Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology

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Abstract

The therapeutic benefit of microencapsulated drugs and vaccines brought forth the need to prepare such particles in larger quantities and in sufficient quality suitable for clinical trials and commercialisation. Very commonly, microencapsulation processes are based on the principle of so-called “solvent extraction/evaporation”. While initial lab-scale experiments are frequently performed in simple beaker/stirrer setups, clinical trials and market introduction require more sophisticated technologies, allowing for economic, robust, well-controllable and aseptic production of microspheres. To this aim, various technologies have been examined for microsphere preparation, among them are static mixing, extrusion through needles, membranes and microfabricated microchannel devices, dripping using electrostatic forces and ultrasonic jet excitation. This article reviews the current state of the art in solvent extraction/evaporation-based microencapsulation technologies. Its focus is on process-related aspects, as described in the scientific and patent literature. Our findings will be outlined according to the four major substeps of microsphere preparation by solvent extraction/evaporation, namely, (i) incorporation of the bioactive compound, (ii) formation of the microdroplets, (iii) solvent removal and (iv) harvesting and drying the particles. Both, well-established and more advanced technologies will be reviewed.

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Keywords: Microencapsulation; Biodegradable microspheres; Solvent extraction; Solvent evaporation; Controlled release; PLA; PLGA; Static mixing; Membrane extrusion; Microchannel micromixer; Jet excitation

Abbreviations: ACN, acetonitrile; BSA, bovine serum albumin; CSTR, continuously stirred tank reactor; CV, coefficient of variation; DCM, dichloromethane; HPMC, hydroxypropylmethylcellulose; OVA, ovalbumin; PEG, poly(ethylene glycol); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PMMA, poly(methyl methacrylate); PTFE, poly(tetrafluoroethylene); PVA, poly(vinyl alcohol); PVP, poly(vinyl pyrrolidone); rhGH, recombinant human growth hormone; sCT, salmon calcitonin; SDS, sodium dodecyl sulfate; SPG, Shirasu Porous Glass

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1. Introduction

Biodegradable microspheres are widely investigated delivery systems for bioactive compounds such as low molecular weight and macromolecular therapeutics, antigens or DNA. As such they may add substantially to the value of therapies and vaccinations. Considered for parenteral, pulmonary, oral or nasal administration, they are capable of providing sustained and controlled release of the encapsulated bioactive compound, while the nonreleased bioactive material may be protected from degradation and physiological clearance. For vaccines, microspheres may provide additional adjuvancy [1,2] and allow for direct targeting to professional antigen-presenting cells [3]. Furthermore, they may be surface-modified to target specific cells [4] and tissues [5].

Owing to their excellent biocompatibility, the biodegradable polyesters poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) are the most frequently used biomaterials for the microencapsulation of therapeutics and antigens [6,7]. Other materials like proteins [5], polymer blends [8], polysaccharides such as chitosan [9], and lipids [10] have also been studied, although at a lower frequency. A large variety of bioactive compounds have been formulated into microspheres, among them are antineoplastic drugs [11,12], narcotics [13], anaesthetic agents [14] as well as therapeutic peptides [15,16] and proteins [17,18], DNA [19,20], viruses [21] and bacteria-derived compounds [22,23]. Preparation technologies capable of producing larger amounts of microspheres in a safe, economic, robust and well-controlled manner are therefore required.

Microspheres have been prepared by various techniques, which feature partly competing, partly complementary characteristics. Many microencapsulation processes are modifications of the three basic techniques: solvent extraction/evaporation, phase separation (coacervation) and spray-drying [24]. Spray-drying is relatively simple and of high throughput but must not be used for highly temperature-sensitive compounds. Moreover, control of the particle size is difficult, and yields for small batches are moderate [25]. Coacervation is frequently impaired by residual solvents and coacervating agents found in the microspheres [26]. Furthermore, it is not well suited for producing microspheres in the low micrometer size range. The use of supercritical gases as phase separating agents was intensively studied to minimise the amount of potentially harmful residues in the microspheres, resulting in processes named, e.g., Precipitation with Compressed Antisolvent (PCA) [27], Gas or Supercritical fluid Anti-Solvent (GAS

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or SAS) and Aerosol Solvent Extraction System (ASES) [28]. Solvent extraction/evaporation neither requires elevated temperatures nor phase separation-inducing agents. Controlled particle sizes in the nanoto micrometer range can be achieved, but careful selection of encapsulation conditions and materials is needed to yield high encapsulation efficiencies and a low residual solvent content.

Microsphere preparation by solvent extraction/evaporation basically consists of four major steps: (i) dissolution or dispersion of the bioactive compound often in an organic solvent containing the matrix forming material; (ii) emulsification of this organic phase in a second continuous (frequently aqueous) phase immiscible with the first one; (iii) extraction of the solvent from the dispersed phase by the continuous phase, which is optionally accompanied by solvent evaporation, either one transforming the droplets into solid microspheres; (iv) harvesting and drying of the microspheres (Fig. 1).

This article reviews the current state of the art in solvent extraction/evaporation-based microencapsulation technology, with a focus on process-related aspects. Issues like materials, microsphere formulation, choice of appropriate solvents or surfactants are not central aspects of this review, although technology and starting materials are interconnected and can by no means be segregated completely. Both well-established and more advanced technologies will be reviewed.

