IJFNPH
5,1/2/3

STABILITY AND MICROSTRUCTURE OF FOOD-GRADE DOUBLE EMULSIONS

Lanny Sapei[1]
Parahyangan Catholic University, Indonesia

Dérick Rousseau[2]
Ryerson University, Canada

Abstract: *Purpose:* Stable, food-grade water-in-oil-in-water double emulsions (DEs) were designed for possible controlled release applications in foods.
Methodology: The primary water-in-oil emulsion prepared with water, canola oil, polyglycerol polyricinoleate (PgPr), sodium chloride (NaCl) and gelatin was stable for months. DEs were developed by dispersing the primary emulsion into a continuous aqueous phase containing different hydrophilic emulsifiers and stabilizers, such as sodium caseinate, gum arabic, polysorbate 80, sodium alginate, and poloxamer 188. Combinations of polysorbate 80 with polysaccharides (gum arabic or sodium alginate) were also investigated.
Findings: Stability of DEs was observed macroscopically via sedimentation tests whereas microstructure were examined using light microscopy. Polysorbate 80 was the most suitable hydrophilic emulsifier for obtaining stable DEs. A hypertonic internal aqueous phase resulted in increased emulsion stability.
Value: Given their long-term kinetic stability, such DEs may potentially be used as controlled release matrices or texture modifiers in foods.

Keywords: *Double Emulsion; Polysorbate 80; Stability; Microstructure; Sedimentation; Microscopy*

International Journal of Food, Nutrition & Public Health
Vol. 5 No. 1/2/3, 2012

[1]Department of Chemical Engineering, Faculty of Industrial Technology, Parahyangan Catholic University, Bandung, West Java, Indonesia, E-mail: lanny.sapei@gmail.com
[2]Department of Chemistry and Biology, Ryerson University, Toronto, Ontario, Canada, E-mail: rousseau@ryerson.ca

INTRODUCTION

Water-in-oil-in-water ($W_1/O/W_2$) double emulsions (DEs) are complex systems that consist of a water-in-oil (W_1/O) emulsion dispersed as droplets within a continuous aqueous phase (Garti, 1997; Sagalowicz & Leser, 2010). Typically, a two-step emulsification process is used to make DEs whereby the primary emulsion (W_1/O) is homogenized using a hydrophobic surfactant with a low hydrophilic-lipophilic balance (HLB) value (typically < 6). In the second step, the primary emulsion is gently emulsified with an external phase that contains a hydrophilic surfactant with a high HLB value (typically > 12) to produce a $W_1/O/W_2$ DE.

Due to the presence of two aqueous domains separated by an oil layer, DE offers great potential for the encapsulation of hydrophilic bioactive ingredients entrapped in the inner aqueous phase and their controlled release towards the outer aqueous phase, particularly when placed in an aqueous release medium (*e.g.*, in the gastrointestinal tract). However, such systems can be more difficult to prepare and stabilize than simple emulsions as they typically consist of relatively large oil and inner aqueous droplets that can coalesce either quiescently or due to destabilization induced by common unit operations (*e.g.*, mixing, sterilization). The end result is a strong tendency to uncontrollably release their entrapped cargo in an uncontrolled manner (Garti, 1997). The development of $W_1/O/W_2$ DEs for food applications is further hampered by the lack of suitable food-grade emulsifiers and biopolymers to stabilize the inner and outer aqueous phases (Sagalowicz *et al.*, 2010).

Nevertheless, $W_1/O/W_2$ emulsions have been successfully used in cosmetics and pharmaceuticals for applications such as drug controlled release and targeted delivery (Gallarate,

Carlotti, Trotta, & Bovo, 1999; Vaziri & Warburton, 1994; Vlaia, Vlaia, Miclea, Olariu, & Coneac, 2009). DEs have also been used for the effective removal of toxic materials (Yan & Pal, 2001). Finally, $W_1/O/W_2$ DEs have been investigated for various food applications, including the encapsulation of vitamin/minerals (Benichou, Aserin, & Garti, 2007; O'Regan & Mulvihill, 2010), aroma and flavour release (Malone, Appelqvist, & Norton, 2003), and the production of low-calorie products (DeCindio & Cacace, 1995; Taki, 2008).

