Development of novel nanoemulsions as delivery systems

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ABSTRACT

In the past decades, emulsions have been widely used as delivery systems for incorporating bioactive compounds into foods. With the advancing of nanotechnology, smaller particles in the nanometric range (i.e. nanoemulsions) can be created with better properties that are more advantageous than conventional emulsions in terms of their stability to gravitational separation, optical clarity and better absorption of nutrients in drug delivery (with increased bioavailability). In particular, emulsification and solvent evaporation method has been used to produce nanoemulsions with optimum results. However, like conventional emulsions, protein-stabilised nanoemulsions become unstable when exposed to certain environmental stresses such as high temperatures, salt addition and extreme pH changes. Additionally, liquid emulsions are difficult to transport and use in some food systems while being susceptible to microbial spoilage. To remedy, a dry, stable emulsion system has to be obtained for their prospective future in food applications.

The objective of this research was to develop nanoemulsions with useful attributes. The thesis consists of three main parts in which the first part studied the formation and properties of nanoemulsions using emulsification and solvent evaporation method; the second part delved into the making of dried nanoemulsion powders and the third part focused on the structural modifications of nanoemulsions and encapsulation of a bioactive compound lutein.

To begin, an experimental study to optimise the conditions for producing nanoemulsions using emulsification and solvent evaporation methodology was performed under different processing conditions (microfluidisation pressures and number of passes), organic phase ratios and materials (oil types and emulsifiers). It was found that smaller oil droplets (around 80 nm in diameter) were achieved when increasing the microfluidisation pressure up to 12000 psi (80 MPa) for 4 passes at an organic phase ratio of 10:90. There was a progressive decrease in particle size with increasing emulsifier concentration up to a 1% (w/w) level for whey protein isolate (WPI) and lactoferrin but it did not decrease further at higher concentration. On the other hand, much larger oil droplets were formed in Tween 20 emulsions (120 – 450 nm). The environmental study showed that lactoferrin and Tween 20 emulsions have

a better stability to pH changes (pH 2 – 12) and salt addition (0 – 500 mM NaCl or 0 – 90 mM $CaCl_2$) than WPI stabilised nanoemulsions.

After successful preparation of nanoemulsions, liquid nanoemulsions were converted to dried powders by spray drying or freeze drying. The nanoemulsions were mixed with different wall materials consisting of maltodextrin alone, trehalose alone or a 1:1 ratio of maltodextrin and trehalose at 10, 20 or 30% (w/w) solid concentration. Results showed that the powders containing 20% trehalose have better powder properties with lower moisture content and water activity, higher bulk density and good reconstitution in water. The freeze-dried powders showed excellent wettability and dispersibility in water but lower encapsulation efficiency than spray dried powders.

In another part of study, nanoemulsions with modified interfacial structure were used to improve their stability to environmental stresses. The interactions between WPI and lactoferrin in aqueous solutions were first studied to explore the feasibility of using these two proteins to form complex interfacial structures at the droplet surface in the emulsions. Based on ζ-potential and turbidity measurements, both proteins were shown to interact with each other via electrostatic interactions at pH values between 6 and 8. The adsorption of protein layers on a gold surface that mimics the hydrophobic oil surface was also confirmed by a quartz crystal microbalance with dissipation (QCM-D) study.

Next, a series of bi-layer nanoemulsions at different pH values and lactoferrin concentrations were prepared so as to determine the best conditions on the overall emulsion stability. It was shown that the stability of emulsions was dependent on both pH and lactoferrin concentration. At pH values close to pI of WPI (around pH 5), the nanoemulsions remained unstable regardless of the lactoferrin concentration used (0.25 – 5% w/w). The nanoemulsions at pH 6 were also unstable at low concentrations (0.5 – 1% w/w) presumably due to “bridging flocculation” and exhibited phase separation. Consequently, a lactoferrin concentration of 3% (w/w) was used to produce bi-layer nanoemulsions at pH 6. At pH 7 – 10, the bi-layer nanoemulsions were stable at all lactoferrin concentrations and formed a bi-layer structure at the interface of droplet.

The formulated nanoemulsions (single layer and bi-layer emulsions) were subjected to a variety of environmental stresses and *in vitro* digestion under simulated gastrointestinal conditions. The emulsion stability to pH changes and salt addition was improved in the bi-layer emulsions containing WPI and lactoferrin when compared to the single layer nanoemulsions stabilised by WPI alone. However, the bi-layer emulsions were more susceptible to destabilisation on heating at temperatures above 60^{o}C. The *in vitro* digestion of bi-layer nanoemulsions was similar to single layer nanoemulsions in which the protein hydrolysis of the interfacial layers results in extensive droplet flocculation.

In subsequent formulations, lutein was incorporated in the emulsions as a model of bioactive compound for the application of nanoemulsions as a novel delivery system. The nanoemulsions well encapsulated lutein in their matrices with an encapsulation efficiency of 80% and contained small oil droplets (70 – 80 nm). All the emulsions were physically stable under the tested conditions up to 28 days at different storage temperatures (5, 20 and 40^{o}C). However, there was a significant decrease in lutein content during storage especially at higher temperatures due to oxidative degradation. Nevertheless, the bi-layer nanoemulsions showed a better stability to lutein degradation. Based on *in vitro* cell toxicity studies on Caco-2 cells using MTT assay, both nanoemulsions did not show toxicity as the cell viability was more than 80% at 10 times or more dilution after 24 hours of incubation. The cellular uptake of lutein was higher in bi-layer nanoemulsions when compared to single layer emulsions.

