International Journal of Peptide Research and Therapeutics (2022) 28:113
https://doi.org/10.1007/s10989-022-10415-z

Curcumin Displays Enhanced Solubility and Antibacterial Activities When Complexed with the Cell Penetrating Peptide pVEC

Ebru Koleoglu[1] · Tayfun Acar[2] · Serap Derman[2] · Berna Sariyar Akbulut[1]

Accepted: 29 April 2022 / Published online: 4 June 2022

Abstract

Curcumin is among phytochemicals with increasing popularity; unfortunately, its therapeutic potential is restricted due to poor water solubility and bioavailability. The current work undertakes the effort to improve the therapeutic potential of curcumin by complexing it with a cell penetrating peptide using copper ions. A mononuclear complex was synthesized from copper(II) acetate and curcumin. Then this complex was conjugated to the cell penetrating peptide, pVEC. The structural characterization of the complexes was achieved using UV-Vis and Fourier transform infrared spectroscopies. Dynamic and electrophoretic light scattering measurements have confirmed the complexation of curcumin with the peptide to form nanoparticles. Both solubility and kinetic stability of curcumin greatly improved upon complex formation with pVEC through copper ions. Then the antibacterial activity of curcumin in the complex was tested. The amount of curcumin in the minimum inhibitory concentration was ~ 30, ~8, and ~ 15 fold lower, respectively for *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* when complexed with pVEC; however, this improvement was specifically noteworthy for the gram-negative *E. coli* since the contribution of pVEC in the complex to the observed activity was negligible in this bacterium. With enhanced solubility and stability, metallo curcumin conjugated pVEC complex possesses potential for different therapeutic applications.

Keywords Curcumin · Cell penetrating peptide · Copper · Solubility · Stability · Antimicrobial activity

Introduction

Antimicrobial resistance, the result of inappropriate or long-term use of antimicrobial drugs, poses serious threat to human health (Genilloud 2019). One key concept of modern antibacterial therapy to fight antimicrobial resistance is the use of small natural molecules, which are lethal for bacteria but are easily and safely administered. These molecules have long been in use as a part of traditional medicine as well as nutrition throughout human history (Nawaz 2011). Over the past century, many natural products have been investigated for their activities in antimicrobial therapies and drug discovery programs (Moloney 2016).

Curcumin or diferuloylmethane, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is the most abundant and biologically active phytochemical of the plant *Curcuma longa*. It has three different types of active functional moieties; two o-methoxy phenolic groups, two enone moieties, and one 1,3-keto-enol moiety (Indira Priyadarsini 2013). Based on its wide range of interesting pharmacological properties (Adamczak et al. 2020), it has attracted the attention of many researchers around the world (Kumavat et al. 2013; Feng et al. 2017). Unfortunately, despite its bioactivity against a multitude of diseases, it has serious limitations as a therapeutic compound. Its primary limitation is based on its very low solubility in water (3×10^{-8} M) (Kharat et al. 2016), which makes its incorporation to many aqueous-based products very difficult (Doktorovova et al. 2018). It is rather unstable at neutral and basic pH and is readily degraded into smaller molecules. The degradation rate increases with increasing pH. Its chemical instability under physical conditions leads to poor bioavailability (Varshosaz et al. 2012). Moreover, although curcumin is resistant to high temperature, it is very sensitive to light (Feng et al.

✉ Serap Derman
serapacar5@gmail.com

[1] Department of Bioengineering, Faculty of Engineering, Marmara University, 34722 Istanbul, Kadikoy, Turkey

[2] Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, 34220 Esenler, Istanbul, Turkey

2017; Kumavat et al. 2013). Altogether, these properties result in its poor absorption in biological systems.

The limitations that reduce the efficacy of curcumin have stimulated the application of different technologies to improve its properties. These include encapsulation of curcumin with different types of nanotechnology-based methods, (Slika et al. 2020; Bechnak et al. 2020) prodrug approaches (Muangnoi et al. 2019), structural modifications (Khatun et al. 2020), and microemulsions (Sha et al. 2021). Such formulations, specifically curcumin conjugated with metals/metal nanoparticles displayed enhanced antimicrobial and anti-oxidant (scavenging) activities (Mouslmani et al. 2015; Khoury et al. 2015; Trigo-Gutierrez et al. 2021). One alternative strategy can be non-covalent attachment of curcumin with cell penetrating peptides (CPPs) (Ratrey et al. 2020).

CPPs are short peptide sequences, typically with less than 30 amino acid residues. They have been reported as promising vectors for the delivery of a variety of cargo-molecules such as small molecule drugs, liposomes, oligonucleotides, imaging agents, peptides, and proteins (Palm et al. 2006; Copolovici et al. 2014). CPPs can pass across not only cellular membranes but also the blood-brain barrier when present at nontoxic concentrations. Their positively charged residues are believed to facilitate electrostatic interactions with the negatively charged cell surface as a first step during membrane translocation (Kristensen et al. 2016). Based on the biochemical and physicochemical properties of a CPP and its cargos, two approaches have been formulated for cargo transport: (i) physical complexation and (ii) covalent conjugation (Kristensen et al. 2016). Among these, the non-covalent strategy is an easier process mainly driven by electrostatic and hydrophobic interactions between the CPP and its cargo, furthermore it allows flexibility in their molar mixing ratios (Munyendo et al. 2012; Guidotti et al. 2017).

In the present work, a non-covalent conjugation strategy using pVEC, an amphipathic CPP derived from murine vascular endothelial cadherin, was pursued with the help of the transition metal, Cu(II). Transition metals can serve as bridge molecules between two different ligand molecules. It has been demonstrated that curcumin can form strong complexes with metals and metal oxides through the α, β-unsaturated β-diketone moiety (Indira Priyadarsini 2013; Vellampatti et al. 2018). The leading curcumin-metal complex can then bind to the peptide via electrostatic and hydrophobic interactions (Ratrey et al. 2020; Li et al. 2018). Thus, this work attempted to enhance the solubility, bioavailability and antimicrobial activity of curcumin by complexing it with a CPP through copper ions.

Materials and Methods

Chemicals

All chemicals and solutions used in this study were purchased from Applichem (Germany), Merck (Germany), Molekula (Germany), Sigma Aldrich (USA), and NovaBiochem (USA). Ultra-pure water was obtained from Millipore Milli-Q system.

Bacterial Strains

Escherichia coli K12 (laboratory stocks), *Bacillus subtilis* 168 (DSM 402), and *Staphylococcus aureus* (MSSA) (ATCC 25,923) were used for antimicrobial susceptibility tests. Growth of *E. coli*, *B. subtilis*, and *S. aureus* was achieved in Luria Bertani broth, nutrient broth, and Mueller Hinton broth, respectively.

Synthesis and Characterization of the Cell-Penetrating Peptide pVEC

The peptide pVEC (LLIILRRRIRKQAHAHSK) was synthesized by the microwave-assisted solid phase peptide synthesis method as described previously (Acar et al. 2019). Resin beads (200 mg, 0.1 mmol) were swelled in ~25 mL dimethylformamide (DMF), and then transferred to a 30 mL reaction vessel in a fully automated peptide synthesis device. Microwave energy used for the deprotection and coupling steps were 55 W to maximum at 45 °C for 2 min and 25 W to maximum at 75 °C for 3 min, respectively. 20 % piperidine solution was used for the deprotection process. The activators of the microwave coupling step were HCTU/HOBt.H_2O (0.5 M in 20 mL DMF) and DIEA/NMP (2 M in 15 mL DMF) solutions. For the activation, activator solutions were added to the Fmoc protected amino acids dissolved in DMF. These procedures (deprotection, activation and coupling) were repeated for each amino acid in the peptide sequence. When coupling of the amino acids residues was finished, the peptide attached resin was cleaned to prepare for the cleavage process by washing three times in DMF and five times in DCM, and then the resin beads were dried under vacuum. A cleavage cocktail prepared with trifluoroacetic acid/thioanisole/1,2-Eethane dithiol/water (90/5/2.5/2.5 (v/v)) was used to cleave the peptide from the resin. The peptide was precipitated by cold diethyl ether (−20 °C) and centrifuged. The obtained peptide was lyophilized (Acar et al. 2019).

Synthesized peptides were characterized by liquid chromatography-mass spectrometry system (Shimadzu 2010 EV) using the Electro Spray Ionization probe. Samples

were injected onto Shimadzu Shim-Pack MRC-ODS HPLC column in the dimensions of 25 cm length and 6 mm inlet diameter. A gradient from eluent A (water, 0.1% (v/v) FA) to eluent B (acetonitrile, 0.1% (v/v) FA) was used for elution at a flow rate of 0.6 ml min^{-1}. The elution program was as follows: 0–5 min, 10 % B; 5–15 min, 10–50 % B; 15–30 min, 50–80 % B; and 30–35 min, 80–10 % B (Ucar et al. 2021).

