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Preparation of a novel bioavailable curcuminoid formulation (Cureit™) using Polar-Nonpolar-Sandwich (PNS) technology and its characterization and applications

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ABSTRACT

Health benefits of curcuminoid are highly limited due to their poor aqueous solubility, very low systemic bioavailability, fast metabolic alterations and rapid elimination. In this study, a novel bioavailable curcuminoid formulation Cureit™ was prepared by using Polar-Nonpolar-Sandwich (PNS) technology with complete natural turmeric matrix (CNTM). The synthesized bioavailable curcuminoid formulation Cureit™ was characterizations by Nuclear magnetic resonance spectroscopy (NMR), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infra-red (IR), current-voltage (I-V) study, Quadrupole Time-of-Flight Mass Spectrometry (Q-TOF), differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA). NMR study showed the presence of hydrogen bonding interactions with curcuminoids, polar and non-polar compounds in the PNS technology. SEM images indicated that Cureit™ was almost spherical and well dispersed with rough morphology, and separated with three layers of PNS formulation. The chemical profile of Cureit™ was analyzed by Q-TOF confirmed the presence of curcuminoids (curcumin, demethoxycurcumin and bismethoxycurcumin), lactones, sesquiterpenes and their derivatives derived from polar layer, aromatic turmerone, dihydroturmerone, turmeronol, curdione and bisacurone derived from non-polar layer. IR, XRD, DSC and TGA also confirmed the presence of curcuminoids with high stability in the PNS formulation. Various biological activities of Cureit™ were also discussed.
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1. Introduction

Curcuminoid is a yellow-colored phenolic natural constituent derived from the rhizome of the spice herb *Curcuma longa* L. widely known as turmeric, and its commercially available natural form (commonly referred to as 'standard curcumin') is a mixture of three curcuminoid: curcumin (72 to 78%), demethoxycurcumin, (DMC, 12 to 18%) and bisdemethoxycurcumin, (BDMC, 3 to 8%) with a purity of ≥95%. Chemically, curcumin is a diferuloyl methane molecule [(1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dion)] containing two ferulic acid residues joined by a methylene bridge. In recent decades, curcuminoid draw great attentions for its broad spectrum of therapeutic actions, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, wound healing, hepatoprotective and potential ability in preventing neurodegenerative diseases [1–4].

Besides, it has a superior safety profile determined by clinical study that as high as 8 g/day of dosage would not induce any observable adverse effects [5]. This safe profile has been reflected by the continuous increase of preparations based on curcuminoid marketed as a food ingredient or constituent of dietary supplements. However, the functional applications of curcuminoid have been seriously limited by its very low systemic bioavailability, attributable to poor absorption, fast metabolic alterations and rapid elimination [6,7]. Available evidence indicates that only minute amounts of curcuminoid reach the circulation after high-dose oral administration in animals and humans. The majority of the orally administered curcuminoid is excreted in the feces and the urine, with very little being detected in the blood plasma [8]. Curcuminoid has very low solubility in aqueous media due to inter and intra hydrogen bonding [9]. Higher solubility was observed in alkaline solution, when dissolved curcuminoid was quickly degraded into vanillin, ferulic acid, and feruloyl methane [10]. Other environmental factors like UV irradiation and heating also contribute to decomposition of this yellowish polyphenolic compound [11–13]. These dramatically affect the absorption and bioavailability of this active molecule with

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consequent unsatisfactory pharmacokinetic profile and reduced efficacy.

To increase its water solubility, stability, bioavailability and potential applications, different methods have been proposed and investigated. Several strategies such as nanoparticles, liposomes, solid dispersions, solid lipid nanoparticles, microemulsions and complexation with phospholipids and cyclodextrins have been developed to improve the bioavailability of curcumin/curcuminoid [14–18]. Phospholipid complexes (phytosomes), which increased the area under the blood concentration-time curve (AUC) of curcumin after oral administration in rats by 5 times [19]; association with cyclodextrin, with a ten-fold increase in curcumin AUC [20]; BCM-95 extract, whose bioavailability in rats was 7.8 times higher than unformulated curcumin [21]; mixing of curcumin with an essential oil obtained from standardized turmeric where AUC was 7–8 times higher than unformulated curcumin [22]. Most of the best performing curcumin formulations produced so far has provided no more than a ten-fold increase bioavailability compared to unformulated curcumin [23].

Nevertheless, it is notable that most of the nano-delivery systems are not readily suitable for food, drug and related applications due to their inherent demerits. Whilst each of these novel delivery strategies offers significant promise, there are still limitations to their potential use in food/medicine. In addition, most of these technologies are not able to accommodate high loading of curcumin/curcuminoid, thus limiting the bioactivity of the finished products. Most of the delivery systems have limited application for use as a powder formulation as their stability will be affected when converted to powder. Moreover, micelle, microemulsion and liposome complexes might be degraded in the stomach before reaching their targeted sites, hence compromising the bioavailability of the active ingredient. In this regard, natural matrix based formulations without sophisticated fabrication and chemical modification have been investigated for delivering curcumin/curcuminoid.

In the present study, Aurea biolabs developed the bioavailable curcuminoid – "Cureit™" based on the recreation of the complete natural turmeric matrix (CNTM) with active curcuminoids (~50%) by a method known as Polar-Nonpolar-Sandwich (PNS) technology, a patent pending formulation. The PNS technology can be used to preserve functional properties, improve the stability of compounds, enhance health benefits, control the release of bioactive compounds at desired time and specific target, and increase the bioavailability of bioactive compounds. The PNS technology is one of the most promising techniques among various techniques used to improve the dissolution of poorly soluble curcuminoid. This is because it is simple, cost effective and commercially attractive for industrial production. The characterizations of Cureit™ are furthermore analyzed by Nuclear magnetic resonance spectroscopy (NMR), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infra-red (IR), current-voltage (I-V) study, Quadrupole Time-of-Flight Mass Spectrometry (Q-TOF) and (thermogravimetric analysis) TGA.

