Microencapsulating Properties of Acacia (sen) SUPER GUM™

John Flanagan(a,b) Jiahong Su(c) Colm O’Brien(b) Brid O’Riordan(b) Harjinder Singh(b) Colum Dunne(b)

(a) Riddet Centre, Massey University
Private Bag 11-222, Palmerston North, New Zealand
(b) Glanbia Innovation Centre, Glanbia Nutritionals
Leggetsrath Business Park, Carlow Road, Kilkenny, Ireland

Summary

Gum arabic has long been used in the beverage industry as an emulsifier and more recently, its potential to be used in microencapsulation technologies has been explored, with positive results. A modified gum arabic (Acacia (sen) SUPER GUM™), marketed as SUPER GUM™, has recently become available on the market. SUPER GUM™ has been modified by an accelerated storage process, and is characterised by increased molecular weight and greater surface activity compared to unmodified gum arabic. In this work, we present two different applications of SUPER GUM™ as an encapsulating agent. The encapsulation efficiencies of multiple emulsions were increased following the use of SUPER GUM™ as an external emulsifier. A second application, for the microencapsulation of a proprietary anti-microbial dairy blend, Glonon, is also reported. Efficiency of the microencapsulation process was indicated by altered sensitivity against Staphylococcus aureus 9518. Hydration time and presence of bile salts also affected the anti-microbial activity of Glonon and microencapsulated Glonon differently, with microencapsulated Glonon retaining greater activity than non-encapsulated Glonon.

1. Introduction

Microencapsulation technologies are currently being utilised in a wide range of industries such as food, cosmetics, pharmaceuticals and agrochemical. Many of the microencapsulation methods used in these industries involve the initial formation of an emulsion before subsequent chemical fixing, spray drying or chill cooling. In all cases, the emulsifier used must possess superior emulsion forming and emulsion stabilising properties to emulsify the hydrophobic or oil phase, and to impart long-term stability to a product which may eventually be redispersed in an aqueous medium. A variety of materials have been studied extensively as emulsifiers; proteins such as caseinate³, whey proteins⁴, soy proteins⁵ and their hydrolyses⁶ and polysaccharides such as pectin⁷, modified starch⁸ and gum arabic⁹. Gum arabic has been extensively used as an encapsulating agent in a coacervate⁹, as the sole emulsifier in beverages⁹, and also as a component in a spray-dried microencapsulated system⁹. A recent introduction to the emulsifier market is a modified gum arabic, marketed as SUPER GUM™. SUPER GUM™ is different from unmodified gum arabic, in that it has undergone an accelerated aggregation process, the results of which are larger molecular weights and higher surface activity. The physicochemical characteristics of SUPER GUM™ have been well described¹⁰–¹⁸. In this work, we describe the use of SUPER GUM™ as a microencapsulating agent in two different applications: stable, high encapsulation efficiency multiple emulsions, and as a microencapsulating agent for a novel anti-microbial.

Multiple emulsions are emulsion systems in which the dispersed phase is itself an emulsion, and they have been extensively studied as a means of encapsulation¹⁹–²⁴. However, despite their immense potential, the application of multiple emulsions in food systems has been limited by their inherent thermodynamic instability, which causes leakage of the encapsulant from the inner aqueous phase, flocculation of the droplets or phase separation during processing and storage²⁵–²⁷.

Glonon is a natural dairy derived anti-microbial with efficacy against a range of microbes. It has been applied into a range of products, such as gels, hand washes, toothpaste and mouth sprays to aid with personal care and oral hygiene. Additionally, Glonon is being evaluated for its ability to act against intestinal tract pathogens in immune-compromised individuals. Colonisation by such intestinal tract pathogens and subsequent infection occurs in response to destabilisation of the indigenous microflora usually by anti-biotic treatment in hospitalised patients. Recurrent infection or relapse following conventional (anti-biotic) treatment is a major problem with intestinal tract pathogen infection. Once in the small intestine, Glonon may be able to successfully prevent opportunistic growth of pathogens. However, the components of Glonon will have to be protected against acidic, enzymatic and complexing forces encountered on passing through the upper gastrointestinal tract.
2. Materials and Methods

