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Original Article

CHARACTERIZATION STUDIES ON A TETRAHYDROCURCUMIN-ZINC COMPLEX

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ABSTRACT

Objective: Preparation and characterization studies on tetrahydrocurcumin complexed with zinc, with particular reference to the location of zinc in the complex.

Methods: Structural characteristics of tetrahydrocurcumin and its complex with zinc were compared using elemental analysis, mass spectrometry (MS), proton, and carbon-13 nuclear magnetic resonance spectroscopy (NMR), ultraviolet-visible (UV) absorption spectroscopy, and Fourier transform infrared (FTIR) spectroscopy.

Results: MS data indicates a Zn molecule holds two THCur components together. NMR data provide evidence that the zinc ion is associated with the 1,3-diketone part of the linker region of the associated THCur. FTIR data is consistent with zinc interaction with the enol tautomer of the 1,3-diketone at the center of the linker region of THCur. UV data indicate that a zinc-dependent shift in absorbance maximum is consistent with changes in the structure of THCur resulting from complexation with zinc. Together, this data indicates the complexation of zinc with tetrahydrocurcumin is consistent with zinc linking two molecules of tetrahydrocurcumin together by binding to the enol forms of the 1,3-diketone moieties located in the linker regions between the aromatic rings.

Conclusion: The spectral properties of the tetrahydrocurcumin-zinc complex are consistent with a structure in which zinc is encased in two tetrahydrocurcumin moieties. Additional studies are needed to determine if this structure results in altered bioavailability, antioxidant activity and other properties important for pharmaceutical development.

Keywords: Tetrahydrocurcumin, Zinc, Complex formation, UV-Vis absorption spectroscopy, Fourier transform infrared spectroscopy, Mass spectrometry, Nuclear magnetic resonance spectroscopy

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INTRODUCTION

General health and a healthy immune system are widely believed to be maintained by supporting antioxidant levels in the body via oral antioxidants. Curcumin, from the turmeric plant (*Curcumin longa*) [1-3] is an example of a natural oral antioxidant that has been widely used as a spice in Indian cuisine for thousands of years. However, curcumin is essentially insoluble in water under acidic or neutral conditions and has poor stability towards oxidation, heat, light, alkalinity, and metabolism. Various approaches to addressing these stability issues have been used [4] as well as complexing curcumin with divalent metal cations [5-8]. Another approach has been the use of tetrahydrocurcumin (THCur), a reduced form of curcumin, which can also be complexed to divalent metal cations to produce complexes. These are expected to exhibit increased stability and may have other improved properties for health-related uses [9]. While better than curcumin for solubility, THCur still is not very water-soluble, which makes both have bioavailable issues and creates challenges for their practical uses as supplements to boost the immune system. This creates a need to develop methods to increase THCur bioavailability and stability.

Complexation of THCur with metal ions is expected to create a complex with aromatic moieties on the exterior in a way that would at least partially encapsulate the metal ion and the carbonyl moieties in the interior. This type of complex would be expected to increase stability and increase bioavailability by uptake in a fat-soluble form by an intestinal lymphatic transport mechanism [10]. The use of divalent zinc ions to complex curcumin has been reported [11], but to our knowledge, preparation and characterization of the zinc complex with THCur have not been reported. Zinc is an essential nutrient with multiple cellular functions, notably those involving gene activation in normal development and the formation of various types of immunity, including innate immunity, neutrophils, and natural killer cells [12, 13].

Zinc ions are expected to complex with THCur in a similar manner as to curcumin, despite the lack of α,β-unsaturations conjugated to the carbonyl moieties [14] (fig. 1). Enol-keto tautomerism of the α,β-unsaturated carbonyl moiety allows the formation of two or more separate coordinate bonds between a polydentate (multiple bonded) ligand and a single central metal atom. The ionic form of the central atom can be critical to the success of chelation to the ligand because divalent ions and trivalent ions do not always bind in the same manner. Curcumin is a ligand that forms stable complexes with certain metal ions and nonmetals. In general, stable structures with 1:1, 2:1 and 3:1 (ligand: metal) stoichiometry can occur. Curcumin is more stable as metal ion complexes and also has better bioavailability. The toxicity of metal ions can be reduced when complexed with curcumin [7]. THCur would be expected to form similar metal ion complexes, which would also be expected to result in greater stability and better bioavailability. The objective of the present study was the preparation and characterization of zinc ion complexed with THCur.

