NOTE:

Simple Green Routes for Metal-Bixin Complexes Synthesis Using Glycerol-Based Deep Eutectic Solvent

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Abstract: *Bixin is a natural dye belonging to the carotenoid group that has been reported to have many health benefits. In this work, for the first time, metal-bixin complexes were synthesized through a simple green route using deep eutectic solvent (DES) as reaction media. DES was produced by stirring choline chloride and glycerol with a molar ratio of 1:3. The metal-bixin complex was prepared by grinding and stirring bixin and divalent metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, separately) in DES at room temperature. DES formed in this work was a homogeneous colorless liquid with a freezing point below -20 °C. It has a pH, density, viscosity, and conductivity of 7.13, 148.99 cP, 1.207 g/cm^3, and 1.8 mS/cm, respectively. UV-Vis spectrophotometric analysis shows that the absorption spectra produced by the Mg(II)-bixin, Ca(II)-bixin, Zn(II)-bixin, and Cu(II)-bixin complexes show an insignificant shift in the direction of a larger wavelength (red shift) compared to pure bixin. Spectral fine structure expressed as %III/II, i.e., the ratio of the height of the longest-wavelength absorption to the middle absorption peak. The value of %III/II for bixin, Mg(II)-bixin, Ca(II)-bixin, Zn(II)-bixin, and Cu(II)-bixin are 16.77, 20.37, 17.39, and 13.52%, respectively. The results of the FTIR spectra analysis confirmed that the bond between bixin and metal ions occurred in the carboxylate acid group, indicated by a decrease in the absorption intensity at wavenumber 3389 and 1716 cm^{-1}.*

Keywords: *bixin; carotenoid; deep eutectic solvent; dye; M(II)-bixin complex*

▪ INTRODUCTION

Bixin is a carotenoid compound that can be extracted from the seeds of Kesumba (*Bixa orellana* L.), one of the most abundant and easy-to-grow plants in tropical countries, including Indonesia. As a natural dye, bixin is safe and healthy and can even provide additional pharmacological benefits to the products to which added by it [1]. Bixin is essential in cell protection and acts as an antioxidant against free radicals due to its long conjugated double-bond system [2]. Abayomi et al. [3] reported that the antioxidant activity of bixin ranged from 5.5 to 48.9%, relatively higher than ascorbic acid (2.9 to 41.5%) at concentrations between 0.25 and 2.5 g/mL, respectively. Several studies on the anti-inflammatory activity of bixin have also been reported. Bixin has been shown to reduce the inflammatory response and atherosclerotic plaque in hypercholesterolemic rabbits [4] and prevent neuroinflammation and demyelination in mice [5]. Rahmalia et al. [6-7] used bixin as a sensitizer, such as a light filter, so it has the potency to protect food products from oxidation due to light.

Regarding the poor thermo- and photostability of bixin, because of its chemical structure (Fig. 1), as well as carotenoids, several researchers have reported increased stability in many ways, such as impregnation, encapsulation, complexation, and incorporation of bixin or norbixin molecules into other materials [8-13]. Our previous research has successfully synthesized the metal-

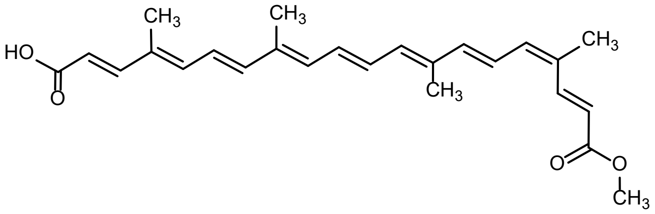

Fig 1. Chemical structure of *cis*-bixin ($C_{25}H_{30}O_4$)

bixin complex through the reflux method using conventional organic solvent [7]. This method can improve the polarity and photostability of bixin, as well as prevent clouding or loss of color from bixin, which is generally caused by the encapsulation method [12]. Color is an essential characteristic of food that adds visual appeal. Metal ions are fundamentally required to perform a series of significant biological functions in the brain, such as nerve transmission, oxygen transport, synthesis, and metabolism of neurotransmitters. Several studies have reported that metal-pigment complexes exhibit much higher antioxidant activity than pure pigments [14-15].

