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Comparison between spray drying and freeze drying techniques for the preparation of microparticles for delivery via a dry powder inhaler to treat cystic fibrosis

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ABSTRACT: Cystic fibrosis is the most common autosomal recessive disease that shortens life expectancy. According to studies, approximately 60 to 70% of adult patients are infected with *P. aeruginosa*. The current work explores the possibility of preparing microparticles using the spray-drying and freeze-drying methods and comparing the results obtained. A combination of ivacaftor and ciprofloxacin was loaded in microparticles of bovine serum albumin with L-leucine by the spray drying and freeze drying approaches to generate microparticles that could be delivered via a dry powder inhaler. The spray-dried microparticles had a particle size of 1.6 ± 0.04 µm with a polydispersity ratio of 0.33. They had a zeta potential of -27.3 ± 1.1 mV. The mass median aerodynamic diameter of the spray-dried microparticles was 3.74 ± 0.08 µm. The freeze-dried microparticles had a particle size of 0.8175 ± 5.6 µm with a polydispersity ratio of 0.33. They had a zeta potential of -23.3 ± 1.1 mV. The mass median aerodynamic diameter of the microparticles was 3.75 ± 0.07 µm. The microparticles produced by the spray-drying process were found to have better aerosol performance.

KEYWORDS: Dry powder inhaler; spray-drying; freeze-drying; microparticles; cystic fibrosis; *Pseudomonas aeruginosa*.

1. INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disease that shortens life expectancy. It has been found that the triad of *Hemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* appears to be most commonly isolated from CF patients' airways [1]. According to studies, approximately 27% of patients with CF aged 2–5 years and 60 to 70% of adult patients are infected with *P. aeruginosa*, a Gram-negative bacterium found in many natural and artificial water sources [2]. Due to its opportunistic nature, this bacterium is regarded as the most critical CF pathogen [3]. The development of new antibiotics or innovative therapeutic approaches for treating *P. aeruginosa* infections is imperatively required for patients whose infections are resistant to currently available antibiotics. Ivacaftor (IVA) was the first FDA-approved cystic fibrosis transmembrane conductance regulator (CFTR) modulator. A synergistic effect between ivacaftor and colistin in eradicating *P. aeruginosa* infection has been reported in previous studies [4].

The pulmonary route has long been used for local and systemic treatment drug administration. It possesses several advantages, which can be categorized into physiological, i.e., large surface area, thin epithelial membrane, highly vascularised, limited enzymatic activity, and patient convenience, i.e., non-invasive, self-administration over oral and systemic routes of drug administration. However, the formulation of dry powder for pulmonary delivery is often challenging due to restrictions on aerodynamic size and the lung's lower tolerance capacity in comparison with an oral route of drug administration. In addition, various physicochemical properties of dry powder play a significant role in aerosolization, deposition, and clearance along the respiratory tract. Therefore, different manufacturing methods have been established to prepare suitable particles with optimal physicochemical properties for inhalation. Preparing dry powder for inhalation is challenging, especially within the most desired particle size range of 1–5 µm [5,6]. Researchers have studied many techniques to achieve this ideal size range, including milling, freeze-drying, spray-freeze-drying, and supercritical fluid-drying. Recently, novel technologies such as particle replication in non-wetting templates, inkjet printing, thin-film freezing, and hot-melt extrusion have emerged as potential technologies for the

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preparation of improved dry powder for inhalation. Among all these mentioned techniques, milling and spray-drying are primarily used in pharmaceutical companies to prepare dry powder for inhalation.
The present work proposes a novel combination between a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator, ivacaftor (IVA) and a fluoroquinolone antibiotic, ciprofloxacin (CIP). Both drugs have been used individually in treating cystic fibrosis, whereas our work aims to use them synergistically by exploring the antibacterial effects of both drugs. The combination of ivacaftor and ciprofloxacin would be loaded into microparticles of L-leucine and bovine serum albumin (BSA). These microparticles would be designed to be delivered by a dry powder inhaler. The microparticles of the combination would be prepared by using spray-drying (SD) as well as freeze-drying (FD) methods. The microparticles would be evaluated for encapsulation efficiency, drug loading, yield, particle size, polydispersity index, zeta potential, mass median aerodynamic diameter, in vitro drug release, and morphology.