Synthesis of Peptide Complexes

Binary Curcumin-Cu(II) Complex (cCUR)

The cCUR complex was synthesized by mixing equimolar curcumin and copper(II) acetate ($Cu(OAc)_2$) in ethanol, as previously described (Barik et al. 2005). $Cu(OAc)_2$ dissolved in ethanol was transferred into a three-neck round bottom flask and heated at 60 °C under nitrogen atmosphere. Then, curcumin dissolved in ethanol was slowly added into the closed system and refluxed for 3 h under nitrogen atmosphere at 60 °C in a dark environment. At the end of 3 h, the precipitated complex was separated by centrifugation. The precipitate was washed two times with cold ethanol and then with water to remove free curcumin and $Cu(OAc)_2$. The pure solid was dried for 24 h in a vacuum oven to achieve a dry powder complex. The powdered complex was stored at room temperature in a dark environment until future use. The highest concentration of curcumin and $Cu(OAc)_2$ in the reaction mixture was 15 mM. Lowering the concentration had no significant effect on the complex formation.

Ternary Curcumin-Cu(II)-Peptide Complex (pCOM)

A procedure similar to the one used to synthesize cCUR complex was used for synthesizing the metallo-curcumin and pVEC ternary complex (pCOM). Equimolar cCUR and pVEC were mixed in ethanol. The synthesis took place in a three-neck round bottom flask for 24 h at room temperature under nitrogen atmosphere in a dark environment (Barik et al. 2005). There were no precipitates in the obtained solution. To achieve dry powder, the obtained solution was dried in a vacuum oven.

All powders obtained were stored at + 4 °C in a dark environment until future use.

Structural Characterization of the Complexes

Analyses with UV-Vis spectrophotometer (UV-1700 pharmaspec, Shimadzu, Japan), Fourier transform infrared (FTIR) (Shimadzu, Japan) spectrometer, and Zetasizer (Zetasizer Nano ZS, Malvern, UK) instrument were carried out for the structural characterization of the complexes.

UV-Vis and FTIR spectroscopy measurements were used to evaluate the complexation. Absorbance of the samples in dimethyl sulfoxide (DMSO) and ethanol were measured in quartz cuvettes of 1 cm path length. The spectrophotometer was scanned within the wavenumber range of 200–800 nm at room temperature. The FTIR spectra of the compounds and the complexes were scanned on the wavenumber range of $4000 - 600\ cm^{-1}$. The resolution was fixed to 4 cm^{-1}.

Zetasizer instrument was used to determine the particle size distribution (Z-Ave), zeta potential (ζ), and polydispersity index of curcumin and the formed complexes. Compounds dissolved in ethanol (curcumin and pCOM) and DMSO (cCUR) were dispersed in water at 1:30 ratio, sonicated, and then mixed well to obtain a homogenous solution. The particle size distribution of each sample was measured by the dynamic light scattering (DLS) technique in triplicate using 0.08872 cP viscosity and 1.330 refractive index (RI). The zeta potential was measured by electrophoretic light scattering (ELS) technique. Measurements were carried out in triplicate at the dielectric constant (ε) of 79 and f(ka), 1.5 (Smoluchowski value) (Derman and Akdeste 2015; Arasoglu et al. 2017).

Solubility of Curcumin and its Complexes

Solubility of curcumin, and cCUR and pCOM complexes were measured using a spectrophotometric method. After adjusting the amount of pure curcumin and curcumin in the complexes to 70 µg mL^{-1}, all compounds were dissolved in distilled water and stirred in a dark environment for 24 h and then centrifuged at 4000 *g* for 10 min. Undissolved crystalline curcumin and its complexes were precipitated by centrifugation. Curcumin in the supernatant was analyzed by measuring the absorbance at 425 nm (Shin et al. 2016).

Stability of Curcumin and its Complexes

In vitro kinetic degradation of pure curcumin and curcumin in cCUR and pCOM was determined by monitoring absorption spectra of freshly prepared solutions. Stock solutions of curcumin (0.05 mg mL^{-1}), cCUR (0.1 mg mL^{-1}) and pCOM (0.35 mg mL^{-1}) were prepared in DMSO. Immediately after mixing 800 µl of a stock solution with 9.2 mL distilled water, monitoring of absorbance at 428 nm started. Absorbance recorded at time zero was considered to be 100 % saturation. The decrease in absorbance implied a reduction in the concentration of curcumin due to its degradation (Wang et al. 1997). Solutions were stirred in a dark environment for 24 h. This procedure was repeated with distilled water and different phosphate buffer solutions (0.1 M) at pH 5.8, 6.8, and 7.8 (Kumavat et al. 2013).

Antimicrobial Assay

Broth dilution method was used to determine the minimum inhibitory concentration (MIC) of curcumin and the synthesized complexes (Amsterdam 1996). Complexes were dissolved in DMSO since their solubilities were low in water. $Cu(OAc)_2$ and pVEC were dissolved in water. For the test, serial two-fold dilutions of freshly prepared solutions were deposited in sterile 96-well U bottomed, microtiter plates. Dilution was achieved with growth media. Then each well was inoculated with 10^5 colony forming unit (CFU) mL^{-1} *B. subtilis*, 10^6 CFU mL^{-1} *E. coli* or *S. aureus* and incubated at 37 °C for 24 h. Finally, viable cells were visualized with 0.5 % (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC) to determine the MIC (Moussa et al. 2013). After the addition of the TTC solution, the test plates were incubated for 1 h at 37 °C to observe a color change. Based on the color change in wells, MIC was defined as the lowest concentration of a compound with which no color change was observed (Amsterdam 1996).

Results

Characterization of the Synthesized pVEC

The CPP pVEC was synthesized by microwave-assisted solid phase peptide synthesis. Quality of the synthesized peptide was assessed following its purification by preparative HPLC. The purity of pVEC was determined to be about 85 % at 210 and 280 nm on the LC-PDA chromatogram (Fig. 1). The molecular weight of pVEC was verified as 2210.87 Da with mass spectrometry (Fig. 2). LCMS: m/z 443.60 (calcd $[M+5\ H]^{5+} = 442.95$), m/z 554.80 (calcd $[M+4\ H]^{4+} = 553.44$), m/z 1103.20 (calcd $[M+2\ H]^{2+} = 1105.86$), $[M]_{average} = 2210.87$ Da $[M]_{theoric} = 2209.72$ Da.

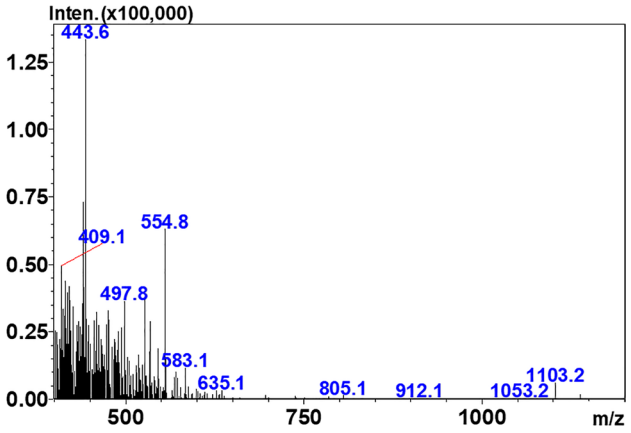

Fig. 2 ESI-MS spectrum of the pVEC peptide

Characterization of Curcumin and its Complexes

Different biophysical tools have been used to monitor the interaction of curcumin not only with metals (Banerjee and Chakravarty 2015), but also with a wide range of molecules including proteins (Gupta et al. 2011). Here, UV-Vis and FTIR spectroscopies have been selected to characterize cCUR and pCOM complexes.

The UV-Vis spectra obtained for curcumin in DMSO showed an absorption maximum (λ_{max}) at 434 nm (Fig. 3a). The spectra of cCUR in DMSO gave λ_{max} at 428 nm and two shoulders at 410 and 450 nm. This showed that due to the $\pi-\pi^*$ transition band of curcumin, λ_{max} was blue-shifted by 6 nm. Furthermore, the shoulders at 410 and 450 nm could be attributed to a curcumin-Cu(II) charge transfer band, an indication of the involvement of the carbonyl group of curcumin in complexation with $Cu(OAc)_2$ (Banerjee 2014). The

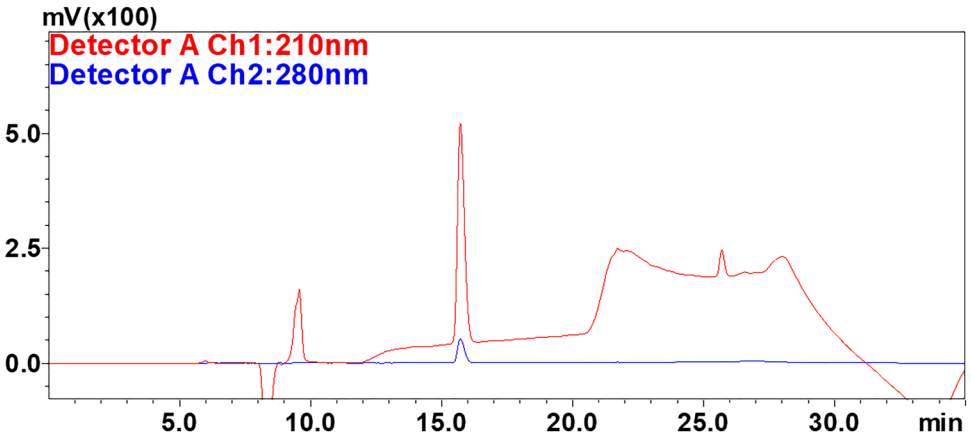

Fig. 1 LC chromatogram of the pVEC peptide (purity: 85%, retention time: 15.6)

