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Stability-limit "Ouzo region" boundaries for poly(lactide-*co*-glycolide) nanoparticles prepared by nanoprecipitation

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ABSTRACT

The introduction of "Ouzo diagrams" has enhanced the applicability of the basic nanoprecipitation process for drug delivery research. The current study investigated the interaction of two relevant polymer/solvent systems, which is thought to impact the location of the stability–limit "Ouzo boundary".

Viscosity measurements (Kurata–Stockmayer–Fixman approach) and static light scattering (Debye method) underlined a distinct interplay of the employed polymer (poly(lactide-*co*-glycolide)) with the utilized organic solvents (acetone and tetrahydrofuran). Both methods indicated that tetrahydrofuran was the "better" solvent for poly(lactide-*co*-glycolide). Thus, nanoprecipitation of this polymer/solvent composition resulted in larger nanoparticles. This observation can be attributed to the chain configuration of poly(lactide-*co*-glycolide) in the organic solvent, which influenced the extent of the break-up of the injected solvent layer. Accordingly, the stability–limit curve of the "Ouzo region" was shifted to lower poly(lactide-*co*-glycolide) fractions for tetrahydrofuran.

Overall, the location of the "Ouzo region", which is an essential tool for drug delivery research, is influenced by the employed organic solvent. The current study described two distinct methods suitable to identify relevant polymer–solvent interactions, which dictate the stability–limit "Ouzo boundary" for relevant poly(lactide-*co*-glycolide).

1. Introduction

Nanomedicine has shown significant potential for the treatment of numerous severe diseases (Farokhzad and Langer, 2009; Shi et al., 2010). The application of nanotechnology in drug delivery is associated with an increased therapeutic index of the administered medication (Couvreur, 2013). Among the diverse colloidal drug delivery systems, nanoparticles composed of degradable polyesters such as poly(lactide-*co*-glycolide) (PLGA) have been most-frequently utilized, in part because of their well-documented biocompatibility and controlled drug release potential (Danhier et al., 2012; Nicolas et al., 2012; Webber et al., 2016). However, a notable issue with significant impact on the biological performance of polymer nanoparticles is their size. It was previously shown, that a preferential organ/tissue accumulation accompanied by a localized action of the encapsulated drug is only achieved when meeting a specific particle size (Beck-Broichsitter et al., 2015a; Moghimi et al., 2012).

Accordingly, special emphasis needs to be placed on the nanoparticle preparation process (Vauthier and Bouchemal, 2009). In this respect, basic nanoprecipitation was recently expanded by the introduction of phase diagrams (i.e., polymer/solvent/non-solvent compositions) depicting the operating window for the production of stable colloidal formulations (Ganachaud and Katz, 2005; Lepeltier et al., 2014). The application of so-called "Ouzo diagrams" represents a valuable tool for drug delivery research and will most-likely replace the "trial-and-error"-approach currently employed for the production of polymer nanoparticles with defined size distributions (Aubry et al., 2009; Beck-Broichsitter et al., 2015b, 2010; Yan et al., 2014). However, only scant information is available defining the "Ouzo boundaries" for relevant polymer (i.e., PLGA)-solvent (i.e., acetone and tetrahydrofuran (THF)) systems (Beck-Broichsitter et al., 2015b, 2010).

Abbreviations: A_2, second virial coefficient; B, polymer–solvent interaction parameter (from Kurata–Stockmayer–Fixman plot); c, concentration; $Đ$, dispersity; d_h, hydrodynamic diameter; η, dynamic viscosity; η_0, dynamic viscosity of solvent; $[\eta]$, intrinsic viscosity; η_{sp}, specific viscosity; f_{PLGA}, mass fraction of PLGA; f_s, mass fraction of solvent; f_w, mass fraction of the aqueous non-solvent; M_n, number-average molecular weight; M_w, weight-average molecular weight; dn/dc, refractive index increment; OD, optical density; PCS, photon correlation spectroscopy; PDI, polydispersity index; PLGA, poly(lactide-*co*-glycolide); R^2, regression coefficient; SD, standard deviation; SLS, static light scattering; THF, tetrahydrofuran.

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The current study identified PLGA/acetone and PLGA/THF interaction parameters, which are (among other factors) thought to influence the stability–limit "Ouzo region" boundary. Therefore, polymer solutions were thoroughly characterized by means of viscosity measurements and static light scattering (SLS). Next, PLGA nanoparticles were formed by nanoprecipitation and the obtained stability–limit "Ouzo region" boundaries were correlated with the determined polymer–solvent interaction parameters.

2. Experimental section

2.1. Materials

PLGA, Resomer® RG502H (#1004596), RG503H (#1025663) and RG504H (#RES0485) were provided by Boehringer Ingelheim (Germany). The physicochemical properties of the PLGA polymers are outlined in Table S1 (Supplementary material). Acetone (ROTISOLV® HPLC) and THF (ROTIDRY®, ≥99.9%, ≤50 ppm H_2O) were stored over a molecular sieve (metal-aluminum silicate spheres (1.6–2.5 mm), pore size: 3 Å (type 564)) (all from Carl Roth, Germany). Poloxamer 188 was purchased from Sigma–Aldrich (Germany). Distilled water was acquired from B. Braun (Germany). All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Preparation and characterization of polymer solutions

PLGA stock solutions were prepared with dry acetone and THF and allowed to equilibrate for 12 h before the measurements. Samples were filtrated prior use (1.2 μm; Cameo 30 N syringe filters, GE Water & Process Technologies, Germany).