Recently our research group has been studied and reported the various biological activities of Cureit™. An anticancer study was carried out by our research group on the cytotoxicity demonstrated by a spectrochemical study using MTT assay on the effect of Cureit™ on cell proliferation such as growth kinetics of MCF-7, LnCAP and HEK 293 T cells. The study clearly revealed that Cureit™ could serve as a good anticancer medication [32]. A cell culture study of the elastase inhibition activity of Cureit™ was measured. The study showed the Cureit™ showed potent elastase inhibiting activity at 100 mg/L concentration. It was observed from the co-culture study of melanocytes and keratinocytes that the cell integrity, morphology and functionality were intact by the Cureit™. Its elastic inhibiting activity in human cell lines inferred that Cureit™ can inhibit elastases activity at high concentration [33]. Another study was conducted on the antioxidant property of Cureit™ by DPPH free radical scavenging activity method. The antioxidant potential of Cureit™ was equivalent to that of ascorbic acid, as a result, it could be a good source of natural antioxidant [34].

Our research group has done a pilot crossover study to assess the human bioavailability of Cureit™. Twelve human healthy male adults in the age group of 18–45 year old, medically healthy were involved in this study. The study compared the bioavailability of Cureit™ in the blood plasma with respect to the control. The safety and tolerability of a single dose of Cureit™ is also established. The study demonstrated that the bioavailability of Cureit™ was 10 fold higher than the 95% curcuminoid as measure by C_{max} as well as AUC data [35]. The effect of Cureit™ on anti-aging study was exhibited by our earlier study with hyaluronidase inhibition through cell culture study. Cureit™ was inhibiting hyaluronidases up to 42%. This confirmed that the Cureit™ could be a useful anti-aging medication [36].

2. Materials and methods

2.1. Materials

The turmeric raw material was purchased from local market and the raw material was subjected to quality analysis. The approved raw material was then subjected to crushing. The crushed raw material was further subjected to extraction using food grade ethanol as solvent. The extract obtained was subjected to crystallization to produce curcuminoid crystals of 95% purity. The residue after extraction of curcuminoid was subjected to aqueous extraction to obtain the polar fractions of turmeric. Organic solvents were of HPLC and LC-MS grade and were purchased from Merck India. Millipore-MilliQ distilled water was employed during the complete study.

2.2. Physical parameters

2.2.1. Water solubility index (WSI) and bulk density

The water solubility index of curcuminoid and Cureit™ was determined using the method described by Anderson et al. [37]. Each sample (2.5 g) and distilled water (30 mL) were vigorously mixed in a 100 mL centrifuge tube, incubated in a 37 °C water bath for 30 min and then centrifuged for 30 min at 10,000 rpm. The supernatant was carefully collected in a pre-weighted Petri dishes and oven dried at a temperature of 103 ± 2 °C. The WSI (%) was calculated as the percentage of dried supernatant with respect to the amount of the original 2.5 g curcuminoid and Cureit™.

Bulk density (g/mL) was determined by adding 10 g of sample into an empty 100 mL graduated cylinder and place the cylinder on a ring stand. The ring stand was adjusted so that, when the base of the cylinder is raised to touch the ring, the bottom surface of the cylinder is exactly one inch from the base of the ring stand. The ratio of the sample mass and the volume occupied in the cylinder determines the bulk density values [38].

2.2.2. Color measurements, moisture content and hygroscopicity

Color measurements of the samples were determined by following ASTA 20.1 method with suitable modifications [39]. Briefly, 0.1 g of the sample was weighed and transferred quantitatively to 100 mL amber colored volumetric flask and the volume was made up with acetone. The samples were shaken and left in a dark room at room temperature for 16 h for color extraction. The absorbance was measured using a spectrophotometer (Shimadzu UV-1800, Japan) at 420 nm wavelength using the solvent acetone as blank. The moisture content was determined based on the AOAC method [40]. Triplicate samples of curcuminoid and Cureit™ (20 mg) were weighed and then dried in a vacuum oven at 70 °C. The drying and weighing processes were repeated until constant weight was obtained.

Hygroscopicity analysis of Cureit™ and curcuminoid was determined by spreading 1 g of the powder evenly on Petri dishes to allow for high surface area between humid air and powder. Each powder

sample in the dishes were placed in a desiccator under the conditions of 23 °C and 76% relative humidity using nitric acid solution. The gain in weight of the samples was considerably lower after 90 min. Although hygroscopicity is based on the equilibrium moisture content, to compare hygroscopicities, the weight increase per gram of powder solids after being subjected to the atmosphere with relative humidity of 76% for 90 min was determined [41,42].

2.2.3. Degree of caking (DC)

The samples were placed in a drying oven at 70 °C. After cooling, the dried samples were weighed and transferred into a sieve of 500 mm size. The sieve was then shaken for 5 min in a shaking apparatus. The weight of the powder remaining in the sieve was measured. The degree of caking was calculated as

$$DC = \frac{a}{b} \times 100 \quad (1)$$

Where DC is the degree of caking (%), 'a' is the amount of the powder used in sieving, and 'b' is the amount of the powder remained on the sieve after sieving [43].

2.3. Characterization and analysis

Chemical composition of Cureit™ was analyzed by Xevo G2-S Q-TOF (Waters Corporation, Milford, USA) via the direct infusion method. Analysis conditions were set at an infusion flow rate-10 μL/min, capillary voltage-3 V, cone voltage-60 V, source temperature-120 °C, Desolvation temperature-500 °C, desolvation gas flow-800 L/h, cone gas flow 60 L/h and ESI modes-both positive and negative. ^{1}H and ^{13}C NMR spectra of both curcuminoid and Cureit™ were recorded by Bruker Avance III 400 MHz, Switzerland, using DMSO-d_6 as a solvent. Fourier transform infra-red (FT-IR) spectra of curcuminoid and Cureit™ were recorded by JASCO FT/IR-460 plus instrument in the range of 4000 to 400 cm^{-1} with 32 scans per samples. Samples of curcuminoid and Cureit™ were fabricated on scanning electron microscopy (SEM) aluminum stubs with double side carbon tape and sputter coated with gold. Surface morphology images were captured with SEM (Vega3Tescan, Germany). Curcuminoid and Cureit™ were tested for the direct current (DC) electrical characteristics using Keithley SCS-4200. The crystalline natures of the curcuminoid and Cureit™ were determined using X-ray diffraction (XRD) (Xpert-Pro). Thermograms of curcuminoid and Cureit™ were recorded using differential scanning calorimeter (DSC) Q10 DSC instrument (Mettler Toledo DSC822e, India). Thermal stability of the Cureit™ was analyzed by thermogravimetric analysis using Thermal Analysis System. Analysis was performed in N_2 gas atmosphere with a flow of 20 mL/min and a scanning rate of 10 °C/min from 725 °C.

