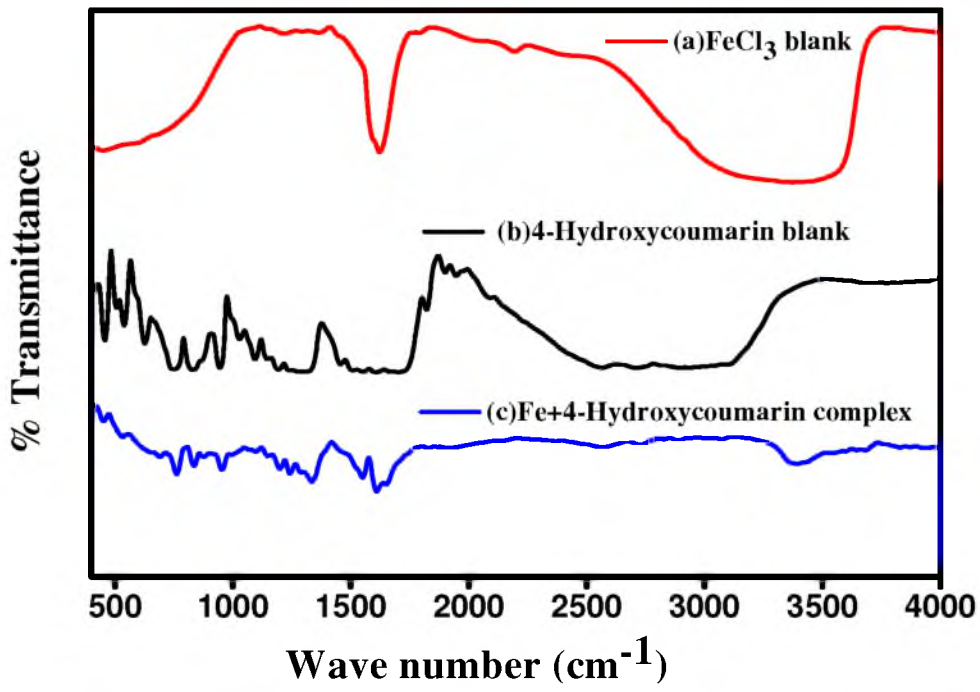


**Fig. 6.4. FT-IR spectra of (a) $FeCl_3$ (b) 4-Hydroxycoumarin (c) Fe(III)-4-hydroxycoumarin complex**

### 6.3.3. UV-Visible spectra

Since absorbance maxima were not observed in the visible region, therefore, spectra were scanned in 250-450 nm range. The concentration ranges of Fe(III)-4-hydroxycoumarin complex was maintained at 5, 10, 15, and 20 ppm. The absorbance of

Fe(III)-4-hydroxycoumarin complex (Fig.6.5) increases with increasing the concentration of 4-hydroxycoumarin. Ferric chloride blank solution shows λmax at 292 nm and 4-hydroxycoumarin (blank) shows the λmax at 282 nm, whereas Fe(III)-4-hydroxycoumarin complex show λmax at 280 nm, which confirm the complex formation. Similar results have been reported in the literature in the study of adsorption and chemical modification of phenols on a silver surface [Sánchez-Cortés *et.al.*, 2000; Belay, 2012]

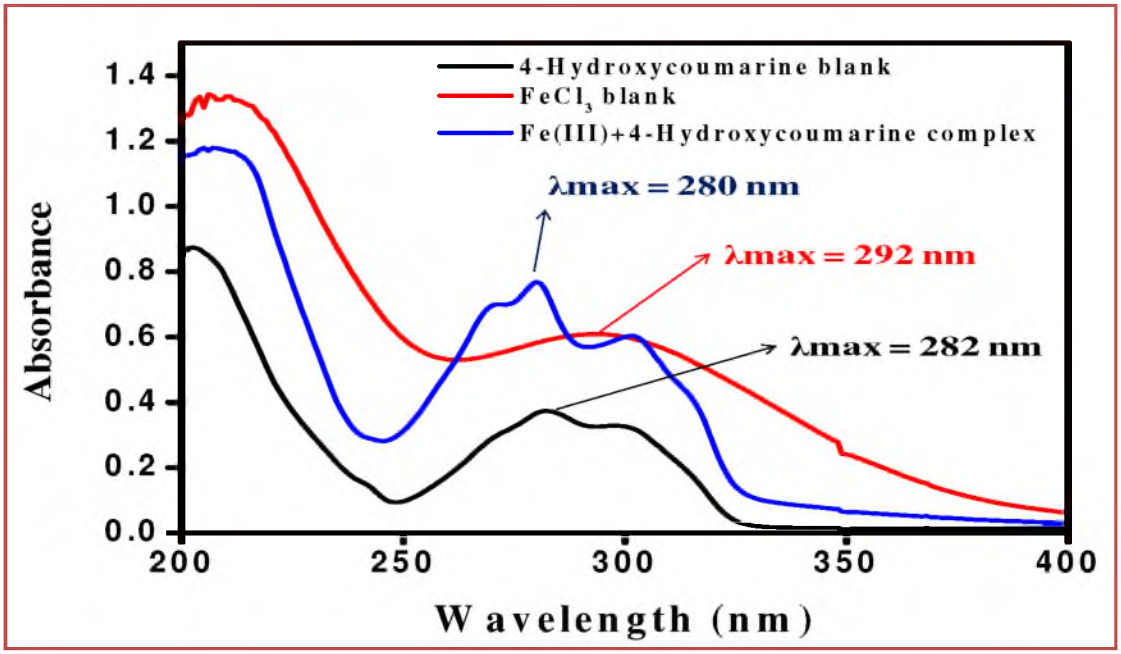


**Fig. 6.5. UV-Visible spectra of ferric chloride, 4-hydroxycoumarin and Fe(III)-4-hydroxycoumarin complex**

### 6.3.4. SEM spectra

The morphology of Fe(III)-4-hydroxycoumarin complex was studied employing the SEM images. The complex of Fe(III) and 4-hydroxycoumarin was prepared at room temperature in double distilled water. These precipitates were dried in the rota vapour. Fig. 6.6 shows the surface morphology of complex which reveals that the surface is mesoporous in both cases. The mesoporous nature of complexity arises due to the formation of the cavity during the chelation process. The free electron scanning microscopy data indicated that the typical diameter of the Fe(III)-complex powder was in the range of 50–250 nm having mesoporous texture. The morphology of complex was looking needle shape.

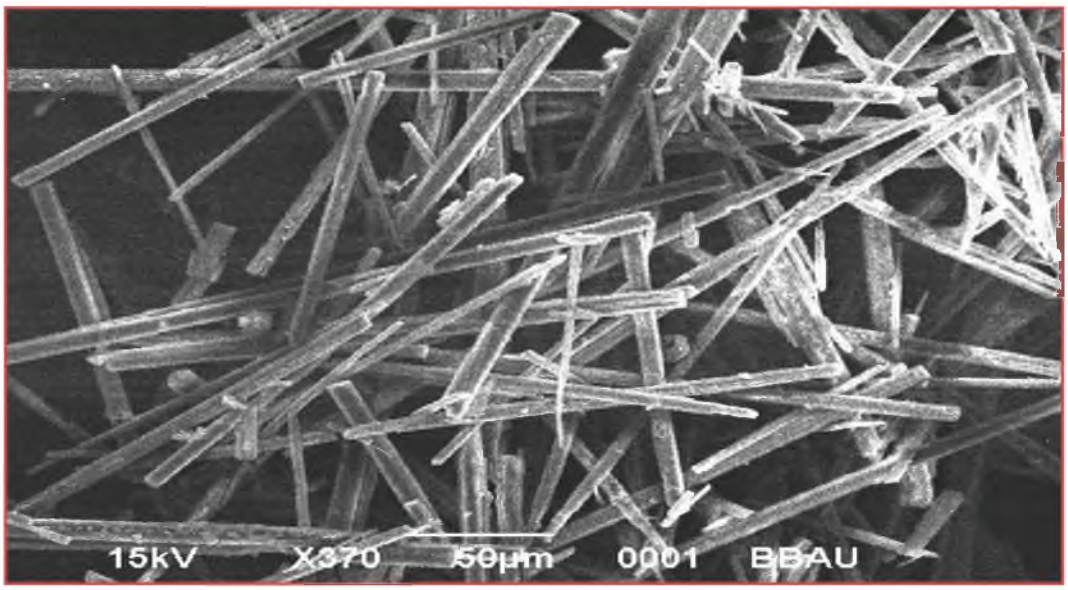


**Fig. 6.6. SEM images of Fe(III)-4-hydroxycoumarin complex**

### 6.3.4. Gustatory properties

The dried compound was only slightly salty with no metallic taste whatever. It dissolved easily and completely in distilled water to give a dark yellow solution at a concentration o.4%, the pH of which was 11.0. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 11.0.

### 6.3.5. pH stability

The stability of the iron/4-hydroxycoumarin chelate was investigated at two stages; first, after the chelate had just been formed, i.e. before the precipitation stage, the chelate was found to be stable over the whole pH range (as judged by no formation of a precipitate). Second, the stability of isolated powdered chelate was investigated. A typical ferric hydroxide precipitate formed at about pH 7.0 as the pH was lowered from pH 11.0.