2. Incorporation of bioactive compounds

Bioactive compounds may be added to the solution of the matrix material by either codissolution in a common solvent, dispersion of finely pulverised solid material or emulsification of an aqueous solution of the bioactive compound immiscible with the matrix material solution [29]. Codissolution may require a

Fig. 1. Schematic overview over the four principal process steps in microsphere preparation by solvent extraction/evaporation.
cosolvent to fully dissolve the drug in the matrix-containing solvent. Dispersion of the solid or dissolved bioactive material in the matrix-containing solution may be achieved by ultrasonication [30], impeller or static mixing [31], high-speed rotor–stator mixing [32] or microfluidisation [30]. The microencapsulation of hydrophilic compounds by dispersion of their aqueous solution in an organic solution of the matrix material was more efficient with finer W/O-emulsions, i.e., at a lower ratio of bioactive material droplet size to microsphere diameter [32,33]. For the entrapment of bovine serum albumin (BSA) into poly(methyl methacrylate) (PMMA) microspheres, a ratio of less than 1:10 was suggested to yield protein loadings of >80% [32]. A higher target load of bioactive material is likely to decrease the encapsulation efficiencies of proteins and peptides in PLGA [33–35] and increase the 24-h (“burst”) drug release [35,36], although some studies report the opposite, e.g., an increase in entrapment efficiency of ovalbumin (OVA) from 40% to 98% with an increase in actual OVA content from 7% to 16% (w/w) [37,38]. Increasing the volume fraction of the internal aqueous phase lowered the encapsulation efficiency due to droplet coalescence and increased probability of contact between the internal drug solution and the external extraction phase resulting in drug loss [39,40]; in addition, an increase in the burst release and microsphere porosity was reported [41,42].

In analogy, entrapment of solid protein particles also improved with decreasing particle size [32,43]. The particle size of drug powders can be reduced by either micronisation of the drug powder prior to its dispersion, or during the dispersion step itself [44,45], or by the use of excipients which are coformulated with the drug so that the blended material dissolves in the matrix’s solvent [46]. Finally, spherically shaped protein particles caused a trend towards more efficient encapsulation than irregular ones [32].

For efficient encapsulation of drugs dissolved in an aqueous phase to be dispersed in an organic matrix solution, stabilisation of the resulting W/O-emulsion may be required. When drug-free microparticles were prepared from emulsions consisting of plain water and PLA dissolved in dichloromethane (DCM) [47], increasing amounts of BSA added to the water as a surfactant stabilised the emulsions and decreased the pore sizes in the resulting microspheres; the latter observation was ascribed to the finer water droplets that were entrapped and left a corresponding void in the matrix. The addition of a surfactant (poloxamer) to the organic phase was found to be much less efficient. Similarly, the model substance indigocarmine was more efficiently entrapped with increasing BSA concentrations in the inner water phase [48]. Other substances, e.g., gelatine [49], poly(vinyl alcohol) (PVA) [35], ovalbumin [50] or combinations of sorbitan esters and polysorbates [51], have also been reported for the stabilisation of such W/O-emulsions. The selection of stabilisers for the W/O-emulsion has to be made with caution, as coencapsulated surfactants can adversely affect drug encapsulation efficiency and release [48,52].

3. Droplet formation

The droplet formation step determines the size and size distribution of the resulting microspheres. Microsphere size may affect the rate of drug release, drug encapsulation efficiency, product syringeability, in vivo fate in terms of uptake by phagocytic cells and biodistribution of the particles after subcutaneous injection of intranasal administration. In the following, the main procedures used for droplet formation in microsphere production are described. Henceforth, the different types of mixtures of bioactive and matrix materials described above will, for simplicity, be referred to as drug/matrix dispersion.

3.1. Stirring

Stirring is the most straightforward method to generate droplets of the drug/matrix dispersion in the continuous extraction phase for subsequent solvent removal. In the simplest approach, extraction phase is filled into a vessel and agitated by an impeller. The drug/matrix dispersion is then added, dropwise or all at once, under agitation at a speed sufficient to reach the desired droplet size.

Obviously, the impeller speed is the main parameter for controlling the drug/matrix dispersion’s droplet size in the continuous phase. Increasing the mixing speed generally results in decreased microsphere mean size [35,53–55], as it produces smaller
emulsion droplets through stronger shear forces and increased turbulence. The extent of size reduction that is attained depends on the viscosity of the disperse and continuous phases, the interfacial tension between the two phases, their volume ratio, the geometry and number of the impeller(s) and the size ratio of impeller and mixing vessel. For example, a 52-mm impeller installed in a 250-ml beaker of 65 mm inner diameter produced microsphere mean diameters decreasing from 38 to 14 μm with impeller speed increasing segmentally from 250 to 1600 rpm, using PLGA dissolved in DCM and an aqueous hydroxypropylmethylcellulose (HPMC) solution as disperse and continuous phases, respectively [53]. In addition to a smaller mean diameter, more vigorous mixing also resulted in lower microsphere polydispersity [53,56].

Increased viscosity of the drug/matrix dispersion yields larger microspheres because higher shear forces are necessary for droplet disruption [16,33,38,41,57]. For PLGA dissolved at 6.25%, 12.5% and 25% in a mixture of acetonitrile (ACN) and DCM and dispersed in liquid paraffin, microsphere mean diameters of 36, 115 and 208 μm were obtained [57]. Such increase in drug/matrix dispersion viscosity, typically caused by higher concentration or molecular weight of the matrix material, may be desirable to restrict the migration of the drug to the continuous phase and thus improve its entrapment.

