



DEVELOPMENT OF MICROSPHERES  
CONTAINING *CASSIA ALATA* EXTRACT

BY

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degree of Master in Pharmaceutical Science  
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## ABSTRACT

*Senna alata* or *Cassia alata* (*C. alata*) is a plant belonging to the family Leguminosae and has been documented to have antifungal and antimicrobial activities. This research aims to extract *C. alata* using known extraction procedure, and to find optimum condition for microencapsulation by employing double emulsion solvent evaporation method. Biodegradable poly (D, L-lactide-co-glycolide) (PLGA) was the polymer of interest to encapsulate the *C. alata* extracts owing to its ability to give a controlled-release profile. Resultant microspheres were characterized for size distribution and external morphology by laser diffraction technique and scanning electron microscopy, respectively. Encapsulation efficiency (EE) was also calculated and the *C. alata* was quantified by UV absorbance. Several parameters have been investigated to optimize the characteristic of fabricated microspheres during the preparation process including different homogenization times for the primary emulsion, different volume ratios of aqueous/oil phases, several types and concentrations of surfactants, co-solvent in aqueous or oil phase and buffer systems (at varying pH and different concentrations of PBS). It was found that, most of the parameters employed resulted in low EE (<11%), however the encapsulation efficiency (64%) was significantly improved when the hardening tank is buffered to pH 7, with minimal effect on particle size. The particle size range obtained was between 6-30  $\mu\text{m}$ . Subsequent in vitro analysis displayed usual release pattern attributed to PLGA that is initial burst release followed by a gradual release phase up to the period of study. Although our *C. alata* extract did not show antimicrobial and antifungal activities against various strains tested but it appeared active against *Escherichia coli*. It is hereby suggested that our *C. alata* microspheres may be useful as preservative and antidote related to food-poisoning commonly caused by *Escherichia coli*.

## خلاصة البحث

كاسيا الاتا او سينا الاتا هي نبتة تنتمي الى عائلة ليغوميناسيا و قد وثقت بأن لها خصائص مضاده للفطور و البكتريا. هذا البحث يهدف الى استخلاص هذه النبتة بطريقة محده وتحديد افضل الشروط الممكنة لاجراء عملية الكبسلة على المستخلص الناتج من عملية الاستخلاص و ذلك بطريقة تبخير المحل. كان البوليمير المستخدم في عملية تحضير المايكروسفير و ذلك كونه يتمتع بخصائص التحرر المديد. **PLGA** المايكروسفيرات الناتجة كانت تصنف حسب الحجم و الشكل السطح و ذلك عن طريق استخدام جهازي مقياس الذرات و المجهر الالكتروني على الترتيب. كمية المستخلص المكبسل كانت تحسب عن طريق مقياس الطيف. عدو متغيرات تم دراستها لتطوير المايكروسفيرات خلال عملية التحضير و منها مدة التحريك للمستحلب الاول, احجام مختلفة من الطور المائي و الزيتي, انواع مختلفة و تركيزات متنوعه من العامل الفعال على السطح, محلات مساعدة في الطور المائي او الزيتي, نظام البفر والذي يشمل بدوره قيم مختلفة لدرجة الحموضة و تراكيز مختلفة. لقد وجدنا انه معظم المتغيرات التي اجرى على عملية التحضير قد اعطت نسب كبسلة قليلة اقل من 11% بينما بوجود البفر بدرجة حموضة 7 ارتفعت نسبة الكبسلة لتصل الى 46%. قياس المايكروسفيرات تراوح بين 6-30 مايكرومتر. عملية التححرر كانت عبارة عن تحرر مبدئي سريع تلاه تحرر بطيء. مع ان مستخلص الكاسيا الاتا لم يبدي خصائص ضد بعض الفطور و البكتريا الا انه كان فعالا ضد الايشيريشيا كولي. ولهذا اقترح ان المستخلص يمكن ان يكون له خصائص حافظه للطعام و مضاده للتسممات الغذائية.

## APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Science (Pharmaceutical Technology).

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## DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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**DEVELOPMENT OF MICROENCAPSULATION OF *CASSIA  
ALATA* EXTRACT INTO BIODEGRADABLE PLGA**

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## TABLE OF CONTENTS

Abstract.....	iii
Abstract in Arabic.....	iv
Approval Page.....	v
Declaration Page.....	vi
Copyright Page.....	vii
Acknowledgements.....	xiii
List of Tables.....	xvi
List of Figures.....	xix
List of Abbreviations.....	xxi

<b>CHAPTER 1: INTRODUCTION .....</b>	<b>1</b>
1.1. Background of Study.....	1
1.1.1. Microencapsulation.....	1
1.1.1.1. Microencapsulation techniques.....	3
1.1.1.2. Microencapsulation by solvent evaporation method.....	4
1.1.1.3. Application of herbal extracts.....	6
1.1.2. Factor affecting encapsulation efficiency.....	7
1.1.2.1 Solubility of PLGA in the organic solvent.....	8
1.1.2.2 Solubility of organic solvent in water.....	9
1.1.2.3 Ratio of dispersed phase to continuous phase (DP:CP ratio).....	10
1.1.3. Polyester microsphere.....	10
1.1.3.1. Physicochemical properties of PLGA.....	11
1.1.3.2. Controlled release profile of microspheres.....	14
1.1.4 Cassia alata.....	17
1.1.4.1. Traditional uses.....	18
1.1.4.2. Antifungal activities of Cassia alata.....	19
1.1.5. Dermatophytes infections.....	20
1.1.5.1. Clinical Features.....	22
1.1.5.2. Treatment approach.....	24
1.2. Objective Of Study.....	26
1.2.1. Problem statement.....	26
1.2.2. Objectives of study.....	27

<b>CHAPTER 2: EXTRACTION OF CASSIA ALATA, FABRICATION AND CHARACTERIZATION OF CASSIA ALATA-LOADED PLGA MICROSPHERES.....</b>	<b>28</b>
2.1. Introduction.....	28
2.2. Materials.....	30
2.3. Method.....	30
2.3.1. <i>Cassia alata</i> extraction.....	30
2.3.2. UV wavelength scan for <i>C. alata</i> aqueous extract.....	30



2.3.3. Standard curve .....	31
2.3.4. Preparation of surfactants.....	31
2.3.5. Fabrication of microsphere.....	32
2.3.5.1. Study on surfactants types and concentrations .....	33
2.3.5.2. Study on homogenisation times of primary emulsion.....	34
2.3.5.3. AQ1-to-oil (AQ1/oil) volume ratios studies.....	35
2.4. Characterization Of Microspheres.....	36
2.4.1. Particle size distribution.....	36
2.4.2. Encapsulation efficiency .....	36
2.4.3. Surface morphology.....	37
2.4.4. Statistical analysis.....	37
2.5. Results And Discussion .....	38
2.5.1. Standard curve of <i>C. alata</i> .....	38
2.5.2. Effect of non-ionic surfactants and homogenisation times on the particle size. ....	38
2.5.3. Effects of the non-ionic surfactants and homogenisation times on encapsulation efficiency (EE). ....	43
2.5.4. Effects of ionic surfactants on particle size and encapsulation efficiency .....	45
2.5.5. Effect of different AQ1 to oil volume ratios on particle size (PS) and encapsulation efficiency (EE).....	46
2.5.6. Surface morphology.....	49
2.6. Conclusion .....	50

**CHAPTER 3: INVESTIGATION OF THE EFFECT OF CO-SOLVENT ON THE SIZE AND ENCAPSULATION EFFICIENCY OF CASSIA ALATA-LOADED PLGA MICROSPHERES..... 52**