3. Results and discussion

3.1. Preparation method of Cureit™

The design of the product, Cureit™ is a very smart design to develop an efficacious and potential bioavailable curcuminoid through the application of the natural physicochemical properties of curcuminoid towards the molecular physiology of the intestinal absorption of a xenobiotic. An orally consumed molecule has to land up at the inner intestinal wall and should pass through the barriers of the cell membrane, before it is to be available in the blood stream for the purported biological action. The molecule needs to be soluble inside the gastrointestinal tract for reaching the inner intestinal walls and permeable through the lipid bi-layer of cell membrane. As curcuminoid is a

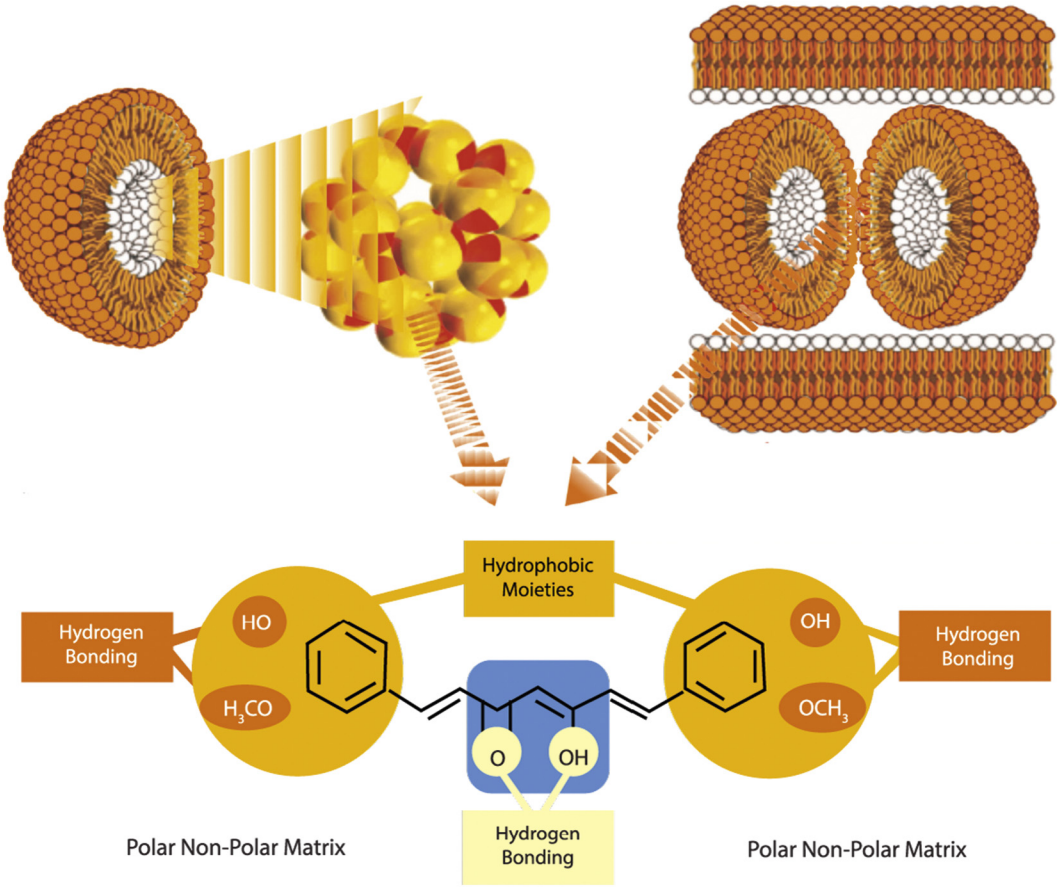

Fig. 1. Schematic representation of the Polar-Nonpolar-Sandwich technology design of Cureit™.

Table 1
Physical properties of curcuminoid and Cureit™.

Properties	Curcuminoid	Cureit™
Water solubility index (%)	1.3 ± 0.5	38.56 ± 2.45
Bulk density (g/mL)	0.45 ± 0.08	0.35 ± 0.04
Color measurement (ASTA color unit)	14,310 ± 16	4019 ± 11
Moisture content (%)	0.8 ± 0.2	4.2 ± 0.8
Hygroscopicity (g/g)	0.12 ± 0.04	0.49 ± 0.13
Degree of caking (%)	3.45 ± 1.2	21.84 ± 1.95

Values are means ± SD of three independent determinations.