MATERIALS AND METHODS

THCur (95% pure) and curcuminoids (70% curcumin, 15% bismethoxycurcumin and 10% demethoxycurcumin) were purchased from Chemill Inc. (Bothell, WA, USA). Anhydrous zinc chloride and methanol were purchased from Sigma-Aldrich, St. Louis, MO, USA. All chemicals and reagents used were of analytical grade.

Preparation of tetrahydrocurcumin-Zn(II) complex

The THCur-Zn complex was prepared by mixing THCur with anhydrous zinc chloride at a molar ratio of 1:1 with both reagents dissolved in methanol. Briefly, THCur (1 mole, 372.4 grams) dissolved in 1 l methanol was added to anhydrous $ZnCl_2$ (1 mole, 136.3 grams) in 1 l of methanol, resulting in a clear solution with a measured pH of 2.0. The mixture was stirred at room temperature

for 12 h. The mixture was dried to a powder under reduced pressure on a rotary evaporator, and the residue placed in a sintered glass funnel. The dried powder was thoroughly washed with copious amounts of water to remove unreacted components. The washed complex was air dried at 50 °C until 422.2 g (82.9% yield) a dry powder was formed and analyzed for changes in the UV, FTIR, NMR, and MS profiles compared to control THCur, which was not complexed with zinc.

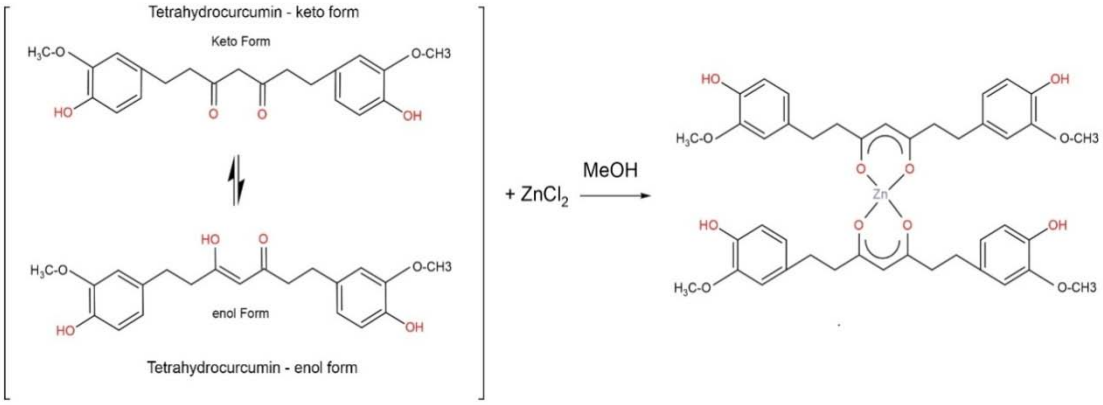

Fig. 1: The reaction that forms the tetrahydrocurcumin-Zn complex

Elemental and spectroscopic analysis

Zinc was analyzed by inductively coupled plasma atomic emission spectrometry (ICP-OES) using US Environmental Protection Agency method 6010D (SW-846) [15] carried out at Minnesota Valley Testing Laboratories (New Ulm, MN, USA). IR spectroscopy of THCur and THCur–Zn complex was performed on a Fourier transformed infrared spectrophotometer (Jasco FT/IR 4600) in KBr pellets. The spectral range was from 4000 to 400 cm-1. Ultraviolet-visible (UV–Vis) absorbance spectroscopy of THCur and THCur-Zn complex was performed on a UV spectrophotometer (Jasco V-730 Spectro-photometer) in quartz cuvettes. Samples were scanned in the range of 210-400 nm. Nuclear Magnetic Resonance spectroscopy (NMR) was carried out on a 500 MHz Bruker NMR spectrometer at NuMega Resonance Labs (San Diego, CA, USA). Mass spectrometry (MS) was carried out on a Perkin Elmer PE-SCIEX API-150 mass spectrometer equipped with an electrospray ionization source at NuMega Resonance Laboratories (San Diego, CA, USA).

