

MICROENCAPSULATION OF *CASSIA ALATA*: FABRICATION AND CHARACTERIZATION

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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*, followed by microencapsulation by solvent-evaporation using biodegradable PLGA. The microsphere is envisaged to release the extract in a controlled manner allowing more convenient mode of administration when treating related skin infection. Several variables had been investigated including different types of surfactants and buffer systems and different homogenization times for primary emulsion. It was found that, most of the surfactants employed resulted in low encapsulation efficiency (<10%). However with 3% poly vinyl alcohol (PVA), slightly higher encapsulation efficiency of 11% was achieved, with size distribution of 7-13 μm . The encapsulation efficiencies were significantly improved (45-64.4%) when the hardening tank was buffered to pH 7, with minimal effect on particle size. Additionally, in-vitro controlled release formulation of *C. alata* microsphere was demonstrated for duration of almost a month.

Keywords: *Cassia alata*; PLGA; Microspheres; Solvent evaporation; Anti-infective.

INTRODUCTION

Microencapsulation by solvent evaporation method has been widely employed in pharmaceutical industries¹. Application of microencapsulated drug delivery systems for medical and pharmaceutical uses have also been widely explored and some successfully used clinically. The micro-carrier systems have clinically demonstrated its ability to improve bioavailability of conventional drugs by prolonging the release of drug from the dosage unit^{2,3}. Such controlled-release manner ensures continuous delivery of the active ingredient thus optimizing therapeutic outcome.

The polymeric matrixes employed in this study is the most commonly used biodegradable, biocompatible, poly-(D,L-lactide-co-glycolide) (PLGA) co-polymer⁴. Its non-toxic degradation products permits its application in drug delivery systems and due to their established safety profile, the FDA (Food and Drug Administration) has long approved its use in human⁵. The lactide/glycolide polymer chains which can be hydrolyzed into natural metabolites (lactic and glycolic acids) are removed from the body by the citric acid cycle. Such cycle is devoid of toxic accumulation in the body. Additionally, its extensive use in wide array of fields is credited to the various degradation rates from months to years it can provide, depending on its composition and molecular weight⁶.

C. alata L. (family: Leguminosae) is a shrub, used in traditional medicine for the treatment of various ailment⁷. Traditionally, it is used to treat superficial fungal infections apart from treating uterine disorders and snakebites⁸. It is also being used as anthelmintic, antibacterial, diuretic and laxative⁷⁻⁹. The water extract of the leaves had been found to be effective against *Staphylococcus aureus* at concentration comparable to standard antifungal agent, Tioconazole⁸. In our lab, we have found that the water extract of the leaves to be positive against *Aspergillus flavus* and *Trichophyton mentagrophytes* (data not published). The results suggest that this *C. alata* could be potentially used as anti-infective to treat various types of cutaneous infection including cutaneous aspergillosis and dermatophytosis. It is envisaged that by having a controlled-release delivery of the active agents, therapeutic outcome can be optimized due to enhancement in compliance towards disease management.

As such, here we aimed to microencapsulate *C. alata* using PLGA as the matrix in order to obtain a prolonged topical delivery of the extract onto the skin. This serves as an alternative to current treatment of cutaneous fungal infection. The controlled release of a drug has many clinical benefits: more convenience and acceptance for patients, reduction in dosing frequency and in certain aspects it can provide a mean of drug targeting to specific locations resulting in a higher efficiency of the therapy^{9,10}. Furthermore, since the plant

extract is a composition of many different compounds, of which, in many cases, the therapeutic utility of a plant extract cannot be attributed to a single compound, the interaction of the different compounds present usually makes a plant extract therapeutically beneficial².

Several variables have been investigated including different types of surfactants (Span 20 and Tween 20), different concentration of stabilizer (polyvinyl alcohol) (PVA) and different processing parameters as a function of particle size distribution, encapsulation efficiency and in-vitro release profiles.

