STUDIES ON PROSPECT OF CURCUMIN AS AN ANALYTICAL REAGENT FOR ALUMINUM

by

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
AWWA	American Waste Water Association
BBB	Blood-Brain Barrier
DME	Dropping Mercury Electrode
DMSO	Dimethyl sulphoxide
FT-IR	Fourier Transform Infrared
HPLC	High Performance Liquid Chromatography
THC	tetrahydrocurcumin
Uv-Vis	Ultra violet-visible Spectroscopy
MIBK	Methyl iso-butyl ketone
K_D	Distribution constant
K_{DR}	Distribution constant of the reagent
K_{DX}	Distribution constant of the chelate
K_f	Overall formation constant
K_i	Formation constant
β	Separation factor

KAJIAN KE ATAS PROSPEK KURKUMIN SEBAGAI SATU REAGEN ANALISIS BAGI ALUMINUM

ABSTRAK

Kajian ini melaporkan kemungkinan menggunakan kurkumin (Cur) sebagai ligan dalam pengekstrakan cecair-cecair aluminum (Al). Pelbagai parameter seperti pelarut, pH, kepekatan ligan, agen penggaraman keluar, zat aktif permukaan dan ligan sekunder telah dikaji menggunakan spectrofotometri ultralembayung-nampak.

Kompleks menghasilkan satu puncak penyerapan maksimum yang jelas di 378 nm, dengan adanya asid pikrik. Komposisi molar 1:2 Al:kurkumin telah diperolehi dalam metil iso-butil keton (MIBK) dan penimbal pH 9.00. Spektrum FT-IR membuktikan asid pikrik tidak terlibat dalam pembentukan kompleks. Analisis aluminum dalam sampel terpilih menunjukkan bahawa kaedah yang dicadang adalah setanding dengan kaedah piawai.

STUDIES ON PROSPECT OF CURCUMIN AS AN ANALYTICAL REAGENT FOR ALUMINUM

ABSTRACT

This study reported on the possibility of using curcumin (Cur) as ligand in the liquid-liquid extraction of aluminum (Al). Various parameters such as solvent, pH, ligand concentration, salting-out agent, surfactant and secondary ligand were studied by UV-Vis spectrophotometry.

The complex had a distinct maximum absorption peak at 378 nm in the presence of picric acid. The 1: 2 Al: curcumin molar composition was obtained in methyl iso-butyl ketone (MIBK) and phosphate buffer pH 9.00. The Fourier transform infrared (FT-IR) spectrum proved that picric acid did not involve in the complex formation. Analyses of aluminum in selected samples had indicated that the proposed method was comparable to the standard method.

CHAPTER 1 − INTRODUCTION

1.1 Aluminum

Aluminum is the third most common element found in the earth's crust, after oxygen and silicon. It is naturally present in soil, water and air and occurs commonly in the form of hydrated oxides which is known as bauxite ore [1]. Since aluminum is too reactive chemically to occur in nature as the free metal, instead, it is always alloyed with other elements. Aluminum had not been isolated as a metal until the year of 1825 and a further 60 years before a commercial production method was developed. The first major commercial use of aluminum was in cooking ware.

With the yield strength of pure aluminum 7-11 MPa, and aluminum alloys yield strength from 200 MPa to 600 MPa, aluminum has about one third the density stiffness of steel. Alloyed with other elements, aluminum is commonly used in the construction of siding, aircrafts, and lightweight utensils because of its good strength and light weight. While aluminum is a reactive metal, and usually occurs bound to other elements or compounds, the naturally occurring forms are stable and do not interact with living organisms [2]. Under acidic conditions, however, aluminum may be released from rocks and soils in a soluble form, which can be absorbed by plants and animals.

1.1.1 Aluminum properties

Aluminum is located in the main sub group III in the periodic table. It is a silvery white, light metal and a good conductor of heat and electricity. The physical properties of aluminum are shown in Table 1.1. Aluminum has nine isotopes, whose mass numbers range from 23 to 30. Only ^{27}Al (stable isotope) and ^{26}Al (radioactive isotope, $t_{1/2}$ = 7.2 × 105 y) occur naturally, however ^{27}Al has a natural abundance of 99.9+ %. ^{26}Al is produced from argon in the atmosphere by spallation caused by cosmic-ray protons.

Table 1.1 The physical properties of aluminum.