Solubility studies

Solubility studies were carried out by placing triplicate samples of curcuminoids, THCur or THCur–Zn(II) at 20 mg/ml in 5 ml of 0.1N HCl (pH 1.2), phosphate buffer (pH 6.8) and phosphate buffer (pH 7.4) separately in 10 ml screw-capped glass tubes, and shaking the tubes for 12 h on a rocking shaker table at room temperature. All solutions were centrifuged and materials dissolved in the supernatants were measured using a UV spectrophotometer at the UV peak absorbance (curcuminoids at 430 nm; THCur and THCur-Zn complexes at 225 nm) with solvent as blank. Averaged relative solubility was calculated by dividing the absorbance by the g/l starting material.

RESULTS

Preparation and characterization of tetrahydrocurcumin-zinc complex

The procedure for preparing the THCur-Zn complex involved mixing equimolar amounts of $ZnCl_2$ and THCur in anhydrous methanol, evaporating, thoroughly washing the residue with water to remove unreacted reagents and then drying. The zinc content in the complex had 46% of the starting zinc remaining, consistent with that much zinc being chelated by THCur. The relative solubility of the THCur-Zn complex in aqueous media at pH 1.2, 6.8 and 7.4 was similar to uncomplexed THCur control and were considerably more soluble than curcuminoid preparations (table 1) at all pH values tested.

Table 1: Relative solubility of control tetrahydrocurcumin, THCur-Zn complex and curcuminoids at different pH values

Analyte	pH 1.2	pH 6.8	pH 7.4
Control THCur	109	110	112
THCur-Zn	110	110	108
Curcuminoids	3	4	8

Mass spectrometry of tetrahydrocurcumin-zinc complex

Both positive and negative ion mass spectra obtained for the THCur-Zn complex contained a preponderance of larger ions than in the corresponding spectra for THCur, indicating that the preparations contained a Zn ion with two associated THCur components held together (fig. 2A-D). Among the assignments in the positive ion mass spectrum for the THCur-Zn complex were *m/e* = 807 (100%) corresponding to $THCur_2\text{-}H^+ + {}^{63}Zn^{++}$, *m/e* = 809 (79%) corresponding to $THCur_2\text{-}H^+ + {}^{65}Zn^{++}$, *m/e* = 435 (66%) corresponding to $THCur\text{-}H^+ + Zn^{++}$, and *m/e* = 395 (37%) corresponding to $THCur + Na^+$. Assignments for the corresponding THCur positive ion mass spectrum were *m/e* = 807 (76%) corresponding to $THCur_2\text{-}H^+ + {}^{63}Zn^{++}$, *m/e* = 809 36(%) corresponding to $THCur_2\text{-}H^+ + {}^{65}Zn^{++}$, *m/e* = 395 (100%) corresponding to $THCur + Na^+$, *m/e* = 373 (7%) corresponding to $THCur + H^+$, and *m/e* = 355 (20%) corresponding to $THCur - H_2O + H^+$. Assignments for the THCur-Zn complex negative ion mass spectrum were *m/e* = 507 (100%) corresponding to $THCur\text{-}H^+ + {}^{63}ZnCl_2$, *m/e* = 509 (72%) corresponding to $THCur\text{-}H^+ + {}^{65}ZnCl_2$, *m/e* = 407 (39%) corresponding to $THCur + Cl^-$, *m/e* = 371 (44%) corresponding to $THCur\text{-}H^+$, and *m/e* = 171 (22%) corresponding to a ring-containing fragment from cleavage adjacent to a carbonyl. Assignments for the THCur negative ion mass spectrum were *m/e* = 507 (46%) corresponding to $THCur\text{-}H^+ + {}^{63}ZnCl_2$, *m/e* = 509 (33%) corresponding to $THCur\text{-}H^+ + {}^{65}ZnCl_2$ with other isotope combinations, *m/e* = 407 (100%) corresponding to $THCur + Cl^-$, and *m/e* = 371 (89%) corresponding to $THCur\text{-}H^+$. The control THCur mass spectra included peaks consistent with that preparation containing plant-derived metal ions, including zinc. Plants are known to accumulate zinc in their roots [16].