On the other hand, one of the highlights in green chemistry, especially for food application, is the development of techniques and procedures with low environmental and health impact, which is become very important. Most conventional organic solvents show severe risks and hazards for handling, storing, or using because they use to be flammable and explosive, and they may form compounds with high toxicity when they break down at high temperatures. Recently, deep eutectic solvents (DES) have become an alternative to conventional organic solvents. DES is a set of new solvents that may be prepared from a combination of solids compounds that become liquids when mixed and heated. They show more accessible preparation methods and lower prices. Many of them are biodegradable and compatible with biological systems. In addition, they show interesting physicochemical properties, high thermal stability, and low volatility and are compatible with water.

Research on using DES as a medium for synthesizing dye-metal complex compounds has not been published. However, due to its physicochemical properties, DES pays special attention to the role of reaction media in organic synthesis [16]. Thus, in this work, we synthesize the metal-bixin complexes using DES. DES was prepared with a simple method by stirring choline chloride with glycerol. Glycerol is a conventional solvent that is defined simply as a polyol (sugar alcohol), and it is used extensively in many industrial applications, especially in the food and pharmaceutical industries. However, the use of glycerol is limited in organic transformations due to its low solubility in organic compounds and the intrinsic reactivity of the polyol backbone, which leads to side product formation. Therefore, to overcome these disadvantages, researchers have been working to enhance the physicochemical properties of glycerol by different methods. One of these methods is preparing DESs containing glycerol as the hydrogen-bound donor (HBD) [17]. Among ammonium salts, choline chloride (ChCl) is one of the most commonly used components. It is used on a large scale in a variety of commodity products, including as an animal feed additive as a pro-vitamin. It is, therefore, biodegradable, available at low cost, has low toxicity and can be accessible from biomass [18].

The bixin complexation step with metal is carried out only by grinding the base material and stirring it at room temperature. The use of room temperature is advantageous when working with natural pigments to prevent their degradation. It can also save energy and not pollute the environment. The choice of metal is also essential to achieve the goal. In this work, some potential metals proposed to corporate into bixin are Ca(II), Mg(II), Cu(II) and Zn(II).

■ EXPERIMENTAL SECTION

Materials

Bixin extracts were obtained from previous work by Rahmalia et al. [6]. Choline chloride ($\geq$ 98%), glycerol ($\geq$ 99%), ethyl acetate ($\geq$ 99.5%), methanol ($\geq$ 99.9%), *n*-hexane ($\geq$ 99%), calcium sulfate ($CaSO_4{\cdot}2H_2O$), magnesium sulfate ($MgSO_4{\cdot}7H_2O$), copper sulfate ($CuSO_4{\cdot}xH_2O$), and zinc sulfate ($ZnSO_4{\cdot}H_2O$) were purchased from Merck.

Instrumentation

Instruments used for the research included glassware sets, oven (Memmert), hotplate stirrer (IKA

C-MAG HS 7), analytical balance (Bel Engineering M164A), spectrophotometer UV-Vis (Shimadzu 1240), and spectrometer FTIR (Thermo Scientific Nicolet iS10).

Procedure

Preparation of deep eutectic solvent

Choline chloride was dried and stored in a sealing chamber. The eutectic mixtures were formed by stirring the two components (3 g choline chloride and 5.94 g glycerol with a molar ratio of 1:3) at 50 °C and normal pressure until a homogeneous colorless liquid was formed. D.E.S. formed was analyzed its freezing point, density, viscosity, pH, and conductivity. To determine the approximate freezing point, D.E.S. was placed in a freezer in the refrigerator with a temperature of -20 °C for 24 h and observed the phase changes. The density, viscosity, pH, and conductivity values were determined at 25 °C using a pycnometer, an Ostwald viscometer, a pH meter, and a conductivity meter, respectively. The functional groups' analysis was also carried out using an FTIR spectrophotometer.