### 6.3.6. Geometrical stability

The isolated complex of Fe(III) and 4-hydroxycoumarin was formed in a tetrahedral geometry. The chelation by ligand with Fe(III) results in chelate complex in a tetrahedral geometry.

### 6.4.0. Kinetics of complex formation of Fe(III) and 4-Hydroxycoumarin

The reaction was followed kinetically by a spectrophotometric method in aqueous solution at pH 11.0 as described in this paper. The initial results of the complex formation showed characteristic absorbance maxima at 280 nm for Fe(III)-4-hydroxycoumarin complex. The complex formation was, therefore, followed by measuring the colour (absorbance λmax at 280 nm) at different interval of time on double beam spectrophotometer. The data on the development of colour was due to complexation of 4-Hydroxycoumarin and Fe(III). To determine effects of each reactant on the observed reaction rate (k), the concentrations of 4-Hydroxycoumarin and Fe(III) were independently varied. The increase in absorbance was monitored at the λmax of Fe(III)-4-hydroxycoumarin complex over time. The slope of the best-fit line through the initial linear sections of the kinetics curves plotted for Fe(III)-4-hydroxycoumarin complex is shown in Fig. 6.8. The value of the rate constant of Fe(III)-4-hydroxycoumarin complex varied with a change in concentration over time was evaluated and such representative data are given in Table.6.1. The complexation reaction was found to be a first-order with rate constants for $k_1$ (formation) $4.35\times10^{-4}$ $min^{-1}$Hynes and Coinceanainn [2004] have studied the interaction of Fe(III) with phenolic acid and found a pseudo-first order kinetics with k = 2560 $M^{-1}.s^{-1}$ quite different from the first-order value found in the present work of $4.35\times10^{-4}$ $min^{-1}$.

### 6.4.1. Effect of concentration of ligands on rate constant

The data illustrates the dependence of complex formation on changing concentration. The reaction mixture of known concentration of 4-hydroxycoumarin and Fe(III) were used at varying concentrations (viz., 10 ppm 20 ppm and 30 ppm) at different interval of time. Corresponding results are plotted in Fig.6.8. The rate constant has been evaluated from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line.

### 6.4.2. Effect of temperature on rate constant

In the previous section, it has been shown that complexation of Fe(III)-4-hydroxycoumarin obeyed first-order kinetic. In this section, the reported data illustrates the dependence of complex formation on temperature. The reaction mixture of the known

and fixed concentration of 4-hydroxycoumarin and Fe(III) were used at a different temperature, viz., 25°C, 35°C and 45°C and the development of colour at λmax 280 nm for Fe(III)-4-hydroxycoumarin complex, was recorded at different intervals of time. The corresponding results are plotted in Fig. 6.9. The rate constant has been evaluated from the first order rate of reaction at different temperatures suggesting that the equation fits the data in straight line. As shown in Fig. 6.7 the apparent activation energy (Ea) was evaluated employing Arrhenius equation and found to be181 kcal/mol for Fe(III)-4-hydroxycoumarin complex, which is reasonable for a complexation reaction. Similar results have also been reported on Fe(III) flavonoid quercetin complex reaction [Bukhari *et.al.*, 2006]

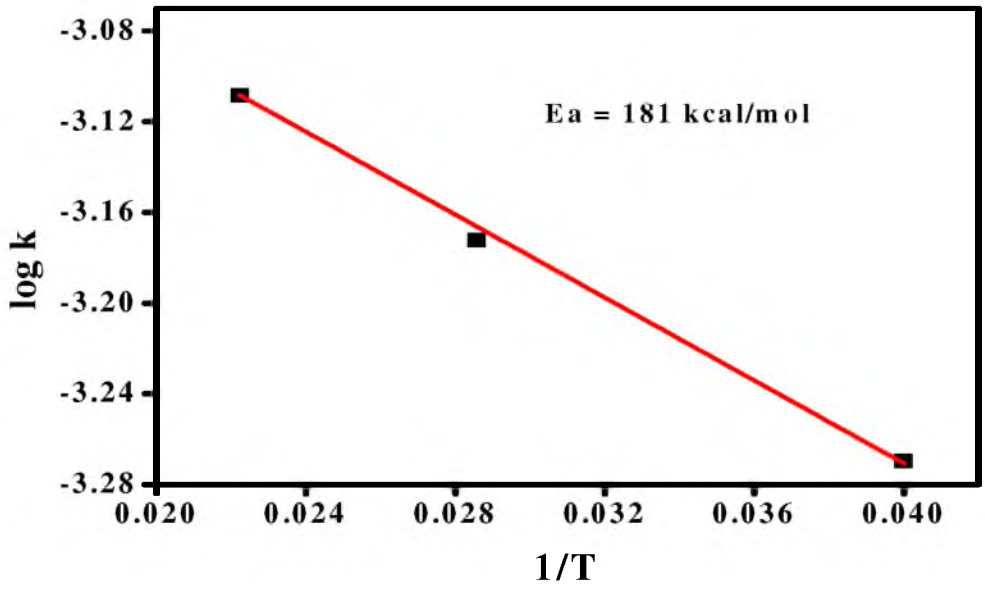


**Fig.6.7. Arrhenius plots for Fe(III)-4-hydroxycoumarin complex**

### 6.5.0. Effect of various parameters on complex formation

### 6.5.1. Effect of concentration

Influence of increasing concentration of 4-Hydroxycoumarin on the fixed concentration of Fe(III) with reference to colour formation was investigated. The absorbance of the complex solution of Fe(III)-4-hydroxycoumarin was recorded at 280 nm, at different interval of time. When Fe(III) (constant concentration) reacts with variable concentration (5, 10, 15 and 20 ppm) of 4-Hydroxycoumarin, its tendency to

complex increases with increasing concentration of 4-hydroxycoumarin, the value of absorbance also increases. Such representative results are shown in Fig. 6.8. Results obtained in the present case are consistent with earlier studies [Leopoldini *et.al.*, 2006].

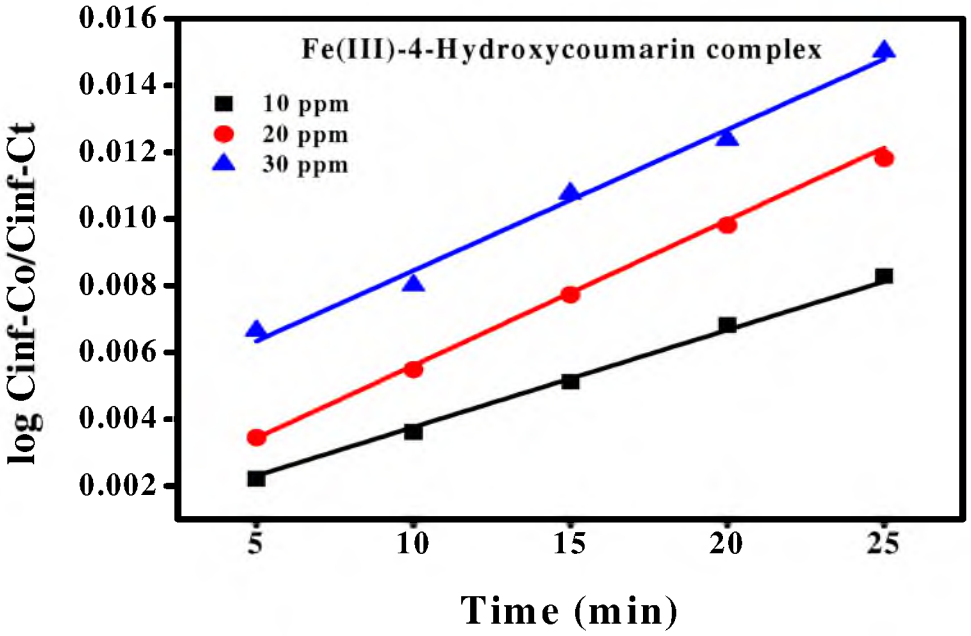


**Fig.6.8. Plot illustrating the effect of change in concentration with time**

### 5.6.2. Effect of temperature

In the previous section, it has been shown that complexation of Fe(III)-4-hydroxycoumarin obeyed first-order kinetic. In this section, the reported data illustrates the dependence of complex formation on temperature. The reaction mixture of the known and fixed concentration of 4-Hydroxycoumarin and iron was used at a different temperature, viz., 25°C, 35°C and 45°C and the development of colour at 280 nm for Fe(III)-chlorogenic acid complex was recorded at different intervals of time. The corresponding results are plotted in Fig. 6.9. The rate constant has been from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line. As shown in Fig. 6.7, the apparent activation energy (Ea) was evaluated employing Arrhenius equation and found to 181 kcal/mol for Fe(III)-4-hydroxycoumarin complex, respectively which is reasonable for a complexation reaction. Similar results have also been reported on iron flavonoid quercetin complex reaction [Leopoldini *et.al.*, 2006].