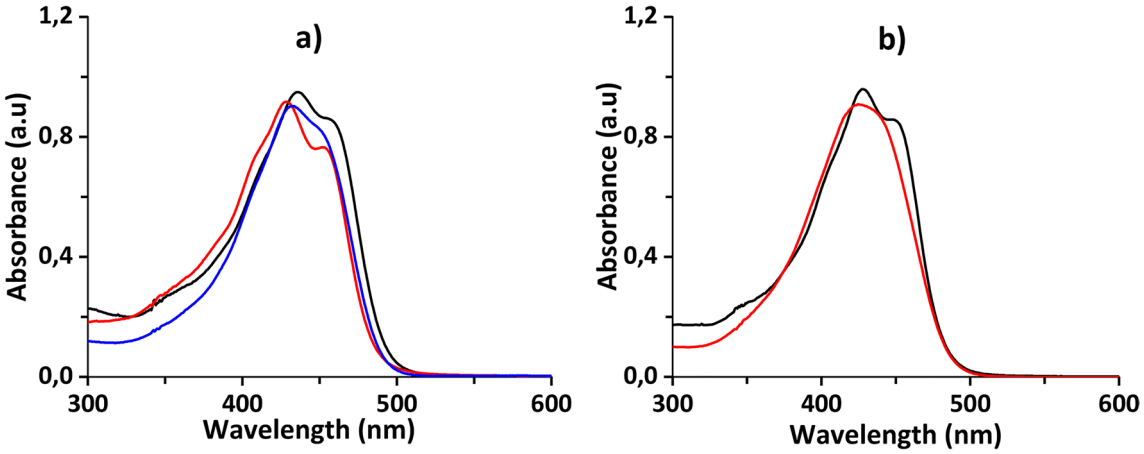

Fig. 3 **a** UV-visible absorption spectra of CUR, cCUR, and pCOM obtained in DMSO, **b** UV-visible absorption spectra of CUR and pCOM obtained in ethanol

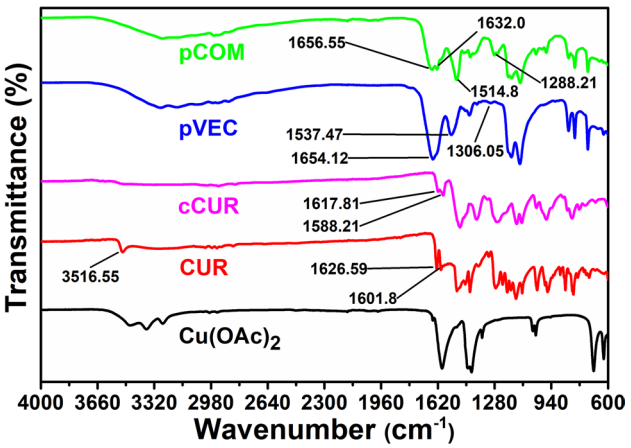

Fig. 4 FTIR spectra of $Cu(OAc)_2$, curcumin (CUR), cCUR pVEC, and pCOM

UV-Vis spectra of pCOM in DMSO gave λ_{max} at 437 nm and a single shoulder at 453 nm.

When ethanol was used as the solvent, due to the $\pi-\pi$ interactions, the UV-Vis spectra of curcumin gave λ_{max} at 425 nm (Fig. 3b). The absorption spectra of pCOM was red-shifted by 2 nm. A shoulder at 445 nm was assigned to the charge transfer between curcumin and the copper ion.

The infrared spectra which showed the changes in the vibrational absorption, wave numbers, peak shape, and peak intensity in cCUR and pCOM as compared to the spectra of curcumin, $Cu(OAc)_2$, and pVEC are presented in Fig. 4.

As cCUR formed, the stretching vibration absorption band in the 3515 cm^{-1} range of the free -OH group of curcumin became weaker with a net decrease in intensity around the same wavenumber. The 1626 and 1601 cm^{-1} peaks, which display a mixed ν(C=O) and ν(C=C) character in curcumin, were red-shifted to 1617 cm^{-1} and 1588 cm^{-1} in the cCUR complex.

The infrared spectrum of pCOM has shown that the strong band of pVEC at 1654 cm^{-1} (amide I) shifted to 1656 cm^{-1} and there was an additional band at 1631 cm^{-1} in this complex. In addition to the above-mentioned shift, the characteristic of the amide I band had also changed, becoming double-headed. The sharp peak seen at 1537 cm^{-1} (amide II) in pVEC shifted to 1514 cm^{-1} in pCOM. The amide III band at 1306 cm^{-1} in pVEC also shifted to 1288 cm^{-1} in pCOM. These phenomena could suggest that the functional groups of pVEC interacted with cCUR when the final complex was synthesized. This interaction could have been established through interaction of the metal with the cationic amino acids such as histidine, arginine, and lysine in pVEC (Moulahoum et al. 2020a, b) or through interaction of the curcumin-metal complex with the N-terminal domains of a histidine residue (Liu et al. 2011).

Measurement of the Solubility of Curcumin and its Complexes

The solubility of curcumin, cCUR and pCOM were investigated using the equilibrium solubility method. This method is based on the addition of the substance under investigation to an aqueous solvent until the solvent is saturated, and then stirring this solution for a prolonged period until equilibrium is attained (Avdeev 2012). After 24 h, absorbances of dissolved curcumin and the complexes were measured at 425 nm. In Fig. 5 given is the image of bottles of curcumin, cCUR and pCOM dissolved in distilled water. The solubility of curcumin was measured as 0.59 ± 0.02 µg mL^{-1} while the solubility of cCUR was measured as 0.53 ± 0.18 µg mL^{-1}, very similar to curcumin. However, the solubility of the pCOM was measured as 10.40 ± 2.26 µg mL^{-1}, which

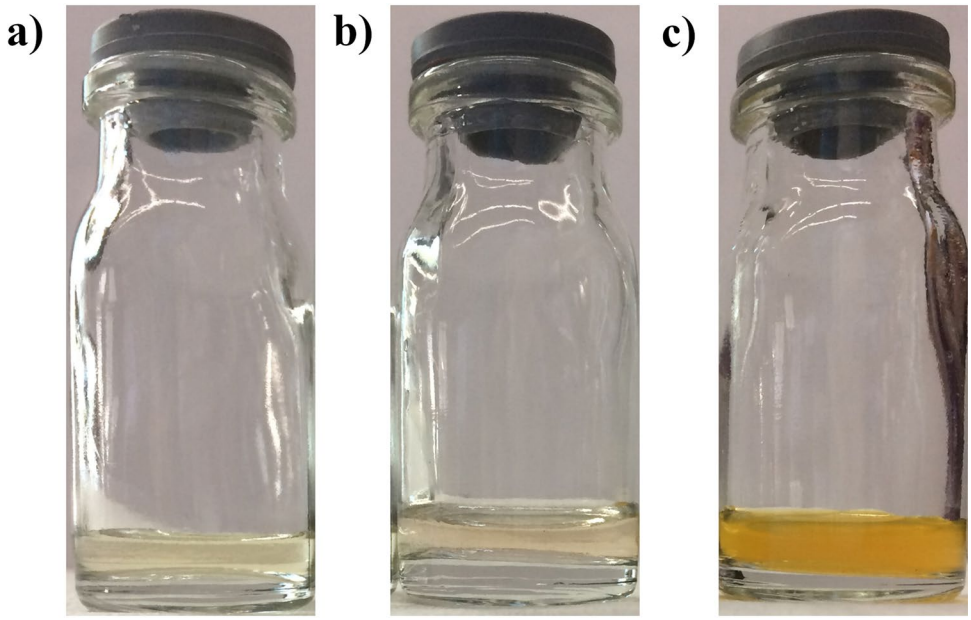

Fig. 5 Solubility of **a** curcumin, **b** cCUR, and **c** pCOM in distilled water