Synthesis M-bixin complexes (M=Ca(II), Mg(II), Cu(II), Zn(II))

$CaSO_4 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $CuSO_4 \cdot xH_2O$, and $ZnSO_4 \cdot H_2O$ were separately heated at 100 °C for 24 h to remove hydrates. The complex of Ca(II)-bixin was prepared as follows. As much as 0.0217 g $CaSO_4 \cdot 2H_2O$ was mechanically mixed in a mortar with 0.05 g bixin (Ca^{2+}: bixin 1/1 mol) for 15 min until a homogenous powder mixture was obtained. Then 2 mL DES was added gradually into the powder while stirring for 24 h at ambient temperature until a homogeneous concentrated combination paste was formed. Then, 5 mL of demineralized water was added to the paste until a precipitate was obtained, filtered, and the residue was dried using N_2 gas. Powder complex of Ca(II)-bixin was obtained. Other complexes derived from another ion sulfate source were prepared by the same method with the weight of $MgSO_4 \cdot 7H_2O$, $CuSO_4 \cdot xH_2O$, and $ZnSO_4 \cdot H_2O$ of 0.0311, 0.0316, 0.0227 g, respectively. The pure bixin, Ca(II)-bixin, Mg(II)-bixin, Cu(II)-bixin, and Zn(II)-bixin complexes were analyzed by a double-beam Shimadzu UV-1800 UV-Vis and a Shimadzu FTIR spectrophotometer.

■ RESULTS AND DISCUSSION

Characteristic of DES

DESs are systems formed from a eutectic mixture of Lewis or Brønsted acids and bases, which can contain a variety of anionic or cationic species. They are usually obtained by the complexation of a quaternary ammonium salt with a metal salt or hydrogen-bound donor (HBD). In this work, choline chloride was used as a hydrogen-bound acceptor (HBA) and glycerol as the hydrogen-bound donor (HBD). The procedure is straightforward and does not produce by-products, so it may be considered an environmentally friendly synthesis since there is no waste and no emissions, and the atom economy is 100% since all the initial atoms are included in the final mixture [16].

To obtain stable DES at room temperature in the liquid phase and without any sediment, the DESs were observed for 30 d; after that, the physical properties (as presented in Table 1) such as pH, freezing point, density, viscosity, and conductivity of DES formed was carried out. DES formed was colorless and transparent liquids with a pH of 7.13. The pH is an important property to consider when developing DESs, and also an actual physical property, and it impacts chemical reactions. Its pH is around 7, which indicates neutral mixtures. Hence, this mixture could be used as a media which does not affect the acidity of the reactant components.

Due to the equipment limitations, we used a freezer in the refrigerator to determine the approximate freezing points of DES. At the temperature of -20 °C in the freezer, DES is still melting in one phase, while the glycerol and choline chloride has frozen. Thus, DES formed in this work has a freezing point below -20 °C. This finding follows several previous studies. Alomar et al. [17] have reported the freezing point of DES formed

Table 1. Physical properties of DES

Parameter	Value
pH	7.13
Freezing point (°C)	< -20
Density (g/cm^3)	1.207
Viscosity (cP)	148.99
Conductivity (mS/cm)	1.8

by a mixture between choline chloride and glycerol with a ratio molar of 1:3 was 248.82 K (-24.33 °C). The melting or freezing points of the DES were lower than that of both of the individual components, where the melting point of the glycerol and choline chloride were 18 and 302.15 °C, respectively. These melting points have complied with the definition of a DES, which is a mixture characterized by significant depression in melting points to those of the neat constituent components. The charge delocalization occurring through hydrogen bonding between a halide ion and the hydrogen-donor moiety is responsible for the decrease in the melting point of the mixture relative to the melting points of the individual components [19]. In organic reactions, the capability of the solvent to remain liquid at low temperatures is sometimes crucial to minimize the side reaction pathway [18].