Physical properties	
Phase	solid
Density (near r.t.)	2.70 $g \cdot cm^{-3}$
Liquid density at m.p.	2.375 $g \cdot cm^{-3}$
Melting point	933.47 K (660.32 °C, 1220.58 °F)
Boiling point	2792 K (2519 °C, 4566 °F)
Heat of fusion	10.71 $kJ \cdot mol^{-1}$
Heat of vaporization	294.0 $kJ \cdot mol^{-1}$
Specific heat capacity	(25 °C) 24.200 $J \cdot mol^{-1} \cdot K^{-1}$

Because of the strong hydrolysis, aluminum is generally insoluble in neutral pH range. But under acidic (pH < 6.0) or alkaline (pH > 8.0) conditions, or in the presence of complexing ligands, the solubility of aluminum is enhanced.

Aluminum metal is likely oxidized, so it is always covered by an oxide film which will protect it from further oxidation [3]. And this oxide film renders aluminum stable to water even at a very high temperature. Electrochemically, aluminum has standard potential closed to the most active metals (alkali and alkaline earth metals); its normal electrode potential is -1.75V [4] in acid solution and -2.35V in alkaline solution [1]. Nevertheless, aluminum is practically insoluble in alcohol, very dilute acid or in concentrated nitric acid, owing to the presence of protective oxide film. However, the metal gradually dissolves in nitric and sulfuric acid solutions of medium concentration. Pure aluminum metal is very slowly dissolved in hydrochloric acid, but aluminum salts or alloys dissolves quite readily.

In an aqueous solution, aluminum is present in a large variety of chemical species , Al^{3+}, $Al(OH)^{2+}$, $Al(OH)^{3+}$, $Al(OH)^{4-}$ and $Al_{13}O_4(OH)_{24}(H_2O)_{12}{}^{7+}$ and the amount of each species present is pH dependent [5].

1.1.2 Determination of aluminum

Until now, a variety of methods for determination of aluminum have been reported, liquid chromatography [6], spectrophotometry [7], fluorimetry [8], atomic spectroscopy [9] and polarography [10]. At low levels, graphite furnace atomic absorption spectroscopy is used, but this is expensive and not feasible for routine analysis.

However, direct voltammetric determination of aluminum is not feasible by its rather negative redox potential. And it is partly because of the presence of neutral interferences in environment and geological samples. Additionally, such kind of samples must be suitably treated to produce a single labile aluminum species which could be easily complexed by any other ligands and reduced at mercury electrode [11, 12]. Thus, indirect voltammetric determination of aluminum, by forming chelate prior to the determination, seems to be more reasonable.

8-Hydroxyquinoline has been used to react with aluminum to form an ion associate complex which can be extracted into an organic solvent. Because the complex has absorption and fluorescent properties it can be used for determination of aluminum spectrophotometrically or fluorimetrically [13].

From the recent study, chrome azuol S [14-16] and eriochrome cyanine R [17, 18] are reported to be the two most sensitive reagents for aluminum in spectrophotometry. And chrome azuol S is employed in the differential spectrophotometric determination of aluminum. The 1:2 complex has a maximum absorption at 545 nm under the optimum pH of the medium 5.7 - 5.8. The optimum pH of the medium is not produced by using buffer solutions. The sensitivity of the method is 0.006 μg Al per ml.

1.1.3 Aluminum and health

Among all metals, aluminum has been more of concern due to its negative roles in the human life. In recent years, interest concerning of aluminum has considerably increased due to the knowledge about the potential toxic effects [19-21]. It has been estimated that the average human body contains, at most, 35 mg of aluminum, of which approximately 50% is in the lungs, and most of the remainder is in the skeleton.

Since aluminum does not appear to be an essential trace element, the body has highly effective barriers to exclude aluminum and similar metals. Only a minimal fraction of aluminum in the diet is taken up from the gut and in healthy individuals the kidneys quickly excrete most of this absorbed aluminum. The brain is vulnerable to many substances, including aluminum. There is a

‘blood-brain barrier’ (BBB), which prevent most of the toxic substances in blood from readily entering this organ [22]. As yet, the way in which aluminum penetrates the brain is incompletely understood.

Evidence accumulated in recent years concerning the neurotoxic action of aluminum as an impairment agent of the BBB function [23]. It has also been demonstrated that aluminum induces alterations of the surface anionic sites in cultured brain microvascular endothelial cells [24]. Aluminum enhances carrier-transport mechanisms without disrupting the BBB or increasing its ‘leakiness’ [25] and acting as a ‘picklock effect’ [26], i.e. aluminum may cross the BBB in many different ways.