$W_1/O/W_2$ DEs for food applications have been developed using several approaches to improve their stability. The effects of different food-grade components (*e.g.* lipid phases, emulsifiers, electrolytes, biopolymers, sugars) (Muschiolik, 2007; Su, Flanagan, Hemar, & Singh, 2006; Su, Flanagan, & Singh, 2008) as well as the influence of dispersing methods (Muschiolik, 2007) have been widely investigated. The enhancement of DE stability using interfacial fat crystals (Garti, Binyamin, & Aserin, 1998) and protein-polysaccharide hybrids (Benichou, *et al.*, 2007) have also been studied and found to be quite effective. However, the formulation of stable food-grade DE using simple approaches still remains a challenge. The objective of this research was to study the stability and microstructure of food-grade DEs generated using a variety of emulsifiers and biopolymers to obtain deeper insight into their preparation, stability and possible uses.

MATERIALS AND METHODS

Materials

Deionized water with a resistivity of >15 MΩ-cm (Barnstead E-Pure, Ottawa, ON, Canada) was used for the aqueous phase. Gelatin (porcine skin, type A, Bloom ~300), polysorbate 80, sodium alginate (viscosity= ~250 cps for 2% solution at 25°C), Na-caseinate

(bovine milk) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Gum arabic was obtained from TIC Gums (Belcamp, MD, USA). Poloxamer 188 was obtained from BASF Canada. Sodium chloride (NaCl) was purchased from Fisher Scientific (Ottawa, ON, Canada). Canola oil (66.7% monounsaturated fatty acids, 27.8% polyunsaturated fatty acids, 5.5% saturated fatty acids; acid value < 0.2) was purchased from a local supermarket (Toronto, ON, Canada). The oil-tending emulsifier polyglycerol polyricinoleate (PgPr) was obtained from Nealanders (Mississauga, ON, Canada) (Grinsted, acid value: 0.65; iodine value: 83; saponification value: 177; hydroxyl value: 99). All chemicals were used without further purification.

Double emulsion preparation

Food-grade $W_1/O/W_2$ emulsions were prepared using a two-stage homogenization process. The inner aqueous phase (W_1) was prepared by adding 3% (w/w) gelatin and 5% (w/w) NaCl to the aqueous phase to help stabilize the primary water-in-oil (W_1/O) emulsion. This solution was hydrated for 30 min followed by mixing at 65°C for 25 min under moderate magnetic stirring. The oil phase (O) containing 6% (w/w) PgPr solution was also mixed at 65°C for 25 min under moderate magnetic stirring. The outer aqueous phase (W2), which contained various water-tending emulsifiers/stabilizers, namely 3% (w/w) polysorbate 80, 2.5% (w/w) sodium alginate, 15% (w/w) gum arabic, 0.5% (w/w) Na-caseinate or 0.5% (w/w) poloxamer 188, was stirred at 25°C for 30 min using moderate magnetic stirring. Combinations of 0.5% (w/w) polysorbate 80 with gum arabic (0.5%, 1%, and 2% (w/w)) and sodium alginate (0.1%, 0.2%, and 0.5% (w/w)) were also investigated. Modifying the osmotic environment was also explored. Namely, isotonic DEs were made by equalizing the NaCl concentration [2% (w/w)] within the DE inner and outer aqueous phases. For this aspect of the study, the concentrations of

polysorbate 80, sodium alginate, sodium caseinate, and poloxamer 188 used for the isotonic DEs were adjusted to 0.5%, 1%, 0.5%, and 0.2% (w/w), respectively.

The primary water-in-oil emulsion (W_1/O) was prepared by pouring the inner aqueous phase (W_1) (40% w/w) into the oil phase (O) (60% w/w). The mixture was equilibrated at 65°C for 25 min and then homogenized using a rotor-stator with a homogenization generator (d = 1.2 cm) (Polytron® PT 10/35, Kinematic, CH-6010, Switzerland) at 27,000 rpm for 3 min. This emulsion was immediately quench-cooled to 4°C in a water-bath to trigger the sol-gel transition of the gelatin in the dispersed aqueous phase. Prior to second-stage homogenization, the temperature was slowly raised to 26°C to improve emulsification efficacy. The secondary emulsion ($W_1/O/W_2$) was prepared by gradually adding the W_1/O emulsion (20% w/w) to the outer aqueous phase (W_2) followed by mixing using the rotor-stator at 27,000 rpm for 3 min. The resulting DE ($W_1/O/W_2$) was stored at room temperature for the sedimentation and microstructural analyses.