Density is a crucial material metric that can provide information about intermolecular interactions in a DES. It is an actual physical property due to its effect on the design and operation of processes. At 25 °C, DES has a density of 1.207 g/cm^3. It was reported that the density of most DES is between 1.0 and 1.35 g/cm^3 at 25 C. This density is higher than the water density because of the organization of molecules or the packaging of the HBA and HBD species. The formation of hydrogen bonds in DES decreases the free volume available, which increases the density [19].

Viscosity is an important property that must be addressed, especially for equipment design and fluid flow calculations. The viscosity of DES is ordinarily high (> 100 cP) [16]. The viscosity of DES formed in this work is 148.99 cP. The high viscosity of DES is related to a significant interaction of hydrogen bonds between HBA and HBD, leading to less mobility of the molecular compounds within the DES. Electrostatic van der Waals force and large ion sizes, and small empty volumes of DES might also contribute to their high viscosity. Due to their relatively high viscosity, DESs have low conductivity. DES formed in this work has a viscosity of 1.8 mS/cm, as well as reported by several studies, the series of choline chloride-based DES have viscosities lower than 2 mS/cm at room temperature.

To detect conformational changes that occur due to the presence of hydrogen bonds formed between the anion of choline chloride with hydroxyl groups of glycerol as HBD, the FTIR spectra of pure compounds, as well as DES, were obtained and analyzed. The FTIR spectra of choline chloride, glycerol, and DES formed are shown in Fig. 2. Pure choline chloride shows several functional groups, such as a vibrational band at 3220 cm^{-1}

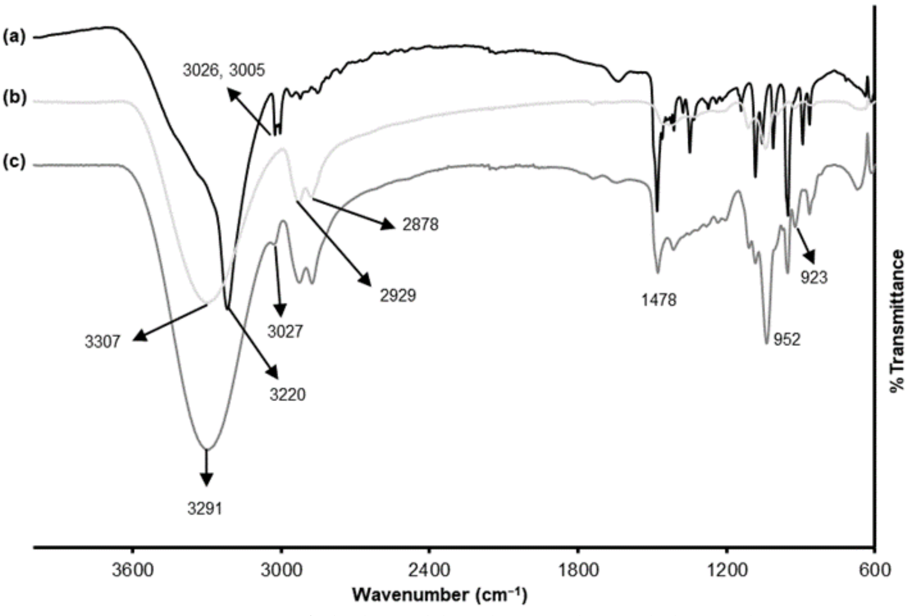

Fig 2. FTIR spectra of choline chloride (a), glycerol (b), and DES (c)

corresponding to a hydroxyl (–OH) stretching, 3026 and 3005 cm^{-1} corresponding to N–H stretching. Glycerol has a broad band between 3500-3000 cm^{-1}, referring to O–H stretching and bending. There are characteristic peaks of C–H bonds in 2929 and 2878 cm^{-1} [20].

