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Preparation and characterization of PLGA particles for subcutaneous controlled drug release by membrane emulsification

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Abstract

Uniformly sized microparticles of poly(DL, lactic-co-glycolic) (PLGA) acid, with controllable median diameters within the size range 40 to 140 microns, were successfully prepared by membrane emulsification of an oil phase injected into an aqueous phase, followed by solvent removal. Initially, simple particles were produced as an oil-in-water emulsion, where dichloromethane (DCM) and PLGA were the oil phase and water with stabiliser was the continuous phase. The oil was injected into the aqueous phase through an array type microporous membrane, which has very regular pores equally spaced apart, and two different pore sizes were used: 20 and 40 microns in diameter. Shear was provided at the membrane surface, causing the drops to detach, by a simple paddle stirrer rotating above the membrane. Further tests involved the production of a primary water-in-oil emulsion, using a mechanical homogeniser, which was then subsequently injected into a water phase through the microporous membrane to form a water-in-oil-in-water emulsion. These tests used a water soluble model drug (blue dextran) and encapsulation efficiencies of up to 100% were obtained for concentrations of 15% PLGA dissolved in the DCM and injected through a 40 micron membrane.

Solidification of the PLGA particles followed by removal of the DCM through the surrounding aqueous continuous phase. Different PLGA concentrations, particle size and osmotic pressures were considered in order to find their effect on encapsulation efficiency. Osmotic pressure was varied by changing the salt concentration in the external aqueous phase whilst maintaining a constant internal aqueous phase salt concentration. Osmotic pressure was found to be a significant factor on the resulting particle structure, for the tests conducted at lower PLGA concentrations (10 and 5% PLGA). The PLGA concentration and particle size distribution influence the time to complete the solidification stage and a slow solidification, formed by stirring gently overnight, provided the most monosized particles and highest encapsulation efficiency.

Keywords

PLGA

Membrane emulsification

Microparticle

Osmotic pressure

Encapsulation efficiency

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Introduction

Controlled release drug encapsulation is one of the leading research fields in the pharmaceutical industry [1]. For treatments that require repeated administration, via ingestion or injection, and for compounds such as proteins with very short half life, the possibility of a single administration followed by a slow and controllable release is an improvement on the usual forms of drug delivery. A vast range of biopolymers have been considered, and the use of different biopolymer combination has been considered, [2–12]. Once the biopolymer enters the body, the environmental conditions cause it to degrade in a predictable manner into monomers that are already present in the body, hence the biocompatibility. This degradation can be controlled through the polymer composition and the characteristics of the administration, to gradually release the encapsulated drugs.

This study considers poly(D,L-lactic-co-glycolic) acid (PLGA), a biopolymer approved by the Food and Drug Administration for human use [13]. By changing the lactic/glycolic acid ratio of the PLGA molecules it is possible to control the degradation rate. PLGA has been used to prepare tablets to be ingested, scaffolds, nanoparticles for inhalers or intravenous injections and microparticles for subcutaneous depot [4–7,9,12,14–17]. The work reported here produced particles in the range of 40 to 140 μm via membrane emulsification followed by the solvent removal method to produce the PLGA microspheres.

The first step is to produce an emulsion where PLGA is dissolved in an organic solvent and dispersed as droplets in a aqueous continuous phase. Then the organic solvent is removed by evaporation and the PLGA solidifies to form particles containing the drug in its matrix. Hydrophobic drugs are easily dissolved together with the PLGA in the organic solvent, while hydrophilic drugs would be poorly encapsulated with this method. For hydrophilic drugs a double emulsion is required: firstly the drug is dissolved in an aqueous phase and dispersed in PLGA and organic solvent. Subsequently, the organic phase is dispersed again into a second water phase giving a Water-Oil-Water (W/O/W) dispersion. With this method both hydrophobic and hydrophilic drugs can be successfully encapsulated [4,5,9,18,19].

