Advances in Colloid and Interface Science 163 (2011) 90–122

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

journal homepage: www.elsevier.com/locate/cis

Influence of process and formulation parameters on the formation of submicron particles by solvent displacement and emulsification–diffusion methods Critical comparison

C.E. Mora-Huertas, H. Fessi, A. Elaissari *

Université de Lyon, F-69622, Lyon, France
Université Lyon 1, Villeurbanne, CNRS, UMR 5007, Laboratoire d'Automatique et de Génie des Procédés, LAGEP-CPE-308G, 43 bd. du 11 Nov.1918, F-69622, Villeurbanne, France

ARTICLE INFO

Available online 3 March 2011

Keywords:
Nanoparticles
Submicron particle
Solvent displacement
Nanoprecipitation
Emulsification–diffusion
Particle size
Zeta-potential

ABSTRACT

Solvent displacement and emulsification–diffusion are the methods used most often for preparing biodegradable submicron particles. The major difference between them is the procedure, which results from the total or partial water miscibility of the organic solvents used. This review is devoted to a critical and a comparative analysis based on the mechanistic aspects of particle formation and reported data on the influence of operating conditions, polymers, stabilizing agents and solvents on the size and zeta-potential of particles. In addition, a systematic study was carried out experimentally in order to obtain experimental data not previously reported and compare the data pertaining to the different methods. Thus the discussion of the behaviors reported in the light of the results obtained from the literature takes into account a wide range of theoretical and practical information. This leads to discussion on the formation mechanism of the particles and provides criteria for selecting the adequate method and raw materials for satisfying specific objectives in submicron particle design.

Contents

* Corresponding author. Tel.: +33 472431841; fax: +33 472431682.
E-mail address: elaissari@lagep.univ-lyon1.fr (A. Elaissari).

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doi:10.1016/j.cis.2011.02.005

1. Introduction

Nowadays, several methods for preparing submicron particles from preformed polymers are available. They can be categorized into two groups depending on the steps involved in their procedure [1]. Examples of the first group are emulsification–diffusion (also called emulsification–solvent displacement), emulsification–evaporation and emulsification–coacervation which are based on two steps: the first is the preparation of an emulsified while the second is based on particle formation by polymer precipitation or cross-linking. The second group of methods does not require emulsion preparation prior to obtaining the particles. They are based on polymer precipitation under conditions of spontaneous dispersion and particle formation from a polymer solution or the self-assembly of macromolecules, or the synthesis of polyelectrolyte complexes. Examples of this type of procedure include solvent displacement (also termed nanoprecipitation, solvent diffusion or interfacial deposition), polymersome preparation and the layer-by-layer technique.

Regardless of the method chosen, the development of biodegradable submicron particles and the assurance of a robust production process require exhaustive knowledge of the process and materials to be used. Consequently, extensive studies have been carried out and different research teams have published reviews on the techniques and initial materials for preparing submicron particles and on particle formation mechanisms [1–11]. As a contribution to updating the state of knowledge, this review provides an in-depth study on the incidence of operating conditions and formulation variables on particle characteristics when particles are prepared by either the solvent displacement technique or emulsification–diffusion method. These methods have been chosen as representative examples of the two major groups mentioned previously for preparing submicron particles since they are those used most often [10,12,13]. In addition, they are characterized by procedural simplicity, high encapsulation efficiency, high reproducibility, low possible contaminant content, low cost and easy up-scaling [9,10,14–18]. Another advantage is that they use preformed polymers as starting materials rather than monomers and toxic solvents [7,19].

Our first aim in this review is to establish an updated view of the two preparation methods for providing readers with consolidated information on research trends in the domain of submicron particle synthesis by using the solvent displacement technique and the emulsification–diffusion method.

We also focus on comparing methods, taking into account the behaviors obtained for the different variables studied. This provides criteria for making decisions on the best starting materials, preparation method and operating variables according to expectations regarding particle performance. Thus the results and conclusions reported by various authors form the starting point and are described in this review through comparisons made using data deduced from the reported results. Taking into account that the information available comes from works carried out with different objectives or reported from a qualitative standpoint, such fragmentation makes it difficult to obtain a complete, comparable and comprehensive survey of all the key variables required to ensure robust process design. To overcome this problem, the results from a systematic study carried out by the authors are included. Submicron spheres have been chosen as model particles to facilitate comparing the methods, since similar materials are used for both particle preparations. Size and zeta-potential have been chosen as the particle characteristics to be studied as they provide simple illustrations of particle behavior. Particle size is a critical parameter as it is directly linked to stability, cellular uptake, biodistribution and drug release [20–22] and the zeta-potential value can influence the stability of particle dispersion as well as particle mucoadhesivity [23–26].

Furthermore, this review is aimed at identifying major advances in the domain to provide understanding of the mechanistic aspects associated with particle formation obtained by each method. However, these research works highlight correlations with particle formation mechanisms, particle characteristics and variables that are limited to the particular experimental conditions used in each work. Thus, in this work, the correlations reported were verified in as many cases as possible in order to investigate their general applicability.

2. Solvent displacement and emulsification–diffusion as methods for preparing biodegradable submicron particles

Submicron biodegradable particles may be defined as solid colloidal particles with a size smaller than 1 μm that contain an active substance [10,27]. However, in the field of pharmaceuticals, there is good agreement that particle size should be in the middle or lower submicronic range (100 to 500 nm) [1–11]. Submicron particles include both spheres and capsules. Submicron spheres can be defined as matrix-type colloidal particles in which a drug is dissolved, entrapped, chemically bound or adsorbed to the constituent polymer matrix [27] while submicron capsules can be defined as vesicular systems that exhibit a typical core–shell structure in which the drug is mainly confined to a reservoir or within a cavity surrounded by a polymer membrane. It can also be carried on the capsule surface or imbibed in the polymeric membrane [13].

The typical procedures for preparing submicron particles by the solvent displacement technique and emulsification–diffusion method were firstly developed by Fessi et al. [19] and Leroux et al. [28], respectively. An outline of the main steps for each method is shown in Figs. 1 and 2, taking sphere preparation as an example (see also Supplementary data for additional illustrations on the procedures).

Sphere synthesis by solvent displacement requires both solvent and nonsolvent phases. The solvent phase essentially consists of a solution of the drug and the polymer. The nonsolvent phase is a nonsolvent or a mixture of nonsolvents for the polymer, supplemented with one or more naturally occurring or synthetic surfactants. In most cases, solvent and nonsolvent phases are respectively called organic and aqueous phases, because the solvent is an organic medium, while the nonsolvent is mainly water. However, it is possible to use either two organic phases or two aqueous phases as long as solubility, insolubility and miscibility conditions are satisfied. Regarding particle preparation, the organic phase is mixed with the stirred aqueous phase in one shot, stepwise, dropwise or by controlled addition rate. Submicron spheres are formed instantaneously and the solvent is removed from the system by using evaporation under reduced pressure.

The method for preparing submicron particles by emulsification–diffusion requires three phases: organic, aqueous and dilution. The organic phase is a solution of the polymer and the active substance in an organic solvent partially miscible with water, that has previously been water-saturated. The aqueous phase comprises the aqueous dispersion of a stabilizing agent prepared by using solvent-saturated water while the dilution phase is usually water.

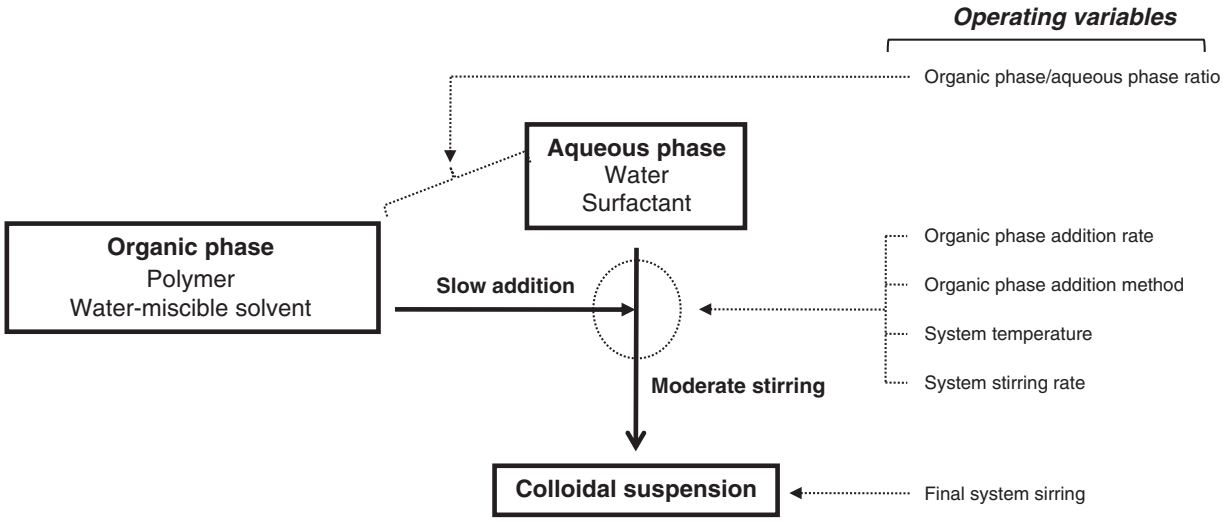

Fig. 1. Preparation of submicron particles by solvent displacement method: schematic procedure and operating variables.

To prepare the particles, as shown in Fig. 2, the organic phase is added rapidly (in less than 5 s) to the aqueous phase and an o/w emulsion is formed immediately by stirring at high speed. The emulsion formed is diluted into the dilution phase by mechanical stirring in order to allow the migration of the organic solvent in the water, leading to particle formation. The solvent and part of the water are then removed by evaporation under reduced pressure.

It must be emphasized that the two methods require mixing two phases (organic and aqueous) as their starting point. Likewise, in both of them the migration of organic solvent in water leads to instantaneous submicron particle formation. However, the procedure differs due to the miscibility of organic solvent in water. Thus the solvent displacement technique (one step procedure) simply requires phase mixing to obtain particles since the organic solvent used is totally miscible in water. Regarding the emulsification–diffusion method (two-step procedure), it requires partially water miscible solvents that must be water saturated. Phase mixing forms a submicron emulsion (first step). Then, the addition of water to dilute the emulsion leads to particle formation (second step). As can be concluded, the simplicity of this procedure and its set-up, the lower consumptions of energy, time and water, and the mild shear forces required by the solvent displacement technique, represent advantages in comparison to the emulsification–diffusion method.

Sahana et al. [22] and Hariharan et al. [26] used a modified emulsification–diffusion method, keeping the basic principles of the Leroux et al. method. Thus the organic and aqueous phases are prepared by using a non-saturated organic solvent and water, by stirring for 3 h to form irregular-sized globules in equilibrium with a continuous phase. The o/w emulsion is formed by high speed stirring and the dilution step is carried out by the addition of water into the emulsion with constant stirring in a water bath set at 40 °C.

With respect to the raw materials used for sphere preparation, Tables 1 and 2 provide a compilation of polymers, stabilizing agents, organic solvents, active substances and other materials reported by

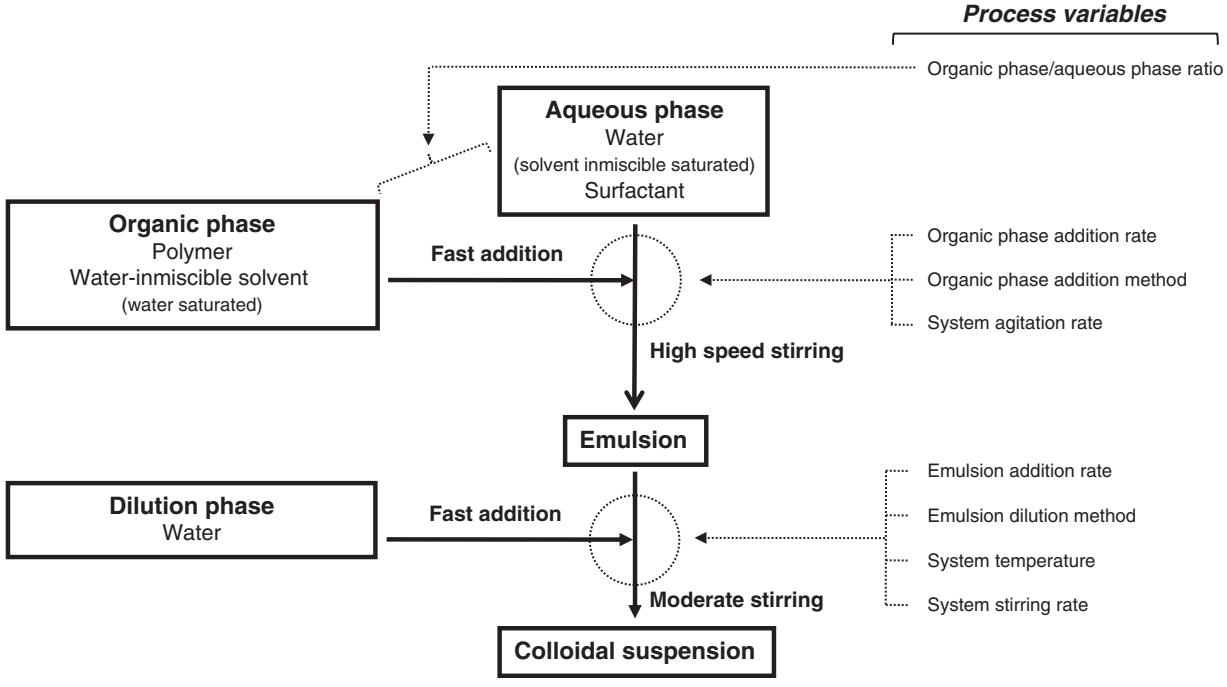

Fig. 2. Preparation of submicron particles by emulsification–diffusion method: schematic procedure and operating variables.

Table 1
Solvent displacement method: examples of raw materials and work conditions used and size and zeta-potential of submicron spheres obtained.

Organic phase			Stabilizing agent/Nonsolvent phase	Work conditions		Size (nm)	Zeta potential (mV)	Drug loading (%)	Drug entrapment efficiency (%)	Reference
Polymer	Solvent	Other		Way of organic phase addition	System stirring					
PCL PDLLA100 PLGA (85:15; 50:50) (0.125 g)	Acetone (20 ml)	Isradipine (2 mg)	PLX 188 0.5% (50 ml)	nr.	nr.	200–210	−20 to −35	nr.	74–97	[14]
PCL Mw: 10 kDa (125 mg)	Acetone:EtOH 6:4 (10 ml)	Rolipram (20 mg)	PVA Mw: 20 kDa; 80% Hydrolyzed, sodium cholate Mw: 430.6 Da (0.1–10%) (100 ml)	Stepwise	400 rpm	150–345	−5 to −55	nr.	2–20	[15]
PDLLA Mw: 90–120 g/mol (100–300 mg)	Acetone, DCM, EtOH (20 ml)	Tyrphostin AG-1295 (0–3 mg)	PLX 188 (20–50 mg) (40 ml)	nr.	Moderate stirring	50–140	nr.	nr.	70	[16]
PLGA 50:50 (Mw: 7, 14, 24, 48 and 63 kDa) PLGA 65:35 (Mw: 114 kDa) PLGA 75:25 (Mw: 92 kDa) PDLLA (Mw: 109 kDa)	Acetone (5 ml)	Haloperidol (0.5 mg/ml)	PVA (Mw: 25 kDa; 88% hydrolyzed) 1% (50 ml)	nr.	nr.	170–230	nr.	0.25–4	nr.	[18]
PDLLA Mw:88 kDa (150 mg)	Acetone (40 ml)	Primaquine (7.5 mg) Soy phospholipid mixture (150 mg)	PLX 188 0.14–0.3%, pH 9.0 (80 ml)	nr.	Magnetic stirring	150–165	−35 to +1.5	nr.	85–94	[29]
PCL Mw: 14 kDa (0.5–15%)	Acetone	nr.	W	nr.	nr.	150–200	nr.	nr.	nr.	[30]
PLGA 50:50 (100–200 mg)	Acetone (20–40 ml)	Cyclosporin A (100 μg/ml)	PVA, PLX 188 (100 mg) (20–40 ml)	Addition rate: 0.1–0.6 ml/s	500 rpm	45–145	nr.	nr.	82–98	[31]
Gliadin (100 mg)	EtOH:W 7:3, MetOH:W 8:2, Acetone:W 5:5, Propan-1-ol:W 5:5, Propan-2-ol:W 5.5:4.5 (20 ml)	All-trans-retinoic acid	PLX 188 (0.5% w/w) in Physiological saline solution (0.9% NaCl) (40 ml)	nr.	250 rpm	460–1000	nr.	0.5–8	75–97	[32]
PCL Mw: 78 kDa, 29.4 kDa; PDLLA 50 Mw: 81.9 kDa; PLGA 75:25 Mw: 96.8 kDa; PLGA 75:25 Mw: 71.6 kDa; PLGA 50:50 Mw: 51.5 kDa (0.5/0.6% of final product)	Acetone	nr.	PLX 188	nr.	Moderate magnetic stirring	110–235	nr.	nr.	nr.	[33]
PDLLA Mw: 90,000 PCL-Mw: 128,000 PLGA 50:75	Acetone (15 ml)	nr.	PLX 188 (38 mg) (15 ml)	nr.	Magnetic stirring	115–220	−20 to −35	nr.	nr.	[34]
PCL (40.75–159.25 mg)	Acetone (8.10–31.89 ml)	Cyclosporin A (1 mg)	PLX (40.75–159.25 mg) (40 ml)	Addition rate: 23 ml/min	Magnetic stirring	110–215	nr.	nr.	90–98	[35]
PLGA 85:15 Mw: 47 kDa (200 mg)	Acetone:DCM (25:0.5 ml)	nr.	PVA different degrees of hydrolyzation (4%w/w) (50 ml)	Addition rate: 2 ml/min	400 rpm	190–315	nr.	nr.	nr.	[36]
PDLLA Mw: 200 kDa (125 mg)	Acetone (25 ml)	Pentamidine (0.5 mg/ml) Soy bean lecithin (0.6–1.25%)	PLX 188 (1.25–3% w/v, pH 7.5–8)	nr.	Magnetic stirring	130–155	nr.	nr.	40–76	[37]
Ethylcellulose (83 kDa)	EtOH	nr.	W	nr.	300–3000 rpm	60–100	nr.	nr.	nr.	[38]
HP55 (1–4%)	Acetone:W (different proportions) (10 ml)	nr.	W (20 ml)	nr.	Moderate magnetic stirring	~300	nr.	nr.	nr.	[39]
Gliadin (0.5% w/v)	EtOH:W (20 ml)	nr.	PLX 188 (0.5% w/w) in physiological saline solution (0.9% NaCl) (40 ml)	Aqueous phase slowly added into organic phase	500 rpm	170–370	nr.	nr.	nr.	[40]
PLGA 75:25 (Mw: 10 kDa) (75 mg)	Acetone (5 ml)	Vancomycin, phenobarbital, valproic acid, cyclosporin A, indomethacin, and ketoprofen (2.5 mg)	PLX 188 (75 mg) (15 ml)	nr.	Moderate stirring	155–170	nr.	nr.	5–94	[41]

(continued on next page)

Table 1 (*continued*)