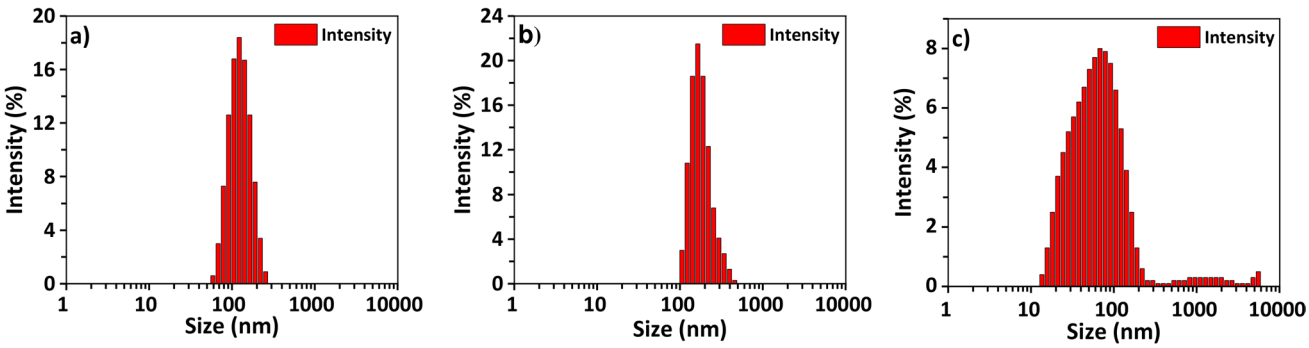

Fig. 6 DLS size profile of **a** CUR and **b** cCUR, **c** pCOM complexes

clearly showed an increase. Indeed, the solubility of the ternary complex was approximately 18-fold higher than that of curcumin alone. The change in the color of the solutions in the bottles (Fig. 5) also indicated increased solubility. While the color of the solutions with curcumin or cCUR were very light yellow, the color of the solution with pCOM was bright yellow, approaching the color of powder curcumin.

Analysis of Particle Size and Surface Charge

Dynamic and electrophoretic light scattering analyses are commonly used techniques to investigate the hydrodynamic mean size and surface charge of the particles, respectively, in solution (Poe 1977; Lee et al. 2016). These techniques were then used to study the complexes.

In Fig. 6 given are the plots of the DLS size profiles obtained for curcumin and its complexes.

The DLS technique has shown that curcumin had a mean particle size of 120 nm with a polydispersity index of 0.08. The average size of the cCUR complex was measured as 190 nm with a polydispersity index of 0.18. Interestingly, the mean particle size of pCOM was 59 nm with a polydispersity index of 0.34.

Then ELS was used to measure the surface charges of curcumin, pVEC, cCUR, and pCOM. The zeta potential of curcumin and pVEC were measured as −0.244 mV and 20.8 mV, respectively. The zeta potential of cCUR was even more negative with a value of −9.51 mV, while the zeta potential of pCOM was 19.3 mV, a positive value, approaching the value obtained for pVEC.

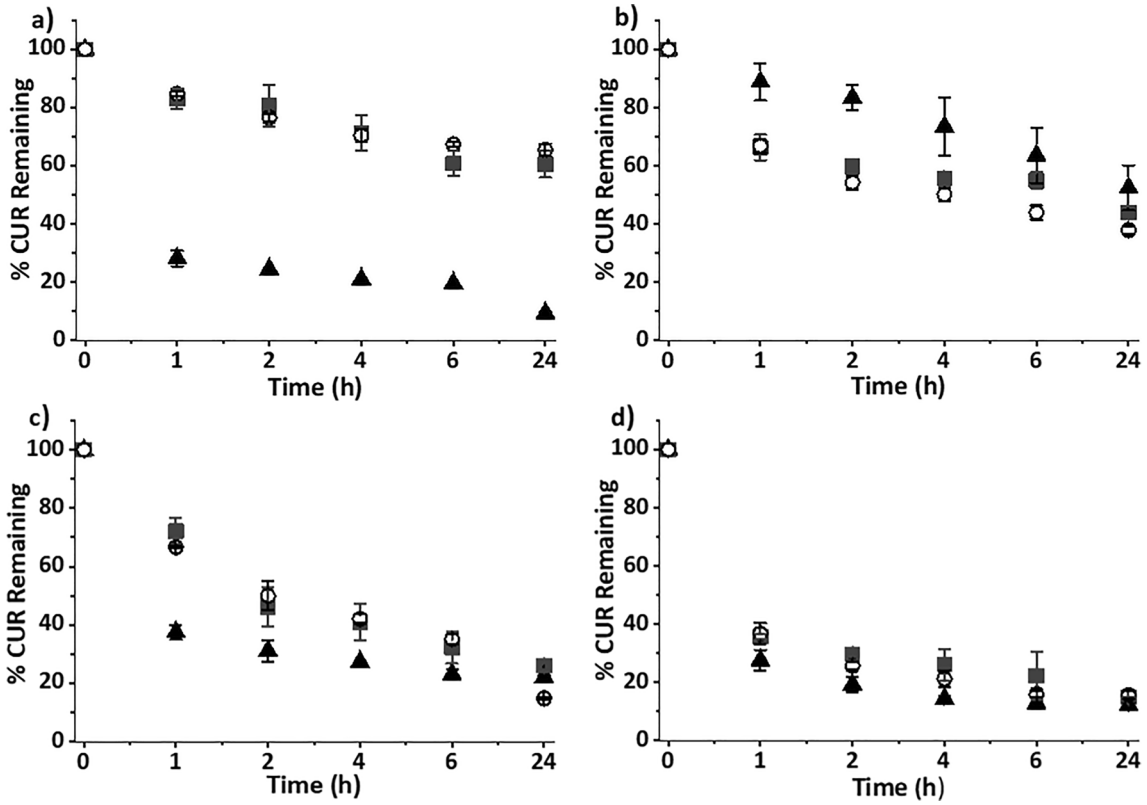

Fig. 7 Kinetic stability of curcumin (CUR), cCUR, and pCOM in **a** water, and in phosphate buffers of **b** pH 5.8, **c** pH 6.8, and **d** pH 7.8. (filled triangle) (▲); CUR (filled rectangle) (■); cCUR (open circle) (○); pCOM

Assessment of the Kinetic Stability of Curcumin and its Complexes

For a comparative investigation, the kinetic degradation of curcumin and the synthesized complexes were analyzed in H_2O and in phosphate buffers of different pH values (Fig. 7).

Stability in distilled water is presented in Fig. 7a. Nearly 80% of curcumin degraded within an hour in distilled water, while approximately only 20% of curcumin in cCUR and pCOM complexes degraded in the same time period. After 24 h, the remaining intact curcumin in the uncomplexed form was 10%. This finding is consistent with previous studies that report the improvement of stability in different formulations (Ratrey et al. 2020; Shin et al. 2016; Zebib et al. 2010; Aboudiab et al. 2020). The comparison of free curcumin with curcumin loaded in polymeric micelles has also shown that stability increased with this formulation (Naksuriya et al. 2016). Curcumin can also interact with metals and be protected against degradation. Zebib et al. have reported that complexation of curcumin with Cu^{2+}, Zn^{2+} or Mg^{2+} decreased curcumin degradation in buffer solutions with varying pH values (Zebib et al. 2010). They have reported 55% residual activity for curcumin after 24 h in a buffer of pH 7.0 when complexed with divalent cations in a glycerol/water system (Zebib et al. 2010). On the other hand, in the complexed forms with pVEC, nearly 60% of curcumin remained intact in distilled water in the same period of time.

In Fig. 7b–d, presented are the stability results obtained in phosphate buffers of pH 5.8, 6.8, and 7.8. Pure curcumin remained slightly more stable than the complexes in the phosphate buffer of pH 5.8 for the first 5 h. After 24 h, approximately 40–55 % curcumin was stable in both free and complexed forms in this pH. The stabilities of the complexes were slightly higher than that of pure curcumin in the phosphate buffer of pH 6.8; nevertheless, decomposition was in general faster when compared to that observed in the buffer of pH 5.8. At the end 24 h, only 15–20 % curcumin was stable in either free or complexed forms. On the other hand, in the phosphate buffer of pH 7.8, decomposition was extremely fast for all species; after 4 h only about 20 % curcumin remained intact in either pure or complexed forms and at the end of 24 h, curcumin that remained in solution was approximately 10 % for all species.

Table 1 Minimum inhibitory concentration (MIC) of tested compounds

Agents	MIC (μg/mL)		
	E. coli	*B. subtilis*	*S. aureus*
curcumin	512	32	256
$Cu(OAc)_2$	512	512	512
pVEC	1024	32	128
cCUR[a]	85/43	43/21	170/86
pCOM[b]	17/9/102	4/2/26	17/9/102

[a]Amount of curcumin/$Cu(OAc)_2$ present in the MIC value of the complex

[b]Amount of curcumin/$Cu(OAc)_2$/pVEC present in the MIC value of the complex

Evaluation of the Antimicrobial Activity of the Curcumin Complexes

Microdilution assay was performed to obtain MIC values of curcumin, $Cu(OAc)_2$, pVEC, and the complexes in *E. coli*, *B. subtilis*, and *S. aureus* cells. In U-bottom 96-well plates, the molecules were 2-fold serially diluted horizontally. The obtained MIC values are summarized in Table 1. For the curcumin complexes cCUR and pCOM, the values given in Table 1 show the amount of each species present in the MIC value of the complex.

