RGC Ref. No.:
UGC/FDS16/M08/20
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

New Development of Rapid and Easy Authentication Method of Single Drug Concentrated Chinese Medicine Granules using Fourier-Transform Infrared Spectroscopy (FTIR) coupled with Chemometrics

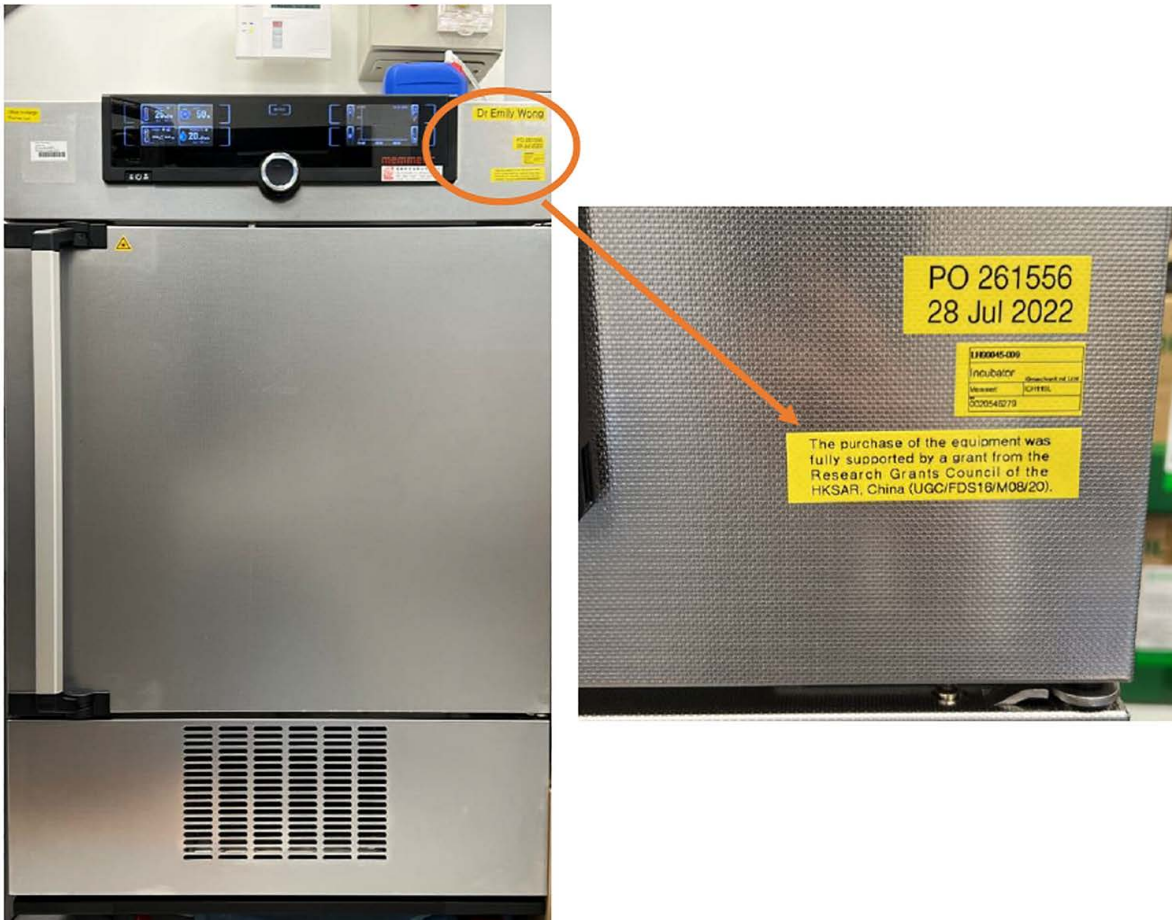
2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	WONG Emily Sze-wan / Assistant Professor	School of Science and Technology / Hong Kong Metropolitan University
Co-Investigator(s)	CHAN Sidney Man-ngai / Assistant Professor	School of Science and Technology / Hong Kong Metropolitan University
	CHU Carlin Chun-fai / Assistant Professor	Department of Computing / The Hang Seng University of Hong Kong
Others		

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	1 January 2021	--	
Project Completion Date	30 June 2022	31 December 2022	30 September 2021
Duration *(in month)*	18	24	30 September 2021
Deadline for Submission of Completion Report	30 September 2022	31 December 2023	30 September 2021

4.3 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.



Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. *To verify the species identity of the selected Chinese Medicine by conventional analytical technique.*
2. *To optimize and standardize the sample preparative conditions of Concentrated Chinese Medicine Granules (CCMG) for FTIR-ATR analysis.*
3. *To validate and discriminate the spectroscopic profiles of CCMG based on the optimized conditions obtained in Objective 2, with Chemometric approach.*
4. *To analyse and evaluate the effects of temperature and moisture on the spectroscopy of CCMG samples.*
5. *To discriminate the impurities or contaminants of CCMG by fingerprint spectroscopic analysis*

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change: NA

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

All objectives were completed smoothly without major problems. The realization of the objectives is summarized as follows,

Objective 1: The identity of Concentrated Chinese Medicine Granules (CCMG) selected in this project was first authenticated with the compound-oriented approach suggested by Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standards. The chemical authentication techniques, including high performance liquid chromatography (HPLC) and thin layer chromatography (TLC), were adopted. All the CCMG involved have been successfully authenticated before further investigations.

Objective 2: Various sample preparative conditions of CCMG for FTIR-ATR analysis were compared, including sample preparation and extraction methods, spectral data preprocessing, and spectral range selection. The generated spectral results were analyzed using Principal Component Analysis (PCA). The preparative conditions were then optimized. In the subsequent studies, the spectral data of CCMG samples without extraction were preprocessed by baseline correction followed by Standard Normal Variate (SNV). A representable spectral range of 1700 – 1150 cm^{-1} was selected for CCMG discrimination.

Objective 3: The spectral data synthesized with the optimal conditions determined in *Objective 2* were analyzed with Chemometric approach. Three machine learning classification models, Support Vector Machine (SVM), A Library for Support Vector Machines (LIBSVM) and Linear Discriminant Analysis (LDA), were compared. SVM and LDA were developed using the MATLAB statistic and machine learning toolbox (MathWorks Inc., MA, US), whereas our self-developed analytical system, LIBSVM,

was implemented in the GNU Octave 7.2.0. To analyze the accuracy of the training and prediction sets among the three classification models, 11 species of CCMG comprising a total of 53 samples from different brands and batches were tested. LIBSVM achieved the highest accuracy in both the training set (where 5 out of 10 spectral data of the sample were trained) with a 100% accuracy, as well as in the prediction set (spectral data without previously training) with 99.5% accuracy. The training and prediction accuracies for SVM were 83% and NA, respectively, while those for LDA were 100% and 98.1%, respectively. Therefore, LIBSVM was chosen for further analysis.

Objective 4: Three species of CCMG, namely *Fritillariae Cirrhosae Bulbus, Panacis Quinquefolii Radix* and *Lonicerae Japonicae Flos* were randomly selected for temperature and moisture analysis. A small quantity of CCMG was placed in a Petri-dish and stored in a climate chamber (ICH 110L, Memmert, Germany) at either 20°C or 30°C, with relative humidity (RH) levels of 20%, 40%, 60%, and 80% for 48 hours. In the prediction set, both *Fritillariae Cirrhosae Bulbus* and *Panacis Quinquefolii Radix* achieved 100% accuracy. The prediction accuracy for *Lonicerae Japonicae Flos* was 25%. The prediction results were correct at both 20 and 30°C of RH 20%. However, for the remaining conditions, the results were incorrectly predicted as the same CCMG of another brand.