Stability evaluation

Sedimentation

Approximately 10 ml of DEs were poured into 15 ml plastic test tubes (VWR International, ON, Canada) for evaluating their kinetic stability macroscopically via sedimentation tests. DE stability were observed within 24 hours after preparation.

Microscopy

Brightfield light microscopy was used to examine DE microstructure. Individual samples were placed onto microscope slides (Fisher Scientific, Nepean, ON, Canada) and gently

covered with a cover slip (Fisher Scientific, Nepean, ON, Canada). A Zeiss Axiovert 200M inverted microscope (Zeiss Inc., Toronto, ON, Canada) with a 20×objective (combined with 1.6× Optivar lens) was used (magnification of 320x). Images captured with a Q-Imaging CCD camera mated to the microscope were analyzed using Northern Eclipse software (version 7.0, Empix Imaging, Mississauga, ON, Canada). DEs (within 24 hours after preparation) were characterized at room temperature (25°C).

RESULTS AND DISCUSSION

Sedimentation tests

Kinetic stability of the DEs was observed macroscopically via sedimentation tests (Fig. 1-3). The primary (W_1/O) emulsion made with PgPr as the hydrophobic emulsifier and which incorporated gelatin and NaCl in the aqueous phase was remarkably stable as no phase separation was observed for at least 6 months at room temperature (results not shown). Once emulsified into a secondary aqueous phase, most of the

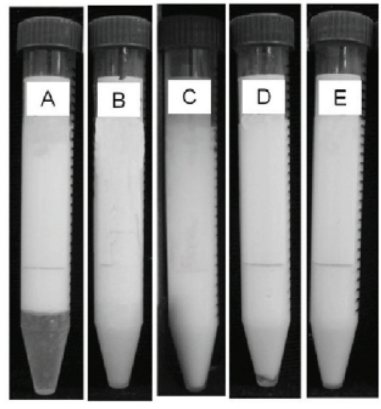

Figure 1: Sedimentation Stability of $W_1/O/W_2$ Double Emulsions Prepared Using Various Hydrophilic Emulsifiers/Stabilizers in the Outer Aqueous Phase. (A) 0.5% Sodium Caseinate; (B) 3 % Polysorbate 80; (C) 0.5% Poloxamer 188; (D) 2.5% Sodium Alginate; (E) 15% Gum Arabic. All % Values are wt%.

resulting $W_1/O/W_2$ DEs showed poor sedimentation stability indicated by the occurrence of phase separation (Fig. 1-3). The kinetic stability of DEs was highly dependent on the judicious combination of ingredients. Amongst the DEs with a hypertonic inner aqueous phase prepared using a single hydrophilic emulsifier or stabilizer, phase separation was only observed in the DE prepared with sodium caseinate (Fig. 1A vs. Fig. 1B-E). This is in spite of published evidence reporting that proteins such as sodium caseinate are excellent emulsifiers (Fechner et al., 2007). Speculatively, a small portion of the salt in the inner aqueous phase was released, which may have affected the surface activity of the sodium caseinate and/or screened the surface charges on the protein-stabilized droplets, leading to droplet-droplet flocculation. DEs prepared using polysaccharide thickeners, namely sodium alginate and gum arabic, were stable against sedimentation (Fig. 1D-E) as they were very viscous and gelled, thus retarding the coalescence of oil globules and phase separation. DEs containing polysorbate 80 and poloxamer 188 (Fig. 1B-C), though pourable, were also kinetically stable.

The combined effect on stability of a monomeric emulsifier (polysorbate 80) and thickeners (gum arabic and sodium alginate) in the external phase was also investigated (Fig. 2). Macromolecular materials such as proteins or polysaccharides can be incorporated both in the internal and external aqueous phase to improve DE stability (Su, Flanagan, & Singh, 2008). Polymeric materials often provide steric stabilization to emulsions by creating a thick film that reduces droplet-droplet flocculation and coalescence. Nevertheless, our results showed that combinations of 0.5% polysorbate 80 with small amounts of thickeners (0.5 - 2% wt gum arabic and 0.1 - 0.5% sodium alginate) did not confer any significant improvement against sedimentation. Unexpectedly, DE stability worsened upon incremental addition of thickener as indicated by the more extensive

phase separation Fig. 2. It is possible that there was limited salt diffusion from the internal to the external aqueous phase, which may have influenced the emulsifying properties of both gum arabic and sodium alginate.