hydrophobic molecule, it cannot dissolve easily in the intestinal tract and also it cannot easily pass through the cellular membrane due its larger structure. The unique product Cureit™ - the bioavailable curcuminoid developed by Aurea Biolabs Pvt. Ltd., Cochin, India, was designed to retain curcuminoid as a single free molecule, inside a turmeric matrix created ex-situ. The turmeric matrix was recreated by extracting three different entities: curcuminoid, turmeric essential oil and water extract of turmeric. Curcuminoid with 95% purity was extracted from dried turmeric rhizomes, using food grade solvent-ethanol and the obtained oleoresin crystallized to get curcuminoid powder. Essential oil was separated by steam distillation. The powdered turmeric was extracted with water to get the carbohydrates (~40%), dietary fiber (~5%) and turmerin protein (~2%). The water soluble protein-turmerin is more efficient to cross over the lipid bilayer. These three components are combined together through a unique process of Polar-Nonpolar-Sandwich (PNS) technology, and the curcuminoid is well protected as a single molecule inside this matrix. The bioactive molecules present in the Cureit™ other than curcuminoid play an important role in the bioavailability of curcuminoid rather than the curcuminoid itself. The bisabolanes and sesquiterpenes present in the Cureit™ help the curcuminoid to make a nonpolar sandwiched matrix while, the water soluble proteins and the carbohydrates make the polar matrix. The Cureit™ also retains the advantages of traditional modified systems such as enhanced physical stability, protection of drug molecules from degradation in the body, controlled drug release, biocompatibility and laboratory to industrial-scalability. The composition of the product has been standardized and a continuous quality control program is initiated to monitor and maintain the quality standards for the product. The PNS technology allows curcuminoid to be delivered to the intestinal walls and pass through the cell membrane by simple diffusion through enhanced solubility and absorption. The schematic representation of the PNS technology design is depicted in Fig. 1.

3.2. Physical parameters

3.2.1. Water solubility index and bulk density

Solubility of bioactive compounds in water is an important physical parameter that affects their absorption and bioavailability. Therefore, the water solubility index of curcuminoid and Cureit™ was determined and the values are given in Table 1. Cureit™ showed significantly higher solubility behavior than the curcuminoid in water. Greater solubility of

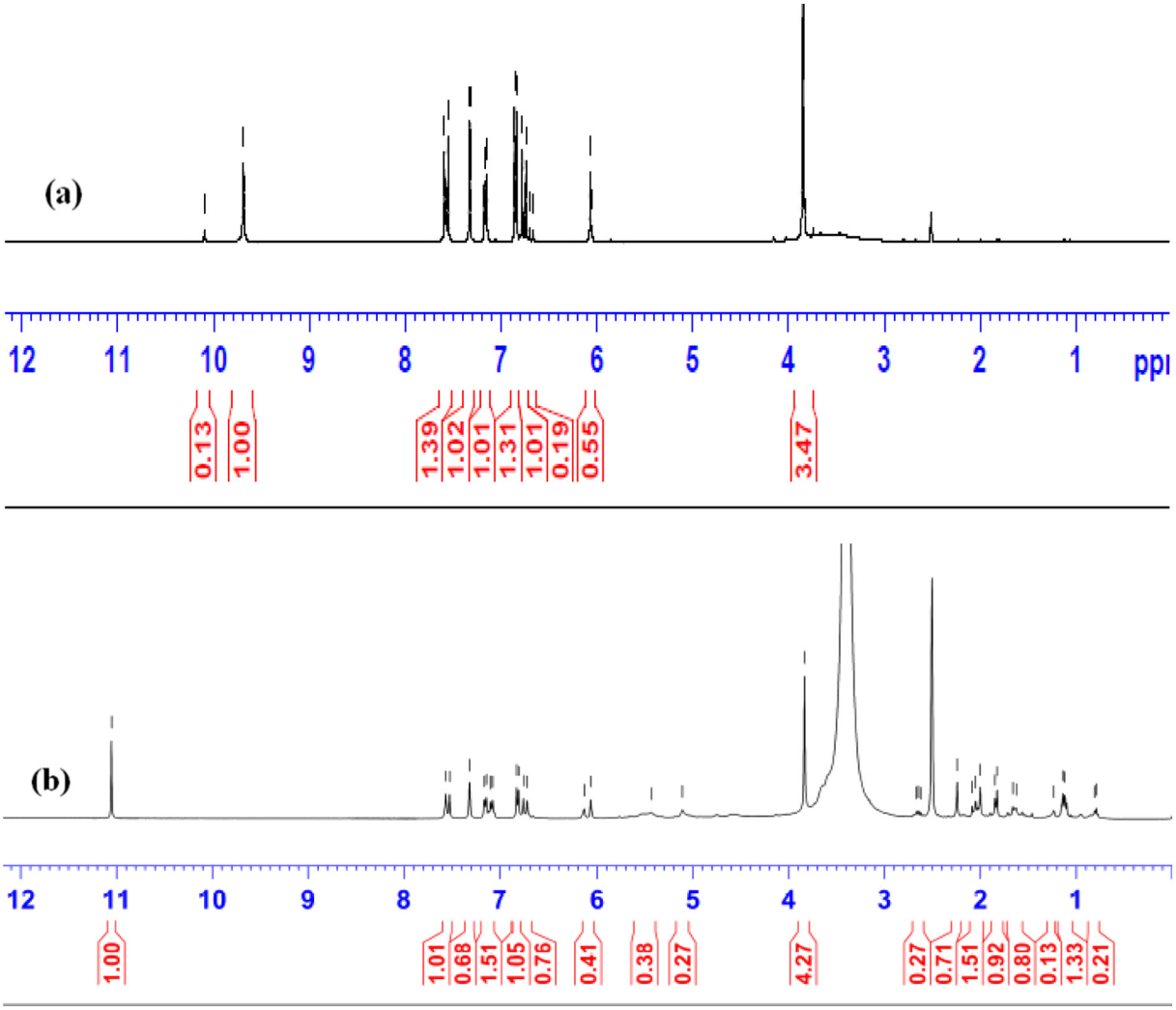

Fig. 2. ^{1}H NMR spectra of (a) curcuminoid and (b) Cureit™.

Cureit™ may be due to the presence of curcuminoid between the polar and non-polar matrix with PNS technology in this unique bioavailable formulation. The bulk density is a significant characteristic of powder/granule products in term of storage and simplicity of transportation. The observed lower value in bulk density of Cureit™ is because the polar fraction contains high molecular weight dietary fiber, carbohydrate and water soluble protein which are apparently bigger than the curcuminoid composition and other non-polar fractions. Cureit™ registered lower bulk density value (0.35 g/mL) than curcuminoid (0.45 g/mL) but higher in the water solubility index. An increase in soluble content decreased the bulk density. This shows an inverse relation between the bulk density and solubility [44].

