

MASTER'S THESIS

Development and application of surfactant-mediated methods for the extraction and analysis of biological and herbal materials

Choi, Pik Kwan

Date of Award:
2002

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**Development and Application of
Surfactant-mediated Methods for the
Extraction and Analysis of Biological
and Herbal Materials**

CHOI Pik Kwan

**A thesis submitted in partial fulfillment of the requirements
for the degree of
Master of Philosophy**

Principal Supervisor: Dr. HUIE Wah Kit, Carmen

Hong Kong Baptist University

October 2002

Abstract

In the first part of this thesis, the feasibility of employing aqueous non-ionic surfactant solutions as an alternative solvent system in accelerated solvent extraction (ASE) is demonstrated for the first time using the roots of American ginseng as model solid samples. When compared to the use of pure water or methanol, the presence of a common non-ionic surfactant (Triton X-100) in water at a concentration above its critical micelle concentration was shown to enhance the amount of pharmacologically active ingredients (ginsenosides) extracted from ginseng roots. The advantages of using aqueous non-surfactant solutions were also demonstrated by comparing extraction performances between ultrasonic-assisted extraction and ASE methods. Furthermore, the combination of ASE and cloud point extraction was shown to be a new and effective approach for the rapid sample preconcentration of herbal materials prior to analysis by high-performance liquid chromatography.

In the second part of this thesis, the cloud point phase separation phenomenon of a non-ionic surfactant, Triton X-100, was used for the extraction and preconcentration of several biological important molecules, i.e., porphyrin carboxylic acids and zinc protoporphyrin, in urine. The effectiveness of cloud point extraction was shown by investigating the effects of different extraction conditions on the extraction of various

urinary porphyrins. It was found that porphyrins could be successfully extracted under acidic medium, with the addition of ammonium sulfate for the induction of cloud point phase separation/preconcentration.

Furthermore, the addition of an anionic surfactant, SDS, as an additive in HPLC mobile phases was shown to be useful in minimizing sample preparation steps, such as protein removal, so that direct injection of the physiological samples in the chromatographic system is possible, and highly reproducible chromatographic peaks can be obtained. The cloud point extraction method with preconcentration using Triton X-100 followed by HPLC with a mobile phase containing SDS was developed for the fluorimetric determination of porphyrins in urine with detectability at the ng/L level.

Table of Contents

	Pages
Declaration	i
Abstract	ii
Acknowledgements	iv
Table of Contents	v
List of Tables	viii
List of Figures	ix
List of Diagrams	xii
List of Abbreviations	xiii
Chapter 1 Introduction	
1.1 Aims of the project	1
1.2 Classification of surfactants	8
1.3 Characteristics and properties of surfactants	10
1.3.1 The aggregation of surfactant molecules	11
1.3.2 Factors influencing the aggregation, CMC and solubilization power of surfactants	12
1.3.3 Cloud point property of nonionic surfactants	17
1.4 Applications of surfactants	20
1.5 Analytical applications of cloud point extraction	22

Chapter 2	Accelerated solvent extraction of active ingredients (ginsenosides) from medicinal plants using aqueous non-ionic surfactant solutions	
2.1	Introduction	27
2.1.1	Working principle of ASE	27
2.1.2	Review on the applications of ASE	32
2.2	Experimental section	34
2.2.1	Chemicals	34
2.2.2	Preparation of ginseng samples	35
2.2.3	Accelerated solvent extraction	35
2.2.4	Ultrasonic-assisted extraction	35
2.2.5	Cloud point extraction and preconcentration	36
2.2.6	Analysis of the ginseng extracts by HPLC and UV absorbance detection	37
2.3	Results and discussion	38
2.3.1	Comparison of different solvent systems for the extraction of ginsenosides	38
2.3.2	Effects of pressure and temperature using methanol and aqueous non-ionic surfactant solutions as the solvent system	44
2.3.3	Comparison of UAE and ASE methods for the extraction of ginsenosides	50
2.3.4	Cloud point preconcentration prior to HPLC analysis of ginsenosides	55
2.4	Concluding remarks	58

Chapter 3	Cloud point extraction / preconcentration and HPLC analysis of zinc protoporphyrin and porphyrin carboxylic acids in urine	
3.1	Introduction	60
3.2	Experimental section	64
3.2.1	Chemicals	64
3.2.2	Cloud point extraction and preconcentration procedures	65
3.2.3	Analysis of cloud point extract of porphyrins by HPLC and fluorescence detection	66
3.3	Results and discussion	67
3.3.1	The thermal stability of porphyrins	67
3.3.2	Effect of pH	69
3.3.3	Effect of the concentration of ammonium sulfate	71
3.3.4	Effect of Triton X-100 concentration	72
3.3.5	Effectiveness of CPE for the preconcentration of porphyrins	74
3.3.6	Working concentration range of CPE of porphyrins	82
3.4	Concluding remarks	84
Chapter 4	References	86
	Curriculum Vitae	94