Food & Function

Curcumin-loaded Pickering emulsion stabilized by insoluble complexes involving ovotransferrin–gallic acid conjugate and carboxymethyldextran

Journal:	*Food & Function*
Manuscript ID	FO-ART-06-2019-001162.R1
Article Type:	Paper
Date Submitted by the Author:	04-Jul-2019
Complete List of Authors:	Wei, Zihao; Rutgers University, Departiment of Food Science Zhang, Hongwei; Rutgers The State University of New Jersey Huang, Qingrong; Rutgers University, Department of Food Science, , 65 Dudley Road, ,

Curcumin-loaded Pickering emulsion stabilized by insoluble complexes involving ovotransferrin–gallic acid conjugate and carboxymethyldextran

Zihao Wei, Hongwei Zhang and Qingrong Huang*

Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901, United States

* To whom correspondence should be addressed. Tel: +1 (848) 932-5514. Fax: +1 (732) 932-6776.

Email: qhuang@sebs.rutgers.edu

ABSTRACT:

The present work aimed to fabricate antioxidant particle-stabilized Pickering emulsions with outstanding protection on encapsulated nutraceuticals. Antioxidant ovotransferrin–gallic acid conjugates (OTGCONJ) were prepared using alkaline method, and electrostatic assembly technique was utilized to construct OTGCONJ–CMD particles with OTGCONJ and carboxymethyldextran (CMD) as building blocks. After investigation of particle size, insoluble character and intermediate wettability of OTGCONJ–CMD particles, OTGCONJ–CMD particles were verified as eligible Pickering stabilizers. Visual appearance showed that stable OTGCONJ–CMD particle-stabilized Pickering emulsion consisted of emulsified phase alone. Rheological analysis revealed that the Pickering emulsion had high viscosity and gel-like structure. In terms of protective effect, OTGCONJ–CMD particle-stabilized Pickering emulsion could significantly retard curcumin degradation against UV light. *In vitro* digestion study revealed that OTGCONJ–CMD particle-stabilized Pickering emulsion improved both extent of lipolysis and curcumin bioaccessibility remarkably, suggesting that OTGCONJ–CMD particle-stabilized Pickering emulsion was an excellent nutraceutical delivery vehicle. The novel findings in this work could have important implications for design of nutraceutical-loaded Pickering emulsions with excellent protective effect and nutraceutical delivery efficiency.

Keywords: antioxidant strategy, Pickering emulsion, ovotransferrin–gallic acid conjugate, protective effect, *in vitro* digestion, bioaccessibility

1. Introduction

Due to potential health benefits such as cancer prevention and neuroprotection, curcumin has been widely used as an attractive food colourant or dietary supplement.[1] However, application of curcumin is seriously limited due to challenges such as low water solubility as well as poor oral bioaccessibility, and susceptibility to degradation during storage is another restraint.[1–4] Photodegradation of curcumin against ultraviolet (UV) light is generally a fast process, leading to loss of color and desirable bioactivies.[3,5] Multiple tools have been developed to overcome these challenges, and encapsulating lipophilic curcumin with emulsion-based delivery systems proves a useful means to improve both storage stability and bioaccessibility of curcumin.[2]

Although emulsifiers at oil-water interfaces may provide physical barrier for photosensitive curcumin, degradation of encapsulated curcumin in emulsions may still proceed at a relatively rapid rate.[2,6] The reason for this phenomenon is that reactive oxygen species in emulsions can accelerate degradation of curcumin.[2,7] Singlet oxygen is one of the most important reactive oxygen species,[7] and contribution of singlet oxygen to photodegradation of curcumin has been confirmed.[5] Since strong antioxidants such as flavonoids and gallic acid can quench singlet oxygen effectively,[8,9] it may be speculated that addition of antioxidants into a proper region may retard photofading of curcumin. Since substantial reactive oxygen species may be located around the oil-water interface,[10] accumulation of antioxidants at emulsion interfaces may significantly improve chemical stability of encapsulated nutraceuticals.[11,12] Considering that emulsifiers are generally located at interfacial regions, immobilization of antioxidants onto emulsifiers may be a feasible approach to improving concentration of antioxidants at the oil-water interface. Choice of appropriate emulsifiers should be made based on physicochemical properties of emulsions. When compared with conventional

nanoemulsions, Pickering emulsions have advantages such as irreversible interface absorption and outstanding stability against coalescence,[13] indicating strong potential to provide long-term protection for curcumin. Therefore, developing Pickering stabilizers composed of antioxidants may be a prospective tool to prevent degradation of curcumin.

Covalent complexations between proteins and polyphenols may serve as an effective means to combine antioxidants and emulsifiers, and protein–polyphenol conjugates may possess advantageous properties of both proteins and polyphenols without introduction of undesirable characteristics.[14,15] Therefore, protein–polyphenol conjugates can be utilized as building blocks to construct antioxidant Pickering stabilizers. Ovotransferrin–gallic acid conjugate (OTGCONJ) are chosen for the concept described above in our study, and that's because ovotransferrin and OTGCONJ have two major advantages. Specifically, the first advantage is that ovotransferrin occupies 12 percent of egg white proteins,[16] indicating abundant material supply. Second, our preliminary experiments have demonstrated that both ovotransferrin and OTGCONJ do not have self-aggregation at most pHs, indicating that OTGCONJ is suitable for precise assembly of particles as Pickering stabilizers. Given that protein–polysaccharide complexation is a bottom-up technique to construct food-grade particles as Pickering stabilizers,[17–19] manipulation of OTGCONJ–carboxymethyldextran complexation can be utilized to fabricate food-grade Pickering stabilizers. Carboxymethyldextran (CMD) is chosen as a polysaccharide model in this study due to narrow molecular weight distribution and high charge density, which is beneficial to precise construction of particles as Pickering stabilizers. It is expected that Pickering emulsions stabilized by OTGCONJ–CMD particles can significantly improve chemical stability and bioaccessibility of curcumin, which helps to design curcumin-loaded delivery platforms rationally.

Thus, the major objectives of this study were to fabricate food-grade Pickering stabilizers with OTGCONJ and CMD as building blocks, characterize Pickering emulsion stabilized by OTGCONJ–CMD particles, study protective effects of the Pickering emulsion on curcumin and investigate bioaccessibility of curcumin in OTGCONJ–CMD particle-stabilized Pickering emulsion.

2. Materials and methods

2.1. Materials

Ovotransferrin (purity > 88%) was purchased from Neova Technologies Inc. (Abbotsford, Canada). According to manufacturer's report, iron content of the ovotransferrin was 1099 μg Fe/g. Gallic acid was obtained from Sigma-Aldrich (St. Louis, USA). Dextran (T10) with an average molecular weight of 10 kDa was provided by Pharmacosmos (Holbaek, Denmark). Medium chain triglyceride (MCT) was kindly provided by Stepan Company (Northfield, USA). Curcumin (85% purity with 11% of demethoxycurcumin and 4% of bisdemethoxy curcumin as impurities) was generously provided by Sabinsa Corporation (East Windsor, USA). Other chemicals were purchased from Sigma-Aldrich (St. Louis, USA), unless otherwise stated. Milli-Q water was used throughout all experiments.

2.2. Preparation of ovotransferrin–gallic acid conjugate (OTGCONJ)

Ovotransferrin–gallic acid conjugate (OTGCONJ) was synthesized as described previously with minor modifications.[15] Ovotransferrin (2 mg/mL) was dispersed in Milli-Q water, followed by stirring overnight. Sodium azide (0.02%, w/v) was added to suppress microbial growth. Gallic acid dissolved in Milli-Q water was added into ovotransferrin solutions to make molar ratio of gallic

acid to ovotransferrin as 19:1, and pH of the mixture was adjusted to 9. To obtain ovotransferrin–gallic acid conjugate (OTGCONJ), the mixture was stirred constantly with free exposure to air at room temperature for 24 h.[15] Thereafter, OTGCONJ was dialyzed (molecular weight cutoff 10000 Da) against Milli-Q water at 4℃ for 48 h to remove unbound gallic acid, followed by freeze-drying. The total phenolic content of OTGCONJ was determined according to the Folin–Ciocalteu method using gallic acid as a standard.[15] ABTS radical scavenging capacity of OTGCONJ was measured and expressed as nmol Trolox equivalents (TE)/mg sample using Trolox as a standard.[20]

To characterize OTGCONJ more clearly, ovotransferrin control (OVTC) was prepared according to the aforementioned procedures but without the addition of gallic acid. Based on result of total phenolic content, ovotransferrin–gallic acid mixture (OTGMIX) was prepared at pH 5.0 according to the aforementioned procedures at molar ratio of gallic acid to ovotransferrin as 17.2:1 without dialysis.

2.3. Characterization of ovotransferrin–gallic acid conjugate (OTGCONJ)

2.3.1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

SDS-PAGE was conducted using 10% acrylamide separating gel in gel electrophoresis apparatus (Bio-Rad Laboratories, Hercules, USA), and the samples (2 mg/mL) were heated at 95 °C for 5 min prior to loading. The electrophoresis was operated at 80 V. Coomassie brilliant blue staining solution was obtained by dissolving Coomassie brilliant blue (0.1 wt%) into solution containing 40% ethanol and 10% acetic acid, and the destaining solution was composed of 25% ethanol and 10% acetic acid. After the the SDS-PAGE run, the gel was stained overnight with

Coomassie brilliant blue staining solution and subsequently destained with destaining solution.

MALDI-TOF-MS was utilized to measure molecular weight of OTGCONJ exactly. The MALDI-TOF-MS analysis was performed using AB Sciex 4800 MALDI-TOF/TOF mass spectrometer (Applied Biosystems, Foster City, USA), and data were acquired after external calibration with bovine serum albumin.