Based on the colour change obtained with the addition of TTC, we have found that MIC of curcumin was higher for *E. coli* as 512 μg mL^{-1}, when compared to 32 μg mL^{-1} for *B. subtilis* and 256 μg mL^{-1} for *S. aureus*. These values were consistent with previous reports (Rai et al. 2008; Sathishkumar et al. 2015; Luo and Yang 2014; Mun et al. 2013). In *E. coli*, the antimicrobial activity of cCUR was 4-fold higher than that of curcumin alone and the amount of curcumin was almost ~ 6-fold lower in this complex. In *S. aureus*, the MIC values obtained with curcumin and cCUR were identical but evidently cells were affected from a lower concentration of curcumin. However, in *B. subtilis* neither the MIC value nor the amount of curcumin improved.

Upon complexation of pVEC with cCUR to form pCOM, the MIC value against *E. coli* remained identical to the MIC value obtained with cCUR. However, when the amount of pVEC was excluded from pCOM, the amount of curcumin in this MIC was calculated to be ~ 30-fold lower when compared to the MIC value of pure curcumin. The activity of pCOM was slightly better than that of cCUR against *S. aureus*, too, and the amount of curcumin was ~ 15-fold lower in the MIC value of pCOM when compared to MIC of pure curcumin. However, here the effect of pVEC in the complex is not negligible since its amount (102 μg mL^{-1}) is pretty close to the MIC calculated with pVEC alone (128 μg mL^{-1}). A similar result was obtained with pCOM against *B. subtilis*. The MIC values obtained with pCOM and pure curcumin were identical. Although the amount of curcumin was very low in the MIC value determined for the complex, the activity displayed by pCOM against *B. subtilis* might be attributed to the presence of pVEC in pCOM. As a summary, complex formation with pVEC improved the MIC values in terms of the amount of curcumin against the bacterial species studied but overall the effect was significantly better for *E. coli*.

Discussion

CPPs are widely investigated to overcome limitations in small molecules' transport across biological membranes. The transport may be achieved through formation of either a chemical linkage or a non-covalent complex with the cargo molecule. In the non-covalent strategy, the molecules are expected to dissociate following internalization. In this context, amphipathic CPPs are commonly used as carriers. Such peptides consist of two domains: a hydrophilic (polar) and a hydrophobic (non-polar) domain. Non-covalent electrostatic interactions and/or hydrophobic-hydrophilic interactions may further stabilize the peptide&cargo complex (Heitz et al. 2009; Kang et al. 2017). In this work, the non-covalent strategy has been pursued to improve the properties of curcumin. To this end, the amphipathic CPP, pVEC, of 18 amino acid residues (Alaybeyoglu et al. 2018) was selected for complexation using copper ion as a bridge molecule for stabilization (Vellampatti et al. 2018; Li et al. 2018). In contrast to cationic and hydrophobic peptides, pVEC was believed to more readily interact with the positively charged copper.

Curcumin displays a characteristic absorption spectrum, a λ_{max} alone with no shoulders, when analyzed with UV-Vis spectroscopy (Kadam et al. 2018). Because this technique is widely used to characterize the structural features in complexes with metals and proteins (Wanninger et al. 2015; Hegde et al. 2013; Razzak et al. 2018) and in nanoparticles (Pandit et al. 2015), analysis with UV-Vis spectroscopy gave clues on the formation of the binary cCUR and ternary pCOM complexes. The normalized ground state UV-Vis spectral features of cCUR complex were consistent with that of the 1:1 cCUR complex reported by (Barik et al. 2007). This confirmed that the binding stoichiometry of curcumin and copper ion in solution was at a molar ratio of 1:1, which was also supported with mass spectrometry (LCMS: m/z 508.4, CUR + $Cu(OAc)^-$+H_2O). The obtained UV-Vis spectrum was distinctly different from that of the 2:1 cCUR complex spectrum found in literature, which gives λ_{max} at 370 nm with no shoulders (Barik et al. 2007). The appearance of the charge transfer band in the 453 nm region in the

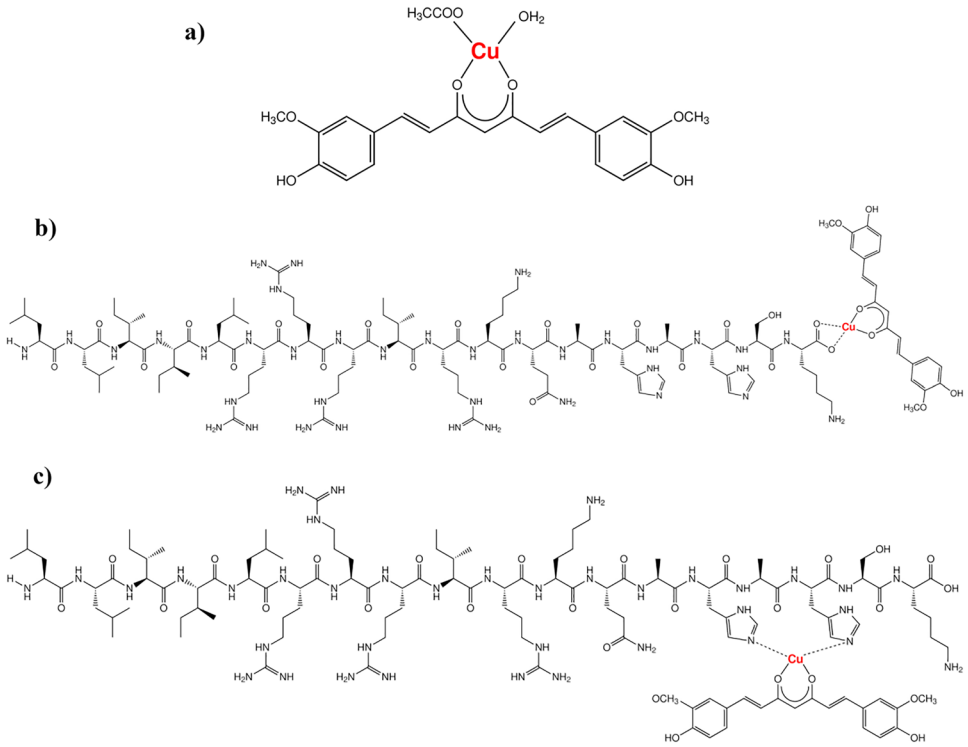

Fig. 8 Proposed structures for the complexes **a** mononuclear cCUR, **b** pCOM via interaction with the carboxyl end, and **c** pCOM via interaction with histidines

spectrum of pCOM also indicated the binding of curcumin to Cu^{2+} as this nanoparticle complex formed (Li et al. 2018).

It was possible to investigate the pCOM complex also in ethanol. The binary curcumin-Cu(II) complex was soluble in DMSO, lipids, and membranes; nevertheless, it was insoluble in organic solvents such as ethanol and methanol. Interestingly, when pVEC was incorporated to cCUR, the newly formed ternary complex was soluble in ethanol. The UV-Vis spectroscopic analysis of pCOM in ethanol, enabled by increased solubility in the presence of pVEC, confirmed the formation of the ternary complex.

The complexes were further confirmed by comparative analyses of the spectra obtained from FTIR spectroscopy for raw curcumin, $Cu(OAc)_2$, bare peptide, cCUR and pCOM. The decrease observed in the vibrational band intensity of the free -OH group of curcumin as cCUR formed was a finding similar to those reported (Zebib et al. 2010) for curcumin-copper and curcumin-magnesium complexes. This phenomenon is a strong indication of the presence of interactions at these sites and their involvement in complexation for the formation of a new link between the metal ion and curcumin (Poe 1977). Reports suggest that the phenolic -OH group of curcumin is commonly not involved in complexation with other metal ions and the -OH stretching of the phenol group exhibits an unaltered vibrational band around 3500 cm^{-1} in the FTIR spectra of these curcumin-metal complexes (Banerjee 2014). The red shifts in the mixed ν(C=O) and ν(C=C) character of curcumin could indicate that the carbonyl group of the ligand in the enol form is coordinating the metal ion in cCUR. The presence of an intense band at 1270 cm^{-1} region, attributed to the bending vibration of the phenolic C-O band, confirmed the absence of the phenolic -OH group in the chelation process (Barik et al. 2005; Refat 2013). The three bands observed in pCOM (1631, 1624 and 1604 cm^{-1}) could belong to curcumin itself and the changes in the vibrational absorption spectrum in complexes could indicate the curcumin-copper ion chelation. In pCOM, the disappearance of the sharp band of pVEC at 1537 cm^{-1} (amide II) and the shift of the amide III band of pVEC at 1306 cm^{-1} could suggest the interaction of the carboxyl or amine groups of pVEC with cCUR via electrostatic interactions along with hydrophobic bonding.

