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(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Development of Smart and Uniform-sized Colloidosomes for Drug Delivery
智能均一膠體體微囊的研制及其在藥物釋放中的應用

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. NGAI, To 魏 濤 教 授	Prof. MA, Guang-hui 馬 光 輝 教 授
Post	Associate Professor 副教授	Professor, Director of National Key Laboratory of Biochemical Engineering 教授, 生化工程国家重点实验室主任
Unit / Department / Institution	Department of Chemistry The Chinese University of Hong Kong 香港中文大学化学系	Institute of Process Engineering, Chinese Academy of Science 中国科学院过程工程研究所
Contact Information	tongai@cuhk.edu.hk Tel: 3943 1222	ghma@home.ipe.ac.cn Tel: 010-82627072
Co-investigator(s) *(with title and institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1-1-2012	/	/
Project Completion date	31-12-2014	/	/

Duration *(in month)*	36	/	/
Deadline for Submission of Completion Report	31/12/2015	/	/

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To synthesize and characterize stimulus-responsive colloidal particles with varying size and hydrophobicity for use as particulate emulsifiers.

2. To prepare uniform-sized and stimulus-responsive colloidosome microcapsules containing a responsive dual shell by the combining of a membrane emulsification technique and a double emulsion–solvent extraction method.
3. To adjust the colloidosome permeability while maintaining the intended response to the release trigger by controlling the size of colloidal particles and by incorporating polymeric materials into colloidosome shells.
4. To evaluate *in vitro* and *in vivo* the feasibility of using produced monodisperse colloidosomes as bio-drugs, such as insulin carriers, for oral administration.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____
_____

1.
2.
3. ....

N/A

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

This has been a fruitful and successful collaboration between a colloidal and interfacial group (led by Prof. Ngai) and a biochemical engineering group (led by Prof. Ma). We were very fortunate to be able to have four excellent and hard-working graduates, namely Dr. Guanqing Sun (Ngai's group), Dr. Feng, Qi (Ma's group), Mr. Man-hin Kwok (Ngai's group), and Miss Fangfang Nan (Ma's group) to work on this project. Four new classes of stimulus-responsive colloidosomes, based on pH-responsive nanoparticle (PMMA-MAA), biocompatible nanoparticle (alginate/chitosan), biodegradable nanoparticle (PLGA), and protein nanoparticle

(BSA) were designed, synthesized and evaluated in this project. Unexpectedly, we also investigated some of the adsorption kinetic of particles and their behavior at water/air surfaces in relation of liquid marbles. This joint grant has generated a total of 15 high-quality publications, including 7 joint publications. Prof. Ngai awarded the young research award 2013 at the CUHK. Prof. Ma has filed 2 patents applications and awarded Hou Debang Chemical Science and Technology Innovation. Dr. Qi won the excellent scholarship of P&G and National Scholarship for Postgraduate. The main findings are summarized as follows:

1. Colloidosome based on PMMA-MAA nanoparticles. We described for the first time of the preparation of uniform-sized colloidosomes stabilized by soft colloidal particles including carboxyl group- or amine group-functionalized microgels and PMMA-MAA particles using Shirasu Porous Glass (SPG) membrane emulsification technique. Narrow polydispersity colloidosomes with tunable size and droplet diameter as small as 10 μm, can be obtained by varying the pore sizes of the SPG membrane. Additionally, we showed that the PMMA-MAA particle-stabilized colloidosomes can be easily destabilized by increasing the pH values which satisfy typical demands by controlled release applications.

2. Colloidosome based on protein BAS nanoparticles. Using BSA proteins as Pickering stabilizers, and subsequently crosslinked the interfacially assembled BSA particles resulted in a protein-based colloidosome. The as-prepared protein colloidosome opens the door for exploring the potential applications in the encapsulation of active pharmaceutical and health ingredients where the delivery of an exact amount is crucial.

3. Colloidosome based on PLGA nanoparticles. We have applied SPG premix membrane emulsification to produce monodisperse PLGA particles and systematically studied the stabilization of Pickering emulsions stabilized by these particles. Our results proved that colloidosomes derived from Pickering emulsions stabilized by PLGA particles had wide potential applications in pharmaceutics and tissue engineering.

4. Colloidosome based on alginate/chitosan nanoparticles. We have successfully prepared monodisperse colloidosomes with a high yield and low permeability by combining the premix membrane emulsification and polymer deposition method. The formulated colloidosomes showed high drug encapsulation efficiency (up to 96.7%) and a pH-triggered release profile. Moreover, in animal testing, these microcapsules achieved a long-term hypoglycemic effect (up to 6 h), indicating their efficacy as an oral drug delivery system.