Organic phase			Stabilizing agent/Nonsolvent phase	Work conditions		Size (nm)	Zeta potential (mV)	Drug loading (%)	Drug entrapment efficiency (%)	Reference
Polymer	Solvent	Other		Way of organic phase addition	System stirring					
PLGA 50:50 Mw: 10 kDa (50 mg)	ACN (5 ml)	Procaine hydrochloride (0–10%)	W pH 9.3 (15 ml)	Dropwise	Magnetic stirring	155–210	−50 to −55	0.2–4.5	28–62	[42]
PLA Mw: 28 kDa PLA-PEG (different molecular weight) (1–20 mg/ml)	Acetone, ACN (5 ml)	Procaine hydrochloride (2–20%)	W (15 ml)	nr.	nr.	50–150	−6 to −50	0.2–3.5	nr.	[43]
PCL Mn: 42.5 kDa PCLLA PDLLA (100 mg)	Acetone (20 ml)	Nimodipine (10 mg)	PLX 188 0.2% (50 ml)	Dropwise	Moderate stirring	80–135	nr.	3.5–9	20–90	[44]
PLA-PEG copolymers (50 mg)	ACN (5 ml)	Procaine hydrochloride (0–20% w/w)	W pH 5.8 (15 ml)	nr.	Magnetic stirring	50–175	−5 to −30	0.2–0.3	6–11	[45]
PLGA 50:50 Mw: 40,000 g/mol, PVA-g-PLGA, SBPVA-g-PLGA (100 mg)	Acetone:EtAc (0–32.5%) (10 ml)	nr.	PLX 188 0.1% w/w (50 ml)	Addition rate: 10 ml/min	250 rpm	100–120	−3 to −25	nr.	nr.	[46]
PLGA 85:15, 75:25, 50:50 PLA 05, 10, 20. (5 g)	Acetone, ACN, EtOH (125 ml)	nr.	PVA 4% w/w (300 ml)	Addition rate: 10 ml/min	400 rpm	200–270	nr.	nr.	nr.	[47]
PLGA (Mw: 22 kDa) PLGA-mPEG (Mw: 37 kDa)	Acetone	^{125}I-CA	Sodium cholate (12 mM) in PBS, pH 7.4	Dropwise	Stirred solution	110–160	−4.0 to −45	nr.	>70	[48]
PLGA 75:25 (25 mg)	Acetone (5 ml)	Rose Bengal (2.5–10 mg)	W (10 ml)	Dropwise	Gentle magnetic stirring.	135–150	−40 to −55	0.3–0.8	1–1.6	[49]
PVM/MA Mw: 200 kDa (100 mg)	Acetone (5 ml)	nr.	EtOH:W (1:1) 10 ml	nr.	nr.	250–280	−25 to +30	nr.	nr.	[50]
PLGA 50:50 (Mw: 6 and 14.5 kDa), 75:25 Mw: 63.3 kDa (100 mg)	Acetone (10 ml)	Paclitaxel (0.4–1 mg)	PLX 188 0.25% (10 or 20 ml)	nr.	Magnetic stirring	115–160	−20 to −35	nr.	15–100	[51]
PLA Mw: 2 kDa (25 mg)	Acetone, EtOH or MetOH (0.3 ml) CHCl3 (1.2–2.0 ml)	Sodium cromoglycate (2.5 mg) PG (150 mg)	EtOH (70%) (5 ml)	nr.	Mixing	200–270	−4 to −8	nr.	nr.	[52]
PCL Mw: 60 kDa PDLLA (1 g) PLGA Mw (75 kDa), PDLLA50 Mw: 42 kDa, PCL	Acetone (270 ml)	Indomethacin (0.150 g)	Polysorbate 80 (0.766 g) (530 ml)	nr.	Moderate magnetic stirring	170–180	nr.	nr.	100	[53]
Mw: 40 kDa, PEG5-PLGA, PEG20-PDLLA, PEG5-PDLLA, PEG5-PCL (20 mg)	Acetone (1 ml)	Antiestrogen RU58668 (2×10^{-5}–10^{-3} M)	PLX 188 (1%) or W (2 ml)	Rapidly dispersed	nr.	75–265	−10 to −65	3.1–3.3	94–100	[54]
PLGA, PLGA-mPEG (different molecular weight)	Acetone	nr.	Sodium cholate (12 mM)	Dropwise	Stirred solution	55–135	−5 to −55	nr.	nr.	[55]
PDLLA Mw: 16 kDa; 109 kDa; 209 kDa (75 mg)	Acetone (20 ml)	Acyclovir (165 mg)	Brij 96, PLX 188, Triton X100 or Polysorbate 80 (0.25–2%) in EtOH:W (1:1 v/v) (40 ml)	nr.	Magnetic stirring	105–265	−10 to −35	2–8	1–3.5	[56]
PCL Mw: 80 kDa PMMA (1 g) PDLLA50 Mw: 42 kDa, PLGA Mw: 75 kDa, PCL	Acetone (267 ml)	Diclofenac (0.1 g)	Polysorbate 80 (0.766 g) (533 ml)	nr.	Moderate magnetic stirring	85–195	nr.	nr.	100	[57]
Mw: 40 kDa, PEG-PDLLA, PEG-PLGA, PEG-PCL (20 mg)	Acetone (1 ml)	Antiestrogen RU 58668 (2×10^{-5} to 10^{-3} M)	PLX 188 1% or W (2 ml)	Rapidly dispersion	nr.	95–260	−5 to −65	3.1–3.3	94–100	[58]
PMMA type C NF/USP (Eudragit L100-55) (360–810 mg)	Acetone, DMSO, Isopropyl alcohol, EtOH, Ethyl lactate (25 ml)	nr.	PVA Mw: 26,000, 88% hydrolyzed (0.4% w/w) (50 ml)	nr.	Stirred magnetically	95–325	nr.	nr.	nr.	[59]
PCL-PEG diblock copolymer	Acetone, THF (4 ml)	All-trans-retinoic acid	W (10 ml)	nr.	nr.	70–460	nr.	2.2–10.8	68–97	[60]

PLGA 74:26 Mw: 50 kDa, PLGA 73:27 Mw: 20 kDa (1% of the organic phase)	Acetone (2–10 ml)	5-Fluorouracil (10 mg)	PLX 188 (1%), PLX F127 (1%), PVA (10%)	nr.	Moderate stirring	75–255	nr.	nr.	66–78	[61]
PMMA type C NF/USP (Eudragit L100-55) (1.44% w/w)	Acetone (25 ml)	Ibuprofen (1.4%)	PVA Mw: 26,000, 88% hydrolyzed (0.8% w/w) (50 ml)	nr.	Stirred magnetically	105–145	nr.	3.2–4.5	40–50	[62]
PCL Mw: 14.8 kDa (1% w/v)	Acetone (50 ml)	Tamoxifen	PLX 188, PLX F108 (0.1–0.5%)	Addition rate: 1 ml/min	Magnetic stirring	180–800	−15 to +25	20	> 90	[63]
PLGA 50:50 Mw: 50–75 kDa (63 mg)	Acetone (10 ml)	XAN or 3-MeOXAN (60 μg/ml)	PLX 0.25% (10 ml)	nr.	Magnetic stirring	150–165	−35 to −40	nr.	26–40	[64]
PCL Mw: 80 kDa (138 mg)	Acetone (25 ml)	Griseofulvin (0–13.8 mg) Span 80 (50 mg)	Polysorbate 80 (100 mg) (50 ml)	Addition rate: 48 ml/min	Magnetic stirring	250–325	nr.	1–7	78–98	[65]
PDLLA Mw: 16 kDa; 109 kDa; 209 kDa PLGA 50:50 (75 mg)	Acetone (20 ml)	Docetaxel (0.5–1% in weight drug/polymer)	Polysorbate 80 0.5% in W:EtOH (1:1 v/v) (40 ml)	nr.	Magnetic stirring	95–175	−2 to −40	nr.	10–23	[66]
PLGA 50:50 Mw: 8 kDa (500 mg)	ACN:EtOH (60:40) (12 ml)	Zinc phthalocyanine (0.5 mg)	PLX 407 5% w/w (50 ml)	nr.	500 rpm	200–210	nr.	nr.	70	[67]
PLGA 75:25 Mw: 98 kDa (90 mg)	Acetone (25 ml)	Flurbiprofen (0.16–1.84 mg/ml)	PLX 188 (6.6–23.4 mg/ml) (50 ml)	nr.	Moderate stirring	150–290	−25 to −30	nr.	74–97	[68]
Hydrophobic derivatives of dextran DexPx, DexC6x, DexC10x (8.5–25%)	THF (1 ml)	nr.	DexP15 (0 and 0.5%) 10 ml	Dropwise	Vigorous magnetic stirring	150–300	nr.	nr.	nr.	[69]
PLGA-b-PEG-COOH (5–50 mg/ml)	Acetone, ACN, DMF, THF	Docetaxel (0–10% of the polymer)	W (2 x organic phase volume)	Dropwise	Stirring	65–295	nr.	nr.	nr.	[70]
PDLLA Mw = 22,600 to 124,800 g/mol (5–20 mg/ml)	THF, Acetone (4.5 ml)	nr.	W (9 ml)	Addition rate: 4.5 ml/min	300 rpm	75–325	nr.	nr.	nr.	[71]
Poly(H2NPEGCA-co-HDCA) (40 mg)	Acetone: EtOH (4 ml)	4-(N)-acyl-gemcitabine derivatives	W (8 ml)	nr.	nr.	150–190	+25 to +35	0.3–10	6–100	[72]
PDLLA Mw: 3 kDa	Acetone:EtOH	Oridonin	PLX 188	Dropwise	400 rpm	105–195	~−15	2.3	92	[73]
PDLLA 0.20 dl/g (25 mg)	Acetone (2 ml)	nr.	PLX 188 (4 ml)	nr.	Mild stirring	240–290	−15 to −40	nr.	nr.	[74]
PCL Mw: 14 kDa (0.25–10 mg/ml) PCL 80 kDa (0.03–4 mg/ml)	Acetone	nr.	W	Addition rate: 3–120 ml/min	nr.	130–630	nr.	nr.	nr.	[75]
PLGA 50:50 (25–150 mg)	Acetone, ACN (5 ml)	Coenzyme Q10 (1/10 mg)	W or PBS (50 ml)	nr.	Magnetic stirring	125–260	~−40	1–19	49–72	[76]
PES (125 mg)	THF (10 ml)	Silymarin (25 mg)	PVA, PLX 188 or Polysorbate 80 in W (25 ml)	Dropwise	Continuous stirring	200–1000	nr.	nr.	20–62	[77]
PLGA (125–175 mg)	Acetone (10 ml)	Carvedilol (5–10 mg)	PLX 188 (250–350 mg) (20 ml)	Addition rate: 10 ml/min	Stirring	130–235	nr.	1.2–4.5	41–56	[78]
PLGA (RG502H, RG503H, RG504H) P(VS-VA)-g-PLGA-4-10 (1–10 mg/ml)	Acetone, ACN, THF	Salbutamol	PLX 188	Addition rate: 10 ml/min	500 rpm	60–190	−25 to −45	1.4–3.2	nr.	[79]
PLA, DexC10$_{164}$ (10–25 g/l of organic phase)	THF, Acetone (4.5 ml)	nr.	DexP20 (2–10 g/l)	Dropwise	Vigorous magnetic stirring	140–240	nr.	nr.	nr.	[80]

nr: non-reported; PLGA: poly(D,L-lactide-co-glycolide); P(VS-VA)-g-PLGA: poly(vinyl sulfonate-co-vinyl alcohol)-graft-poly(D,L-lactide-co-glycolide); ACN: Acetonitrile; THF: Tetrahydrofuran; 125I-CA-labeled: 125I bound to cholesterylaniline; PLGA-mPEG: poly(lactide-coglycolide) monomethoxy(polyethyleneglycol); LA: D,L-lactide; GA: glycolide; PBS: phosphate buffered saline; PEG: poly(ethylene glycol); PCL: Poly-e-caprolactone; PLGA-b-PEG-COOH: carboxy-terminated poly(D,L-lactide-co-glycolide)-block-poly(ethylene glycol); Brij 96: decaethylenglycol oleyl ether; PES: polyethylene sebacate; PCL-PEG diblock copolymer: poly(e-caprolactone)/poly(ethylene glycol); SB-PVA-g-PLGA: poly(2-sulfobutyl-vinyl alcohol)-g-poly(lactide-co-glycolide); CHCl3: Chloroform; PG: Propylene glycol; Poly(H2NPEGCA-co-HDCA): poly[aminopoly(ethylene glycol)cyanoacrylate-co-hexadecyl cyanoacrylate]; XAN: Xanthone; 3-MeOXAN: 3-methoxyxanthone; HP55: hydroxypropyl methylcellulose phthalate; PVM/MA: Poly(methyl vinyl ether-co-maleic anhydride); DexPx, DexC6x, DexC10x: hydrophobic derivatives of dextran where x is the substitution ratio, i.e. the average number of grafted phenoxy, C6 or C10 alkyl chains respectively per 100 glucose units; PCLLA: copolymer of e-caprolactone and L-lactide; PDLLA: poly(D,L-lactic acid); PLA: poly(L-lactic acid); PMMA: Poly(methyl methacrylate); W: Table 2. Emulsification–diffusion method: examples of raw materials and work conditions used and size and zeta-potential of submicron spheres obtained.

Table 2
Emulsification–diffusion method: examples of raw materials and work conditions used and size and zeta-potential of submicron spheres obtained.

Organic phase			Stabilizing agent–aqueous phase	Dilution phase	Work conditions		Size (nm)	Zeta potential (mV)	Drug loading (%)	Drug entrapment efficiency (%)	Reference
Polymer	Solvent	Other			Emulsification	Diffusion					
PLGA 50:50 (50 mg)	Acetone, CHCl3, DCM, EtAc (2.5 ml)	Estradiol (5 mg)	DMAB, PVA Mw: 30 kDa (1%, 5 ml)	W	15,000 rpm, 5 min	Constant stirring	95–585	+70 to +95	nr.	48–95	[22]
PLGA 50:50 (50 mg)	EtAc (2.5 ml)	Estradiol (5 mg)	DMBA, PVA (5 ml)	W	15,000 rpm, 5 min	Constant stirring on a water bath set at 40 °C	100–655	−1 to +70	nr.	46–73	[26]
PDLLA 100DL, PLGA 85:15, PCL, PMMA S100 (3 g)	BA (21 g)	Chlorambucil	PVA 26 kDa, gelatin (10–28%, 40 g)	W or buffer (660 g)	1200 rpm, 10 min	nr.	70–1000	nr.	5.5–8.5	60–63	[28]
PMMA L100-55 (3 g)	BA (21 g)	nr.	PVA Mw: 26 kDa (7–21%, 30 g)	W (660 g)	2000 rpm, 15 min	nr.	105–715	nr.	nr.	nr.	[59]
PMMA L100-55 (3 g)	BA (21 g)	Ibuprofen (1.4%)	PVA Mw: 26 kDa (12%, 40 g)	W (660 g)	2000 rpm, 15 min	nr.	310–430	nr.	5.5–8	62–86	[62]
PDLLA100 (200 mg)	PC (10 ml)	nr.	PVA Mw: 26 kDa, 30–70 kDa, PLX 188 (5%, 20 ml)	W (80 ml)	8000 rpm, 10 min	Stirring	100–450	nr.	nr.	nr.	[81]
PDLLA100 (200 mg)	EtAc (20 ml)	nr.	PVA Mw: 26 kDa (5%, 20 ml)	W (200 ml)	8000 rpm, 10 min	Stirring	~174	nr.	nr.	nr.	[82]
PLGA 75:25 Mw: 75–120 kDa (1–4 mg)	PC (10 ml)	17b-estradiol benzoate (3 mg)	DMAB (1.0–4.0%), PVA Mw: 30–70 kDa (2.5–10%, 20 ml)	W (80 ml)	4800–15,000 rpm, 7 min	Moderate magnetic stirring	75–350	nr.	nr.	67	[83]
PLGA 50:50 Mw: 12 kDa, PMMA S100 (5%)	BA (2.1 g)	Enalaprilat (42 mg)	PVA Mw: 27 kDa (10–20%, 4 g)	W (66 g)	15,000 rpm, 5 min	nr.	180–615	−30 to −60	7–13	24–46	[84]
PLGA 75:25 Mw: 75–120 kDa (200 mg)	MEK, EtAc, PC, BA (10 ml)	nr.	PLX 188 (20 ml)	W (500 ml)	12,000 rpm, 7 min	Moderate magnetic stirring	120–270	nr.	nr.	nr.	[85]
PLGA 50:50 Mw: 12 kDa, PLGA 75:25 Mw: 12 kDa, PDLLA Mw: 22 kDa	BA (6 g)	p-THPP (0–20%)	PVA 4-88 Mw: 26 kDa (17%, 8 g)	W (500 ml)	2000 rpm, 15 min	2000 rpm	90–160	−4 to −8	3.5–13	47–91	[86]
PDLLA50 Mw: 30 kDa (0.4–2 g)	EtAC (20 ml)	nr.	PLX 188 (0.5–5%, 40 ml)	W (215 ml)	8000 rpm, 5 min	Moderate stirring	230–560	nr.	nr.	nr.	[87]
PLGA 70:30 (200 mg)	EtAc (10 ml)	nr.	PVA (100 mg) and chitosan (30 mg) (10 ml)	W	13,500 rpm, 10 min	Stirring	100–180	+10 to +30	nr.	nr.	[88]
PLGA RG502 Mw: 8 kDa, PDLLA Mw: 2 kDa, CAP Mw: 2.5 kDa (400 mg)	EtAc, MEK (20 ml)	Triclosan (0–33%)	PVA (5%, 40 ml)	W (160 ml)	1700 rpm, 10 min	nr.	175–450	nr.	0.8–24	63–89	[89]
PLGA 75:25 Mw: 75–120 kDa (100 mg)	DCM, EtAc, PC, Acetone (10 ml)	nr.	DMAB, PVA Mw: 9–10 kDa, PLX 188 (1%, 20 ml)	W (80 ml)	1 min, sonicator operating 40% amplitude intensity	Moderate magnetic stirring	50–460	nr.	nr.	nr.	[90]
PCL Mw: 42.5 kDa (0.5 g)	EtAc (10 ml)	Magnetite (0.3 g), gemcitabine.HCl (150 mg)	PVA 15–20 kDa (20 ml)	W	Probe sonicator at 200 W, 10 min	Moderate stirring	130–170	nr.	0.1–0.7	3.4–8	[91]
PLGA 50:50 (50 mg)	EtAc, DCM, CHCl3, EtAc:DCM 20:80 (2 ml)	Cyclosporine (10 mg)	DMAB (0.1%, 3 ml)	W (30 ml)	Sonication, 1 min	1000 rpm	60–270	nr.	nr.	16–23	[92]
PLGA 50:50 (Mw: 14.5; 45; 85; 137; 213 kDa)											
PLGA 65:35 (Mw: 97 kDa), PLGA 85:15 (Mw: 87 kDa) (50 mg)	EtAc (10 ml)	Estradiol (5 mg)	DMAB (1%, 20 ml)	W (80 ml)	15,000 rpm, 5 min	Constant stirring	90–155	+70 to +105	nr.	35–68	[93]
PHBHV Mw: 23, 300 kDa. (20 mg)	$CHCl_3$:EtOH (different proportions) (4 ml)	nr.	PVA Mw: 200 kDa (0.025%, 80 ml)	PVA 0.01% (200 ml)	17,500 rpm, 5 min	Moderate magnetic stirring	250–890	nr.	nr.	nr.	[94]
Propyl-starch derivatives (degrees of substitution: 1.05 and 1.45) (1 mg)	EtAc (1 ml)	nr.	PVA (0–1%, 4 ml)	W (5 ml)	14,000 rpm, 15 min	nr.	150–185	−5 to −9	nr.	nr.	[95]
PLGA 50:50 Mw 5–70 kDa, PLGA-mPEG (10 mg/ml)	EtAc (10 ml)	Tacrolimus (10 mg)	PLX 188 (2%, 20 ml)	W (90 ml)	20,000 rpm, 10 min.	Magnetic stirring	215–220	−20 to −30	nr.	50–60	[96]

nr: non-reported; BA: Benzyl alcohol; CAP: cellulose acetate phthalate; DMAB: didodecyldimethyl ammonium bromide; EtAc: Ethyl acetate; MEK: Methyl ethyl ketone; PC: Propylene carbonate; PHBHV: poly(3-hydroxybutyrate-co-hydroxyvalerate); PDLLA: poly(D,L-lactic acid); PLA-PEG: methoxy PEG-(D,L-lactide); PLGA: Poly(D,L-lactide-co-glycolide); PLGA-mPEG: Poly(lactide-co-glycolide)-methoxy poly(ethylene glycol); PLX 188: Poloxamer 188; PMMA: poly (methyl methacrylate); p-THPP: Mesotetra(p-hydroxyphenyl)porphyrin; PVA: polyvinyl alcohol; W: Water.

different authors. The quantity used of each material, the operating conditions worked and the results of size and zeta-potential obtained in each study are also included. Briefly, poly-ε-caprolactone (PCL), poly(D,L-lactic acid) (PDLLA) and poly(D,L-lactic-co-glycolic acid) (PLGA) are the polymers used most often. However, alternatives such as starch and cellulose derivatives, polyethylene sebacate, hydrophobic dextranes, poly(methyl methacrylates), poly(methyl vinyl ether-co maleic anhydride), poly-cyanoacrylates, poly(3-hydroxybutyrate-co-hydroxyvalerate) and copolymers based on polyethylene glycol or polyvinyl alcohol have also been investigated. The use of poloxamer and polyvinyl alcohol as stabilizing agents predominates. Certain research works have reported the use of sodium cholate, polysorbate 80, Brij 96, triton and hydrophilic dextrans, and a few studies have been performed without stabilizing agent or using buffering agents. The organic solvents are chosen for each method as a function of their specific solvent requirements. Thus water-miscible solvents such as acetone, tetrahydrofuran, acetonitrile, methanol, ethanol, isopropanol, ethyl lactate, dimethyl sulfoxide and dimethyl formamide have been used for particle preparation by the solvent displacement method. Also, partially water-miscible solvents such as ethyl acetate, benzyl alcohol, propylene carbonate and methyl ethyl ketone have been chosen in studies of the emulsification–diffusion method. In addition, water is the dilution phase commonly used in this latter method while buffer solutions or stabilizing agent solutions at low concentration have been used with this purpose, though more rarely.

It can be concluded that solvent displacement and emulsification diffusion are versatile methods from the standpoint of the polymers and stabilizing agents that can be used. Thus synthetic, semi-synthetic and natural starting materials can be investigated. However, research into new materials is limited to those soluble in the few organic solvents capable of satisfying total or partial water miscibility requirements. This variety is even more limited when submicron particles are intended as carriers of active molecules due to the safety requirements for organic solvents, such as their low toxicity. On the other hand, it is interesting to note that research on new starting materials and on the encapsulation of active molecules is more intensive for the solvent displacement technique than for emulsification–diffusion method. This is probably due to the advantages associated with the ease of implementing this method. In addition, low amounts of stabilizing agent are used which can facilitate subsequent purification steps.

Lipophilic-like active substances are generally used when submicron spheres are prepared by the two methods (Tables 1 and 2). However, they have also been modified for loading hydrophilic molecules such as peptides and proteins by the solvent displacement technique [97–99] or for using other starting materials such as lipid substances, in order to obtain solid lipid particles by emulsification–diffusion [100,101].

The two methods allow active substance loading higher than 10% and entrapment efficiencies higher than 70% (Tables 1 and 2). However, wide ranges are also reported for these parameters in both solvent displacement [15,41,42,44,51,72,77] and emulsification–diffusion studies [22,86,93]. Unfortunately, there are no works have been published providing comparisons of the two methods when the same active substance is used. In addition, as shown in Table 3, contradictory conclusions have been obtained by researchers when the same variable was investigated, possibly due to the purification and concentration of the particles after their preparation (e.g., washing [15,26,32,56,63,66,67,73,93], dialysis [72,83], ultrafiltration–centrifugation [29,37,53,57,68], ultracentrifugation [31,35,41–45,54,58,92], cross-flow filtration [28,86], filtration by 0.1 μm filter [65,84], centrifugation [22,62,64,76,77,89,91,96] and separation by gel filtration [14,48]).