Finally, the influence of isotonicity on the kinetic stability of DEs was studied by equalizing the NaCl concentration (2%) in both aqueous phases. All isotonic DEs phase-separated to a greater extent in comparison to those with an hypertonic inner aqueous phase, except for the isotonic DE prepared with polysorbate 80 (Fig. 3). It was obvious that the presence of a relatively high salt concentration (at least 2%) induced a detrimental effect on the emulsifying properties of sodium caseinate, sodium alginate, and poloxamer 188. In contrast, the efficacy of polysorbate 80 was minimally affected by the presence of salt. A hypertonic inner aqueous phase conferred greater kinetic stability to the DEs compared to their isotonic counterparts, presumably as a result of an osmotic gradient. The presence of a hypertonic inner aqueous phase led to water migration from the external to the internal aqueous phase, resulting in the swelling of the inner water droplets and

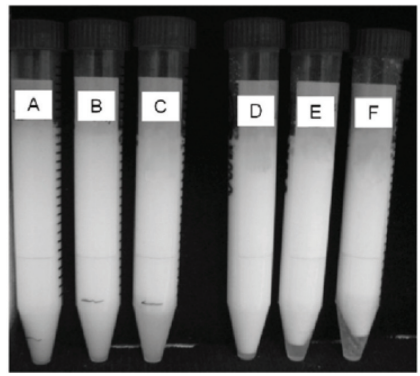

Figure 2: Sedimentation Stability of $W_1/O/W_2$ Double Emulsions Prepared Using a Combination of 0.5% Polysorbate 80 With Various Concentrations of Thickeners in the Outer Aqueous Phase. (A) 0.5% Gum Arabic; (B) 1% Gum Arabic; (C) 2% Gum Arabic; (D) 0.1% Sodium Alginate; (E) 0.2% Sodium Alginate; (F) 0.5% Sodium Alginate. All % Values are wt%.

thus oil globules. In turn, the overall packing density within the DE was increased leading to a decrease in phase separation. This has been also confirmed by our previous investigation (Sapei et. al., 2012). Overall, these results indicated that polysorbate 80 was a suitable hydrophilic emulsifier to obtain $W_1/O/W_2$ DEs with considerable stability.

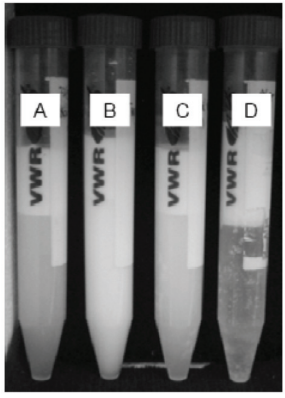

Figure 3: Sedimentation Stability of isotonic $W_1/O/W_2$ Double Emulsions Containing 2% NaCl in both the inner and Outer Aqueous Phases with Various Hydrophilic Emulsifiers/Stabilizers. (A) 0.5% Sodium Caseinate; (B) 0.5% Polysorbate 80; (C) 0.2% Poloxamer 188; (D) 1% Sodium Alginate. All % Values are wt%.

Microstructure

Composition had a significant impact on DE microstructure and stability against flocculation and coalescence (Fig. 4). The choice of surfactants also impacted the number of emulsion 'compartments' and the volume that the inner phase occupies. Based on Florence and Whitehill's nomenclature (Florence & Whitehill, 1981), there exist 3 types of DEs. Type A DEs consist of oil globules that contain one large internal droplet; Type B DEs contain several small internal aqueous droplets within the globules and type C DEs consist of oil globules with large numbers of internal droplets. Type C systems are also known to confer the highest long-term stability (Florence &Whitehill, 1981).

The least stable DE, which was prepared with sodium caseinate, was of DE type B (Fig. 4A). This was in contrast to the remaining stable DEs (Fig. 4B-E), which contained large numbers of internal droplets (DE type C). Additionally, DEs prepared with thickeners (either sodium alginate or gum arabic) had oil globules 2-3 times smaller in diameter compared to the other DEs (Fig. 4D-E vs. Fig. 4A-C). This was due to their high viscosity and/or gelled state that slowed the coalescence of the oil globules. Interestingly, DEs prepared with poloxamer 188 (Fig. 4E) showed an unusual microstructure with an almost invisible external aqueous phase. This may have been due to its strong emulsification capacity.