5. Particle behavior at the water/air interfaces. We report the fabrication of silica particle-based liquid marbles with the encapsulating particle shells not only acting as protecting layers to provide a confined environment, but also providing the reactive substrate surfaces to regulate the classical silver mirror reaction. Besides, micron-sized silica particles were used for the preparation of monolayer-stabilized liquid marbles, which demonstrated great potential in fabricating Janus particles from superhydrophobic particles, which is not attainable from Pickering emulsions.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

In this project, four new classes of stimulus-responsive colloidosomes were designed, synthesized and evaluated. For colloidosomes based on PMMA-MAA particles, they are stable at low pH, but dissolve at high pH. In this way, this kind of colloidosome has the potential to be used in the pharmaceutical field to protect active ingredients against the harsh acidic environment in the stomach by delaying their release until they reach the milder environment of the intestine. For the emulsions stabilized by protein BSA and biodegradable PLGA nanoparticles, locking the interfacially assembled particles into a robust particle shell and can be subsequently dried to colloidosomes. Therefore, the as-prepared emulsion droplets are excellent templates for production of monodisperse colloidosomes/microcapsules, which opens the door for exploring the potential applications in the encapsulation of active pharmaceutical and health ingredients where the delivery of an exact amount is crucial. The uniform-sized colloidosomes based on alginate/chitosan showed high drug encapsulation efficiency (up to 96.7%) and a pH-triggered release profile. Moreover, in animal testing, these microcapsules achieved a long-term hypoglycemic effect (up to 6 h), so that they will enable us to develop a novel, non-parenterally, orally administered drug carrier. Furthermore, we showed that silica stabilized liquid marbles could be employed as a substrate to carry out interfacial silver mirror reaction. Since silver has extensive utilization in various fields, these microreactors can be used to fabricate *in-situ* platforms for further applications, such as catalysis, surface-enhanced Raman spectroscopy (SERS), and bactericidal action. We believe that the ability to use liquid marbles as microreactors will be particularly attractive and competitive, especially when the transportation of the liquids on solid substrate without leakage is a necessity.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Colloidosomes are microcapsules that consist of a hollow core coated by a shell composed of self-assembled colloidal particles. They have recently received considerable attention because of their great potential as vehicles for the controlled delivery of active ingredients in medicine, home and personal care products, agrochemicals, and cosmetics. However, even though examples of the practical usefulness of colloidosomes have been shown, their use as delivery systems has hitherto been extremely limited because of the low encapsulation efficiency, high permeability, low mechanical strength, and broad size distribution. In this project, we have designed and successfully synthesized four new classes of stimulus-responsive colloidosomes based on Pickering emulsion and membrane emulsification technique. Narrow polydispersity colloidosomes with tunable size (10 – 50 μm), permeability, mechanical strength, and stimulus-triggered release have been obtained using different kinds of polymeric nanoparticles. Moreover, we have evaluated the potential application of the formulated colloidosomes as oral insulin delivery vehicles. The

results were encouraging since the uniform colloidosomes showed high drug encapsulation efficiency. More importantly, these microcapsules achieved a long-term hypoglycemic effect (up to 6 h) in animal testing, which will enable us to develop a novel, non-parenterally, orally administered drug carrier.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2013				**Man-hin Kwok**, Zifu Li and **To Ngai***	"Controlling synthesis and characterization of micron-sized PNIPAM microgels with tailored morphologies", *Langmuir*, **2013**, 29, 9581-9591.	30/6/2013	Yes	Yes	Yes
2013				Zifu Li, Karen Geisel, Walter Richtering, and **To Ngai***	"Poly(*N*-isopropylacrylamide) microgels at the oil-water interface: adsorption kinetics", *Soft Matter*, **2013,** 9, 9939-9946.	30/6/2013	Yes	Yes	Yes
2013				**Feng Qi**, Jie Wu, Qingze Fan, Fan He, Guifang Tian, Tingyuan Yang, **Guanghui Ma***, and Zhiguo Su	"Preparation of uniform-sized exenatide-loaded PLGA microspheres as long-effective release system with high encapsulation efficiency and bio-stability" *Colloid Surf. B-Biointerfaces* **2013**, 112, 492-498.		Yes	Yes	Yes

2014				Xiaodong Li, **Guanqing Sun**, Yecheng Li, Jimmy C Yu, Jie Wu, **Guang-Hui Ma***, and **To Ngai***	"Porous TiO_2 materials through Pickering high-internal phase emulsion templating", *Langmuir*, **2014**, 30, 2676-2683.		Yes	Yes	Yes
2014				Xiangjun Gong, Zhaohui Wang, and **To Ngai***	"Direct measurements of particle-surface interactions in aqueous solutions with total internal reflection microscropy", *Chem. Commun*. **2014**, 50, 6556-6570.		Yes	Yes	Yes
2014				**Guanqing Sun**, **Feng Qi**, Jie Wu, **Guang-Hui Ma***, and **To Ngai***	"Preparation of uniform particle-stabilized emulsions using SPG membrane emulsification", *Langmuir*, **2014**, 30, 7052-7056.		Yes	Yes	Yes
2014				**Guanqing Sun**, Yifeng Sheng, Jie Wu, **Guang-Hui Ma***, and **To Ngai***	"Liquid marbles stabilized by charged polymer latexes: how does the drying of the latex particles affect the properties of liquid marbles" *Langmuir*, **2014**, 30, 12503-12508.		Yes	Yes	Yes
2014				**Guanqing Sun**, Min Liu, Xi Zhou, Liangzhi Hong*****, and **To Ngai***	"Influence of asymmetric ratio of amphiphilic diblock copolymers on one-step formation and stability of multiple emulsions", *Colloid Surf. A-Physicochem. Eng. Asp.* **2014**, 454, 16-22.		Yes	Yes	Yes