Mass spectrometry of tetrahydrocurcumin-Zn complex

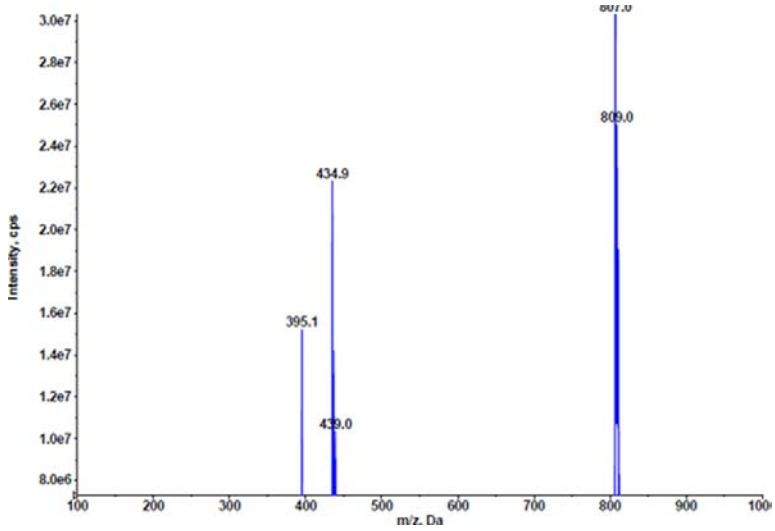

Fig. 2A: Positive ion mass spectrum of tetrahydrocurcumin-Zn complex

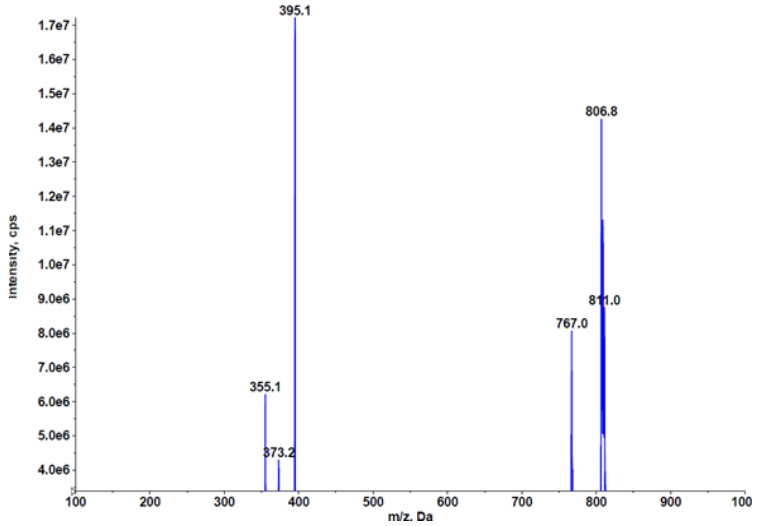

Fig. 2B: Positive ion mass spectrum of control tetrahydrocurcumin

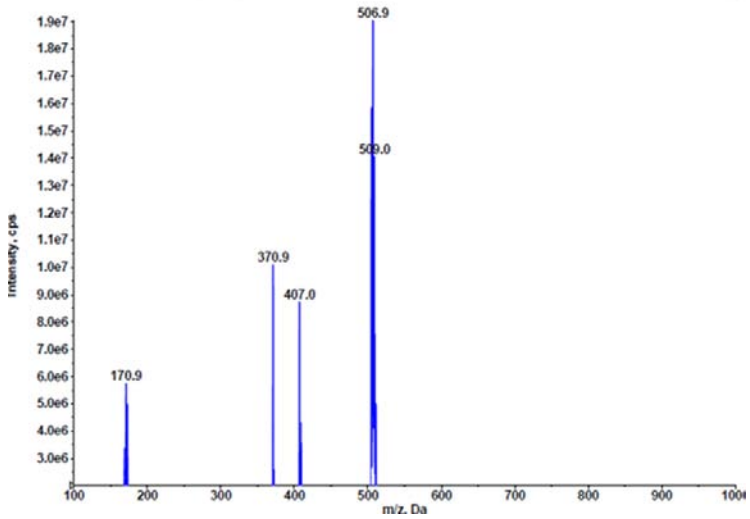

Fig. 2C: Negative ion mass spectrum of tetrahydrocurcumin-Zn complex

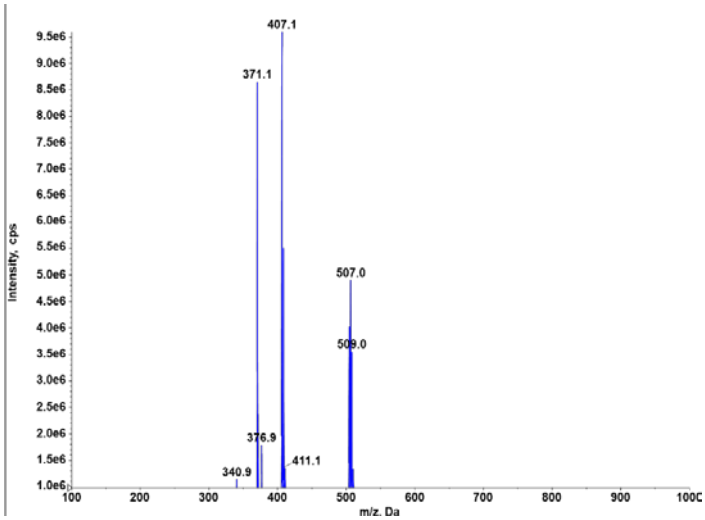

Fig. 2D: Negative ion mass spectrum of control tetrahydrocurcumin

Proton nuclear magnetic resonance spectroscopy of tetrahydrocurcumin-zinc complex

Differences between the proton NMR spectra for the THCur-Zn complex preparation vs for the control THCur provide evidence that the zinc ion is associated with the 1,3-diketone part of the linker region of the associated THCur molecules (fig. 3 as overlays). The differential proton NMR spectra show the following two notable differences between the spectra of control THCur and of the THCur-Zn complex preparation: (i) there are absorbance bands at 7.35 ppm (Ar-H benzenoid absorbances) and at 5.1 ppm in both spectra, but there is notable band broadening in the THCur-Zn complex preparation spectrum, which can be interpreted as due to slower tumbling due to the larger molecular weight associated with being complexed to a zinc ion; and (ii) there is a substantial broad singlet band in the THCur-Zn complex preparation spectrum, which has been assigned to Zn^{++}ion-associated H_2O, although unusual ROH and ArOH absorbances have not been rigorously excluded.

