

# Novel and Sensitive Spectrophotometric Method for the Determination of a Cosmeceutical: Tetrahydrocurcumin

Syeda Ayesha

Department of Chemistry, Government First Grade College, Kuvempunagar, Mysore, India

## ABSTRACT:

Tetrahydrocurcumin (THC), a cosmeceutical is one of the major metabolite and reduced form of curcumin. Simple, sensitive, selective, rapid and reliable method for the spectrophotometric determination of tetrahydrocurcumin has been developed. The method is based on the reaction of THC with iron (III) and subsequent reaction with ferricyanide to yield a Prussian Blue product with a maximum absorption at 720 nm. The method obeys Beer's law. As many as ten each of anions and cations listed do not interfere.

Keywords: Tetrahydrocurcumin; Prussian Blue; Cosmeceutical; Nutraceutical

## INTRODUCTION

The resurgence for alternate medicine globally supported by safe and cost effective and more accessible health produces has resulted the emergence of new field of activities of nutraceuticals and cosmeceuticals. Nutraceuticals encompass a large group of preventive and curative health ingredients derived from herbs, especially those with a well-established use as foodstuff and the design molded on pharmaceuticals. Cosmeceuticals are nutraceuticals, which display cosmetic properties.

Tetrahydrocurcumin, a cosmeceutical is colourless reduced form of yellow curcuminoids extracted from the roots of *Curcuma longa*, commonly called turmeric root [1]. Tetrahydrocurcumin exhibits strongest antioxidant activity among curcuminoids studied in several vitro systems [2,3]. They may therefore be used in colour - free foods and cosmetic products, which currently employ conventional synthetic antioxidants such as butylated hydroxytoluene. An antioxidant used in a cosmetic application should have the capability of efficiently quenching any radicals on the surface of the skin. Tetrahydrocurcumin displays free radical scavenging ability and skin lightening actions [3]. It also displays anti-inflammatory [3] and anti-cancer activity [4]. Protective role of THC against erythromycin estolate induced hepatotoxicity has also been reported [5]. The structural studies of THC have been established [6]. The molecule is non-planar and the benzene rings positioned at the ends of heptane chain are orthogonally placed, with a dihedral angle of  $84.09(7)^\circ$  between them. The study was carried out to confirm the reports that the p-hydroxy functional groups are responsible for the antioxidant and chemo preventive action of the compound [7].

This paper is an attempt to meet an ever-increasing demand for the analytical control of commercialized health care products by developing simple, sensitive, selective, rapid and reliable spectrophotometric procedure for the determination of newly introduced cosmeceutical product. Survey of the literature revealed that no analytical method has been reported for the determination of the cosmeceutical. We report first ever spectrophotometric method for the determination of THC in pure and laboratory prepared formulation. The method is based on the reaction of THC involving the use of iron (III) salts. The reduced iron (III) in the presence of potassium ferricyanide produces a blue colour complex called Prussian Blue.

The proposed method has distinct advantages of sensitivity and stability and offers flexibility for direct and extractive spectrophotometry. Also, the method does not require heating or distillation and exhibits reliability due to reproducibility.

## EXPERIMENTAL

### Apparatus

UV-VIS spectrophotometer UVIDEK-610 type with 1.0-cm matched cell was employed for measuring the absorbance values.

### Reagents

Tetrahydrocurcumin (100mg) was dissolved in isopropyl alcohol in a 100-ml volumetric flask and made up to mark. The solution was further diluted with distilled water to get solutions of required strength. Aqueous solutions of 0.05% w/v iron (III) chloride containing few drops of 2N (v/v) hydrochloric acid and 0.05% w/v potassium ferricyanide was prepared in doubled distilled water.

### Procedures

Aliquots of standard solutions of tetrahydrocurcumin and 2.0 ml each of iron (III) chloride and potassium ferricyanide were taken in 25-ml calibrated flask. The contents were mixed well and kept aside for 10 min at room temperature. It was then diluted to 25-ml mark with distilled water and the absorbance was measured at 720 nm against the corresponding reagent blank and calibration graph was constructed. The optical characteristics for the determination of tetrahydrocurcumin are presented in Table I.

**Table 1: Spectral data for the determination of tetrahydrocurcumin**

Parameters	
Colour	Blue
$\lambda_{\max}$ (nm)	730
Stability (h)	3
Beer's law ( $\text{ng ml}^{-1}$ )	0.1-1.6
Recommended drug concentration ( $\mu\text{g ml}^{-1}$ )	0.6

Molar absorptivity ( $L \text{ mol}^{-1} \text{ cm}^{-1}$ )	$11.70 \times 10^4$
Sandell's sensitivity ( $\mu\text{g cm}^{-2}$ )	0.002
Regression equation*	
Slope (a)	0.1654
Intercept (b)	-0.0021
Correlation coefficient	0.9974
R.S.D. %**	$\pm 1.06$

\* $y=ax+b$  where x is the concentration of tetrahydrocurcumin in  $\mu\text{g ml}^{-1}$

\*\* relative standard deviation(n=5)

## RESULTS AND DISCUSSION

### Reaction mechanism

The procedure involves the reaction of tetrahydrocurcumin with iron (III) salts in the presence of potassium ferricyanide in neutral medium to produce a blue colour with maximum absorption at 720 nm. The reaction involves the reduction of iron (III) chloride by tetrahydrocurcumin to form iron (II) which subsequently reacts with ferricyanide to form a Prussian Blue product. Addition of a few drops of 2N HCl is necessary to prevent precipitation of iron (III) as hydrated ferric oxide. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law were investigated.