3.1. Introduction.....	52
3.2. Material.....	55
3.3. Method.....	55
3.3.1. Fabrication of microspheres .....	55
3.3.1.1. Fabrication of microspheres by adding co-solvent (ethyl acetate) to the oil phase.....	56
3.3.1.2. Fabrication of microspheres with addition of co-solvents into the AQ1 phase. ....	57
3.3.2. Microspheres characterization.....	58
3.3.2.1. Particle size analysis .....	58
3.3.2.2. Encapsulation efficiency .....	58
3.3.2.3. Statistical analysis .....	58
3.4. Results And Discussion .....	58
3.5. Conclusion .....	62

**CHAPTER 4: EFFECTS OF INCORPORATING BUFFER SYSTEM DURING THE SYNTHESIS OF MICROSPHERES ..... 63**

4.1. Introduction.....	63
4.2. Materials .....	65
4.3. Methods .....	65

4.3.1. Preparation of PBS in 1% w/v PVA .....	65
4.3.2. Fabrication of microspheres .....	66
4.3.2.1. Study of different pH of PBS buffer .....	67
4.3.2.2. Effects of different PBS concentration at different stages of microsphere synthesis.....	67
4.3.2.3. Tween 20 concentration study using PBS buffer.....	68
4.3.2.4. Evaluation of addition of 0.066 M PBS buffer to AQ1 and/or AQ2.....	69
4.3.2.5 Effect of employing 0.06 M PBS at different surfactant types and Comparison study between presence and absence of PBS in the fabrication process .....	70
4.4. Characterisation of microspheres .....	70
4.4.1. Particle size distribution.....	70
4.4.2. Encapsulation efficiency .....	70
4.4.3. Surface morphology.....	70
4.4.4. Statistical analysis.....	70
4.5. Results and discussion .....	71
4.5.1. Evaluation of different pH of PBS buffer on PS and EE of microspheres .....	71
4.5.2. The effect of concentration of PBS buffer added in AQ2 and HT on encapsulation efficiency and particle size.....	74
4.5.3. The effect of different concentration of Tween 20 with the presence of PBS in HT or in HT and AQ2, on particle size and encapsulation efficiency. ....	77
4.5.4. Evaluation of addition of 0.066 M PBS buffer to AQ1 and/or AQ2 .....	80
4.5.5. Comparison between five formulations in regard to the presence and absence of PBS buffer using different types of surfactants on C. alata content.....	82
4.5.6. Surface morphology.....	86
4.6. Conclusion .....	88

**CHAPTER 5: IN-VITRO RELEASE AND ANTIMICROBIAL STUDY ..... 89**

5.1. Introduction.....	89
5.2. Materials .....	91
5.3. Method.....	91
5.3.1. Fabrication of microspheres.....	91
5.3.2. In vitro release study .....	92
5.3.3. Antifungal assay .....	93
5.3.3.1. Sabouraud dextrose agar preparation.....	93
5.3.3.2. Sabouraud dextrose broth preparation.....	93
5.3.3.3. Preparation of fungal Culture .....	93
5.3.4. Antimicrobial assay .....	94
5.3.4.1. Agar Preparation .....	94
5.3.4.2. Broth Preparation .....	94
5.3.4.2. Extraction Of C. alata Extract From Microspheres.....	95
5.3.5. Disc Diffusion Method.....	95
5.3.6. Microdilution Method (MIC) .....	96

5.4. Results and discussion .....	96
5.4.1. In vitro-release study.....	96
5.4.2. Antifungal and antimicrobial study .....	98
5.5. Conclusion .....	101
<b>CHAPTER 6: GENERAL DISCUSSION.....</b>	<b>103</b>
<b>CHAPTER 7 .....</b>	<b>109</b>
<b>BIBLIOGRAPHY.....</b>	<b>109</b>
<b>APPENDEX.....</b>	<b>120</b>

## LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
1.1	Different techniques used for microencapsulation (Ghosh, 2006).	3
1.2	Microencapsulation processes and their applicability.	4
1.3	Clinical presentation of dermatophytosis.	24
2.1	Formula for the <i>C. alata</i> -loaded microspheres for the study on surfactants (example shown here is for AQ1/oil volume ratio of 1:10). Asterisk indicates varying quantity of <i>C. alata</i> depending on ratio of oil to AQ1 phase.	34
2.2	Formula for the <i>C. alata</i> -loaded microspheres for different volume ratios AQ1/oil study.	35
2.3	The effect of surfactants and homogenisation times for the primary emulsion on the particle size of the microspheres. Data were analysed by multiple comparison Tukey post-hoc test.	40
2.4	Effect of surfactant and homogenisation time for primary emulsion on encapsulation efficiency.	44
2.5	The effect of surfactant concentration on particle size and encapsulation efficiency. Independent t-test was performed for 1% versus 5% concentration of each surfactant.	46
2.6	Characteristics of the microspheres prepared with different AQ1/oil volume ratio using 1% w/v PVA as surfactant. Data were analysed with Tukey test.	47
3.1	Properties of commonly used organic solvents, including the boiling point temperature ( $T_{bp}$ ), hydrogen bonding capability and water solubility (Hongkee, 1997; Morello, et al., 2007).	54
3.2	General formula for the effect of co-solvent in oil phase study of <i>C. alata</i> -loaded microspheres.	56
3.3	General formula for the effect of adding the co-solvents in AQ1 phase of <i>C. alata</i> -loaded PLGA microspheres.	57

3.4	Characteristics of microspheres using different co-solvent in the DCM phase. Dunnett's test was used to analyze data comparing against control.	59
3.5	Characteristics of microspheres using different organic solvents in the AQ1 phase. Data was analyzed using Dunnett's test comparing against control.	61
4.1	General recipe for PBS preparation.	66
4.2	General formula for the <i>C. alata</i> -loaded-PLGA microspheres at different pH of PBS study.	67
4.3	General formula for the <i>C. alata</i> -loaded PLGA microspheres at different stages of buffering with PBS.	68
4.4	General formula for the <i>C. alata</i> -loaded PLGA microspheres synthesized in a buffered environment at different Tween 20 concentrations.	69
4.5	Effect of pH for PBS on encapsulation efficiency of <i>C. alata</i> -loaded PLGA. Data presented as the mean $\pm$ standard deviation (S.D) of triplicate samples.	72
4.6	The effect of varying the pH in PBS buffer based on the particle size. Data is expressed as the mean $\pm$ standard deviation (S.D) of triplicates.	74
4.7	The effect of PBS concentration on particle size and encapsulation efficiency of microspheres. Data presented as the mean $\pm$ standard deviation (S.D) of triplicate samples.	77
4.8	Particle size for Tween 20 concentration study. Data presented as the mean $\pm$ standard deviation (S.D) of triplicate samples.	79
4.9	The effect of addition of 0.066 M of PBS buffer AQ1 and/or AQ2 on particle size and encapsulation efficiency. Data presented as the mean $\pm$ standard deviation (S.D) of triplicate samples.	81
4.10	Comparison of particle size between five types of surfactant according to the presence or absence of PBS buffer. Data presented as the mean $\pm$ standard deviation (S.D) of triplicate samples.	84
5.1	General formula for the <i>C. alata</i> -loaded microspheres for different types of surfactants with the presence of PBS buffer.	92
5.2	Antifungal and antimicrobial of <i>C. alata</i> leaf extract.	99