2.3.2. Small-angle X-ray scattering (SAXS)

SAXS analysis of OTGCONJ, OVTC and OTGMIX at different pHs was performed at the BioCAT 18-ID beamline of Advanced Photon Sources (Argonne National Laboratory, Lemont, USA) as previously described.[21] The scattering data were acquired using a detector located at 3.5 m from the sample. The wavelength and short exposure time of X-ray radiation were 1.033 Å and 1 s, respectively. The final scattering data were obtained by subtracting corresponding solvent background.

2.4. Synthesis of carboxymethyldextran (CMD)

Carboxymethyldextran (CMD) was synthesized using dextran (T10) based on a previously reported method,[22] followed by dialysis (molecular weight cutoff 3500 Da) against Milli-Q water to remove impurities and spray drying process.

2.5. Preparation and characterization of OTGCONJ–CMD particles

2.5.1. Turbidity titrations

OTGCONJ (0.4 mg/mL) and CMD (0.4 mg/mL) solutions were prepared by dispersing OTGCONJ and CMD in Milli-Q water, followed by thorough stirring to obtain homogeneous solutions. The same volume of OTGCONJ and CMD solutions were mixed together, and pH of the

mixture was adjusted to 8 with NaOH.

The pH-dependent turbidity was determined employing a Brinkmann PC910 colorimeter (Metrohm, Riverview, USA). The colorimeter was calibrated to 100% transmittance (*T*) with Milli-Q water, and turbidity was defined as 100– *T*%.[23] The direction of turbidity titration was from high pH to low pH, and HCl solutions with concentration gradients were used to adjust the solution pH to minimize the dilution effect. Titrations of individual OTGCONJ (0.2 mg/mL) and CMD (0.2 mg/mL) were conducted as controls.

2.5.2. Zeta potential

Zeta potential of OTGCONJ, CMD and OTGCONJ–CMD mixture over the pH range of 2 to 8 was measured from their electrophoretic mobilities employing Zetasizer Nano-ZS90 instrument (Malvern Instruments, Worcestershire, UK). Smoluchowski model was used for zeta potential analysis. Zeta potential data were acquired by averaging 3 measurements, and the result was expressed as mean value ± standard deviation.

2.5.3. Dispersion stability test of OTGCONJ–CMD particles and particle size analysis

OTGCONJ–CMD particles (0.2 wt%, calculated by total biopolymer concentration) within a narrow pH range of 3.5–4.0 were freshly prepared at mass ratio of 1 to 1 as mentioned above, and these OTGCONJ–CMD particles were stored at room temperature for 1 week. Sodium azide (0.02%, w/v) was added to suppress microbial growth. Based on the result of storage stability, selected OTGCONJ–CMD particles were further studied for particle size analysis. The hydrodynamic diameter and polydispersity index (PdI) of OTGCONJ–CMD particles at pH 3.7 were measured using Zetasizer Nano-ZS90 instrument.

2.5.4. Contact angle

OTGCONJ–CMD particles applied in the following research were prepared at pH 3.7 and mass ratio of 1 to 1. Homogeneous OTGCONJ–CMD particle film was deposited onto smooth glass substrate by drying OTGCONJ–CMD particle dispersion (2.5 wt%, calculated by total biopolymer concentration) in an oven at 50 °C. Static water-in-air contact angle was determined by dropping water droplets (2 μL) onto OTGCONJ–CMD particle film using VCA (video contact angle) optima setup (AST Products Inc., Billerica, USA).[24]

2.6. Preparation of curcumin-loaded Pickering emulsion stabilized by OTGCONJ–CMD particles

Curcumin was dissolved in MCT under agitation and heating to a final concentration of 1 mg/mL The minimal amount of OTGCONJ–CMD particles required to formulate stable Pickering emulsions with homogenous emulsified phase only was investigated in preliminary experiments. OTGCONJ–CMD particle dispersions (2.5 wt%, calculated by total biopolymer concentration) at pH 3.7 were mixed with curcumin-loaded MCT at a volume ratio of 8 to 17, and oil fraction of the mixture was 0.68. To obtain Pickering emulsion stabilized by OTGCONJ–CMD particles, an IKA Ultra-Turrax T25 homogenizer (IKA-Werke GMBH & CO., Germany) was utilized to homogenize the mixture at 10000 rpm for 3 min. The emulsion type (water-in-oil or oil-in-water) of Pickering emulsion stabilized by OTGCONJ–CMD particles was tested by checking dispersibility of the emulsion in water or MCT.[17,25] Oil-in-water (water-in-oil) Pickering emulsions dispersed readily in water (MCT oil), but stayed immiscible in MCT oil (water). Characterization of OTGCONJ–CMD particle-stabilized Pickering emulsions was generally conducted within 24 h after fresh preparation, unless otherwise stated.

2.7. Preparation of curcumin-loaded Pickering emulsion stabilized by OTGMIX–CMD particles

To compare Pickering emulsifier performance of OTGCONJ–CMD particles and OTGMIX–CMD particles, Pickering emulsion stabilized by OTGMIX–CMD particles was also prepared. OTGMIX–CMD particles were assembled as follows. OTGMIX (2.5 wt%) and CMD (2.5 wt%) solutions were prepared by dispersing OTGMIX and CMD in Milli-Q water, followed by thorough stirring to obtain homogeneous solutions. The same volume of OTGMIX and CMD solutions were mixed together, and pH of the mixture was adjusted to pH 3.7. Afterwards, OTGMIX–CMD particles (2.5 wt%, calculated by total biopolymer concentration) were acquired. Upon fresh preparation of OTGMIX–CMD particles, OTGMIX–CMD particle dispersions (2.5 wt%, calculated by total biopolymer concentration) at pH 3.7 were mixed with curcumin-loaded MCT at a volume ratio of 8 to 17, and oil fraction of the mixture was 0.68. To obtain Pickering emulsion stabilized by OTGMIX–CMD particles, an IKA Ultra-Turrax T25 homogenizer (IKA-Werke GMBH & CO., Germany) was utilized to homogenize the mixture at 10000 rpm for 3 min.

Since OTGMIX–CMD particle-stabilized Pickering emulsion was not stable with obvious phase separation and a significant amount of curcumin could not be encapsulated into emulsions, only OTGCONJ–CMD particle-stabilized Pickering emulsion was studied during the rest of this study.

2.8. Characterization of OTGCONJ–CMD particle-stabilized Pickering emulsion

2.8.1. Measurement of emulsified phase volume fraction

The height of emulsified phase (H_e) and total emulsion height (H_t) of Pickering emulsion were measured, and emulsified phase volume fraction of emulsions was calculated as:

$$\text{emulsified phase volume fraction} = (H_e/H_t) \times 100 \quad (1)$$

2.8.2. Microscopic observation

Microstructure of OTGCONJ–CMD particle-stabilized Pickering emulsion was captured employing 10× objective microscope (Nikon Eclipse TE 2000-U, Nikon Corporation, Tokyo, Japan). The droplet size of Pickering emulsion was estimated employing Image J software (National Institutes of Health, Bethesda, MD), and average emulsion droplet size was obtained after analysis of at least 500 emulsion droplets.[17]

Fluorescence microscopy was applied to verify adsorption of OTGCONJ–CMD particles at emulsion interfaces. Nile red and rhodamine B were used to stain oil phase and OTGCONJ–CMD particles, respectively. The Pickering emulsions labeled with individual fluorescence dye (Nile red or rhodamine B) were subject to optical microscopy observation under fluorescence field.[26]

2.8.3. Rheological measurements

The rheological properties of OTGCONJ–CMD particle-stabilized Pickering emulsion were analyzed employing a Discovery HR-2 rheometer (TA Instruments, New Castle, USA) with a parallel plate geometry (diameter 25 mm, gap 1 mm). The emulsion was placed onto the plate and five-minute rest was allowed to reach thermal equilibrium prior to analysis. The plate edge was covered with silicone oil to inhibit drying of emulsions. Apparent viscosity of Pickering emulsion as function of shear rate was measured during steady-state flow measurements.[27] Frequency-dependent storage modulus (G′) and loss modulus (G″) were obtained during dynamic

frequency sweep test at a fixed strain amplitude of 1% (within the linear viscoelastic region).

2.9. Stability of encapsulated curcumin in Pickering emulsion against ultraviolet (UV) light exposure

To study protective effect of OTGCONJ–CMD particles at emulsion interfaces on encapsulated curcumin against UV radiation treatment, curcumin-loaded Pickering emulsion stabilized by OTGCONJ–CMD particles was exposed to UV radiation (6 W, 365 nm) via a UV radiation equipment in a lightproof cabinet, and curcumin-loaded MCT oil was applied as control.[6] To better clarify protective effect of OTGCONJ–CMD particle-stabilized Pickering emulsion, based on similar procedures as described in 2.6, Pickering emulsion stabilized by ovotransferrinn–CMD particles (non-antioxidant particles withoout gallic acid) was prepared as control. The Pickering emulsion stabilized by non-antioxidant particles (ovotransferrinn–CMD particles) was also exposed to UV radiation. At designated time points a 200 μL aliquot was sampled from the Pickering emulsion and control MCT oil, respectively. To recover curcumin from the emulsion and MCT oil, 2.4 mL of methanol was mixed with the 200 μL aliquot using vortex mixer, followed by centrifugation (12000 rpm, 10 min) to separate the supernatant layer.[6] The curcumin recovery procedures were repeated three times, and concentration of curcumin was determined using an UltiMate 3000 HPLC system (Dionex, Sunnyvale, USA) as previously described.[28] The residual curcumin level was determined as $(C/C_0)\times100\%$, where C_0 and C were the initial concentration of curcumin and the concentration of curcumin at designated time point during UV radiation, respectively.