To prevent coalescence of the drug/matrix dispersion droplets, a surface-active or viscosity-enhancing stabiliser such as PVA is generally added to the continuous phase. Increasing the stabiliser concentration frequently leads to decreased microsphere sizes [20,35,37,53,58]. For instance, when microspheres were prepared from PLGA dissolved in DCM and emulsified in an aqueous PVA solution, the mean diameter decreased from 8.3 to 3.7 μm when the PVA concentration was increased stepwise from 1% to 10% [37]. When HPMC was used as a stabiliser, an increase of its concentration in the continuous phase from 0.4% to 2.4% resulted in an almost linear decrease of the microsphere size from 29 to 13 μm, along with a reduced width of the size distribution [53]. Higher stabiliser concentrations will yield a larger excess of material that adsorbs on the surface of newly formed droplets, thus preventing coalescence [35,53]. With macromolecular stabilisers, the viscosity of the continuous phase will also increase, amplifying—for a given stirring rate—the shear forces acting upon the drug/matrix dispersion droplets and thus minimising their size.

Reports about the impact of the volume ratio between drug/matrix dispersion and continuous phase on the size of the resulting microspheres are conflicting. Various studies reported a reduction in the mean microsphere size with decreasing continuous phase volume [16,37,59,60], while in other studies, no significant effect was observed [53,54].

In an attempt to predict the mean diameter of microspheres prepared in a so-called continuously stirred tank reactor (CSTR), an empirical equation was derived [61]. In a vast number of experiments, the size of PLGA and PMMA particles was correlated with reactor parameters and fluid properties, using dimensional analysis. In agreement with previous reports, the equation predicted a strong correlation of the microsphere mean diameter with stirring speed, impeller diameter (decreased diameter) and polymer concentration (increased diameter) as well as moderate correlation with continuous phase viscosity (decreased diameter) and interfacial tension (increased diameter). Disperse and continuous phase volumes did not significantly influence microsphere size. The equation reproduced and predicted the microsphere diameter with good accuracy for different types of extraction fluids and for microspheres without and with protein loading. Also, in scaled-up equipment (from 1 to 3, 10 and 100 l), the deviation of the predicted diameter from the experimentally obtained one was less than 20%. However, no prediction on the width of the particle size distribution could be made.

### 3.2. Static mixing

Static mixers consist of baffles or other flow obstacles installed in a tube. The baffle arrangement repeatedly splits and recombines the stream of fluid passing through the tube. Recombination occurs through impingement of the substreams, creating turbulence and inducing back-mixing.

In a comprehensive study, static mixers of different baffle design, length (4–76 cm) and diameter (0.6–2.5 cm) were examined for microsphere production involving concentrated solutions (18% and 30%, w/ w) of PLGA and PMMA in DCM dispersed in aqueous PVA solutions [62]. Using continuous phase
flow rates of 36 to 320 l/h yielded microsphere mean diameters of 35 to 90 μm. For each of the three mixer designs, an empirical equation relating microsphere size to fluid properties, mixer geometry and flow rate was derived by dimensional analysis. Correlation between the equations and experimental data was good, as was the predictive power, with the calculated mean diameter deviating less than 10% from that experimentally determined. Analysis of the equations revealed that increasing the interfacial tension, polymer concentration and mixer diameter produced larger microspheres, while increasing the flow rate, continuous phase viscosity and length of the mixer resulted in smaller particles. Moreover, the authors concluded that the mean size of the microspheres would not change during scale-up if the flow velocity inside the mixer could be maintained. However, no statement about retention of the particle size distribution was made, which is of equal interest in a scale-up. For the three mixer designs studied, a ranking with respect to emulsification efficiency was established and explained with respect to baffle geometry. A comparison of the static mixers with a CSTR for emulsification efficiency revealed that static mixers generate the same degree of mixing at much lower Reynolds numbers. Uniformity of the particle size distribution was not improved by static mixing. The authors concluded that static mixing scores over CSTR-based microencapsulation with respect to process continuity, mixing efficiency and scalability.

A convenient way to scale-up microencapsulation by static mixing is the parallel installation of several small-diameter mixers, with outflows that are recombined downstream, rather than using a single mixer of larger diameter (Fig. 2) [63]. A preblending mixer preceding the mixer manifold ensures that a uniformly composed preemulsion of drug/matrix dispersion and extraction phase enters each mixer of the manifold. Furthermore, it was observed that the uniformity and symmetry of the microspheres’ size distribution was improved by increasing the emulsion’s residence time in the static mixer manifold, i.e., by increasing the manifold’s length.

As an alternative to classical static mixing, a tube of very small diameter was suggested for the formation of an emulsion of the drug/matrix dispersion in the continuous extraction phase [64]. The two phases to be mixed were pumped through such a tube at flow rates high enough to yield Reynolds numbers exceeding values of 4000 to induce intense turbulent mixing. As an example of conditions applicable for microsphere preparation, a 3.7-m-long poly(tetrafluoroethylene) (PTFE) tube of 1.65 mm inner diameter and drug/matrix dispersion and continuous phase flow rates of 70 and 240 to 900 ml/min, respectively, are given. The resulting microsphere size distribution displayed rather polydispersed particles, i.e., with particle diameters ranging from below 10 to 200 μm.

Generally, the fact that the droplet size is a function of the flow rate constitutes a drawback in the use of static mixers for microencapsulation because microsphere size and throughput cannot be controlled separately.