Commercial soybean oil was purchased from AMCO Ltd., Auckland, New Zealand. Polyoxyethylene glycerol ester of polyricinoleic acid (PGPR 4150, Palsgaard Ltd., Denmark) was purchased from Hawkins Watt Ltd., Auckland, New Zealand. Commercial sodium caseinate was obtained from Fonterra Cooperative Ltd., Palmerston North, New Zealand, and contained approximately 93% protein, 1.2% sodium, and 0.06% calcium. Violet dye poly R-478 was purchased from Sigma Ltd., San Louis, USA, and Nile Blue was from BDH Ltd., Poole, UK. Glovan and whey protein isolate (Pronova 190, containing 90-95% protein) was obtained from Glanbia Nutritional, Kilkenny, Ireland. All microbiological media was from Oxoid (Basingstoke, Hampshire, England) and Staphylococcus aureus 9518 strain was from the National Collections of Industrial Food and Marine Bacteria (NCIMB), Aberdeen, Scotland. SUPER GUM™ was kindly gifted from San-Ei Gen F.F.I., Inc. Osaka, Japan, and contained approximately 1.7% salt.

2.1. Preparation of multiple emulsions

Multiple emulsions were prepared by a two-step process with a two stage homogeniser (APV 2000, Denmark) with a slight modification to the method described previously. For preparation of primary water-in-oil (W/O) emulsions, 20% (w/v) of the hydrophobic emulsifier PGPR was initially mixed with soybean oil. Subsequently, the emulsifier/oil mixture was mixed in a 1:4 ratio with 0.1 M sodium phosphate buffer at pH 6.6. The aqueous phase contained either sodium caseinate (0.5% w/v) or varying concentrations of SUPER GUM™. In addition, the internal aqueous phase contained a water soluble dye (Poly R-478) which was used for measurement of stability of the emulsion. The mixtures were homogenised at 800/80 bar (first-stage pressure/second stage pressure) twice to obtain the primary W/O emulsions. The second step involved the dispersion of the primary emulsions into solutions (sodium caseinate, 0.5% w/v or SUPER GUM™, 0.2% w/v), prepared in 0.1 M sodium phosphate buffer at pH 6.6, using the same homogeniser, at a pressure of 100/0 bar twice. The ratio of primary W/O emulsion: buffer solution was 1:4.

2.2. Characterisation of multiple emulsions

2.2.1. Confocal laser microscopy

Confocal scanning laser microscopy (CSLM) was used to observe the multiple emulsion samples (Leica TCS 4D confocal microscope, Leica Lasertecnik GmbH, Heidelberg, Germany). Samples (1 ml) of emulsions were stained with 0.1 ml of 1% (w) Nile Blue, and the stained samples were dropped on concave glass slides, covered by cover slide and examined with a 100× magnifications lens using an Ar/Kr laser with an excitation line of 488 nm; the resolution of this techniques is >0.2 μm.

2.2.2. Determination of average droplet size of multiple emulsions

The droplet sizes of secondary emulsions were measured by a Malvern Mastersizer MSE (Malvern Instruments Ltd, Malvern, Worcestershire, United Kingdom). Volume average diameter (dV) and surface average diameter (dS) of multiple emulsions were determined. The lens range was set at 45 mm and the active beam length at 2.4 mm. The presentation code was 2NAD, the relative refractive index, i.e. the ratio of the refractive index of emulsion droplets to that of the dispersion medium was 1.065 and the absorbance value of the emulsion droplets was 0.001.

2-2-3. Determination of encapsulation efficiency (EE)

The EE is defined as the percentage of the water soluble dye (Poly R-478) retained inside the internal aqueous phase, measured immediately after multiple emulsion preparation and after one month's storage at 20°C.