2. RESULTS

2.1. Evaluation of ivacaftor and ciprofloxacin-loaded microparticles

Table 1. Results of in vitro assessment of IVA-CIP microparticles prepared by spray-drying and freeze-drying techniques

Evaluation parameter	Spray-dried microparticles		Freeze-dried microparticles		p-value	p-value summary	Significant?	R^2
	Mean	S.D.	Mean	S.D.				
Encapsulation efficiency (IVA)	89.26	0.2	68.56	1.2	<0.0001	****	Yes	0.9954
Encapsulation efficiency (CIP)	85.25	0.1	65.25	1.13	<0.0001	****	Yes	0.9957
Drug loading (IVA)	44.9	0.63	27.2	0.9	<0.0001	****	Yes	0.9949
Drug loading (CIP)	47	0.43	25.6	0.6	<0.0001	****	Yes	0.9984
Yield (%)	14.2	2.08	68.56	1.96	<0.0001	****	Yes	0.9963
Particle size (µm)	1.6	0.04	0.817	0.0056	<0.0001	****	Yes	0.9965
Polydispersity index	0.33	0.02	0.36	0.03	0.223	ns	No	0.3418
Zeta potential	-27.3	1.1	-23.3	1.1	0.0112	*	Yes	0.8322
In vitro mucoadhesion strength	90.18	1.25	75.9	2.42	0.0008	***	Yes	0.9537
Capsule retention	10.5	0.5	11	0.75	0.3911	ns	No	0.1875
% Emitted (IVA)	87.92	0.75	97	0.5	<0.0001	****	Yes	0.987
% Emitted (CIP)	88.59	1.05	95	0.6	0.0008	***	Yes	0.9547
Fine particle fraction (IVA)	83.2	0.46	45.8	2.2	<0.0001	****	Yes	0.9952
Fine particle fraction (CIP)	85.21	0.57	43.6	1.6	<0.0001	****	Yes	0.9978
Mass median aerodynamic diameter	3.74	0.08	3.75	0.07	0.8785	ns	No	0.006593

****p-value <0.0001, ***p-value <0.001, **p-value<0.01, *p-value<0.05, ns: p-value > 0.05, S. D. – standard deviation

The in vitro release of both the drugs in their pure state and from the microparticles prepared by both methods is depicted in Figure 1. Either way, the release of IVA and CIP was prolonged from the microparticles prepared. There was no significant difference observed, affirming that the method of preparation of the microparticles did not influence the amount of drug released and their release profiles.

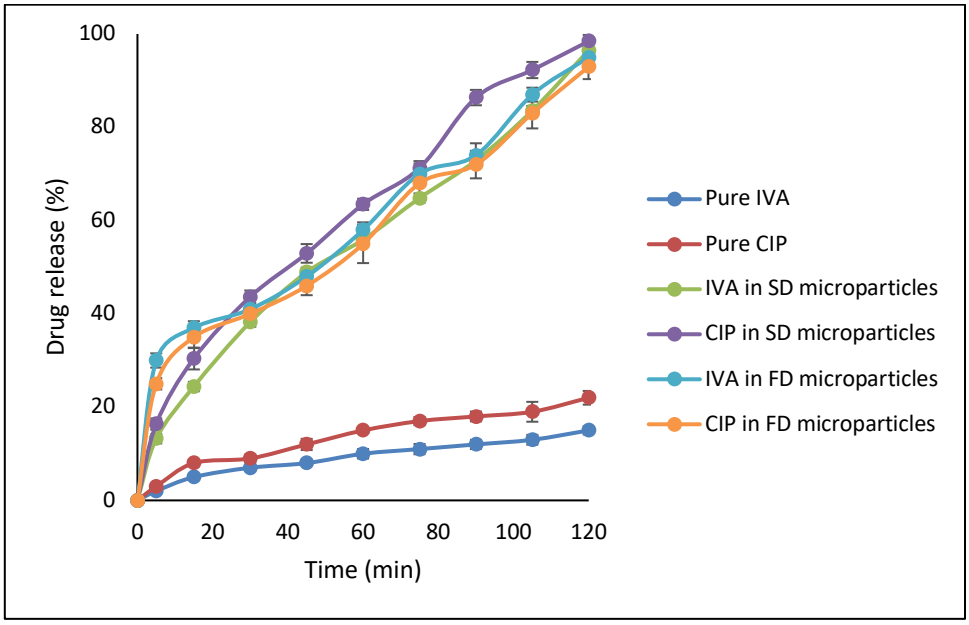

Figure 1. In vitro drug release profiles of pure drugs and the prepared microparticles

The SEM scans of the spray-dried and freeze-dried microparticles are shown in Figure 2.