Objective 5: To discriminate the impurities or contaminants of CCMG, testing samples of CCMG were mixed with either an excipient, Dextrin, or toxic CCMG such as *Radix Aconiti Lateralis Radix Praeparata* in varying ratios of 5, 10, 20, 40, 50, 60, 80, 90 and 95%. The testing samples could be accurately predicted at concentrations ranging from 40 – 95%, 60 – 95% and 80 – 95%.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To verify the species identity of the selected Chinese Medicine by conventional analytical technique.	√	100%
2. To optimize and standardize the sample preparative conditions of Concentrated Chinese Medicine Granules (CCMG) for FTIR-ATR analysis.	√	100%
3. To validate and discriminate the spectroscopic profiles of CCMG based on the optimized conditions obtained in Objective 2, with Chemometric approach.	√	100%
4. To analyse and evaluate the effects of temperature and moisture on the spectroscopy of CCMG samples.	√	100%
5. To discriminate the impurities or contaminants of CCMG by fingerprint spectroscopic analysis	√	100%

6. Research Outcome

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

In this project, several achievements were made in the authentication of CCMG. Chemical authentication techniques, such as HPLC and TLC, were employed to successfully authenticate the identity of the CCMG samples. This ensured the reliability and accuracy of the subsequent investigations.

The project focused on optimizing and standardizing the sample preparative conditions for FTIR-ATR analysis of CCMG. Through a systematic comparison of various sample preparation and extraction methods, spectral data preprocessing techniques, and spectral range selection, our research team was able to identify the most effective parameters. Principal Component Analysis (PCA) was utilized to analyze the spectral data, leading to the optimization of the preparative conditions. Baseline correction and Standard Normal Variate (SNV) preprocessing were applied to spectral data without extraction, and a representative spectral range of 1700 – 1150 cm^{-1} was selected for accurate discrimination of CCMG.

The spectral data generated with the optimized conditions were analyzed using three machine learning classification models: SVM, LIBSVM, and LDA. Among these models, LIBSVM demonstrated superior performance, achieving the highest accuracy in both the training set and prediction set. Consequently, LIBSVM was chosen as the preferred model for further analysis, showcasing its potential for accurate classification and prediction of CCMG samples.

The project also investigated the effects of temperature and moisture on CCMG samples. Three specific species of CCMG were subjected to different temperature and relative humidity conditions. The results revealed that accurate predictions could be achieved under specific temperature and relative humidity settings. However, it was observed that incorrect predictions occurred when the conditions varied. Further investigations were suggested.

Furthermore, the discrimination of impurities or contaminants in CCMG were studied. Testing samples of CCMG were mixed with an excipient (Dextrin) or toxic CCMG (Radix Aconiti Lateralis Radix Praeparata) at various concentrations. The presence of impurities or contaminants at concentrations ranging from 40 – 95%, 60 – 95% and 80 – 95% could be predicted correctly.

In conclusion, our research team has developed a new authentication method for CCMG using FTIR-ATR spectroscopy in conjunction with our self-developed tailor-made LIBSVM classification model. This authentication method has been successfully granted a patent in Hong Kong. By combining accuracy with efficiency, simplicity, and cost-effectiveness, this authentication method offers a reliable, fast, easy, and economical choice for CCMG authentication.

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

The sample preparation procedures for CCMG have been optimized and standardized, ensuring consistent and reliable results. Additionally, we have developed an analytical machine learning system that plays a crucial role in creating a comprehensive FTIR

fingerprint spectra library for CCMG. This library enables the straightforward identification of unknown CCMG samples by comparing their spectra with the reference spectrum.

The application of FTIR technique in pharmaceutical industry, hospitals, and clinics holds immense potential. It offers a reliable, rapid, easy and cost-effective method for authenticating CCMG and conducting on-site inspections. By utilizing this technique, we can enhance the safety of CCMG administration.

7. Layman's Summary

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

Our research team has achieved significant progress in the authentication of CCMG. We have developed a new method that combines FTIR-ATR spectroscopy and our tailor-made LIBSVM classification model. This novel authentication method has been granted a patent in Hong Kong. The method offers a reliable, fast, easy, and cost-effective solution for authenticating CCMG.