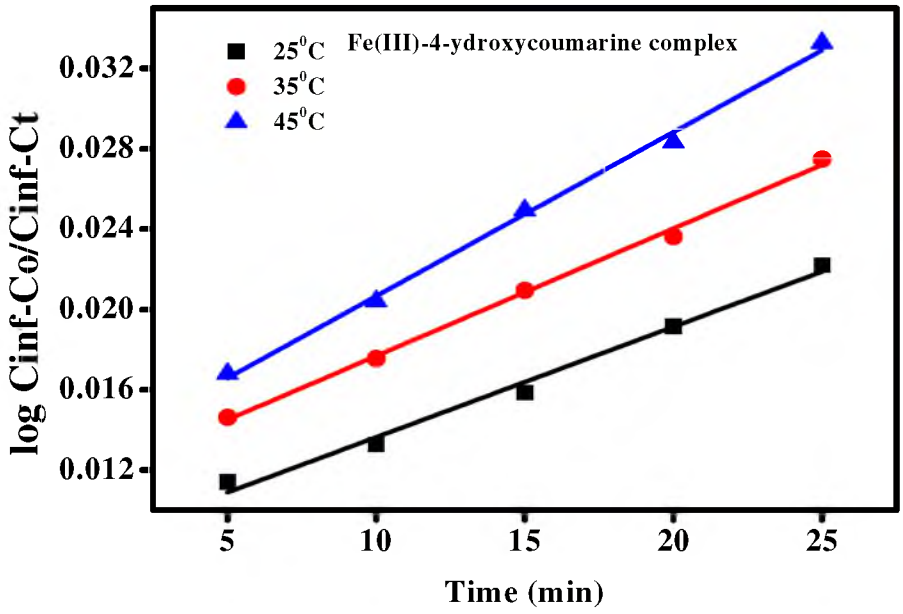


**Fig.6.8. Plot illustrating the effect of change in temperatures with time**

### 6.6.0. DFT study of Fe(III) and 4-hydroxycoumarin complex

With the help of DFT method, various parameters of Fe(III) and the 4-Hydroxycoumarin complex was evaluated by Gaussian09 software in Table 6.3.

**Table 6.3 Showing different parameters of Fe(III)-4-hydroxycoumarin complex**

| S.No. | Name of parameters of complex | DFT values of complex |
|---|---|---|
| 1. | Total energy | $0.889\times10^{-9}$ eV |
| 2. | Dipole moment | 11.03 Debye |
| 3. | Hardness | 0.1532 |
| 4. | Softness | 6.52 |
| 5. | Electronegativity | 0.484 |
| 6. | Chemical potential | -0.484 |
| 7. | Electrophilicity Index | 0.7643 |
| 8. | Point group | C1 |

### 6.7.0. Conclusion

In this paper, the kinetic study on the complexation of Fe(III) with 4-hydroxycoumarin was performed in aqueous solution at pH 11.0. The complex formation between Fe(III) and 4-hydroxycoumarin has been confirmed by electron spectroscopy. The Fe(III)-4-hydroxycoumarin complex exhibit maximum absorbance $\lambda$max at 280 nm at which neither of ligand (blank) nor Fe(III) which give assurance for complex formation between Fe(III) and chosen antioxidant phenolic acid. The FT-IR spectra of Fe(III)-4-hydroxycoumarin complex showed the absorptions at 1550.2.4 $cm^{-1}$ shows alkenyl C=C stretching vibrations and at 1650.4 $cm^{-1}$ can be attributed to aromatic –C=O stretching vibrations of 4-hydroxycoumarin with Fe(III). The broad peak at 3873.0 $cm^{-1}$ can result from stretching vibration of -OH groups of 4-hydroxycoumarin. The SEM images of Fe(III)-4-hydroxycoumarin complex with Fe(III) demonstrated the mesoporous nature which was needle shape. Under the experimental conditions, the studied complexation reaction was found to follow a first-order kinetics with rate constants for $k_1$ (formation) $4.35\times10^{-4}$ $min^{-1}$. The value of the colour of the complex was found to increase with increasing the temperature suggesting that complex undergoes dissociation and different products are formed. The apparent activation energy of the complexation reaction was evaluated to be 181 kcal/mol. The DFT study shows that the isolated complex of Fe(III) and 4-hydroxycoumarin was formed in a tetrahedral geometry. DFT study used for evaluation of various parameters of the studied complex showed hardness (0.153), electronegativity (0.484), softness (6.52), total energy ($0.889\times10^{-9}$ eV), dipole moment (11.03 Debye), chemical potential (-0.484), electrophilicity index (0.764) and point group symmetry (C1).

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# CHAPTER-7

## Kinetics of complex formation of Fe(III) with 7-Hydroxy-4-methylcoumarine: Experimental and theoretical study


**Abstract**

Kinetic study on the complexation of 7-hydroxy-4-methylcoumarine with Fe(III) was described in aqueous solution at pH 10.0 together with a reappraisal of spectral evidence for chelate formation. The complexation reaction was found to be a first-order with rate constants for $k_1$ (formation) $6.1\times10^{-4}$ $min^{-1}$. The dried compound was only slightly salty with no metallic taste whatever. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 10.0. The apparent activation energy of the complexation reaction was evaluated to be 995 kcal/mol. The isolated complex showed tetrahedral geometry. DFT study shows that the isolated complex of Fe(III) and 7-hydroxy-4-methylcoumarine was formed in tetrahedral geometry. Various parameters of the isolated complex such as hardness, electronegativity, softness, total energy, dipole moment, chemical potential, electrophilicity index and point group symmetry were evaluated using DFT method and were found as 0.2264, 0.4793, 4.41, $0.179\times10^{-9}$ eV, 9.78 Debye, -0.4793, 0.5066 and C1, respectively.

## 7.0. Introduction

Coumarins are a very large group of 1, 2-benzopyrones derivatives that are widely distributed in a variety of natural plant sources [Borges *et.al.*, 2005]. They comprise substances with a very different chemical structure including even hepatotoxic aflatoxins and photosensitive. However, other coumarins are considered to be interesting compounds for pharmacological research because of their wide spectrum of potentially positive pharmacological activities [Borges *et.al.*, 2005; Bhattacharyya *et.al.*, 2009; Campos-Toimil *et.al.*, 2002; Braccio *et.al.*, 2004]. Especially their antioxidant, antiaggregant, lipid-lowering, anti-inflammatory and vaso relaxing effects may predetermine them for the treatment and/or prevention of cardiovascular diseases [Borges *et.al.*, 2005; Fylaktakidou *et.al.*, Kostova *et.al.*, Kostova, 2006] and, in fact, rare in vivo studies with coumarins suggested their positive role in some cardiovascular pathologies [Hoult, 1996; Baccard *et.al.*, 2000; Chang *et.al.*, 1994]. Iron is an essential element participating in many vital processes. However, in the organism, it has to be firmly stored in proteins and its metabolism has to be meticulously regulated due to possible participation of free iron in reacting oxygen species generation [Halliwell, 1999; Mladenka *et.al.*, 2005]. Coumarin derivatives are of interest because of their physiological, photodynamic, anticoagulant, spasmolytic and bacteriostatic activity. They are also extensively used as analytical reagents. 7-Hydroxycoumarin is known for its antibiotic and antifungal activities [Sardari et. al., 1999]. 8-Substituted-4-methyl-7-hydroxycoumarin [Teotia et. al., 1973; Issa *et.al.*, 1992; El-Ansari *et.al.*, 1988] and 6-substituted-4-methyl-7-hydroxycoumarin [Tyagi *et.al.*, 1975], have been reported for complexing ability. These derivatives of coumarin have been reported to exhibit anticoagulant and plant growth regulating properties [Luzzatto *et.al.*, 1986]. Racemic sodium warfarin is widely used in the prevention of thromboembolic disease [Handler *et.al.*, 1998]. Coumarin derivatives are known to have good complexing ability [Singh, 1980]. The presence of phenolic hydroxyl group or carboxylic acid happened to be the key factor for higher activity of coumarin against iron chelation. A number of dioxouranium(VI) and titanium(IV) complexes with hydroxycoumarins, *e.g.*, 3-hydroxycoumarin and 7, 8-dihydroxy-4-methylcoumarin, *etc.*, have been reported spectrophotometrically and it has been observed that depending upon pH, 1:1, 1:2 and 1:3 complexes are formed in the solution [Singh, 1983; Singh *et.al.*, 1976]. This paper reports the solution structure of the Fe(III) phenolic acids interactions. Because the metal ion

coordination resulted in simultaneous deprotonation of the phenolic functions of the aromatic ring, the spectra of the formed metal species resembled those of the anions of the parent molecule, with a bathochromic shift due to the metal ion [Fiallo *et.al.*, 1999]. The kinetics and mechanism of Fe(III) with various polyphenolic compounds have been investigated [Hynes, 2004] and it has been reported that caffeic and chlorogenic acid are generally consistent with the formation of a 1:1 complex that subsequently decays through an electron transfer reaction. However, in the present case, an attempt has been made to corroborate the experimental findings by a theoretical study of the isolated complex. Herein, we have critically examined the structure relationship of 7-Hydroxy-4-methylcoumarin interaction with Fe(III) at pH 10.0. The objective of this work, therefore, is to substantiate the mechanistic aspects of the interactions between Fe(III) and 7-hydroxy-4-methylcoumarin employing experimental and theoretical study.

### 7.2.0. Experimental

### 7.2.1. Chemicals

7-Hydroxy-4-methylcoumarin was purchased from Himidea Ltd., India. Ferric chloride was obtained from Fisher Scientific India (Fig. 7.1). All the reagents and solvents were of analytical grade and chemically pure and were used as received.