Based on the spectroscopic findings that (i) the binding stoichiometry of curcumin and copper ion in solution is at a molar ratio of 1:1 and (ii) a mononuclear cCUR complex formed, the structure of cCUR proposed is presented in Fig. 8a. For a pCOM complex with 1:1 binding ratio of cCUR and pVEC, equimolar pVEC and cCUR were mixed. With the available spectroscopic information, two potential binding models in the complexation of cCUR and pVEC were proposed: (i) interaction of the metal with the carboxyl end of the peptide (Fig. 8b) and (ii) interaction of the metal with the imidazole side chains of the two histidines (Fig. 8c) (Li et al. 2018). In the first binding model, we assume that there would be attraction between the negatively charged carboxyl end of the peptide and the positively charged metal cation. The second binding model is based on the fact that copper binds exclusively to histidine residues

(Barber-Zucker et al. 2017; Kulon et al. 2008; Moulahoum et al. 2020a, b). A site that resembles one of the three signature copper-binding motifs, the bis-His motif (Conklin et al. 2017), is present in pVEC. We hypothesize that this is the HAH motif located towards the C-terminal end of the peptide. The two histidine residues are separated by one of the smallest amino acids, alanine; thus, the effect of alanine may be considered to be negligibly small to alter the binding of copper.

A critical factor limiting the application of pure curcumin of crystalline powder in clinical settings is its extremely poor water solubility. Literature suggests different approaches including encapsulation of curcumin particles or complexes with various carriers such as polymers, liposomes, nanospheres, metals, and proteins and formation of covalent or non-covalent conjugates with peptides to enhance its potential (Ratrey et al. 2020; Banerjee and Chakravarty 2015; Manju and Sreenivasan 2011; Dutta and Ikiki 2013; Li et al. 2005; Gosangari and Dyakonov 2013; Jain et al. 2011; Silva et al. 2018). The current study reports that the complexation of curcumin with pVEC using copper ion as a linker increases solubility. Increase in the polarity of the molecule could be an explanation for the observed behavior (Sareen et al. 2016). The results from the Zetasizer instrument also support this finding. Size measured for curcumin here was rather big for such a small molecule, a direct consequence of its insolubility. Curcumin molecules gathered together to form aggregates, which resulted in larger particles. The size of cCUR was even larger than curcumin alone. The attachment of a small copper ion to curcumin should theoretically not increase the size of this molecule to this extent. The decrease in size as pCOM formed should be directly correlated with increased solubility and thereby bioavailability (Ubeyitogullari and Ciftci 2019). However the polydispersity index indicated pCOM to be considered as a moderately polydisperse particle (Lee et al. 2016). Furthermore, unlike curcumin and cCUR, the surface charge of pCOM was positive, just like pVEC. This abrupt change from a negative value to a high positive value in the zeta potential clued the binding of pVEC to form the ternary complex pCOM.

Increased solubility is an important factor that influences bioavailability; however, it is not sufficient. The compound should also be stable in solution (Wang et al. 1997). The decomposition of curcumin is believed to be a consequence of the removal of a proton from its phenolic group (Zebib et al. 2010). This was also demonstrated in our results for curcumin and its complexed forms at pH values approximately at or above neutral. The behavior of the complexes and curcumin were pretty similar at these pH values, therefore it might possible for curcumin to dissociate from the complex and to decompose following release. Interestingly, in distilled water after 24 h, more than half of the curcumin exhibited stability only in the complexed forms. It is well documented that curcumin can act as a chelating agent for transition metals and form metallo-curcumin complexes. The formation of such complexes has been shown to improve the properties of free curcumin (Sareen et al. 2016). Consequently, the presence of copper might contribute to the observed stability of the complexes. Although, proteins, peptides, and amino acids have also been used to improve the solubility, stability, and bioavailability of curcumin (Li et al. 2018; Yamashita et al. 2016; Mohammadian et al. 2019), the contribution of pVEC to stability here is questionable since the binary and ternary complexes displayed similar behaviors.

Many studies report the improvement of a wide range of activities upon conjugation to a CPP (Zhang et al. 2020; Lee et al. 2019, 2020; Lindgren et al. 2006; Habault and Poyet 2019). Thus; we have tested the antimicrobial activity of the formed complex based on the fact that curcumin displays antimicrobial activity against a wide range of microbes such as Gram-positive *B. subtilis*, *B. cereus*, and *S. aureus*, and Gram-negative *Pseudomonas aeruginosa* and *E. coli* (Brezden et al. 2016). Commonly, Gram-negative bacteria display higher resistance to raw curcumin than Gram-positive bacteria, which is explained by the high content of hydrophilic lipopolysaccharides in the outer membrane of gram-negative bacteria. These act as a permeability barrier against a variety of hydrophobic antimicrobial compounds (Shakeri et al. 2014). The MIC values of raw curcumin found here against different bacteria was consistent with previous reports. Upon complexation, solubility and stability greatly improved, which was also reflected in the MIC values or the curcumin amount in the MIC values. The improvement was specifically significant against the Gram-negative *E. coli*, which was consistent with the data reported by Lee et al. (2019). A more recent study has shown that a complex with the water-soluble octaarginine peptide (a CPPs) improved the antimicrobial activity in both Gram-negative and Gram-positive bacteria by enhancing aqueous dispersibility and bioactivity of curcumin (Ratrey et al. 2020). However, the significance of copper in the complex should not be disregarded since reports are already available on the activity improvement with transition metals (Chandrasekar et al. 2014; Zorofchian Moghadamtousi et al. 2014). Kumar et al. (2021) have reported that upon complexation with Cu^{2+}, curcumin displayed enhanced antimicrobial activity against both *E. coli* and *S. aureus* with improved stability. A similar increase in antimicrobial activity has been observed in complexes with Mn^{2+} and Co^{2+} (Saha et al. 2020; Girish et al. 2019).

Significant improvement in antimicrobial activity has also been reported with nanotechnology strategies (Gao et al. 2020). When curcumin was encapsulated together with berberine, a synergistic effect was reported and activity against MRSA was higher when compared to that

of free curcumin (Bhatia et al. 2021). Curcumin-silver nanoparticles have also displayed both higher antimicrobial and antibiofilm activities against Gram-negative and Gram-positive species with better stability (Jaiswal and Mishra 2018). Overall, available data suggest that different systems can be used to overcome curcumin's limitations, primarily low solubility and stability. Here in this study, the synthesized cCUR and pCOM displayed better antimicrobial activity with improved stability and solubility when compared to free curcumin.

Conclusions

In the present study, the copper ion and the well-known cell-penetrating peptide, pVEC, were evaluated with an attempt to improve curcumin's solubility and consequently its antimicrobial activity. The complex of curcumin with copper acetate and pVEC was successfully prepared and characterized in terms of solubility and stability utilizing UV-Vis spectrophotometer, FT-IR, and Zetasizer. It was concluded that complexation of curcumin with copper acetate and peptide enhanced solubility. The stabilities of cCUR, pCUR, and pCOM were significantly better than of free curcumin in water. The complex formation with copper and pVEC apparently protected curcumin against chemical degradation in water for a significant period (24 h), which raised hope for further pharmaceutical applications. The size of the complex obtained from DLS measurements confirmed the molecule to be on the nanoscale. The different surface charges of pVEC and cCUR was a strong indication of complex formation with the peptide. The formed complex (pCOM) displayed enhanced antibacterial activity against not only against gram-positive but also against gram-negative bacterial cells.

In conclusion, the constructed metallo curcumin conjugated pVEC complex exhibited enhanced properties; with copper altering more the stability and pVEC altering more the solubility. Thus, the ternary complex possesses the potential for future applications. After evaluation of its cytotoxicity, this complex can be proposed as a new form to administer curcumin.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aboudiab B, Tehrani-Bagha AR, Patra D (2020) Curcumin degradation kinetics in micellar solutions: Enhanced stability in the presence of cationic surfactants. Colloids Surf A 592:124602

Acar T, Arayıcı PP, Ucar B, Karahan M, Mustafaeva Z (2019) Synthesis, characterization and lipophilicity study of Brucella abortus' immunogenic peptide sequence that can be used in the future vaccination studies. Int J Pept Res Ther 25(3):911–918

Adamczak A, Ożarowski M, Karpiński TM (2020) Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals 13(7):153

Alaybeyoglu B, Sariyar Akbulut B, Ozkirimli E (2018) pVEC hydrophobic N-terminus is critical for antibacterial activity. J Pept Sci 24(6):e3083

Amsterdam D (1996) Susceptibility testing of antimicrobials in liquid media. Antibiotics in laboratory medicine

Arasoglu T, Derman S, Mansuroglu B, Yelkenci G, Kocyigit B, Gumus B, Acar T, Kocacaliskan I (2017) Synthesis, characterization and antibacterial activity of juglone encapsulated PLGA nanoparticles. J Appl Microbiol 123(6):1407–1419

Avdeev A (2012) Solubility, permeability and Charge state. Absorption and Drug Development. John Wiley & Sons, Hoboken, New Jersey

Banerjee R (2014) Inhibitory effect of curcumin-Cu (II) and curcumin-Zn (II) complexes on amyloid-beta peptide fibrillation. Bioinorganic chemistry and applications 2014