2.10. *In vitro* gastrointestinal digestion of OTGCONJ–CMD particle-stabilized Pickering emulsion

Simulated gastric fluid was prepared by adding 2 g of sodium chloride into 1 L of Milli-Q water and adjusting pH to 1.2 with HCl.[29] The samples (MCT oil and Pickering emulsion stabilized by OTGCONJ–CMD particles) containing 2 g of oil were thoroughly mixed with 16 mL of simulated gastric fluid and incubated in a temperature-controlled water bath (37.0±0.2 °C) with continuous agitation.[26] To start gastric digestion process, freshly dissolved pepsin in 2 mL of simulated gastric fluid was added to the mixture to make a final pepsin concentration of 1.6 mg/mL (equivalent to enzyme activity of 4800 FCC U/mL).[30] After digestion in gastric juice for 120 min, pHs of the samples were raised to 7.5 to inactivate pepsin, and gastric digestion was terminated.[26]

Simulated intestinal fluid was obtained by adding 10 mM $CaCl_2$ and 10 mg/mL bile salt into Tris-maleate buffer (pH 7.5), and pancreatin was added into simulated intestinal fluid to make a lipase concentration of 3.2 mg/mL (equivalent to enzyme activity of 179.2 U/mL).[30] To initiate intestinal digestion, the gastric digesta were collected and mixed with an equal volume of simulated intestinal fluid, followed by incubation in a water bath (37.0±0.2 °C) for 120 min. Adding 0.25 M NaOH manually was applied to maintain pH of mixtures at 7.5 during lipolysis, and the volume of added NaOH solution over time was recorded throughout the intestinal digestion. The digesta were centrifuged (at 10000 g for 30 min) after gastrointestinal digestion, and the centrifuged samples included a clear micelle phase as well as an opaque sediment phase. The clear micelle phase was collected, and curcumin amount in it was determined as previously described.[28]

The release of free fatty acids (FFA) was investigated to understand lipolysis of samples. It was supposed that digestion of 1 mol of triglycerides could release 2 mol of FFA and consumed 2 mol of NaOH.[31] Thus, the fraction of FFA released could be determined using the following equation:

$$\%\mathrm{FFA} = 100 \times \frac{m_{\mathrm{NaOH}} \times V_{\mathrm{NaOH}} \times M_{\mathrm{lipid}}}{2 \times w_{\mathrm{lipid}}} \qquad (2)$$

Here m_{NaOH} is the molarity of NaOH solution (in mol/L), V_{NaOH} is the volume of NaOH solution required to neutralize the released FFA (in L), M_{lipid} is the molecular mass of the triacylglycerol oil (in g/mol) and w_{lipid} is the total mass of initially present triacylglycerol oil (in g).[31]

After HPLC quantification of the micelle phase, the bioaccessibility of curcumin was calculated as follows:

$$\%\mathrm{bioaccessibility} = \frac{\text{amount of curcumin in the micelle phase}}{\text{total amount of curcumin in the formulations}} \times 100\% \qquad (3)$$

2.11. Statistical analysis

All experiments were conducted at least in triplicate. All statistical analysis was performed using OriginPro 2018. The statistical differences were determined employing one-way analysis of variance (ANOVA) with Fisher LSD test, and differences were regarded statistically significant with $p < 0.05$.

3. Results and discussion

3.1. Characterization of ovotransferrin–gallic acid conjugate (OTGCONJ)

Complexations between proteins and polyphenols are feasible to attach polyphenols to proteins,[14,32] and protein–polyphenol conjugates may generally lead to higher interfacial accumulation of polyphenols while applied as emulsifiers.[15] Given that sufficient polyphenols at the oil-water interface might inhibit degradation of encapsulated nutraceuticals and lipid oixdation,[10–12] ovotransferrin–gallic acid conjugates (OTGCONJ) were synthesized as building blocks to construct Pickering stabilizers with strong antioxidant potential. SDS-PAGE was utilized to confirm

successful preparation of ovotransferrin–gallic acid conjugates. Ovotransferrin–gallic acid mixture (OTGMIX) was prepared as control group here, and it was assumed that non-covalent bonds such as hydrogen bonds, Van der Waals forces and hydrophobic interaction could exist between ovotransferrin and gallic acid in OTGMIX.[14,15] As shown in Fig. S1, OTGCONJ had larger molecular weight than OVTC (ovotransferrin control) and ovotransferrin–gallic acid mixture (OTGMIX). Since SDS could break non-covalent protein–polyphenol interactions and OTGMIX had the same molecular weight as OVTC,[33] SDS-PAGE band at higher molecular weight could confirm the covalent coupling between ovotransferrin and gallic acid in OTGCONJ. MALDI-TOF-MS was further applied to obtain exact molecular weight of OTGCONJ. As indicated in Fig. 1, molecular weight of OVTC and OTGCONJ was 77152.66 and 78697.90, respectively. The increase in molecular mass confirmed that gallic aicd was covalently bound to ovotransferrin.

The total phenolic content of OTGCONJ was measured to estimate the amount of gallic acid bound to ovotransferrin. As Table S1 (Supporting Information) shows, the total phenolic content of OTGCONJ was 36.52 mg/g, suggesting that substantial gallic acid was covalently attached to ovotransferrin. The antioxidant activity of OTGCONJ was evaluated using ABTS assay. Table S2 shows that antioxidant capacity of OTGCONJ was as high as 1343.9 mmol TE/mg sample, and it was believed that hydroxyl groups of gallic acid contributed mainly to superior antioxidant activity of OTGCONJ.[34] As shown in Table S2, although antioxidant capacity of OTGCONJ was a little lower than that of OTGMIX, there were no significant differences between antioxidant capacities of OTGCONJ and OTGMIX.

Protein–polyphenol interactions may lead to emergence of large aggregates,[35] which may not be beneficial to precise assembly into particles using protein–polyphenol complexes as building

blocks. Thus, protein–polyphenol complexes without large aggregates are expected to be prepared, and gallic acid is chosen in this research due to its weaker tendency to form aggregates with biomacromolecules than many polyphenols (tannic acid, (–)-epigallocatechin-3-gallate, etc). SAXS technique was employed to investigate size of OTGCONJ. Fig. 2a presents SAXS profiles of OTGCONJ at different pHs, and Fig. 2b shows Guinier plots of OTGCONJ at different pHs. The R_g (radius of gyration) values were extracted from Guinier plots in Fig. 2b by general Guinier relation:

$$\ln \frac{I(q)}{I(0)} = -\frac{1}{3}(qR_g)^2 \qquad (4)$$

The data to be used for Guinier analysis should satisfy qR_g <1.57.[21] The estimated R_g values of OTGCONJ were shown in Table 1. No large aggregates were observed in OTGCONJ, and OTGCONJ mainly dispersed in solution as monomer or dimer. To better understand impact of covalent bound gallic acid on conformation of ovotransferrin, SAXS profiles and corresponding Guinier plots of OVTC as well as OTGMIX were shown in Fig. S2 and Fig. S3, and R_g values of OVTC and OTGMIX were shown in Table S3. It was observed that both covalent and non-covalent ovotransferrin–gallic acid complexations could alter conformation of OVTC, and OTGCONJ had larger R_g than OVTC and OTGMIX at many pHs (pH 5.5, 6.5, 7.4, etc), which was possibly due to protein unfolding upon covalent modification with gallic acid.[15] Given that antioxidant OTGCONJ without existence of larger aggregates was prepared, OTGCONJ was further employed to fabricate antioxidant Pickering stabilizers.

3.2. Preparation and validation of OTGCONJ–CMD particles as effective Pickering stabilizers

Electrostatic attractions between proteins and polysaccharides can be utilized to construct

protein–polysaccharide particles,[19,23] and electrostatic assembly is expected to prepare OTGCONJ–CMD particles as antioxidant Pickering stabilizers. Considering that turbidity is closely linked with particle size and the number of particles,[23,36] turbidimetric titrations can be employed to analyze formation of OTGCONJ–CMD particles. Fig. 3 shows turbidimetric titration curve of OTGCONJ–CMD mixtures, and Fig. S4 depicts turbidity of OTGCONJ and CMD solution. It was noteworthy that the turbidity baseline in Fig. 3 was interfered with color of OTGCONJ, which was supported by turbidity baseline in Fig. S4a. During covalent modification of ovotransferrin with gallic acid, gallic acid was oxidized into quinone products with characteristic colors and the covalent reaction might lead to further color darkening, resulting in OTGCONJ with characteristic green color.[15,32,33] Since SAXS data confirmed that no large aggregates existed in OTGCONJ solutions and Brinkmann PC910 colorimeter was very sensitive to colored solution, it could be speculated that the turbidity baseline with relatively high value in Fig. 3 and Fig. S4a was attributed to color interference from OTGCONJ. The actual turbidity from OTGCONJ should be around zero, and Fig. S4b depicts that CMD showed negligible turbidity, suggesting that the turbidity rise in Fig. 3 should be triggered by OTGCONJ–CMD complexation. The critical pH transition points (pH_c, $pH_{\varphi1}$ and $pH_{\varphi2}$) in Fig. 3 were determined as the intersection point of two curve tangents, and corresponding phase diagram was marked as previously described.[23,36] Because the aim of this research was to obtain insoluble particles as Pickering stabilizers,[13] insoluble OTGCONJ–CMD complexes in the region of $pH_{\varphi1}$–$pH_{\varphi2}$ were mainly studied.

Zeta potential measurement was performed to support that formation of insoluble OTGCONJ–CMD complexes was mainly driven by electrostatic attractions. As shown in Fig. 4, OTGCONJ and CMD carried opposite charges in $pH_{\varphi1}$–$pH_{\varphi2}$ region, which resulted in strong

attractions to induce molecular assembly. As CMD almost lost charges at around $pH_{\varphi 2}$, insoluble OTGCONJ–CMD complexes totally dissociated into co-soluble polymers. Based on these understandings, although CMD could bind polyphenols weakly,[37] electrostatic attractions between OTGCONJ and CMD were the major driving force of OTGCONJ–CMD complexation.