6.1	Top five pathogens contributing to domestically acquired foodborne illnesses resulting in hospitalization.	107
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## LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
1.1	A schematic diagram of different structural orientation of microparticles: those with well-defined shell/wall structure are preferentially called as microcapsules (simple & multi-wall), whereas those with undefined shell/wall structure are more accurately called as microspheres (multi-core & matrix).	2
1.2	Scheme of the microencapsulation by solvent evaporation method.	5
1.3	Factor affecting encapsulation efficiency.	8
1.4	Shows the chemical structure of Poly (D, L-lactide-co-glycolide).	13
1.5	Controlled-release system by diffusion mechanism.	16
1.6	Leaves and flowers of <i>Cassia alata</i> growing in the state of Pahang in Malaysia (March, 2011). (Photo by Mulham Alfatama).	17
1.7	Dermatophytes species.	21
2.1	Wavelength scan of <i>C. alata</i> aqueous extract.	31
2.2	Schematic diagram of fabrication of PLGA <i>C. alata</i> microspheres using double emulsion solvent evaporation method.	33
2.3	Standard curve for <i>C. alata</i> aqueous extract.	38
2.4	SEM micrographs of microencapsulated <i>C. alata</i> .	50
4.1	Effect of different concentrations of PBS on encapsulation efficiency of <i>C. alata</i> microspheres.	75
4.2	The Effect of Tween 20 concentration on encapsulation efficiency of <i>C. alata</i> -loaded PLGA microspheres prepared by adding iphosphate buffer to HT only or to HT and AQ2	80
4.3	Comparison of encapsulation efficiency regarding to the presence and absence of PBS buffer using different types of surfactants.	83
4.4	SEM micrographs of microencapsulated buffered <i>C. alata</i> with different types of surfactants: (A) 3%Span20; (B) 3% Tween20; (C) 3% SDS; (D) 3% CTAB.	85

4.5	SEM micrographs of microencapsulated <i>C. alata</i> at different pH values of: pH 2 (A&B); pH 7 (C&D); pH 8 (E&F); pH 12 (G&H).	87
5.1	In vitro release profile of <i>C. Alata</i> from the microspheres containing different types of surfactants.	97
5.2	(A) The zone of inhibition of naked <i>C. alata</i> aqueous extract against <i>Escherichia coli</i> (23.5mm±0.5), (B) The positive control chloramphenicol marked with (CMC), as well as the PBS marked with (PBS) and of water marked with (water).	100
5.3	The zone of inhibition of <i>C. alata</i> microspheres against <i>Escherichia coli</i> (8.75 mm ±0.35).	101



## LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
AQ	Aqueous
CTAB	Cetyl trimethylammonium bromide
CFU	Colony Forming Unit
E.coli	Escherichia coli
DCM	Dichloromethane
EE	Encapsulation efficiency
HT	Hardening tank
HLB	Hydrophile-Lipophile Balance
MIC	Minimum Inhibition Concentration
PS	Particle size
PBS	Phosphate Buffer Saline
PGA	polyglycolic acid
PLA	Poly (D, L-lactic) acid
PVA	Poly vinyl alcohol
PLGA	Poly (D, L-lactide-co-glycolide) acid
S.D	Standard Deviation
SDS	Sodium dodecyl sulfate
SEM	Scanning Electron Microscopy
US FDA	United State Food Drug Administration

# CHAPTER 1

## INTRODUCTION

### 1.1 BACKGROUND OF STUDY

#### 1.1.1 Microencapsulation

The term microencapsulation refers to the technologies for enveloping micron-sized particles of solids or droplets of liquids or gasses in an inert shell; the latter is basically a natural or synthetic polymer (Ghosh, 2006; Rodrigues and Grosso, 2008). The yield obtained by microencapsulation is labelled as microparticles, microcapsules or microspheres, which are different in terms of their internal structure and morphology. One of the earliest microspheres that reached the market has been used only in agriculture and cosmetic industry. However, the current trend permits it to be used as a drug delivery system for humans. One example of products that has been marketed since 1989 is Lupron Depot®, which is a delivery system for luteinizing hormone releasing, encapsulated in microspheres of biodegradable poly (DL-lactide-co-glycolide) copolymer. By using different techniques of microencapsulation, a wide range of diameters can be achieved for the particles. When the diameter of a particle is below 1  $\mu\text{m}$ , it is known as nanoparticles, nanocapsules or nanospheres. On the other hand, particles with diameters between 3–800  $\mu\text{m}$  are known as either microparticles, microcapsules or microspheres. The particles that have diameters larger than 1000  $\mu\text{m}$  are known as macroparticles (Jyothi, Prasanna et al., 2010). The encapsulated material is often called as core, internal phase or fills, whereas the encapsulating matrix is frequently called as shell, coating, wall material or membrane. The wide range of materials, like active pharmaceutical ingredients,