2-3. Preparation of microencapsulated Glovan

A 10% (w/v) SUPER GUM™ solution was prepared, adjusted to pH 7.0 and allowed to hydrate fully overnight. A 10% (w/v) whey protein isolate solution was also prepared, pH unadjusted (pH 6.7) and allowed to hydrate overnight. The following day, a 25% oil emulsion was prepared using SUPER GUM™ as the emulsifier and Glovan's proprietary blend of fatty acids as the oil phase. The pre-emulsion was blended on a Silverson (LAR Model, Chesham, HP51PQ, UK) for 2 mins at 4,500 rpm. Subsequently, the pre-emulsion was twice passed through a two-stage APV high pressure homogeniser (APV 1000, Albertslund, Denmark) at 450/50 bar (first-stage pressure/second stage pressure), care was taken to prevent unhomogenised material and semi-homogenised material coming into contact with the final product. Fifteen minutes after emulsion formation, the emulsion was mixed 9 parts to 1 with the 10% WPI solution. The emulsion-WPI mixture was frozen at −8°C until fully frozen. Samples were subsequently lyophilised using a Freezezone 4.5 lab-scale freeze dryer (Labconco, Kansas, MO) until a fine powder was obtained.

2.4. Characterisation of microencapsulated Glovan (MEG)

2.4.1. Assessing the antimicrobial activity of Glovan and MEG

Increasing concentrations (0-1%) of both MEG and Glovan were resuspended in diluted overnight Staphylococcus aureus 9518 culture. 100 μl aliquots were taken at time 0, 3, 10, 30 and 60 min, serially diluted and spread plated on Tryptic Soya agar (TSA) plates. Plates were incubated at 37°C for 24 hours and the resulting colonies counted.

2.4.2. Effect of hydration on Glovan and MEG

To determine the effect of hydration on Glovan and MEG the above experiment was repeated with the following modifications: prior to commencing the experiment Glovan and MEG were hydrated in Tryptic soya broth (TSB) for 1 hour. Timepoints were taken at 0, 10, 30 and 60 min.

2.4.3. Effect of 5% and 7.5% bile salts on Glovan and MEG

To determine the effect of bile salts on Glovan and MEG, 1% Glovan and 1% MEG were resuspended in dilute overnight 9518 culture containing either 5% or 7.5% bile salts, mixed and the stopwatch started. Controls included (a) broth containing just culture (b) broth containing culture and 5% bile salts and (c) broth containing culture and 7.5% bile salts. 100 μl aliquots
were taken at time 0, 10, 30 and 60 min, serially diluted and spreadplated on Tryptic Soya agar (TSA) plates. Plates were incubated at 37°C for 24 hours and the resulting colonies counted.

2-4-4. Simulated gastric juice studies
A 10% resuspension of MEG and Glovon were added to an equal volume of simulated gastric juice (at pH 2) and incubated at 37°C for 2 hours. Samples were taken at time 0 and at regular intervals thereafter up to 5 h. The pH values of the samples were adjusted to pH 7 prior to assaying and activity was determined by the agar well diffusion assay with Lactobacillus gasseri as the indicator strain.

2-4-5. Simulated ileum juice studies
A 10% resuspension of MEG and Glovon were added to an equal volume of simulated ileum juice (pH 7) and incubated at 37°C for 5 hours. Samples were taken at time 0 and regular intervals thereafter up to 5 h. Antimicrobial activity was determined by the agar well diffusion assay with Lactobacillus gasseri as the indicator strain.

2-4-6. Simulated colon juice studies
A 10% resuspension of MEG and Glovon were added to an equal volume of simulated colon juice (pH 7) and incubated at 37°C for 24 hours. Samples were taken at time 0 and at intervals thereafter up to 24 h. Antimicrobial activity was determined by the agar well diffusion assay with Lactobacillus gasseri as the indicator strain.

3. Results and Discussion
3-1. Effect of addition of SUPER GUM™ to the internal aqueous phase of multiple emulsions
The presence of multiple emulsion oil droplets (containing 0.5% (w/v) sodium caseinate in the external aqueous phase) was confirmed by confocal microscopy (Figure 1). The addition of SUPER GUM™ to the internal aqueous phase of these systems did not result in observable changes to the appearance of the multiple emulsion droplets. However, some insoluble material was evident in SUPER GUM™ solutions prepared in the sodium phosphate buffer.