In DES, the OH stretching mode associated with the choline chloride $OH(Ch^+)–Cl^-$ hydrogen bond at 3220 cm^{-1} seems to have disappeared. Nevertheless, characteristic absorptions originating from the $(CH_3)_3N^+$ group of choline chloride are observed at 3027 and 1478 cm^{-1}. The bands at 952 and 923 cm^{-1} are attributed to the asymmetric and symmetric stretching modes of C–N, respectively. The alcohol moiety of the glycerol delocalizes the charge of the choline chloride through interactions with the chloride ion. This phenomenon follows the hypothesis of several researchers who described the structure of glycerol and choline chloride-based DES as shown in Fig. 3 [20-21].

Characteristics of M-bixin Complexes

One of the interests of our group is to develop new “green routes” to protect and stabilize natural bixin. In this work, we investigate a complexation of bixin with divalent ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cu^{2+}, separately) by mechanical mixture without conventional organic solvents. The preparation of the metal-bixin complexes was carried out in the presence of deep eutectic solvent/DES (mixture of choline chloride and glycerol with 1:3 of molar ratio) at 25 °C. When used as reaction media, the high solubility of DES in water allows the separation of metal-bixin complexes as an insoluble layer in water. Hence, with the addition of some water that dissolves the DES, avoiding the typical extraction of organic solvents at the end of the reaction. DES could also be recovered by evaporating water from the aqueous layer to obtain the starting DES again.

Metal-bixin complexes obtained were characterized by UV-Vis spectrophotometry and FTIR methods. Fig. 4 shows the spectra of metal-bixin complexes compared to pure bixin. Based on this figure, pure bixin has three characteristic peaks at wavelengths of 486, 456, and 433 nm in the absorption spectrum that can be formed due to the existence of several vibrational levels (v_0, v_1, v_2).

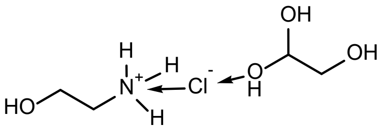

Fig 3. Structure of glycerol and choline chloride based-DES

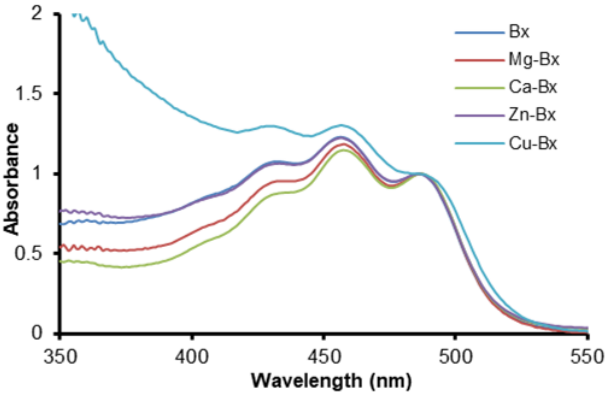

Fig 4. Absorption spectra of bixin and Metal-bixin complexes

The conjugated double bond in bixin acts as a chromophore group that can absorb light and produces strong absorption spectra at wavelengths above 400 nm. The absorption peak formed shows the electronic transition that occurred from π→π* from HOMO→LUMO. The electronic transition that can also occur in the bixin structure is n→π*, which shows the characteristic peak for the carbonyl compound [22].

The absorption spectra produced by the metal-bixin complexes show relatively overlapping spectra. In this case, there is no shift in the absorption peak in the metal-bixin complexes spectra compared to the pure bixin spectrum. This effect could be because the shape and position of the peak of the absorption spectra of carotenoids were only influenced by the chromophore group and the number of conjugated double bonds in the carotenoid structure. Metal binding to the carboxylate or ester groups of the bixin ligand will not affect the chromophore group of the ligand, so it will not affect the shape of the absorption spectra produced [23].