3.3. Extrusion

Extrusion denotes feeding the drug/matrix dispersion through a single or a plurality of pathways
directly into the continuous extraction phase. Upon leaving the pathway(s), discrete droplets of the drug/matrix dispersion are formed within the slowly flowing continuous phase, which also transports the droplets away from the site of their formation.

Extrusion is distinguished from static mixing by the droplet-forming mechanism and the prevailing flow regime. In extrusion, the flow is mainly laminar and the droplets are formed directly at the site of introduction of the dispersed phase into the continuous phase and do not change their dimension thereafter (given that coalescence is negligible). On the contrary, static mixing relies mainly on turbulent flow, which constantly acts on the disperse phase and thus causes the size of the droplets to change over the whole length of the mixer. Therefore, extrusion is considered to allow for more uniform and better-controlled microsphere sizes than static mixing.

3.3.1. Single pathway systems

The continuous injection of a drug/matrix dispersion (hydrocortisone/PLA codissolved in DCM) via a hypodermic needle into a coaxial stream of continuous extraction fluid (mineral oil) was studied for microsphere formation [65]. The microsphere size (mean diameter of 145–400 μm) was controlled by the needle diameter (510 and 710 μm) and by the flow rate of the mineral oil at the needle tip, with smaller particles being obtained from smaller needle diameters and higher oil flow rates. Downstream inlets were used to further add mineral oil for efficient extraction of the solvent independent of the flow rate at the needle tip. Particle size distributions were considerably polydispersed (coefficient of variation [CV]=15–40%), and the drug/matrix dispersion flow rate was 3.6 ml/h, representing a very low process productivity.

In a slightly different approach, a stainless steel blunt-ended needle was used to inject a solution of PLGA in DCM into a perpendicular flow of an aqueous PVA solution used as continuous phase [66]. With a PLGA solution flow rate of 30 ml/h, process productivity was considerably higher than with the aforementioned technique. Mean microsphere size varied between 68 and 295 μm (CV=5–35%). Measures to decrease the mean particle diameter comprised of increasing the continuous phase flow velocity, reducing the needle diameter (from 457 to 254 μm) and decreasing the adhesion between needle and polymer solution (e.g., by using PTFE or silicone coated needles [67]). The width of the size distribution narrowed when one of the two prominent forces prevailed, i.e., either the shear force exerted by the extraction phase on the growing droplet or the adhesion force between the droplet and the needle tip. Changing the angle between needle and extraction phase flow from 90° to 45° did not significantly influence the microsphere size distribution [67].

Generally, the single pathway extrusion systems have turned out to be unsuitable for the production of small microspheres (<50 μm), and their throughput was quite low. Scale-up may be feasible through parallel employment of a plurality of needles, which, however, might be difficult to implement without considerably perturbing the flow of the extraction phase and causing interactions between the outflows from the different needles.

3.3.2. Multichannel systems

Recently, a micromixer consisting in essence of an array of fine channels (25 or 40 μm in width; 300 μm in depth; Fig. 3a–c) was employed for microsphere preparation [68]. PLGA dissolved in DCM, into which an aqueous BSA solution was emulsified, and an aqueous PV A solution used as extraction phase were separately fed into the microchannel array from opposite sides and discharged through an outlet slit (60 μm wide), which was micromachined in the mixer housing’s top plate perpendicular and central to the channel array (Fig. 3b). Upon entering the outlet slit, alternating fluid lamellae of the two fluid phases formed. Owing to the much faster flow rate of the extraction fluid, the microsphere-forming phase disintegrated into droplets (Fig. 3d) [68,69]. The mean microsphere diameter was tuned from 8 to 29 μm by simply varying the flow rates of the two fluids pumped through the mixer (Fig. 3e). Relatively wide particle size distributions were obtained, e.g., ranging from 4 to 60 μm for a mean diameter of 16 μm. Interestingly, both the microsphere mean size and size distribution remained largely unaffected by varying PLGA solution concentrations (2–10%, w/w), drug load and polymer type. On the contrary, switching the polymer solvent from DCM to ethyl formate yielded considerably smaller microspheres (7 μm mean diameter instead of 16 μm for DCM), which was attributed to decreased interfacial tension. Scale-up
can be comfortably achieved by the so-called number-
ing-up, i.e., by employing a large number of micro-
mixers in parallel. Owing to its simple design and
because it may be easily sterilised, the micromixer
was suggested for aseptic microsphere manufactur-
[68].

Another simple and ingenious microchannel sys-
tem, etched into a silicon chip (Fig. 4a,b) [70], was
examined intensively for the formation of monodis-
perse emulsions and, more recently, for the solvent
evaporation-based preparation of uniform lipid micro-
particles [71]. The channels measure only a few
micrometers in height and width and open up to a
terrace that descends to a well through which the
continuous phase slowly passes (Fig. 4b,c). The
device is covered by a glass plate to allow for
observation by a camera system. The disperse phase,
flowing out of the microchannel, spreads into the
space between the terrace and the glass cover in a
disk-like shape until it reaches the rim of the well.
When flowing over the rim and into the well,
interfacial forces contract the fluid to form a droplet