Banerjee S, Chakravarty AR (2015) Metal complexes of curcumin for cellular imaging, targeting, and photoinduced anticancer activity. Acc Chem Res 48(7):2075–2083

Barber-Zucker S, Shaanan B, Zarivach R (2017) Transition metal binding selectivity in proteins and its correlation with the phylogenomic classification of the cation diffusion facilitator protein family. Sci Rep 7(1):1–12

Barik A, Mishra B, Shen L, Mohan H, Kadam R, Dutta S, Zhang H-Y, Priyadarsini KI (2005) Evaluation of a new copper (II)–curcumin complex as superoxide dismutase mimic and its free radical reactions. Free Radic Biol Med 39(6):811–822

Barik A, Mishra B, Kunwar A, Kadam L, Dutta S, Padhye S, Satpati AK, Zhang HY, Priyadarsini KI (2007) Eur J Med Chem 42 (4):431

Bechnak L, Khalil C, Kurdi RE, Khnayzer RS, Patra D (2020) Curcumin encapsulated colloidal amphiphilic block co-polymeric nanocapsules: Colloidal nanocapsules enhance photodynamic and anticancer activities of curcumin. Photochem Photobiol Sci 19(8):1088–1098

Bhatia E, Sharma S, Jadhav K, Banerjee R (2021) Combinatorial liposomes of berberine and curcumin inhibit biofilm formation and intracellular methicillin resistant Staphylococcus aureus infections and associated inflammation. J Mater Chem B 9(3):864–875

Brezden A, Mohamed MF, Nepal M, Harwood JS, Kuriakose J, Seleem MN, Chmielewski J (2016) Dual targeting of intracellular pathogenic bacteria with a cleavable conjugate of kanamycin and an antibacterial cell-penetrating peptide. J Am Chem Soc 138(34):10945–10949

Chandrasekar T, Pravin N, Raman N (2014) Biosensitive metal chelates from curcumin analogues: DNA unwinding and anti-microbial evaluation. Inorg Chem Commun 43:45–50

Conklin SE, Bridgman EC, Su Q, Riggs-Gelasco P, Haas KL, Franz KJ (2017) Specific histidine residues confer histatin peptides with copper-dependent activity against Candida albicans. Biochemistry 56(32):4244–4255

Copolovici D, Langel K, Eriste E, Langel Ü (2014) Cell-penetrating peptides: design, synthesis, and applications. ACS Nano 8:1972–1994

da Silva AC, de Freitas Santos PD, Prado Silva JT, Leimann FV, Bracht L, Goncalves OH (2018) Impact of curcumin nanoformulation on its antimicrobial activity. Trends in Food Sci Technol 72:74–82

Derman S, Akdeste ZM (2015) Particle size and zeta potential investigation of synthetic peptide-protein conjugates/Sentetik peptid-protein konjugatlarının parçacık boyutu ve zeta potensiyel analizi. Turkish J Biochem 40(4):282–289

Doktorovova S, Souto EB, Silva AM (2018) Hansen solubility parameters (HSP) for prescreening formulation of solid lipid nanoparticles (SLN): In vitro testing of curcumin-loaded SLN in MCF-7 and BT-474 cell lines. Pharm Dev Technol 23(1):96–105

Dutta AK, Ikiki E (2013) Novel drug delivery systems to improve bioavailability of curcumin. J Bioequiv Availab 6(1):001–009

El Khoury E, Abiad M, Kassaify ZG, Patra D (2015) Green synthesis of curcumin conjugated nanosilver for the applications in nucleic acid sensing and anti-bacterial activity. Colloids Surf B 127:274–280

Feng T, Wei Y, Lee RJ, Zhao L (2017) Liposomal curcumin and its application in cancer. Int J Nanomed 12:6027

Gao M, Long X, Du J, Teng M, Zhang W, Wang Y, Wang X, Wang Z, Zhang P, Li J (2020) Enhanced curcumin solubility and antibacterial activity by encapsulation in PLGA oily core nanocapsules. Food Funct 11(1):448–455

Genilloud O (2019) Natural products discovery and potential for new antibiotics. Curr Opin Microbiol 51:81–87

Girish K, Channu B, Baba A (2019) Synthesis and antibacterial activity of cobalt (II) complex of curcumin. Indian J Pharm Sci 81(1):150–155

Gosangari S, Dyakonov T (2013) Enhanced dissolution performance of curcumin with the use of supersaturatable formulations. Pharm Dev Technol 18(2):475–480

Guidotti G, Brambilla L, Rossi D (2017) Cell-penetrating peptides: from basic research to clinics. Trends Pharmacol Sci 38(4):406–424

Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ, Priyadarsini IK, Aggarwal BB (2011) Multitargeting by curcumin as revealed by molecular interaction studies. Nat Prod Rep 28(12):1937–1955

Habault J, Poyet J-L (2019) Recent advances in cell penetrating peptide-based anticancer therapies. Molecules 24(5):927

Hegde AH, Sandhya B, Seetharamappa J (2013) Investigations to reveal the nature of interactions of human hemoglobin with curcumin using optical techniques. Int J Biol Macromol 52:133–138

Heitz F, Morris MC, Divita G (2009) Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. Br J Pharmacol 157(2):195–206

Indira Priyadarsini K (2013) Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Design 19(11):2093–2100

Jain N, Bhawana R, Basniwal H (2011) Buttar and VK Jain. J Agric Food Chem 59:2056

Jaiswal S, Mishra P (2018) Antimicrobial and antibiofilm activity of curcumin-silver nanoparticles with improved stability and selective toxicity to bacteria over mammalian cells. Med Microbiol Immunol 207(1):39–53

Kang MH, Yoon HY, Choi YW (2017) RIPL peptide as a novel cell-penetrating and homing peptide: Design, characterization, and application to liposomal nanocarriers for hepsin-specific intracellular drug delivery. Nanostructures for Cancer therapy. Elsevier, pp 129–157

Kadam PV, Yadav KN, Bhingare CL, Patil MJ (2018) Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. J Pharmacognosy Phytochemistry 7(5):1913–1918

Kharat M, Du Z, Zhang G, McClements D (2016) Physical and chemical stability of curcumin in aqueous solutions and emulsions: Impact of pH and temperature. J Agric Food Chem 65:1525–1532

Khatun B, Baishya P, Ramteke A, Maji T (2020) Study of the complexation of structurally modified curcumin with hydroxypropyl beta cyclodextrin and its effect on anticancer activity. New J Chem 44(12):4887–4897

Kristensen M, Birch D, Mørck Nielsen H (2016) Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos. Int J Mol Sci 17(2):185

Kulon K, Valensin D, Kamysz W, Valensin G, Nadolski P, Porciatti E, Gaggelli E, Kozłowski H (2008) The His–His sequence of the antimicrobial peptide demegen P-113 makes it very attractive ligand for Cu2+. J Inorg Biochem 102(4):960–972

Kumar P, Saha T, Behera S, Gupta S, Das S, Mukhopadhyay K (2021) Enhanced efficacy of a Cu2 + complex of curcumin against Gram-positive and Gram-negative bacteria: Attributes of complex formation. J Inorg Biochem 222:111494

Kumavat S, Chaudhari YS, Borole P, Mishra P, Shenghani K, Duvvuri P (2013) Degradation studies of curcumin. Int J Pharm Rev Res 3(2):50–55

Lee J-Y, Termsarasab U, Park J-H, Lee SY, Ko S-H, Shim J-S, Chung S-J, Cho H-J, Kim D-D (2016) Dual CD44 and folate receptor-targeted nanoparticles for cancer diagnosis and anticancer drug delivery. J Controlled Release 236:38–46

Lee H, Lim SI, Shin S-H, Lim Y, Koh JW, Yang S (2019) Conjugation of cell-penetrating peptides to antimicrobial peptides enhances antibacterial activity. ACS omega 4(13):15694–15701

Lee J, Kwon M, Oh N, Park J, Park S, Seo J, Roh S (2020) Cell-Penetrating peptides enhance the activity of human fibroblast growth factor 2 by prolonging the retention time: a new vision for drug-delivery systems. Int J Mol Sci 21(2):442

Li L, Braiteh FS, Kurzrock R (2005) Liposome-encapsulated curcumin: in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. Cancer: Interdisciplinary International Journal of the American Cancer Society 104(6):1322–1331

Li Y, Zou Q, Yuan C, Li S, Xing R, Yan X (2018) Amino acid coordination driven self-assembly for enhancing both the biological stability and tumor accumulation of curcumin. Angew Chem Int Ed 57(52):17084–17088

Lindgren M, Rosenthal-Aizman K, Saar K, Eiríksdóttir E, Jiang Y, Sassian M, Östlund P, Hällbrink M, Langel Ü (2006) Overcoming methotrexate resistance in breast cancer tumour cells by the use of a new cell-penetrating peptide. Biochem Pharmacol 71(4):416–425