There are many membrane emulsification techniques available, but most are appropriate for the production of fine drops, less than 20 microns, and the intention of the project was to produce particles significantly bigger than this. Recently, a membrane technique operating in the required size range has been detailed [20,21]. The Micropore Technologies Ltd Dispersion Cell provides the ability to tailor the droplet size and size distribution, by changing operating conditions and the chemical properties of the phases. The ability to make dispersions of a known, and controlled, size distribution is important for controlled release drug encapsulated particles as knowing the exact size of the particles facilitates modelling the drug release and controls aspects such as the initial burst. The latter effect could lead to problematic side effects. The Dispersion Cell technique minimises the shear, and other operating conditions, experienced by the drugs, which may be perishable. This reduces losses in encapsulation efficiency that may occur when not operating under such mild operating conditions. This work presents operating parameters involved in the stages of emulsion production and particle solidification. Droplet and particle size, size distributions and the encapsulation efficiency of a water soluble model drug are reported.

Materials and Methods

The method selected to produce the PLGA particles is membrane emulsification followed by solvent evaporation. Both simple PLGA particles and an encapsulated water-soluble model drug were investigated. Initially, the simple particles were created to test the conditions needed to produce particles in the desired size range. It is unlikely that these conditions would change significantly in the case of dissolving a hydrophobic drug in the PLGA oil phase. For a hydrophilic drug encapsulation is required, in which the water soluble drug is encapsulated by the oil phase, which is then dispersed in to water to form a W/O/W emulsion. The latter process is significantly more technically demanding than forming a O/W emulsion, as it is important that the primary emulsion does not break during the process of secondary emulsion formation. In both sets of tests the chemicals used were: Resomer RG 503H (d,l-lactide glycolide ratio 50/50) obtained from Boehringer Ingelheim, Poly Vinyl Alcohol (PVA MW 25000, 88% degree of hydrolysis) and sodium chloride came from Fisher Scientific, Di-ChloroMethane (DCM) from Acros and blue dextran 2000 from Pharmacia Fine Chemicals. Reverse osmosis water was obtained from a Millipore RO unit. The membrane emulsification

apparatus, a stirred Dispersion Cell, was provided by Micropore Technologies Ltd., Leicestershire, UK. In the Dispersion Cell the discontinuous phase is injected at the base of the cell, where it passes through the membrane, and the droplets emerge into the continuous phase. The continuous phase is agitated by a simple two-bladed paddle controlled by a DC motor. The membrane is a thin flat metal disc with monosized circular pores distributed in a highly regular array and is chemically treated in order to make the surface hydrophilic, see Figure 1. Extensive validation of the Dispersion Cell as a means to provide reproducible drop sizes was performed in the work described previously [20,21], using the system of sunflower oil injected in to water, where multiple sets of experiments were performed for each reaction condition tested. A similar set of experiments was not performed here, due to the cost of the materials.

1. Simple Particle Production

Table 1 summarises the composition and the operating parameters used in the following experiments. When simple particles are produced, 10 ml of DCM with different concentrations of PLGA (5, 10, 15, 20 and 30%) were injected at a rate of 0.5 ml/min into 150 ml of water containing 1% w/v PVA using a 40 μ m pore diameter membrane. The continuous phase was agitated at a stirrer speed of 600 rpm and the cell was immersed in a cold bath at 4°C. Once the injection phase was finished, the droplets were solidified by different methods. The solidification is a result of the solvent (DCM) leaving the system. It is possible to influence this stage by controlling the diffusivity of the solvent out of the drops as they solidify to form particles. This control can be achieved by: temperature, surface area of the liquid free surface from which the DCM evaporates into the atmosphere and DCM concentration in the water phase. After producing the emulsion in the stirred cell it was poured into a beaker and stirred constantly at 120 rpm at room temperature for DCM removal. Four different solidification methods were tested changing evaporation areas and adding continuous phase to change DCM aqueous phase concentration.

The four different methods investigated, and different solidification times, are shown in Table 2. The solidification is the result of the DCM evaporation through the water phase and into the outside environment, so changing the DCM solubility in water controls the solidification rate.