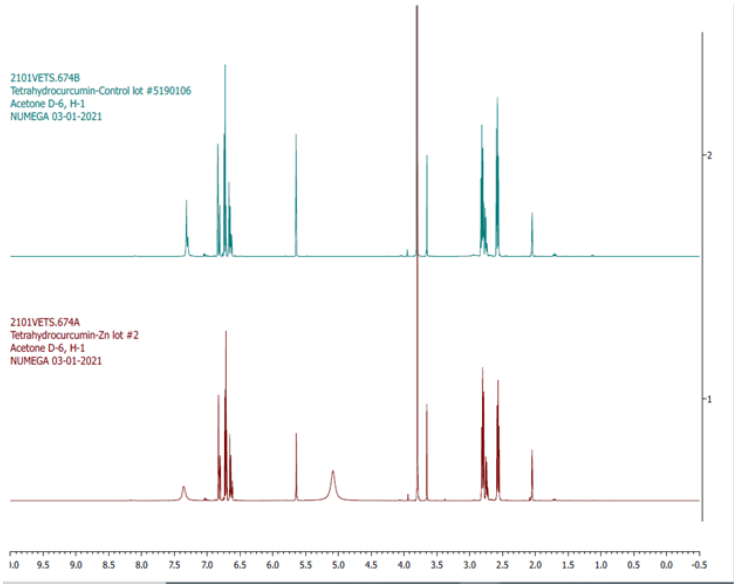

Fig. 3: Differential proton NMR of control tetrahydrocurcumin and tetrahydrocurcumin-Zn complex

C-13 nuclear magnetic resonance spectroscopy of tetrahydrocurcumin-zinc complex

The differential C-13 NMR spectra between the THCur-Zn complex preparation and the THCur control (fig. 4 as overlays) show the following two notable differences: (i) there is a shift in the absorbance for the carbonyl of a ketone in the middle of the linker between the two aromatic rings from 206.1 ppm in the control THCur spectrum to 206.7 ppm in the spectrum for the THCur-Zn complex preparation with an increase in intensity, which could be attributed to the association with a zinc ion in the THCur-Zn complex; and (ii) the absorbance at 41.0 ppm (alkyl C-H) in the THCur spectrum exhibits a decreased intensity in the spectrum of the THCur-Zn complex, which can be attributed enolization associated with zinc ion complexation at the adjacent carbonyl.

Ultraviolet absorption spectroscopy of tetrahydrocurcumin-zinc complex

An absorption maximum in the ultraviolet absorption spectrum of THCur at 283 nm was shifted to 286 nm in the ultraviolet absorption spectrum of THCur-Zn complex resulting from slight shoulder formation (fig. 5A, 5B). The zinc-dependent shift in absorbance maximum is consistent with changes in the structure of THCur resulting from complexation with zinc.

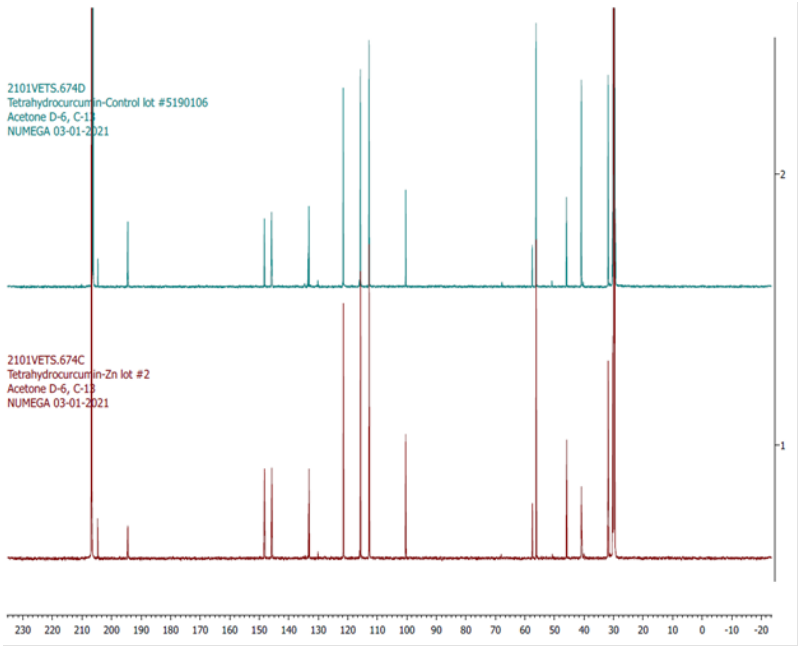

Fig. 4: Differential C-13 NMR of control tetrahydrocurcumin and tetrahydrocurcumin-Zn complex