proteins, peptides, volatile oils, food materials, pigments, dyes, monomers, catalysts, and pesticides, can be encapsulated with different types of materials, namely ethylcellulose, hydroxyl propylmethyl cellulose, sodium carboxy methyl cellulose, sodium alginate, PLGA, gelatine, polyesters, and chitosans (Yates, Birnbaum et al., 2000). The active agent may form a core within a shell of the polymeric materials, but, usually, the active agent is dispersed into the polymeric matrix. The particles may be spherical or may have an irregular shapes (which the term ‘microparticles’ is the most suitable).

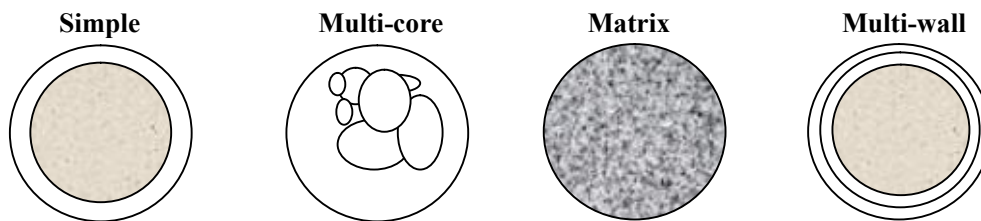


Figure 1.1: A schematic diagram of different structural orientation of microparticles: those with well-defined shell/wall structure are preferentially called as microcapsules (simple & multi-wall), whereas those with undefined shell/wall structure are more accurately called as microspheres (multi-core & matrix).

There are various reasons to microencapsulation in relation to the agents that are encapsulated. Microencapsulation can be used to control the release of the encapsulated materials (Rama, Senapati et al., 2005), reduce drug dosage and the frequency of dosage forms (Lecaroz, Gamazo et al., 2006), protect active ingredients (Yeh, Chen et al., 2007), reduce nutritional loss, mask or preserve flavours, as well as deliver drugs to specific locations and make handling encapsulated material easier (Y. Özalp, 2001). Carbonless paper was the earliest commercial application of microencapsulation (Martins, Rodrigues et al., 2009). In the food industry,

microencapsulation is extensively used to encapsulate oils, enzymes, flavours, and fat. One of the reasons is to protect the encapsulated ingredients from environmental conditions, such as light, moisture, and oxygen, in order to obtain higher durability and lower volatility (Bae and Lee, 2008). In pharmaceutical industry, microencapsulation protects drugs from the environment, stabilizes sensitive drug substances, eliminates incompatibilities, masks unpleasant taste, as well as controls and sustains the release of drugs (Melnick, Jameson et al., 1987).

### ***1.1.1.1 Microencapsulation techniques***

The primary aims of microencapsulation are to stabilize a drug and to prolong its therapeutic effect by enclosing it into a polymeric matrix (Conti, Panico et al., 1997). The microspheres are prepared by various techniques, which allow different characteristics of the final product to be obtained (Freitas, Merkle et al., 2005). Most of the techniques applied for microencapsulation are basically modifications of the three basic methods: phase separation (coacervation), spray-drying, and solvent extraction/evaporation (Hai and et al., 2009). Many techniques are applicable for the encapsulation of core materials. These methods can be divided into three types, as listed in Table 1.1.