Despite this, an all-embracing view of the conclusions reported by different authors on the effect of operating variables and starting materials on the entrapment of active molecules (Table 3) allows us extending the general statements suggested by Sahana et al. [22] in the case of emulsification–diffusion method to the solvent displacement technique. Thus, the highest entrapment efficiency is reached at the lowest molecule solubility in the aqueous phase, the fastest rate of polymer precipitation/solidification, the largest solid-state solubility of the molecule in the polymer and the highest affinity between the organic solvents and the aqueous phase. Consequently, the nature and concentration of the stabilizing agent, the pH of the aqueous phase,

Table 3
Influence of operating variables and starting materials on the entrapment efficiency and on the loading of active substances into submicron spheres prepared by solvent displacement and emulsification–diffusion methods.

Variable	Solvent displacement	Emulsification–diffusion
Operating variables		
Stirring rate	The lowest stirring rate the largest EE [31]	nr.
Method for preparing organic phase	There is influence on EE [51]	
Aqueous to organic phase volume	The lowest aqueous phase volume the smallest EE [16,18] The highest organic/aqueous phase ratio the lowest EE [77]	
Starting materials		
Drug nature	Hydrophilic molecules show the lowest EE [45,79]	nr.
Drug initial amount	EE increases as drug initial amount increases till a maximum value. After drug precipitation occurs.	The largest drug initial amount the largest EE [26,91].
	Therefore, it is common that the largest initial concentration the largest DL but, the largest initial concentration the lowest EE [18,29,42,43,49,64,65,68,76].	There is influence but without a particular trend [89]
	Drug/polymer ratio has incidence on EE [32,77,78]	The largest drug initial amount the largest DL but, the largest initial concentration the lowest EE [86].
	Non significant influence on EE [16]	Non significant influence on EE [28]
Polymer nature	The best drug–polymer affinity the largest EE [41,44,56,60,66,72,79].	The best drug–polymer affinity the largest EE [84,89,93].
	Non significant influence on EE [14,51,54,58,64,66].	Non significant influence on EE [86,96].
Polymer concentration	The largest polymer concentration the largest EE [31,35,68,77].	Non significant influence on EE [93].
	Non significant influence on EE [56,79].	
Stabilizing agent nature	Non significant influence on EE [15,77].	There is influence on EE [22]
Stabilizing agent concentration	The largest stabilizing agent concentration the largest EE [15].	The largest stabilizing agent concentration the largest EE [26].
	The largest stabilizing agent concentration the lowest EE [35].	The lowest stabilizing agent concentration the largest EE [92].
	Non significant influence on EE [18].	Non significant influence on EE [84,96]
Aqueous phase pH	Significant influence on EE [42,68].	Non significant influence on EE [28]
Solvent nature	Significant influence on EE [32,60].	The highest solvent water solubility the largest EE [22].
		Non significant influence on EE [92]

EE: Entrapment efficiency; DL: Drug loading; nr.: None reported information.

and the natures of the polymer and the solvent prove to be the key variables in governing the entrapment of active substances. Also, the initial amount of active substance turns out to be particularly important when the solvent displacement technique is used, which in turn might be linked to active substance–polymer affinity.

3. Mechanistic aspects related to particle formation by solvent displacement and emulsification–diffusion methods: The state of the art

First of all knowledge of the mechanistic aspects related to particle formation is necessary in order to obtain deeper understanding of the factors influencing the characteristics of submicron particles prepared by the solvent displacement technique and emulsification–diffusion method. Following this, the different approaches taken by each of the methods will be discussed.

3.1. Solvent displacement technique

Different approaches derived from the spontaneous emulsification process have been proposed in order to explain particle formation when the solvent displacement technique is used. Stainmesse et al. demonstrated in 1995 that submicron particles only can be formed at certain proportions of polymer, solvent and nonsolvent, characterized by a low concentration of polymer and small amount of organic solvent [30]. Afterwards, in 1998, Quintanar et al. proposed a mechanistic approach based on interfacial phenomena due to variations of surface tension between solvent/nonsolvent phases [3] while more recently, in 2005, Ganachaud and Katz [102] correlated the findings of Stainmesse with the "ouzo effect" proposed by Vitale and Katz [103] for homogeneous liquid–liquid nucleation.

In this review the two main approaches taken up-to-now are grouped as those based on mechanical mechanisms (dispersion mechanisms or spinodal decomposition) and those due to system chemical instability (condensation mechanism or nucleation), which is in line with the categorization adopted by other researchers [4,104–106].

Fig. 3 provides an illustration from a ternary phase diagram for the polymer/solvent/nonsolvent system. The particle dispersions are formed in the metastable region located between the binodal (miscibility-limit curve) and the spinodal (stability-limit curve) compositions. The mechanical mechanisms involve all the phenomena occurring from the spinodal region towards the metastable region and the nucleation approach ranges from the binodal curve towards the metastable region.

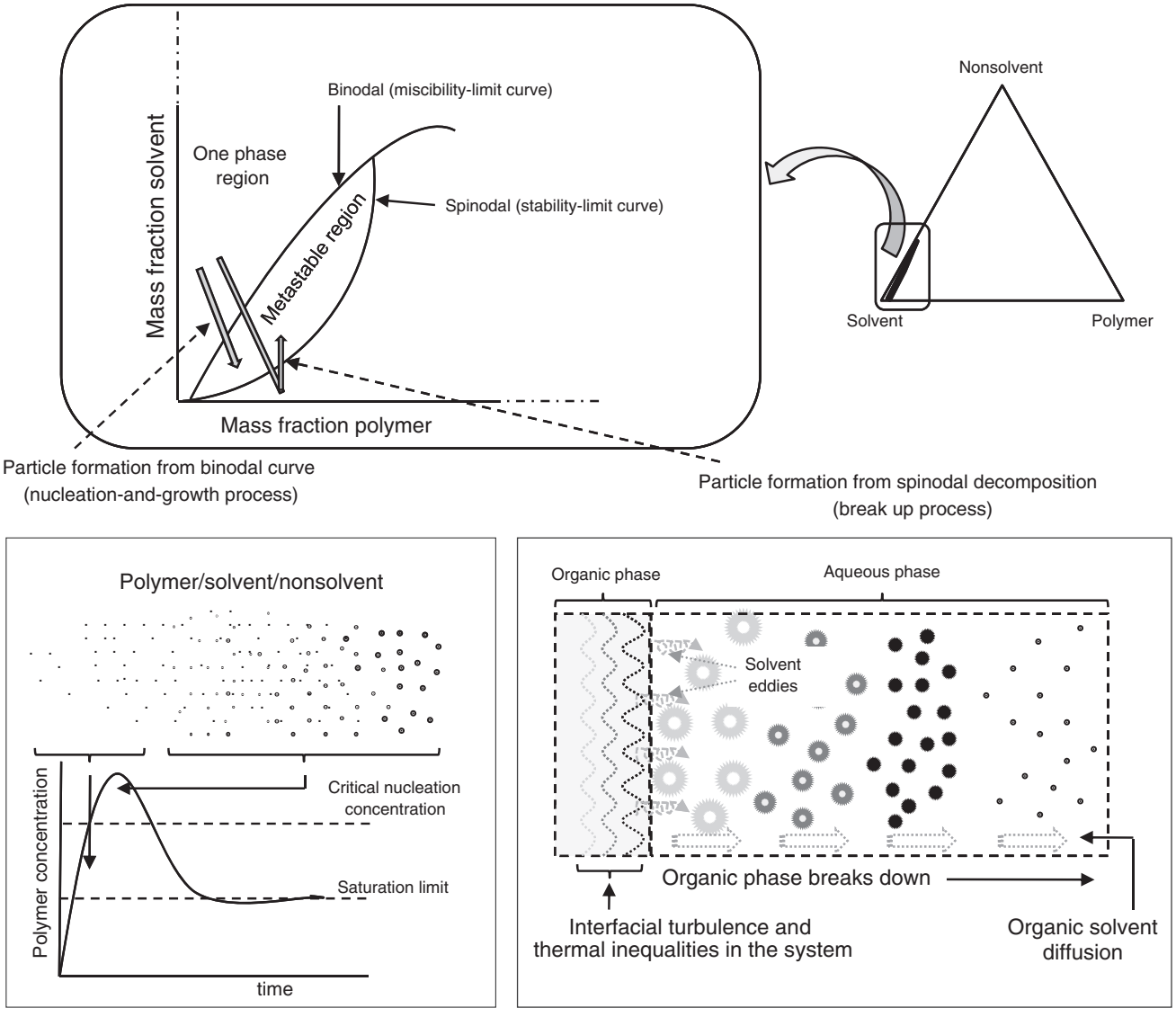

Fig. 3. Schematic representation of the mechanistic aspects related to the particle formation by solvent displacement method.

3.1.1. Mechanical mechanisms

The mechanical mechanisms for particle formation involve breaking up of the organic phase and dispersing it as drops in the aqueous phase. Thus Quintanar et al. and Galindo et al. [3,59] proposed the formation of submicron particles via interfacial turbulence or the Gibbs–Marangoni effect, taking into account the differences in surface tension between the solvent and nonsolvent used. Since a liquid with a high surface tension (aqueous solvent) pulls more strongly on the surrounding liquid than one with a low surface tension (organic solvent), this difference between surface tensions causes interfacial turbulence and thermal inequalities in the system, leading to the continuous formation of eddies of solvent at the interface of both liquids which generates interfacial convective flows. These flows contribute towards renewing the interfacial surface and are capable of sharply increasing the mass-exchange rate between the phases [107,108]. Consequently, violent spreading is observed due to mutual miscibility between the solvents which breaks down the organic phase into small droplets which again break down into smaller droplets and so on until forming "submicron droplets". Then the solvent flows away from regions of low surface tension and the polymer precipitates, forming submicron particles (Fig. 3).

The intensity of the interfacial tension gradients can be estimated by the Marangoni number (*Ma*). To guarantee system instability, *Ma* must be larger than a critical value that is specific for each solvent/nonsolvent system [109,110]. In the particular case where the surface tension gradient is caused by concentration gradients, the Marangoni number can be defined as follows [111]:

$$Ma = \frac{\Delta\gamma \cdot \Delta C}{\eta \cdot D_{AB}} \tag{1}$$

where: $\Delta\gamma$ is the rate of change of interfacial tension; ΔC is the concentration gradient, η is the viscosity of the organic phase and D_{AB} the diffusion coefficient of the organic phase in the aqueous phase. Thus it is obvious that in addition to surface tension, the viscosity of the aqueous phase plays a critical role. Research by Ostrovsky and Ostrovsky [108] showed that $\Delta\gamma$ decreases as the concentration of organic solvent in water increases. This reduces the intensity of the pulsations and, as a consequence, the Marangoni effect. Also, they highlighted differences in the intensity and frequency of the pulsations according to the organic solvent/aqueous phase system.

Although the Marangoni effect appears to be the most popular mechanical approach in view of the experimental research carried out, another mechanism for breaking up the organic phase into the aqueous phase was proposed by Montasser et al. [4]. This is a theoretical approach based on the results of spontaneous emulsification previously reported for ternary systems: toluene/ethanol/water or toluene/ethanol/water-surfactant. In this case, the driving force for breaking up the organic phase is the development of transient negative values of interfacial tensions that cause spontaneous interfacial expansion, generating a crowd of solvent droplets in the nonsolvent. The main argument in favor of this mechanism is the fact that the stabilizing agent used could inhibit the Marangoni effect without having any impact on spontaneous emulsification.

Research on spontaneous emulsification for oil–water systems has highlighted other kinds of instabilities possibly involved in spinodal decomposition [112]. For instance, Miller referred to Rayleigh–Taylor instability or the phase fragmentation phenomena governed by the drop curvature of the dispersed phase [105]. In fact, according to Ostrovsky and Ostrovsky [108] it has been demonstrated that mass transfer by Marangoni effect could not show total agreement with dependence on $\Delta\gamma$. In these cases, the intensity of the mixing process is also influenced by natural convection and forced mixing [108]. The Rayleigh number ($\overline{R}$) describes the natural convection intensity, which is proportional to both the mass transfer coefficient for a stable surface (K_D) and the expression on the right:

$$\overline{R} \approx K_D \approx (d\rho / dc)\Delta C\left(\sqrt{D_{AB}} / \eta\right) \tag{2}$$

where $d\rho/dc$ is the change in density of the aqueous phase with the concentration of organic phase added; ΔC is the change of concentration at the surface of the organic/aqueous phase; D_{AB} is the diffusion coefficient of the organic phase in the aqueous phase and η the organic phase viscosity [108]. As can be seen, the interaction between organic and aqueous phases and mixture density could also influence the efficiency of phase mixing.

3.1.2. Mechanism based on the chemical instability of the system

Chemical instability in polymer precipitation has been investigated by Beck et al. [79], Ganachaud and Katz [102] and Aubry et al. [106] who took into consideration that particles are formed both with and without surfactant. This suggests that interfacial tension variations that support Gibbs–Marangoni theory may not be critical for particle formation [79]. In this case, when the polymer solution is in contact with water, the solvent diffuses into the aqueous phase, creating a local supersaturation of polymer molecules which leads to spontaneous nucleation in the form of small particles ("protoparticles") that grow with time (nucleation-and-growth process) [105] (Fig. 3). This scenario presumes that the blending rate and the associated process of molecular diffusion are extremely rapid, in comparison to the nucleation rate [113]. Thus, when phases are mixed the free energy of the system changes in such a way that phase separation is energetically more favorable and the polymer molecules coalesce forming nuclei [5].

As shown by Lince et al. [75] in the case of solvent displacement process, the nucleation rate (J) can be calculated by the following expression:

$$J = \frac{2D}{d^5}\exp\left(-\frac{16\pi\gamma^3\,\tilde{v}^2}{3k_B^3T^3[\ln(S)]^2}\right) \tag{3}$$

where D is the molecular diffusion of the polymer molecule, d is its molecular diameter, k_B and T are the Boltzmann constant and absolute temperature respectively, γ is the interfacial tension between the already formed particles and the solution, $\tilde{v}$ is the polymer molecular volume and S is the super-saturation defined as the ratio of the actual polymer concentration and the solubility of the polymer in the solvent mixture.

If the nuclei radius is higher than the critical nucleus radius (r^*), the "protoparticle" can grow until the system reaches equilibrium (Fig. 3). r^* depends on the surface tension between the two phases (γ) and their difference in free energy per unit volume [5]:

$$r^* = -\frac{2\gamma}{\Delta g_v} \tag{4}$$

The particle growth rate is governed by the molecular weight of the polymer (M_w), its density (ρ), the mass transfer coefficient (k_m), the polymer concentration (c) and the super-saturation as follows [75]:

$$G = \frac{2k_m M_w c}{\rho}(S-1) \tag{5}$$

Particle aggregation can also occur via the Ostwald ripening phenomenon as explained by Horn and Rieger [5]. However, Lince et al. and Aubry et al. [75,106] stated that the aggregation phenomena depend on the size of the particles and their probability of encounters due to Brownian motion (perikinetic aggregation) and fluid motion (orthokinetic aggregation). The rate at which perikinetic aggregation occurs can be estimated from the dynamic viscosity of the dispersive medium (η), temperature (T), the Boltzman constant and the radii of the colliding particles (a_i, a_j) [114]:

$$k = \frac{8k_BT}{3\eta}\frac{\left(a_i + a_j\right)^2}{a_i a_j} \tag{6}$$

In turn, orthokinetic aggregation is influenced by particle size and shear rate (velocity gradient, G). Thus the collision rate coefficient is:

$$k_{ij} = \frac{4}{3}G\left(a_i + a_j\right)^3 \tag{7}$$

Also the number of aggregates can be estimated from the mass fraction of the solvent (f_s), the initial mass fraction of the polymer in the solvent (f_p^i), the densities of the dispersive medium and the particles (ρ_{sol} and ρ_p respectively), and the mean particle diameter (d) by the expression:

$$n = \frac{6 f_s f_p^i \rho_{sol}}{\rho_p \pi d^3} \tag{8}$$

Finally, it is possible to know the variation of particle size as a function of aggregation time, as shown by Aubry et al. [106]:

$$d^3 = \frac{8 k_B T \rho_{sol} f_s f_p^i}{\pi \rho_p \eta} \times t. \tag{9}$$

As can be seen, according to the nucleation-and-growth mechanism final particle size is governed by the growing process, the aggregation phenomena and the performance of the stabilizing agent during the nucleation process. In addition, since polymer precipitation obeys the classical nucleation theory, it can be either homogeneous or heterogeneous depending on the composition of the system [5]. Thus in the particular case of submicron particles as carriers of active substances, the interaction between the polymer and active substance may play an important role during particle formation.

Cluster structure could also affect nucleation rate, as was reported by Ruckenstein et al. [115]. Clusters are built by successively adding layers around the central molecule. Thus, for example, icosahedral configurations are recognized as being more preferred, energetically, by small clusters than amorphous or face-centered cubic (fcc) configurations. This is due to the number of bonds of the surface molecules located on the vertices, edges and facets of each structure, the way the cluster is formed and the number of nearest neighbors for interaction. The local molecular order in the icosahedral cluster exhibits an almost crystalline structure.

It is noteworthy that the main difference between mechanical mechanisms in particular, the Gibbs–Marangoni effect, and the nucleation-and-growth process is the driving force underlying particle formation. The Gibbs–Marangoni effect is referred to as "surface tension-driven flow" and, as mentioned above, variations in interfacial tension at the solvent/nonsolvent interface cause disturbances in mechanical equilibrium, resulting in low free energy [3]. As quantitative description of this phenomenon shows, the factors governing particle formation are the physicochemical properties of the organic phase and its interaction with the aqueous medium. Furthermore, nucleation and growth is a spontaneous process that is strongly dependent on the composition of the polymer/solvent/nonsolvent system, the interaction between the particles formed and the physicochemical properties of the dispersive medium [75,116].

At present, there is not enough experimental evidence with permission that favors a specific mechanistic approach. It appears that particle formation by using the solvent displacement technique occurs via the nucleation and growth process at low organic/aqueous phase ratios and low polymer concentrations. It is probable that the Gibbs–Marangoni effect is the prevailing mechanism when precipitation occurs at the highest polymer concentration and organic/aqueous phase ratio (between the composition ranges leading to efficient particle formation by using this method, as demonstrated by Stainmesse et al. [30]).

3.2. Emulsification–diffusion method

For this method, the first step of particle preparation is organic phase dispersion of globules in aqueous phase at high stirring speed. Taking into account that the organic solvent used is partially water soluble, mutual saturation of the phases is required in order to obtain an emulsion in thermodynamic equilibrium. Once the emulsion is formed, the submicron droplets are then diluted in water and the interaction between the emulsion droplets and the dilution phase is referred to as a "modification of phase equilibrium and solvent diffusion", which leads to polymer precipitation since the polymer is in poor solvent [3,83]. Two approaches to particle formation can be taken with this method (Fig. 4). The first is based on the Marangoni

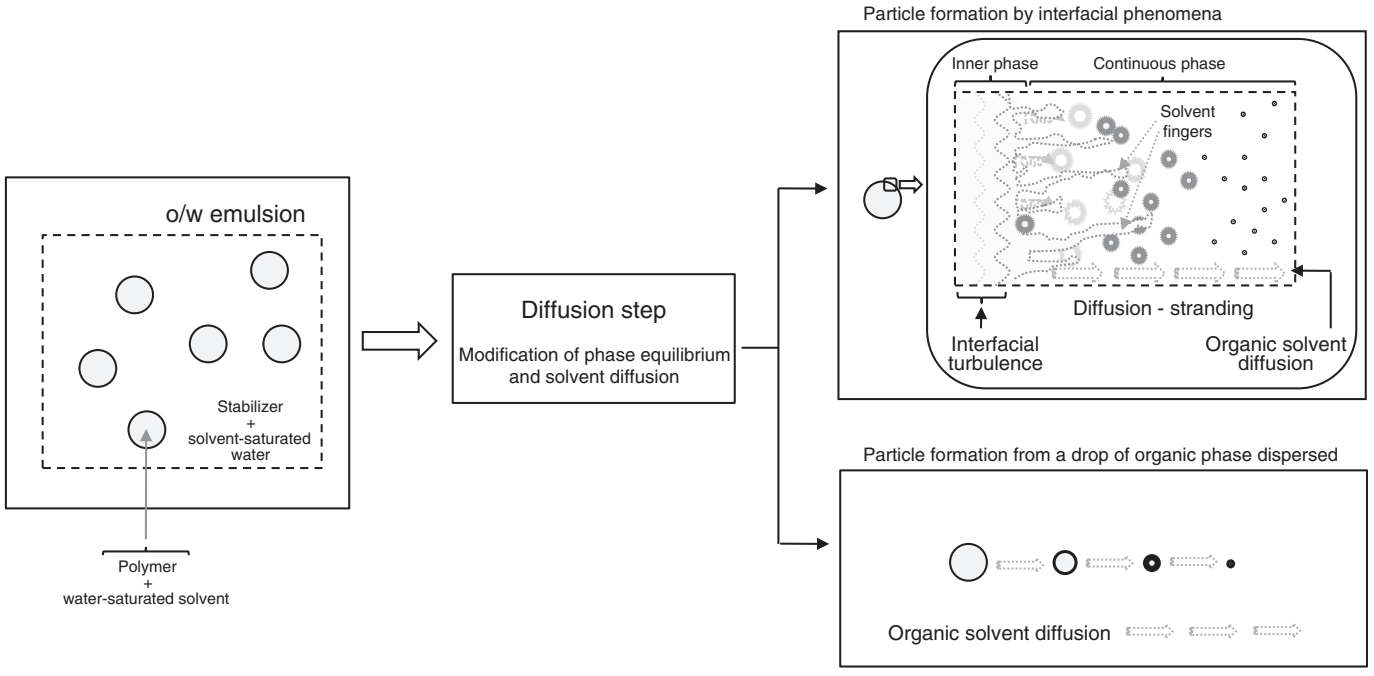

Fig. 4. Schematic representation of the mechanistic aspects related to the particle formation by emulsification–diffusion method.

effect (mechanical mechanism) and the second involves particle formation from droplets of emulsion.