MATERIALS

All chemicals used in this study were of analytical grade and was used as received without any further purification. Dichloromethane (DCM) was obtained from Fisher Scientific (United Kingdom), PVA (MW 115 kD) was purchased from BDH Laboratory Supplies (England) and PLGA (Purasorb PDLG 5004A) was purchased from PURAC (Holand). Tween 20 and Span 20 were purchased from MERCK (Germany). Phosphate-buffered saline (PBS) was supplied by Sigma-Aldrich.

Plant material and Extraction

Leaves used in the present investigation were purchased from Nilam Enterprise (Kuantan, Pahang, Malaysia). The botanical identification of the plant was done by the Institute of Bioscience, University Putra Malaysia. Voucher specimen is conserved at the Phytomedicinal Herbarium of the Institute of Bioscience under the accession number ACP 0047. The leaves were grounded after they were oven dried at 45 °C overnight. One kilogram of coarsely powdered dry leaves was successively extracted using distilled water as a solvent at 60 °C for 2 h. The resultant extract was then concentrated to dryness using freeze dryer (Christ Alpha 1-4LD). The stock solution containing 100% w/v of freeze-dried extract in distilled water was prepared and stored at 4 °C.

Fabrication of Microsphere

A modified multiple emulsion-solvent evaporation method employed elsewhere¹¹ was adopted. Briefly, 100 μl of *C. alata* stock solution was mixed with 12 μl of surfactant (or PVA) and vortexed to prepare the aqueous phase. This aqueous phase was injected into 5% w/v PLGA previously dissolved in DCM. This primary emulsion was homogenized at 14,500 rpm (IKA® T10 basic Ultra-Turrax) for 2 min and immediately injected into 12 ml of secondary continuous phase composed 1% w/v PVA only (for preparation without buffer) and with 0.06 M PBS (for preparation with buffer), homogenized at 14,500 rpm for 3 min. This multiple emulsion was then transferred

into a continuously stirred hardening tank constituted of 1% w/v PVA only (for preparation without buffer) and with 0.06 M PBS (for preparation with buffer). The stirring was continued for 2 hr to allow complete evaporation of DCM. The hardened microsphere was collected by centrifugation, washed 3 times with distilled water and lyophilized (Christ Alpha 1-4LD) for 24 h. Lyophilized microspheres were kept at 4°C in air-tight container with silica gel.

Characterisation of microspheres

Particle Size Analyzer BT-9300H (Bettersize Instrument Ltd. China) which is a laser diffractometry was used to determine the size distribution of the microspheres prior to lyophilisation, dispersing the microspheres in water until a laser obscuration around 10% was reached. The size distribution (PS) was expressed as volume median diameter (VMD) as adopted by other study¹¹. Data presented were the $d(v,50)$ which is equivalent volume diameters at 50% cumulative volume.

The encapsulation efficiency (EE) was calculated according to the method described elsewhere¹² with some modification. 5 mg of lyophilized microspheres was accurately weighed. Dichloromethane (3 ml) was added to solubilize the extract-containing microspheres, followed by addition of distilled water (3 ml) to partition out the *C. alata*. After rotating end to end for one hour, the mixture was centrifuged (4000 rpm) for 20 minutes and the upper layer was collected and subjected to UV absorbance measured at 264 nm. Background UV readings were obtained using supernatant from blank microspheres of corresponding surfactant. The concentration of encapsulated *C. alata* was derived from a standard curve of *C. alata* (correlation coefficient 0.999). The EE is the ratio of actual content of *C. alata* extracted from microspheres to theoretical content expressed as percentage.

Approximately 1-2 mg of lyophilized microspheres were mounted onto an aluminium stub and coated with gold. The samples were imaged on a Carl Zeiss Evo® 50 Scanning Electron Microscope (Germany), operating at 10kV, 20°C and 10⁻⁵ Torr.