Aluminum can accumulate both inside and outside of the vascular endothelial cells in experimental animals supporting this idea [26, 27]. So when the natural barriers which limit the absorption of aluminum are bypassed via intravenous administration, or when the ability of kidneys to excrete aluminum is impaired, accumulation of aluminum compounds in the body may occur. And the presence of aluminum in drinking water and foods has given rise to discussion of its possible adverse effects. Moreover, the effects of aluminum in the environment are highly dependent upon the chemical form in which this element enters the considered biological system.

In an aqueous solution, aluminum is present in a large variety of chemical species whose amounts are definitely pH dependent [28]. Consequently, a different pH inside or outside the cell characterizes the type or the quantity of metal uptake.

There are many common sources of aluminum in our body [29, 30]. The American Waste Water Association (AWWA) has estimated that drinking water (including treated water) provides about 5% of overall aluminum in human diets. Most aluminum consumed by humans comes from food and beverages other than plain water. This includes: inhalation (especially in certain industrial settings), soil clinging to unwashed fruits and vegetables, processed foods, baked goods, brewed drinks, over-the-counter anti-acid preparations, anti-perspirants, cooking utensils and containers. Under these conditions, people get more chances to absorb aluminum.

Currently, adverse effects of aluminum are known to be far more chronic than acute. It has been shown to be a neurotoxic compound if it is allowed to enter the bloodstream. Aluminum has also been suggested as a cause of Alzheimer's disease, Lou Gehrig's disease and other forms of senile dementia [19, 20, 31].

So far, a number of links between aluminum and Alzheimer's disease have been claimed. It has been claimed that [31] the brain content of aluminum is increased in Alzheimer's disease. And various investigations have suggested that

Alzheimer’s disease is more common in areas where the aluminum content is highest. There have also been many experimental studies on animals and on isolated cells showing that aluminum has toxic effects on the nervous system, but in almost all cases the doses of aluminum used were much higher than those occurring naturally in tissues.

At present, the health effects of aluminum are still under investigation. It is certain that aluminum has no direct, positive effects. However, there are certainly very positive, indirect effects on humans. In this aspect, prevention of toxicity is the major objective of the public policies.

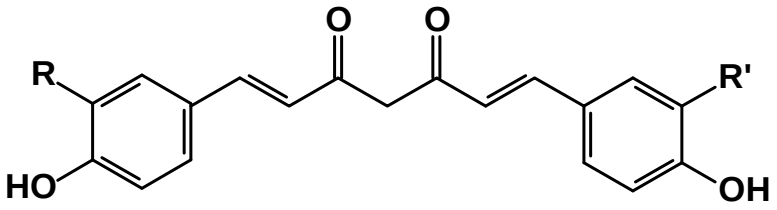
1.2 Curcumin

Natural plant products have been used throughout human history for various purposes and its role in human healthcare cannot be underestimated. In developing countries, approximate 80% of individuals depend primarily on natural products to meet their healthcare needs [32].

Curcumin ($C_{21}H_{20}O_6$), a natural product in use for thousands of years, is an active constituent of the perennial herb *Curcuma longa* (turmeric) [33]. Figure 1.1 shows the physical look of *Curcuma longa*. The main component turmeric is from the rhizome of this plant. The 2% of curcumin is extracted in 95% ethanol for 24 hours, then filter and dry.

Figure 1.1 The physical look of *Curcuma longa*.
(Adopted from: http://en.wikipedia.org/wiki/Curcuma_longa)

The yellow pigmented fraction of turmeric contains curcuminoids, which are chemically related to its principal ingredient, curcumin. The major curcuminoids present in turmeric (about 3-5%) are demethoxycurcumin (curcuminII), bisdemethoxycurcumin (curcuminIII), and the recently identified cyclocurcumin [7]. The chemical structure of curcuminoids is illustrated in Figure 1.2. The major components of commercial curcumin are curcumin I (77%), curcumin II (17%), curcumin III (3%) [34].



R, R' = OCH_3	curcumin	I
R = OCH_3, R' = H	demethoxycurcumin	II
R, R' = H	bisdemethoxycurcumin	III

Figure 1.2 The major components of curcuminoids.

Curcumin was first isolated in 1815, obtained in the crystalline form in 1870 [35, 36]. It is also known as C.I. 75300, or Natural Yellow 3. The systematic chemical name of curcumin is (1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione or diferuloylmethane. Then the feruloylmetnane skeleton of curcumin was confirmed and synthesized by Lampe [37].