3.2.2. Color measurement, moisture content and hygroscopicity

The color measurement value for curcuminoid (14310 ASTA color unit) was 3.6 times higher than the Cureit™ (4019 ASTA color unit) when acetone was used as solvent. The lower color measurement value of Cureit™ could be attributed the presence of 50% of curcuminoid combined with other polar and non-polar turmeric matrix. The pure curcuminoid was highly soluble in acetone, hence higher color measurement value was obtained. The moisture content of curcuminoid and Cureit™ was found 0.8% and 4.2% respectively. Higher moisture content obtained for the Cureit™ than the curcuminoid is attributed to the presence of polar fraction in the Cureit™. The higher hygroscopicity result of Cureit™ (0.49 g/g) than the curcuminoid (0.12 g/g) also agrees well with this result.

3.2.3. Degree of caking

Degree of caking was higher in the case of Cureit™ (21.84%) than the curcuminoid (3.45%). Moisture absorbs on particle surfaces formed saturation with moisture and thereby making the particle sticky and capable of forming liquid bridges. Degree of caking is a direct relationship with moisture content and hygroscopic nature of the product. Moreover the degree of caking could be comparatively increased depending upon the presence of high molecular weight substance like dietary fiber, carbohydrates and water soluble proteins [42]. The presence of turmeric dietary fiber in the Cureit™ is the main factor for the enhance degree of caking property.

3.3. Chemical analysis and characterization of Cureit™

The chemical analysis of Cureit™ with Q-TOF was done to obtain the molecular profile of Cureit™. The identity and the presence of curcuminoid viz., curcumin, demethoxycurcumin and bisdemethoxycurcumin were confirmed by mass spectra (Fig. S1). The mass spectra of non-curcuminoid fraction, present in the Cureit™ has shown in Fig. S2. The spectra distinctly demonstrate the presence of sesquiterpenes and their derivatives, and lactones in polar layer. Presence of aromatic turmerone, dihydroturmerone, turmeronol, curdione and bisacurone in the spectra indicated the occurrence of these compounds in non-polar layer of Cureit™. This analysis clearly confirmed that the Cureit™ is completely designed by the polar-nonpolar fractions of turmeric with curcuminoid.

The NMR study was investigated to clarify the PNS technology of the Cureit™ (Fig. 2). For curcuminoids (Fig. S3) ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H), 9.69 (2H, OH,s), 7.57 (2H, d, J = 15.6 Hz), 7.32 (2H, s), 7.15 (2H, d, J = 8.4 Hz), 6.84 (2H, d, J = 8.4 Hz), 6.74 (2H, d, J = 16.0 Hz), 6.05 (1H,s), 3.83 (OCH_3, 6H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 183.17, 149.32, 147.97, 140.68, 126.34, 123.05, 121.07, 115.91, 111.34, 100.89, 55.65 (Fig. S4). For Cureit™ (Fig. S5), ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.05 (s, 1H), 7.54 (2H, d, J = 15.6 Hz), 7.31 (2H, s), 7.15 (2H, d, J = 8.0 Hz), 6.83 (2H, d, J = 8.0 Hz), 6.75 (2H, d, J = 16.0 Hz), 6.07 (1H, s), 3.84 (OCH_3, 6H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 183.15, 149.50, 148.01, 140.67, 126.21, 123.10, 120.99, 115.83, 111.31, 100.82, 55.66 (Fig. S6).

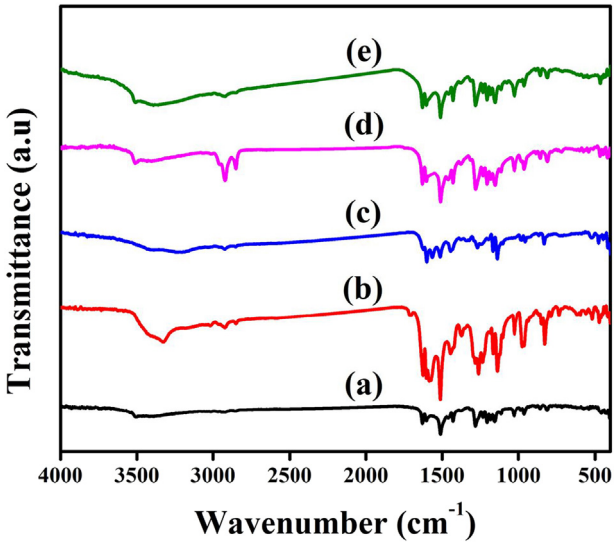

Fig. 3. FT-IR spectra of (a) curcumin, (b) demethoxycurcumin (DMC), (c) bisdemethoxycurcumin (BDMC), (d) curcuminoid and (e) Cureit™.

^{1}H NMR spectrum of curcuminoid (Fig. 2(a)), shows a sharp singlet peak at 9.69 ppm, indicating the presence of hydroxyl groups while a small singlet peak at 10.09 ppm indicates the presence of intramolecular H-bonding in curcuminoids [24]. In Fig. 2(b) a new signal at 11.05 ppm was observed, due the shifting of the peaks at 9.69 and 10.09 downfield. This is attributed to the presence of strong interaction between the –OH group of curcuminoids viz., phenolic –OH and intramolecular/enol form of –OH, and polar and non polar environment. Moreover, the interaction of polar and non polar entities with the hydrogen of –OH group deshielded the electron density in the hydrogen. This NMR data are clear evidence for the presence of hydrogen bonding interactions with curcuminoids, polar and non polar compounds. The presence of other peaks in Fig. 2(b) from 2.67 ppm to 1.12 ppm indicate the existence of other polar and non polar compounds in the PNS technology.