Table 1.1  
Different techniques used for microencapsulation (Ghosh, 2006)

Chemical processes	Physico-chemical processes	Physic-mechanical process
Interfacial polymerization	Coacervation and phase separation	Spray drying and congealing
In situ polymerization	Sol-gel encapsulation	Fluid bed coating
Poly condensation	Supercritical CO <sub>2</sub> assisted microencapsulation	Solvent evaporation

Many pharmaceuticals can be microencapsulated using the above-mentioned techniques (Table 2). Examples of these techniques include fluidized bed or air suspension method, coacervation and phase separation, spray drying and spray-congealing, as well as pan coating and solvent evaporation, which are the methods that are commonly used. The variation of the microencapsulation techniques are related to the nature of the core material.

Table 1. 2  
Microencapsulation processes and their applicability

Microencapsulation process	Nature of the core material	Approximate particle size ( $\mu\text{m}$ )
Solvent evaporation	Solids and liquids	5-5000
Coacervation and phase separation	Solids and liquids	2-5000
Multi-orifice centrifugation pan coating	Solids and liquids	1-555
Spray drying and congealing	Solids	600-5000
Air suspension	Solids	5-5000

#### ***1.1.1.2 Microencapsulation by solvent evaporation method***

As the name suggests, solvent evaporation method employs volatile solvent that is evaporated at the end of a microencapsulation process. Amongst the most commonly used microencapsulation techniques by solvent-evaporation is the double emulsion or W/O/W method, especially for encapsulating water-soluble drugs (Ogawa, Yamamoto et al., 1988; Alonso, Gupta et al., 1994; Boury, Marchais et al., 1997; Leach and Mathiowitz, 1998; Pistel and Kissel, 2000; Yeo, Baek et al., 2001; Sun, Jeong et al., 2003). This technique is accomplished by emulsifying an aqueous solution of a drug into an organic solution of a polymer (e.g. PLGA). The primary water-in-oil emulsion

is, then, dispersed in a second aqueous phase to acquire a double water-in-oil-in-water (w/o/w) emulsion; hence, evaporation of the organic solvent occurs with the generation of solid microspheres. This technique is applied in the present work for the preparation of polylactide-co-glycolide (PLGA) microspheres that are loaded with *C. alata*.

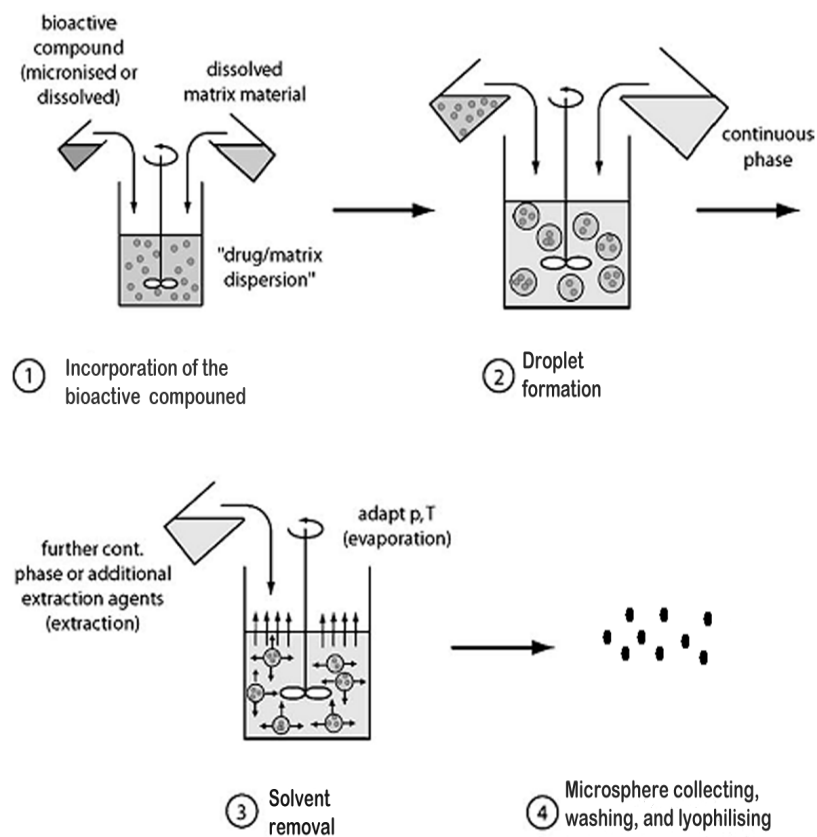


Figure 1.2: Scheme of the microencapsulation by solvent evaporation method.