3.2.1. Mechanical mechanism

The mechanical approach to particle preparation by using the emulsification–diffusion method was proposed by Quintanar et al. [3], based on polymer precipitation theories and interfacial phenomena, as explained previously for the solvent displacement method. However, in this case strong interfacial tension gradients cannot be driven by variations of interfacial concentrations since the solvent is partially water-miscible and it is water-saturated beforehand in order to maintain thermodynamic equilibrium during the emulsion step. In addition, higher stabilizing agent concentrations are used for the emulsification–diffusion method than for the solvent displacement procedure (usually 1.0% and 0.25%, respectively) which could drastically reduce the interfacial phenomena that govern the breakup of emulsion globules.

In addition, it is important to note that the interface between organic and aqueous phases was subjected to shear force during the emulsification step. According to Sternling and Scriven [109], energy will be dissipated because the molecules must be reoriented and that energy increases with the rate of shearing and the presence of surface-active agents. Thus, for the emulsification–diffusion method it might be expected that surface tension gradients can be due to the thermal effects associated with heat transport during organic solvent diffusion. Typically, the thermal Marangoni effect results in fingering instability [117] where the low interfacial tension difference and the drop curvature less than its spontaneous one allow that the flexible drop surface develops multiple undulations generating long fingers as the organic solvent diffuses towards the aqueous medium. In this process, the solvent carries polymer molecules into the aqueous phase. Then, if spontaneous curvature favors an organic phase-in-water arrangement, it could be expected that many drops of smaller diameter detach from the fingers and become dispersed in the aqueous phase. Thus new globules or polymer aggregates (not totally desolvated) are formed and stabilized by the stabilizing agent (protoparticles). The submicron particles will be formed after the complete diffusion of the solvent, if the stabilizing agent remains at the liquid–liquid interface during the diffusion process and if its protective effect is adequate (Fig. 4). The works of Moinard et al. [9] suggest that solvent diffusion from the droplets takes place too quickly (duration less than 20 ms), leading to the rapid formation of particles. The theoretical analysis of similar phenomena carried out for Miller supports this mechanistic approach [105].

As in the solvent displacement technique, the intensity of interfacial tension gradients can be estimated by the Marangoni number, but in this case thermal *Ma* is defined by the expression [111]:

$$Ma = \left|\frac{\partial\gamma}{\partial T}\right| \frac{\Delta\gamma \cdot \Delta T}{\eta \cdot \alpha} \tag{10}$$

where: $\left|\frac{\partial\gamma}{\partial T}\right|$ is the temperature coefficient of surface tension, $\Delta\gamma$ is the rate of change of interfacial tension; ΔT is the temperature gradient, η is the viscosity and α the thermal diffusivity. System instability occurs in this way if *Ma* is higher than the critical Marangoni number which is specific for each system [109].

3.2.2. Mechanism based on particle formation from an emulsion droplet

The second approach to submicron particle formation by using the emulsification–diffusion method is supported by the research performed by Guinebretière et al., Galindo et al., Moinard et al. and Hassou et al. [9,62,118,119]. It is strongly suggested that particles prepared after solvent diffusion are formed from an emulsion droplet (Fig. 4). Moinard et al. [118] demonstrated that mean particle size is always smaller than that of the emulsion droplets. Thus emulsion droplet size governs final particle size and consequently, it is directly influenced by all the operating variables linked to the preparation of the emulsion and their colloidal properties. Although the mathematical model developed by Moinard et al. [118] takes into account submicron capsules as model particles, a similar approach could be taken for submicron spheres. Thus the ratio between mean particle diameter (d_p) and mean diameter of the primary emulsion drop (d_{ed}) is determined by particle volume (V_p) and emulsion drop volume (V_{ed}), as in the following:

$$\frac{d_p}{d_{ed}} = \left[\frac{V_p}{V_{ed}}\right]^{\frac{1}{3}} \tag{11}$$

The way droplets in the organic phase are formed can be explained by binary break-up or by capillary break-up mechanisms [120]. With the binary break-up mechanism, droplets are continuously broken up into two fragments, until the drop size is small enough to survive the prevailing hydrodynamic conditions. With the capillary break-up mechanism, the droplet is stretched to produce a long filament that will fragment due to the action of capillary waves into a relatively large number of fragments during a single break-up. The prevalence of a particular mechanism depends on the capillary number (*Ca*) which is the ratio between the viscous stress that causes the droplet fragmentation and the restoring stress from surface forces [118]. *Ca* can be defined by the expression [120]:

$$Ca = \frac{r_{ed}\eta\dot{\gamma}}{2\gamma} \tag{12}$$

where r_{ed} is the drop emulsion radius, η is the aqueous phase viscosity, $\dot{\gamma}$ the shear stress and γ the interfacial tension between the organic and aqueous phases. The organic phase fragmentation occurs at a critical value of *Ca*, which in turn depends on the viscosity ratio between the organic phase and aqueous phases and on the presence of other components such as surfactants, as reported by Briscoe et al. [120]. Then, if the operating conditions or the physicochemical properties of the liquids lead to a capillary number just above the critical capillary number, the droplet breaks up via the binary break-up mechanism. If the capillary number is increased to a value well above the critical value, the capillary break-up mechanism prevails [120].

According to Galindo et al. [62], the maximum stable drop size of the droplets ($d_{ed\ max}$) depends on the stirring rate ($r_{stirring}$), the stirrer diameter ($d_{stirrer}$), the interfacial tension (γ) and the density of the aqueous phase (ρ) as follows:

$$d_{ed\,\max} \approx r_{stirring}^{-6/5} d_{stirrer}^{-4/5} \gamma^{3/5} \rho^{-3/5} \tag{13}$$

From the latter, it has been possible to express the evolution of the droplet mean size according to the stirring rate and establish their relationship with mean particle size [62].

Up-to-now, experimental research focused on mechanistic aspects associated with the emulsification–diffusion method are based on the assumption that particle formation stems from emulsion droplets [62,118]. In fact, the high shear stress due to the emulsification step may guarantee submicron droplet formation. In this phase, physicochemical properties and system stirring govern both the ease with which the emulsion is formed and its stability. There is no reported works evidencing that the Marangoni effect is the driving force during particle formation. However, although Galindo et al. [62] demonstrated the relationship between stirring rate and mean particle size, they reported discrepancies between the theoretical model and experimental data. From our standpoint, the high thermal energy of the emulsification process released in the aqueous phase during the dilution step, may have an impact on particle formation, thereby practically explaining these deviations.

As can be concluded from the discussion of mechanistic aspects related to the solvent displacement and emulsification–diffusion techniques, the theoretical and experimental evidence suggests that submicron particle formation depends on the successful combination of operational conditions and starting materials. In the following, this review will focus in these aspects, by making a comparative analysis between the preparation methods.

4. Influence of the operating conditions on submicron sphere size

The study of the operating conditions related to the submicron particle preparation methods can be investigated from different angles such as their influence on the up-scaling procedure [38,62,75] or on particle characteristics, in particular size [81,121]. In this review, we have adopted the second approach to compile useful information for handling variables to obtain specific particle sizes and discuss the behaviors obtained from the mechanistic aspects of the particle formation described previously for each method.

4.1. Solvent displacement process

When considering particle formation mechanisms, the organic/aqueous phase ratio, the organic phase addition method, the stirring system, the temperature and the final stirring time prove to be interesting operating variables for studying the solvent displacement method (Fig. 1).

As is shown in Table 4, the research performed up to now has focused on the phase mixing method, the organic phase addition rate, the organic/aqueous phase ratio, the type of stirring used, the system stirring rate and the system temperature. Nevertheless, contradictory behaviors are reported regarding the organic phase addition rate and the organic/aqueous phase ratio which might be due to differences in experimental conditions or in the materials used. To overcome this problem, Fig. 5 summarizes a controlled study of the solvent displacement method in which the following operating variables were investigated: organic/aqueous phase ratio, organic phase injection rate, method of organic phase addition (dropwise-out and dropwise-in continuous medium), system stirring rate, experimental temperature and final stirring time (for methodological aspects to see Supplementary data).

An all-embracing view of the results reported in the literature and those from the systematic study allows explaining the previously mentioned conflicting results. Thus the organic phase addition rate can influence particle size but is dependent on the organic/aqueous phase ratio (Fig. 5B). The highest injection rate produces the largest particle mean size, particularly at the highest organic/aqueous phase ratios. This behavior suggests that submicron particle formation due to solvent diffusion is time-dependant. Therefore, the particles can continue to grow if the diffusion time is insufficient due to an excessively fast organic phase injection rate. However, this increase in particulate growth can be offset by increasing the continuous medium stirring rate until phase mixing is significantly faster than particle formation. This was demonstrated in an additional study in which the operating conditions of the organic phase injection rate — system stirring rate were 300 μl/min–825 rpm, 300 μl/min–750 rpm and, 225 μl/min–750 rpm. In these cases particle sizes ranged from 160 to 190 nm.

Table 4
Summary of reported studies on the influence of operating variables on the size of submicron spheres prepared by solvent displacement method.

Variable	System composition		Work conditions	Particle size (nm)	Reference
	Organic phase	Aqueous phase			
Organic phase addition rate	PCL–acetone	PLX–W	6–270 ml/min	100–165 nm	[35]
	PCL–acetone	W	3 ml/min	611	[75]
			40 ml/min	474	
			60 ml/min	363	
			80 ml/min	312	
			120 ml/min	340	
	PLGA–acetone	PLX–W	3.5 ml/min	142	[79]
			10.6 ml/min	121	
Organic/aqueous phase ratio	PCL–acetone	W	0.1	115	[30]
			0.2	130	
			0.3	150	
	Ethylcellulose–EtOH	W	0.12–0.34	70–85	[38]
	PLGA–acetone	PVA–W	0.2	181	[18]
			0.4	220	
			0.8	270	
	PLGA-b-PEG–acetone	W	0.1	118	[70]
			0.2	115	
			0.5	122	
			1.0	149	
	PLGA–ACN	W	0.1	126	[76]
			0.2	155	
			0.6	164	
	PES–THF	PLX–W	0.2	581	[77]
			0.4	298	
			0.6	258	
	PLGA–acetone	PLX–W	0.1	149	[79]
			0.2	145	
Type of stirrer	Ethylcellulose–EtOH	W	Rushton turbine	75–90	[38]
			Four pitched 45° blade turbine	70–90	
System stirring	PCL–acetone	W	Flow rate on the organic and aqueous phases: 3–120 ml/min	Smaller particle sizes are promoted working the largest conditions on phases flow rate.	[75]
Method of phases mixing	PMMA–acetone PMMA–THF	W	Adding in one shot the aqueous phase into the organic phase	PMMA–acetone: 74 PMMA–THF: 131	[105]
			Dropwise addition of the aqueous phase to the organic phase	PMMA–acetone: 116 PMMA–THF: 183	
			Dropwise addition of the organic phase to the aqueous phase	PMMA–acetone: 129 PMMA–THF: 142	
Temperature	Ethylcellulose–EtOH	W	10–40 °C	80–90	[38]

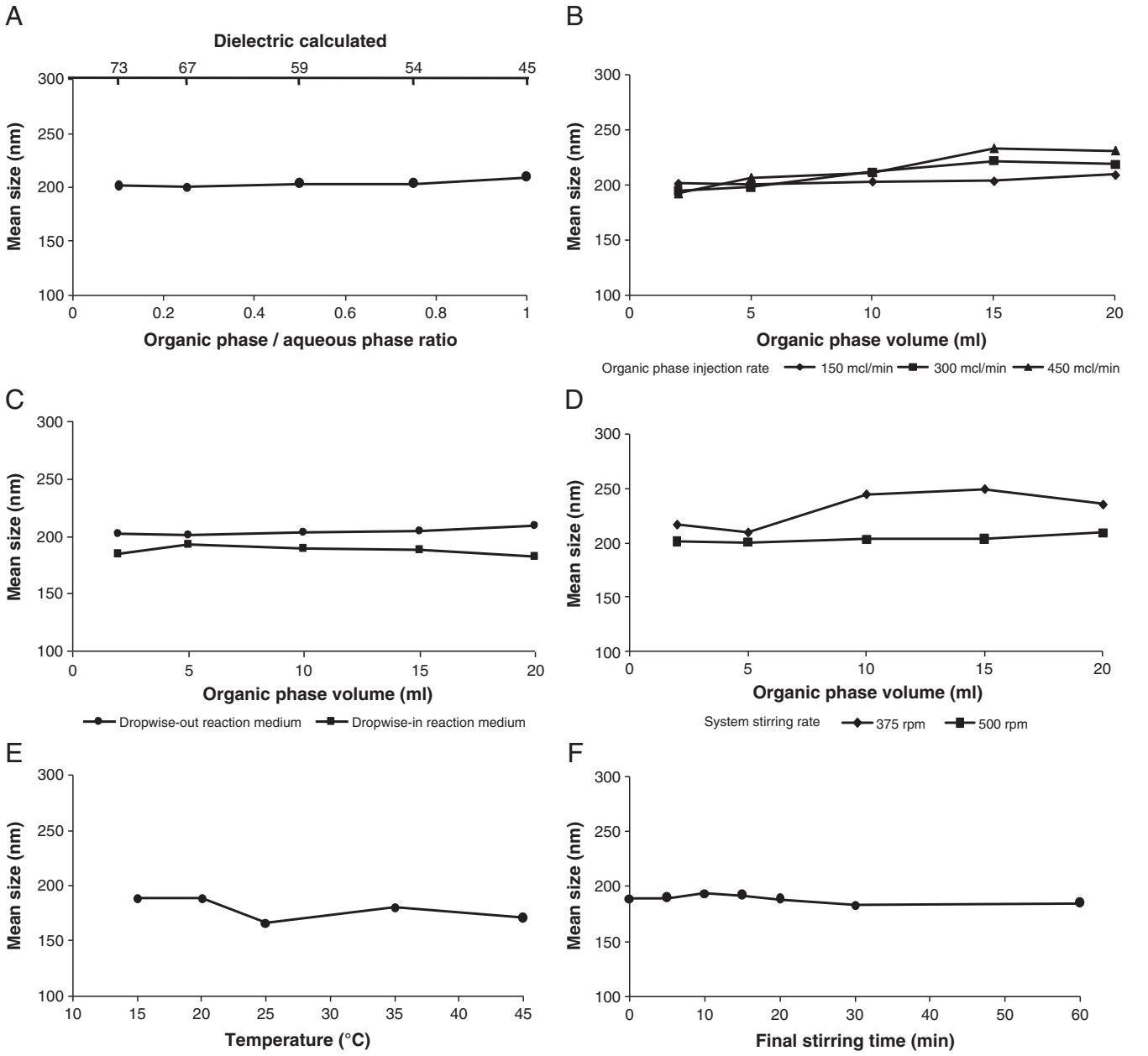

Fig. 5. Preparation of submicron particles by solvent displacement method: influence on mean size of operating variables. A. Organic phase/aqueous phase ratio; B. organic phase injection rate; C. method of organic phase addition; D. system stirring rate; E. system temperature; and F. final stirring time.

Aubry et al. [106] reported that the organic phase addition method can also influence particle size. This might depend on the order of phase mixing (organic phase into aqueous phase or vice versa) or the nature of the organic solvent. In our experiments, for example, when the organic phase is added dropwise into a continuous medium (i.e. organic phase added drop by drop into the continuous medium) instead of dropwise-out of a continuous medium, the particle size obtained is smaller (Fig. 5C). An initial approximation allows stating that drop size is smaller when the dropwise into continuous medium is used due to stirring shear strength. This facilitates the nucleation of smaller particles and consequently smaller particle sizes are obtained.

It was found that the system stirring rate is another factor influencing particle size but it depends on the volume of the organic phase (Fig. 5D). This may clarify the conclusions reported by Lince et al. on the smaller particle size obtained at the highest stirring rates of organic and aqueous phases [75]. In addition, it shows that close attention is needed for setting stirring conditions precisely when submicron particles are prepared by the solvent displacement process. As shown in Table 1, terms such as “moderate stirring”, “magnetic stirring” and “gentle magnetic stirring” are frequently used to refer to the stirring rate of the system, omitting its effect on fluid dynamics and neglecting its possible influence on polymer supersaturation phenomena, solvent migration, system micromixing and particle aggregation [122].

The absence of impact of the organic/aqueous phase ratio (Fig. 5A), system temperature (Fig. 5E) and final stirring time (Fig. 5F) on particle mean size was confirmed. The relative standard deviations (RSD) of the particle sizes obtained in these cases are between 3 and 8% which is common for submicron particle dispersions prepared by the solvent displacement process [38,75]. The organic/aqueous phase ratio was studied using an organic phase addition method with an organic injection rate of 150 μl/min, a system stirring rate of 500 rpm and a dropwise-in continuous medium. Adequately balanced operating conditions can be achieved in this case, leading to efficient solvent

diffusion and particle nucleation. On the other hand, the non-effect of temperature suggests that this variable is not significant if maximum solvent diffusion is achieved. With regard to the behavior observed when the final stirring time was examined, this suggests that particle formation is associated with stirring speed during organic phase addition. Therefore additional stirring is not necessary.

4.2. Emulsification–diffusion method

Table 5 shows published data on the impact of operating variables on the size of submicron particles prepared by the emulsification–diffusion method, which is in good agreement with the results obtained in our systematic study (Fig. 6).

In general terms, the emulsification–diffusion technique is a robust process and the emulsification rate governs particle size (Fig. 6B). The highest values of this variable lead to exhaustive fragmentation in the organic phase, forming small emulsion droplets. Consequently, smaller particle sizes are obtained. Also, as reported by Leroux et al. and Poletto et al. [28,94], the organic/aqueous phase ratio appears to have an influence on particle size, highlighting non-homogeneity in the emulsion when low phase ratios are used (Fig. 6A). In addition, emulsification time has less effect than emulsification speed, while the phase ratio (Fig. 6C) and organic phase/aqueous phase mixing method do not have any effect (198 ± 1.4 and 199 ± 7.3 nm for controlled addition at 1.25 ml/min and for total addition in one step, respectively).

The operating variables related to the solvent diffusion step do not seem to affect particle size (Table 5, Fig. 6D–H). Indeed, unlike the solvent displacement process, the operating conditions of the emulsification–diffusion method guarantee free solvent diffusion as long as the organic solvent solubility condition is satisfied. This explains the seemingly contradictory results reported by Song et al. [90] in which the highest particle size is obtained at the lowest volumes of water for dilution In their study, the lowest volumes of water used did not lead to complete solubility of the organic solvent. In addition, difficulty in solvent diffusion can be expected due to the barrier effect of the stabilizing agent on the emulsion droplet. This could explain the data

Table 5
Summary of reported studies on the influence of operating variables on the size of spheres prepared by emulsification–diffusion method.

Variable	System composition		Work conditions	Particle size (nm)	Reference
	Organic phase	Aqueous phase			
External/internal phase ratio	PMMA–BA	PVA–W	1.4	178	[28]
			2.8	162	
			4.7	153	
			8.5	139	
	PHBHV–$CHCl_3$	PVA–W	0.25	896	[94]
			0.35	691	
			0.4	606	
			0.5	629	
			0.6	481	
			0.8	458	
Emulsification stirring rate	PMMA–BA	PVA–W	1200 rpm	244	[28]
			1200 rpm and concomitant sonication	244	
			5000 rpm	141	
	PDLLA–PC	PLX–W	1500–2460 rpm	>1000	[81]
			9000 rpm	166	
			13,500 rpm	149	
	PMMA–BA	PVA–W	1000 rpm	427	[62]
			1250 rpm	375	
			1500 rpm	351	
			1750 rpm	323	
			2000 rpm	312	
	PLGA–PC	PVA–W	4800 rpm	348	[83]
			8000 rpm	285	
			11,200 rpm	205	
			13,600 rpm	200	
			15,000 rpm	197	
Type of stirrer	PDLLA–PC	PLX–W	High speed homogenizer (9000 rpm)	166	[81]
			Propeller stirrer (2500 rpm)	211	
Volume of water for dilution	PLGA–EtAc	DMAB–W	20 ml	190	[90]
			40 ml	106	
			80 ml	67	
			160 ml	56	
	PLGA–PC	DMAB–W	20 ml	194	[90]
			40 ml	63	
			80 ml	46	
			160 ml	41	
Temperature of adding water	PLGA–PC	PVA–W	25 °C	204	[83]
			47 °C	173	
			60 °C	170	
	PLGA–PC	DMAB–W	25 °C	78	[83]
			47 °C	68	
			60 °C	65	
Adding rate of water for dilution	PLGA–PC	PVA–W	0.03 ml/s	220	[83]
			16 ml/s	204	
	PLGA–PC	DMAB–W	0.03 ml/s	76	[83]
			16 ml/s	78	
Stirring rate for the dilution	PLGA–PC	PVA–W	0 (arbitrary units)	206	[83]
			2 (arbitrary units)	204	
			8 (arbitrary units)	195	
			10 (arbitrary units)	193	

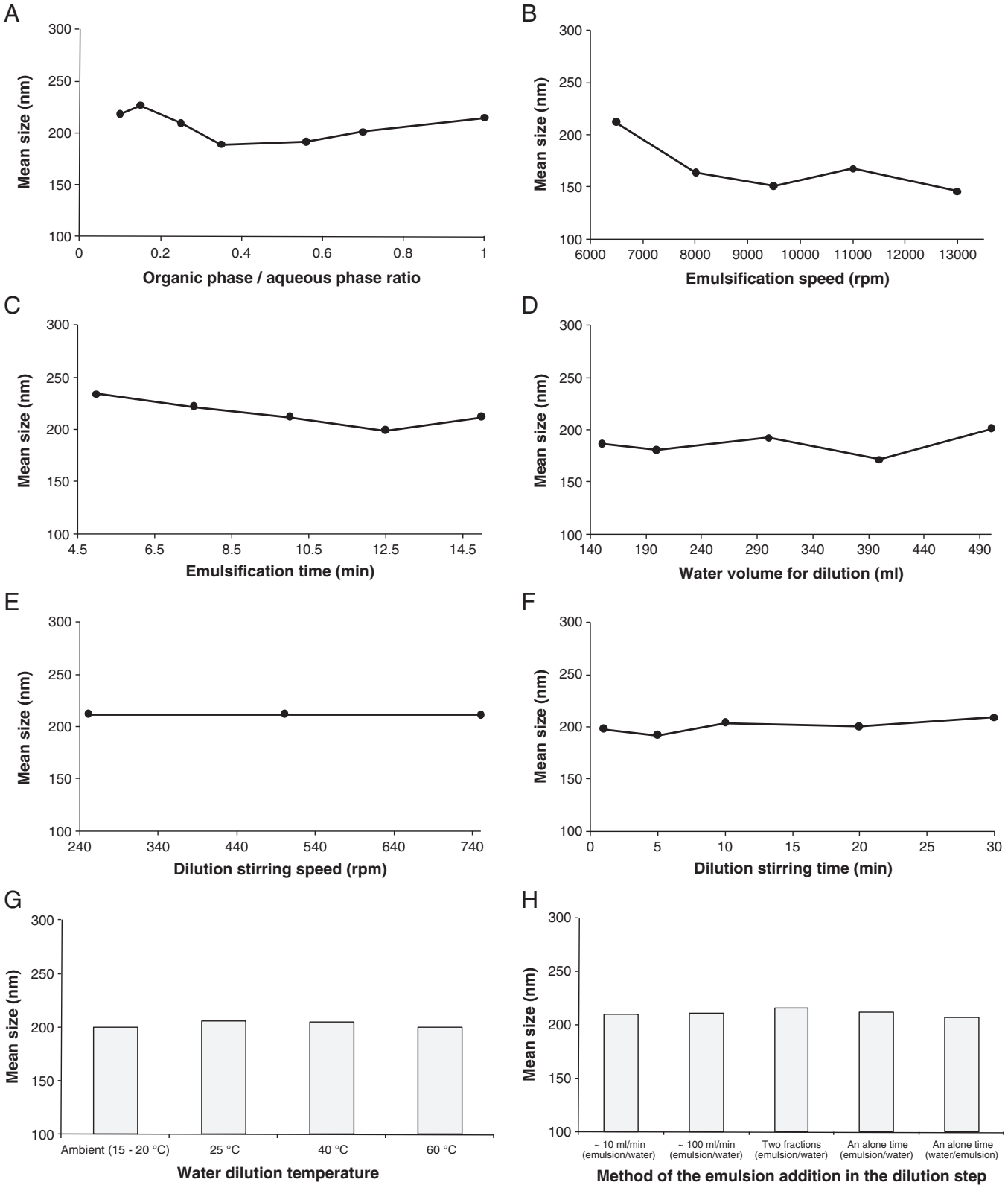

Fig. 6. Preparation of submicron particles by emulsification–diffusion method: influence on mean size of operating variables. A. Organic phase/aqueous phase ratio; B. emulsification stirring speed; C. emulsification time; D. water volume for dilution; E. dilution stirring speed; F. dilution stirring time; G. water dilution temperature; and H. method of emulsion addition in the dilution step.

reported by Kwon et al. where the size of submicron particles prepared using PVA as a stabilizing agent is influenced by the temperature of the dilution water [83]. In this case, reducing the viscosity of the external phase could facilitate solvent diffusion.