In a fast solidification the emulsion (160 ml) was poured into a beaker with a free surface of 150 cm² together with one litre of reverse osmosis water containing 1% PVA to act as a stabiliser and keep the droplets-particles apart. The system was continuously stirred and the complete solidification process took approximately two hours. In a gradual (Grad) solidification 450 ml of new continuous phase was gradually added to the stirred emulsion at a rate of 7.5 ml/min, for one hour, followed by stirring for approximately 24 hours. In a slow solidification process no other phase was added, the emulsion was stirred in a beaker with a free surface of 50 cm² for approximately 24 hours. In a very slow solidification process the top of the beaker was sealed with only the entrance for the overhead stirrer open, the solidification process took three days.

2. Encapsulated Particle Production

Firstly, 20 ml of reverse osmosis water, 1000 ppm of blue dextran 2000 and 40 g/l salt were emulsified with 50 ml of DCM and different concentrations of PLGA (5, 10 and 15%) using a mechanical homogenizer (Silverson Machines Ltd.), for three minutes at 8600 rpm. These operating conditions were established by observation using a microscope: at 8600 rpm there was a noticeable difference between the distribution given by homogenising for 60 and 90 seconds, but no further difference was observable beyond this time. Hence, a total time of 180 seconds was used to ensure uniformity of the primary emulsion between the different tests. The primary emulsion, formed in this way, was completely stable showing no signs of coalescence and did not require a surfactant for stabilisation. This primary emulsion became the discontinuous, or injected, phase for the secondary emulsification. For the second emulsification, 10 ml of the discontinuous phase was injected in the dispersion cell into 150 ml of reverse osmosis water containing 1% PVA and different salt concentrations (40, 33, 26, 16 g/l). The injection rate was 0.5 ml/min, the stirrer agitation speed was either 600 or 860 rpm and membrane pore diameters of 40 and 20 µm respectively were used. The emulsification and the solidification stages were conducted at room temperature. A small amount of salt and water saturated in DCM was required to slow down DCM diffusion into the water during the emulsification. Different solidification methods were tested for the simple particles and from

those results, the solidification method chosen for encapsulated particles was an overnight stir at 120 rpm.

The optimal concentration of PVA chosen for both types of experiments was 1%, determined from the literature, in order to provide the best particle size distribution, [22], and encapsulation efficiency, [23]. Blue dextran 2000 was selected as the water-soluble model drug because it can be easily measured spectrophotometrically and it has already been used as a marker when producing smaller PLGA microparticles [23]. Preparation temperature effects were thoroughly studied. It is reported that the emulsification temperature affects solvent removal rate, and therefore influences the surface morphology and the size of the final product, but not the encapsulation efficiency [24,25].

Particle Size and Encapsulation Efficiency Analyses

Three pictures were taken of the newly formed emulsion using sampling and analysis under an optical microscope. The emulsion was not stable enough to be analyzed using an instrumental technique, such as laser light diffraction. Up to 600 droplets were size analyzed using an image analysis system running Image J software. Once the solidified particles were obtained, their size and size distribution were measured using a Malvern Mastersizer. A comparison of Image J and Malvern data showed that the two methods provide very similar results.

To determine the amount of blue dextran 2000 released, or not encapsulated, during the secondary emulsification process a sample from the outer water phase was filtered using Whatman filter paper number 3 and analyzed by an ultraviolet spectrophotometer at 620 nm (UV-1201 Shimadzu).

Results and Discussion

Since the method chosen is membrane emulsification followed by solvent removal, oil droplet and solidified particle size are closely linked. It is important to control the emulsification stage, and the solidification stage to achieve and maintain the required size and size distribution.

Other operating characteristics influence the surface morphology and hence the encapsulation efficiency.

