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Letter to Editor Open Access

Vibration Technology for Microencapsulation: The Restrictive Role of Viscosity

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Abstract

Microencapsulation employed in a broad range of applications, including those with a strict demand on standardization, requires to understand the limitations of respective encapsulation technologies. Among the most frequently used technologies, vibration technology has gained a significant interest due to high capacity and capability to produce uniform and monodisperse microspheres. In this contribution, the restrictive role of viscosity and practical consequences regarding the production of microspheres using vibration technology are reported and discussed.

Keywords: Microencapsulation; Vibration technology; Viscosity; Microsphere size

To the Editor

For the past decades, microencapsulation has been widely utilized in a variety of biotechnological, agricultural, pharmaceutical and medical applications as a versatile technology allowing immobilization, protection, release and functionalization of diverse chemical and biological materials [1]. In the current era of cellular-based therapies, special attention is given to cell encapsulation as a modern and promising strategy for treating various types of diseases [2,3]. With respect to the specific therapeutic application, strict requirements are imposed on the selection of suitable biocompatible polymers with well-defined physico-chemical properties that are primarily determined by chemical structure and molar mass distribution. Beside the development of suitable materials for cell encapsulation, there is a distinct demand for technologies that meet the requirements of production capacity, minimal batch-to-batch variability and, eventually, meet the regulatory standards necessary for the GMP grade products for testing in clinical setting.

In the recent years, several technologies applying various physical and chemical principles have been established for production of microspheres aimed at cell encapsulation [4]. Vibration technology based on breaking up a laminar liquid stream into droplets by a superimposed vibration has gained a significant interest mainly due to the capability to produce uniform and monodisperse microspheres [5]. For the purposes of operator-free, standardized and reproducible production of microspheres with defined sizes, the automatization of microencapsulation process is highly needed.

One of the commercially available encapsulators for standardized microspheres production is the semi-automated Buchi Encapsulator B-395 Pro. This device employs vibration technology for controlled manufacture of microspheres with size approximately twice the diameter of the used nozzle. The provided set of 8 single nozzles (80 µm; 120 µm; 150 µm; 200 µm; 300 µm; 450 µm; 750 µm; 1000 µm) enables production of microspheres ranging from 150 to 2000 µm in size with a declared size distribution of less than 5%. Beside the set of nozzles, a number of adjustable parameters such as (i) liquid flow rate (0.01-50.00 mL/min), (ii) vibration frequency (40-6000 Hz), and (iii) electrode tension (250-2500 V) enable a variety of individual set-ups and allow for fine-tuning of size and shape of produced microspheres.

Despite detailed description of microspheres production using vibration technology, only minimal attention has been paid to the impact of viscosity [5-7], while no practical consequences have been directly disclosed. Viscosity is, however, a key property of a polymer solution intended for encapsulation, and, as detailed below, is a crucial factor determining the controllable production of microspheres using this technology.

In this study, the Buchi Encapsulator B-395 Pro was tested to evaluate the role of viscosity in microspheres production. With respect to the applicability of this encapsulator, declared by the manufacturer (http://www.buchi.com[I,II]), the impact of viscosity and the practical aspects behind the production of microspheres using vibration technology are reported and discussed in this paper.

Experiments were performed using alginate solutions (Protanal LF 10/60, FMC Biopolymer, Norway, Batch No. S 19905) of concentrations 0.5-4.0% (w/v) to cover a broad range of viscosities (10-2100 cP). The viscosity curves for Protanal solutions, as well as for alginate solutions provided in the Buchi operation manual (http://www.buchi.com[I]) (Buchi alginate, Batch No. GQ5608003) are shown in Figure 1.

Alginate microspheres were prepared according to the manufacturer's instructions specified in the Buchi operation manual by (i) following the set-ups within the declared *optimal working ranges* (http://www.buchi.com[I,III]), (ii) reproducing the set-ups within the referred *working ranges* (http://www.buchi.com[I,II]), and (iii) applying conditions outside the defined *working ranges* (Figure 2). All experiments were performed using alginate solutions of the defined viscosities and single nozzles, respectively, whereas the produced microspheres were hardened in a gelling solution containing 1.0 % (w/v) $CaCl_2$ and 0.9% (w/v) NaCl for 5 minutes. The size and shape of prepared microspheres were evaluated by optical microscopy using the Prover ImageForge software (Prover s.r.o., Slovakia). The calculated

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coefficient of variation (*Cv*) was used to compare the size dispersity of microspheres produced by different set-ups.

The experiments performed within the *optimal working range* for a given viscosity led to reproducible results with a narrow size distribution and spherical shape of microspheres. On the other hand, experiments performed outside the *optimal working range* led to unsatisfactory results in terms of increased size dispersity, non-spherical shape or inconsistent microspheres. Within the *working range* defined for a particular viscosity, fine-tuning of adjustable parameters may be applied towards improved size dispersity and shape of produced microspheres. However, as these parameters are mutually interdependent [6] (http://www.buchi.com[I,IV]) and the required values for a given nozzle diameter are determined empirically, finding the proper combination of these values appears to be difficult [7,8]. Most importantly, outside the defined *working range*, the production of microspheres was significantly restricted by the viscosity of the polymer solution. It is of extreme importance to note, that working conditions outside the *working ranges* are not specified in detail in the operation manual, therefore, it is difficult to draw clear conclusions regarding the applicability of the encapsulator for viscosities and nozzles within this area.

These limitations were observed for all the tested viscosities. For a demonstration of the effect of viscosity on microspheres production, the obtained data are presented *via* one representative viscosity (90 cP) and are, together with the declared *working ranges*, shown in Figure 2.