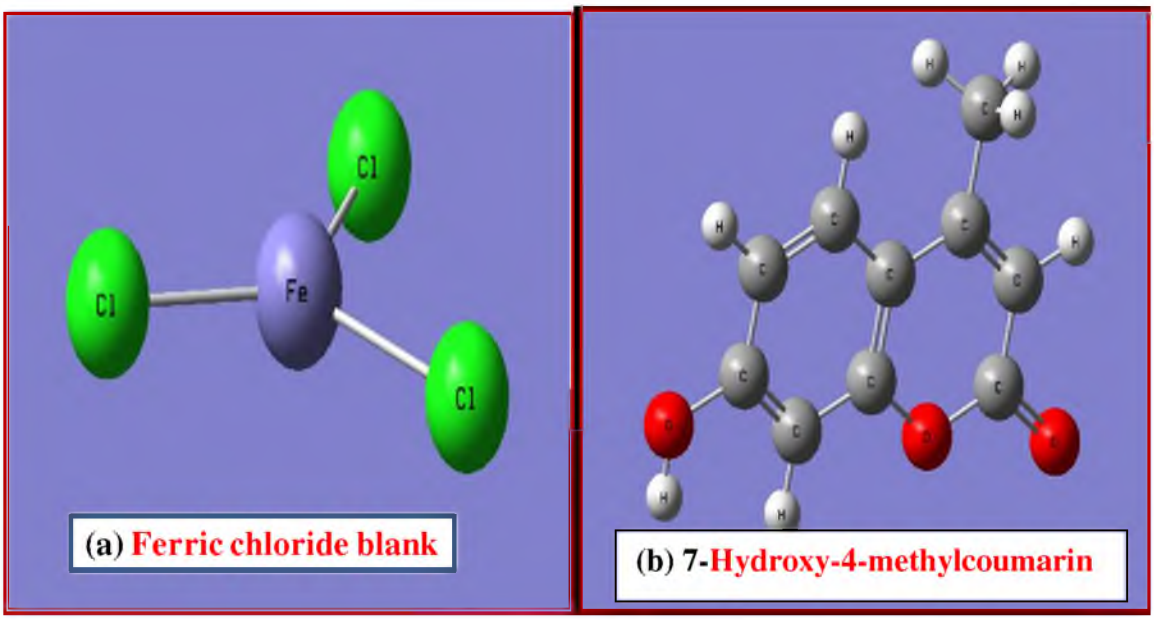


**Fig. 7.1. Molecular structure of (a) Ferric chloride (b) 7-Hydroxy-4-methylcoumarin**

### 7.2.3. Synthesis of Fe(III)–7-hydroxy-4-methylcoumarin complex

All experiment was performed in aqueous solution at pH 10.0. A stock solution of Fe(III), **7-**Hydroxy-4-methylcoumarin were prepared in (10 ppm) in 100 ml volumetric flask. Ethanol (10 ml) was added to 7-hydroxy-4-methylcoumarin stock solutions for complete dissolution. When the equimolar concentration of Fe(III) reacts with 7-hydroxy-4-methylcoumarin, the dark brown coloured complex was formed. After 45 minutes, the precipitate of 7-hydroxy-4-methylcoumarin was formed at room temperature. The precipitates were filtered and dried for further analysis.

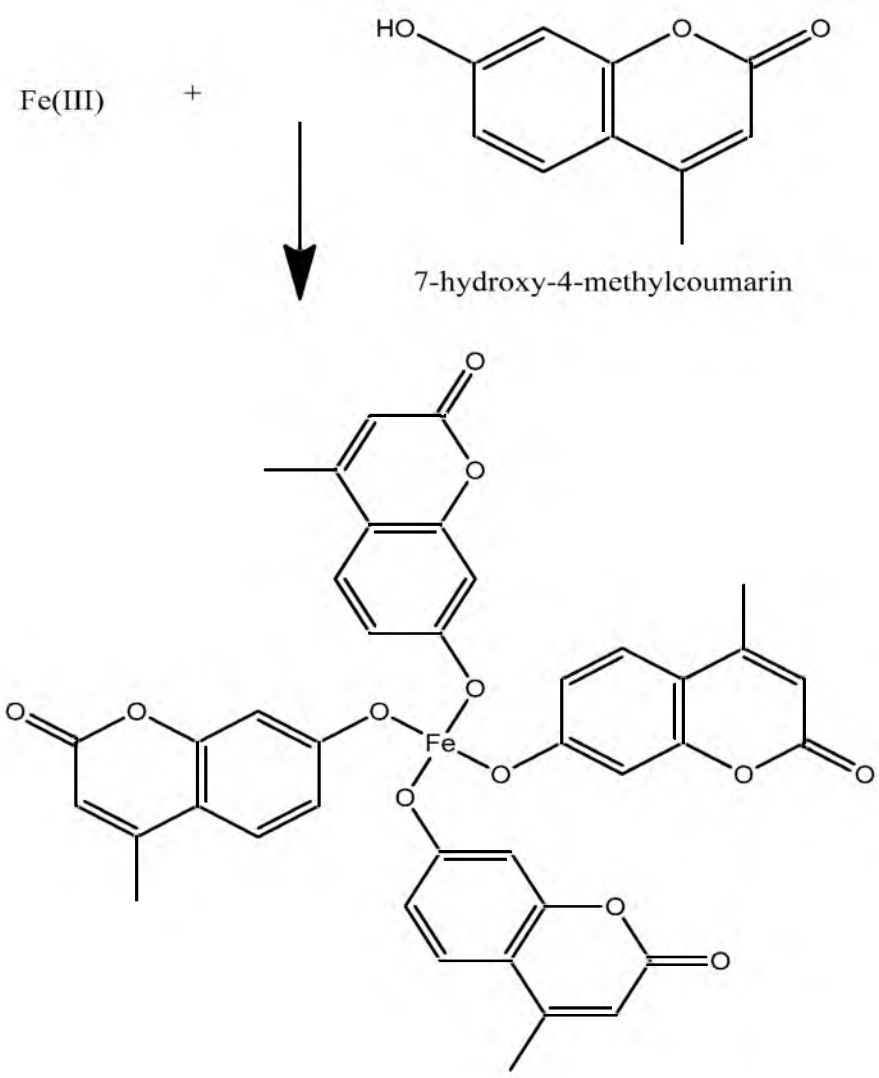


**Fig. 7.2. Showing reaction between Fe(III) and 7-Hydroxy-4-methylcoumarin**

### 7.2.4. Analysis of the chelate

The iron content of the chelate and gustatory properties of the dried powder and aqueous solution of the isolated chelate (Fig. 7.3) was considering prime importance, therefore analysis of the chelate was investigated.

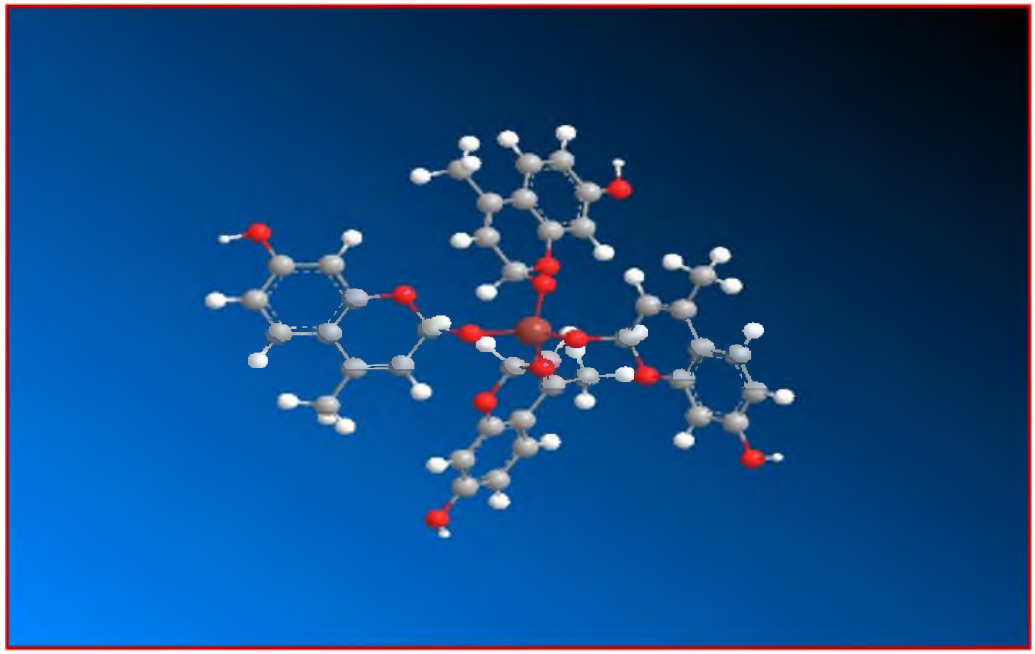

**Fig. 7.3. Showing the reaction between Fe(III) and 7-hydroxy-4-methylcoumarin**

### 7.2.5. Theoretical study

With the help of DFT method, various parameters of Fe(III) and 7-hydroxy-4-methylcoumarin complex were evaluated employing Gaussian09 software.

### 7.2.6. Characterization

The absorbance and maximum wavelength of blank and complexes were recorded using UV–visible spectrophotometer (Carry 100 make). The functional group identification of Fe(III)-7-hydroxy-4-methylcoumarin complex was determined by Fourier Transform Infra-Red Spectrometer (FTIR) (Thermo-Scientific, Nicole 6700). The morphological analysis of Fe(III)-7-hydroxy-4-methylcoumarin complex was studied by Scanning Electron Microscope (SEM) by employing JEOL JSM 7610F model.