Dispersion stability of particles as Pickering stabilizers is generally beneficial to outstanding stability of Pickering stabilizers.[25] Since enough surface charge of OTGCONJ–CMD particles may be a key contributor to maintain dispersion stability, it is expected that OTGCONJ–CMD particles should not carry too few charges. Based on analysis of zeta potential result in Fig. 4, OTGCONJ–CMD particles within pH 3.5–4.0 were further investigated to obtain stable particle dispersions. Fig. 5a shows that OTGCONJ–CMD particles within pH 3.7–4.0 remained stable after 1-week storage, while precipitates existed at the bottom of vials for pH 3.5 and 3.6. Therefore, OTGCONJ–CMD particles within pH 3.7–4.0 were regarded as potential Pickering stabilizers. Since larger particle size of stabilizers could lead to improved stability of Pickering emulsions via increasing desorption energy of colloidal particles,[13] OTGCONJ–CMD particles with larger particle size should be selected as stabilizers. Our previous study reveals that increasing turbidity of particle dispersion is associated with rise of particle size,[38] thus based on turbidity in Fig. 5a OTGCONJ–CMD particles at pH 3.7 were eventually chosen as Pickering stabilizer in this research. Fig. 5b shows particle size distribution of OTGCONJ–CMD particles at pH 3.7, and average particle size of the monodisperse particles was around 153.0 nm.

Qualified Pickering stabilizers should be partially wetted by both aqueous and oil phases,[13] and contact angle analysis was conducted to verify that OTGCONJ–CMD particles owned intermediate wettability.[25] Fig. 6 shows that air-water contact angle of OTGCONJ–CMD particles was 65.2°.

Since particles with air-water contact angle between approximately 53° and 82° may have intermediate wettability (partially wetted by both water and oil),[39] it may be induced that OTGCONJ–CMD particles can be partilly wetted by both water and oil. Based on aforementioned discussion, OTGCONJ–CMD particles met all prerequisites (proper particle size, insoluble character and intermediate wettability) of eligible Pickering stabilizers, and OTGCONJ–CMD particles were subsequently applied in fabrication of food-grade antioxidant Pickering emulsions.

3.3. Characterization of antioxidant Pickering emulsion stabilized by OTGCONJ–CMD particles

Fig. 7 shows photograph of Pickering emulsion stabilized by OTGCONJ–CMD particles, and it was observed that the Pickering emulsion had emulsified phase alone and no aqueous phase, suggesting excellent Pickering stabilization performance of OTGCONJ–CMD particles. No gravitational separation and oiling-off were observed in the emulsion after 30 days, indicating that Pickering emulsion stabilized by OTGCONJ–CMD particles could maintain stable during long-term storage. As shown in Fig. S5, ready dispersion of the Pickering emulsion in water but not in MCT indicated that OTGCONJ–CMD particle-stabilized Pickering emulsion was oil-in-water emulsion.[25] It was noteworthy that OTGCONJ–CMD particle-stabilized Pickering emulsion had a high oil fraction of 0.68, indicating that OTGCONJ–CMD particle-stabilized Pickering emulsions could load more curcumin than conventional oil-in-water emulsions at the same emulsion volume. Specifically speaking, curcumin-loaded conventional emulsions can stabilize emulsions with a maximal oil fraction of 0.10.[40] If curcumin concentration in MCT oil is fixed as 1 mg/mL, curcumin loading in OTGCONJ–CMD particle-stabilized Pickering emulsions (oil fraction =0.68) and conventional emulsions (oil fraction =0.10) is 0.68 mg/mL and 0.10 mg/mL, respectively, which

indicates that curcumin loading in OTGCONJ–CMD particle-stabilized Pickering emulsions is 5.8 times higher than that in conventional emulsions. Since OTGCONJ–CMD particle-stabilized Pickering emulsions can load more curcumin than conventional emulsions, OTGCONJ–CMD particle-stabilized Pickering emulsions can be applied as delivery systems with higher nutraceutical loading and improved bioefficacy.

It was intriguing to compare visual appearance of OTGCONJ–CMD particle-stabilized Pickering emulsion and OTGMIX–CMD particle-stabilized Pickering emulsion. Fig. S6 shows visual appearance of OTGMIX–CMD particle-stabilized Pickering emulsion, and it was observed that upper oil phase existed in OTGMIX–CMD particle-stabilized Pickering emulsion. Table S4 shows that emulsified phase volume fraction of OTGMIX–CMD particle-stabilized Pickering emulsion was below 92.5%. Considering that OTGMIX–CMD particle-stabilized Pickering emulsion was oil-in-water emulsion and a significant amount of upper oil phase existed in OTGMIX–CMD particle-stabilized Pickering emulsion, OTGMIX–CMD particle-stabilized Pickering emulsion could be regarded unstable. The poor stability of OTGMIX–CMD particle-stabilized Pickering emulsion could be explained by sedimentation of OTGMIX–CMD particles. As shown in Fig. S7, significant precipitation was observed in OTGMIX–CMD particle dispersion, and that was probably because non-covalent complexations among ovotransferrin, gallic acid and CMD could lead to precipitation.[35] The great tendency to precipitatie indicated that OTGMIX–CMD particles could not adsorb at oil-water interfaces well, which could lead to poor stability of OTGMIX–CMD particle-stabilized Pickering emulsion.

Considering that upper oil phase existed in OTGMIX–CMD particle-stabilized Pickering emulsion and a significant amount of curcumin could not be encapsulated into the emulsion,

OTGMIX–CMD particle-stabilized Pickering emulsion was not a suitable emulsion system for curcumin delivery. Besides, since OTGMIX–CMD particle-stabilized Pickering emulsion was unstable and the emulsion was a mixture of oil phase as well as emulsified phase, it was infeasible and unreasonable to characterize physicochemical properties of OTGMIX–CMD particle-stabilized Pickering emulsion. Based on these understandings, only OTGCONJ–CMD particle-stabilized Pickering emulsion was investigated in the rest of this study.

Fig. 8a depicts optical microscopic image of OTGCONJ–CMD particle-stabilized Pickering emulsion. No obvious flocculation and coalescence of emulsion droplets were observed, and the average emulsion droplet size was 47.7±3.9 μm. Fluorescence images of the Pickering emulsion were shown in Fig. 8b and 8c. Red round regions corresponded to dyed oil phase of emulsion droplets, and green fluorescence glowing layers corresponded to dyed OTGCONJ–CMD particles at the emulsion interface. The fluorescence pictures clearly indicated that the emulsion was indeed stabilized by OTGCONJ–CMD particle at the oil-water interface.[26]

The rheology is vital to stability of emulsions and a valuable tool to shed light on complex structures of the Pickering emulsions,[13,17,26] thus rheological properties of Pickering emulsion stabilized by OTGCONJ–CMD particles were investigated. Fig. 9a and 9b show that the Pickering emulsion had relatively high viscosity and storage modulus, and transcend of G′ over G″ indicated gel-like structures of the Pickering emulsion. The high viscosity and entangled gel-like structures could contribute to outstanding physical stability of the emulsion.[41] Fig. 9c shows that OTGCONJ–CMD particle-stabilized Pickering emulsion could be employed to create soft structures with controlled shapes, which confirmed gel-like property of the emulsion.

3.4. Protective effect of OTGCONJ–CMD particle-stabilized Pickering emulsion on curcumin

UV photostability was studied to evaluate protective effect of OTGCONJ–CMD particle-stabilized Pickering emulsion on curcumin. To better clarify protective effect of OTGCONJ–CMD particle-stabilized Pickering emulsion, protective effect of MCT oil and Pickering emulsion stabilized by ovotransferrin–CMD particles (non-antioxidant particles without gallic acid) was investigated as control. Fig. 10 shows that curcumin in OTGCONJ–CMD particle-stabilized Pickering emulsion degraded much slower than that in bulk MCT oil and ovotransferrin–CMD particle-stabilized Pickering emulsion, and degradation rate constant was calculated using the first-order kinetic model:

$$\ln C / C_0 = -kt \qquad (5)$$

where C is concentration of curcumin after UV radiation, C_0 is the initial concentration of curcumin, k is reaction rate constant and t is UV treatment time.[42] As Table S5 shows, degradation rate constant of curcumin in MCT oil and ovotransferrin–CMD particle-stabilized Pickering emulsion was 16.3 times and 12.1 times larger than that in OTGCONJ–CMD particle-stabilized Pickering emulsion, respectively. Protective effect of OTGCONJ–CMD particle-stabilized Pickering emulsion on curcumin was much better than that of kafirin particle-stabilized Pickering emulsion in our previous study.[6] Several explanations may account for outstanding chemical stability of curcumin in OTGCONJ–CMD particle-stabilized Pickering emulsion. First, OTGCONJ–CMD particles at the emulsion interface may provide excellent physical barrier to UV light, which can significantly reduce exposure of curcumin to UV light. Second, due to composition of antioxidant polyphenols, OTGCONJ–CMD particles have strong antioxidant activity and quench singlet oxygen effectively. Since singlet oxygen is the major contributor to curcumin decomposition, curcumin degradation can be effectively retarded by OTGCONJ–CMD particles.[5] Plenty of singlet oxygen may exist in the

tested samples. Specifically, curcumin may act as a photosensitizer, which can induce formation of singlet oxygen.[5,7] In addition, photosensitized oxidation of oils leads to formation of singlet oxygen in the presence of UV light,[43] and substantial amount of singlet oxygen can be produced from other mechanisms (Russell mechanism from peroxy radicals, metal-catalyzed Haber-Weiss reaction, etc).[7] Based on these understandings, quenching singlet oxygen is essential in reducing curcumin degradation, and quenching singlet oxygen with OTGCONJ–CMD particles is believed to play a vital role in protection of curcumin.