Particle suspension concentration under reduced pressure was examined and found to have no effect on particle size (mean size differences less than 10 nm). This may be attributed to total solvent diffusion from the emulsion droplet during the diffusion stage and,

consequently, the complete formation of submicron particles during this step. However, additional discussion on this subject from the standpoint of polymer–solvent interaction will be included below.

4.3. Mechanistic approaches and operating conditions: Comparative analysis between methods

As was shown above, the data reported highlights that particles prepared by the solvent displacement technique can be formed via either nucleation and growth or the Marangoni effect. Therefore the non effect of operating conditions on particle size when the lowest phase ratios are used show that nucleation and growth is the prevailing mechanism. Furthermore, the incidence of the stirring rate on particle size and the method used for adding the organic phase reveal that the Marangoni effect is predominant for particle formation, but only when the highest phase ratios are investigated.

On the other hand, the formation of submicron particles by the emulsification–diffusion method is governed by the emulsion step, particularly the rate and time of emulsification. At first sight, this is in agreement with the mechanistic approach based on the formation of a particle from an emulsion drop. However, the modest effect of the phase ratio suggests that additional mechanistic considerations should be included. It is risky assume that thermal Marangoni effect fully explains this behavior, because, as mentioned above, an effect of the solvent concentration may be present. Whatever the case, it is a potential starting point for investigating unknown factors in further studies.

From a comparative standpoint, the emulsification–diffusion method appears to be robust. Basically two variables determine particle size, which supposes easy up-scaling. However, difficulties can be expected with the emulsion dilution step, which has to take place very quickly, since Ostwald ripening phenomenon may occur. On the other hand, the solvent displacement technique does not allow general statements on its robustness. According to our previous discussions, if particle formation is obtained by the nucleation and growth mechanism the robustness of the method should be better than that of emulsification–diffusion. In fact, particle size is no affected by any operating variable. However, if particles are formed via the Marangoni effect, their size depends on a complex combination of variables which can make up-scaling difficult.

5. Influence of the materials from which the submicron spheres are prepared

The technical literature regarding the preparation of submicron particles by solvent displacement and emulsification–diffusion methods provides many examples illustrating the incidence of different composition variables on particle characteristics, such as their morphology, size, size distribution and zeta-potential. Thus our aim under this subheading is to perform a comparative analysis of the methods described in the literature and those used in our experimental study, taking into consideration how the particularities of the different polymers, stabilizing agents and solvents employed determine particle behavior, and how can this behavior determine decision-making regarding the development of products based on submicron particles.

5.1. Influence of polymer

Two points are usually recognized as critical with respect to the influence of the polymer on the size and zeta-potential of the submicron particles, namely the nature and the concentration used.

5.1.1. Behavior of the nature of polymer

Data reporting the influence of the nature of the polymer used on the size and zeta-potential of submicron particles is summarized in Tables 6 and 7. In general terms, different conclusions can be drawn from the information reported: (1) the particle size obtained by the two methods is in the same range (50–300 nm); (2) submicron

Table 6
Summary of reported studies on the influence of polymer nature on the size of submicron spheres prepared by solvent displacement and emulsification–diffusion methods.

Polymer order according to the particle size	Particle size range (nm)	Organic solvent	Reference
Solvent displacement method			
$PLGA_{50:50} < PLGA_{75:25} < PDLLA < PCL$	110–235	Acetone	[33]
$PLGA < PDLLA \ll PCL$	118–220	Acetone	[34]
$PDLLA < PLGA_{50:50} = PLGA_{85:15} \ll PCL$	109–208	Acetone	[14]
$PLA:PEG_{15:5} < PLA:PEG_{45:5} < PLA:PEG_{75:5} = PLA < PLA:PEG_{110:5}$	50–157	Acetone	[43]
$PCL:LA_{6:4} = PCL:LA_{2:8} < PDLLA < PCL$	81–132	Acetone	[44]
$SB\text{-}PVA\text{-}g\text{-}PLGA = PVA\text{-}g\text{-}PLGA = PLGA$	104–120	Acetone	[46]
$PLGA_{50:50\ 6\ kDa} = PLGA_{50:50\ 14.5\ kDa} < PLGA_{75:25}$	117–159	Acetone	[51]
$PDLLA = PCL$	169–182	Acetone	[53]
$PMMA \ll PCL$	84–195	Acetone	[57]
$PEG:PLGA < PEG\text{-}PCL < PEG\text{-}PLA < PCL = PDLLA < PLGA$	78–262	Acetone	[58]
$PLGA\text{-}PEG_{34} = PLGA\text{-}PEG_{70} < PLGA\text{-}PEG_{495} < PLGA$	58–134	Acetone	[55]
$PDLLA_{209\ kDa} < PDLLA_{109\ kDa} < PDLLA_{16\ kDa}$	51–131	Acetone	[56]
$PLGA = PDLLAR_{203} < PDLLAR_{207}$	98–138	Acetone	[66]
$PLGA_{50:50\ 7\ kDa} = PLGA_{5050\ 63\ kDa} = PLGA_{65:35} = PLGA_{75:25} = PDLLA$	175–194	Acetone	[18]
$PDLLA_{22\ kDa} = PDLLA_{52.3\ kDa} < PDLLA_{124.8\ kDa}$	185–260	Acetone	[71]
$PCL_{14\ kDa} \ll PCL_{80\ kDa}$	295–395	Acetone	[75]
$PLA_{05} = PLA_{20} < PLGA_{75:25} = PLGA_{85:15}$	201–258	Acetone:EtOH	[47]
$PCL\text{-}PEG_1 = PCL\text{-}PEG_2 < PCL\text{-}PEG_3$	71–93	THF	[60]
$DexP_{130} = DexC_{6\text{–}85} < DexC_{6\text{–}300} < DexP_{210} \ll DexC_{10\text{–}52}$	145–300	THF	[69]
$PDLLA_{22.6\ kDa} = PDLLA_{32.1\ kDa} < PDLLA_{52.3\ kDa} \lll PDLLA_{124.8\ kDa}$	104–322	THF	[71]
$PLA\text{-}PEG_{15:5} = PLA\text{-}PEG_{30:5} < PLA\text{-}PEG_{75:5} < PLA:PEG_{110:5}$	55–152	ACN	[45]
Emulsification–diffusion method			
$PLGA < PLGA$	176–219	EtAc	[89]
$Propyl\text{-}starch_{subst.\ 1.05} < Propyl\text{-}starch_{subst.\ 1.45}$	150–183	EtAc	[95]
$PLGA = PLGA\text{-}PEG$	218–220	EtAc	[96]
$PMMA \ll PLGA = PCL$	140–265	BA	[28]
$PLGA_{50:50} = PLGA_{75:25} = PDLLA$	112–132	BA	[86]

Criterion for classifying the nanoparticle size difference: =: difference smaller than 20 nm; <: difference between 21 and 70 nm; ≪: difference between 71 and 120 nm; ⋘: difference larger than 121 nm.

Table 7
Summary of reported studies on the influence of polymer nature on the zeta-potential of submicron spheres prepared by solvent displacement and emulsification–diffusion methods.

Polymer	Organic solvent	Zeta potential (mV)	Reference
Solvent displacement method			
PCL	Acetone	−22 to −29	[14,54,58]
PLA	Acetone	−6 to −50	[43]
PDLLA	Acetone	−20.3 to −67	[14,54,58,66]
PDLLA R206	Acetone	−6 to −10	[66]
PLGA 85:15	Acetone	−23 to −54	[14,51,55]
PLGA 50:50	Acetone	−6 to −10	[46,54,58,66]
PCL-PEG	Acetone	−11	[54,58]
PLA:PEG (low PLA:PEG ratios)	Acetone or ACN	−6 to −14	[43,45]
PLA:PEG (high PLA:PEG ratios)	Acetone or ACN	−18 to −28	[43,45,54,58]
PLGA-PEG (different PLGA:PEG)	Acetone	−4 to− 9	[54,55,58]
PVA-g-PLGA	Acetone	−3.2	[46]
SB-PVA-g-PLGA 10 (different)	Acetone	−18	[46]
Emulsification–diffusion method			
PDLLA	BA	−6	[86]
PLGA 50:50	BA	−5	[86]
PLGA 50:50	EtAc	−28	[96]
PLGA-PEG	EtAc	−24	[96]
Propyl-starch	EtAc	−5 to −8.3	[95]

particles are spherical (Fig. 7 shows typical TEM and AFM micrographs); (3) regardless of the preparation method, the zeta-potential of particles prepared using non-ionic stabilizing agents is always negative due to the presence of terminal carboxylic groups in the polymer molecule; (4) the nature of the polymer influences the size and zeta-potential of particles prepared either by solvent displacement or by emulsification–diffusion; and (5) the size and zeta-potential of particles prepared from the same polymer or the same series of polymers is influenced by other starting materials and operating conditions. The latter conclusion makes it difficult to perform in-depth analysis and make general statements on influence of the polymer on size and zeta-potential of submicron particles on the basis of the data reported. Therefore we performed a controlled study of the impact of the nature of the polymer used on particle size and zeta-potential. The polymers chosen were those commonly used in submicron sphere preparation (PCL, PLGA, and PDLLA).

5.1.1.1. Influence of the nature of polymer on particle size. The nature of polymer influences particle size (Fig. 8). The differences found could be explained by the crystalline and amorphous character of the polymers when re-precipitated after having been solubilised in organic solvent. According to the X-ray diffraction and differential scanning calorimetry studies performed by Leroueil-Le Verger et al. [14], the precipitation by solvent displacement of PLGA and PDLLA exhibit amorphous character while PCL exhibits amorphous as well as crystalline domains.

Although crystalline grade and particle structure depend on specific precipitation conditions (taking into account the analysis carried out by Rastogi and Terry [123] on the behavior of other polymers like polyesters and poly-hydroxy alkanoates), it can be assumed that the semi-crystalline behavior of PCL will produce a larger precipitation nucleus than that obtained from amorphous polymers (PLGA and PDLLA). This is mainly due to the molecular ordering of semicrystalline and amorphous structures. In the case of PCL, the thin crystalline lamellae are separated by amorphous regions and the chains that emerge at the crystalline surfaces with a high degree of molecular alignment must either fold back into the crystallite or stay in the amorphous matrix. This results in a three-phase model consisting of the crystalline and the rigid and mobile amorphous fractions, where the specific volume of the rigid amorphous phase is larger than that of the mobile amorphous phase.

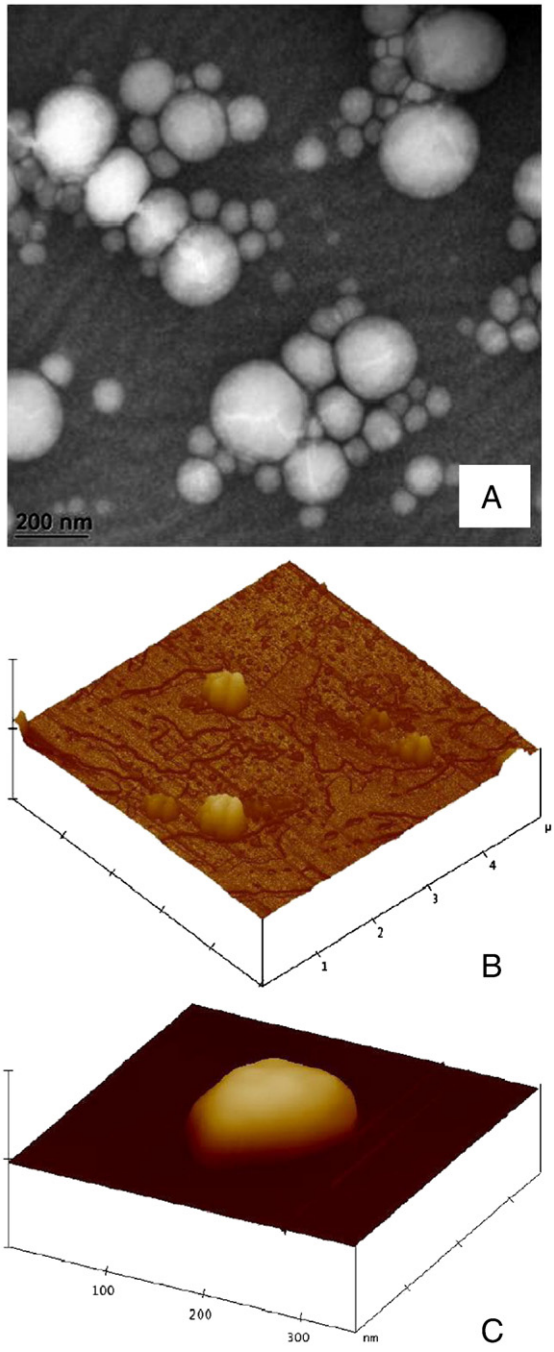

Fig. 7. TEM micrograph of typical PCL spheres prepared by solvent displacement process (A); AFM micrograph of typical spheres prepared by emulsification–diffusion method: from PDLLA (B), from PLGA (C).

It is important to note that when PCL is used for submicron particle preparation by solvent displacement, there is no difference between sphere sizes when different molecular weights are used (Fig. 8A). This is in agreement with the results reported by Lince et al. [75] in their research relating to PCL. Likewise, this conclusion could be inferred for PLGA, in agreement with the results reported by Leroueil-Le Verger et al. [14] for different PLGA (PDLLA85GA15 and PDLLA50GA50) when using acetone as a solvent and PLX 188 0.5% as a stabilizing agent. This behavior suggests that the semi-crystalline or amorphous nature of the re-crystallized polymers predominates more than the difference in polymer molecular weight when the solvent displacement method is used.

Regarding the emulsification–diffusion method, DSC analysis of PDLLA and PLGA submicron particles shows the precipitation of the polymers in

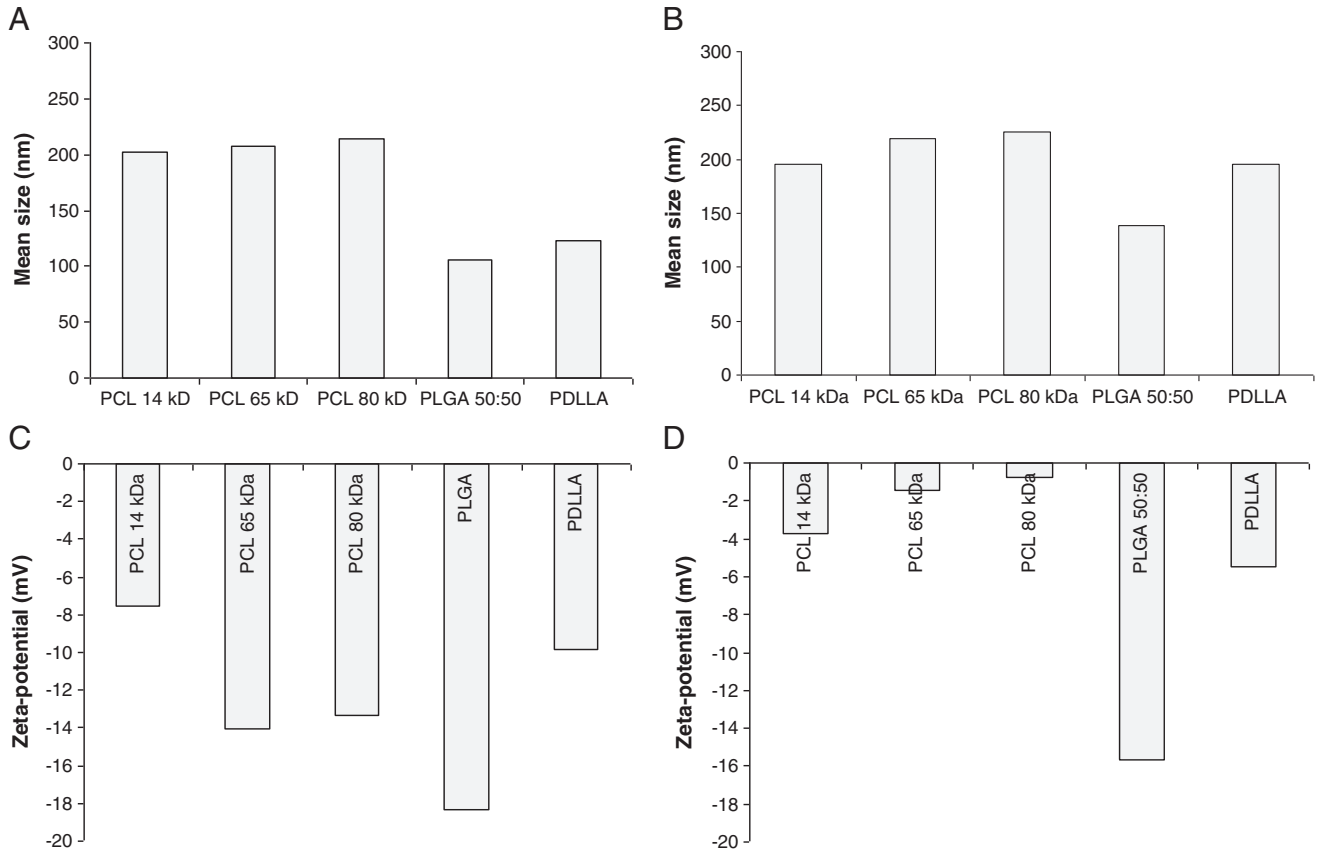

Fig. 8. Influence of polymer nature on the size and the zeta-potential of submicron spheres. A and C: spheres prepared by solvent displacement: polymer concentration 3.5 mg/ml, stabilizing agent PLX 0.4%; B and D: spheres prepared by emulsification–diffusion: polymer concentration 10 mg/ml, stabilizing agent PLX 1%.

amorphous state. However, certain crystallization phenomena are associated with PDLLA precipitation due to the formation of polymer crystallites after 24–72 h [89]. This might explain the larger mean size of PDLLA particles in comparison to PLGA particles. Unfortunately, to our knowledge no works have been reported in which PCL is re-precipitated from ethyl acetate by the emulsification–diffusion method, thus it is not possible to express any opinion on the influence of polymeric arrangements on particle size.

As shown in Fig. 8B, the behavior trends of particle size using different polymers are more marked when the emulsification–diffusion method is used. This is probably due to the polymer concentration used, which is almost three times that used in the solvent displacement method. Thus, when the polymer concentration is high, different behaviors can be observed as a function of the molecular weight of PCL due to molecule size and molecular arrangement during polymer precipitation. Additional study of this aspect showed that mean particle size is similar when using the same polymer concentration regardless of the preparation method used (Fig. 9). Aggregate formation by using the solvent displacement method highlights its limitation regarding the maximum polymer concentration to be used. This was predicted by Stainmesse et al. [30] and explains why comparison between methods is not adequate under the same conditions of polymer concentration. In addition, this limitation entails a disadvantage for the solvent displacement method as the presence of aggregates implies difficulties related to particle yield and purity.