To ensure reliable results, we have optimized and standardized the sample preparation procedures for CCMG. In addition, we have created an analytical machine learning system that plays a crucial role in building a comprehensive library of CCMG fingerprint spectra using FTIR. This library enables us to easily identify unknown CCMG samples by comparing their spectra with the reference spectrum.

The application of FTIR technique in the pharmaceutical industry, hospitals, and clinics holds great promise. It provides a dependable, rapid, easy, and cost-effective approach for authenticating CCMG and performing on-site inspections. By utilizing this technique, the safety of CCMG administration can be enhanced. This is particularly important for protecting individuals from potentially harmful or counterfeit substances. Overall, our research contributes to improved authentication methods for CCMG and advances the use of FTIR in healthcare sector, benefitting both healthcare providers and patients.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

(Please attach a copy of the 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)** *(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 RGC** *(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)*						
	2023			S.H. Fung, E.S.W. Wong*, C.Y. O, S.M.N. Chan, E.T.P. Sze, W.F.F. Tang, C.H. Li, F.W.F. Lee.	Sample Optimization of Fast Authentication of concentrated Chinese medicine granules using FTIR-ATR with Chemometrics. *Lecture Notes in Networks and Systems*	**No**	**Yes [Attachment 1 & 2]**	**Yes**	**Yes**
		√		X. Qu, J. Xu, H. Li, K. Meehan, B. Chen, Z. Chen, L. Cai, H. Zhu, S.W. Cheng, J.Z. Zuo, J.Y.K Chan*, E.S.W. Wong*, L.L. Leung*	Aqueous extracts of Dendrobium Officinale have anti-tumor effects on oral squamous cell carcinoma. *Int. J. Mol. Sci.*	**No**	**Yes [Attachment 3]**	**Yes**	**Yes**
			√	X. Qu, J. Xu, H. Li, K. Meehan, B. Chen, Z. Chen, L. Cai, H. Zhu, S.W. Cheng, J.Z. Zuo, J.Y.K Chan*, E.S.W. Wong*, L.L. Leung*	Aqueous extracts of Dendrobium Nobile Lindl evoke anti-tumor effects in oral squamous cell carcinoma. *American Journal of Chinese Medicine*	**No**	**Yes [Attachment 4]**	**Yes**	**Yes**

			√	S.H. Fung, C.Y. O, S.M.N. Chan, F.W.F. Lee*, E.S.W. Wong*,	FTIR-ATR spectroscopy coupled with machine learning approaches for Rapid Authentication of Concentrated Chinese Medicine Granules. *Int. J. Mol. Sci.*	**No**	**No**	**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 conference abstract)

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 RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Aug / 2022 / Hong Kong, China	Rapid Authentication of Concentrated Chinese Medicine Granules (CCMG) by Fourier transform Infrared Spectroscopy Attenuated Total Reflection (FTIR-ATR) with Support Vector Machine (SVM)	International Conference on Environment and Human Health: Challenges and Opportunities in the 21st Century	No	Yes [Attachment 5]	Yes	No
Aug / 2023 / London, UK	Sample Optimization of Fast Authentication of concentrated Chinese medicine granules using FTIR-ATR with Chemometrics.	World Conference on Smart Trends in Systems, Security & Sustainability (7th International Conference, WorldS4 2023)	No	Yes [Attachment 6]	Yes	No

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

We have trained undergraduate student helpers to conduct the sample preparation experiments using FTIR-ATR, HPLC and TLC.

11. 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
[redacted]	MPhil	1 November 2020	28 February 2023 [Attachment 7]

12. Other Impact
(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

1. A patent was awarded with details as follows:
   單味中藥濃縮顆粒的認證方法與認證裝置; Short-term patent; Application number: 32023081152.5 [Attachment 8]
2. The results generated from this project were reported by Tai Kung PAO (大公報):
   都大研製光譜圖識別藥材 中藥顆粒難辨認 「親子認證」驗真身. *大公報*, 10 Dec 2023. [Attachment 9]

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
No. of outputs arising directly from this research project	4	2	0	1	Type	No.
					MPhil thesis	1
					Media interview	1

14. Public Access Of Completion Report
(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
N/A	N/A