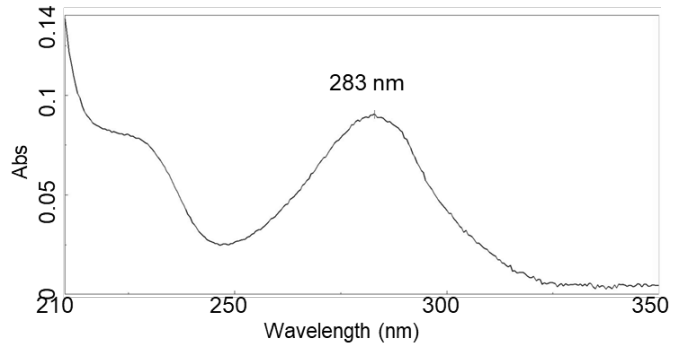

Fig. 5A: Ultraviolet absorption spectrum of tetrahydrocurcumin

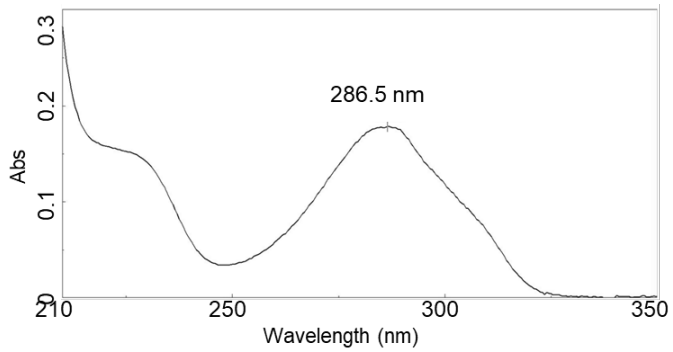

Fig. 5B: Ultraviolet absorption spectrum of tetrahydrocurcumin-Zn complex

Infrared absorption spectroscopy of tetrahydrocurcumin-zinc complex

Fourier-transform infrared (FTIR) absorption spectra were obtained for the THCur-Zn complex preparations and the starting material control THCur (fig. 6A, 6B). Changes in wavenumber and the amount and breadth of absorptions are consistent with zinc interaction with the enol tautomer of the 1,3-diketone at the center of the linker region of THCur. A redshift and deep broadening of the absorption band occurred around 3400 cm^{-1} in the THCur-Zn complex preparations, consistent with stretching vibrations of hydroxyl groups of the enol tautomer of the 1,3-diketone at the center of the linker region of THCur being altered by zinc interaction, although interaction with a phenolic OH cannot be excluded. Similar FTIR spectrum changes have been reported with curcumin when divalent cation metals bind to the enol structure [17]. A zinc-associated FTIR spectral shift of the absorption band from 1600 cm^{-1} in THCur to 1614 cm^{-1} (carbonyl stretch) in THCur-Zn also occurred, consistent

with zinc interacting with the 1,3-diketone at the center of the linker region of THCur. Additional zinc-associated FTIR spectral absorption band changes occurred at wavenumbers associated with the aromatic rings, including ~1033 cm^{-1}, 680 cm^{-1} to 860 cm^{-1}, 1116 cm^{-1}, and 800 cm^{-1} to 400 cm^{-1}, consistent with zinc-associated structural alterations affecting the aromatic rings.