In vitro release study

Approximately, 30-mg amount of dried microspheres was suspended in a 1.5 ml microcentrifuge tube containing 1 ml of PBS and 0.1% Tween 20. The tubes were left undisturbed in a controlled temperature of 37 °C incubator. At a pre-determined time (1h, 8h, and 24hrly for two weeks, followed by 72 hourly for subsequent two

weeks), the tubes were centrifuged for 3 min at 6000 rpm (PRO-6k, Eppendorf Centrifuge). The supernatant was then collected and its *C. alata* content was analyzed using UV spectrophotometer at 264 nm. The tubes were returned to the incubator after the dissolution media were replenished with equal volume of fresh buffer solution.

Statistical Analysis

All data were taken in triplicates and reported as mean ± standard deviation. Data were statistically analyzed either using independent t-test (with 95% confidence interval) or one way analysis of variance with Tukey's HSD post-hoc test using SPSS (ver. 18) software.

RESULTS

Effect of different types of surfactants and surfactant concentrations on *C. alata*- loaded PLGA microsphere.

Two types of surfactants (Tween 20 and Span 20) and a stabilizer, PVA were selected to be investigated on the basis of they are commonly employed in other fabrication of microspheres by solvent-evaporation. The concentrations of surfactants (Tween 20 and Span 20) were varied from 1-5% and corresponding particle size and encapsulation efficiency were compared against the "control". PVA fabricated microspheres were regarded as control due to 1% PVA usually yield the best characteristics of microspheres notably for particle size and encapsulation efficiency. Based on Table 1, it can be clearly seen that the EE were extremely low (less than 6%) in all formulation. Notwithstanding that PVA-prepared microspheres have always been the gold standard, in this case the results suggest otherwise. Particularly for Tween 20-prepared microspheres, at 3% concentration, it appeared to be the best surfactant in terms of yielding the highest EE that was statistically significant to that of PVA-prepared microspheres. Besides, it also yielded acceptable particle size distribution at similar concentration. In contrast, for Span 20-prepared microspheres, the EE obtained for all concentrations did not appear to be statistically significant to the control. However, the particle size of Span 20-prepared microspheres displayed statistically significant different to that of PVA-prepared microspheres. The relationship observed between the particle size and concentration of Span 20 was that of inverse relation. Similarly, regardless of types of surfactants, the particle size distribution appeared to decrease with increase in concentration of surfactant.

Table 1: Effect of different types of surfactants and surfactant concentrations on the particle size (PS) and encapsulation efficiency (EE) of the *C. alata* loaded PLGA microsphere. Data represented as mean ± SD.

Formulation	Surfactant (% v/v)	PS (µm) ± SD	EE (%) ± SD
1	1% PVA	8.89 ±0.01	2.9 ±0.29
2	1% Span 20	10.6 ±0.17 ^a	3.54 ±0.74
3	3% Span 20	7.43 ±0.01 ^a	2.4 ±0.38
4	5% Span 20	6.71 ±0.01 ^a	2.61 ±0.31
5	1% Tween 20	12.36 ±2.06 ^a	1.63 ±0.06 ^a
6	3% Tween 20	8.69 ±0.01	5.49 ±0.55 ^a
7	5% Tween 20	8.69 ±0.03	2.88 ±0.24

^astatistically significant (p<0.05) compared to the "control" (Formulation No.1).

For external morphology, we had selected only 3% concentration of surfactants (Span 20 & Tween 20) and 3% PVA [Fig. 1 (A), (B) & (D)] to be published here as representatives of *C. alata*-loaded PLGA microspheres fabricated with other surfactant concentrations. In addition, we also enclosed here the external morphology of a 'blank' (unloaded *C. alata*) prepared with 3% PVA [Fig. 1 (D)]. As clearly depicted (Fig.1), the microspheres produced were perfectly spherical implying substantial stability of the PLGA-constituted oil droplets (as precursor of the microspheres) conferred by PVA and Tween 20. The presence of *C. alata* appeared to favour formation of microspheres by supporting spherical integrity of microspheres as depicted in Fig 1 (A) as compared to Fig. 1 (C). The smooth surface morphology was also consistent to that of *C. alata*-loaded, Tween 20-prepared microspheres. In contrast, Span 20-prepared microspheres

yielded highly porous particles with some population showing spherical identity. The results of microimages may well demonstrate that surfactant (or stabiliser) that yielded spherical shaped particle without visible porosity normally had comparatively high EE. This is particularly exemplified by *C. alata*-loaded, Span 20-prepared microspheres [Fig. 1 (B)] that had lower EE compared to Tween 20-prepared microspheres [Fig.1(D)] at same concentration.