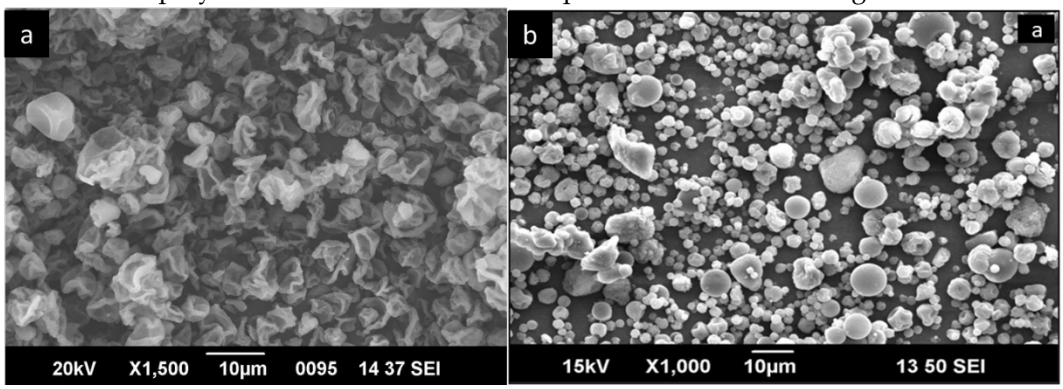

Figure 2. SEM scan of IVA-CIP microparticles prepared by a) spray-drying and b) freeze-drying

3. DISCUSSION

3.1. Preparation of ivacaftor and ciprofloxacin-loaded microparticles

The polymer selected for this work was bovine serum albumin (BSA). This was done taking into consideration the various benefits offered by BSA. They included its natural origin, abundance, inexpensiveness, biocompatibility, safety and clinical approval, excellent stability, versatility in encapsulating drugs with various physicochemical properties, and ability to bind with many substances to allow for the fabrication of surface-engineered microparticles [7–10]. Although it is a protein, its stability during spray drying has been demonstrated by various researchers [11–13]

Several studies have demonstrated that amino acids such as L-leucine lubricate particles and improve their dispersibility [14]. Because of its hydrophobicity, leucine stays in the interface between the medium and the polymer, thereby getting coated onto the surface of the spray-dried microparticles. This would result in a decrease in the aggregation potential of the carriers and improve the stability and performance of the formulated DPI [15]. Various researchers have quoted a similar benefit of L-leucine, thereby improving the aerosolization ability of the microparticles [16–18]. Leucine is included in the monographs of various pharmacopoeias and has been given the Generally Regarded as Safe (GRAS) status. It is already found in different food as well as pharmaceutical preparations. Although it has still not been approved for inhalation route studies, have revealed that even at concentrations as high as 20% w/w, no cytotoxicity was observed on lung epithelial cell lines A549 and Calu-3 [19] and neither in NR8383, which is the alveolar cell line [20]. Several DPI containing leucine are already in clinical trials [21].

3.2. Evaluation of ivacaftor and ciprofloxacin-loaded microparticles

The encapsulation efficiency and loading of the drugs were found to be higher in the case of microparticles prepared by SD compared to that observed with the microparticles prepared by the freeze-drying method. This could be attributed to the use of cryoprotectants in the case of the freeze-drying method, as they are known to occupy most of the weight ratio [22]. In addition, studies of higher drug loading with spraying-drying over freeze-drying are available [23].

The product yield of the freeze dryer experiments was generally higher than the spray dryer experiments; since the product loss in the freeze dryer method was only due to the adhesion of the emulsions to the equipment's glass plate surface [24]. Due to the fine particles not settling on the cyclone collector chamber in the spray dryer, the yield was low, whereas, in the freeze dryer, the yield was relatively high [25].