The reported results indicate several important practical implications. When considering the use of a specific polymer solution for production of microspheres with a defined size, first of all, its viscosity has to be taken into account. This, in fact, primarily determines the range of applicable nozzles and thus defines the achievable size range of produced microspheres. If too high, viscosity may cause gradual clogging of particular nozzles, which consequently determines the minimal applicable nozzle diameter and, therefore, the minimal achievable microsphere size (Figure 2A). Moreover, as we reported previously, the higher the viscosity, the lower the effect of superimposed vibration on microspheres production [8]. On the other hand, if too low, viscosity might lead to limitations in terms of inability to produce spherical microspheres using particular nozzles (Figure 2E), which in turn determines the maximal achievable microsphere size for a given polymer solution. Furthermore, within the range of applicable nozzles, only a limited number of nozzles allows for reproducible production of spherical microspheres with low size dispersity (Figure 2C), as deviations in terms of non-sphericity (Figure 2D) or significant size dispersity (Figure 2B) can occur.

Interestingly, on the official website (http://www.buchi.com[II,V]), Buchi Labortechnik AG declares only minor influence of physico-chemical properties of the polymer mixture on microsphere size, whereas major impact is attributed to the nozzle size. However, the effect of superimposed vibration [8], the range of applicable nozzles, and thus the size range of achievable microspheres, clearly depend on the viscosity of the polymer mixture. This means that despite the fact that the Buchi Encapsulator B-395 Pro allows for adjustment of several parameters to tune the size and shape of produced microspheres, it is the viscosity of the polymer solution that predominantly determines the applicability of vibration technology for the production of microspheres of desired size. The role of viscosity was outlined also by Heinzen et al. [6,7], Prüsse et al. [4] and Mazzitelli et al. [9] who reported on its impact on achievable microsphere sizes using vibration technology. Also, Whelehan et al. [5] suggest that viscous polymer

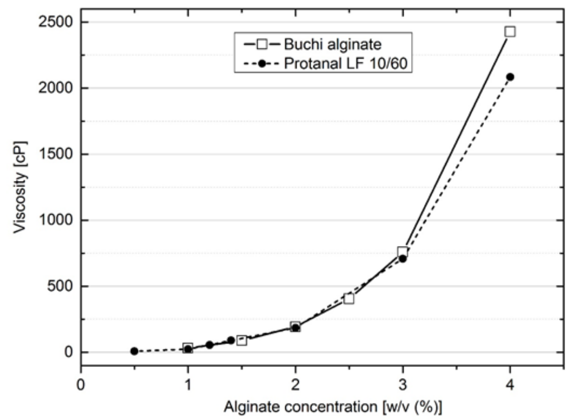
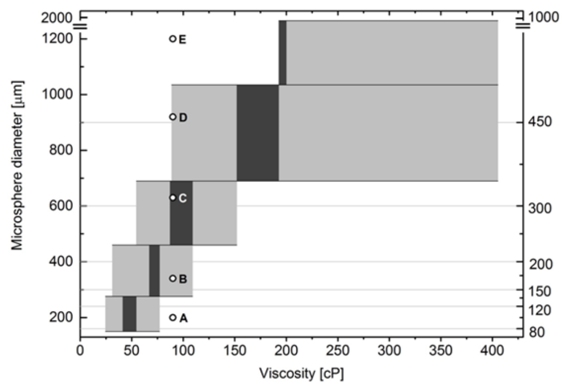
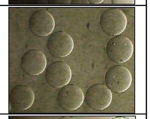
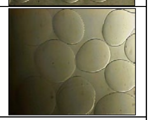


Figure 1: Solid line (empty squares) stands for Buchi alginate; dashed line (solid circles) stands for Protanal LF 10/60. All viscosities were determined at 25°C



Point	Range	Nozzle [μm]	Microsphere diameter [μm]	*Cv* [%]	Shape
A	Outside *working range*	< 150	n/a[a]	n/a[a]	n/a[a]
B	*Working range*	150; 200	340	18	
C	*Optimal working range*	300	630	5	
D	*Working range*	450	920	15	
E	Outside *working range*	> 450	n/a[b]	n/a[b]	n/a[b]

n/a – not available: [a]viscosity of the solution is too high; [b]viscosity of the solution is too low

Figure 2: (Top) Individual *working ranges* declared by the manufacturer and described in the Buchi operation manual (http://www.buchi.com[I,III]): white – outside *working range*, grey – *working range*, dark grey – *optimal working range*. A-E stand for representative experimental data for viscosity of 90 cP highlighting the principal impact of viscosity on microspheres production. (Bottom) Real experimental data associated to A-E

solutions with non-Newtonian dynamics constrain the applicability range of this technology. However, none of these studies have critically discussed the practical consequences of the reported restrictions.

In this paper, the restrictive role of viscosity is demonstrated and clear practical implications are reported. This study highlights the fact, that the viscosity of the polymer mixture is the key parameter determining the range of applicable nozzles and thus the size of spheres that could be produced by vibration technology. In other words, viscosity may restrict the preparation of microspheres of the desired size using the intended polymer solution.

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Notes

I. http://www.buchi.com/en/system/files/downloads/B-395_OM_en_C.pdf

II. http://www.buchi.com/en/products/spray-drying-and-encapsulation/encapsulator-b-395-pro

III. pages 52 (Table 6-3) and 68 (Table 6-7)

IV. pages 46 (6.8.1: Note #2); 66-68 (6.15)

V. section FAQ/What influences the final bead size?

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