The FT-IR spectra of curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), curcuminoid and Cureit™ are shown in Fig. 3(a–e) respectively. The bands at 1510 and 1428 cm^{-1} are due to the stretching vibrations of C—C of the benzene ring and olefin bending vibration of the C═C group bound to the benzene ring respectively [25]. The peak at 1629 cm^{-1} is attributed to the carbonyl (C═O) stretching of the conjugated ketone. The sharp absorption peaks at 1602 cm^{-1}

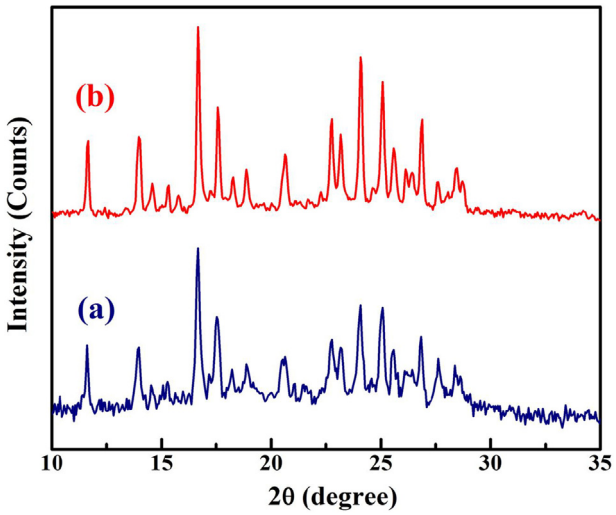

Fig. 4. XRD pattern of (a) curcuminoid and (b) Cureit™.

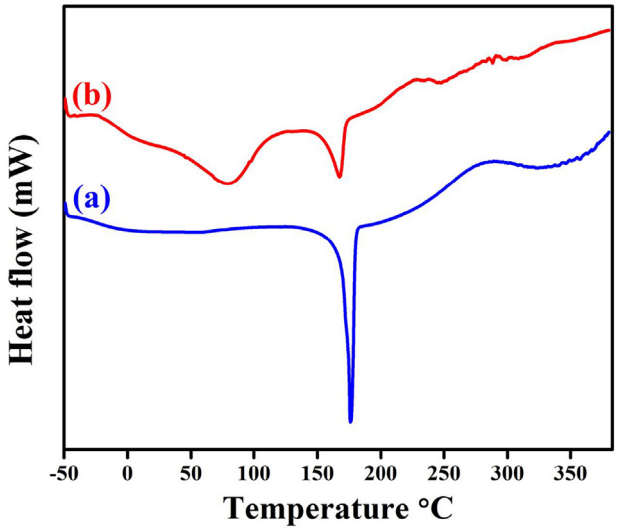

Fig. 5. DSC thermogram of (a) curcuminoid and (b) Cureit™.

corresponds to the benzene ring stretching [26] while, the peak at 1281 cm^{-1} corresponds to aromatic C—O stretching vibrations. In addition, the peak at 811 cm^{-1} is attributed to the stretching vibration of C—O in —C—OCH_3 while, the peak at 1027 cm^{-1} correspond to the C—O—C stretching of ether [27,28]. The peak for the phenyl ring was also observed at 857 cm^{-1} While, the peak at 714 cm^{-1} corresponds to aromatic in plane bending of curcuminoids [29]. Furthermore, a characteristic absorption bands was observed in all the spectra in the range between 3390 and 3500 cm^{-1}. This is attributed to the phenolic O—H stretching vibration [30]. However, in the Cureit™ (Fig. 3(e)) this band became broad. This broadness clearly indicated that the band does not due only to the presence of phenolic O—H, but also due to the hydrogen bonding of curcuminoids between the Polar-Nonpolar layer. Furthermore, the prepared Cureit™ showed characteristic peaks which were very close to the bands of curcumin analogs confirming the presence of curcuminoid in the PNS technology with weak hydrogen bonding. These data are in very good agreement with NMR analysis, and further confirmed the presence of hydrogen bonding interactions with curcuminoids, polar and non-polar compounds.

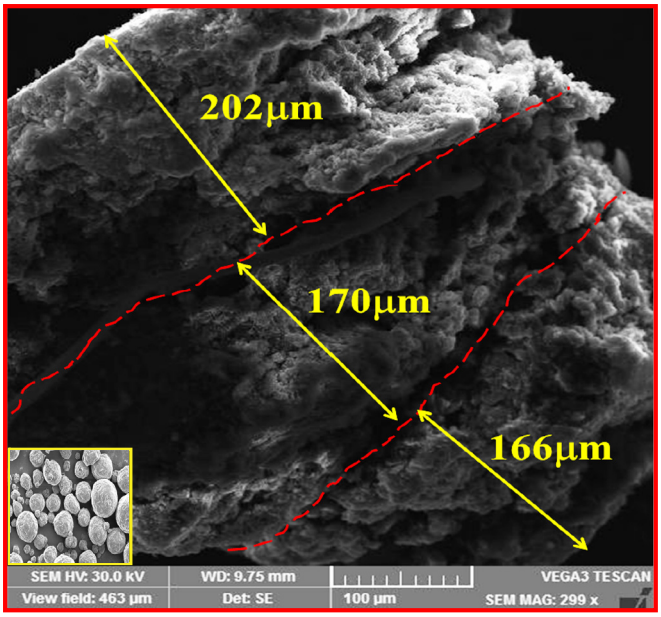

Fig. 7. SEM image of Cureit™ with three layers of Polar-Nonpolar-Sandwich form. [Insert: Cureit™: almost spherical and well dispersed with rough morphology].

The XRD pattern of curcuminoid and Cureit™ are shown in Fig. 4. In the XRD pattern of both curcuminoid and Cureit™, a number of peaks are seen in the region of 5–30 °C, without any significant difference between the two samples. This also confirmed the presence of curcuminoid in the polar-nonpolar-sandwich design without any modifications.