3.5. Lipolysis and bioaccessibility of curcumin in Pickering emulsion stabilized by OTGCONJ–CMD particles

In vitro digestion model was applied to estimate extent of lipolysis and curcumin bioaccessibility. As shown in Fig. 11a, the final amount of FFAs released from the Pickering emulsion after simulated intestine digestion was higher than that from MCT oil, implying higher extent of lipolysis in OTGCONJ–CMD particle-stabilized Pickering emulsion. Several theories may explain this phenomenon. First, as evidenced by steeper increase initially in Fig. 11a, the Pickering emulsion had faster lipolysis rate than MCT. The Pickering emulsion has larger interfacial area in contact with digestive fluids than MCT, and sufficient interfacial area is beneficial to fast lipolysis.[44] Second, since stability of OTGCONJ–CMD particles is largely dominated by pH, OTGCONJ–CMD particle-stabilized Pickering emulsion is a pH-responsive emulsion.[45] Destabilization of OTGCONJ–CMD particle-stabilized Pickering emulsion in digestive fluids facilitates release of FFAs. It is noteworthy that improved lipolysis of food-grade Pickering emulsion is not always the case, and a previous study has demonstrated some food-grade Pickering emulsions may delay digestion of lipids.[46] Thus, improved lipolysis is an attractive feature of

OTGCONJ–CMD particle-stabilized Pickering emulsion as delivery vehicle.

Fig. 11b shows that curcumin bioaccessibility in OTGCONJ–CMD particle-stabilized Pickering emulsion was significantly higher than that in MCT oil, and higher extent of lipolysis in OTGCONJ–CMD particle-stabilized Pickering emulsion could explain the bioaccessibility result. The hydrophobic curcumin mainly becomes bioaccessible via solubilization in the micelle cores, and generation of micelles comes from lipolysis of lipid-based formulations during digestion.[28] The larger number of micelles in the digested Pickering emulsion may make solubilization of more curcumin possible, which leads to higher curcumin bioaccessibility. It is interesting to compare nutraceutical bioaccessibility of OTGCONJ–CMD particle-stabilized Pickering emulsion with that of other food-grade Pickering emulsions. As shown in a previous study, when compared with bulk oil, nutraceutical bioaccessibility of genipin-crosslinked particle-stabilized emulsion increased by only 13.6%.[30] In this study, in comparison with bulk oil, nutraceutical bioaccessibility of OTGCONJ–CMD particle-stabilized Pickering emulsion increased by 106.4%, indicating that OTGCONJ–CMD particle-stabilized Pickering emulsions could function as outstanding delivery vehicles with high nutraceutical bioaccessibility.

4. Conclusion

In summary, antioxidant OTGCONJ without existence of large aggregates was prepared, and OTGCONJ–CMD particles were constructed via assembly of OTGCONJ–CMD complexes. OTGCONJ–CMD particles met all prerequisites (proper particle size, insoluble character and intermediate wettability) of qualified Pickering stabilizers, and oil-in-water Pickering emulsions with long-term stability could be stabilized solely by OTGCONJ–CMD particles. OTGMIX–CMD particle-stabilized Pickering emulsion was not stable with obvious phase separation.

OTGCONJ–CMD particle-stabilized Pickering emulsion could be used to create soft structures with controlled shapes due to high viscosity and gel-like structure. It was noteworthy that OTGCONJ–CMD particle-stabilized Pickering emulsion significantly retarded curcumin degradation against UV light. OTGCONJ–CMD particle-stabilized Pickering emulsion could improve both extent of lipolysis and curcumin bioaccessibility remarkably. The knowledge gained from this work may have important implications for design of nutraceutical-loaded Pickering emulsions with excellent protective effect and delivery efficiency.

Acknowledgements

This work was supported by United State Department of Agriculture, National Institute of Food and Agriculture (grant No. 2019-67017-29176). Use of the Advanced Photon Source was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

Abbreviations used

CMD, carboxymethyldextran; FFA, free fatty acids; MCT, medium chain triglyceride; OVTC, ovotransferrin control; OTGCONJ, ovotransferrin–gallic acid conjugate; OTGMIX, ovotransferrin–gallic acid mixture; SAXS, small-angle X-ray scattering

References

1. T. Esatbeyoglu, P. Huebbe, I. M. A. Ernst, D. Chin, A. E. Wagner and G. Rimbach, Curcumin—From molecule to biological function, *Angew. Chem. Int. Ed.*, 2012, **51**, 5308–5332.

2. A. Araiza-Calahorra, M. Akhtar and A. Sarkar, Recent advances in emulsion-based delivery approaches for curcumin: From encapsulation to bioaccessibility, *Trends Food Sci. Technol.*, 2018, **71**, 155–169.

3. B. H. Lee, H. A. Choi, M.. Kim and J. Hong, Changes in chemical stability and bioactivities of curcumin by ultraviolet radiation, *Food Sci. Biotechnol.*, 2013, **22**, 279–282.

4. L. C. Price and R. W. Buescher, Decomposition of turmeric curcuminoids as affected by light, solvent and oxygen, *J. Food Biochem.*, 1996, **20**, 125–133.

5. H. H. Tønnesen, J. Karlsen and G. B. van Henegouwen, Studies on curcumin and curcuminoids VIII, photochemical stability of curcumin, *Z. Lebensm. Unters. Forsch.*, 1986, **183**, 116–122.

6. J. Xiao, C. Li and Q. Huang, Kafirin nanoparticle-stabilized Pickering emulsions as oral delivery vehicles: Physicochemical stability and in vitro digestion profile, *J. Agric. Food Chem.*, 2015, **63**, 10263–10270.

7. E. Choe and D. B. Min, Chemistry and reactions of reactive oxygen species in foods, *Crit. Rev. Food Sci. Nutr.*, 2006, **46**, 1–22.

8. I. Lagunes, F. Vázquez-Ortega and A. Trigos, Singlet oxygen detection using red wine extracts as photosensitizers, *J. Food Sci.*, 2017, **82**, 2051–2055.

9. C. Tournaire, S. Croux, M.. Maurette, I. Beck, M. Hocquaux, A. M. Braun and E. Oliveros, Antioxidant activity of flavonoids: efficiency of singlet oxygen ($^1\Delta_g$) quenching, *J. Photochem. Photobiol. B, Biol.*, 1993, **19**, 205–215.

10. T. Waraho, D. J. McClements and E. A. Decker, Mechanisms of lipid oxidation in food dispersions, *Trends Food Sci. Technol.*, 2011, **22**, 3–13.

11. Z. Wei and Y. Gao, Physicochemical properties of β-carotene emulsions stabilized by chitosan–chlorogenic acid complexes, *LWT-Food Sci. Technol.*, 2016, **71**, 295–301.

12. Z. Wei and Y. Gao, Physicochemical properties of β-carotene bilayer emulsions coated by milk proteins and chitosan–EGCG conjugates, *Food Hydrocolloids*, 2016, **52**, 590–599.

13. J. Xiao, Y. Li and Q. Huang, Recent advances on food-grade particles stabilized Pickering emulsions: fabrication, characterization and research trends, *Trends Food Sci. Technol.*, 2016, **55**, 48–60.

14. T. Ozdal, E. Capanoglu and F. Altay, A review on protein–phenolic interactions and associated changes, *Food Res. Int.*, 2013, **51**, 954–970.

15. Z. Wei, W. Yang, R. Fan, F. Yuan and Y. Gao, Evaluation of structural and functional properties of protein–EGCG complexes and their ability of stabilizing a model β-carotene emulsion, *Food Hydrocolloids*, 2015, **45**, 337–350.

16. E. Abeyrathne, H. Y. Lee and D. U. Ahn, Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents–A review, *Poult. Sci.*, 2013, **92**, 3292–3299.

17. Z. Wei and Q. Huang, Edible Pickering emulsions stabilized by ovotransferrin–gum arabic particles, *Food Hydrocolloids*, 2019, **89**, 590–601.

18. Z. Wei and Q. Huang, Assembly of protein–polysaccharide complexes for delivery of bioactive ingredients: A perspective paper, *J. Agric. Food Chem.*, 2019, **67**, 1344–1352.

19. A. Ye, Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications–a review, *Int. J. Food Sci. Technol.*, 2008, **43**, 406–415.

20. Z. Wei and Y. Gao, Evaluation of structural and functional properties of chitosan–chlorogenic acid complexes, *Int. J. Biol. Macromol.*, 2016, **86**, 376–382.

21. Y. Li, Q. Xia, K. Shi and Q. Huang, Scaling behaviors of α-zein in acetic acid solutions, *J. Phys. Chem. B*, 2011, **115**, 9695–9702.

22. R. Huynh, F. Chaubet and J. Jozefonvicz, Carboxymethylation of dextran in aqueous alcohol as the first step of the preparation of derivatized dextrans, *Die Angew. Makromol. Chem.,* 1998, **254**, 61–65.

23. Q. Ru, Y. Wang, J. Lee, Y. Ding and Q. Huang, Turbidity and rheological properties of bovine serum albumin/pectin coacervates: Effect of salt concentration and initial protein/polysaccharide ratio, *Carbohydr. Polym.,* 2012, **88**, 838–846.

24. W. Jin, J. Zhu, Y. Jiang, P. Shao, B. Li and Q. Huang, Gelatin-based nanocomplex-stabilized Pickering emulsions: Regulating droplet size and wettability through assembly with glucomannan, *J. Agric. Food Chem.,* 2017, **65**, 1401–1409.

25. J. W. J. de Folter, M. W. M. van Ruijven and K. P. Velikov, Oil-in-water Pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein, *Soft Matter,* 2012, **8**, 6807–6815.

26. Z. Wei and Q. Huang, Developing organogel-based pickering emulsions with improved freeze-thaw stability and hesperidin bioaccessibility, *Food Hydrocolloids,* 2019, **93**, 68–77.

27. Z. Wei, J. Cheng and Q. Huang, Food-grade Pickering emulsions stabilized by ovotransferrin

fibrils, *Food Hydrocolloids,* 2019, **94**, 592–602.