### 7.3.0. Results and discussion

### 7.3.1. Fe(III)–7-hydroxy-4-methylcoumarin complex formation

The theoretical structure of Fe(III) and 7-hydroxy-4-methylcoumarin were obtained from optimizing by DFT method in which Fe(III) attacked oxygen atom due to the availability of pi-electron. Each valency of Fe(III) will attack oxygen of the 7-hydroxy-4-methylcoumarin (Fig.7. 4).

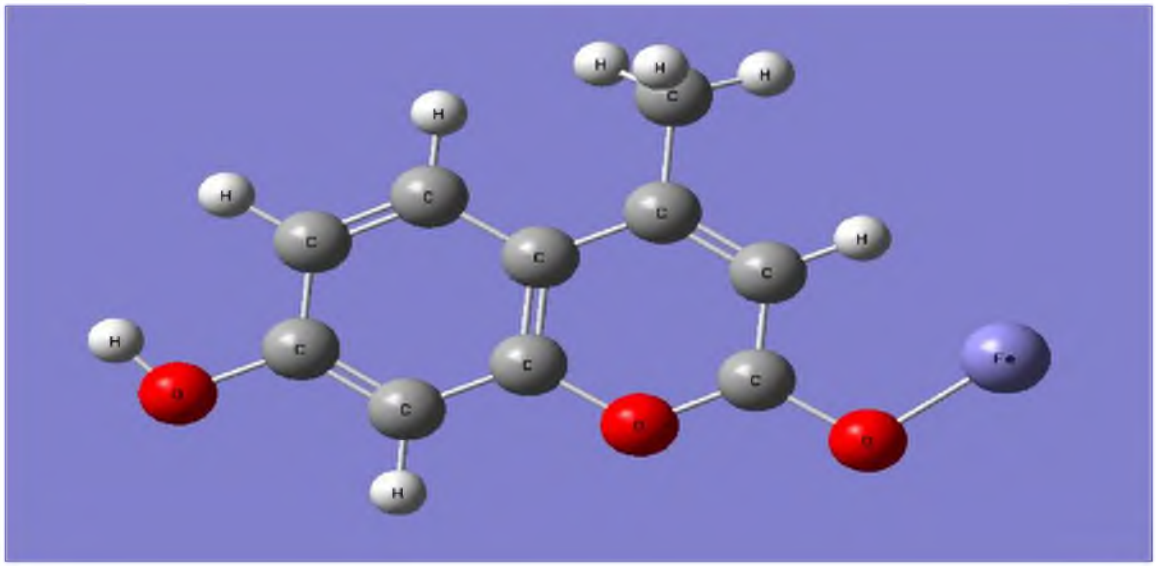

**Fig. 7.4. Complex formation of Fe(III) and 7-hydroxy-4-methylcoumarin by DFT method**

### 7.3.2. FT-IR spectra

The FT-IR spectra of Fe(III)-7-hydroxy-4-methylcoumarin complex shown in Fig. 7. 5 confirmed the complex formation of Fe(III) with phenolic acid. Fig.4 illustrates that peaks were sharp in the complex as compared to 7-hydroxy-4-methylcoumarin (blank). The absorptions at 1513.2 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1670.3 $cm^{-1}$ can be attributed to aromatic -C=O stretching vibrations of 7-hydroxy-4-methylcoumarin with Fe(III). The broad peak at 3721.0 $cm^{-1}$ can result from stretching vibration of -OH groups of 7-Hydroxy-4-methylcoumarin. Some peaks of Fe(III) and 7-hydroxy-4-methylcoumarin disappeared and some new peaks appeared, when the complex was formed.

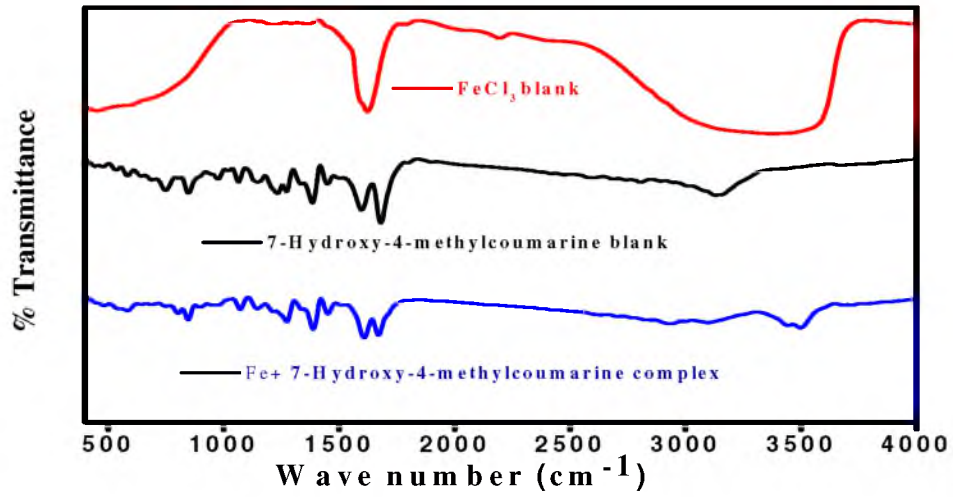


**Fig. 7.5. FT-IR spectra of (a) $FeCl_3$ (b) 7-Hydroxy-4-methylcoumarin (c) Fe(III)-7-hydroxy-4-methylcoumarin complex**

### 7.3.3. UV-Visible spectra

Since absorbance maxima were not observed in the visible region, therefore, spectra were scanned in 250-450 nm range. The concentration ranges of Fe(III)-7-hydroxy-4-methylcoumarin complex was maintained at 5, 10, 15, and 20 ppm. The absorbance of Fe(III)-7-hydroxy-4-methylcoumarin complex (Fig.7.6) increases with increasing the concentration of 7-hydroxy-4-methylcoumarin. Ferric chloride blank solution shows λmax at 292 nm and 7-hydroxy-4-methylcoumarin (blank) shows the λmax at 320 nm, whereas Fe(III)-7-hydroxy-4-methylcoumarin complex show λmax at 318 nm, which confirm the complex formation.

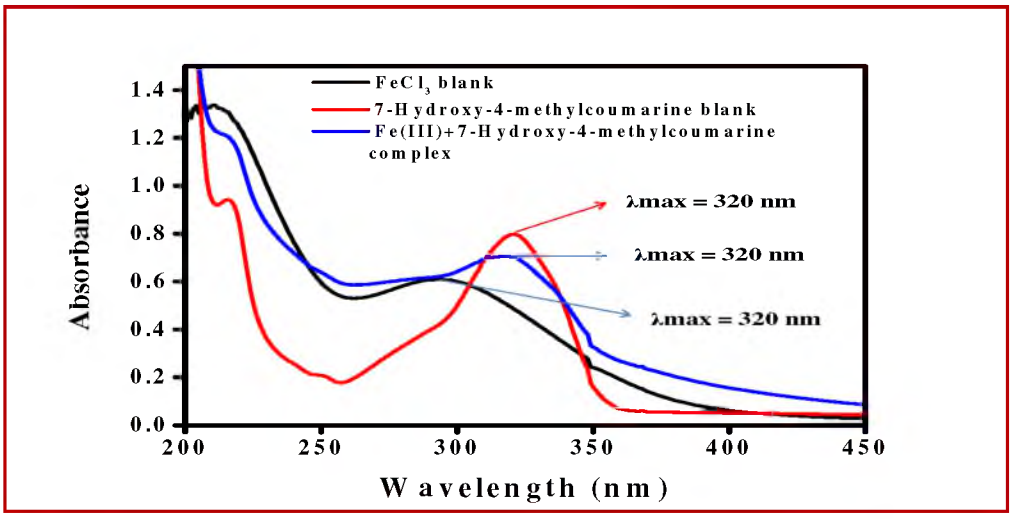


**Fig. 7.6. UV-Visible spectra of (a) $FeCl_3$ (b) 7-Hydroxy-4-methylcoumarin (c) Fe(III)-7-hydroxy-4-methylcoumarin complex**

### 7.3.4. SEM spectra

The morphology of Fe(III)-7-hydroxy-4-methylcoumarin complex was studied employing the SEM images. The complex of Fe(III) and 7-hydroxy-4-methylcoumarin was prepared at room temperature in double distilled water. These precipitates were dried by the rota vapour. Fig. 7.7 shows the surface morphology of complex which reveals that the surface is mesoporous in both cases. The mesoporous nature of complexity arises due to the formation of the cavity during the chelation process. The free electron scanning microscopy data indicated that the typical diameter of the Fe(III)-complex powder was in the range of 50–250 nm having mesoporous texture. The morphology of complex looks like rhomboidal shape.