Stability, crystallinity and curcuminoid-polar-nonpolar interactions were evaluated by differential scanning calorimeter (DSC) analysis of curcuminoid and Cureit™ and the corresponding thermograms are shown in Fig. 5. DSC analysis showed a sharp endotherm peak at 176.7 °C for curcuminoid (Fig. 5(a)) corresponding to the melting point of crystalline region [13]. In the case of Cureit™, the endothermic peak was at 167.7 °C for curcuminoids. This peak is broader with reduced intensity and shifted to lower temperature due to the presence of polar and non-polar matrix (Fig. 5(b)). The thermogram of Cureit™

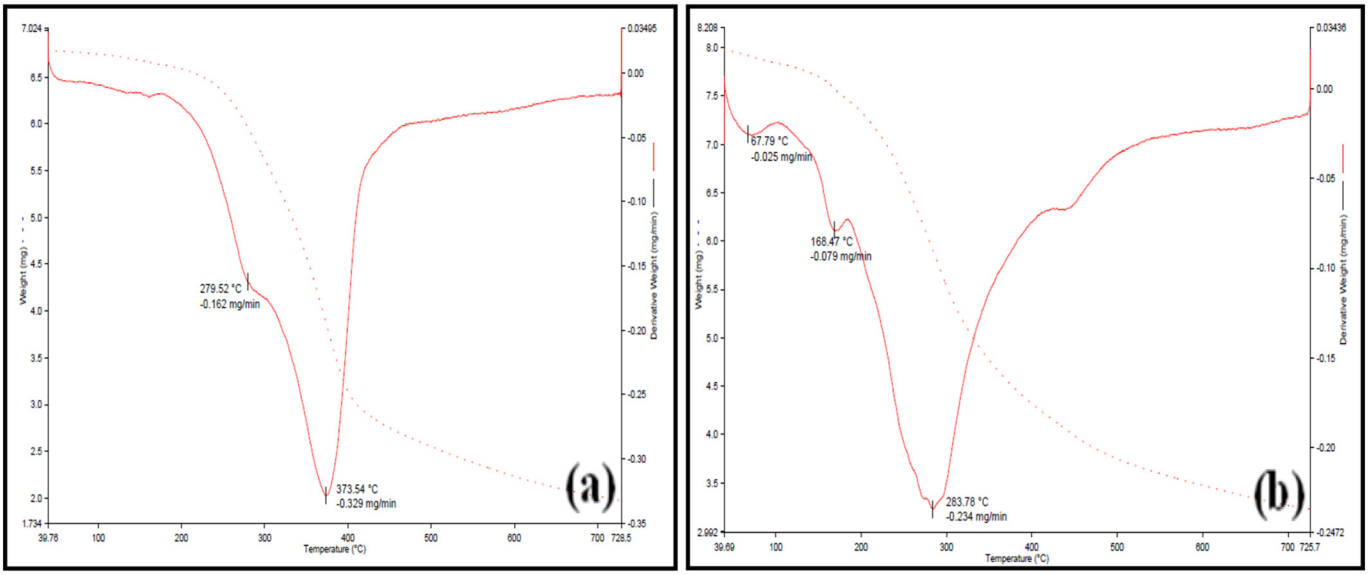

Fig. 6. TGA curve of (a) curcuminoid and (b) Cureit™.

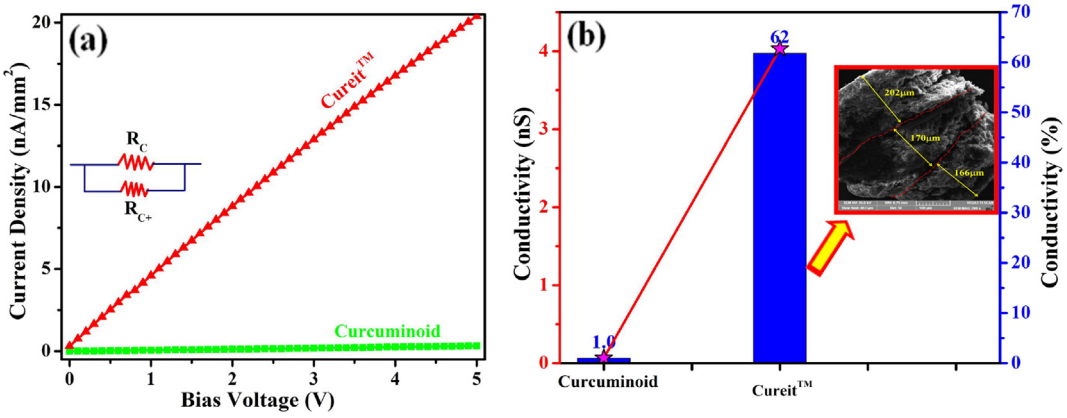

Fig. 8. Current-voltage study of (a) curcuminoid and Cureit™ [insert: equivalent current] (b) conductivity and percentage of increase in conductivity of curcuminoid and Cureit™ [insert: SEM micrograph of Cureit™].

also showed a small broad endothermic peak at around 79.3 °C corresponds to the melting of polar and non-polar matrix and loss of residual moisture.

Additional characterization was performed by thermogravimetric analysis (TGA). TGA curves of curcuminoid and Cureit™ are shown in Fig. 6. Curcuminoids (Fig. 6(a)) does not show any stage of water loss up to 200 °C due to its high hydrophobicity. However, it shows a gradual weight loss with first weight loss at 279.5 °C and second at 373.5 °C [18]. In Fig. 6(b), the first degradation step due to elimination of moisture from the polar matrix occurs at 67.8 °C while the second degradation step at 168.5 °C corresponds to the breakage of protein chain [18,31] present in the polar layer in the Cureit™. The third weight loss is recorded at 283.8 °C and corresponds to the degradation of available curcuminoid in the Cureit™ [18]. Curcuminoid and Cureit™ show almost similar weight loss pattern in the range between 279 and 284 °C. This also confirmed that curcuminoid encapsulated PNS technology does not alter thermal degradation pattern.

SEM analysis was conducted to investigate the morphology of Cureit™. It was evident from SEM image that the formulation was almost spherical and well dispersed with rough morphology (Fig. 7 (insert)). The mean diameter is in the range of 400–600 μm. Fig. 7 clearly indicated the presence of three different layers with slight morphological modifications. It might be the characteristic morphology of Polar-Non-polar-Sandwich technology.