Fig. 3. Multilamination micromixer. (a) Assembled micromixer. (b) Dismantled mixer with extracted mixing tool. (c) Close-up of the
microchannel array. Channel width is 40 μm. (d) Formation and disintegration of fluid lamellae in the mixer’s outlet slit. (e) Control of the
microsphere size by variation of the flow rates. Extraction phase flow rate was 1200, 900, 600 and 420 ml/h (size distributions from left to
right); drug/matrix dispersion flow rate was adapted at 1/50 of the extraction fluid rate. (a)-(c) With kind permission of Institut für Mikrotechnik
Mainz (www.imm-mainz.de); (d, e) Reproduced from Ref. [68] with permission.
The interfacial area of the disperse phase, as spread on the terrace, is large compared to that of the droplet in the well, driving the fluid to leave the terrace and adopt a spherical form. On a micrometer scale, interfacial forces dominate over other forces like gravity, inertia and viscosity [70]. Therefore, droplet formation was governed by this single force only, leading to monodisperse droplets (CV<5%; Fig. 4d) [72]. Droplets of a few, up to 100 μm, were produced [73,76]. Because the produced droplets were, in general, significantly larger than the channels’ dimensions, production devices for low micro-meter-scaled microspheres may be susceptible to clogging. Droplet size increased with channel height and terrace length but was largely independent of channel width and length, although longer and narrower channels accommodated a wider range of disperse phase pressures still producing monodisperse droplets [74]. An empirical equation predicted the droplet size as a function of microchannel height and terrace length with good accuracy [75]. Unfortunately, the achievable throughput of such devices is limited to just a few millilitres per hour, even when using several hundred channels in parallel [76]. Increasing the throughput by augmenting the pressure applied to the disperse phase produced more polydispersed and...
larger droplets, as interfacial tension no longer dominated over the viscous force.

3.3.3. Membranes

Microporous glass membranes of well defined pore size were used for nitrogen-driven extrusion of polystyrene dissolved in chloroform [77] and PLA/PLGA dissolved in DCM [78] into a continuous slowly circulating aqueous surfactant solution, followed by subsequent solvent evaporation. This method, also named Shirasu Porous Glass (SPG) emulsification technique [79], produced very uniform PLGA microspheres of 1.2, 1.8 and 2.9 µm (number-averaged) mean diameters from membranes with pore sizes of 0.7, 1.1 and 2.4 Å, respectively. Generally, the particles produced were slightly larger than the pores from which they were manufactured. The continuous phase preferably contained anionic surfactants like sodium dodecyl sulphate (SDS), while cationic and nonionic surfactants (polysorbates) and protective colloids like PVA or poloxamer were inappropriate [78]; cationic surfactants interacted electrically with the negatively charged glass membranes, the nonionic surfactants were soluble in both the aqueous phase and DCM so that they did not adsorb sufficiently at the interface, and PVA was assumed to partition to slowly to the interface upon droplet formation. Furthermore, uniform microspheres were only obtained when the aqueous continuous phase was presaturated with the polymer solvent. When progesterone was codissolved in the PLA/PLGA solutions to yield particles with a payload of up to 50%, no changes in the size and uniformity of the resulting microspheres were observed. A SPG membrane of larger pore size (5.2 µm) was also used to produce PLA microparticles [79]. PLA was dissolved in DCM at high concentrations of 10% to 20% (w/w), along with dodecyl alcohol or hexadecane as cosurfactant, which were used to reduce the solution’s hydrophilicity and, thereby, its wetting of the polar glass pores to yield more uniform microspheres. An aqueous solution of PVA and SDS was employed as a continuous phase. The resulting microspheres were considerably larger (mean diameters of 10–25 µm) than the membrane pores and moderately polydispersed (CV=10–15%). No consistent relationship between particle size or polydispersity and polymer or cosurfactant concentrations was observed. Moreover, the microspheres were not perfectly spherical but elliptical and hemispherical when made with dodecyl alcohol and hexadecane, respectively.

A hydrophilic polycarbonate membrane [80] and a micromachined silicon chip (Fig. 5a,b) [81], both featuring uniformly sized pores or holes, have also been studied for emulsion formation. Although the emulsions were not used to form microspheres, an interesting insight into droplet formation with such devices was achieved. Membranes of both materials with 10-µm circular pores yielded polydispersed droplets of up to about 100 µm for the emulsification of soybean oil in an aqueous surfactant solution flowing parallel to the membrane. With the polycarbonate membrane [80], the droplet mean size (along with polydispersity) was lowered from approximately 70 to 20 µm by increasing the continuous phase flow velocity from 0.02 to 0.54 m/s. In agreement with observations on glass membranes [78], anionic surfactants were superior to nonionic ones, while cationic surfactants hampered droplet formation. Silicon chips with oblong holes of 17.3 µm equivalent diameter yielded highly uniform (CV<1.5%) droplets of 32.5 µm average diameter (Fig. 5c) [81]. Here, droplet size and polydispersity remained unaffected by variations in the very low (0–9.2 mm s⁻¹) continuous phase velocity. Hence, it was concluded that the microdroplets detach spontaneously from the oblong channels due to instability of the elongated interface at the channel outlet without the need of the continuous phase shearing action. The productivity per channel plate (5000 channels) amounted to 6.5 ml/h of the disperse phase.