The droplet size distributions of multiple emulsions prepared with SUPER GUM™ in the internal aqueous phase are presented in Figure 2a. Droplet size distributions of all

![Figure 1. Confocal microscopy images of multiple emulsions with 2% (w/v) PGPR in oil phase, 0.5% (w/v) sodium caseinate in external aqueous phase and varying concentration of SUPER GUM™ in the internal aqueous phase. a: 0%, b: 2%, c: 8% (w/v). Scale bar represents 5 μm.](image)

![Figure 2. Particle size distributions (a) and yield (b) of multiple emulsions prepared with 2% (w/v) PGPR in oil phase, 0.5% (w/v) sodium caseinate in the external aqueous phase and varying (1-10%) w/v) SUPER GUM™ concentrations in the internal aqueous phase.](image)
emulsions exhibited a bimodal distribution, typical of all multiple emulsions prepared under the current set of conditions and were similar to the control emulsion prepared with 0.5% sodium caseinate in the internal emulsion phase.

The encapsulation efficiencies (EE) of multiple emulsions prepared with varying SUPER GUM™ concentrations (1 to 10% w/v) in the internal aqueous phase were determined (Figure 2b). Overall, significantly increased EE of multiple emulsion were obtained after the addition of SUPER GUM™ to the internal aqueous phase, at all concentrations of SUPER GUM™ examined, compared to the yields of emulsion prepared without SUPER GUM™ in the internal aqueous phase.

3.2. Effect of Addition of SUPER GUM™ to the External Aqueous Phase of Multiple Emulsions

The external aqueous phase of the multiple emulsions containing 0.125-2% (w/v) SUPER GUM™ were analysed by confocal microscopy (results not shown). The presence of coalesced and flocculated oil droplets in the multiple emulsion prepared at low concentrations of SUPER GUM™ (0.125-0.5%) were detected, while flocculation was less evident in multiple emulsions prepared with a 1% (w/v) SUPER GUM™ in the external aqueous phase. Again, some insoluble material was observed in the emulsion sample.

The droplet size distributions of multiple emulsions prepared with SUPER GUM™ in the external aqueous phase are presented in Figure 3a. Droplet size distributions of all emulsions again exhibited a typical bimodal distribution, and were similar to the control emulsion prepared with 0.5% sodium caseinate in the external aqueous phase.

At 0.125-2% (w/v) SUPER GUM™, some of the oil droplets were outside the range of the Mastersizer’s range, indicating coalesced oil droplets of size greater than 50 μm (Figure 3a). This result is in agreement with the confocal microscopy image. Flocculation was not observed in multiple emulsions prepared with a 2% (w/v) SUPER GUM™ in the external aqueous phase.

The EEs of multiple emulsions prepared with varying SUPER GUM™ concentrations (0.125 to 2% w/v) in the external aqueous phase were determined (Figure 3b). Overall, the yields of multiple emulsions increased after the addition of SUPER GUM™ in the external aqueous phase, compared to the yields of emulsion prepared with 0.5% sodium caseinate in the external aqueous phase. Yields of multiple emulsions containing 2% (w/v) SUPER GUM™ in the external aqueous phase displayed almost 100% EE. However, under the present conditions, 2% SUPER GUM™ was unable to form a stable multiple emulsion in terms of particle size. Higher concentration of SUPER GUM™ may be required in the external aqueous phase to achieve a more stable multiple emulsion.

Under the current experimental conditions, the addition of increased concentration of SUPER GUM™ dissolved in sodium phosphate buffer did not significantly improve the yield and stability of multiple emulsions when it was added to the internal aqueous phase of multiple emulsions. It is unclear in this adverse effect is due to the presence of some insoluble material in the SUPER GUM™ material in sodium phosphate buffer. Subsequent work to this has shown that when SUPER GUM™ was dissolved in distilled water, it had a positive effect on both the yield and stability of multiple emulsions when applied in either internal or external aqueous phase.

3-3. Microencapsulation of Glovon

As an indication of Glovon’s anti-microbial activity, S. aureus 9518 was incubated with different concentrations of microencapsulated and non-encapsulated Glovon for up to 60 mins. Glovon and microencapsulated Glovon (MEG) were tested at identical concentrations (0.25, 0.5 and 1.0%); however, it must be noted that the MEG contained 77.7% Glovon, due to the presence of the microencapsulation material.