Liu BR, Huang Y-w, Winiarz JG, Chiang H-J, Lee H-J (2011) Intracellular delivery of quantum dots mediated by a histidine-and arginine-rich HR9 cell-penetrating peptide through the direct membrane translocation mechanism. Biomaterials 32(13):3520–3537

Luo J, Yang M (2014) Demethoxycurcumin: a potential antimicrobial agent. J Therm Anal Calorim 115(3):2331–2338

Manju S, Sreenivasan K (2011) Conjugation of curcumin onto hyaluronic acid enhances its aqueous solubility and stability. J Colloid Interface Sci 359(1):318–325

Mohammadian M, Salami M, Momen S, Alavi F, Emam-Djomeh Z, Moosavi-Movahedi AA (2019) Enhancing the aqueous solubility of curcumin at acidic condition through the complexation with whey protein nanofibrils. Food Hydrocolloids 87:902–914

Moloney MG (2016) Natural products as a source for novel antibiotics. Trends Pharmacol Sci 37(8):689–701

Moulahoum H, Ghorbani Zamani F, Timur S, Zihnioglu F (2020a) Metal binding antimicrobial peptides in nanoparticle bio-functionalization: new heights in drug delivery and therapy. Probiotics and antimicrobial proteins 12(1):48–63

Moulahoum H, Zamani FG, Timur S, Zihnioglu F (2020b) Metal binding antimicrobial peptides in nanoparticle bio-functionalization: new heights in drug delivery and therapy. Probiotics and antimicrobial proteins 12(1):48–63

Mouslmani M, Rosenholm JM, Prabhakar N, Peurla M, Baydoun E, Patra D (2015) Curcumin associated poly (allylamine hydrochloride)-phosphate self-assembled hierarchically ordered nanocapsules: size dependent investigation on release and DPPH scavenging activity of curcumin. RSC Adv 5(24):18740–18750

Moussa SH, Tayel AA, Al-Hassan AA, Farouk A (2013) Tetrazolium/formazan test as an efficient method to determine fungal chitosan antimicrobial activity. Journal of Mycology 2013

Muangnoi C, Ratnatilaka Na Bhuket P, Jithavech P, Supasena W, Paraoan L, Patumraj S, Rojsitthisak P (2019) Curcumin diethyl disuccinate, a prodrug of curcumin, enhances anti-proliferative effect of curcumin against HepG2 cells via apoptosis induction. Sci Rep 9(1):1–9

Mun S-H, Joung D-K, Kim Y-S, Kang O-H, Kim S-B, Seo Y-S, Kim Y-C, Lee D-S, Shin D-W, Kweon K-T (2013) Synergistic antibacterial effect of curcumin against methicillin-resistant Staphylococcus aureus. Phytomedicine 20(8–9):714–718

Munyendo WL, Lv H, Benza-Ingoula H, Baraza LD, Zhou J (2012) Cell penetrating peptides in the delivery of biopharmaceuticals. Biomolecules 2(2):187–202

Naksuriya O, van Steenbergen MJ, Torano JS, Okonogi S, Hennink WE (2016) A kinetic degradation study of curcumin in its free form and loaded in polymeric micelles. AAPS J 18(3):777–787

Nawaz A (2011) Curcumin: a natural product of biological importance. Gomal Univ J Res 27(1):7–14

Palm C, Netzereab S, Hällbrink M (2006) Quantitatively determined uptake of cell-penetrating peptides in non-mammalian cells with an evaluation of degradation and antimicrobial effects. Peptides 27(7):1710–1716

Pandit RS, Gaikwad SC, Agarkar GA, Gade AK, Rai M (2015) Curcumin nanoparticles: physico-chemical fabrication and its in vitro efficacy against human pathogens. 3 Biotech 5(6):991–997

Poe M (1977) Acidic dissociation constants of folic acid, dihydrofolic acid, and methotrexate. J Biol Chem 252(11):3724–3728

Rai D, Singh JK, Roy N, Panda D (2008) Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. Biochem J 410(1):147–155

Ratrey P, Dalvi SV, Mishra A (2020) Enhancing aqueous solubility and antibacterial activity of curcumin by complexing with cell-penetrating octaarginine. ACS omega 5(30):19004–19013

Razzak MA, Lee JE, Park HH, Park TH, Choi SS (2018) Exploring binding mechanisms between curcumin and silkworm 30Kc19 protein using spectroscopic analyses and computational simulations. Biotechnol Bioprocess Eng 23(5):605–616

Refat MS (2013) Synthesis and characterization of ligational behavior of curcumin drug towards some transition metal ions: Chelation effect on their thermal stability and biological activity. Spectrochim Acta Part A Mol Biomol Spectrosc 105:326–337

Saha T, Kumar P, Sepay N, Ganguly D, Tiwari K, Mukhopadhyay K, Das S (2020) Multitargeting antibacterial activity of a synthesized Mn2 + complex of curcumin on gram-positive and gram-negative bacterial strains. ACS omega 5(27):16342–16357

Sareen R, Jain N, Dhar K (2016) Curcumin–Zn (II) complex for enhanced solubility and stability: an approach for improved delivery and pharmacodynamic effects. Pharm Dev Technol 21(5):630–635

Sathishkumar P, Hemalatha S, Arulkumar M, Ravikumar R, Yusoff ARM, Hadibarata T, Palvannan T (2015) Curcuminoid Extraction from Turmeric (C urcuma Longa L.): Efficacy of Bromine-Modified Curcuminoids Against Food Spoilage Flora. J Food Biochem 39(3):325–333

Sha K, Ma Q, Veroniaina H, Qi X, Qin J, Wu Z (2021) Formulation optimization of solid self-microemulsifying pellets for enhanced oral bioavailability of curcumin. Pharm Dev Technol 26(5):549–558

Shakeri A, Khakdan F, Soheili V, Sahebkar A, Rassam G, Asili J (2014) Chemical composition, antibacterial activity, and cytotoxicity of essential oil from Nepeta ucrainica L. spp. kopetdaghensis. Ind Crops Prod 58:315–321

Shin GH, Li J, Cho JH, Kim JT, Park HJ (2016) Enhancement of curcumin solubility by phase change from crystalline to amorphous in CUR-TPGS nanosuspension. J Food Sci 81(2):N494–N501

Slika L, Moubarak A, Borjac J, Baydoun E, Patra D (2020) Preparation of curcumin-poly (allyl amine) hydrochloride based nanocapsules: Piperine in nanocapsules accelerates encapsulation and release of curcumin and effectiveness against colon cancer cells. Mater Sci Engineering: C 109:110550

Trigo-Gutierrez JK, Vega-Chacón Y, Soares AB, Mima EGdO (2021) Antimicrobial activity of curcumin in nanoformulations: a comprehensive review. Int J Mol Sci 22(13):7130

Ubeyitogullari A, Ciftci ON (2019) A novel and green nanoparticle formation approach to forming low-crystallinity curcumin nanoparticles to improve curcumin's bioaccessibility. Sci Rep 9(1):1–11

Ucar B, Acar T, Arayici PP, Derman S (2021) A nanotechnological approach in the current therapy of COVID-19: model drug oseltamivir-phosphate loaded PLGA nanoparticles targeted with spike protein binder peptide of SARS-CoV-2. Nanotechnology 32(48):485601. doi:https://doi.org/10.1088/1361-6528/ac1c22

Varshosaz J, Ghaffari S, Khoshayand MR, Atyabi F, Dehkordi AJ, Kobarfard F (2012) Optimization of freeze-drying condition of amikacin solid lipid nanoparticles using D-optimal experimental design. Pharm Dev Technol 17(2):187–194

Vellampatti S, Chandrasekaran G, Mitta SB, Lakshmanan V-K, Park SH (2018) Metallo-Curcumin-conjugated DNA complexes induces preferential prostate cancer cells cytotoxicity and pause growth of bacterial cells. Sci Rep 8(1):1–11

Wang Y-J, Pan M-H, Cheng A-L, Lin L-I, Ho Y-S, Hsieh C-Y, Lin J-K (1997) Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Anal 15(12):1867–1876

Wanninger S, Lorenz V, Subhan A, Edelmann FT (2015) Metal complexes of curcumin–synthetic strategies, structures and medicinal applications. Chem Soc Rev 44(15):4986–5002

Yamashita R, Oshima T, Baba Y (2016) A hydrophobic peptide fraction that enhances the water dispersibility of curcumin. Asian J Pharm Sci 11(5):631–640

Zhang W, Taheri-Ledari R, Hajizadeh Z, Zolfaghari E, Ahghari MR, Maleki A, Hamblin MR, Tian Y (2020) Enhanced activity of vancomycin by encapsulation in hybrid magnetic nanoparticles conjugated to a cell-penetrating peptide. Nanoscale 12(6):3855–3870

Zebib B, Mouloungui Z, Noirot V (2010) Stabilization of curcumin by complexation with divalent cations in glycerol/water system. Bioinorganic chemistry and applications 2010

Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K (2014) A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed research international 2014