3.4. Dripping

3.4.1. Single droplet formation

Microspheres have been prepared by dripping 10% and 15% (w/w) solutions of poly(ethylene-co-vinyl acetate) in DCM, containing dispersed protein particles, from a needle into an electric field (Fig. 6) [82]. In this process, the forming droplets were detached from the needle by electrostatic forces. Particle collection and solvent removal occurred in a bath of cold (−75 °C) methanol. The electric field was generated by connecting the needle to electric potentials of up to 4 kV and the collection bath to ground. Very large microspheres of 500 to 1500 µm
average diameter were obtained, whereby the largest particles formed with voltage-free dripping. Droplets disrupted by the electric field upon detachment from the needle tip resulted in highly polydispersed size distributions, ranging from 600 to 1200 μm or sometimes even from 200 to 1200 μm. Productivity was low with 30 ml/h of processed polymer solution. Dripping PLGA dissolved in ACN from a needle into a collection bath of light mineral oil, in which a ring-shaped anode was submerged, resulted in much smaller microspheres of 50–100 μm mean diameter, using voltages of 1.25–1.85 kV [83].

3.4.2. Jet excitation

The vibration of a liquid jet for its disruption into droplets was originally studied by Lord Rayleigh as early as in the late 19th century [84,85]. A longitudinal oscillation imposed on a liquid stream causes periodic surface instabilities, which break up the liquid into a chain of uniform droplets. Lord Rayleigh found that uniform droplets are produced from a range of excitation wavelengths corresponding to 7 to 36 times the liquid jet radius.

This principle was recently used to produce uniform PLGA microparticles [86,87]. A 5% (w/v) solution of PLGA in DCM was fed through a nozzle to form a cylindrical jet while the nozzle was excited...
by an ultrasonic transducer of adjustable frequency (Fig. 7a). The particles were collected in 1% (w/v) PVA solution for solvent extraction/evaporation. Very uniform microspheres of 45 to 500 μm diameter were produced by jetting the polymer solution from nozzles of different orifice size (Fig. 7b,c). Generally, 95% of the microspheres was within 1.5 μm of the average diameter. At a fixed feed rate (2–3 ml/min; 60 μm nozzle), the microsphere size could be adjusted between 70 and 130 μm by decreasing the frequency from 70 to 19 kHz. Augmenting the feed rate at fixed excitation frequency from 2 to 3 ml/min resulted in a 30% increase in the microsphere diameter. Predetermined size distributions were obtained by switching the excitation frequency during production. Generally, the size of the microspheres was slightly larger than the diameter of the nozzle. Therefore, particle sizes below 25 μm are difficult to achieve with this technique as the pressure drop across the orifice opening rapidly increases, as does the risk of orifice clogging. Scale-up is achieved using multiorifice nozzles (e.g., Ref. [88]). Multiorifice nozzles with nonuniform openings were designed to yield desired microsphere size distributions [89].

The jet of drug/matrix dispersion may be surrounded by an annular stream of extraction fluid or any other suitable fluid immiscible with the drug/matrix dispersion (Fig. 7a). The biphasic jet is then again vibrated and disintegrated into biphasic droplets [86,90]. The outer layer of fluid around the droplets of drug/matrix dispersion protected the latter from deformation upon impact with the collection/extraction fluid bath [91,92]. Feeding the outer stream at a higher velocity than the inner stream of drug/matrix dispersion stretched and thinned the latter due to the friction between the two phases. Subsequent vibration of the biphasic jet yielded uniform particles as small as 5 μm produced from a nozzle of much larger diameter [86]. The combined control of exciting frequency and annular sheath stream velocity allowed

![Diagram](image_url)

**Fig. 7.** Microencapsulation by jet excitation. (a) Schematic representation of the process. (b) Size distributions and (c) SEM picture of PLGA microspheres produced by jet excitation. Scale bar in panel (c) represents 100 μm. Panel is (a) adapted from Ref. [86]. Panels (b) and (c) are reproduced from Ref. [87] with permission.
for a wide range of particle sizes manufactured from a single nozzle. The annular stream may alternatively be employed to dissolve a second matrix material, allowing for the manufacture of core/(multi)shell microspheres [90,92].

4. Solvent removal

In both solvent extraction and evaporation, the solvent of the disperse phase, i.e., the drug/matrix dispersion, must be slightly soluble in the continuous phase so that partitioning into the continuous phase can occur leading to precipitation of the matrix material. In solvent evaporation, the capacity of the continuous phase is insufficient to dissolve the entire volume of the disperse phase solvent. Therefore, the solvent must evaporate from the surface of the dispersion to yield sufficiently hardened microspheres. In solvent extraction, the amount and composition of the continuous phase are chosen so that the entire volume of the disperse phase solvent can be dissolved.

Generally, a continuous phase that is a nonsolvent for the microencapsulated bioactive compound is favourable. While for lipophilic compounds, aqueous solutions may be comfortably chosen, the use of hydrophobic, organic liquids as continuous phase for the encapsulation of hydrophilic compounds (e.g., Refs. [57,93,94]) is more delicate. Hydrophobic extraction fluids may not be readily removed from the final product, potentially causing undesired residues. Therefore, aqueous solutions are frequently used as continuous phase, even for the microencapsulation of hydrophilic compounds. Here, loss of bioactive compound is typically prevented by increasing the concentration of the matrix material solution; the resulting higher viscosity restricts the migration of the bioactive compound from the solidifying microspheres to the external phase by means of lowered diffusion and increased stability of the drug/matrix dispersion [33,48,95]. Other means of preventing loss of bioactive material into the continuous phase encompass the adaptation of the continuous phase pH to lower the solubility of the bioactive compound [50] or the addition of electrolytes to increase the osmotic pressure of the continuous phase [96–98].