The present work demonstrated that nanoemulsions can be formed using emulsification and a solvent evaporation method. Dried microcapsules of nanoemulsions were formed with similar properties as their original nanoemulsions after reconstitution in water. The nanoemulsions with bi-layer interfacial structure have better stability to environmental changes than single layer emulsions. Nanoemulsions did not show more toxicity than their corresponding conventional emulsions with large oil droplets produced without the use of organic solvent. These have important implications in the use of nanoemulsions for encapsulation lutein or other bioactive compounds for applications in foods and beverages.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

C	Mass sensitivity constant
D	Translational diffusion coefficient
$D_{3,2}$	Surface weighted mean
$D_{4,3}$	Volume weighted mean
f(κa)	Henry's function
g	Gravitational acceleration
h	Thickness
K	Boltzmann's constant
k	Rate constant
n	Overtone number
r	Radius
T	Temperature
U_E	Electrophoretic mobility
v	Creaming velocity
$t_{½}$	Half-life
ΔD	Dissipation shift
Δf	Frequency shift
Δm	Adsorbed mass
ΔP	Laplace pressure
γ	Interfacial tension
δ	Interfacial layer thickness
η	Viscosity
λ	Wavelength
ε	Dielectric constant
μ	Shear elastic modulus
ρ	Density
ρ_{eff}	Effective surface density
ϕ	Volume fraction
ω	Angular frequency of oscillation

LIST OF ABBREVIATIONS

AMD	Age-related macular degeneration
AMY	Amylase
ANOVA	Analysis of Variance
APS	Ammonium persulphate
BHT	butylated hydroxyl toluene
$CaCl_2$	Calcium chloride
CC	Coconut oil
CCP	Colloidal calcium phosphate
CIE	Commission Internationale de L'Eclairage
CR	Corn oil
DE	Dextrose Equivalence
DLS	Dynamic Light Scattering
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
EE	Encapsulation efficiency
GI	Gastrointestinal
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
pI	Isoelectric point
kBar	Kilobar
kDa	Kilodalton
L	Lipase
LBL	Layer-by-layer
LCT	Long chain triglycerides
LF	Lactoferrin
LO	Lemon oil
LSCM	Laser Scanning Confocal Microscopy
MCT	Medium chain triglycerides
MD	Maltodextrin
MPa	Mega pascal
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Na_3N	Sodium azide
NaCas	Sodium caseinate
NaCl	Sodium chloride
nm	nanometre
O.D.	Optical Density
O/W	Oil-in-water
PDI	Polydispersity Index
PIT	Phase inversion temperature
pmol	picomol
psi	Pound force per square inch
QCM-D	Quartz Crystal Microbalance with Dissipation
RNase	Ribonuclease
ROS	Reactive oxygen species
SAM	Self-assembled monolayer
SCT	Short chain triglycerides
S.D.	Standard Deviation
SDS	Sodium dodecyl sulphate
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscopy
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SLS	Static Light Scattering
SNEDDS	Self-Nanoemulsifying Drug Delivery System
SOR	Surfactant to oil ratio
SPR	Surface plasmon resonance
TEM	Transmission Electron Microscopy
TEMED	N,N,N',N'-tetramethylethylene diamine
TR	Trypsin
TRE	Trehalose
WI	Whiteness Index
WPC	Whey protein concentrate
WPH	Whey protein hydrolysate
WPI	Whey protein isolate

W/O	Water-in-oil
α-lac	Alpha-lactalbumin
β-lg	Beta-lactoglobulin
ζ-potential	Zeta-potential

LIST OF PUBLICATIONS & CONFERENCE PROCEEDINGS

1. Teo, A., S. Dimartino, S. J. Lee, K. K. T. Goh, J. Wen, Indrawati, O., S. Ko & H. S. Kwak (2016). Interfacial structures of whey protein isolate (WPI) and lactoferrin on hydrophobic surfaces in a model system monitored by quartz crystal microbalance with dissipation (QCM-D) and their formation on nanoemulsions. *Food Hydrocolloids*, 56, 150-160.

2. Teo, A., Goh, K. K. T., Wen, J., Oey, I., Ko, S., Kwak, H. S. & Lee, S. J. (2016). Physicochemical properties of whey protein, lactoferrin and Tween 20 stabilised nanoemulsions: effect of temperature, pH and salt. *Food Chemistry*, 197(Part A), 297-306.

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6. Teo, A., Lee, S. J. & Goh, K. K. T. (2014). Modulation of interfacial composition on the physico-chemical stability and lipid digestibility of nanoemulsions stabilised by whey protein isolates (WPI) and lactoferrin, Food Structure and Functionality Forum Symposium from Molecules to Functionality, 30 March-2 April, Amsterdam, The Netherlands.

7. Teo, A., Lee, S. J. & Goh, K. K. T. (2012). Nanotechnology in emulsions: preparation and characterisation of protein-stabilised nanoemulsions and their stability against heat, ionic strength and pH changes, NZIFST Annual Conference, 26-28 June, Hamilton, New Zealand.