The Micropore Dispersion Cell has already been tested using other systems: sunflower oil in water [20], paraffin wax in water and water in kerosene [21]. The current challenge was to test the control parameters (membrane pore size, shear at the membrane surface, chemical properties) on the PLGA system and to preserve the size and size distribution properties during the solidification stages, whilst maintaining a good encapsulation efficiency.

Simple Particle Production - Droplet Formation

The effect of agitation speed on the droplet size was tested by simple particle production, i.e. O/W emulsions. Figure 2 presents a similar trend to those already reported, the droplet size decreases as the agitation speed (or shear) increases. This is due to a higher drag force at the membrane surface, which directly depends on the rotation speed [20]. In the previous work with the stirred cell it was shown that the particle size uniformity of the drops formed is a function of the viscosity of the dispersed phase used. In the system studied here dispersed phase viscosity was changed by a variation in the PLGA content within the DCM, between 5 and 30% PLGA dissolved in DCM was used. The results are shown in Figure 3, the volume distribution improves with an increase in the PLGA concentration, as the width of the distribution curves can be seen to become narrower with increasing PLGA concentration and the peak height increases, hence with a higher discontinuous phase viscosity.

Simple Particle Production - Solidification Stage

After forming the drops, the next step was to maintain them during the solidification. During this stage, the solvent leaves the system, so particle shrinkage is to be expected. The droplets are very unstable and during this stage they may collide and coalesce, or be broken by the shear. The amount of PLGA used for these tests was 5%, in 95% DCM. As shown in Figure 4, all the final solidified particles are smaller than the original droplets and they all shrank by the same amount. The “slow” procedure produced the best particle size distribution, actually improving it from the 5% PLGA droplet size distribution.

It appeared that when the solidification time is too long, the droplets display a very long unstable period and their final shape is no longer spherical but elongated by the mild stirring, Figure 5a. All the other particles are spherical and with an orange peel surface effect, see Figure 5b for an example image. The solidification methods chosen did not appear to influence the outer surface morphology, as also obtained in [26]. In both Figure 5a and 5b there is a scale bar in the bottom right hand corner of the image, illustrating a distance of 100 microns. One of the project aims was to produce micro particles with a diameter ranging from 50 up to 100 μm and this appears to be successfully achieved for the simple PLGA particles.

Encapsulated Particle Production

In this case the aim is to encapsulate an internal aqueous phase within the PLGA oil phase in a W/O/W double emulsion. The primary emulsion is obtained by homogenization. Very small drops are required in order for them to spread evenly in the PLGA matrix. Using the homogenizer for three minutes produced a sufficiently small droplet size that the emulsion was stable during the secondary emulsification without the need for any surfactant. The higher the amount of PLGA, the more stable the primary emulsion became.

To obtain particles of 100 μm , a 40 μm pore diameter membrane was used, and a stirrer agitation speed of 600 rpm. To obtain smaller particles of 50-60 μm , a membrane with a pore diameter of 20 μm and a higher agitation speed of 860 rpm was used. One of the main properties of membrane emulsification is the possibility to link droplet size to pore size and operating conditions, see Figure 2 and [22]. The PLGA concentration also influenced the particle size. Contrary to reports in the literature [23,27], an increase in PLGA concentration caused a decrease in particle size. This is a consistently observable trend in Figure 6 and may be related to the porous nature of the resulting PLGA microsphere and the swelling it may experience.

One important aspect in the degree of uniformity of the encapsulated particles formed was the salt concentration used in the external aqueous phase during the secondary emulsification and how it relates to the salt concentration of the internal aqueous phase used in the primary emulsification. This influences the osmotic pressure across the oil phase, which acts as a

barrier between the two aqueous phases, [28]. The degree of uniformity of the size distribution can be measured by the 'span', which is defined as follows:

$$\text{span} = \frac{x_{90} - x_{10}}{x_{50}}$$

Where x_{90} , x_{50} and x_{10} are the particle sizes at which 90, 50 and 10% of the distribution fall below. Hence, the nearer the value of the span to 0 the more monosized the distribution is. In general, a distribution is considered to be monosized if its span value is less than unity.