Effect of different homogenization times for primary emulsion (HTPE) on *C. alata*-loaded PLGA microspheres

To determine the influence of homogenization time on encapsulation efficiency and particle size distribution, 5% Span 20 was selected for it gave the smallest particle size distribution (Table

1). Microspheres (Sample A, B, & C) were prepared by varying the homogenization time (1, 2 & 3 min) of the primary emulsion (Table 2), of which the surfactant was incorporated in this stage. As shown in Table 2, the homogenization time did not significantly affect encapsulation efficiency ($p > 0.05$) of *C. alata* although slight

increase in EE was observed for both Sample B and C. Similarly, there is no clear relationship between homogenization times with size of particles. However, prolonging homogenization time for more than 1 min appeared to improve EE due to probably larger particle size generated.

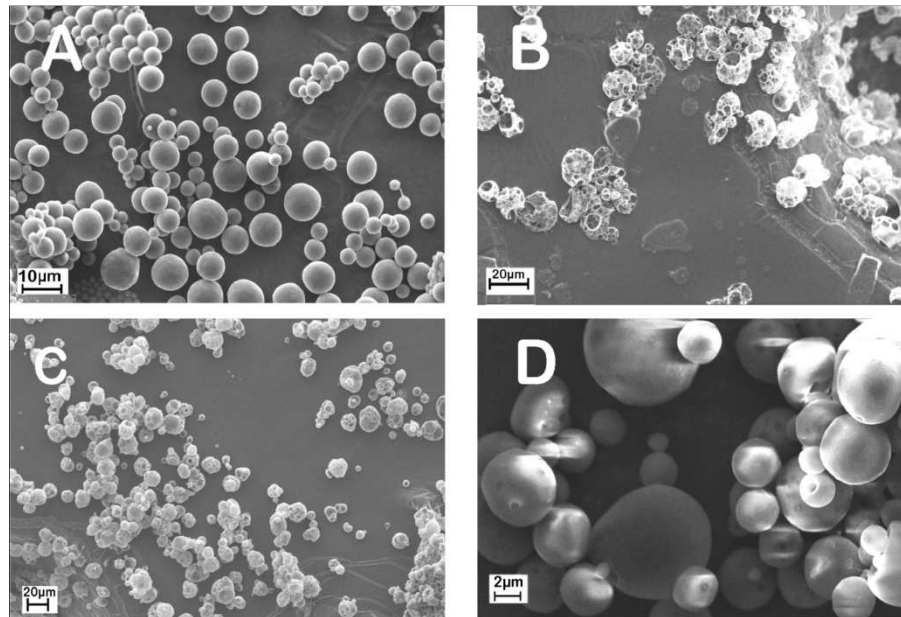


Fig. 1: SEM micrographs of microencapsulated *C. alata* with different types of surfactants. (A) 3%PVA; (B) 3% Span; (C)3% PVA blank; (D) 3% Tween 20.

Table 2: Effect of different homogenization times for primary emulsion on the PS and EE of the *C. alata* using 5% Span 20 as surfactant. Data represented as mean ± SD.

Sample	Homogenization Time (min)	PS (μm)	EE (%)
A	1	6.71 ±0.18	2.61 ±0.31
B	2	8.02 ±0.02 ^a	3.34 ±0.51
C	3	7.61 ±0.01 ^a	2.96±0.63

^astatistically not significant ($p > 0.05$) compared to the control (sample A).