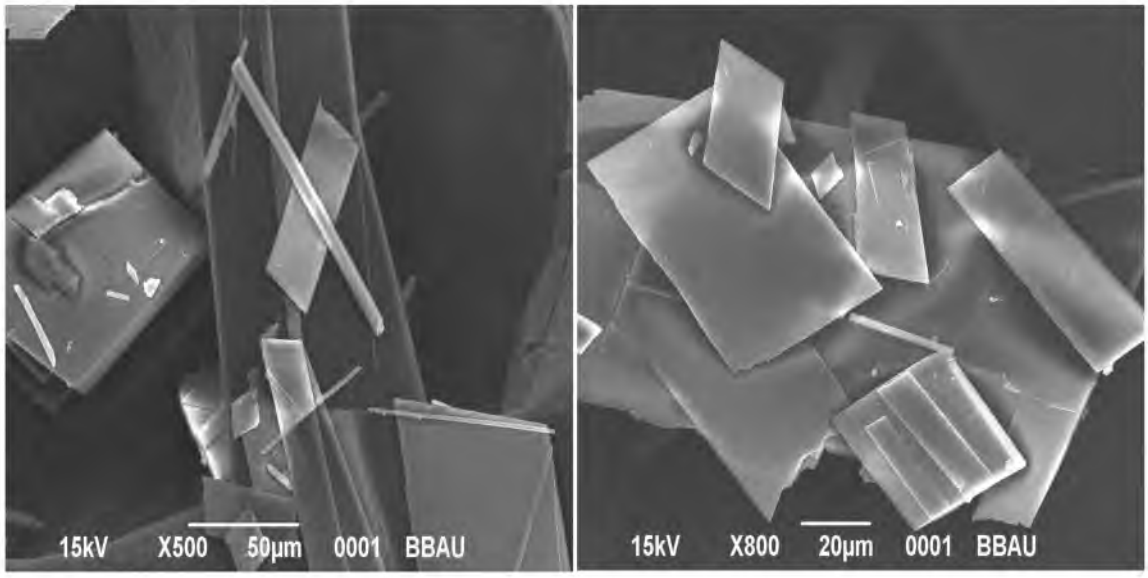


**Fig. 7.7. SEM images of Fe(III)-7-hydroxy-4-methylcoumarin complex**

### 7.3.5. Gustatory properties

The dried compound was only slightly salty with no metallic taste whatever. It dissolved easily and completely in distilled water to give a dark yellow solution at a concentration 0.4%, the pH of which was 10.0. This solution had a salty and slight caramel taste but again no metallic taste. The gustatory properties of the isolated complex were investigated and complex showed no metallic taste. The isolated complex was stable at pH 10.0.

### 7.3.6. pH stability

The stability of the iron/7-Hydroxy-4-methylcoumarin complex chelate was investigated at two stages; first, after the chelate had just been formed, i.e. before the precipitation stage, the chelate was found to be stable over the whole pH range (as judged by no formation of a precipitate). Second, the stability of isolated powdered chelate was investigated. A typical ferric hydroxide precipitate formed at about pH 7.0 as the pH was lowered from pH 10.0.

### 7.3.7. Geometrical stability

The isolated complex of Fe(III) and 7-hydroxy-4-methylcoumarin was formed in tetrahedral geometry (Fig. 7.2). The chelation by ligand with Fe(III) results in a chelated complex stable in a tetrahedral geometry.

### 7.4.0. Kinetics of complex formation of Fe(III) and 7-hydroxy-4-methylcoumarin

The reaction was followed kinetically by a spectrophotometric method in aqueous solution at pH 10.0 as described in this chapter. The initial results of the complex formation showed characteristic absorbance maxima at 318 nm for Fe(III)-7-hydroxy-4-methylcoumarin complex. The complex formation was, therefore, followed by measuring the colour (absorbance λmax at 318 nm) at different interval of time on double beam spectrophotometer. The data on the development of colour was due to complexation of 7-hydroxy-4-methylcoumarin and Fe(III). To determine effects of each reactant on the observed reaction rate (k), the concentrations of 7-hydroxy-4-methylcoumarin and Fe(III) were independently varied. The increase in absorbance was monitored at the λmax of Fe(III)-7-hydroxy-4-methylcoumarin complex over time. The slope of the best-fit line through the initial linear sections of the kinetics curves plotted for Fe(III)-7-hydroxy-4-methylcoumarin complex in Fig. 7.7. The value of rate constant of Fe(III)-7-hydroxy-4-methylcoumarin complex varied with a change in concentration over time was evaluated The complexation reaction was found to be a first-order with rate constants for k (formation) $4.35\times10^{-4}$ $min^{-1}$. Hynes and O'Coinceanainn [2004] have studied the interaction of Fe(III) with phenolic acid and found a pseudo-first order kinetics with k = 2560 $M^{-1}.s^{-1}$ quite different from the first-order value found in the present work of

$6.1\times10^{-4}$ $min^{-1}$. In addition, in this present work first-order kinetics, was formed in contrast with previous findings.

### 7.4.1. Effect of concentration on rate constant

The data illustrates the dependence of complex formation on changing concentration. The reaction mixture of known concentration of 7-hydroxy-4-methylcoumarin and Fe(III) were used at varying concentrations(viz., 10 ppm 20 ppm and 30 ppm) at different interval of time. Corresponding results are plotted in Fig.7.7. The rate constant has been evaluated from the first order rate of reaction at infinite concentration suggesting that the equation fits the data in straight line.

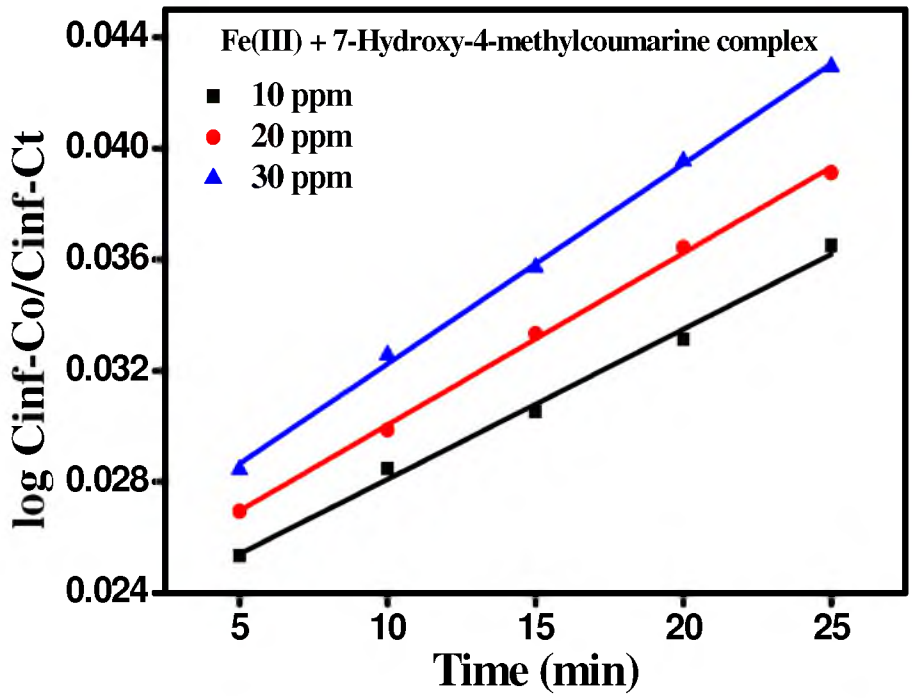


**Fig. 7.8. Plot illustrating the effect of change in concentration with time**

### 7.4.2. Effect of temperature on rate constant

In the previous section, it has been shown that complexation of Fe(III)-7-hydroxy-4-methylcoumarin obeyed first-order kinetic. In this section, the reported data

illustrates the dependence of complex formation on temperature. The reaction mixture of the known and fixed concentration of 7-hydroxy-4-methylcoumarin and Fe(III) was used at a different temperature, viz., 25°C, 35°C and 45°C and the development of colour at λmax 318 nm for Fe(III)-7-hydroxy-4-methylcoumarin complex, was recorded at different intervals of time. The corresponding results are plotted in Fig. 7.8. The rate constant has been evaluated from the first order rate of reaction at different temperatures suggesting that the equation fits the data in straight line. As shown in Fig.7.9 the apparent activation energy (Ea) was evaluated employing Arrhenius equation and found to be 995 kcal/mol for Fe(III)-7-hydroxy-4-methylcoumarin complex, which is reasonable for a complexation reaction. Similar results have also been reported on Fe(III) flavonoid quercetin complex reaction [Bukhari *et.al.*, 2006].