The particle size of the spray-dried particles was almost double that obtained by the freeze-dried particles. It is typical for droplet mass median diameters in pharmaceutical spray dryers to range from less than 10 µm to upwards of 100 µm, which translates to specific dry particle diameters of 0.5 to 50 µm. Our particles were within the said range [26]. Various studies have identified that particles of sizes between 1- 5 µm have the ideal deposition pattern in the airways [27]. However, particles with sizes on either side of the proposed range have a cohesiveness that is not perfect for aerosolization [28]. Of the two methods under consideration, the particles yielded via spray drying fulfilled the criteria mentioned above for the particle size. Furthermore, both methods yielded particles with uniform distribution, as revealed by the polydispersity index values, which were found to be less than 0.4 [29]. The low polydispersity index shows the narrow particle size distribution of the microparticles as it would enable all the particles to target a specific location in the airways, thereby leading to higher deposition of the drugs in the area of interest.

The zeta potential of the IVA-CIP microparticles prepared by SD was found to be -27.3 ± 1.1 mV, and that of the ones prepared by FD was -27.3 ± 1.1 mV. A zeta potential value close to ± 30 mV indicates good physical stability of the microparticles on account of electrostatic repulsion [30]. However, the negative charge was developed due to the polymer used – BSA. A zeta potential of -25 mV was reported by Tarhini et al., who had prepared nanoparticles of human serum albumin as carriers for proteins [31]. The negative sign proves that the polymer was a polyanion, which has been reported to have greater mucoadhesion than polycations and non-ionic polymers [32]. Furthermore, negative charges may facilitate penetration into the mucus layer [33].

The particles prepared by SD displayed a significantly greater ($p<0.001$) mucoadhesion strength than those designed by FD. This could be attributed to the wrinkles on the particles observed in the SD case. Due to the large surface area of wrinkled particles, they will be able to adhere more effectively to mucus membranes [34].

The in vitro aerosol deposition was studied using an Anderson cascade impactor. The powder loss by capsule retention did not show any statistically significant difference. However, the amount emitted was significantly higher for particles prepared by FD, which could be owing to the spherical nature of the particles. A significant difference was observed in the case of fine particle fraction. The FPF of particles prepared by SD is significantly higher ($p< 0.0001$) than those designed by freeze-drying (83 to 85 % with SD microparticles against 43 to 45 % with FD microparticles). This could be due to the grooves observed on the surface of particles prepared by SD (Figure 2). The aerosol performance of powders with little surface corrugations is reported to be significantly improved [35]. Particle-particle interactions are decreased by the surface corrugation in two ways; firstly, wrinkles prevent particles from being close to each other and effectively increase the distance between them; secondly, irregularities reduce the surface available for interaction.

Microparticles prepared by the freeze-drying method were spherical, as seen from the SEM scans shown in Figure 2b. They had a smooth surface. On the other hand, the ones prepared by the spray-drying technique were cylindrical and wrinkled (Figure 2a). Various researchers' previous studies concurred with distorted particle formation [36,37]. It is possible that wrinkled particles are a result of low heat and mass transfer coefficients, as described by the Ranz-Marshall equation [38]. It is expected that spray-dried cylindrical particles will have a hollow character and, thus, a lower density, which would improve their aerodynamic performance [39]. This goes hand-in-hand with the observations made during the assessment of the free particle fractions. The FPF values for both drugs are significantly higher than those observed in the microparticles prepared by the freeze-drying method. The in vitro drug release profiles of both microparticles revealed that the method of preparation did not have a significant impact on the rate and profile of release of both drugs. The similarity factor (f2) values for the release profiles of IVA from the SD and FD microparticles were found to be 57, and that for CIP were 56. Both of these values lie in the prescribed range of 50 – 100, indicating that the release of the drugs from microparticles prepared by either method were similar, thereby

confirming that the method of preparation did not have any statistically significant impact on the release of the entrapped drugs.

4. CONCLUSION

The characteristics and topography of microparticles, such as particle size, polydispersity, shape, and degree of surface roughness, were found to be significantly influenced by the preparation technique and subsequently affected in vitro aerosol performance. IVA-CIP microparticles produced by the spray-drying process were found to have better aerosol performance than those prepared by FD. The SD microparticles were shown to possess higher emission percentages and FPF. Thus, careful selection and optimization of preparation methods are likely to enhance the deposition of IVA and CIP into the lower airways as needed in CF. The other performance indicators, such as encapsulation efficiency, drug loading and mucoadhesion strength, were observed to be affected by the method of preparation too. In this way, manipulation of the features of the prepared IVA-CIP microparticles by altering their fabrication process may allow for new applications of treatment for pulmonary and extrapulmonary diseases.