Effect of 0.06 M phosphate- buffered saline on *C. alata* loaded PLGA

High encapsulation efficiency of an encapsulated agent of a microsphere delivery system is one of the most important criteria in producing high quality microspheres; thereby ability to control this is crucial. Based on the previous results (Table 1), the encapsulation efficiency (EE) for all surfactants was extremely poor. Therefore, this part of works was done to improve the EE. One effective way of optimizing EE for water-soluble encapsulated agent is to use a suitable buffer system in the continuous phase. Similar approach was applied here whereby 0.06 M phosphate buffer with 1% w/v

PVA were employed as the secondary aqueous phase as well as the hardening phase. Previously, we had tested several concentration of phosphate buffer with varying pH. It was found that pH 7 gave the highest EE at 0.06 M concentration.

Table 3 displays the particle size and EE of five formulations of microspheres prepared using five types of surfactants (ionic and non-ionic), with and without the buffer. It is clearly shows that, regardless of types of surfactants, the EE were statistically significantly ($p < 0.001$) improved when phosphate buffer was employed as the external and hardening phases.

Table 3: Different characteristics of particles with and without the inclusion of PBS. Data represented as mean ± SD.

Formulation	Surfactant	PS (μm)	EE (%)	PS (μm)	EE (%)
		Without PBS		With PBS	
1	3% PVA	16.97 ±0.06	10.12 ±0.59	12.82 ±0.01 ^c	60.91 ±2.00 ^d
2	3% Span 20	9.09 ±0.13	3.41 ±1.54	13.98 ±1.08 ^c	48.34 ±1.77 ^d
3	3% Tween 20	13.92 ±0.06	5.88 ±0.2	13.67 ±0.79	52.22 ±3.56 ^d
4	3% SDS ^a	10.89 ±0.008	4.84 ±0.22	12.71 ±0.005 ^c	45.10±2.79 ^d
5	3% CTAB ^b	11.55 ±0.09	6.14 ±0.22	20.46 ±0.08 ^c	64.40±1.86 ^d

^a sodium dodecyl sulphate

^b cetyl trimethyl ammonium bromide

^cstatistically significant ($p < 0.05$) when comparing PS without and with buffer.

^d statistically extremely significant ($p < 0.001$) when comparing EE without and with buffer.

On the other hand, the use of buffer significantly ($p < 0.05$) increased the particle size distribution of the *C. alata*-loaded microspheres for some ionic surfactants (SDS & CTAB) and for relatively hydrophobic surfactant (Span 20). Particle size remain non-statistically significant different for relatively hydrophilic surfactant, Tween 20-prepared microspheres in the presence or absence of buffer. In contrast, particle size distribution was significantly ($p < 0.05$) decreased for PVA-prepared microspheres to comparatively similar value when the Tween 20 was employed as surfactant in the primary emulsion.

In vitro release profile

Figure 2 shows the effect of different types of surfactants on the release behaviour of *C. alata* from the microspheres. We assumed that the release of *C. alata* was 100% within 29 days. As anticipated,

the *in vitro* release profile showed that more than 50% of encapsulated *C. alata* was released within 48 h. The release continued to be at slower rate for the next subsequent hours until it reached plateau level (100% release) after almost a month (696 h). These release profile are typically reported to occur from microparticles fabricated with biodegradable PLGA. However, slight differences can be detected at the duration of 'burst-release' which dependent on the co-polymer intrinsic viscosity (IV). In this case, the PLGA IV was 0.4 dL/g which is expected to show a faster and yet shorter duration of the 'burst-release' than a higher IV of PLGA (e.g IV 1.0 dL/g). Overall, the release rate fabricated from all types of surfactants tested here was not significantly different to each other, with Span 20-prepared microsphere appeared to release *C. alata* at the slowest rate amongst others.