5.1.1.2. Influence of nature of polymer on particle zeta-potential. In general terms, the absolute values of the particle zeta-potential follow the order: PLGA > PDLLA > PCL, which is directly linked to the carboxylic group/alkyl chain ratio per polymer monomer unit. Fig. 8C and D show that the zeta-potential values obtained for submicron particles prepared by solvent displacement are more negative than those obtained from particles prepared by the emulsification–diffusion method. Although these conclusions can be logically supported by the study of the stabilizing agent, as will be discussed below, they only appear valid for polymers such as PCL. The research by Trimaille et al. and Hirsjärvi et al. using the emulsification–diffusion method and the solvent displacement technique respectively [74,87], showed that PDLLA behaves differently. The zeta-potential of particles prepared by solvent displacement is always the lowest. This suggests that the hydrophilic/hydrophobic moiety ratio of the polymeric molecule could influence the electrostatic behavior of particles.

5.1.2. Behavior of polymer concentration

The influence of polymer concentration on submicron particle size is of considerable importance. Fig. 10 shows the behavior of polymer

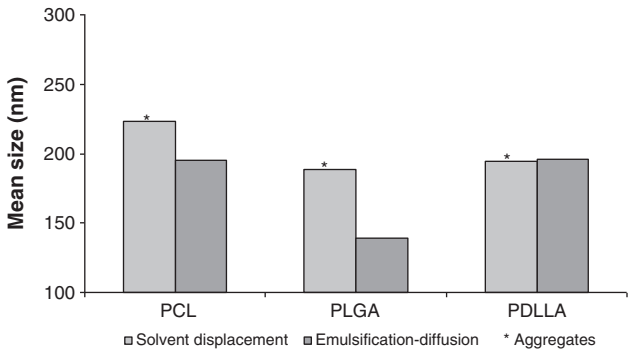

Fig. 9. Influence of polymer nature on size of submicron spheres prepared by solvent displacement and emulsification–diffusion methods (polymer and the stabilizing agent concentrations: 10 mg/ml and 1% respectively).

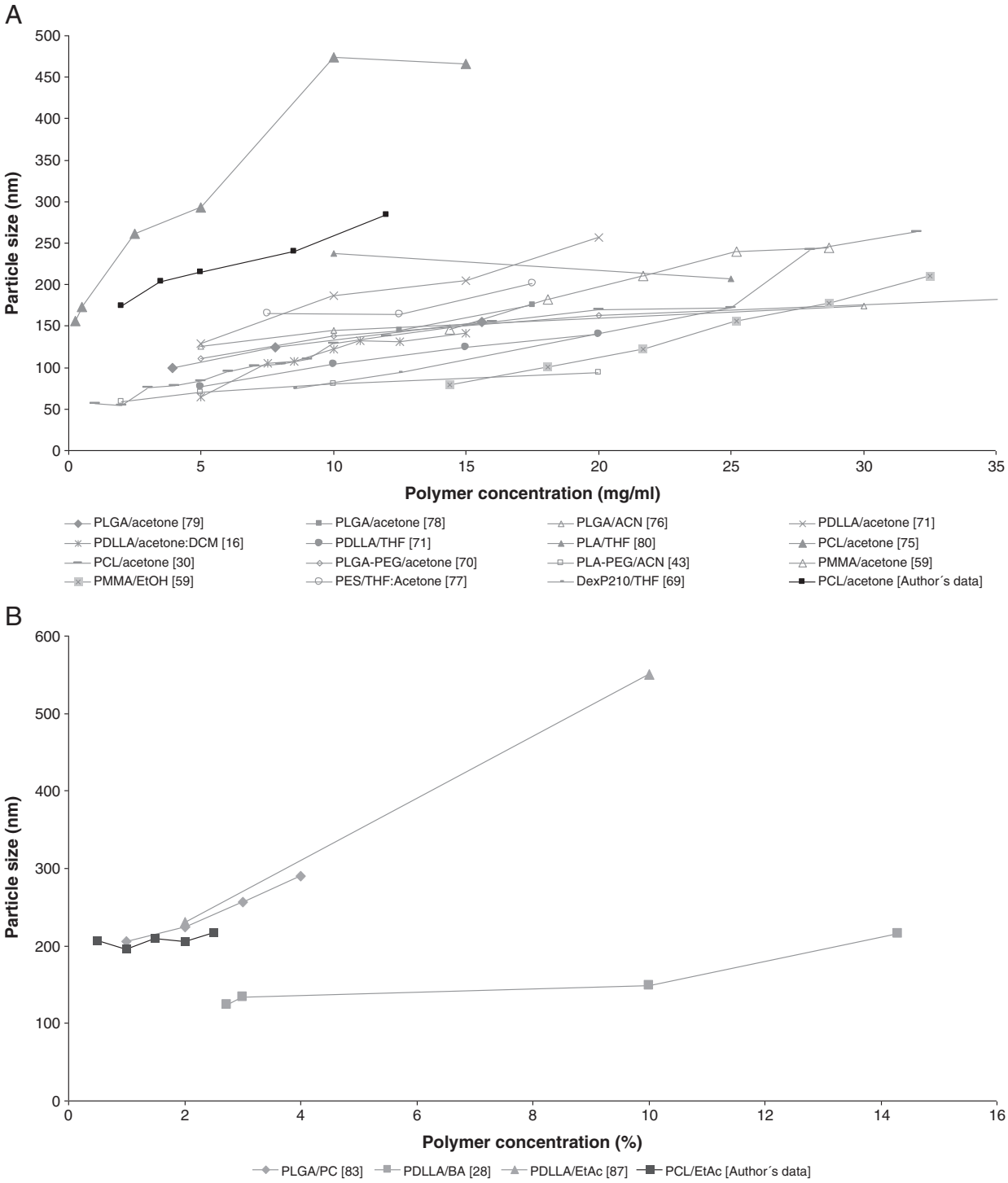

Fig. 10. Influence of polymer concentration on size of submicron spheres prepared by A. solvent displacement process; B. emulsification–diffusion method.

concentration according to sphere size taken from published data and those obtained from our experimental study.

It is obvious that the solvent displacement process is highly sensitive to changes in polymer concentration regardless of the nature of polymer, the other initial materials used or the operating conditions (e.g., in our study, particle sizes are ~170 and 300 nm for the lowest and highest polymer concentrations, respectively). On the other hand, the size of particles prepared by emulsification–diffusion does not undergo significant variations at concentrations lower than 2.5%. Above this value, particle size increases as polymer concentration increases.

Particle size behavior obtained as a function of the method used can be interpreted from two angles: droplet formation and particle formation.

Regarding droplet formation, in the emulsification–diffusion method this depends on high-shear stirring that guarantees droplet formation regardless of the composition of the organic phase. However, if the polymer solution is too concentrated, it can impede solvent diffusion due to higher viscosity in the organic phase and promote the Ostwald ripening phenomenon in the emulsion, leading to an increase in particle size.

Also, in the solvent displacement method the viscosity of the organic phase is highly dependent on polymer concentration even at the lowest

Table 8
Summary of reported studies on the influence of stabilizing agent nature on the size of submicron spheres prepared by solvent displacement and emulsification–diffusion methods.

Stabilizing agent order according to the particle size	Particle size range (nm)	Organic phase	Reference
Solvent displacement method			
$PVA_{98.5\%\ Hydrolyzed} < PVA_{88\%\ Hydrolyzed} < PVA_{80\%\ Hydrolyzed}$	225–290	PLGA:acetone	[36]
Polysorbate 80 < PLX 188 = Triton X100 < Brij 96	131–194	PLA:acetone	[56]
$PLX_{F68} = PLX_{F108}$	180–190	PCL:acetone	[63]
Polysorbate 80 ≪ PVA = PLX 188	220–300	PES:THF	[77]
Emulsification–diffusion method			
DMAB ⋘ PVA	102–260	PLGA:EtAc	[22]
DMAB ⋘ PVA	145–410	PLGA:EtAc	[26]
DMAB < PLX 188 ≪ PVA	67–213	PLGA:EtAc	[90]
PVA ⋘ gelatin	270–730	PMMA:BA	[28]
$PVA_{26\ kDa} < PLX\ 188 = PVA_{30–70\ kDa}$	123–179	PLA:PC	[81]

Criterion for classifying the nanoparticle size difference: =: difference smaller than 20 nm; >: difference between 21 and 70 nm; ≫: difference between 71 and 120 nm; ⋙: difference larger than 121 nm.

values. This has been demonstrated by Thioune et al. [39] and might be explained by the increase in polymer chain association as the polymer concentration increases. However, since solvent displacement is a spontaneous process without additional mechanical energy, polymer chain association could govern nucleation and growth rates. In addition, rapid solvent diffusion towards the aqueous phase could be hindered.

5.2. Influence of the stabilizing agent

Usually, stabilizing agents are recognized as key factors for guaranteeing the physical stability of dispersions of submicron particles; however, this depends on their properties and their role in particle synthesis. Therefore in the following, we consider the performance of the stabilizing agent from the standpoint of the particle preparation method, paying great attention to the impact of typical variables such as the nature and the concentration used on the size and zeta-potential of the particles.

5.2.1. Behavior of the nature of stabilizing agent

Table 8 summarizes data taken from the literature on the effect of the nature of stabilizing agent on the size of submicron particles prepared by the solvent displacement technique and the emulsification–diffusion method. In general terms, these conclusions are in agreement with our experimental results in which PCL was chosen as polymer while PVA, PLX and polysorbate 80 (non-ionic surfactants), and SDS and DTAB (negatively and positively charged surfactants respectively) were the stabilizing agents investigated (Fig. 11). Although poly(ethylene glycol) (2000, 4600 and 10,000) and dextran (T500 and T2000) have been used as steric stabilizing agents for obtaining poly(alkylcyanoacrylates) (PACA) particles [34], they did not exhibit any stabilizing effects in our study. They perhaps require a higher concentration or synergistic effect with another steric or "electro-steric" stabilizing agent.

5.2.1.1. Influence of the nature of stabilizing agent on particle size. The mean sizes of spheres are significantly dependent on the nature of stabilizing agent, with similar trends for the two preparation methods. In addition, in all cases adequate stabilization was obtained for the particles as aggregates were not detected.

It should be taken into account that the role of the stabilizing agent differs as a function of preparation method. In the solvent displacement method, the stabilizing agent prevents aggregation during particle formation without significantly affecting droplet formation. This is due to the high initial spreading coefficient of the organic solvent (e.g., 42.4 dyn/cm at 20 °C for acetone [124]) which

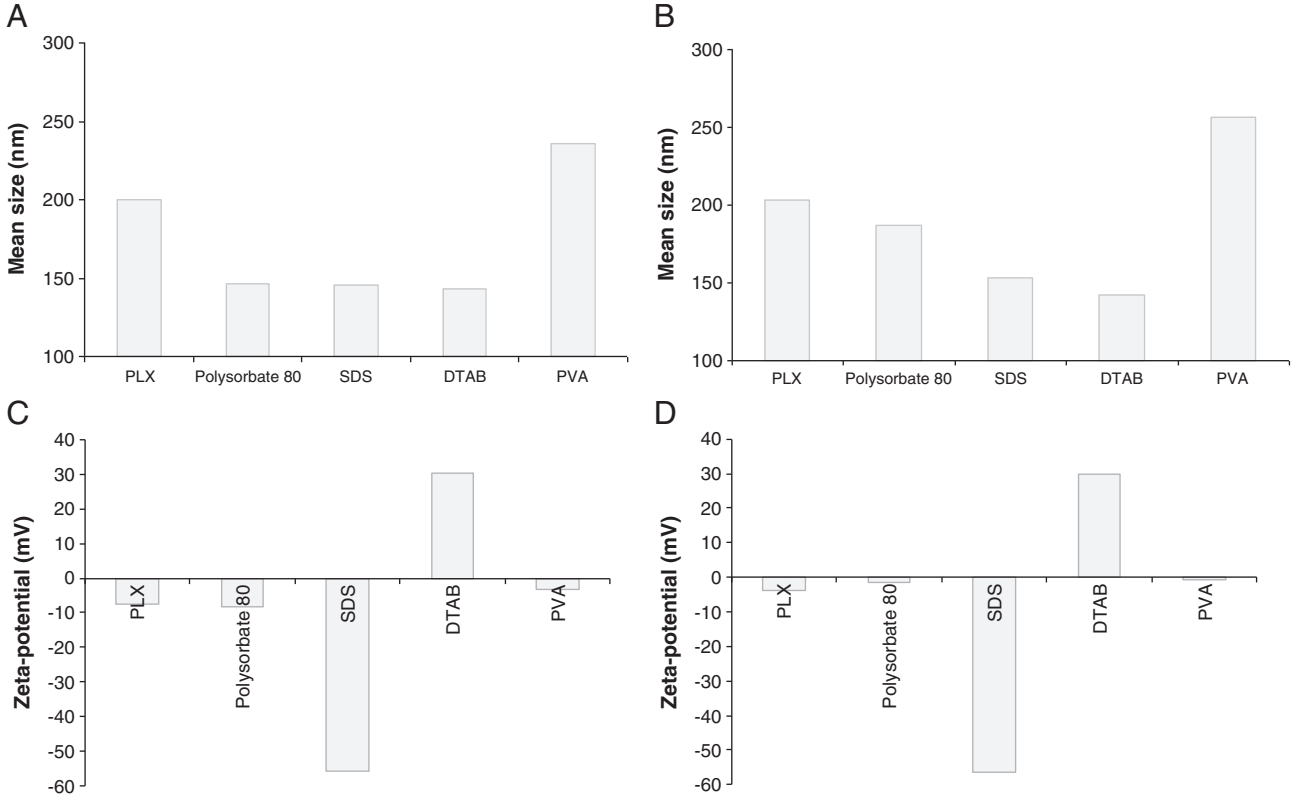

Fig. 11. Influence of the stabilizing agent nature on mean size and zeta-potential of submicron spheres. A and C: spheres prepared by solvent displacement: polymer concentration 3.5 mg/ml, stabilizing agent 1%; B and D: spheres prepared by emulsification–diffusion: polymer concentration 10 mg/ml, stabilizing agent 1%.

guarantees efficient solvent–water interaction when organic and aqueous phases are brought into contact.

Therefore the performance of stabilizing agents is governed by their electrostatic, steric and electro-steric effects [125]. As shown in Fig. 11, the size of the particles prepared by solvent displacement decreases, following the order PVA>PLX>Polysorbate 80 = SDS = DTAB. PVA, PLX and polysorbate 80 have a predominantly steric effect, whereas SDS and DTAB exhibit an electro-steric effect. This suggests that stabilizing agents with an electro-steric effect are adequate for obtaining smaller particle sizes. In addition, the steric effect might delay solvent diffusion, thereby favoring particle growth. In fact, in the particular case of PVA, Murakami et al. [36] suggest the localized gelatinization of PVA due to a kind of acetone–PVA interaction. Such interaction occurs preferentially on the surface of particles, delaying solvent migration.

Unlike the solvent displacement process, the stabilizing agent in the emulsification–diffusion method acts as a surfactant in droplet formation and as a stabilizer of particles during their formation. Thus the stabilizing agent is adsorbed on the solvent–water interfacial area formed during the emulsification step, while the remaining quantity contributes towards preventing particle aggregation in the dilution step. Consequently, the performance of a stabilizing agent is governed by its ability to lower the interfacial tension between aqueous and organic phases, which in turn depends on the ability of the hydrophobic moiety of the molecule to bind to the organic phase and on that of its hydrophilic part to remain in the aqueous medium. In addition, the steric, electrostatic and electro-steric effects are also important for preventing polymer aggregation. The behavior of the submicron particles obtained confirms that efficient reduction of interfacial tension combined with electro-steric effects permits obtaining smaller particle sizes. Therefore particle size decreases as follow: PVA>PLX>Polysorbate 80>SDS = DTAB.

Regardless of the method for preparing submicron particles and taking into account the surfactant absorption mechanisms studied by Zhang and Somasundaran [126], the hydrophobic segment of the polymer and the hydrophobic moieties of the stabilizing agent interact via hydrophobic interaction when the stabilizing agents are non-ionic (PLX, polysorbate 80 and PVA) or negatively charged (SDS). Positively charged molecules such as DTAB exhibit both attractive electrostatic and hydrophobic interactions with the polymer.

5.2.1.2. Influence of the nature of stabilizing agent on particle zeta-potential. The data in the literature leads to the sole assumption that non-ionic stabilizing agents have no impact on the zeta-potential of particles prepared by solvent displacement (Table 9). However, comparative analyses between the methods for the particular case of particles prepared from PCL can be established on the basis of our experimental work (Fig. 11C and D). The values obtained from the submicron spheres prepared by the solvent displacement process by using non-ionic stabilizing agents are more negative than those obtained from the emulsification–diffusion method. As mentioned above, a similar result was obtained when investigating the nature of the polymer (Fig. 8). There are two possible explanations for this. The first is based on the polymer–stabilizing agent ratio while the second takes into account the role of the stabilizing agent in the droplet formation as a function of the preparation method.

Table 9
Summary of reported studies on the influence of stabilizing agent nature on the zeta-potential of submicron spheres prepared by solvent displacement and emulsification–diffusion methods.

Stabilizing agent	Organic phase	Zeta potential (mV)	Reference
Solvent displacement method			
PLX 188	PLA:acetone or PCL:acetone	−15 to −34.4	[56,63]
PLX 188	PLA:acetone	−25	[74]
Without stabilizer		−34	
Triton X100	PLA:acetone	~−32.9	[56]
Brij 96	PLA:acetone	~−30.4	[56]
Polysorbate 80	PLA:acetone	~−31.3	[56]
Emulsification–diffusion method			
PVA	PLGA:EtAc	−1.4 to −5.8	[22,26]
PVA	Eudragit S100:BA	~−50	[84]
DMAB	PLGA:EtAc	+75 to +80	[22,26]

It was found that the polymer–stabilizing agent ratio was 1:2.3 for the solvent displacement method and 1:4 for the emulsification–diffusion process. It is known that stabilizing agents such as PLX and PVA are adsorbed on the particles, stabilizing the polymer–water interface during preparation [87,127,128] and the charge and potential distribution of the electric double layer surrounding the particle may be affected by the presence of the polymer adlayer [127]. The work reported by Hirsjärvi et al. [74] highlights a difference in zeta-potential between stabilizing agent–free PDLLA particles and those prepared from PLX aqueous dispersion (Table 9). Thus the higher quantity of stabilizing agent used in the emulsification–diffusion method in comparison to the solvent displacement process could form a dense steric barrier making it difficult to detect the negative polymer charge.

By taking into consideration the role of the stabilizing agent as a function of the preparation method in the emulsification–diffusion method, as mentioned above, it can be seen that the stabilizing agent takes part in droplet formation, perhaps leading to stronger polymer–stabilizing agent interaction. It can be presumed that as a result of this interaction, some stabilizing agent molecules can be mechanically trapped by the structure of the particle, particularly at its surface, masking the negatively charged polymer and reducing the negative electrical behavior of the particles. When the solvent displacement method is used, polymer–stabilizing agent interaction is probably less due to the major role of the stabilizing agent which is to prevent particle aggregation. Thus, the negative polymer groups can be highly exhibited. Additional considerations regarding this point will be expressed below from the standpoint of the influence of the solvent on particle zeta-potential.

5.2.2. Behavior of stabilizing agent concentration

Although submicron particles can be prepared without stabilizing agents as they are stabilized by the electrostatic repulsion of their surface charge [74,97], the use of a stabilizing agent is strongly advised since it prevents aggregate formation and contributes to system stability [34,58,129]. Consequently, determining the optimal concentration becomes a variable of interest in the study of solvent displacement and emulsification–diffusion methods.

As shown in Fig. 12, it is clear that the concentration of the stabilizing agent does not have a significant effect on the mean size of particles when the solvent displacement process is used. On the contrary, the concentration of the stabilizing agent affects particle size when using the emulsification–diffusion method.