Table 3 provides values of the span for the encapsulated particles using a variety of ratios of internal phase to external phase salt concentration. In the experiments the inner phase contained 40 g/l salt and outer/inner salt ratios used were between 1:1 (same concentration of salt in the inner and outer water phase) to a ratio of 1:3 (outer phase salt concentration of 13 g/l, inner phase salt concentration of 40 g/l). Figure 6 illustrates the variation in the median size, based on the number distribution of the particles, as a function of the ratio of the salt concentration outer:inner. There is no osmotic pressure when the salt concentration is equal between the phases. At lower ratios it is likely that water enters the drop, which then solidifies, so a larger diameter particle results from the process than given by a ratio of 1:1. This effect is apparent for all concentrations of PLGA used in the process and for the two membrane pore sizes tested.

Shrinkage, which occurs due to the removal of the solvent from the beads, and solidification of blank and encapsulated particles has been extensively studied, [29]. In the study reported here the intention is to consider the effect of osmotic pressure and PLGA concentration, whilst keeping constant the amount of inner water phase. PLGA concentration does not appear to affect the particle size or shrinkage, and this behaviour is characteristic of the encapsulated particles. For a given amount of inner water phase the same amount of shrinkage occurs despite the fact that the amount of PLGA is lower when a lower concentration is used. It is

likely that this has an influence on the structure of the PLGA matrix, which is likely to be an important factor in the drug release.

Figure 7 illustrates the shrinkage that occurs between the as-formed drops and the final solid particles. Shrinkage is due to the removal of the DCM solvent, leaving the PLGA matrix and, in the case of the encapsulated particles, the inner water phase. As reported in [28] when the difference of salt concentration is high between the inner and outer phases, the osmotic pressure acts in order to equalize them, so for higher salt concentration in the inner phase water from the outer phase enters the particles, which explains why the apparent shrinkage is reduced at the lower salt concentration in the outer phase.

Encapsulation Efficiency

The encapsulation efficiency for the different operating conditions is shown in Figure 8. An encapsulation efficiency of less than 100% may be due to two effects: disruption of the primary W/O emulsion which is being emulsified into a W/O/W emulsion, and by the leaching out of material from the inner aqueous phase after the encapsulated particles have been formed. Forming large encapsulated particles, particle diameters bigger than approximately 10 microns, is quite challenging as the larger drops tend to be more easily ruptured. Hence, it may be possible to create a W/O emulsion using a mechanical homogeniser, but the subsequent creation of the secondary emulsion (to form a W/O/W emulsion) is unlikely to have a high encapsulation efficiency if a mechanical homogeniser is again used. As the high shear will break the primary emulsion releasing the internal water phase. Thus, a gentle technique for the formation of the secondary emulsion is required, that will not give rise to rupture of the primary emulsion. Conventional membrane emulsification uses tortuous pore channel type membrane structures, which provide lower shear conditions than a mechanical homogeniser, but still provide significant opportunities for disruption of the primary emulsion which is being injected through these tortuous pore channels. The membrane used in this study, and illustrated in Figure 1, does not use a tortuous pore channel and provides a very short and gentle shear path for the primary emulsion to flow through to the surface of the membrane, where it is broken in a controlled manner by the shear imposed at the membrane

surface arising from the stirrer. Hence, very high encapsulation efficiencies can be provided, as illustrated in Figure 8.

The second effect, leaching of the inner water phase material, may partially be influenced by the water transport due to osmotic gradients. When osmotic transport is high it is possible that pathways for transfer between the two aqueous phases (internal and external) become available. When 15% PLGA is used, the encapsulation efficiency does not depend on the osmotic pressure and it is higher for larger particles, provided by the 40 micron pore size membrane. Generally, a higher PLGA concentration gives a higher entrapment efficiency, when all the other variables are constant. When a lower PLGA concentration is used, the different salt concentration becomes important and the encapsulation efficiency is lower when less salt is used in the outer phase. This effect is very noticeable with the smaller particles, formed using the 20 micron pore size membrane.