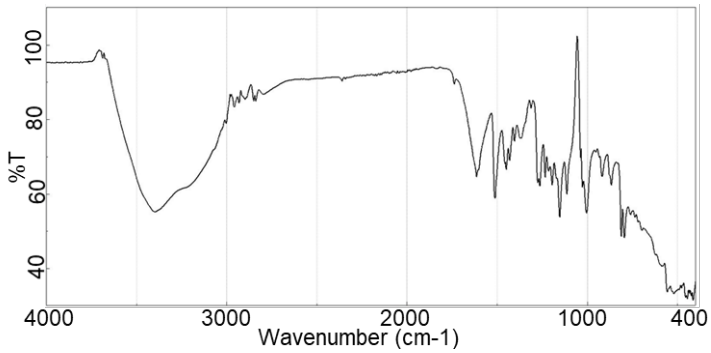

Fig. 6A: FTIR of tetrahydrocurcumin-Zn complex

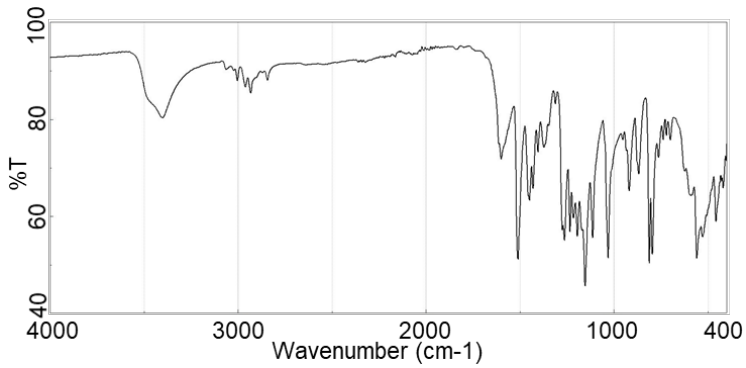

Fig. 6B: FTIR of control tetrahydrocurcumin

DISCUSSION

Formation of a complex between zinc ions and THCur in which the zinc is chelated by the enol form of the 1,3-diketone moiety in the middle of the linker between the two aromatic rings is consistent with published reports on the interaction of multivalent transition metal cations with curcuminoids [17, 18]. THCur would be expected to undergo similar chemistry because the enol form of the 1,3-diketone moiety can readily form even if it is not as favorable as in curcumin which has double bonds in conjugation with the 1,3-diketone moiety. Mass spectrometry evidence is consistent with zinc complexing with two THCur molecules. Molecular models of this type of complex suggest a structure in which the zinc forms a central core around which is an outer, relatively hydrophobic layer. This type of structure would be expected to result in enhanced solubility in oils (e. g., in olive oil) for zinc and make possible oil-based pharmaceutical formulations. A complex with this type of structure would not be expected to be absorbed into enterocytes by the transporters used in normal uptake of dietary zinc [19], but it may make possible an alternate zinc plus THCur uptake mechanism by intestinal lymphatic transport [8], when administered in an oil-based formulation. Given that this type of structure would place the oxygenated parts of the aromatic rings pointing outward at the surface of the outer shell, complexation with zinc would be expected to increase antioxidant potential relative to uncomplexed THCur, or at minimum, not reduce it. Additional studies are needed to determine if complexation with zinc results in altered bioavailability [20], antioxidant activity and other properties important for pharmaceutical development.

While the solubility of curcumin has been an issue for using it as a pharmaceutical lead molecule, THCur may offer a better lead molecule. The solubility of THCur is known to be relatively greater than curcumin or curcuminoids, which was confirmed in these studies, but the addition of zinc to the THCur did not alter the solubility of THCur under the tested conditions. Future studies on THCur-Zn will have to be undertaken to confirm the possibility of better bioavailability.

CONCLUSION

Mixing anhydrous zinc chloride with THCur in methanol yields a complex with spectral properties consistent with a structure in which two molecules of THCur are bridged by a zinc atom associated with the enol forms of the two 1,3-diketone moieties in the center of the linkage regions between the two aromatic rings. A complex of this type would be expected to retain most of the desirable properties of THCur, as well as enable novel pharmaceutical approaches in future studies.

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Nil

AUTHORS CONTRIBUTIONS

Daniel J. DuBourdieu prepared samples, results and a manuscript draft. W. Thomas Shier interpreted results and carried out manuscript revisions. Sarath Nalla carried out analysis and interpreted results. Jamil Talukdar carried out manuscript review.

CONFLICT OF INTERESTS

Declared none

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