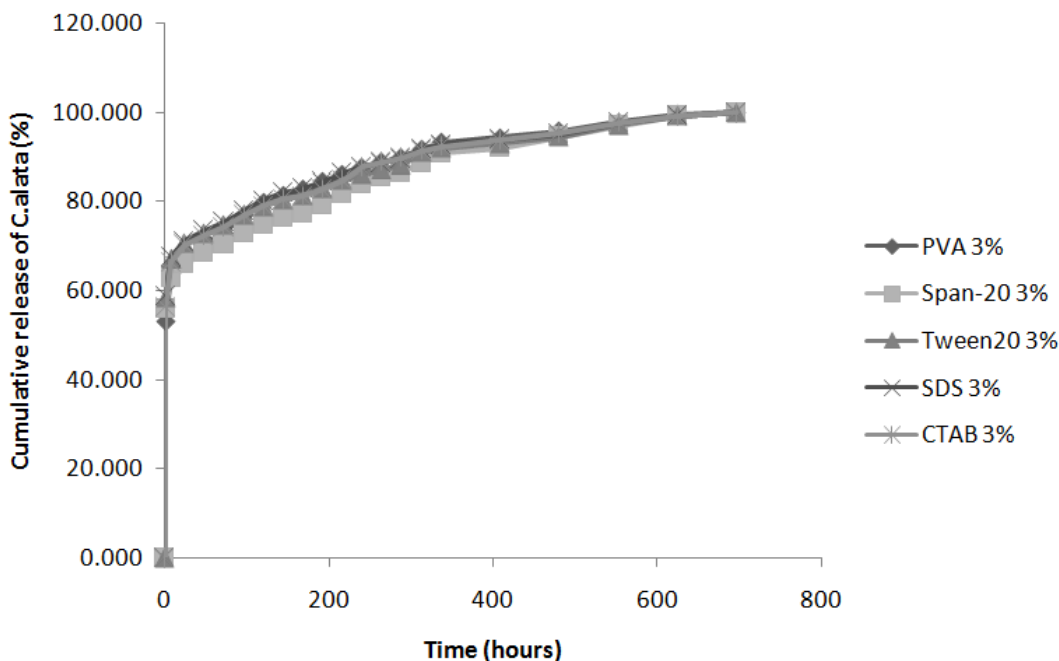


Fig. 2: In vitro release profile of *C.alata* from the microspheres prepared with different types of surfactants.

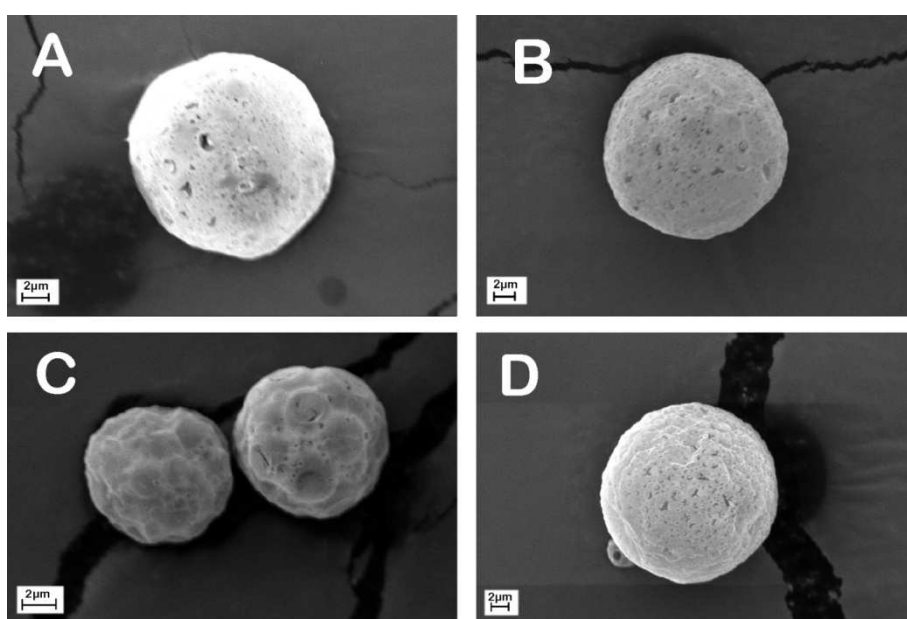


Fig. 3: SEM micrographs of microencapsulated *C. alata* prepared with inclusion of PBS in the secondary phase and hardening tank. Different types of surfactants were included in the primary emulsions: (A) 3%Span20; (B) 3% Tween20; (C) 3% SDS; (D) 3% CTAB.