The density, refractive index and viscosity of polymer solutions were measured using an oscillating density meter (DMA 4100 M, Anton Paar, Austria), a refractometer (DR201-95, Krüss, Germany) and a temperature controlled capillary viscosimeter of the Ubbelohde type (Capillary No. 0 (Type No. 53100), Schott, Germany) at 25.0 ± 0.1 °C (equilibration time of ≥5 min).

SLS was performed (single scattering angle of 173°, λ = 633 nm) on a Zetasizer NanoZS/ZEN3600 (Malvern Instruments, Germany) (Lee et al., 2004). The background light intensity and scattering intensity of the standard toluene (Wu, 2010) (ROTIDRY®, ≥99.5%, ≤50 ppm H_2O; Carl Roth, Germany) was checked prior to the samples. All measurements were done in glass cuvettes at 25.0 ± 0.1 °C (equilibration time of ≥5 min).

2.3. Preparation and characterization of polymer nanoparticles

Polymer nanoparticles were prepared by a nanoprecipitation technique (Beck-Broichsitter et al., 2015b, 2010). Briefly, PLGA dissolved in acetone and THF was injected (injection needle: Fine-Ject® 0.6 × 30 mm) into magnetically stirred (500 rpm) aqueous phase containing 0.1% poloxamer 188 (flow rate: 10.0 ml/min). After injection of the organic phase, the resulting colloidal dispersion was stirred for 10 min under a fume hood before removing the organic solvent by rotary evaporation (Rotavapor®, Büchi, Switzerland). The actual mass concentration of polymer nanoparticles in the aqueous phase was determined as previously described (Beck-Broichsitter et al., 2013). Nanosuspensions were characterized directly after preparation.

The hydrodynamic diameter (d_h) and size distribution (i.e., polydispersity index (PDI)) of polymer nanoparticles were measured by photon correlation spectroscopy (PCS) (Zetasizer NanoZS/ZEN3600) (Varenne et al., 2015). All measurements were performed at a temperature of 25.0 ± 0.1 °C (equilibration time of ≥5 min) using appropriately diluted samples.

2.4. Construction of ternary phase diagrams

As a map of compositions a right triangle, three-component phase diagram ("Ouzo diagram") was chosen and constructed as previously described (Aubry et al., 2009; Beck-Broichsitter et al., 2015b, 2010; Yan et al., 2014). Therefore, organic polymer solutions were added to the aqueous non-solvent phase to reach the desired final mass fractions in the ternary system. The mass fraction of PLGA (f_{PLGA}) was plotted on the abscissa and the mass fraction of solvent (f_s) can be found on the ordinate. The mass fraction of the aqueous non-solvent (f_w) is obtained by the difference $f_w = 1 - f_{PLGA} - f_s$.

2.5. Determination of "Ouzo region" boundaries

The "Ouzo region" is surrounded by the miscibility-limit boundary (for "low" f_{PLGA}), where the presence of polymer nanoparticles became apparent by a sudden increase in sample turbidity, and by the stability–limit boundary (for "high" f_{PLGA}), where nanoprecipitation forms nanoparticles and microparticles. The stability–limit curve was determined by comparing the optical density (OD, λ = 600 nm; Ultrospec® 3000, Pharmacia Biotech, Germany) of raw and filtered (1.2 μm) nanosuspensions (separation of microparticles, reduction in OD of the sample) (Aubry et al., 2009; Beck-Broichsitter et al., 2015b).

2.6. Statistics

All measurements were carried out in triplicate and values are presented as the mean ± standard deviation (SD) unless otherwise noted. Statistical calculations were performed with Origin 8.5 (OriginLab, USA).

3. Results and discussion

Nanotechnology has revolutionized the biomedical field, owing to the versatile novel biological features of nanoscale drug carriers (Farokhzad and Langer, 2009; Shi et al., 2010). Compared to "simple" drug solutions, application of said vehicles enabled a distinct medicament distribution pattern throughout the body (Beck-Broichsitter et al., 2015a). As an example, polymer nanoparticles achieved a size-dependent accumulation within distinct target organs, which is thought to contribute to the development of a more specialized therapy of life-threatening diseases (e.g., cancer) (Couvreur, 2013; Moghimi et al., 2012). Furthermore, nanomedicine offers the chance to overcome a short duration of drug action, drug resistance and significant toxicities, due to random drug distribution.

Although rather convenient, the basic nanoprecipitation process offers only poor control over particle properties (Mora-Huertas et al., 2011). In order to provide polymer nanoparticles with defined particle sizes, nanoprecipitation was recently amplified by introduction of "Ouzo diagrams" in order to obtain defined colloidal formulations (Aubry et al., 2009; Beck-Broichsitter et al., 2015b, 2010; Lepeltier et al., 2014; Yan et al., 2014). Beside the diffusion coefficient (Beck-Broichsitter et al., 2010) in and interplay (Beck-Broichsitter et al., 2015b; Galindo-Rodriguez et al., 2004) of the employed organic solvent with the aqueous non-solvent phase, especially the interaction of the polymer and solvent is of significant relevance for the stability–limit "Ouzo boundary", a parameter that has so far not been studied in detail (Mora-Huertas et al., 2011). However, a deeper understanding of the underlying mechanisms involved in nanoparticle formation by nanoprecipitation would clearly enhance our knowledge on the applicability and potential limitations of "Ouzo diagrams" to further support drug delivery research.