28. H. Yu and Q. Huang, Improving the oral bioavailability of curcumin using novel organogel-based nanoemulsions, *J. Agric. Food Chem.*, 2012, **60**, 5373–5379.

29. A. Sarkar, K. K. T. Goh, R. P. Singh and H. Singh, Behaviour of an oil-in-water emulsion stabilized by β-lactoglobulin in an *in vitro* gastric model, *Food Hydrocolloids*, 2009, **23**, 1563–1569.

30. Z. Wei, Y. Cheng, J. Zhu and Q. Huang, Genipin-crosslinked ovotransferrin particle-stabilized Pickering emulsions as delivery vehicles for hesperidin, *Food Hydrocolloids,* 2019, **94**, 561–573.

31. Y. Li and D. J. McClements, New mathematical model for interpreting pH-stat digestion profiles: impact of lipid droplet dharacteristics on in vitro digestibility, *J. Agric. Food Chem.,* 2010, **58**, 8085–8092.

32. M. Ali, T. Homann, J. Kreisel, M. Khalil, R. Puhlmann, H. Kruse and H. Rawel, Characterization and modeling of the interactions between coffee storage proteins and phenolic compounds, *J. Agric. Food Chem.*, 2012, **60**, 11601–11608.

33. J. Kroll, H. M. Rawel and S. Rohn, Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds, *Food Sci. Technol. Res.*, 2003, **9**, 205–218.

34. C. A. Rice-Evans, N. J. Miller and G. Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids, *Free Radic. Biol. Med.*, 1996, **20**, 933–956.

35. A. J. Charlton, N. J. Baxter, M. L. Khan, A. J. G. Moir, E. Haslam, A. P. Davies and M. P. Williamson, Polyphenol/peptide binding and precipitation, *J. Agric. Food Chem.,* 2002, **50**, 1593–1601.

36. Z. Wei, P. Zhu and Q. Huang, Investigation of ovotransferrin conformation and its complexation with sugar beet pectin, *Food Hydrocolloids*, 2019, **87**, 448–458.

37. Y. Cai, S. H. Gaffney, T. H. Lilley and E. Haslam, Carbohydrate–polyphenol complexation, in R. W. Hemingway, J. J. Karchesy and S. J. Branham (Eds.), Chemistry and Significance of Condensed Tannins, Springer US, Boston, 1989, pp. 307-322.

38. B. Hu, S. Wang, J. Li, X. Zeng and Q. Huang, Assembly of bioactive peptide–chitosan nanocomplexes, *J. Phys. Chem. B,* 2011, **115**, 7515–7523.

39. J. W. Grate, K. J. Dehoff, M. G. Warner, J. W. Pittman, T. W. Wietsma, C. Zhang and M. Oostrom, Correlation of oil–water and air–water contact angles of diverse silanized surfaces and relationship to fluid interfacial tensions, *Langmuir*, 2012, **28**, 7182–7188.

40. X. Wang, Y. Jiang, Y. Wang, M. Huang, C. Ho and Q. Huang, Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions, *Food Chem.*, 2008, **108**, 419–424.

41. T. Tadros, Application of rheology for assessment and prediction of the long-term physical stability of emulsions, *Adv. Colloid Interface Sci.*, 2004, **108**, 227–258.

42. D. Dutta, A. Dutta, U. Raychaudhuri and R. Chakraborty, Rheological characteristics and thermal degradation kinetics of beta-carotene in pumpkin puree, *J. Food Eng.*, 2006, **76**, 538–546.

43. E. Choe and D. B. Min, Mechanisms and factors for edible oil oxidation, *Compr. Rev. Food Sci. Food Saf.*, 2006, **5**, 169–186.

44. L. Salvia-Trujillo, C. Qian, O. Martín-Belloso and D. J. McClements, Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions, *Food Chem.,* 2013, **141**, 1472–1480.

45. J. Tang, P. J. Quinlan and K. C. Tam, Stimuli-responsive Pickering emulsions: recent advances and potential applications, *Soft Matter*, 2015, **11**, 3512–3529.

46. A. Sarkar, B. Murray, M. Holmes, R. Ettelaie, A. Abdalla and X. Yang, In vitro digestion of Pickering emulsions stabilized by soft whey protein microgel particles: influence of thermal treatment, *Soft Matter*, 2016, **12**, 3558–3569.

Figure captions:

Fig. 1. MALDI-TOF-MS spectra of OVTC and OTGCONJ.

Fig. 2. Scattering intensity profiles of OTGCONJ solutions at different pHs (a), Guinier plots of OTGCONJ at different pHs (b).

Fig. 3. Turbidity as a function of pH for OTGCONJ–CMD mixture. The turbidity baseline was interfered with color of OTGCONJ.

Fig. 4. Zeta potential of OTGCONJ solutions, CMD solutions and OTGCONJ–CMD mixture.

Fig. 5. (a) Visual observation of OTGCONJ–CMD particles at different pHs. (b) Particle size distribution of OTGCONJ–CMD particles at pH 3.7.

Fig. 6. Water-in-air contact angle determined by dropping water droplet onto OTGCONJ–CMD particle film in air environment.

Fig. 7. Visual appearance of freshly prepared Pickering emulsion stabilized by OTGCONJ–CMD particles and the emulsion after 1-month storage at room temperature.

Fig. 8. (a) Optical microscopic image of Pickering emulsion stabilized by OTGCONJ–CMD particles (b) Fluorescence image of Pickering emulsion stabilized by OTGCONJ–CMD particles. Nile Red was used to stain the oil phase (=red color). (c) Fluorescence image of Pickering emulsion stabilized by OTGCONJ–CMD particles. Rhodamine B was used to stain OTGCONJ–CMD particles (=green color). The scale bar in all images is 100 μm.

Fig. 9. (a) Apparent viscosity of Pickering emulsion stabilized by OTGCONJ–CMD particles. (b) Storage modulus (G′) and loss modulus (G″) of Pickering emulsion stabilized by OTGCONJ–CMD particles. (c) Photograph of the shape of alphabets constructed by OTGCONJ–CMD

particle-stabilized Pickering emulsion using a plastic syringe at ambient temperature. The photograph was taken after a waiting period of 60 min.

Fig. 10. Residual curcumin level in bulk MCT oil, Pickering emulsion stabilized by ovotransferrin–CMD particles (non-antioxidant particles without gallic acid) and Pickering emulsion stabilized by OTGCONJ–CMD particles during UV radiation.

Fig. 11. (a) Release profile of FFA in MCT oil and Pickering emulsion stabilized by OTGCONJ–CMD particles. (b) The bioaccessibility of curcumin in MCT oil and Pickering emulsion stabilized by OTGCONJ–CMD particles after *in vitro* digestion.

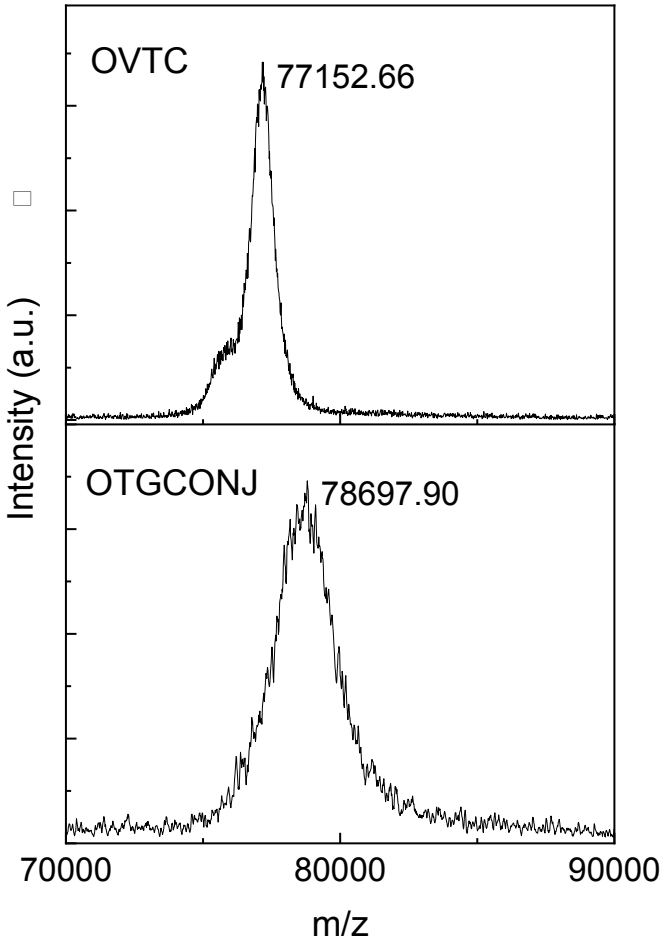
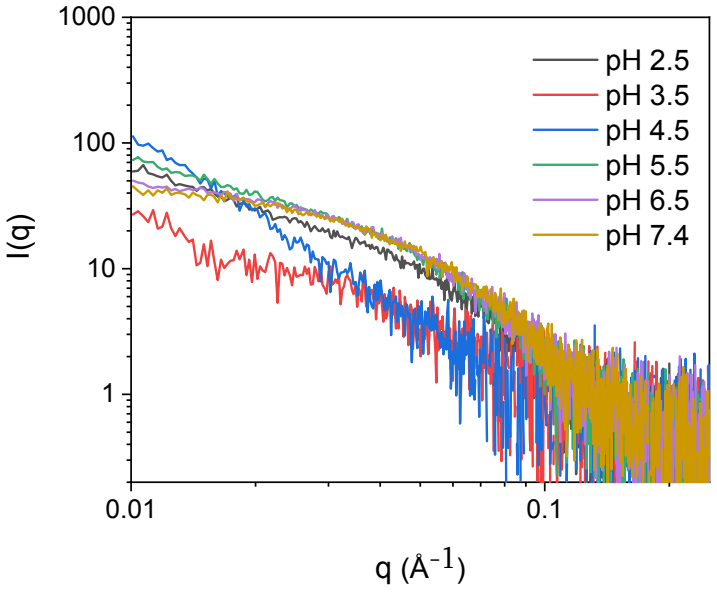
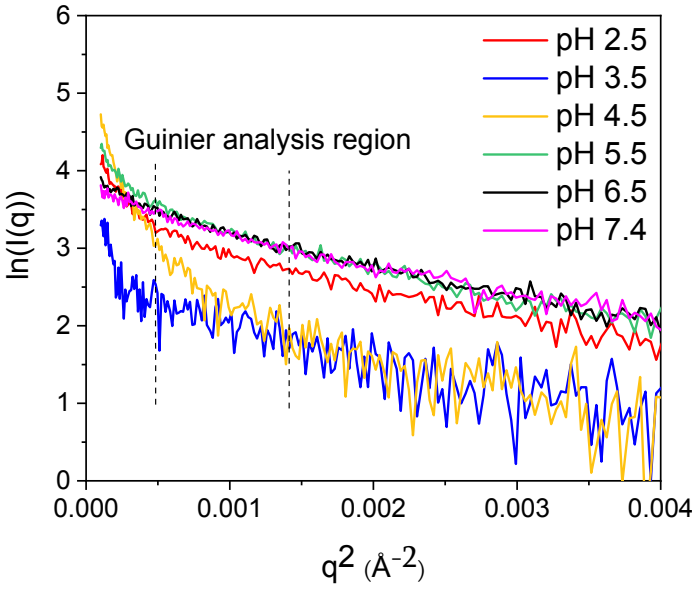