Curcumin is a yellow-orange powder which is insoluble in water but very soluble in organic solvents, such as ethanol, dimethylsulfoxide, and acetone [38]. It has a melting point of 183.8°C. The maximum absorption of curcumin in methanol is at 430 nm and in acetone at 415-420 nm [39]. Curcumin appears brilliant yellow color at pH 2.5 - 7.0 and red at pH > 7.0. It can exist at least in two tautomeric forms, keto and enol, which is shown in Figure 1.3.

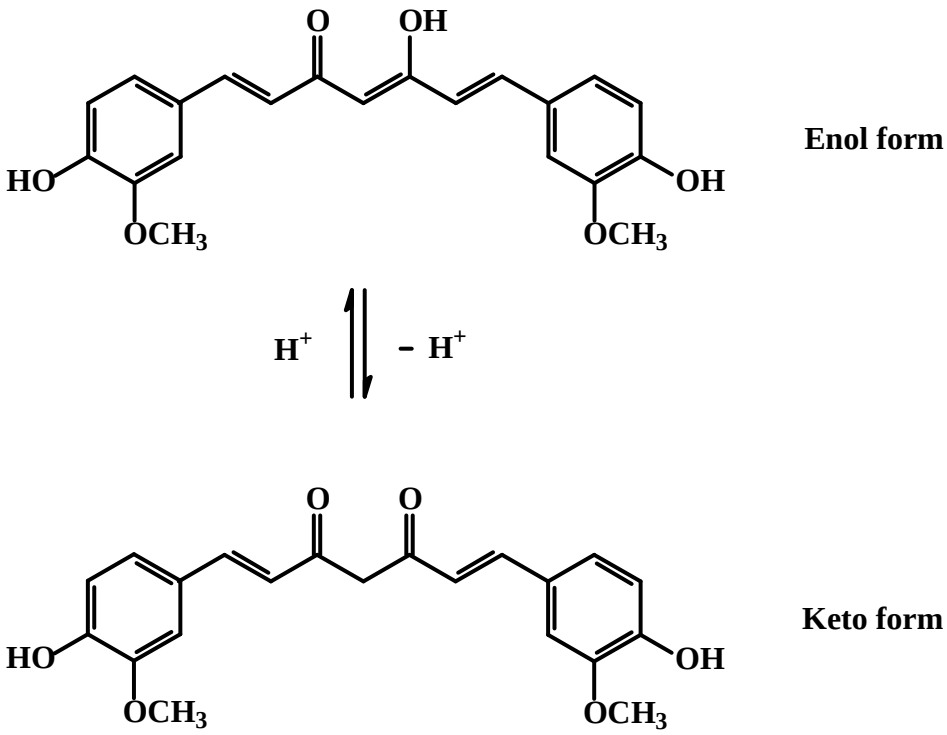


Figure 1.3 The equilibrium of keto-enol formations of curcumin.

In solutions, curcumin exists primarily in its enolic form [40], and the stability of curcumin in aqueous media increases at high pH (> 11.7) [41, 42]. By varying the pH of the solution, curcumin exists in different forms. Figure 1.4 shows the various forms of curcumin in different pH [37, 38, 40].