2014				**Feng Qi**, Jie Wu, **Guanqing Sun**, Fangfang Nan, **To Ngai***, and **Guanghui Ma***	"Systematic studies of Pickering emulsions stabilized by uniform-sized PLGA particles: preparation and stabilization mechanism", *J. Mater. Chem. B.* **2014**, 2, 7605-7611.		Yes	Yes	Yes
2014				**Fangfang Nan**, Jie Wu, **Feng Qi**, Qingze Fan, **Guanghui Ma***, and **To Ngai***	"Preparation of uniform-Sized colloidosomes based on chitosan-coated alginate particles and its applications for oral insulin delivery", *J. Mater. Chem. B.* **2014**, *2*, 7403-7409.		Yes	Yes	Yes
2014				**Fangfang Nan**, Jie Wu, **Feng Qi**, Yan Liu, **To Ngai*** and **Guang-Hui Ma***	"Uniform chitosan-coated alginate particles as emulsifiers for preparation of stable pickering emulsions with stimulus dependence", *Colloid Surf. A-Physicochem. Eng. Asp.* **2014**, *456*, 246-252.		Yes	Yes	Yes
2014				**Guanghui Ma***	"Microencapsulation of protein drugs for drug delivery: Strategy, preparation, and applications", *J. Controlled Release*, **2014**, 193, 324-340.		Yes	Yes	Yes

2014				**Feng Qi**, Jie Wu, Tingyuan Yang, **Guanghui Ma***, and Zhiguo Su	"Mechanistic studies for monodisperse exenatide-loaded PLGA microspheres prepared by different methods based on SPG membrane emulsification" Acta Biomaterialia, **2014**, 10, 4247-4256.		Yes	Yes	Yes
2014				**Feng Qi**, Jie Wu, Dongxia Hao, Tingyuan Yang, Yu Ren, **Guanghui Ma***, and Zhiguo Su	"Comparative studies on the influences of primary emulsion preparation on properties of uniform-sized exenatide-loaded PLGA microspheres" Pharm Res. **2014**, 31, 1566-1574.		Yes	Yes	Yes
2015				Yifeng Sheng, **Guanqing Sun**, Jie Wu, **Guanghui Ma***, and **To Ngai***	"Silica-based liquid marbles as microreactors for the silver mirror reaction" *Angew. Chem. Int. Ed.* **2015**, 54, 7012-7017.		Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

March/2013/ Italy	One-Step Formation of W/OW Multiple Emulsions Stabilized by Single Amphiphilic Block Copolymers	Third International Conference on Multifunctional, Hybrid and Nanomaterials		Yes	Yes	Yes
April/2013/ Xiamen, China	Uniform-Sized Colloidosomes for Functional Molecules Encapsulation	3rd International Colloids Conference		Yes	Yes	Yes
June/2013/ South Korea	Design, Synthesis and Application of Microgel Particles with Tailored Morphologies	Collaborative Conference on 3D & Materials Research (3DMR) 2013.		Yes	Yes	Yes
August/2014 /Fukuoka/Japan	Tailor-Made Microgel Particles: New Preparation Routes and Applications	The 15th IUMRS-International Conference in Asia (IUMRS-ICA 2014)		Yes	Yes	Yes
October/2014/Beijing/China	Colloidal Particles at Liquid Interfaces	Sino-German Symposium on Colloid and Interface Materials Meeting		Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
SUN, Guanqing	Ph.D in Chemistry	Aug. 2009	July 2014
KWOK, Man-hin	Ph.D in Chemistry	Aug. 2012	July 2016

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

The involved students, Dr. Sun Guanqing and Mr Kwok Man-hin awarded Hong Kong Ph.D Fellowship in 2011 and 2012, respectively.
Prof. Ngai To, Young Research Award 2013, Faculty of Science, The Chinese University of Hong Kong.
Prof. Ma Guanghui, Hou Debang Chemical Science and Technology, 2014.
Dr. Qi Feng, Excellent Scholarship of P&G and National Scholarship for Postgraduate 2014.
The student Dr. SUN Guanqing has conducted a cooperation research at the Institute of Process Engineering, Chinese Academy of Sciences, Beijing, during June 30 2012 to August, 31, 2012 (about 63 days), under supervision of Prof. Guanghui MA. Dr. Qi Feng has conducted the research at the department of Chemistry under Prof. Ngai's supervision in August 2013.