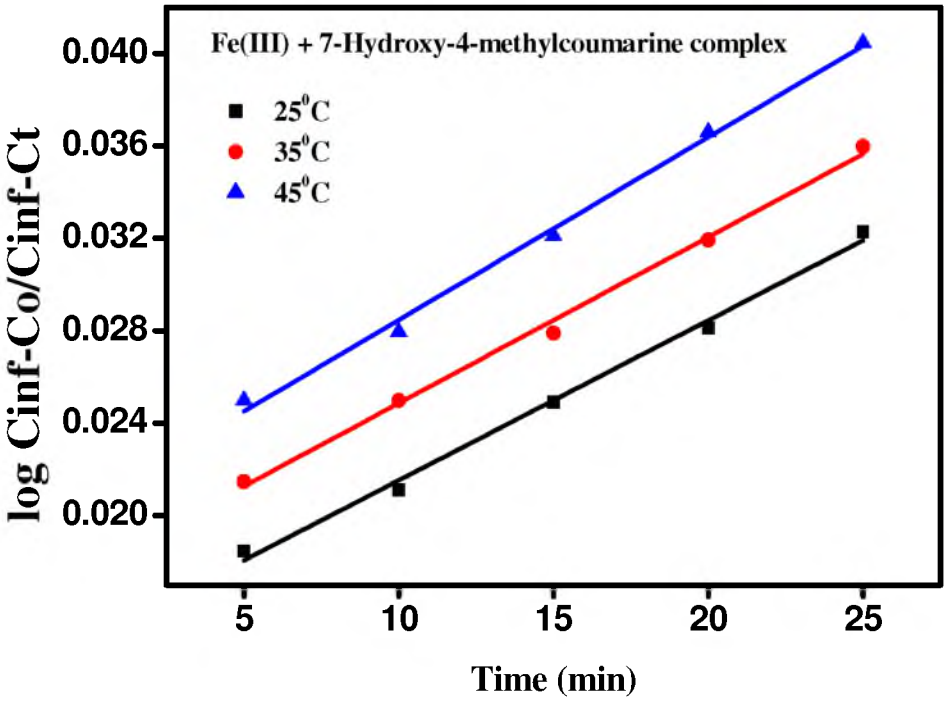


**Fig. 7.9. Plot illustrating the rate of complex formation between Fe(III) and 7-hydroxy-4-methylcoumarin with respect to temperature**

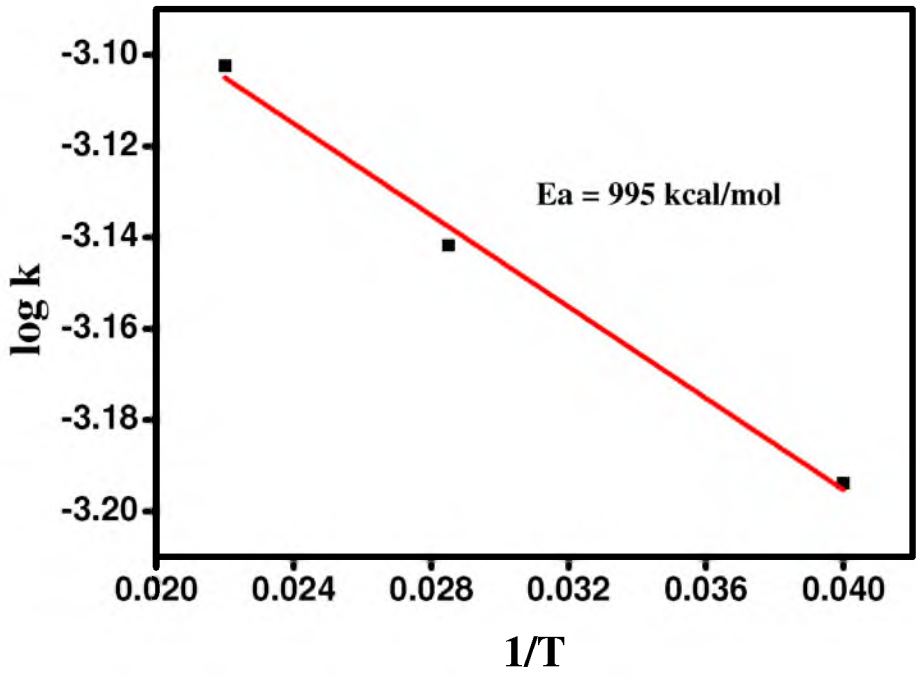


**Fig. 7.10. Arrhenius plots for Fe(III)-7-hydroxy-4-methylcoumarin complex**

### 7.6.0. DFT study of Fe(III) and 7-hydroxy-4-methylcoumarin complex

With the help of DFT method, various parameters of Fe(III) and the 7-hydroxy-4-methylcoumarin complex was evaluated by Gaussian09 software in Table 3.

**Table 7.3. Showing different parameters of Fe(III)-7-hydroxy-4-methylcoumarine complex**

| S.No. | Name of parameters of complex | DFT values of complex |
|---|---|---|
| 1. | Total energy | $0.179\times10^{-9}$ eV |
| 2. | Dipole moment | 9.78 Debye |
| 3. | Hardness | 0.0.2264 |
| 4. | Softness | 4.41 |
| 5. | Electronegativity | 0.479 |
| 6. | Chemical potential | -0.479 |
| 7. | Electrophilicity Index | 0.5066 |
| 8. | Point group | C1 |

### 7.7.0. Conclusion

In this paper, the kinetic study on the complexation of Fe(III) with 7-hydroxy-4-methylcoumarin was performed in aqueous solution at pH 10.0. The complex formation between Fe(III) and 7-hydroxy-4-methylcoumarin has been confirmed by electron spectroscopy. The Fe(III)-7-hydroxy-4-methylcoumarin complex exhibit maximum absorbance $\lambda$max at 318 nm at which neither of ligand (blank) nor Fe(III) $^{absorbs}$ which give assurance for complex formation between Fe(III) and chosen antioxidant phenolic acid. The absorptions at 1513.2 $cm^{-1}$ show alkenyl C=C stretching vibrations and at 1670.3 $cm^{-1}$ can be attributed to aromatic -C=O- stretching vibrations of 7-hydroxy-4-methylcoumarin with Fe(III). The broad peak at 3721.0 $cm^{-1}$ can result from stretching vibration of -OH groups of 7-hydroxy-4-methylcoumarin. The SEM images of Fe(III)-7-hydroxy-4-methylcoumarin complex with Fe(III) demonstrated the mesoporous nature which was rhomboidal and tetrahedral geometry. Under the experimental conditions, the studied complexation reaction was found to follow first-order kinetics with rate constants for k (formation) $6.1\times10^{-4}$ $min^{-1}$. The extent of the colour of the complex was found to increase with increasing the temperature suggesting that complex undergoes dissociation and different products are formed. The apparent activation energy of the complexation reaction was evaluated to be 995 kcal/mol. In this paper, the isolated complex showed tetrahedral geometry. DFT study shows that the isolated complex of Fe(III) and 7-hydroxy-4-methylcoumarine was formed in tetrahedral geometry. Various parameters of the isolated complex such as hardness, electronegativity, softness, total energy, dipole moment, chemical potential, electrophilicity index and point group symmetry evaluated DFT method and were found as 0.2264, 0.4793, 4.41, $0.179\times10^{-9}$ eV, 9.78 Debye, -0.4793, 0.5066 and C1, respectively.

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# Publication

**PUBLICATIONS**

**Published paper in referred journals/proceedings**

1. Kaman Singh, Bhuwan Chandra, Satya Prakash Gupta and **Ajay Kumar,** Application and potential of a pulsed magnetic field in controlling scale formation in cane-sugar processing., *Proc. Int. Soc. Sugar Cane Technologists'*.29 **(2016)** 566-571
2. Kaman Singh, Manisha Gautam, Bhuwan Chandra, **Ajay Kumar,** Removal of Pb(II) from its aqueous solution by activated carbon derived from Balam Khira (Kigelia Africana), *Des. Water Treat.,* 57 **(2016)** 1–11.
3. Kaman Singh, **Ajay Kumar**, Satya Prakash Gupta, Effect of ultrasonic on morphology of precipitated calcium carbonate (PCC) for controlling scale formation in sugar processing: Laboratory scale experiment. *Proc. Ann. Conv. Sugar Tech. Ass.* India, 74 **(2016)** 579-587.
4. Kaman Singh and **Ajay Kumar**, Iron-chlorogenic acid interaction and development of colour during sugar processing, Proc. Ann. Conv. *Sugar Tech. Ass.* India 75 **(2017)** 643 – 654

**Papers present in conferences/seminar and workshop attended**

1. Poster presented in International Seminar on Advances in Bio- & Nano-materials (ISABNM-2013) held from 17 Nov 2013 at Department of Physics, University of Lucknow, Lucknow (India).

2. Poster presented in National Conference on Thermodynamics of Chemical, Biological and Environment Systems (TCBES-2013), held from 25-26 Nov 2013 at Department of Applied Chemistry, BB Ambedkar Central University, Lucknow (India).

3. Participated in Awareness Seminar on MATLAB for Data Processing & Application Development, held on 9 Feb 2015 at Department of Chemistry, University of Lucknow, Lucknow (India).

4. Participated Hands-on-Training on SEM, FTIR, FPLC and Ion Chromatography, (USIC), held from 18-20 Feb 2015 at University Science Instrumentation Centre (USIC), BB Ambedkar Central University, Lucknow (India).

5. Poster presented paper in "National Conference on the Frontiers of Chemical Sciences and potential Interfaces" held from 10-11 April 2015 at School of Chemical Sciences, Central University of Gujarat, Gandhinagar, Gujarat (India).

6. Poster presented in "National Conference on Food Safety and Consumer Awareness," held from 21-22 Feb 2016 at Innovation Centre on Food Processing and Food Technology, University of Lucknow, Lucknow (India).

7. Poster presented in "Recent Advance and Innovation in Chemical and Materials Science-RAICMS-2017," held from 23-24 Feb 2017 at J.N.P.G. College, Lucknow, U.P. (India).

8. Oral research paper presented on "Iron-Chlorogenic Acid Interaction and Development of Colour during Sugar Processing" in National Conference on the Sugar Technologies Association of India, held from 3-5 Aug, 2017 at Kochi, Kerala (India).