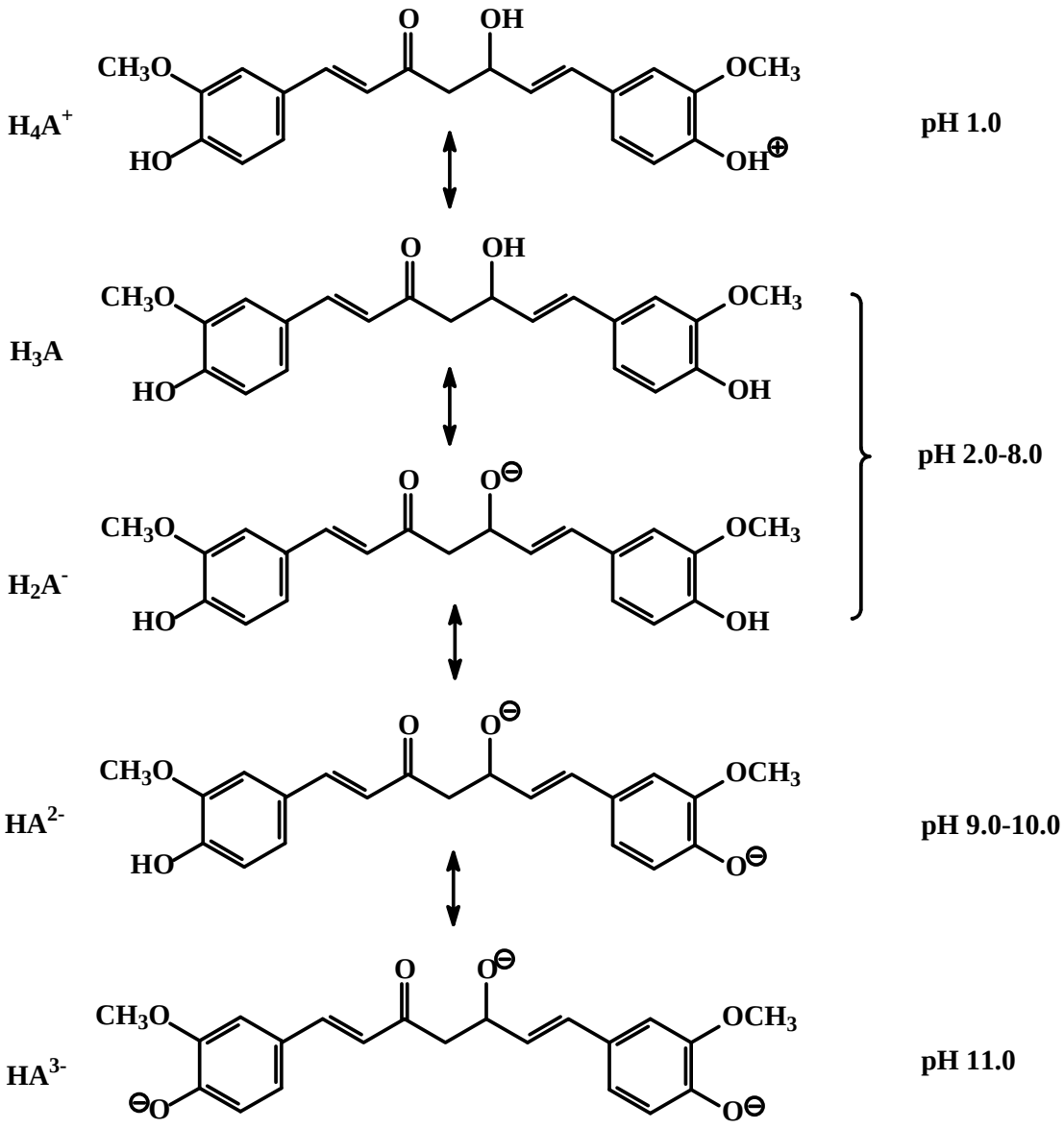


Figure 1.4 Chemical forms of curcumin in different pH values. (Ref. 37, 38, 40)

Usually, curcumin is stable at acidic pH but unstable at neutral and basic pH, under which conditions it is degraded to ferulic acid feruloylmethane [41-43]. In contrast, one of curcumin's major metabolites (tetrahydrocurcumin, or THC) is quite stable at neutral or basic pH and still possesses antioxidant activities [41-44]. Curcumin is more stable in human blood and no more than 20% of curcumin

being degraded within 1 hour and approximately 50% by 8 hours [41]. The major degradation product of curcumin is trans-6-(40-Hydroxy-30-methoxyphenyl)-2, 4 -dioxo-5-hexenal, while the minor degradation products are vanillin, ferulic acid and feruloylmethane.

1.2.1 Curcumin as a chelating reagent

Curcumin is a classical reagent for the determination of boron. The color reaction between borates and curcumin is used within the spectrophotometrical determination and quantification of boron present in food or materials [45].

Rosocyanine and rubrocurcumin are two red colored materials, which are formed by the reaction between curcumin and borates. The reaction is very sensitive in that smallest quantities of boron can be detected at 540 nm.

The formation of rosocyanine depends on the reaction conditions. The reaction is carried out preferentially in acidic solutions containing hydrochloric or sulfuric acid. The color reaction also takes place under different conditions, but in alkaline solution however gradual decomposition is observed. The reaction might be disturbed at higher pH values due to interference with other compounds.

Rosocyanine is formed as 2:1-complex from curcumin and boric acid in acidic solutions. Curcumin possesses a 1, 3-diketone structure and can therefore consider as a chelating agent. The formed boron complexes are called dioxaborines. The chemical structure of rosocyanine is demonstrated (Figure 1.5).