5. MATERIALS AND METHODS

The drugs ivacaftor and ciprofloxacin were kindly gifted by MSN laboratories (Hyderabad, India) and Medreich (Bengaluru, India). Bovine serum albumin (BSA) and L-leucine were purchased from Sigma-Aldrich, USA. All the chemicals and reagents used were of analytical grade.

5.1. Preparation of ivacaftor and ciprofloxacin-loaded microparticles

L-leucine and BSA (L-Leu-BSA) were added to distilled water under continuous stirring at 1000–1200 rpm. The polymer BSA concentration was maintained between 20 – 50 % w/w. Next, the drugs were added to the above polymeric mixture with constant mixing, and stirring was continued with a magnetic stirrer for a further 30 min. After that, the resulting mixture was halved and used to prepare microparticles by spray-drying as well as freeze-drying method.

A spray dryer (LU222, Labultima, India) was used for microparticles' formation, spraying the fluid through a co-current spray gun having a nozzle size of 0.7 mm into a drying chamber maintained under vacuum (110 mm Wc). The liquid film was atomized with compressed air in the spray gun. Then, the droplets were introduced into the heated drying chamber, wherein the solvent evaporated, and dried microparticles were collected at the bottom of the collector. Air was used as the drying gas. The temperature of the inlet air was varied between 120 – 140 °C, and the outlet air temperature was aimed at 80 to 90 °C. The feed introduction rate was 4 to 6 mL/min, the atomization pressure was 2 kg/cm^2, and the aspiration rate was maintained at 45 to 50 %[40].

In the alternative method, the aforementioned mixture was frozen at -20°C for 24 hr and then subjected to lyophilization using a bench-top freeze dryer (Buchi, Switzerland) at -70 to -75°C. Finally, the cycle was run for eight days by applying a vacuum at 76 mTorr. Mannitol (1 %w/w) was used as the cryoprotectant.

5.2. Evaluation of ivacaftor and ciprofloxacin-loaded microparticles

5.2.1. Encapsulation efficiency and drug loading

Fifty mg of the IVA-CIP microparticles prepared by both methods were accurately weighed and dispersed in an equal quantity of deionized water. The mixture was sonicated for 15 mins and stirred for 2 hours using an overhead stirrer operating at 100 rpm. The resultant dispersion was centrifuged (Remi, India) for 10 mins at 2000 rpm. The supernatant was additionally filtered and suitably diluted, and the absorbance was noted using a UV-visible spectrophotometer (UV-1700, Shimadzu, Japan). The wavelengths were adjusted at 313 and 278 nm for IVA and CIP, respectively. The encapsulation efficiency (%) and drug loading (%) were calculated by formulae 1 and 2. The experiment was run in triplicate, and the results are expressed as mean ± standard deviation (S.D.).

$$Encasulation\ efficiency\ (\%) = \frac{Initial\ weight\ of\ drug - Free\ drug}{Initial\ weight\ of\ drug}\ X\ 100 \text{ ---------- } 1$$

$$Drug\ loading\ (\%) = \frac{Initial\ weight\ of\ drug - Free\ drug}{Total\ weight\ of\ microparticles}\ X\ 100 \text{ ---------- } 2$$

5.2.2. Product yield

The microparticles prepared by both methods were weighed, and the yield was calculated using formula 3. The experiment was run in triplicate, and the results are expressed as mean ± S.D.

$$Yield\ (\%) = \frac{Weight\ of\ microparticles\ obtained}{Total\ weight\ of\ raw\ materials}\ X\ 100 \text{ ---------- } 3$$