In general, MEG exhibited less anti-microbial activity than the non-encapsulated form (Figure 4). Following 10 mins incubation of 9518 with a 1% solution of MEG, 9518 was undetectable (Figure 4b), whereas in less than 3 mins, 9518 was undetectable in the presence of 1% Glovon (Figure 4a). At lower concentrations of Glovon, a time dependent decrease in
9518 was observed up to 60 mins, with 1.3 and 2.4 log reductions in the presence of 0.25 and 0.5% Glovon, respectively. After 60 mins incubation, log reductions of 1.1 and 2.2 were observed with the 0.25 and 0.5% MEG samples, respectively (Figure 4b), which was less than the log reduction noted for the non-encapsulated Glovon (Figure 4a). However, the reduced activity of MEG compared to Glovon was not as great as expected - if all of the active components of Glovon were fully microencapsulated, no activity would be expected. Thus, either there must be some breakdown in the microencapsulation system over time or all of the Glovon must not have been microencapsulated to start with. A further experiment was conducted to determine the effect of hydration on Glovon and MEG on the ingredients' consequent ability to reduce the viability of Staphylococcus aureus 9518.

Solutions of Glovon and MEG were hydrated in TSB for 1 hour, and subsequently added to stock solutions of Staphylococcus aureus 9518 (Figure 5). As observed previously, Glovon and MEG reduced the viability of Staphylococcus aureus 9518 by 2.6 and 4.2 logs, respectively. While hydration had little effect on Glovon, it did enhance the anti-microbial activity of MEG, inferring that the microencapsulation coating was dissolving over time, and thus the active components of Glovon were free to act. The effects of 5 and 7.5% bile salts on anti-microbial activity of 9518 were also determined to give an indication of Glovon activity upon entry into the small intestine (Figure 6). The presence of 5 and 7.5% bile salts alone had little effect on 9518 viability after incubation for 60 mins (results not shown). While Glovon completely eliminated Staphylococcus aureus 9518 after 10 mins, increasing bile salts concentration dramatically reduced the ability of Glovon to reduce the viability of Staphylococcus aureus 9518 (Figure 6a). This indicated that the bile salts effectively complex the active components of Glovon, resulting in reduced anti-microbial activity. The anti-microbial activity of MEG, on the other hand, did not appear affected by the presence of 5% bile salts (Figure 6b). However, the

![Figure 4](image-url) Effect of incubation time on anti-microbial activity of different concentrations of Glovon (a) and microencapsulated Glovon (MEG; b) against S. aureus 9518.

![Figure 5](image-url) Effect of incubation time on anti-microbial activity of Glovon, microencapsulated Glovon (MEG) used immediately or after hydration for 1 hour against S. aureus 9518.

![Figure 6](image-url) Effect of incubation time on anti-microbial activity of Glovon (a) and microencapsulated Glovon (MEG; b) in the absence and presence of 5 and 7.5% bile salts against S. aureus 9518.
presence of 7.5% bile salts greatly reduced the activity of Glown to a level similar to that observed in the non-encapsulated Glown, also at 7.5% bile salts. A likely explanation of this effect is that the free Glown in the MEG sample (which increases in concentration the longer the sample is hydrated) was immediately bound by the high level of bile salts.

A second series of experiments focussed on the activity of MEG and non-encapsulated Glown in simulated gastric, ileum and colon juice against Lactococcus lactis HP, Lactobacillus gasseri and Lactobacillus gasseri, respectively. Both MEG and non-encapsulated Glown retained full activity against the pathogens tested (results not shown). This indicates that the active components of Glown are available to act against pathogens in simulated gastrointestinal conditions.

4. Conclusion

SUPER GUM™ successfully aided in the increased encapsulation efficiencies of multiple emulsions when incorporated into the external aqueous phase. However, the stabilities or encapsulation efficiencies of multiple emulsions were not improved on addition of SUPER GUM™ to the internal aqueous phase. This may be due to the partial insolubility of SUPER GUM™ in a sodium phosphate buffer system. On the other hand, SUPER GUM™ successfully microencapsulated Glown, resulting in decreased activity against S. aureus 9518 compared to non-encapsulated Glown. In addition, microencapsulated Glown retained greater activity against S. aureus 9518 in the presence of bile salts, compared to non-encapsulated Glown.