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# Application and potential of a pulsed magnetic field in controlling scale formation in cane-sugar processing

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Abstract The deposition of calcium carbonate ($CaCO_3$), commonly known as scale, costs billions of dollars to the world-wide economy. This is also a common problem for sugar refineries that use the carbonation process. However, carbonate scale is unusual in plants using the sulphitation process; hence, it would be useful to know the composition of the scale formed. A suitable magnetic field pulse can effectively reduce scale formation and also change the nature of the scale formed. Examining the physical characteristics of scale samples showed that the scale after a PMF treatment was of a loose, soft sludge type and easily removed. Scanning electron micrograph (SEM) images, FT-IR spectrum and X-ray diffraction pattern (XRD) provides definitive proof of the existence of $CaCO_3$ scale in the sulphitation process of sugar manufacture.

Key words $CaCO_3$ scale, sugar evaporator, pulsed magnetic field, energy saving

## INTRODUCTION

Calcium carbonate is one of the most ubiquitous minerals in nature. Among the three anhydrous crystal polymorphs, rhombohedral calcite, orthorhombic aragonite and hexagonal vaterite, calcite is the most stable phase at ambient temperature, aragonite is metasable polymorph, while vaterite is thermodynamically unstable at all external conditions. The unwanted precipitation of $CaCO_3$ has been a common problem in heat exchangers and it is one of the most important scale forming minerals in oil and gas production (Andreassen 2005). This is also a likely problem for sugar refineries using the carbonation process. As a result of the progressive accumulation of scale on heated surfaces, the heat transfer coefficient (HTC) considerably declines over time causing significant economic losses (Hu *et al.* 2006). Hence, there is considerable interest in finding methods that effectively control the formation of this deposit.

This work is essentially an extension of our earlier work (Singh *et al.* 2016) on the effect of a pulsed magnetic field (PMF) on the viscosity of cane molasses and massecuite. In this paper we show that application of a suitable magnetic field pulse can significantly reduce scaling tendency and changes the nature of the scale in sugar evaporators.

## PMF TREATMENT

The general operating principle for a proposed PMF has been described elsewhere (Singh *et al.* 2016). For the application of a PMF on evaporators, the cell design of the PMF generator given in Figure 1 was used. All experiments were performed using a PMF intensity in the range of 0.15 T to 1.5 T and pulse duration between 5 and 720 s. The chemical composition of the scale samples was tested in the Ita Lab, Mumbai.

Our study was undertaken in a sugar mill (5000 t of cane per day) in western India that produces plantation-white sugar using the sulphitation process. The PMF generator was installed in the evaporators as shown in Figure 2 for 15 days and scale collected at the fifth body was examined to assess the effect of the PMF on the scale deposits. Scale formed after 15 days without PMF treatment was collected for comparison. All other process parameters remained unchanged in both the cases. The cane throughput was the same (juice analyses were similar). To assess the reproducibility, tests were done consecutively with and without the technology in a season or even at different times in the season.

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# Removal of Pb(II) from its aqueous solution by activated carbon derived from Balam Khira (*Kigelia Africana*)

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ABSTRACT

A low cost activated carbon derived from the fruit of *Kigelia Africana* (KA) was characterized by using Powder X-ray diffraction, Fourier transform infrared spectroscopy, and Brunauer–Emmett–Teller techniques for effective removal of Pb(II) from its aqueous solution. The derived activated carbon from *Kigelia Africana* (CKA) had micro porous and meso porous pore size distribution with high surface area (799 $m^2 g^{-1}$) and high carbon content (79.42%). The batch mode experiments are carried out to investigate the effect of process parameters such as solution concentration, pH, temperature, contact time, CKA amount on adsorption. The maximum adsorption was found at pH 5.0 (97% for 2.5 g/l CKA in 50 mg/l Pb(II) initial concentration). The Langmuir, Freundlich, and Temkin models were modeled to evaluate the equilibrium adsorption data, and the results describe the best representation of the Langmuir isotherm model with adsorption capacity 79.87 mg/g ($R^2$ = 0.99) at 30°C. Thermodynamic study demonstrates spontaneous and endothermic nature of the adsorption. Kinetic studies were examined using different kinetic models (Lagergren first-order and pseudo-second-order) and found pseudo-second-order kinetic data are well fitted for adsorption process. It is observed that adsorption of Pb(II) ions followed Lagergren-second-order kinetics. These results suggest that the synthesized CKA is a promising adsorbent for the removal of Pb(II) ions from wastewater.

*Keywords:* Low cost activated carbon (CKA); Pb(II); Isotherm models; Lagergren-first-order; Pseudo-second-order

## 1. Introduction

Lead is the one of such highly toxic element that is found both naturally and as introduced contaminant in the environment. In recent times, Pb has been introduced into natural water from a variety of sources such as acid battery manufacturing, metal plating and finishing, ammunition manufacturing, tetraethyl lead (TEL) manufacturing, and ceramic and glass industries [1]. Lead poisoning in humans causes severe damage

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Kaman Singh, Ajay Kumar & Satya Prakash Gupta
Proceedings of 74th Annual Convention of STAI: 579 – 587 2016

# EFFECT OF ULTRASONICS ON MORPHOLOGY OF PRECIPITATED CALCIUM CARBONATE (PCC) FOR CONTROLLING SCALE FORMATION IN CANE SUGAR PROCESSING : LABORATORY SCALE EXPERIMENT

Kaman Singh[1]*, Ajay Kumar[2] & Satya Prakash Gupta[2]

***ABSTRACT***

*The deposition of calcium carbonate commonly known as scale costs billions of dollars to the world wide economy. This is also a customary global problem for cane sugar processing. The present work is essentially an extension of our earlier work on the effect of power ultrasound on cane sugar processing. Application of ultrasonics can effectively reduce scale formation and also changes the nature of the precipitated calcium carbonate (PCC). The FTIR spectrum of the CaCO3 after PUS treatment presents the characteristic absorption peaks of vaterite at 1112 and 1134 $cm^{-1}$. No characteristic peaks belonging to calcite at 713 and 876 $cm^{-1}$ were detected after treatment. However, partial characteristic peaks belonging to aragonite were appeared after treatment at 649.4 $cm^{-1}$. The Scanning electron micicrograph (SEM) images clearly demonstrates the vaterite (thermodynamically least stable polymorph of $CaCO_3$) image after PUS treatment. Thus, extension of these laboratory scale model experiments to industrial scale could lead to an energy saving process for cane sugar manufacturing.*

***Keywords:*** Precipitated calcium carbonate (PCC), power ultrasound (PUS), characterization, sugar evaporator, energy saving.

## INTRODUCTION

Calcium carbonate is one of the most ubiquitous minerals in nature. Among

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Kaman Singh & Ajay Kumar
Proceedings of 75th Annual Convention of STAI: 643 – 654 2017

# IRON-CHLOROGENIC ACID INTERACTION AND DEVELOPMENT OF COLOUR DURING SUGAR PROCESSING

Kaman Singh* & Ajay Kumar

***ABSTRACT***

*Colour formation study of the complexation of chlorogenic acid with ferric chloride was performed using UV–Vis absorption spectroscopy. The colour complexation reaction was found to be a first-order with rate constants for $k_1$ (formation) 2.4 × 10-2 $min^{-1}$ for chlorogenic acid. The effect of concentration and temperature on the complexation reaction was also investigated. The apparent activation energy of the complexation reaction was evaluated to be 0.085eV.*

***Keywords:*** Iron-chlorogenic acid interactions, rate constant, activation energy".

## 1. INTRODUCTION

There is a general update that fast caramalisation catalysed by iron and iron-polyphenol interaction is only hypothesis without experimental data therefore in the present study CA (a major polyphenols found in cane juice) was chosen as a representative selection of polyphenols and its interaction with iron has been investigated with an objective of colour development in a pure chemical system which can be extended to explain the development of colour during sugar processing. Colour of sugar is an important commercial parameter and sugar mills are quite vigilant to comply with market requirements. There is no colour of pure sucrose, but it appears colourful due to its involvement in lots of complex compounds. Therefore, colourant is the common word that is used to cover various components which contribute to the colour of sugar. The complexity of sugars is very complex in nature and the volume is not easy to measure. Numerous processes have been developed over the years to achieve efficient and cost-effective removal of colour in order to produce low

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This is to certify that Prof./Dr./Mr./Ms. Ajay Kumar from B.B.A.U, Lucknow attended the "Hands-on-Training" on SEM, FTIR, FPLC and Ion Chromatorgraphy organized by the University Science Instrumentation Center, Babasaheb Bhimrao Ambedkar University, Lucknow (Uttar Pradesh), during 18th – 20th February 2015.

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**23rd & 24th February, 2017**

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This is to certify that Prof./Dr./Mr./Ms. AJAY KUMAR, Department of Chemistry from BABA SAHEB DR. B.R. AMBEDKAR UNIVERSITY, LUCKNOW delivered an Invited/ Oral Lecture/ Presented Poster/ Participated in the National Conference on Recent Advances and Innovations in Chemical and Materials Science (RAICMS-2017) February, 23-24, 2017 held at Chandra Shekhar Azad Auditorium, JNPG College Lucknow.

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# The Sugar Technologists' Association of India

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It is hereby certified that

AJAY KUMAR

Participated in the 75th Annual Convention & Sugar Expo 2017 and

Presented Research Paper entitled

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