5.2.3. In vitro mucoadhesion study

For the determination of mucoadhesion strength, the previously reported methodology was applied [41]. To determine mucoadhesion strength, goat mucosa was used, while pH 7.4 simulated lung fluid (SLF) was used to maintain moisture [42]. A surgical blade was used to cut the mucosa of goat lungs into clean pieces with standard measurements. To maintain humidity in the mucosa lining, SLF pH 7.4 was used. On each mucosa slice, 10 mg of the IVA-CIP microparticles was accurately weighed and kept undisturbed for 5 mins. As a consequence, adhesive bonds are formed between the microparticles and mucosa. The IVA-CIP microparticles-loaded mucosa was bound tightly to a glass slide (5.5 × 1.5 cm) using cotton fibres. SLF was dripped onto the mucosa at a rate of 100 drops/min. The volume of SLF needed to dislodge the IVA-CIP microparticles was noted and considered as the mucoadhesion strength. The force of mucoadhesion was calculated using formula 4. The experiment was run in triplicate, and the results are expressed as mean ± S.D.

$$Force\ of\ mucoadhesion\ (N) = \frac{Mucoadhesive\ strength\ X\ 9.81}{100} \text{ ---------- } 4$$

5.2.4. Particle size and zeta potential

The microparticles' particle size and zeta potential were determined using Zetasizer (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK). The sample was dispersed in phosphate buffer (pH 7.4), and an aliquot of 700 µL was introduced to the cell to analyze the IVA-CIP microparticles. The experiments were run in triplicate, and the results are expressed as mean ± S.D.

5.2.5. Aerodynamic behaviour

Microparticle aerodynamics was studied using an Anderson cascade impactor (ACI) (Copley Scientific, Nottingham, UK). It works on the principle of size fractionation based on the size of the nozzles at each stage. It is designed with an induction port and a pre-separator to sort particles by their aerodynamic diameter. The Size "2" hard gelatin capsule (Coni-Snap® Gelatin DPI) was used as a carrier for 15 mg of microparticles. The filled capsule was inserted into the sample chamber of Rotahaler® (Cipla, India). During the duration of the experiment (T = 240/Qout, in seconds), the flow rate of the air was maintained at 30 L/min over the Rotahaler®, corresponding with the evacuation of 4 L of air from the mouthpiece of the inhaler within the ACI. Through the piercing of the hard gelatin capsule, drug content was discharged from the Rotahaler®, and the amount retained in each of the seven stages of the ACI was estimated. The free particle fraction (FPF) collected was rinsed with distilled water, appropriately dissolved, filtered and analyzed for IVA and CIP content employing a UV-visible spectrophotometer (UV-1700, Shimadzu, Japan) at 313 and 278 nm. The experiments were run in triplicate, and the results are expressed as mean ± S.D. The Mass Median Aerodynamic Diameter (MMAD) of the microparticles was determined using linear interpolation from the two-point interpolation of the data points corresponding to the cumulative mass percentage above and below the halfway mark (i.e. 50%) on a plot of cumulative mass percentage vs calculated cut-off aerodynamic diameter on a logarithmic scale.

5.2.6. In vitro drug release

The in vitro drug release of the pure drugs and the drugs from the IVA-CIP microparticles was determined using a Franz diffusion cell [43] using a cellophane membrane. The reservoir of the cell had a capacity of 16 mL and was filled with phosphate buffer saline (PBS) pH 7.4 with 0.2 % sodium dodecyl sulphate to mimic blood. The assembly was maintained at 37 ± 0.5 °C and constantly stirred at 25 rpm throughout the study period. Aliquots were withdrawn at predetermined time intervals of 15 mins till more than 90 % w/v drug was released. Then, the aliquots were filtered and suitably diluted, and the absorbance was measured using the UV-visible spectrophotometer (UV-1700, Shimadzu, Japan). The experiment was run in triplicate, and the results are expressed as mean ± S.D.

5.2.7. Morphology

The morphology of the IVA-CIP microparticles was evaluated using a scanning electron microscope (SEM). To generate the SEM scan, two mg of the sample was spread on the double-sided carbon adhesive taps attached to aluminium stubs. The excess particles were removed by gentle tapping, followed by platinum coating at a current intensity of 20 mA using a fine auto coater (JFC1600, JEOL, Japan) and scanned using Scanning 210 Electron Microscopy (JSM 6390, JEOL, Japan).

5.2.8. Statistical analysis

The results of the evaluations performed on both sets of microparticles were compared using GraphPad® Prism 8.2. In addition, the two-sampled unpaired t-test was used [44].

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