The ideal rate of solvent removal depends on a variety of factors like the type of matrix material, drug and solvent as well as the desired release profile of the microspheres. For example, fast microsphere solidification will be preferred if the drug easily partitions into the continuous phase. On the other hand, slow solidification favours denser over more porous microspheres, affecting the drug release.

4.1. Evaporation

The rate of volatile solvent removal from the solidifying microspheres can be controlled by the temperature of the microsphere dispersion. Higher temperatures will facilitate the evaporation of the solvent from the continuous phase and thereby maintain a high concentration gradient for the solvent between the microspheres and the continuous phase. In two similar studies on the encapsulation of BSA in a PLGA–poly(ethylene glycol) (PEG) blend [99] and in pure PLGA [100], both dissolved in DCM, and using an aqueous PVA solution as continuous phase, the influence of the temperature (4–42 °C) at which the resulting dispersion was stirred for 30 min was examined. Maintaining the temperature, the dispersion was thereafter diluted with an additional continuous phase until a defined volume was attained. The PLGA microspheres tended to be larger when prepared at higher temperatures (38 and 42 °C), showed wider size distributions and decreased particle density compared to those prepared at lower temperatures (4–33 °C). As 38 and 42 °C are close to or even above the boiling point of the solvent DCM (b.p. ≈ 40 °C), these findings were attributed to very rapid microsphere solidification with insufficient mixing time to reduce droplet size. With PLGA, the morphology of the particle interior (honeycomb-like) and BSA encapsulation efficiency (53% to 63%) were unaffected by the preparation temperature, while for the PLGA–PEG blend, BSA encapsulation appeared to be temperature-sensitive with a minimum efficiency of 15% obtained at 22 °C, which steadily improved (up to 52%) for lower and higher temperatures (Fig. 8). For both polymers, the burst (24 h) release was highest at intermediate preparation temperatures, while values continuously decreased for higher and lower temperatures (Fig. 8). For the PLGA–PEG
microspheres, these phenomena were explained by a fast skin formation at the extremes of temperature range studied, restricting BSA transport to the microspheres’ periphery and loss of the protein. At high temperatures, rapid solvent evaporation obviously leads to fast solvent depletion in the microspheres. The authors’ hypothesis for the low temperature effect was an increased DCM solubility in water.

When salmon calcitonin (sCT) was encapsulated into PLGA, using a temperature gradient to remove the solvent, hollow microspheres with porous walls were obtained [101]. An aqueous solution of sodium oleate was used as a continuous phase, and the temperature of the resulting dispersion was increased from 15 to 40 °C. A rapid temperature increase within 30 min led to particles with a large empty core and a thin wall, while a gradual or a stepwise increase over 200 min resulted in increased wall thickness. Peptide incorporation, however, was largely unaffected by the solvent removal conditions. The formation of the hollow core, which was not found when the solvent was removed by extraction, was attributed to the slow removal of methanol in the evaporation process; methanol was used as cosolvent for the dissolution of sCT in the polymer solvent DCM.

As an alternative to elevated temperatures, reduced pressure is sometimes used to promote the evaporation of the solvent, as in the encapsulation of lidocain [14] or albumin [102] in small (0.7–1.2 μm) PLA microspheres. In both studies, an aqueous PVA solution was employed as the continuous phase. Evaporation of the polymer solvent DCM was accomplished within 6 h at 760 mm Hg or 2 h at 460 or 160 mm Hg at 25 °C. Irrespective of the encapsulated drug, i.e., lidocain or BSA (lidocain was codissolved in the polymer solution for encapsulation, and BSA was dissolved in an aqueous phase, which was subsequently emulsified in the organic polymer solution), both the microsphere mean size and encapsulation efficiency decreased at reduced pressure, whereas the drug release profile remained unaffected. With the encapsulation of progesterone in PLA, however, drug release was slower for microspheres prepared at reduced pressure (200 mm Hg) as compared to those manufactured at atmospheric pressure [103]. The slow solvent removal at atmospheric pressure favoured the formation of a crystalline over an amorphous polymer matrix, which prevailed at reduced preparation pressure. In the amorphous state, data indicated a molecular dispersion of polymer and drug, lowering the release rate of the latter. Drug encapsulation efficiency was not affected by the mode of solvent removal.

4.2. Liquid extraction

Solvent extraction is frequently performed as a two-step process. First, the drug/matrix dispersion is mixed with a small amount of continuous phase to yield an emulsion of desired droplet size (distribution). Then, a further continuous phase and/or additional extraction agents are added at an amount sufficient to absorb the entire solvent leaching from the solidifying microspheres. Nonetheless, a patent application [104] teaches a one-step solvent extraction process. Without prior emulsification step, the drug/matrix dispersion is immediately homogenised with such a quantity of continuous phase that is capable of dissolving the total amount of the disperse phase solvent at once. However, this process requires careful settings of the physicochemical parameters during the homogenisation step in order to yield homogeneously dispersed particles.
A number of publications have reported that the drug substance can be more efficiently retained in the microspheres if the amount of continuous phase strongly exceeds that theoretically necessary for dissolving the disperse phase solvent (e.g., Ref. [105]). The rapid formation of a skin on the microspheres’ periphery reduces the loss of drug to the continuous phase, which is of special importance when the latter is a good solvent for the drug. For example, the use of tenfold the amount of fluid necessary to extract all the disperse phase solvent is suggested for the encapsulation of substances that are sparingly to freely soluble (>10 mg/ml) in the continuous phase [105].