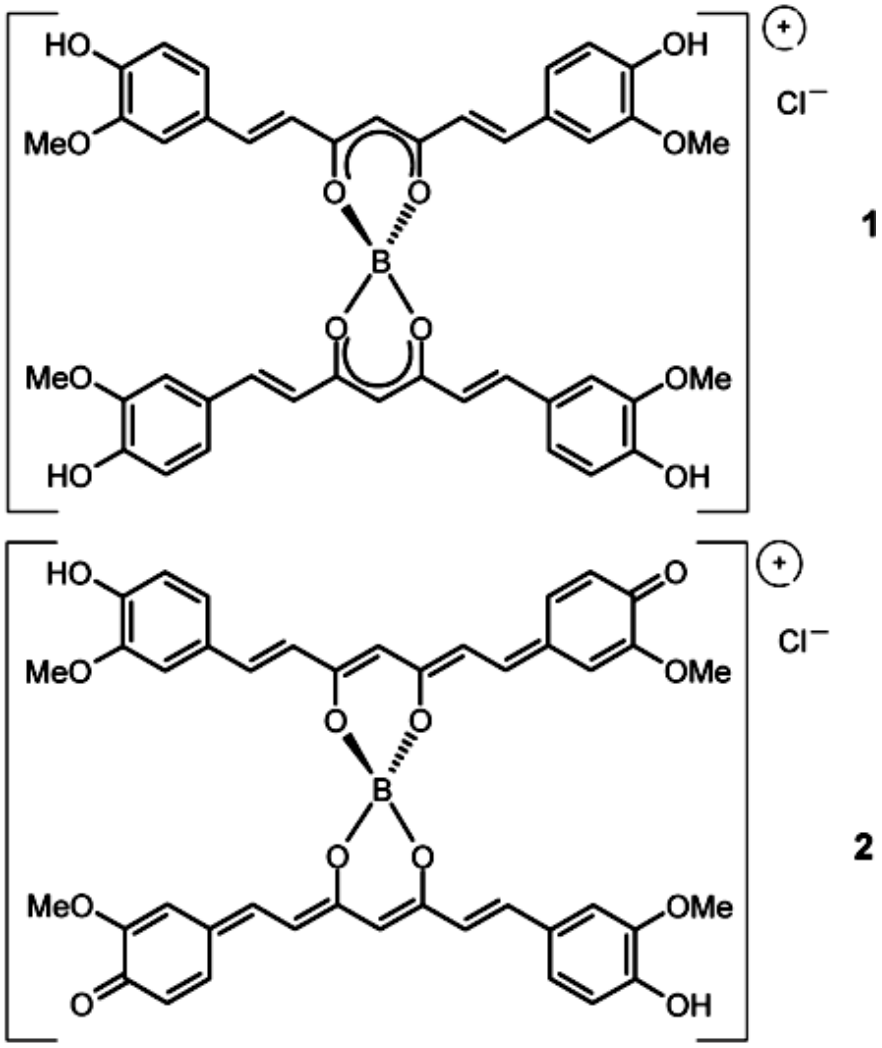


Figure 1.5 Structure of rosocyanine in two formulas.
(Adopted from: http://en.wikipedia.org/wiki/Rosocyanine)

The structural formula 1 given for rosocyanine (a cationic dicurcuminatoboron complex) is idealized. Investigations on the structure show that, the positive charge is distributed all over the molecule, and therefore a structure as indicated in formula 2 rather describes the molecule condition.

In rosocyanine the two curcumin molecules halves are not planar within the same level, but are twisted against each other.

The reaction of curcumin with borates in presence of oxalic acid produces 1:1:1 rubrocurcumin (Figure 1.6).The complex is measured spectrophotometrically at 556 nm.

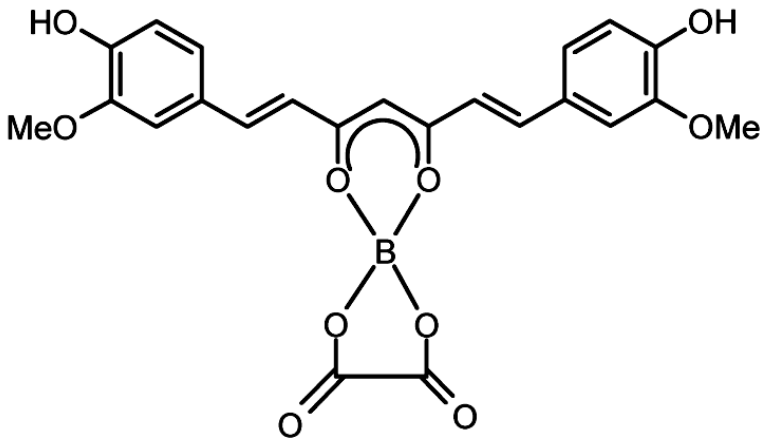


Figure 1.6 Structure formulas of rubocurcumin.