However, at wavelengths below 450 nm, there was a significant increase in absorption area in the Cu(II)-bixin spectrum and a slight increase in the Zn(II)-bixin spectrum compared to pure bixin. While for the spectra of Mg(II)-bixin and Ca(II)-bixin, the absorption area tends to decrease. Metal ions Cu^{2+} and Zn^{2+} include metal ions in the borderline metal group that can interact with ligands from hard or soft bases. Bixin ligands have an O atom that acts as an electron donor and can bind in coordination with metal ions as electron acceptors. Based on the HSAB theory according to Pearson [24], the O atom of the carbonyl group (CO) will act as a soft Lewis base which can then be easily bound to metal ions Cu^{2+} and Zn^{2+} compared to Mg^{2+} and Ca^{2+}, which are hard metals. The HSAB theory states that soft acids react faster and form stronger bonds with soft bases, whereas hard acids react faster and form stronger bonds with hard bases. The widening of the spectra and the increase in absorbance values indicate the increased ability of light absorption from the Cu-Bixin complex. In addition, metals with greater electronegativity will more readily accept electron pairs donated by ligands [25]. The electronegativity values of metal ions Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+}, are 1.00, 1.31, 1.65, and 1.90, respectively, so the order of metal that interacts the most with bixin is Cu(II), followed by Zn(II), Mg(II), and Ca(II).

Another characteristic of absorption spectra that can be analyzed to identify the shape of the peak produced is fine spectral structure (%III/II). The %III/II is the ratio of the height of the longest-wavelength absorption peak, designated III, and that of the middle absorption peak, designated II, taking the minimum between the two peaks as the baseline, multiplied by 100 [26]. The spectra of most carotenoids have a fine vibrational structure; they show not just a single absorption band but three more or less distinct peaks. The %III/II value should show the characteristic shape or fine structure of the spectrum [27]. The difference in the value of %III/II in the metal-bixin complexes compared to pure bixin (Table 2) indicates the interaction of the carbonyl functional group or chromophore group in bixin with metal [28].

Table 2. Spectral fine structure (%III/II) of bixin and bixin-metal complexes

Sample name	%III/II
Bixin	16.77
Mg-Bx	20.37
Ca-Bx	24.01
Zn-Bx	17.39
Cu-Bx	13.52

The bixin spectrum from the analysis using FTIR (Fig. 5) shows a band in the region of wavenumber 3389 cm^{-1}, which shows the vibrations for stretching –OH bands; at 2950, 2919, and 2852 cm^{-1} indicate bending vibrations H–C–H; 1716 cm^{-1} shows vibrations for C=O carboxylic acid; 1609 and 1436 cm^{-1} show vibrations for stretching C=C alkenes; 1379 cm^{-1} shows the bending vibration of C–H for the methyl group; 1161 cm^{-1} shows symmetric and asymmetric vibrations of the C–O–C ester group; 850 cm^{-1} shows out-of-plane wagging vibrations for C–H and is characteristic for *cis*-carotenoid isomers [22,29].

The infrared spectra produced for each metal-bixin complex show similarities but differ from the spectra produced for bixin. In the spectra of the metal-bixin complexes, the absorption band for –OH carboxylate at a wave number of 3389 cm^{-1} widens, along with a decrease in peak intensity. The C=O carboxylate absorption band also changes shape to become sharper, indicating the occurrence of a new bond on the carboxylic acid functional group. This finding suggests that the carboxylic groups of bixin ligands interact to form coordination bonds with metals [30]. The O atom in the carbonyl group donates lone pairs to the metal. The bond between the C and O atoms will get weaker, or the bond will lengthen. The longer the bond, the more vibrational energy decreases, so there will be a shift to the lower wave number [30-31].