Once again, these results might be due to the role of the stabilizing agent as a function of the droplet formation mechanism and the extent to which the stabilizing agent participates in it. Since the stabilizing agent does not take part in droplet formation by solvent displacement, its effect on sphere size is neglected. However, regarding the emulsification–diffusion method, the extent to which the stabilizing agent takes part in emulsion formation might be governed by the extent of organic phase–stabilizing agent affinity, in addition to the emulsifying capacity of the stabilizing agent. For instance, Quintanar et al. [81] reported drastic particle size reduction (from 450 nm up 160 nm) as a function of stabilizing agent concentration in a system composed of PDLLA as polymer, propylene carbonate as organic solvent and PLX (0.5–15%) as stabilizing agent, at 8000 rpm for 10 min in the emulsification step. Nevertheless, in our results using PCL/PLX (0.5–5%)/EtAc, particle size reduction was only from 140 nm up 90 nm. Unlike ethyl acetate, propylene carbonate has a major solubility parameter (δ_{EtAc}: 18.2 $MPa^{1/2}$; δ_{PC}: 27.2 $MPa^{1/2}$

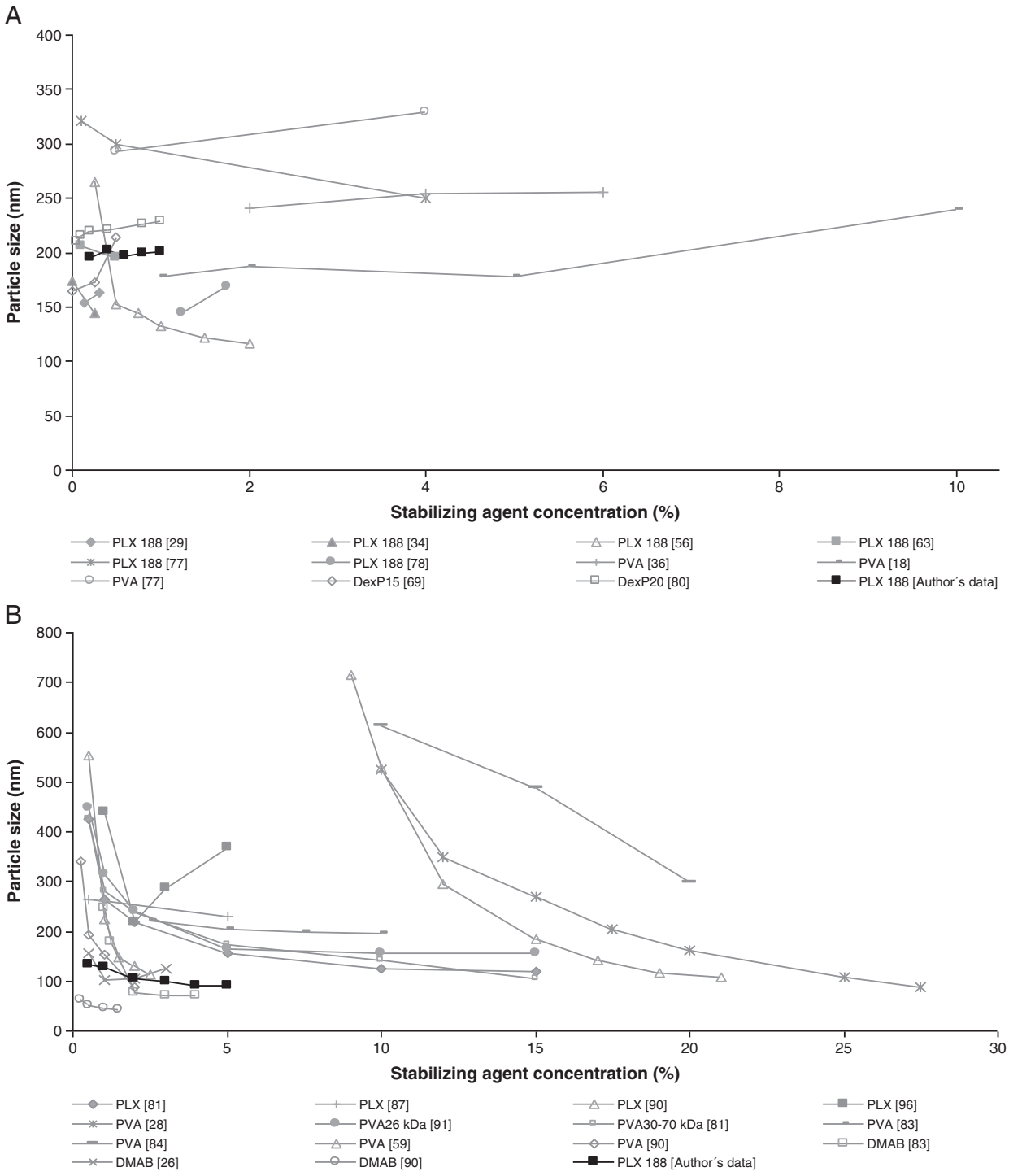

Fig. 12. Influence of stabilizing agent concentration on size of submicron spheres prepared by: A. solvent displacement method; B. emulsification–diffusion method.

[130]), which may facilitate solvent–stabilizing agent interactions and make the emulsification process more efficient.

Leroux et al. [28] also reported drastic reduction of particle sizes (from 500 nm to 100 nm) when the emulsion was prepared from PDLLA/benzyl alcohol/PVA (10–30%) at 1200 rpm stirring speed. In this case, the low emulsion stirring speed could be compensated by both the solvent–stabilizing agent interactions favored ($\delta_{\text{Benzyl alcohol}}$: 23.7 $MPa^{1/2}$ [130]) and the high concentration of stabilizing agent, which reduces the interfacial tension between the organic and aqueous phases [62]. Other examples of this can be found in the works reported by Kwon et al. [83] who used a PLGA–propylene carbonate–PVA (2.5–10%) system at a high emulsification rate (non-specified) for 7 min; Galindo et al. [62], who used PMMA L100-55–benzyl alcohol–PVA (8–20%) at 2000 rpm for 15 min; and Song et al. [90] who used PLGA–propylene carbonate and PVA or PLX as stabilizing agents (0.5–2.5%) and an emulsification process using the ultrasound technique.

5.3. Influence of the solvent

In this review the study of the solvent's influence on the size and zeta-potential of submicron particles has taken a global view of the

polymer/stabilizing agent/solvent system. In what follows, despite certain constraints, this approach provides us with very interesting evidence that contributes to elucidating the mechanistic aspects relating to particle formation.

5.3.1. Influence of the nature of solvent on particle size

Different approaches from the physicochemical point of view have been investigated in order to understanding the particle size behavior obtained when polymer, organic phase and water interact during the particle preparation. For instance, for the solvent displacement method, Stainmesse et al. used the organic solvent dielectric constant [30]; Galindo et al. used the solvent/water interactions [62]; Ganachaud and Katz used solvent/water solubility parameter difference [102], and Legrand et al. and Thioune et al. used polymer–solvent interactions [39,71]. Regarding the emulsification–diffusion method, the effect of solvents was analyzed from the standpoint of solvent–water solubility [90], solvent–polymer interactions, the solvent–water diffusion coefficient [85] and from that of molecular descriptors of solvent hydrophilicity [131]. In both solvent displacement and emulsification–diffusion methods, researchers have found certain correlations between particle size and the physicochemical parameters chosen. In addition, Murakami et al. [132] demonstrated that polymer–solvent affinity influences the size and yield of particles, by using a method that combines solvent displacement and emulsification–diffusion.

In this review we have chosen polymer/solvent/nonsolvent interactions and the physicochemical properties of organic solvent to obtain an overall view of the data available in the literature. Both solubility parameter difference ($\Delta\delta$) and interaction parameter (χ) were used for illustrating the behaviors reported in the largest number of cases possible. We are aware that this approach may lead to misinterpretations of behavior, mainly due to the different experimental conditions and formulations used in each study reported and to the assumption that polymers/solvents/nonsolvents are the most important components, whereas the effect of other starting materials such as the active substance or the stabilizing agent are omitted. However, we run this risk because it is offset by the possibility of obtaining evidence on the influence of thermodynamic properties on submicron particle characteristics, thus making a contribution to the discussion on the mechanisms proposed for particle formation.

The solubility parameters of the solvents (δ), solvent mixtures and polymers were searched in the literature or recalculated. From these values, $\Delta\delta$ and χ were estimated for the pair polymer–solvent and solvent–water (Tables 10 and 11). The method of group contribution proposed by van Krevelen for determining the polymer solubility parameter [133,134], the calculation of the solubility parameter of solvent mixtures assuming additive behavior, as proposed by Martin and Bustamente [135], and the calculation of the interaction parameter according to Peppas procedure [116], were used in this review. The approximations involved in each of these methods have been shown to be valid and are good tools for obtaining practical information. The results are shown graphically to facilitate analysis (Figs. 13 and 14). Certain differences were detected between our results and those reported by other teams. They are due to differences in the bibliographical sources used for data such as solubility parameters and do not substantially modify the general conclusions reported.

As can be seen in Figs. 13A–B and 14A–B, $\Delta\delta_{\text{polymer–solvent}}$ between 1 and 15 $MPa^{1/2}$ and $\Delta\delta_{\text{solvent–water}}$ between 20 and 40 $MPa^{1/2}$ can be used for preparing submicron particles by the two methods. It seems that the thermodynamic criterion required for polymer solubility and solvent diffusion are satisfied in these wide ranges of $\Delta\delta$. However, none of these physicochemical parameters clearly interacts with particle size.

Regarding χ, a theoretical view is necessary in order to facilitate the interpretation of the results. Lower $\chi_{\text{solvent–water}}$ values mean better solvent–water affinity which is favorable for solvent diffusion. On the other hand, higher $\chi_{\text{polymer–solvent}}$ values can also facilitate solvent diffusion. According to the above, we could expect that in terms of particle size, lower $\chi_{\text{solvent–water}}$ values and higher $\chi_{\text{polymer–solvent}}$ values lead to the smallest size.

As shown in Fig. 13C, the behavior of $\chi_{\text{polymer–solvent}}$ estimated for the systems used by the solvent displacement technique do not correlate clearly with particle size, probably because of the lower polymer concentration commonly used by this method. On the other hand, in some cases $\chi_{\text{solvent–water}}$ displays interaction with particle size, although this trend generally does not highlight any correlation (Fig. 13D). Indeed, it was difficult to suggest any mechanistic interpretation. Some explanation could be given in terms of total solvent–water miscibility which guarantees fast phase mixing making the impact of solvent diffusion irrelevant. Therefore, particle size is governed by parameters related to the polymer and stabilizing agent as mentioned above. However, taking into account that particle size depends on the organic phase/aqueous phase ratio, particularly at the highest values of this variable (see Section 4.1), it can also be suggested that in these cases, the ease of solvent diffusion is a critical factor. Thus, given that the nature of the solvent has no influence in some cases, whereas the amount of solvent does have an impact in others, it is possible to propose once again that particle formation by the solvent displacement technique could be carried out simultaneously via the two mechanistic approaches (i.e. nucleation and mechanically), the most relevant mechanism depends on the phase ratio and the composition of the system.

In the emulsification–diffusion method, both $\chi_{\text{polymer–solvent}}$ and $\chi_{\text{solvent–water}}$ appear to maintain a correlation with particle size (Fig. 14C and D). Thus major polymer–solvent and solvent–water affinities lead to the largest particle sizes. This appears logical from the point of view of the mechanistic approach which proposes particle formation from one emulsion droplet. Therefore higher polymer–solvent affinity causes solvent diffusion difficulties that might lead to incomplete solvent migration towards the external phase. Consequently, the particle sizes are the largest. Coincidentally, the two largest particle sizes seen in the charts were obtained by using DCM as a solvent. Its very low water solubility compared with other solvents can make complete solvent dissolution in water difficult, promoting the Ostwald ripening phenomenon. If these results are removed, it is can be seen that polymer–solvent interactions govern particle size without major influence on solvent–water interaction.

In addition, the influence of the solvent on the size of submicron particles prepared by the two methods can also be analyzed through the physicochemical properties of the organic solvent. To this end the data obtained in our systematic study was used to guarantee that organic solvent was the sole experimental variable and that the effect of the polymer could be neglected due to its constant concentration in the organic phase. Table 12 compiles the solvent properties that can affect particle formation (density, viscosity, surface tension and water solubility) and includes a preliminary qualitative analysis facilitating discussion. As can be seen, particle size does not correlate with solvent properties when the solvent displacement method is used. However, good agreement between solvent physicochemical properties and particle size is observed for the emulsification–diffusion method. Thus the lowest values of density, viscosity and surface tension provide the smallest particle sizes. For the particular case of PC, its water solubility can overcome the difficulties associated with high density, viscosity and surface tension values. Therefore these results further support the idea that solvent physicochemical properties do not have a critical impact on particle formation when using the solvent displacement procedure and working with low phase ratios. It also supports the hypothesis that the ease of emulsion formation is the critical factor for obtaining

Table 10
Some physicochemical parameters related to the solvent/polymer/nonsolvent systems used for the sphere preparation by solvent displacement method.

Solvent	Polymer	Solvent molar volume (ml/mol)[a]	Solvent solubility parameters ($MPa^{1/2}$)[b]				Polymer solubility parameters ($MPa^{1/2}$)[c]				$\Delta\delta_{polymer-solvent}$ ($MPa^{1/2}$)[d]	$\Delta\delta_{solvent-water}$ ($MPa^{1/2}$)[d]	$\chi_{polymer-solvent}$[e]	$\chi_{solvent-water}$[e]	Size (nm)	Reference
			δ	δd	δd	δh	δ	δd	δd	δh						
Acetone:EtOH (99.5:0.5)	PCL 14 kDa		20.1	15.5	10.4	7.1					6.0	35.8			100	[30]
Acetone:EtOH (97:3)			20.3	15.5	10.4	7.4					5.9	35.5			99	
Acetone:EtOH (93:7)			20.6	15.5	10.3	7.9					5.8	35.0			98	
Acetone:EtOH (90:10)			20.8	15.5	10.2	8.2	19.7	17.2	4.8	8.3	5.7	34.6			105	
Acetone:EtOH (85:15)			21.1	15.5	10.2	8.9					5.6	34.0			115	
Acetone:EtOH (80:20)			21.4	15.6	10.1	9.5					5.7	33.4			162	
Acetone:EtOH (75:25)			21.7	15.6	10.0	10.1					5.7	32.9			215	
EtOH:W (7/3 v/v)	Gliadin		33.0	15.7	11.0	26.3									470	[32]
MetOH:W (8/2 v/v)			33.3	15.2	13.0	26.3									742	
Acetone:W (5/5 v/v)			34.0	15.5	13.2	24.	34.5[f]								466	
Propan-1-ol:W (5/5 v/v)			36.3	15.8	11.4	29.9									1000	
Propan-2-ol:W (5.5/4.5 v/v)			34.5	15.7	10.6	28.1									772	
EtOH : W Mixture solubility parameter	Gliadin		32.9				34.5[f]								173	[40]
			34.0												182	
			34.5												157	
			35.0												338	
			36.0												278	
			37.8												374	
Acetone	SB-PVA-g-PLGA	74.0	20.1	15.5	10.4	7.0									88	[46]
Acetone:EtAc (99:1)			20.1												75	
Acetone:EtAc (98:2)			20.1												73	
Acetone:EtAc (95:5)			20.0												75	
Acetone:EtAc (91:9)			20.0												88	
Acetone:EtAc (84:16)			19.9												108	
Acetone:EtAc (75:25)			19.7												138	
Acetone:EtAc (72:28)			19.7												293	
Acetone:EtAc (70:30)			19.6												402	
Acetone:EtAc (67:33)			19.6												550	
Acetone:EtOH (6:4)	PLGA 85:15		22.7	15.6	9.8	12.0					1.1	31.1			261	[47]
Acetone:MetOH (6:4)			23.9	15.3	11.2	13.1					1.7	29.7			266	
ACN:EtOH (50:50)			21.0	16.9	7.5	22.0	23.0	16.5	10.4	12.2	10.2	22.2			244	
ACN:EtOH (60:40)			19.8	17.1	7.2	22.5					10.8	21.8			240	
ACN:EtOH (70:30)			18.7	17.3	6.9	23.0					11.4	21.5			247	

Acetone: DCM (19.5:0.5)	PDLLA			20.1	15.6	10.3	7.0					4.6	35.9			114	[16]
Acetone:DCM:EtOH (19.0:0.5:0.5)				20.3	15.6	10.3	7.3					4.3	35.6			105	
Acetone:DCM:EtOH (18.5:0.5:1.0)				20.4	15.6	10.2	7.6					4.0	35.3			97	
Acetone:DCM:EtOH (17.5:0.5:2.0)				20.8	15.6	10.1	8.2					3.4	34.7			90	
Acetone:DCM:EtOH (16.5:0.5:3.0)				21.1	15.6	10.1	8.8					2.8	34.1			64	
Acetone:DCM:EtOH (15.5:0.5:4.0)				21.4	15.6	10.0	9.5					2.2	33.5			54	
CHCl3: acetone	PLA			24.0	17.0	6.5	12.0					2.7	31.9			260	[52]
CHCl3:MetOH				25.6	17.0	6.8	14.6	21.7	16.2	8.0	11.3	4.0	29.3			200	
CHCL3:EtOH				25.1	17.1	6.2	14.1					4.0	30.0			270	
Ethyl lactate	PMMA	115.0		21.7	16.0	7.6	12.5							31.1	32.2	178	[59]
Acetone		74.0		20.1	15.5	10.4	7.0							35.8	23.4	146	
Isopropyl alcohol		76.8		23.5	15.8	6.1	16.4	18.6 to 26.4						27.8	18.8	101	
DMSO		71.3		26.6	18.4	16.4	10.2							32.3	13.4	97	
EtOH		58.5		26.6	15.8	8.8	19.4							24.1	11.1	79	
Acetone	PCL-PEG	74.0		20.1	15.5	10.4	7.0					6.2	35.8	0.4	23.4	336	[60]
THF		81.7		19.4	16.8	5.7	8.0	19.6	17.1	4.6	8.5	1.2	38.9	0.4	27.1	458	
DMF		77.0		24.8	17.4	13.7	11.3					9.5	31.2	1.2	16.9	276	
DMF	PLGA-b-PEG	77.0		24.8	17.4	13.7	11.3					5.0	31.2	0.5	16.9	83	[70]
Acetone		74.0		20.1	15.5	10.4	7.0	22.7	17.3	8.7	11.8	5.4	35.8	0.6	23.4	138	
ACN		52.6		24.6	15.3	18.0	6.1					11.1	36.4	0.4	11.9	165	
THF		81.7		19.4	16.8	5.7	8.0					4.9	35.9	0.7	27.1	144	
Acetone	PLGA 50:50	74.0		20.1	15.5	10.4	7.0	23.0	16.5	10.4	12.2	5.3	35.8	0.6	23.4	165	[76]
ACN		52.6		24.6	15.3	18.0	6.1					9.8	36.4	0.4	11.9	164	
Acetone	PLGA	74.0		20.1	15.5	10.4	7.0					5.3	35.8	0.6	23.4	140	[79]
ACN		52.6		24.6	15.3	18.0	6.1	23.0	16.5	10.4	12.2	9.8	36.4	0.4	11.9	148	
THF	PLA	81.7		19.4	16.8	5.7	8.0					6.3	35.9	0.8	27.1	185	[80]
THF		81.7		19.4	16.8	5.7	8.0	21.7	16.2	8.9	11.3	4.6	35.9	0.5	27.1	237	
Acetone		74.0		20.1	15.5	10.4	7.0					4.6	35.8	0.4	23.4	220	
Acetone	PCL	74.0		20.1	15.5	10.4	7.0					6.0	35.8	0.4	23.4	203	[Author's data]
THF		81.7		19.4	16.8	5.7	8.0					1.0	35.9	0.4	27.1	192	
ACN		52.6		24.6	15.3	18.0	6.1	19.7	17.2	4.8	8.3	13.5	36.4	0.9	11.9	197	
DMF		77.0		24.8	17.4	13.7	11.3					9.4	31.2	1.2	16.9	180	

[a] Reference: Van Krevelen and te Nijenhuis [133].

[b] Data for pure solvents from Grulke [130]; data for solvent mixtures $= \sum(f_s\ \delta_s)$; where f_s: volume solvent fraction and δ_s: solubility parameter of solvent.

[c] Solubility parameter calculated by group contribution method according to van Krevelen–Hoftyzer procedure [133].

[d] Solubility parameter difference between substanceA and substanceB $(\Delta\delta) = [(\delta_{d,A} - \delta_{d,B})^2 + (\delta_{p,A} - \delta_{p,B})^2 + (\delta_{h,A} - \delta_{h,B})^2]^{1/2}$ where substanceA and substanceB refer to any polymer, solvent or water that correspond.

[e] Interaction parameter $\chi_{\text{substanceA}-\text{substanceB}} = 0.35 + [V_{\text{solvent}}/(RT)](\delta_{\text{substanceA}-\text{dsubstanceB}})^2$ where substanceA and substanceB refer to any polymer, solvent or water as correspond, V is the molar volume of the organic solvent, R is the gas constant, T is the temperature, and $\delta_{\text{substanceA}}$ and $\delta_{\text{substanceB}}$ are the total solubility parameters of any polymer, solvent or water that correspond.

[f] Data reported by Duclaroir et al. [40].

Table 11
Some physicochemical parameters related to the solvent/polymer/nonsolvent systems used for the sphere preparation by emulsification–diffusion method.