Beside boron, curcumin was also used to determine pentavalent arsenic and

beryllium by spectrophotometric [46, 47].

1.2.2 Applications

Traditionally, turmeric has been used as an additive in foodstuff, cosmetic, and medicine. It is also used as spice with the distinctive yellow color and flavor in food [44, 48].

In non-Indian recipes, turmeric is sometimes used as a coloring agent. It has found application in canned beverages, baked products, dairy products, ice cream, yogurt, yellow cakes orange juice, biscuits, popcorn-color, sweets, cake icings, cereals, sauces, gelatins, etc. It is a significant ingredient in most commercial curry powders.

1.2.2.1 Natural colorant

Turmeric (coded as E100 when used as a food additive) is used to protect food products from sunlight. In combination with annatto (E160b), turmeric has been used to color cheeses, yogurt, dry mixes, salad dressings, winter butter and margarine. Turmeric is also used to give a yellow color to some prepared mustards, canned chicken broths and other foods (often as a much cheaper replacement for sunset yellow saffron).

Pure curcumin is not ideal for direct use by the food industry as a coloring agent. Since it is insoluble in water and has poor stability towards light and heat. Light was the most destructive agent while temperature was less effective in promoting loss of curcumin color in aqueous solutions but was found to be more stable in dry powder. This factor i.e. light is one that usually limits its application in foods.

Photochemical properties of curcumin are also strongly influenced by solvent types. Additions of oxidizing agents such as sulphur dioxide, peroxide or oxygen at certain amount, reduces the stability of curcumin. The presence of SO_2 at over 100 ppm in 2% of curcumin aqueous solution reduces its color intensity.

Previous studies have indicated that several metal ions have the ability to retain the stability of curcumin towards temperature. Curcumin in water solution is stable at $\leq 80°C$ in the presence of Cu^{2+}, Zn^{2+}, Sn^{2+} and Al^{3+}. The color lasting of curcumin can also be achieved by complex curcumin with basic amino acid (e.g. L-arginine, L-lysine) and alkylamines for the purpose of stabilization in aqueous solution [49].

Modern research has found that, curcumin is not only used as spice and food coloring agent but also well known for its good therapeutic effects, including antioxidant [50], anti-inflammatory [43,51,52], anticarcinogenic and antimicrobial [53-55], hepatoprotective [55], thrombosuppressive [56],

cardiovascular (i.e., as protection against myocardial infarction) [52, 57, 58], hypoglycemic [59-61], and antiarthritic [62]. Until now, the therapeutic use of curcumin is extremely safe, and no studies in either animal [63, 64] or humans [65] have discovered any toxicity associated with the use of curcumin.

1.2.2.2 Free-radical scavengers

Free radicals are produced by the body to aid in the metabolic processes, such as digestion and the conversion of food into energy. Literally, the body can handle free radicals. They are purposefully created by the body's immune system's cells to neutralize viruses and bacteria. However, if antioxidants are unavailable, or if the free-radical production becomes excessive, damage to the body such as cancer would occur and this aggravates with age.

Generally, our bodies try to protect us from free-radical damage by producing enzymes that neutralize them. However, they are not capable of dealing this without the help of antioxidants provided by our diets. Such antioxidants are protective molecules also referred to as free-radical scavengers, helping to prevent cell and tissue damage. Antioxidants neutralize free radicals by donating one of their own electrons.

We can prevent free radical damage by supplementing our diets with antioxidants, e.g. Vitamins A, C and E other antioxidants can also work synergistically to help the body cope with free radicals, like N-acetyl cysteine, bilberry, rosemary, turmeric, green tea, grape seed, and lutein.

The turmeric plant is a powerful antioxidative and anti-hepatotoxic herb. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids which include curcumin, demethoxycurcumin and bisdemethoxycurcumin. Continuing laboratory and clinical research indicates that turmeric and its phenolics compound have unique antioxidant properties. It is a powerful ingredient for immune support. Furthermore, curcumin helps to maintain cholesterol levels already in the normal range, helps to suppress free radicals and increase antioxidants in the kidneys. Diets rich in curcumin may help explain why rates of memory loss are much lower among the elderly in India compared with their western peers [66-68]. Curcumin supports and promotes brain health which is essential to minimize dementia.