The microstructure of DEs prepared with polysorbate 80 and different concentrations of gum arabic and sodium

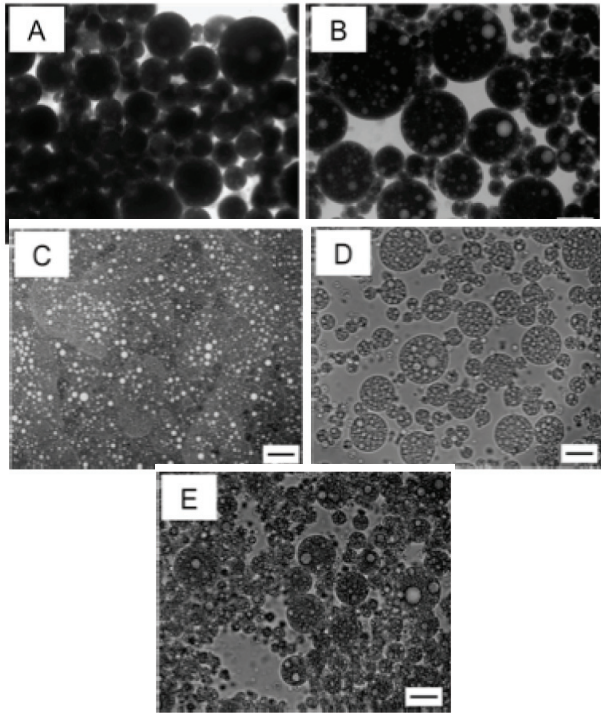

Figure 4: Microstructure of $W_1/O/W_2$ Double Emulsions Prepared Using Various Hydrophilic Emulsifiers/Stabilizers in the Outer Aqueous Phase. (A) 0.5% Sodium Caseinate (bar= 40 μm); (B) 3% Polysorbate 80 (bar= 40 μm); (C) 0.5% Poloxamer 188 (bar= 40 μm); (D) 2.5% Sodium Alginate (bar= 12 μm); (E) 15% Gum Arabic (bar= 20μm). All % Values are wt%.

alginate are shown in Fig. 5. In general, they all showed similarities with numerous internal droplets, even though sedimentation tests indicated that the DEs destabilized with an increase in thickener concentrations (0.5 - 2 wt% for gum arabic and 0.1 - 0.5 wt% for sodium alginate).

Finally, the microstructure of isotonic DEs prepared with various hydrophilic emulsifiers/thickeners is shown in Fig. 6. Internal droplets were hardly visible in all isotonic DEs, except when the DE was prepared with polysorbate 80 (type C DE). This explains why isotonic DEs with polysorbate 80 remained stable whereas the others destabilized. The primary emulsions not stable in an isotonic environment tended to coalesce and in turn likely 'released' the internal droplets towards the external continuous phase, thus inducing more rapid phase separation. As such, the presence of 2% salt in both the external and internal aqueous phases was detrimental to DE stability in contrast to the presence of 5% salt in the internal droplets (Fig. 6 vs. Fig. 4). This corroborated previous

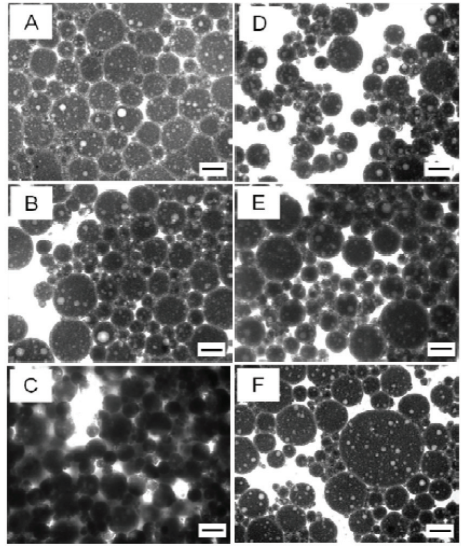

Figure 5: Microstructure of $W_1/O/W_2$ Double Emulsions Prepared Using a Combination of 0.5% Polysorbate 80 with Various Concentrations of Thickeners. (A) 0.5% Gum Arabic; (B) 1% Gum Arabic; (C) 2% Gum Arabic; (D) 0.1% Sodium Alginate; (E) 0.2% Aodium Slginate; (F) 0.5% Sodium Alginate. All % Values are wt%. Size bars= 40 μm.