The most common problem encountered with the microencapsulation of hydrophilic substances applying aqueous emulsion techniques is the drug loss into the continuous phase during the hardening process of the microspheres, such as water-in-

oil-in-water (WOW) or solid-in-oil-in-water (SOW) techniques (Alex and Bodmeier, 1990; Pistel and Kissel, 2000; Weidenauer, Bodmer et al., 2003). However, in order to overcome this kind of problems, many adequate studies have been done, including the inclusion of surfactant and an antisolvent, such as dimethyl sulfoxide, (Bao et al., 2006) and the use of supercritical CO<sub>2</sub> subscript, with the '2' as the solvent (Bao, Zhou et al., 2006). The unique importance of solvent evaporation method is its ability of handling the properties of the produced microspheres, such as the encapsulation efficiency and the particle size.

#### ***1.1.1.3 Application of herbal extracts***

Even though from delivery systems, such as micelles, liposomes, nanoemulsions, and biopolymeric nanoparticles, numerous applications in the pharmaceutical sector are found (Pertuit, Moulari et al., 2007), and their uses as vehicles for the delivery of natural bioactive extracts is relatively new in the pharmaceutical industry (Obidike and Emeje, 2011). Nowadays, there is an increase in the attraction in natural antimicrobial compounds, and numerous studies on the antimicrobial activity of a wide range of natural compounds have been reported (Ayala-Zavala, Del-Toro-Sanchez et al., 2008; Carraminana, Rota et al., 2008). A huge number of herbal extracts, such as garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, laurel, ginger, rosemary, and peppermint, among others, are studied as antimicrobial natural products to fight both bacteria and fungus (Edris and Farrag, 2003; Arcila-Lozano, Loarca-Pina et al., 2004; Jugl-Chizzola, Ungerhofer et al., 2006; Ravi, Prakash et al., 2007; Senhaji, Faid et al., 2007; Zeller and Rychlik, 2007; Ayala-Zavala, Oms-Oliu et al., 2008; Ayala-Zavala, Soto-Valdez et al., 2008; Del Toro-Sánchez, Ayala-Zavala et al., 2010). Therefore, there is a need to protect these plant

extracts from environmental factors, namely oxygen, light, and moisture, which contribute to the deterioration of these plants. Microencapsulation is the applicable solution to tackle this problem. These encapsulated extracts are prepared by locking the natural core material into a special matrix, resulting in an improved shelf life and controlled release profile (Krishnan, Bhosale et al., 2005).

It was previously reported that herbal extracts are successfully encapsulated using different techniques of microencapsulation with varying types of polymers. Ayala-Zavala and his team microencapsulated cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in *b*-cyclodextrin for antimicrobial and antifungal activities (Ayala-Zavala, Soto-Valdez et al., 2008).

Therefore, the design of a *C. alata* drug delivery system may turn it into an antifungal drug, which serves as a topical treatment for skin infections. Furthermore, the development of a drug delivery system may improve the solubility and, as a result, contribute to the bioavailability and release of *C. alata* in a sustained manner over a longer period of time.

### **1.1.2 Factor affecting encapsulation efficiency**

Most attempts to prevent the drug loss into the continuous phase and hence low encapsulation efficiency were based on a common principle that fast polymer precipitation could increase the encapsulation efficiency (Bodmeier & McGinity, 1988). Fig. 1.3 depicts two set of variables influencing encapsulation efficiency.