As described previously, [27], the particles are formed by progressive loss of the organic solvent, so an increase in the PLGA concentration of the oil phase leads to a shorter time needed for solidification. The dominating loss of inner phase is believed to be due to transport to the external phase, and if the unstable time is shorter, then the loss of the inner phase will be lower. Also, higher PLGA concentrations result in a higher viscosity of the oil phase, which restricts the transport of the inner phase material towards the outer phase [30]. The diffusion of the inner phase material is also influenced by the size of the particle [14]. These conditions help explain why smaller particles have a lower encapsulation efficiency. It is also notable that when the droplets are smaller the interfacial area between the emulsion droplets and the external water phase is larger, hence the drug contained in the inner water phase has more area over which to diffuse. Moreover, the smaller the size, the shorter is the distance for the drug to reach the drop/particle surface.

As shown for PLA and PLA/PLGA mixtures, when the osmotic pressure is high, water from the outer phase tends to enter the particles, leaving behind pores which provide exit routes for the inner phase once the particle has completely solidified [3,28]. At the same time, two other effects play an important role. Increasing salt concentration increases microsphere drug

loading by reducing drug aqueous solubility, and at the same time it may decrease microsphere drug loading by depressing organic solvent solubility in the aqueous phase [31]. However, in the case where all the organic solvent is removed the latter effect is one appropriate to the kinetics of the solidification process as, at equilibrium, all the solvent will be removed regardless of the solvent solubility. Other researchers on PLA have shown that the necessity to control the osmotic pressure is greater when membrane emulsification is used. It has been suggested that increasing encapsulation efficiency can be obtained by preparing the initial emulsion by using a membrane, rather than a homogeniser, and controlling the osmotic pressure [32]. A very comprehensive review of further developments in membrane based capsule and solid particle production, and factors influencing these appropriate to medical diagnostics and healthcare, has been published [33].

Conclusions

For subcutaneous drug delivery biocompatible particles with diameters in a size range between 20 to 100 microns are required. These are of sufficient size to contain a reasonable amount of active ingredient, but not too big as to cause discomfort in administration and use. The production of particles in this size, and without the existence of material much smaller and bigger than these sizes, is challenging and often classification of the produced material is required to remove the under- and over-sized material. A more effective method would be to generate the required size, with minimal off-size material. Furthermore, encapsulation efficiencies should be as high as possible, so that the active ingredient is going to the product rather than to waste from the process. Membrane emulsification is an effective method for producing drop sizes, and hence particle sizes after solidification, in a controlled way. The membrane type used in this study is ideal for the production of subcutaneous drug delivery particles as it is possible to produce particles in the size range required and with encapsulation efficiencies (for a water in oil in water system) shown to be as high as 100%.

The regular array of pores in the membranes studied had pore diameters of 20 and 40 microns, producing a range of encapsulated particles with median diameters between 60 and 140 microns. Membranes of this type are available with pore diameters down to 7 microns

and the resulting particle diameter is a significant function of pore diameter. In this study, and for the production of encapsulated PLGA particles, it was shown that for a salt ratio inner:outer water phases of 1:1 the 40 micron pore size gave a median diameter of 100 microns and under the same operating conditions the 20 micron membrane gave a size of 60 microns. The uniformity of these particles was very good, with calculated span values of 0.30, under conditions of 15% PLGA and a salt ratio of 1:1. The same operating conditions gave encapsulation efficiencies close to 100%.