OVTC
77152.66
OTGCONJ
78697.90
Intensity (a.u.)
70000
80000
90000
m/z

Fig. 1


1000
100
10
1
I(q)
0.01
0.1
q (Å-1)
pH 2.5
pH 3.5
pH 4.5
pH 5.5
pH 6.5
pH 7.4

(a)


6
5
4
3
2
1
0
ln(I(q))
0.000
0.001
0.002
0.003
0.004
q2 (Å-2)
Guinier analysis region
pH 2.5
pH 3.5
pH 4.5
pH 5.5
pH 6.5
pH 7.4

(b)

Fig. 2

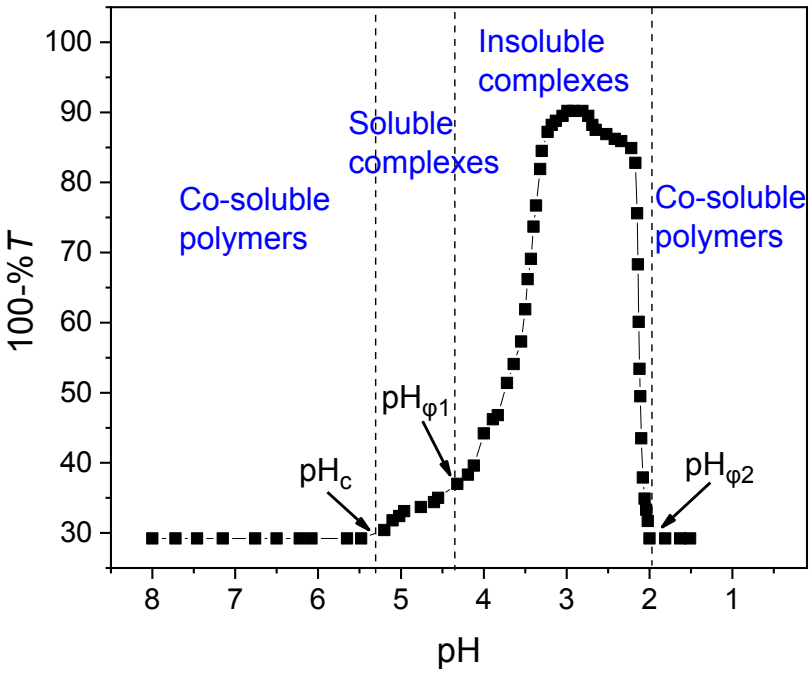

100
90
80
70
60
50
40
30
100-%T
8
7
6
5
4
3
2
1
pH
Co-soluble
polymers
Soluble
complexes
Insoluble
complexes
Co-soluble
polymers
pH$_c$
pH$_{\varphi 1}$
pH$_{\varphi 2}$

Fig. 3

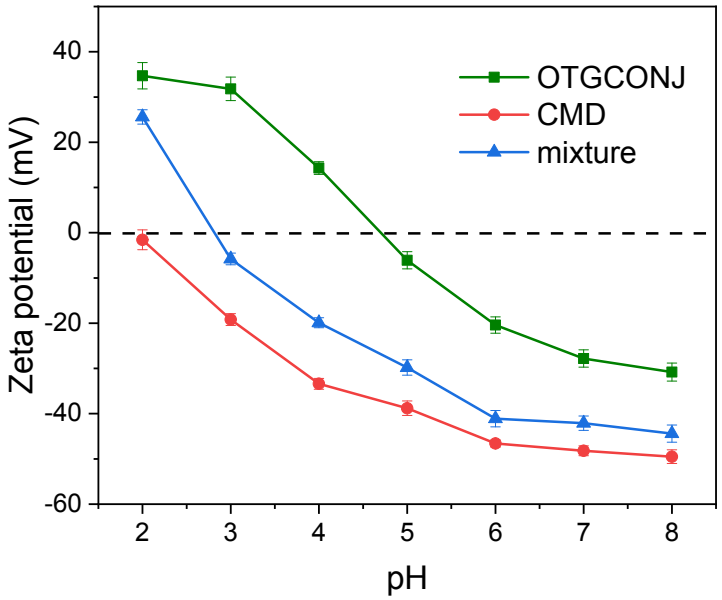
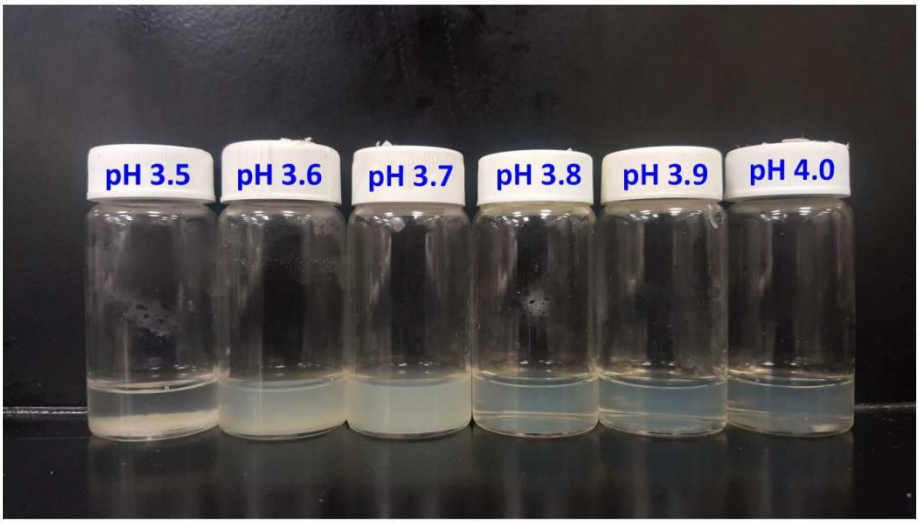
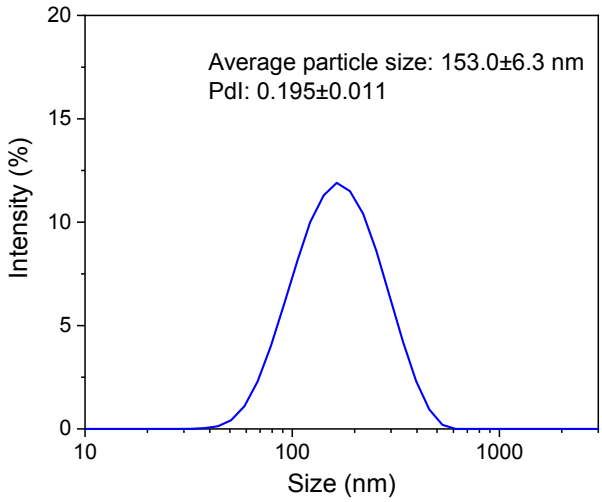

40
20
0
-20
-40
-60
Zeta potential (mV)
2
3
4
5
6
7
8
pH
OTGCONJ
CMD
mixture

Fig. 4


pH 3.5
pH 3.6
pH 3.7
pH 3.8
pH 3.9
pH 4.0

(a)


Average particle size: 153.0±6.3 nm
PdI: 0.195±0.011
Intensity (%)
Size (nm)
0
5
10
15
20
10
100
1000

(b)