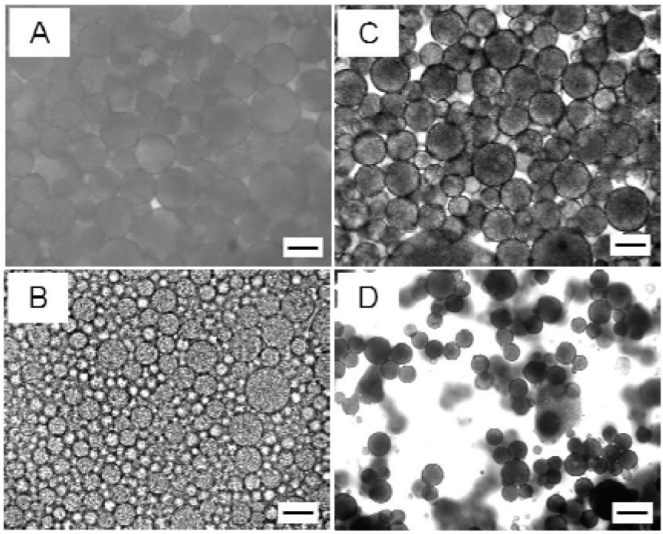

Figure 6: Microstructure of Isotonic $W_1/O/W_2$ Double Emulsions Containing 2% NaCl in Both Aqueous Phases With Various Hydrophilic Emulsifiers/Stabilizers. (A) 0.5% Sodium Caseinate (bar= 20 μm); (B) 0.5% Polysorbate 80 (bar= 12 μm); (C) 0.2% Poloxamer 188 (bar= 20 μm); (D) 1% Sodium Alginate (bar= 40 μm). All % Values are wt%.

research indicating that the presence of salt in only the internal phase was important for DE stability (Kawashima et. al., 1992; Rosano et. al., 1998).

CONCLUSIONS

The stability and microstructure of food-grade double emulsions were highly influenced by the selection of hydrophilic emulsifiers/stabilizers present in the external phase. Polysorbate 80 was the most suitable external emulsifier for obtaining highly-stable DEs but still pourable. The combination of polysorbate 80 and thickeners such as gum arabic and sodium alginate did not confer any significant improvement on overall DE stability. Finally, isotonic DEs rapidly

phase-separated in contrast to the DEs with a hypertonic inner aqueous phase. The higher stability of DEs with a hypertonic inner aqueous phase was due to an increase in the packing density of oil globules triggered by the swelling of inner aqueous droplets. Stable DEs are a promising avenue for use in foods where novel textures and/or possibly controlled release properties are desired. Such foods include salad dressings, mayonnaise and pudding enriched with bioactive ingredients encapsulated within the inner aqueous phase. However, further investigations related to sensory perception and long-term stability (*i.e.*, many months) are important prior to the use of such DEs in food applications.

BIOGRAPHY

Lanny Sapei earned her Ph.D. in Biomaterials at Max-Planck Institute of Colloids and Interfaces Potsdam/ Potsdam University, Potsdam, Germany in 2007. After completing her doctoral research related to silica in *Equisetum hyemale*, she received a postdoctoral fellowship from Food Research Lab at Ryerson University, Toronto, Canada in 2008. She was responsible for formulating food-grade water-in-oil-in-water (W/O/W) double emulsion and study of their controlled release.In 2011, she joined the Chemical Engineering Department, Faculty of Industrial Engineering at Catholic Parahyangan University, Bandung, Indonesia as a lecturer. Her research interests cover utilization and characterization of natural resources, bio-waste utilization, formulation of highly nutritious and healthier foods, and bio-based materials for medical applications, health, and wellness.

Dérick Rousseau is a professor in the Department of Chemistry and Biology at Ryerson University in Toronto, Canada. He obtained his Ph.D. in Food Science from the University of Guelph in 1997 and his B.A.Sc. in Food Science and Technology from Université Laval in 1993. His research

efforts focus on understanding the physical and chemical factors that control the formation and stability of colloidal systems. His group has made important contributions in a number of areas, including emulsion stabilization in food and non-food applications, chocolate functionality, dairy product fortification, lipid crystallization and the development of controlled release matrices.