Current-voltage curve (I-V curve) is a relationship represented as a graph between the electric current through a material and the corresponding voltage difference across it. The direct current (DC) electrical characteristics study, (the I-V study) shows that the conductivity of the Cureit™ is being increased by 1:62 ratio than the curcuminoid (Fig. 8(a)) due to the presence of layered structure (Fig. 8(b)). Current-voltage studies on different layers of Cureit™ showed that is highly positive, that is Cureit™ conducts electricity. This is attributed to the incorporation of polar and non-polar matrix, which is an evidence of the PNS technology, whereas curcuminoid is very poor conducting.

3.4. Comparison of various biological activities of different formulation with Cureit™

The maximum bioavailability of Cureit™ with various commercially available and different formulations of the bioavailable curcuminoid reported in the literature was summarized in Table 2. Antony et al. [21] and Benny et al. [45] prepared a formulation comprising of curcumin with essential oil of turmeric to increase the bioavailability. The study showed that the formulation showed 7 fold enhanced bioavailability than normal curcuminoid. The ability of the formulation to prevent Alzhemier's disease was also investigated by Baum et al. [46]. In 2009, Shaikh et al. [47] studied the possibility of nanoparticle formulations to enhance the bioavailability of curcuminoid. Curcumin loaded nanoparticle gel formulation showed increased bioavailability of 9 fold compared to native curcumin. Xie et al. [48] developed a formulation of poly (lactic-*co*-glycolic acid) nanoparticles loaded with curcumin and studied the mechanisms underlying the enhancement of bioavailability of curcumin. The formulation which included poly (lactic-*co*-glycolic acid) nanoparticles of curcumin showed relative bioavailability of 5.6 fold and had a longer half-life compared with that of native curcumin. A silica-coated flexible liposomes as a nanohybrid delivery system for enhanced oral bioavailability of curcumin was studied by Li et al. [49]. The bioavailability of curcumin was increased in curcumin-silica coated liposomes by 7.76 fold higher compared to normal curcuminoid. The formulation also showed sustained drug release in the intestinal fluid.

Table 2
Comparison of various biological activities of different formulation with Cureit™.

Formulations	Technology involved	Bioavailability (no. of fold)	Study involved	Reference
Curcumin with turmeric oil	Blending technique	7.0	Human bioavailability, Alzheimer's disease studies	[21,45,46]
Curcumin loaded nanoparticle	Encapsulation	9.0	Pharmacokinetic study carried out by using Sprague Dawley rat model	[47]
PLGA nanoparticles loaded with curcumin	Encapsulation	5.6	Pharmacokinetics & bioavailability study carried out by both in vitro & in vivo	[48]
Silica coated flexible liposomes loaded with curcumin	Sol gel process	7.8	In vitro curcumin release assay	[49]
Curcumin with cyclodextrin	Gel encapsulation	1.8	Preclinical study	[50]
Nano curcumin emulsion	Emulsion	6.0	In vitro blood brain barrier model using Tg2576 mice	[51]
Curcumin phospholipid formulation	Emulsion	7.9	A randomized, double-blind, crossover human study in healthy volunteers	[52]
Curcumin loaded BSA dextran emulsion	Emulsion	4.8	In vivo pharmacokinetics study	[53]
Cureit™	Unique PNS technology	10.0	Bioavailability, elastase inhibition, immune booster, angiogenesis, antioxidant and hyaluronidase inhibition	[32–36]

A nanoformulation was prepared by the complexation of curcumin with β-cyclodextrin to enhance the curcumin bioavailability. The study showed that the nanoformulation increased the bioavailability of curcumin by 1.8 folds [50]. Cheng et al. [51] prepared a highly stabilized curcumin nanoparticles tested in an in vitro blood-brain barrier model and in Alzhemier's disease Tg2576 mice. The pharmacokinetic studies showed that the nanoparticle formulation significantly improved curcumin bioavailability with a much greater plasma concentration and 6 fold higher AUC and MRT in the brain. A phospholipid formulation of curcumin was reported by Jager et al. in 2014 [52]. The curcumin phospholipid formulation showed 7.9 fold enhanced bioavailability than normal curcuminoid. Wang et al. [53] studied a formulation comprising of BSA-dextran emulsion with curcumin to enhance the bioavailability of curcumin. The pharmacokinetics studies demonstrated that curcumin-loaded BSA-dextran emulsion could increase curcumin oral bioavailability in mice by 4.8-fold compared with curcumin/Tween 20 suspension.

From these observations, this study strongly suggested that the consumption of curcuminoid in the form of Cureit™ formulation using PNS technology with complete natural turmeric matrix instead of plain curcuminoid for greater beneficial effects without side effects. Moreover, Cureit™ is completely made by natural and the bioavailability of curcuminoid 10 folds higher than the pure curcuminoid.

4. Conclusion

A novel bioavailable curcuminoid formulation, Cureit™ was prepared by using Polar-Nonpolar-Sandwich technology with complete natural turmeric matrix. The PNS technology of Cureit™ was confirmed by various instrumental techniques. SEM images clearly indicated that Cureit™ was almost spherical and well dispersed with rough morphology and separated with three layers of PNS formulation. The metabolic profile of Cureit™ was analyzed by Q-TOF and confirmed the presence of curcuminoids, lactones, sesquiterpenes and their derivatives in polar layer, aromatic turmerone, dihydroturmerone, turmeronol, curdione and bisacurone in non-polar layer. NMR data clearly confirmed the presence of hydrogen bonding interactions with curcuminoids, polar and non-polar compounds in the PNS technology. IR, XRD, DSC and TGA analysis further confirmed the presence of curcuminoids with high stability in the PNS formulation. All the analyses confirm the presence of curcuminoid inside the PNS design without any alterations. The earlier studies of Cureit™ ensured that the enhancement of bioavailability of curcuminoid and the PNS technology can be a promising curcuminoid delivery system for various biological activities without any side effect.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2017.02.068.

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