Solvent	Polymer	Solvent molar volume (ml/mol)[a]	Solvent solubility parameters ($MPa^{1/2}$)[b]				Polymer solubility parameters ($MPa^{1/2}$)[c]				$\Delta\delta_{polymer-solvent}$ ($MPa^{1/2}$)[d]	$\Delta\delta_{solvent-water}$ ($MPa^{1/2}$)[d]	$\chi_{polymer-solvent}$[e]	$\chi_{solvent-water}$[e]	Size (nm)	Reference
			δ	δd	δp	δh	δ	δd	δp	δh						
BA	PLGA 50:50	103.6	23.7	18.4	6.3	13.7	23.0	16.5	10.4	12.2	4.8	30.4	0.4	24.8	263	[85]
PC		85.0	27.2	20.1	18.0	4.1					11.7	38.6	1.0	15.1	152	
MEK		90.1	19.0	16.0	9.0	5.1					7.3	38.0	0.9	30.7	126	
EtAc		98.5	18.2	15.8	5.3	7.2					7.2	36.8	1.3	35.4	119	
EtAc	PLGA 50:50	98.5	18.2	15.8	5.3	7.2	23.0	16.5	10.4	12.2	7.2	36.8	1.3	35.4	64	[92]
DCM		63.9	20.3	18.2	6.3	6.1					7.5	37.7	0.5	20.0	502	
CHCl3		80.7	19.0	17.8	3.1	5.7					9.9	39.0	0.9	27.6	201	
EtAc:DCM (20:80)			19.9	17.7	6.1	6.3					7.4	37.5			266	
PC	PLGA 75:25	85.0	27.2	20.1	18.0	4.1	23.0	16.5	10.4	12.2	11.7	38.6	1.0	15.1	213	[90]
EtAc		98.5	18.2	15.8	5.3	7.2					7.2	36.8	1.3	35.4	117	
Acetone		74.0	20.1	15.5	10.4	7.0					5.3	35.8	0.6	23.4	221	
DCM		63.9	20.3	18.2	6.3	6.1					7.5	37.7	0.5	20.0	461	
CHCl3:EtOH (100:0)	PHBHV 23 kDa.		19.0	17.8	3.1	5.7	20.1	16.6	6.3	9.5	5.1	39.0			896	[94]
CHCl3:EtOH (90:10)			19.8	17.6	3.7	7.1					3.7	37.5			780	
CHCl3:EtOH (70:30)			21.3	17.2	4.8	9.8					1.6	34.5			540	
CHCl3:EtOH (60:40)			22.0	17.0	5.4	11.2					2.0	33.0			421	
CHCl3:EtOH (50:50)			22.8	16.8	6.0	12.6					3.1	31.5			356	
CHCl3:EtOH (40:60)			23.6	16.6	6.5	13.9					4.4	30.0			335	
CHCl3:EtOH (30:70)			24.3	16.4	7.1	15.3					5.8	28.6			253	
EtAc	PLGA 50:50	98.5	18.2	15.8	5.3	7.2	23.0	16.5	10.4	12.2	7.2	36.8	1.3	35.4	257	[22]
DCM:EtAc (50:50)			19.3	17.0	5.8	6.7					7.2	37.2			414	
DCM:EtAc (60:40)			19.5	17.2	5.9	6.5					7.3	37.3			452	
CHL:EtAc (50:50)			18.6	16.8	4.2	6.5					8.5	37.9			372	
Acetone:EtAc (50:50)			19.2	15.7	7.9	7.1					5.8	36.2			230	
Acetone:EtAc (60:40)			19.3	15.6	8.4	7.1					5.6	36.1			255	
EtAc	PCL	98.5	18.2	15.8	5.3	7.2	19.7	17.2	4.8	8.3	1.8	36.8	0.4	35.4	203	[Author's data]
PC		85.0	27.2	20.1	18.0	4.1					14.2	38.6	2.3	15.1	215	
EMK		90.1	19.0	16.0	9.0	5.1					5.4	38.0	0.4	30.7	115	
Water		18.0	47.9	15.5	16.0	42.4										

[a] Reference: Van Krevelen and te Nijenhuis [133].
[b] Data for pure solvents from Grulke [130]; data for solvent mixtures $= \sum (f_s\ \delta_s)$; where f_s: volume solvent fraction and δs: solubility parameter of solvent.
[c] Solubility parameter calculated by group contribution method according to van Krevelen-Hoftyzer procedure [133].
[d] Solubility parameter difference between substanceA and substanceB $(\Delta_d) = [(\delta_{d,A} - \delta_{d,B})^2 + (\delta_{p,A} - \delta_{p,B})^2 + (\delta_{h,A} - \delta_{h,B})2]^{1/2}$ where substanceA and substanceB refer to any polymer, solvent or water that correspond.
[e] Interaction parameter $\chi_{substanceA-substanceB} = 0.35 + [V_{solvent}/(RT)](\delta_{substanceA} - \delta_{substanceB})^2$ where substanceA and substanceB refer to any polymer, solvent or water that correspond, V is the molar volume of the organic solvent, R is the gas constant, T is the temperature, and $\delta_{substanceA}$ and $\delta_{substanceB}$ are the total solubility parameters of any polymer, solvent or water that correspond.

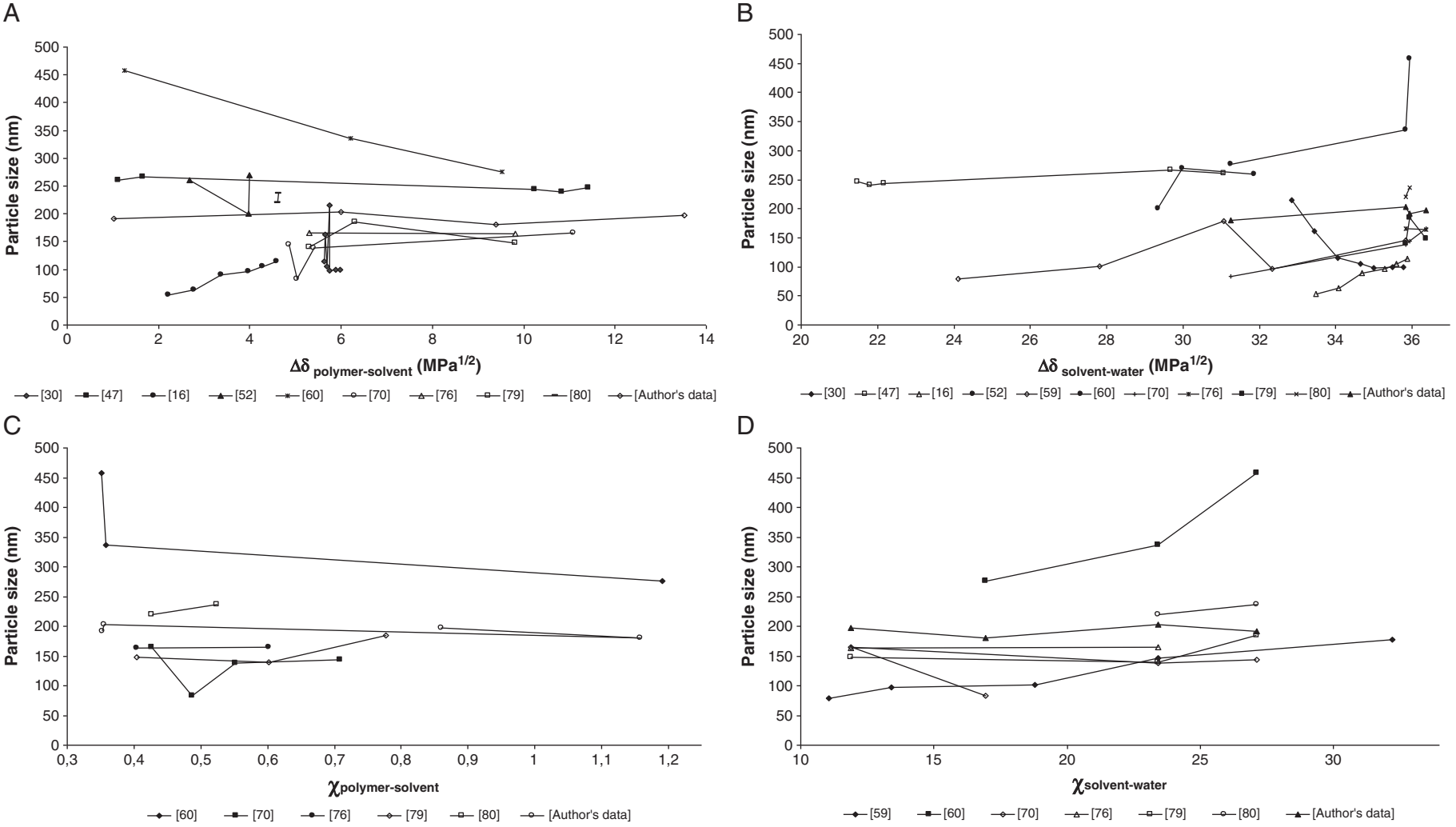

Fig. 13. Influence of some physicochemical parameters related to the solvent/polymer/nonsolvent systems on the size of particles prepared by solvent displacement.

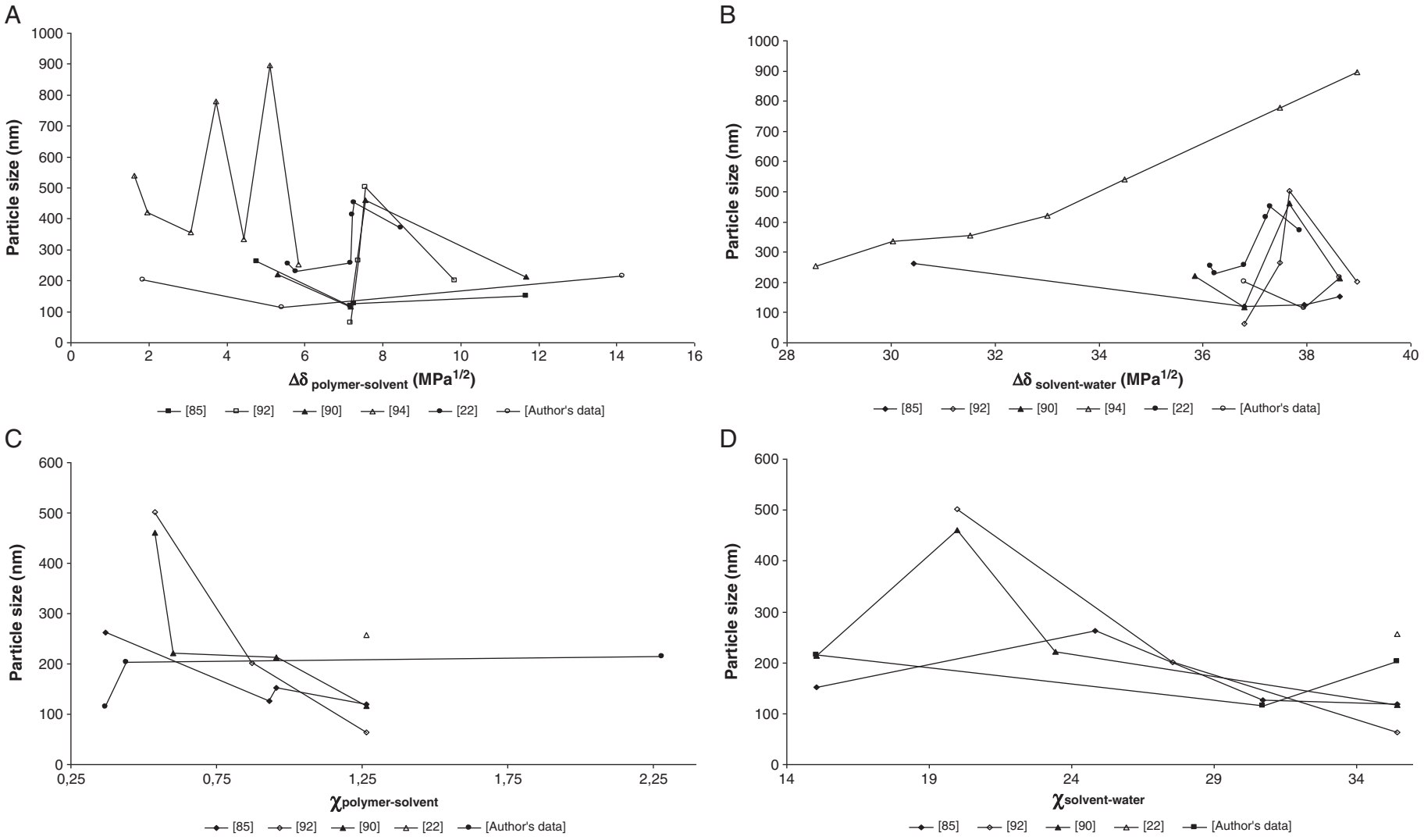

Fig. 14. Influence of some physicochemical parameters related to the solvent/polymer/nonsolvent systems on the size of particles prepared by emulsification–diffusion.

Table 12
Properties of the solvents commonly used in the solvent displacement and emulsification–diffusion methods and preliminary comparative analysis respect to the particle size behavior.

	Solvent displacement method				Emulsion–diffusion method		
	Acetone	THF	ACN	DMF	EtAc	PC	MEK
ρ (g/cm^3; 20 °C)[a]	0.792	0.888	0.783	0.949	0.901	1.201	0.805
η (mPa s; 25 °C)[a]	0.32	0.36	0.35	0.80	0.44	2.8	0.42
γ (10^{-3}; N/m; 20 °C)[a]	23.7	26.4	29.3	~36.76/~38	23.9	40.5	~24.3
Water solubility (%, 25 °C)	Miscible[b]	Miscible[b]	Miscible[b]	Miscible[b]	8.2[c]	21.7[c]	27.5[b]
ρ solvent order	DMF>THF>ACN≈Acetone				PC>EtAc>MEK		
η solvent order	DMF>THF≈ACN≈Acetone				PC>>EtAc≈MEK		
γ solvent order	DMF≫ACN>THF>Acetone				PC>>EtAc≈MEK		
Particle size order	Acetone≈ACN≈THF≈DMF				PC>EtAc≈MEK		

[a] Reference: [133].
[b] Reference: [136].
[c] Reference: [137].

specific particle sizes when the emulsification–diffusion method is used.

5.3.2. Influence of the nature of solvent on particle zeta-potential

The results compiled under the subheadings devoted to the analysis of natures of polymer and stabilizing agent suggest that the nature of the organic solvent might play a role in the electrostatic behavior of submicron particles, particularly when PCL is used as the polymer. Therefore Fig. 15 shows the PCL particle zeta potential as a function of the solvent and the preparation method. It is clear that the solvent influences the particle zeta-potential, particularly when the emulsification–diffusion method is used. One possible explanation for this is that the monomer structure of PCL has one hydrophilic carboxylic group and one hydrophobic alkyl chain (five monomer units). Thus different molecular arrangements of the polymeric chains could be obtained, depending on the nature of the solvent used to ensure re-precipitation. When PCL is precipitated from PC, a hydrophilic solvent (dielectric constant: 64.8 at 25 °C [138]), during the solvent diffusion to water phase, the polar parts of the PCL are predominantly exhibited in the vicinity of the water–polymer interface. Taking into consideration the randomness and speed of polymer precipitation, it is possible that only a few alkyl chains are positioned facing the aqueous phase.

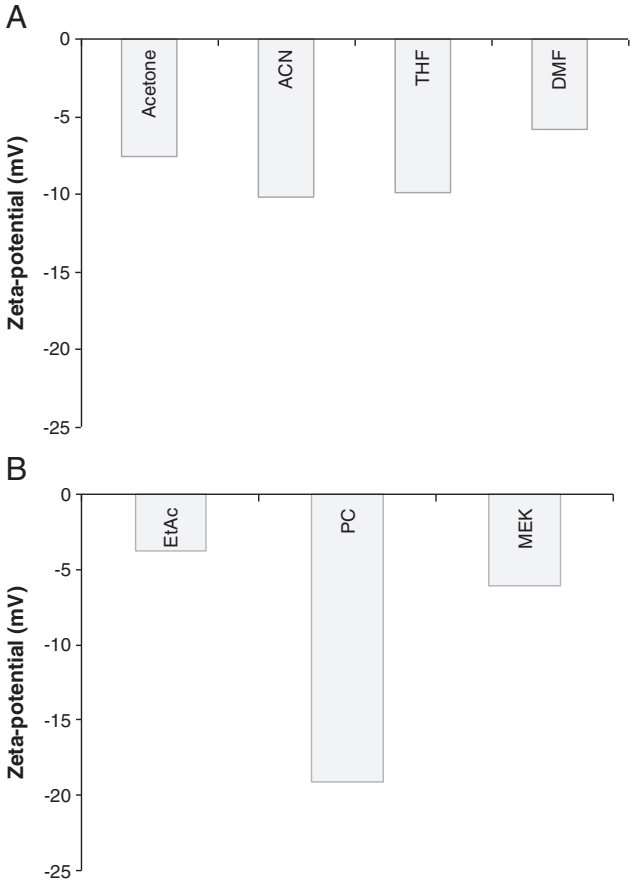

Fig. 15. Influence of solvent nature on the zeta-potential of particles. A: Prepared by solvent displacement: PCL 14 kDa (3.5 mg/ml), stabilizing agent PLX 0.4%; B: prepared by emulsification–diffusion: PCL 14 kDa (10 mg/ml), stabilizing agent PLX 1%.

In the same way, when EtAc is used as a solvent due to its hydrophobic nature (dielectric constant: 6.27 at 20 °C [133]), the PCL alkyl chains are located near the particle surface in the solvent diffusion step and carboxylic groups can be positioned facing the external phase. MEK, a solvent with intermediate polarity (15.45 to 18.51 [133]), permits the preparation of particles with intermediate zeta-potential.

The investigations of the surface properties of PCL films performed by Tang et al. [139] lend credence to this approach to particle formation. Their results from contact angle, surface morphology and attenuated total reflection–Fourier transform infrared spectroscopy (ATR–FTIR) prove that polymer arrangement depends on the nature of the solvent.

Regarding the solvent displacement method, in spite of the significant difference between the dielectric constants of the solvents used for submicron particle preparation, there is no tangible difference between the solvents with respect to particle zeta-potential. This might be because the lower concentration of the polymer used did not permit the detection of a clear tendency. However, seen from another angle, the difference observed between the two methods might suggest a critical influence on particle electrostatic behavior of the organic solvent present in the solvent-saturated aqueous phase when using emulsification–diffusion method. Unfortunately, to our knowledge there is no experimental evidence to support this approach, thus it is not possible to deal with this issue in-depth.

6. Concluding remarks

There is increasing interest in investigating submicron particles due to their potential capacity for carrying drugs, targeting systems and overcoming the typical problems of conventional drug delivery systems due to the stability, dissolution, gastrointestinal mucosa irritation or the disagreeable organoleptic properties of the active substances used. Consequently, the preparation method is a key step for ensuring that particles behave according to the use intended. As can be seen in this review devoted to the study of the solvent displacement procedure and emulsification–diffusion technique, the operating variables and starting materials used influence the size and

zeta-potential of particles as well as their capacity to entrap and load active molecules.

The study of mechanistic aspects reveals that the formation of submicron particles depends on the combination of operating conditions, the composition of the organic and aqueous phases (since it determines their physicochemical properties) and the physicochemical interactions between phases. The extent of their participation is unclear at present, though it appears that one prevails over another depending on their interrelationship.

The emulsification–diffusion technique is the more robust method from the experimental standpoint. In this case, the emulsification rate and time are the key variables for obtaining specific particle size without any influence being introduced by the dilution step. On the contrary, the size of submicron particles prepared by solvent displacement is strongly determined by the interrelated effects of operating variables such as the method for mixing the organic and aqueous phases, the system stirring rate and the organic phase volume. Nevertheless, the use of large volumes of material, i.e. water for dilution, and high power consumption due to emulsification through high mechanical shear strength, are disadvantages for the emulsification–diffusion method that contrast with the very simple procedure and assembly required for the solvent displacement technique.

The size of submicron particles can be influenced by the materials used in their preparation. Thus the nature of the polymer and the stabilizing agent influences the size of the particles prepared by the two methods. Likewise, polymer concentration is a critical factor for obtaining specific particle size by solvent displacement, whereas the concentration of the stabilizing agent may influence the sizes of particles prepared by emulsification–diffusion. The nature of the organic solvent also plays a key role but only in the emulsification–diffusion method.

Regarding zeta-potential, it is influenced by the nature of the polymer and stabilizing agent chosen. Zeta-potential behavior depending on solvent polarity was observed for PCL submicron particles prepared by the emulsification–diffusion method. This opens the door for new research work focusing on, for example, the molecular ordering of polymeric chains depending on the ratio of hydrophilic and hydrophobic groups and its incidence on particle properties.

The literature suggests, that regardless of the method, the efficient entrapment of active molecules depends on their partition between the aqueous and the organic phases, molecule–polymer affinity and polymer precipitation rate. Therefore, taking into consideration and comparing the mechanisms governing particle formation by each method, it might be expected that molecule–polymer affinity would be more critical when the solvent displacement technique is used and molecule partition between phases would be the predominant factor in the emulsification–diffusion method. As particles are formed immediately phases are mixed, the solubility of the active substance in the polymer governs its entrapment by the solvent displacement technique. Regarding the emulsification–diffusion method, the emulsification step could facilitate the migration of the active molecule towards the aqueous phase, thus it governs the amount of active substance to be encapsulated. Therefore additional factors associated with the starting materials used for preparing the particles, such as operating conditions, might influence the loading and entrapment of active substances. Unfortunately, the literature does not allow in-depth analysis or making general statements on this subject.

On the other hand, in addition to the identification of general trends and correlations between variables and particle behavior, studying the method requires taking into account the mechanistic aspects related to particle formation. Thus this review contributes to discussion by analyzing the available data from the physicochemical standpoint, i.e. polymer/solvent/water molecular interactions and organic solvent properties.

In brief, taking into consideration that neither solvent–polymer–water interactions nor solvent physicochemical properties seem to govern the size of particles prepared by the solvent displacement technique, it is possible that nucleation and mechanical phenomena occur simultaneously. Apparently, their respective importance as the main mechanisms for particle formation depends on the organic phase/aqueous phase ratio. Thus mechanical phenomena predominate at the highest phase ratios.

For the emulsification–diffusion method, particle size has some correlation with $\chi_{\text{polymer–solvent}}$ which suggests that particle formation from a drop of emulsion is the most convincing mechanistic approach. In addition, there is good agreement between the density, viscosity and surface tension of the solvents and particle size, thereby supporting the hypothesis that emulsion formation is the critical factor for obtaining specific particle sizes.

Furthermore, these findings allow us to explain the main drawback of the solvent displacement procedure compared with the emulsification–diffusion method. Since particle formation by the emulsification–diffusion method is governed by the emulsion step, the high mechanical force used for obtaining the emulsion facilitates processing larger quantities of polymer for obtaining particles of a specific size. On the contrary, the solvent displacement technique can only be carried out with low polymer concentrations, due to the limitation of working in the “metastable region”, which means low particle yields. Research on this subject is underway to determine the industrial applicability of this procedure [62,75,140,141].

In conclusion, the results compiled and analyzed in this review give a complete background on the incidence of the method variable on submicron particle properties when the solvent displacement and emulsification–diffusion methods are used. By taking a logical approach from the outset, they can be used as the starting points for defining the work conditions and choosing the starting materials according to the aims of each research team. Also, they can be used for implementing versatile strategies for submicron particle production as the statistical design of experiments [31,35,61,142,143]. However, the comprehensive study carried out highlights the importance of making progress regarding research into the mechanistic aspects related to particle formation. Basic understanding of how particles are formed leads to flexible process manipulation achieved by varying process parameters and using suitable starting materials.

Acknowledgements

The authors are grateful to Dr. Magdy Ayoub from National Research Center Cairo for helpful discussions, to Serge Buathier for the transmission electron microscopy performed at the Université Claude Bernard Lyon 1 and to Hassan Saadaoui for the atomic force microscopy performed at the Centre de Recherche Paul Pascal, France. C.M was supported by a grant from Departamento Administrativo de Ciencia, Tecnología e Innovación — Colciencias (Colombia). She also acknowledges the Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Farmacia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.cis.2011.02.005.

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