DISCUSSION

C. alata-loaded PLGA microsphere had been successfully prepared by solvent- evaporation, that among the microencapsulation techniques, the multiple emulsion water-in-oil-in-water (w/o/w) method is one of the most frequently employed methods for encapsulating water soluble agents^{13,14}. Despite the fact that the method is conceptually simple, many factors have been shown to affect the final properties of the resultant PLGA microsphere^{15,16}. DCM was chosen as an organic solvent due to its abundant uses in polymeric microencapsulation¹⁷.

Apart from SEM data, the determination of the encapsulation efficiency as a function of surfactants confirms the significant influence of the surfactant (or emulsifier) on the microspheres. The resultant primary emulsions are complex systems consisting of reverse micelles with entrapped solubilised *C. alata* in the polar cores, surrounded by DCM-solvated random coiled PLGA chains. With thermal and mechanical agitation due to homogenization, the w/o interface massively increases, contributing to entropy gain of the system, and the requirement to decrease the surface tension in order to maintain emulsions stability¹⁸. Addition of non ionic surfactants (Span 20 & Tween 20) or emulsifier (PVA) were expected to reduce the interfacial tension of which the extent were reflected by morphology of microspheres. In the absence of sodium chloride molecules, none of the surfactants (or emulsifier) seems to sufficiently prevent the escape of *C. alata* from the nascent microsphere. The transient secondary emulsion, when being homogenized shall facilitate the incorporation of *C. alata* into the internal droplet of nascent microspheres, however appeared unable to do so without the presence of phosphate and sodium chloride molecules. As the drugs or encapsulated agents had been demonstrated to mainly diffuse out and loss towards the droplets surface during the first minutes after the second emulsification step¹³ therefore, the addition of a buffered salt at this phase is crucial.

It appears that all surfactants (and emulsifier) employed yielded statistically significant difference size distribution in the presence of buffered-salt. The hydrophobic surfactant Span 20 (Sorbitan monolaurate, HLB 4.3) is found to produce smaller particle size microspheres compared to hydrophilic surfactant Tween 20 (Polysorbate 20, HLB 14.9), which is in good agreement with previous data¹⁹. Span 20 which has lower HLB is oil soluble and generally produces a stable primary emulsion when the dispersion medium is oil (in this case, DCM). This may explain a smaller particle size generated when Span 20 was employed as the surfactant in the primary emulsion. On the other hand, the lower HLB value of Tween 20 may have facilitated collision of small emulsified droplets to form larger particles. With respect to concentration of surfactants, our findings are in parallel to other study²⁰ that also observed inverse relationship between concentration and particle size distribution of the microspheres, regardless of surfactant types. The effect was essentially due to a better stabilization of the thermodynamically unstable internal droplets with the increase of surfactant concentration, which also increased the viscosity of the system, preventing coalescence and facilitating the sizing of the primary emulsion²¹. In summation, it leads to size reduction of the nascent microspheres.

In addition, the influence of homogenization time of the primary emulsion on the encapsulation efficiency and size distribution of the microsphere was studied without buffering the 1% w/v PVA phases of secondary continuous phase and hardening phase. The only surfactant being studied was 5% w/v Span 20 and it was found that, under all conditions, a low encapsulation efficiency of *C. alata* was obtained. While prolonging the homogenization time appeared to have no significant effect on the encapsulation efficiency, except a slightly decreased EE in Sample C as compared to Sample B, but the particle size seem to be affected with unclear relationship between the speed and size. The decrease in encapsulation efficiency with homogenization time can be explained in terms of the effect of homogenization time on particle size. In fact, a prolonged homogenization time reduced the mean diameter of the microspheres simply because of a sustained homogenization

duration leads to an increase in the droplet energy causing droplet breakdown, which in turn increases the shear stress resulting in decreased size of particle. Our results are in good agreement with those obtained by Song et al. (2008), who studied the influence of many preparation variables on the mean diameter of PLGA and found that the mean diameter decreases with increasing the homogenization time. However, a further increase in homogenization time lead to a maximum size of microspheres, reflected by the plateau level of the particle size vs. mean diameter plot²². This study however, did not explore the probable effect that homogenization time could have on particle size and EE with the presence of phosphate and sodium chloride molecules.