### Spectral characteristics

A blue product with maximum absorption at 720 nm was formed when tetrahydrocurcumin was allowed to react with iron (III) chloride in the presence of ferricyanide.

### Optimization of analytical variables

It was found that iron (III) chloride (0.05% w/v) in the range of 1.0-3.5 ml and potassium ferricyanide (0.05% w/v) in the range of 1.0-4.0 ml were necessary to achieve maximum colour intensity. Hence, 2.0 ml each of iron (III) chloride and potassium ferricyanide solutions are recommended. The order of addition of iron (III) chloride, ferricyanide and tetrahydrocurcumin was studied via the formation of the blue complex. There was no appreciable change in the absorbance or colour of the product, when the order of addition of these reactants was varied.

### Effect of solvents

Development of the coloured product was carried out at room temperature. The coloured product was stable for 3 h. Isopropyl alcohol was the preferred solvent for preparing stock solution of tetrahydrocurcumin as ethyl alcohol and methyl alcohol interfered in the development of colour. Ethyl alcohol and methyl alcohol interfered only, if added before the development of the colour. Subsequently, both the solvents do not interfere in the reaction.

Conversely, isopropyl alcohol can be used for dilution purposes. however, the use of isopropyl alcohol is discouraged, as it is more costly let alcohol and methyl alcohol. Ethyl alcohol was preferred to methyl alcohol as it is nontoxic.

### Calibration and spectral data

The blue colour complex obeyed Beer's law. The optical characteristics, such as optimum range, as evaluated from a Ringbom plot, molar absorptivity, sandell's sensitivity, slope, intercept, correlation coefficient is shown in Table 1.

### Interference

The effect of various anions and cations on the determination of tetrahydrocurcumin was studied as per the proposed procedure and the results are presented in Table 2 and 3. In general, 100mg of the salt was added individually to aliquots containing  $0.6 \mu\text{g ml}^{-1}$  of tetrahydrocurcumin.

**Table 2: Effect of anion on the determination of tetrahydrocurcumin**

Salt of the anion added	Salt added mg	% Recovery of tetrahydrocurcumin* $\pm$ RSD**
Ammonium phosphate	100	99.7 $\pm$ 0.97
Calcium carbonate	100	99.3 $\pm$ 1.08
Potassium bromate	100	101.0 $\pm$ 0.65
Potassium chloride	100	99.8 $\pm$ 0.43
Potassium iodate	100	99.5 $\pm$ 0.99
Potassium sulphate	100	99.9 $\pm$ 0.76
Sodium fluoride	100	100.9 $\pm$ 0.76
Sodium nitrate	100	98.8 $\pm$ 0.90
Sodium phosphate	100	100.9 $\pm$ 0.43
Sodium sulphate	100	99.4 $\pm$ 0.57

\* $0.6 \mu\text{g ml}^{-1}$  of tetrahydrocurcumin

\*\* relative standard deviation(n=5)

**Table 3: Effect of cation on the determination of tetrahydrocurcumin**

Salt of the cation added	Salt added mg	% Recovery of tetrahydrocurcumin* $\pm$ RSD**
Copper sulphate	100	100.8 $\pm$ 0.61
Barium sulphate	100	99.5 $\pm$ 0.76
Cadmium sulphate	100	100.7 $\pm$ 0.73
Lead nitrate	100	99.1 $\pm$ 1.02
Magnesium sulphate	100	100.2 $\pm$ 0.88
Manganese sulphate	100	99.7 $\pm$ 0.94
Potassium chromate	100	99.4 $\pm$ 0.72
Strontium nitrate	100	98.5 $\pm$ 1.02
Tin chloride	100	100.7 $\pm$ 0.53
Zinc sulphate	100	99.8 $\pm$ 0.92

\* $0.6 \mu\text{g ml}^{-1}$  of tetrahydrocurcumin

\*\* relative standard deviation(n=5)

## CONCLUSION

With increasing consumer awareness, the health care department have been interested in development of simple and sensitive methods for the assay and evaluation of health care products in bulk and dosage forms to assure high standard of quality control. The present trend is in the direction of improvement of physico-chemical methods of analysis. It is envisaged that simple methods based on spectrophotometry will become an accepted analytical tool for the assay and evaluation of health care products. The procedure described in this paper meets most of the demands of analytical chemists namely selectivity, sensitivity, simplicity, rapidity reliability and cost of analysis.

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