Fig. 5

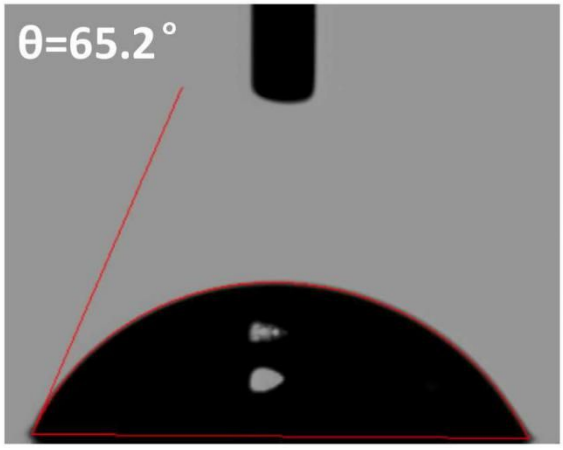


Fig. 6

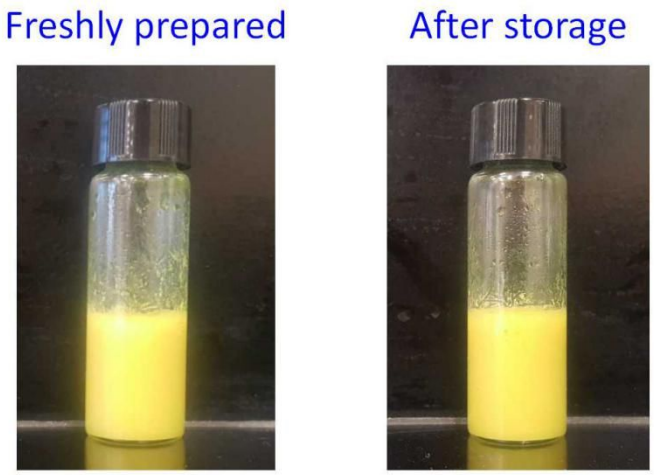
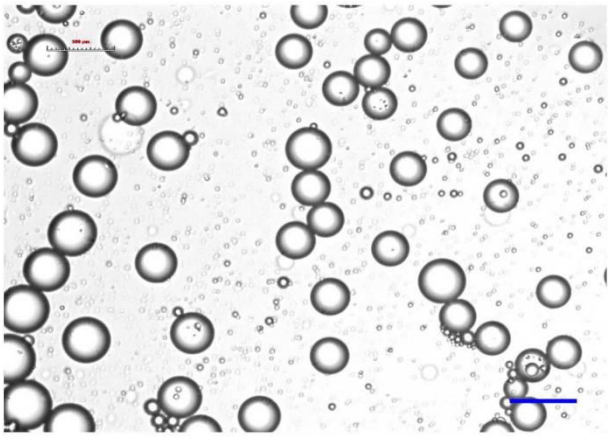
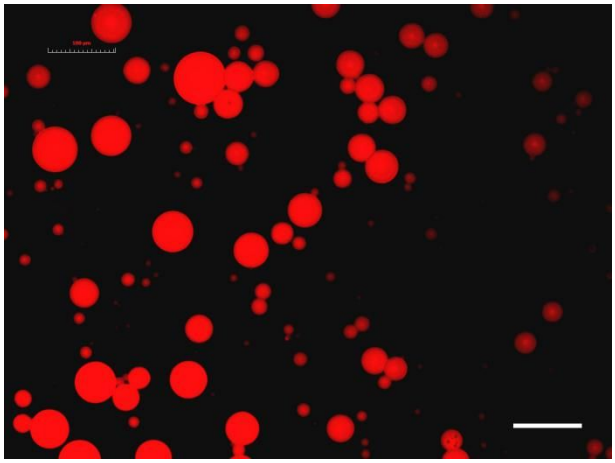


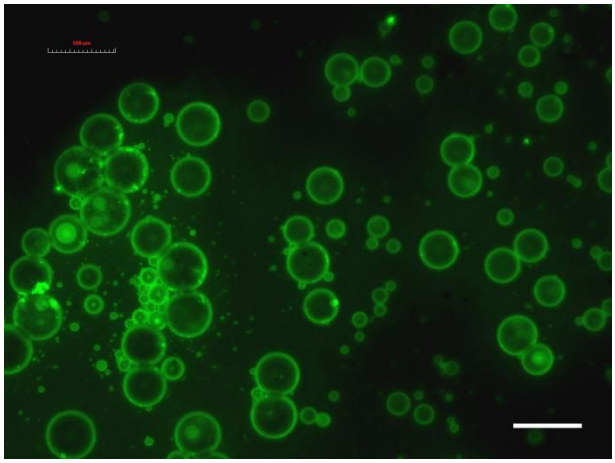
Fig. 7



(a)

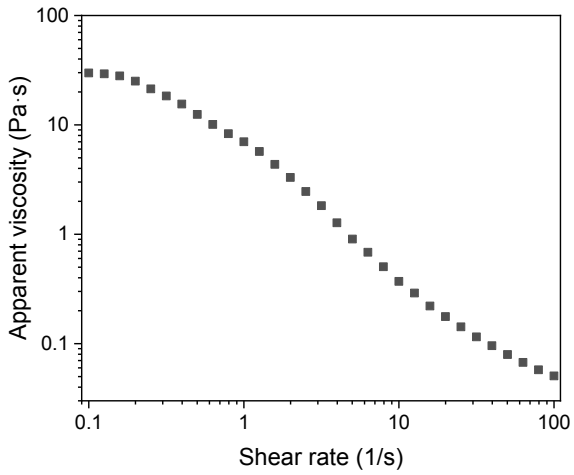


(b)

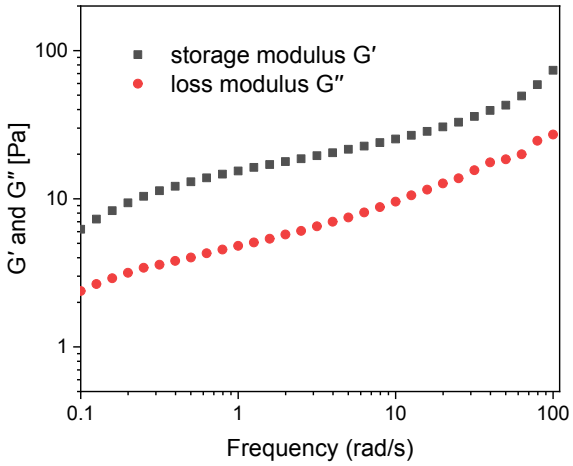


(c)

Fig. 8


Apparent viscosity (Pa·s)
100
10
1
0.1
0.1
1
10
100
Shear rate (1/s)

(a)


storage modulus G′
loss modulus G″
G′ and G″ [Pa]
100
10
1
0.1
1
10
100
Frequency (rad/s)

(b)


RUTGERS

(c)

Fig. 9

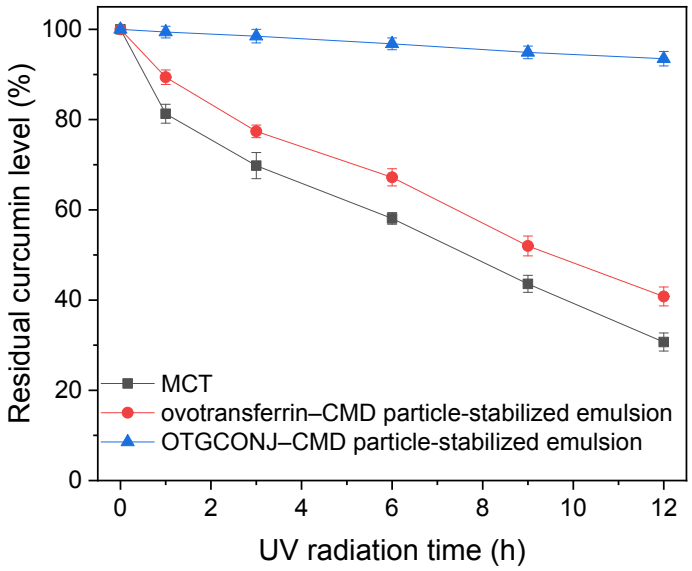
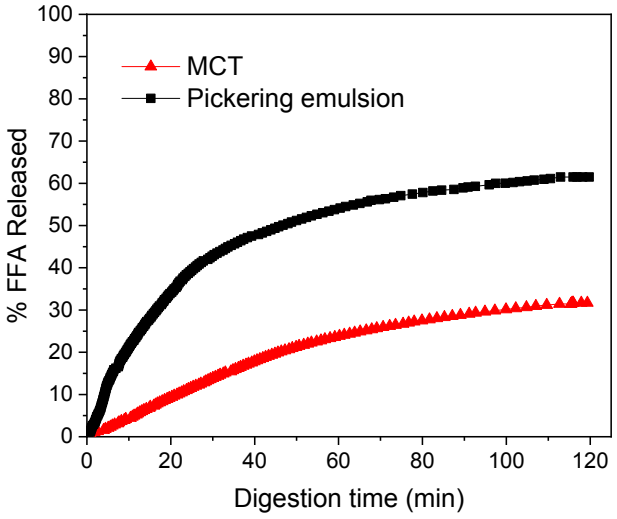
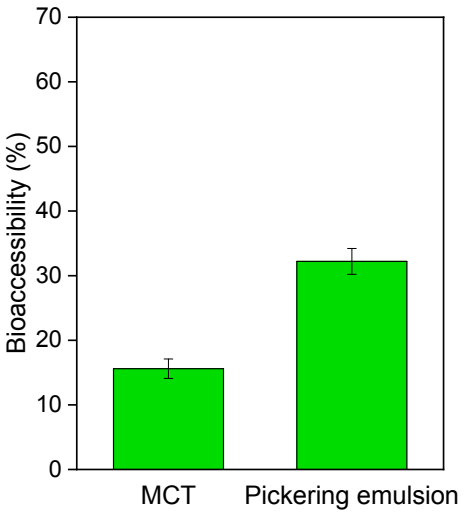

Residual curcumin level (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
UV radiation time (h)
MCT
ovotransferrin–CMD particle-stabilized emulsion
OTGCONJ–CMD particle-stabilized emulsion

Fig. 10


MCT
Pickering emulsion
% FFA Released
Digestion time (min)
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120

(a)


Bioaccessibility (%)
0
10
20
30
40
50
60
70
MCT
Pickering emulsion

(b)

Fig. 11

Table 1. Radius of gyration (R_g) of OTGCONJ at different pHs fitted from SAXS intensity profiles

OTGCONJ	R_g (Å)
pH 2.5	43.8±0.1[c]
pH 3.5	39.1±0.1[a]
pH 4.5	77.1±0.2[d]
pH 5.5	44.0±0.1[c]
pH 6.5	41.8±0.1[b]
pH 7.4	39.0±0.1[a]

Values are means±SD (n=3). Different superscript letters for the same column indicate significant differences ($p < 0.05$).

Table of contents