As previously reported, the encapsulation efficiency of the drug into microspheres was increased with addition of sodium chloride to the external aqueous PVA solution^{23,24}. The presence of such additives was reported to be advantageous for preparing a stable water-in-oil-water (w/o/w) emulsion²⁵ of which similar approach was applied here. Surprisingly, the addition of 0.06 M PBS appeared essential to efficiently incorporate *C. alata* extract into PLGA microspheres. Uchida et al. (1996) reported that the addition of sodium chloride into the external aqueous phase in the preparation process significantly improved the loading efficiency of brilliant blue as a water-soluble model dye²⁶. The addition of phosphate buffer increased *C. alata* encapsulation efficiency by as much as 16-fold from the initial microspheres fabricated in the absence of buffer. The improvement perhaps was mainly achieved by the increase in partition coefficient of *C. alata* into DCM phase as suggested earlier¹⁵. In addition, the increase in osmotic pressure of the external phase was suggested to act as a barrier for *C. alata* to be expelled from the primary emulsion water-in-oil to the external aqueous phase during the secondary emulsification²⁷. Besides, the phenomena also explain the formation of less porous surface morphology for microspheres exclusively prepared with the additional presence of buffer, most obviously observed for Span 20-prepared microspheres [Fig. 1 (B) & Fig. 3 (A)]. In the absence of buffer, Span 20 (HLB 8), which favours stabilising the water-in-oil emulsion appeared to produce unstable emulsion droplets that lead to the formation of highly visible pores of fused and aggregated microspheres¹³. This atypical behaviour of hydrophobic surfactant may be explained due to the highly volatile DCM solvent used for the oil phase, as compared to less volatile hydrocarbon oil, the latter being the prime oil being investigated for the construction of HLB scale²⁸.

In addition to desorption of encapsulated agents (in this case is *C. alata*) from the surface of microspheres that partially caused the burst release, several other factors have been progressively discussed. Surface porosity has naturally been suggested as an apparent determinant to the drug release behaviour and some studies have demonstrated this experimentally^{29,30} and by computer simulation³¹. As most of fabricated microspheres here, especially when fabricated in the presence of buffered saline, yielded porous surface, the high release rate observed within 48 h may well be due to a combination effect between surface porosity and presence of *C. alata* at the surface of microspheres. Relatively hydrophilic surfactant employed in the primary emulsion had been suggested to accelerate the burst release¹⁹, which is parallel to our findings; however this was contrary to findings by other study³². The latter demonstrated that Tween 20 could reduce burst effect without prior control on primary emulsion stability. Subsequent sustained and slower rate of *C. alata* release was observed following the burst-effect. At this stage, the release of *C. alata* had been suggested to be due to diffusion through the channels or pores that were formed in the polymer coat prior to bioerosion of the polymeric matrix^{33,34}. As the release ensued thereafter seems to approach a steady-state, and is afforded by polymer bulk erosion, it is conceivable that this is likely to follow the release kinetics of Higuchi, analogous to description of drug release from a homogenous polymeric matrix. It is interesting to note that the release rate of *C. alata* was higher in the ionic surfactants-fabricated microspheres compared to non-ionic surfactant. The faster the release of the CTAB and SDS-fabricated microsphere may be caused by the presence of more visible surface porosity that allows easier hydration of the polymeric coat and the permeation out of *C. alata*¹³.

In summary, the release profile obtained here demonstrated a favourable delivery of anti-infective agent that usually required a sufficiently high enough concentration (provided by the burst-effect) to effectively kills the microbes. Subsequent sustained release depicted by *C. alata* microspheres may provide the so called 'steady-state' concentration of drug and envisaged to completely kill the microbes causing the skin diseases.

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