References


[日本語訳（要旨）]
“スーパーガム”のマイクロカプセル化による特性

John Flanagan*1 Jiahong Su*2 Coim O’Brien*3 Brid O’Riordan*4 Harjinder Singh*4 Colum Dunne*5

*1Riddet Centre, Massey University
*2Private Bag 11-222, Palmerston North, New Zealand
*3Glennia Innovation Centre, Glennia Nutritional
Leggatereath Business Perk, Carlow Road, Kilkenny, Ireland

スーパーガムは長年にわたり食品産業において乳化剤として利用されてきた。近年、マイクロカプセル化技術にアラビアガムを使用する（可能頸）の研究が進み、肯定的な結果を収めている。"スーパーガム"の名で販売されている改質アラビアガムが最近になって市場に出された。"スーパーガム"は加速潰製工法によって製造されており、未改質のアラビアガムに比べると分子量が増大し、界面活性度も強化されている。本研究は、"スーパーガム"のカプセル化物質として、2つの異なるアプレサーション（適用）を紹介している。"スーパーガム"は水相系に乳化剤として利用することによって、多様な乳化液のカプセル化効率が向上した。2つ目のアプレサーションとして独自開発された添加剤抗菌加工製品であるグローボンのマイクロカプセル化について報告している。グローボンのマイクロカプセル化がなぜ必要かといえば、それが腸管を通じて運ばれる病原体の抑制に効力を発揮できるよう小腸内に分布するようになるためである。マイクロカプセル化されたグローボンが黄色プドウ球菌BS18に対し活性を低下させることが観察され、グローボンがマイクロカプセル化されていることを示した。本報の長と短の両存を否定することができ、マイクロカプセル化グローボンの抗菌性に異なる影響を及ぼし、マイクロカプセル化グローボンはカプセル化されていないグローボンよりも強い活性を維持することを示した（図6）。

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**Profile**

**John Flanagan**  
Riddet Centre, Massey University  
Glänbia Innovation Centre  
Research Scientist  
Ph. D.

Dr. Flanagan completed his doctorate studies in 2002 at the University of Limerick, Ireland, focusing on improving the functional properties of sodium caseinate through the use of enzymatic modification. He then accepted a three year post-doctoral position at the Riddet Centre, Massey University, New Zealand where his experience with emulsion systems was expanded. Other areas of research, such as microemulsions, multiple emulsions, liposomes, covalent and non-covalent protein-polyaccharide interactions were also developed. Dr. Flanagan subsequently joined Glänbia Nutritional in June 2005 to initiate a new microencapsulation platform for the business.

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**Jiahong Su**  
Riddet Centre, Massey University

**Colm O'Brien**  
Glänbia Innovation Centre

**Brid O'Riordan**  
Glänbia Innovation Centre

**Harjinder Singh**  
Riddet Centre, Massey University  
Director

Professor Harjinder Singh currently holds a Chair in Dairy Science and Technology at Massey University. He is also a Director of the Riddet Institute (New Zealand Centre of Research Excellence in Foods and Biologics). His main areas of expertise include milk protein structure and functionality, functional foods and gels and encapsulation and delivery of bioactive compounds. He has published over 200 papers in international journals, and is one of ISI highly cited researchers. He was elected Fellow of Royal Society of New Zealand (2002) and Fellow of the International Academy of Food Science and Technology (2006). He was awarded MarshBall Rhodia International Dairy Science Award (2001) by the American Dairy Science Association, USA for his contributions to the dairy protein research. He is member of the editorial boards of six international journals.

**Colum Dunne**  
Glänbia Innovation Centre  
Research and Development Director

Colum Dunne (B. Sc., Hon., Ph.D., MBA) has held responsibilities as Manager of multi-centre clinical trials focused on intestinal health at Ireland's National Food Biotechnology Centre; General Manager of Ireland's only research centre dedicated solely to cancer research, where the teams became focused on the prophylactic and therapeutic potential of functional foods, in addition to the emerging area of medical devices and controlled gene therapy. Most recently, Colum joined Glänbia Nutritional where he is now Research and Development Director. Colum has published more than 50 scientific articles and patents. He is currently guest executive editor of the journal Current Pharmaceutical Design.