Rather than adding the entire amount of continuous phase at once, it may be added continuously over an extended period of time. However, in a system composed of aqueous BSA dispersed in a solution of PLA in DCM and 0.05% aqueous PVA solution, stirred at constant rate in a beaker for 30 min, further addition of continuous phase at constant rates ranging from 1.5 to 9 ml/min exerted no significant influence on the microspheres’ characteristics [99]. Likewise, in a similar process using a fixed addition rate but different final volumes of continuous phase, no significant influence on the resulting sCT-loaded microspheres was observed [101]. A continuously operated alternative to the batch-mode metering of continuous phase into a beaker consists in introducing further continuous phase or additional extraction-promoting agents through a series of feed streams into a continuous flow of dispersed nascent microspheres [64]. A conduit featuring a number of down-stream inlets can be employed for this purpose. A static mixer installed at the entrance of the conduit may be used for emulsion formation.

A combination of solvent evaporation and extraction is suggested to improve the economic efficiency of the microencapsulation process [64]. After emulsion formation, a sufficient quantity of an extraction fluid is added to induce skin formation on the microspheres’ periphery while the remaining solvent is removed by evaporation. The brief skin-forming extraction step prior to evaporation minimises the loss of drug during the following evaporation procedure, while the volume of extraction fluid consumed is reduced as compared to an extraction process alone.

The two steps of solvent extraction and evaporation may be combined by using a mixed solvent system [57]. For example, a system has been studied consisting of an aqueous protein solution, which was dispersed in a solution of PLGA in a mixture of ACN and DCM (Fig. 9). The drug/matrix dispersion was emulsified in liquid paraffin containing sorbitan mono-oleat. The production vessel was then purged with air and thereafter put stepwise under reduced pressure (300/50 mm Hg). The moderately polar DCM is extracted by the paraffin, whereas the strongly polar ACN, which is not soluble in paraffin, is evaporated during the purging and evacuating steps. BSA and lysozyme were very efficiently encapsulated, i.e., at 93% and 91%, respectively, while lower values were obtained for gelatin (71%) and a decapeptide (25–46%). After the extraction of DCM, the remaining ACN is miscible, with the water dissolving the protein causing precipitation of BSA and lysozyme, while the decapeptide remained dissolved enhancing its potential to escape encapsulation.

Solvent extraction, evaporation and a combined procedure were compared for the entrapment of ovalbumin (OVA) in PLGA microspheres [106]. Aqueous OVA solution was intensely homogenised in a solution of PLGA in DCM. The drug/matrix dispersion was further emulsified in either water (solvent evaporation), a 1:1 water–methanol mixture (combined mechanism) or solely methanol (extraction) as continuous phases, using poly(vinyl pyrrolidone) (PVP) as an emulsifier. OVA entrapment was approximately 10% with the combined and the pure extraction processes but only 7.5% with the evaporation method. As DCM is much more soluble in methanol than in water, the presence of the alcohol led to faster solvent removal and thus improved drug entrapment.

Two patents [107,108] teach methods for in-process reprocessing and recycling of the continuous phase to minimise waste. A portion of continuous phase rich in disperse phase solvent is repeatedly or continuously withdrawn from the suspension of nascent microspheres, deprived of part of the solvent and refed to the microsphere suspension. Solvent removal is achieved by exposing the said portion of continuous phase to either a gas separation membrane [107], on which a vacuum is applied, or to an...
absorption fluid of high dissolution capacity for the disperse phase solvent, using a liquid–liquid column [108].

5. Microsphere harvest and drying

Separation of the solidified microspheres from the continuous phase is usually done either by filtration or centrifugation. The particles may then be rinsed with appropriate liquids to remove adhering substances such as dispersion stabilisers or nonencapsulated drugs. Rinsing may involve elevated temperatures or the use of extraction agents to reduce the amount of residual solvent in the microspheres [109]. Finally, the microspheres are dried either at ambient conditions or under reduced pressure, heat or by lyophilisation to yield a free-flowing powder. The drying procedure removes not only continuous phase and wash fluid adhering to the microspheres’ surface but also traces of solvents and continuous phase from the interior of the particles. Thus, the conditions and rate of drying influence the amount of solvent and moisture residue [110], microsphere morphology and porosity as well as drug recrystallisation inside the spheres, and are therefore likely to affect the release behaviour of the final product.

6. Conclusions

The widespread interest in microencapsulated drugs brought forth the need to prepare such particles in larger quantities and in sufficient quality suitable for clinical trials and commercialisation. The most frequently described solvent extraction/evaporation-based technology using simple beaker/stirrer setup is inappropriate for producing larger amounts of microspheres in an economic, robust and well-controled manner. Static mixers warrant continuous production and simple scale-up, while the extrusion through porous membranes or microchannels, inte-
Electrotropically in small-scaled equipment that is easy to operate and sterilise, additionally offers improved control of the microsphere size distribution as compared to classical mixing processes. Further, jet excitation is powerful in combining productivity and microsphere size control. Solvent removal by evaporation may be accelerated using elevated temperatures or reduced pressure. The rapid solvent extraction may require relatively large amounts of processing fluids and their subsequent recycling. Therefore, combined extraction and evaporation represents a compromise in terms of both time- and waste-efficient microsphere production.

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