In the bixin-Mg complex, the group of C=O shows a shift to the lower wave number, 1705 cm^{-1}. In other bixin-metal complexes, there is a shift in wavenumbers that are small or almost the same. This phenomenon can also be due to the back donation from atom O to atom C, so there is no change in the wavenumber. The C atom in the carbonyl can not only donate electrons to the ligand but also accept electrons from the metal. In addition to shifting wave numbers, the interaction between carbonyl groups in bixin and metal ligands also decreases the intensity of the carbonyl group [30].

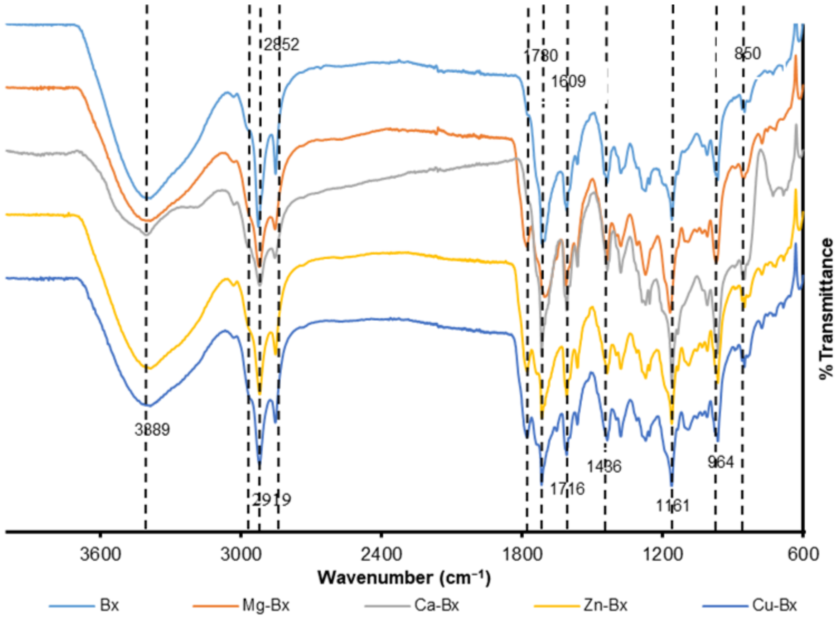

Fig 5. FTIR spectra of bixin and metal-bixin complexes

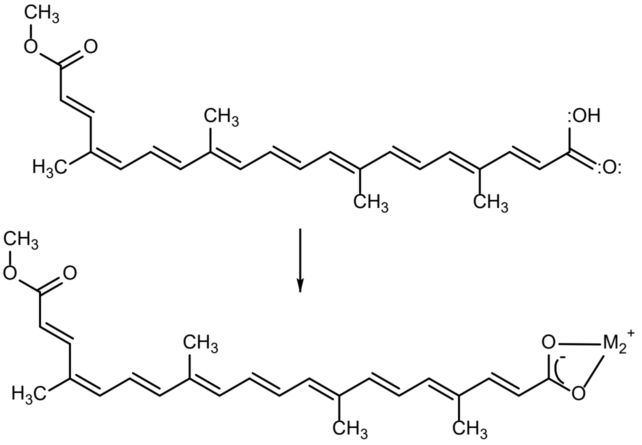

Fig 6. Proposed mechanism of M-bixin complexation

Based on the results of UV-Vis and FTIR analysis, the M-bixin complex was formed. This formation is possible because, in the bixin structure, there is a carboxylate group that can act as a ligand for metal ions. The formation of a precipitate indicates the complex formation process after a mixture of DES, bixin, and metal was added to distilled water. The proposed mechanism is presented in Fig. 6. However, stoichiometrically, the reaction is uncertain.

CONCLUSION

Deep eutectic solvents prepared by a mixture of glycerol and choline chloride have ideal characteristics as a medium for organic and inorganic The metal-bixin complex was successfully synthesized using this solvent at room temperature by grinding and stirring. This way, an environmentally friendly and nontoxic production process could be achieved.

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