During the solidification process the organic solvent transfers from the drops formed by the emulsification process to the external water phase and then to the water-air interface, where it then transfers to the air. As the organic solvent constitutes such a large amount of the organic phase, up to 95% by volume, the shrinkage of the drops as they solidify is substantial. Volume reductions of up to 75% are shown to be possible, for the particles using a 1:1 salt ratio between the inner and outer aqueous phases. As the shrinkage is less than the content of organic solvent it may be concluded that the PLGA encapsulated particles may have a structure swollen by water imbibition from the external phase. The minimum shrinkage between the drops formed and the solidified particles was 8% (volume reduction of 8% in Figure 7), despite the presence of 85% solvent which is removed during solidification. Hence the water imbibition would appear to be very significant with these particles. The conditions for minimum shrinkage existed when the salt ratio was such that there was a much higher salt concentration in the internal water phase in the W/O/W emulsion. Hence, there was a strong osmotic pressure driving the water from the external phase and in to the internal water phase. Despite this effect, the encapsulation efficiency was still approximately 100% for the 15% PLGA particles produced using the 40 micron pore size membrane.

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Table 1 Overview of emulsion compositions

1. PLGA particle production:		
Emulsion type:	Oil (PLGA & DCM) in water; i.e. O/W emulsion	
Discontinuous phase:	5, 10, 15, 20, 30% PLGA, remaining material was DCM	
Continuous phase:	1% PVA dissolved in reverse osmosis water	
Emulsification condition:	agitation speed:	600 rpm stirrer speed
	injection rate:	0.5 ml/min
	membrane pore size:	40 µm
2. PLGA encapsulated particle production:		
W/O/W Emulsion:		
Emulsion type:	Water in Oil (PLGA & DCM) in water; i.e. W/O/W emulsion	
Inner water phase:	1000 ppm blue dextran 40 g/l sodium chloride reverse osmosis water	
Oil phase:	5, 10, 15% PLGA, remaining material was DCM	
Outer water phase:	1% PVA dissolved in reverse osmosis water, together with: 13, 26, 33, or 40 g/l sodium chloride and saturated with DCM	
Emulsification condition:	Primary emulsion:	By a mechanical homogeniser
	Secondary emulsification:	By membrane emulsification using:
	Agitation speed:	600, and 860 rpm stirrer speed
	Injection rate:	0.5 ml/min
	Membrane pore size:	40, 20 µm

Table 2 Solidification methods employed for the PLGA particles

Method:	Duration (hours)	Evaporation area (cm ²)	Continuous phase added (ml)	Agitation speed (rpm)
A - Fast	2	150	1000	120
B - Grad	24	150	450	120
C - slow	24	50	-	120
D - Very slow	72	1.7	-	120

Table 3 **Different number size distribution spans obtained for PLGA encapsulated particles at different PLGA concentration, size and outer phase salt concentrations**

PLGA concentration (%)	Ratio water phase salt concentration outer to inner	Resulting Span of PLGA particles produced
Pore size of membrane: 20 µm		
5	1	0.33
5	0.83	0.35
5	0.67	3.85
5	0.33	3.66
10	1	0.30
10	0.83	0.32
10	0.67	0.36
10	0.33	0.34
15	1	0.31
15	0.83	0.30
15	0.67	0.35
15	0.33	0.35
Pore size of membrane: 40 µm		
5	1	1.2
5	0.83	0.51
5	0.67	0.52
5	0.33	0.63
15	1	0.38
15	0.83	0.46
15	0.67	0.38
15	0.33	0.40

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Figure 1 Micropore Technologies Ltd Dispersion Cell and pore array membrane

Figure 2 PLGA droplet size dependence on stirrer agitation speed

Figure 3 PLGA drop size distribution dependence on PLGA concentration whilst using a 40 micron membrane for the emulsification

Figure 4 PLGA particle size distribution dependence on solidification method

(a) Very slow solidification

(b) Slow solidification

Figure 5 PLGA particles obtained by (a) method D - very slow and (b) method C - slow solidification processes

Figure 6 PLGA encapsulated particle size dependence on outer salt concentration, expressed as a ratio of the inner concentration

Figure 7 PLGA encapsulated particle shrinkage dependence on outer salt concentration expressed as ratio over inner water phase salt concentration

Figure 8 PLGA encapsulated particle encapsulation efficiency dependence on outer water phase concentration expressed as a ratio of the inner water phase