In conclusion, by consuming more food, which contains these antioxidant powerhouses, can help reduce the signs of aging and strengthen virtually all aspects of health.

1.2.3 Determination of curcumin in food products

As curcumin is an antioxidant and non-toxic, it is preferred as a preservative as compared to synthetics colorant. Some of the food manufactures get away with synthetic colorant such as sunset yellow, in place of turmeric in their products. Its quality is dubious upon times unless checked pending degradation of foodstuffs. Hence, the determination of curcumin in turmetric, curry and food, is of significant important. So far, several techniques have been developed.

The qualitative determination of curcumin in spice is done by spot test. Sample is treated with petroleum ether to remove oil. The residue is then mixed with diethyl ether and allows standing for 15 minutes. The extract is filtered and concentrated to near dryness. On clean filter paper, spot a few drop of the filtrate and is allowed to dry. After treatment with boric solution, filter paper is heated for at least 10 minutes. A characteristic rose-red color indicates the presence of curcumin [69].

HPLC method was also used for the quantitative measurement by extraction of curcumin using methanol. The compound is monitored at 420 nm. The limit of detection limit of curcumin is 2×10^{-10} g [70].

Fluorometry detection was done by extracting curcumin with 50 ml of ether, followed by back extraction with 10 ml 1% NaOH. Curcumin in NaOH phase is then re-extracted with 20 ml ether at acidic pH. Finally ether is evaporated and the residue is dissolved in 10 ml alcohol. Curcumin is determined fluorometrically using 525 nm excitation and 435 nm emission with the detection limit 0.1 ppm [71].

Another method to determine curcumin in curry and turmeric powder is by oscillopolarography technique with using three electrode system and second derivative method. Before the analysis, sample solution was treated with tartaric acids-sodium tartarate buffer, 0.01% gelatine, 0.09% boric acids solution (pH 4.5). The calibration graph was linear for 0.1 to 1.6 μg/mL and 2.0 to 12 μg/mL and the recoveries were 95.1 to 101.1% with coefficient of variation (n = 5) was 5% [72].

The electrochemical study of curcumin has been reported recently, based of cyclic and differential pulse voltammetric methods, using carbon paste and hanging mercury drop electrodes. Curcumin yields well-defined differential pulse voltammetric responses with well-defined oxidation (in the potential region of 0.3-0.6 V, vs. Ag/AgCl) and reduction (at 0.3V) peaks using carbon paste electrode [73].

1.3 Analytical techniques

1.3.1 Solvent extraction

Solvent extraction, also known as liquid-liquid extraction, is a method to separate compounds based on their relative solubilities in two different immiscible solvents. Generally, the extraction processes are, the formation of the extractable species, the partition of extractable species between two immiscible phases and the interaction occurring in the organic phase. Therefore, in order to separate two ions, it is necessary to make one electrically neutral. Thus, the extractable species, which are formed in the metal-extraction system, are realized either an electrically neutral complex (chelate) or an ion association complex.

The chelate extraction system is known to include only neutral chelates. Since the charged chelates may pair with oppositely charged extractable species through ion association, the neutral chelates are most easily extracted into organic solvents.

In solvent extraction, a distribution ratio is often quoted as a measure of how well-extracted a species is. A good extractability is obtained by controlling several parameters, such as pH control, solvent, surfactant or even synergist. It has been reported that high extractability was observed when a large amount of counter anions (picrate anion, dinitrophenolate or tetraphenylborate anions) are used [74].

The extractability is reflected by the term percent extracted, %E and the distribution ratio D. They give the equation that:

$$\%E = \frac{100D}{D + (V_w/V_0)} \tag{1.1}$$

Where, V_0 and V_w represent the volumes of the organic and aqueous phase respectively.

Among the more commonly encountered metal extractions is that involving a weakly acidic chelating agent dissolved in an organic solvent. Writing the formula for the chelating agent as HR, the case of coordination may be described by,

$$M^{n+} + nR^- \rightleftharpoons MR_n \tag{1.2}$$

Where M^{n+} is an n-valent metal ion and R^- is an anion of a suitable chelating or coordinating agent.

We can describe the above reaction involved by the following equation:

$$D = \frac{K_f K_{DX} K_i^n}{K_{DR}^n} \cdot \left\{ \frac{[HR]_o}{[H^+]} \right\}^n = K^* \left[\frac{[HR]_o}{[H^+]} \right]^n \tag{1.3}$$

Where K_f is the overall formation constant, K_{DX} and K_{DR} are distribution constant of the chelate and distribution constant of the reagent, respectively.