ACKNOWLEDGEMENTS

Financial support from the Natural Science and Engineering Research Council (NSERC) of Canada, the Advanced Foods and Materials Network (AFMNet), and Ryerson University is acknowledged.

REFERENCES

Benichou, A., Aserin, A., & Garti, N. (2007). W/O/W double emulsions stabilized with WPI-polysaccharide complexes. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 294*(1-3), 20-32.

DeCindio, B., & Cacace, D. (1995). Formulation and rheological characterization of reduced-calorie food emulsions. *International Journal of Food Science and Technology, 30*(4), 505-514.

Fechner, A., Knoth, A., Scherze, I., & Muschiolik, G. (2007). Stability and release properties of double-emulsions stabilised by caseinate-dextran conjugates. *Food Hydrocolloids, 21*(5-6), 943-952.

Florence, A. T., & Whitehill, D. (1981). Some features of breakdown in water-in-oil-in-water multiple emulsions. *Journal of Colloid and Interface Science, 79*(1), 243-256.

Gallarate, M., Carlotti, M. E., Trotta, M., & Bovo, S. (1999). On the stability of ascorbic acid in emulsified systems for topical and cosmetic use. *International Journal of Pharmaceutics, 188*(2), 233-241.

Garti, N. (1997). Double emulsions - Scope, limitations and new achievements. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 123*, 233-246.

Garti, N., Binyamin, H., &Aserin, A. (1998). Stabilization of water-in-oil emulsions by submicrocrystalline alpha-form fat particles. *Journal of the American Oil Chemists Society, 75*(12), 1825-1831.

Kawashima, Y., Hino, T., Takeuchi, H., & Niwa, T. (1992). Stabilization of water/oil/water multiple emulsion with hypertonic inner aqueous phase. *Chemical Pharmaceutical Bulletin, 40*(5), 1240-1246.

Malone, M. E., Appelqvist, I. A. M., & Norton, I. T. (2003). Oral behaviour of food hydrocolloids and emulsions. Part 2. Taste and aroma release. *Food Hydrocolloids, 17*(6), 775-784.

Muschiolik, G. (2007). Multiple emulsions for food use. *Current Opinion in Colloid & Interface Science, 12*, 213-220.

O'Regan, J., & Mulvihill, D. M. (2010). Sodium caseinate-maltodextrin conjugate stabilized double emulsions: Encapsulation and stability. *Food Research International, 43*(1), 224-231.

Rosano, H. L., Gandolfo, F. G., & Hidrot, J. D. P. (1998). Stability of $W\text{-}_1/O/W\text{-}_2$ multiple emulsions - Influence of ripening and interfacial interactions. *Colloids and Surfaces*

a-Physicochemical and Engineering Aspects, 138(1), 109-121

Sagalowicz, L., & Leser, M. E. (2010). Delivery systems for liquid food products. *Current Opinion in Colloid & Interface Science, 15*(1-2), 61-72.

Sapei, L., Naqvi, M.A., & Rousseau, D. (2012). Stability and release properties of double emulsions for food applications. *Food Hydrocolloids, 27*(2), 316-323.

Su, J. H., Flanagan, J., Hemar, Y., & Singh, H. (2006). Synergistic effects of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilisation of water-oil-water emulsions. *Food Hydrocolloids, 20*(2-3), 261-268.

Su, J. H., Flanagan, J., & Singh, H. (2008). Improving encapsulation efficiency and stability of water-in-oil-in-water emulsions using a modified gum arabic (Acacia (sen) SUPER GUM (TM)). *Food Hydrocolloids, 22*(1), 112-120.

Taki, J. (2008). $W_1/O/W_2$-Type double emulsion dressing and method for production thereof. WO 2007/043678.

Vaziri, A., & Warburton, B. (1994). Slow-release of chloroquine phosphate from multiple taste-masked W/O/W multiple emulsions. *Journal of Microencapsulation, 11*(6), 641-648.

Vlaia, L., Vlaia, V., Miclea, L. M., Olariu, I., & Coneac, G. (2009). Topical W/O/W double emulsions of Piroxicam: *in vitro* drug release study. *Farmacia, 57*(5), 639-647.

Yan, J., & Pal, R. (2001). Osmotic swelling behavior of globules of W/O/W emulsion liquid membranes. *Journal of Membrane Science, 190*(1), 79-91.