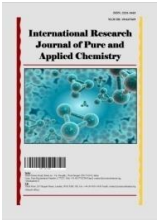

International Research Journal of Pure & Applied Chemistry

21(17): 18-31, 2020; Article no.IRJPAC.61485
ISSN: 2231-3443, NLM ID: 101647669

Aerosol Performance of Beta-carotene Supplementation Prepared by Spray and Spray-Freeze Drying

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Authors' contributions

This work was carried out in collaboration among all authors. Author MNL designed the study, performed the statistical analysis, wrote the protocol and wrote the manuscript. Author MDS helped in designing the study and analyses of the study. Author JAM reviewed the manuscript and helped in correction of manuscript. Author CA provided the concept and supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2020/v21i1730262
Editor(s):
(1) Dr. Wolfgang Linert, Vienna University of Technology Getreidemarkt, Austria.
(2) Dr. Hao-Yang Wang, Shanghai Institute of Organic Chemistry, China.
Reviewers:
(1) Adekanmi, Daniel Gbenga, Nigerian Defence Academy, Nigeria.
(2) George Gonçalves dos Santos, Universidade Federal da Bahia (UFBA), Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61485

Original Research Article

Received 20 July 2020
Accepted 24 September 2020
Published 06 October 2020

ABSTRACT

The goal of the present work is to find a suitable method for developing β-carotene aerosols by varying core (active material) to wall (excipient) ratios (1:10, 1:25, and 1:50). For this, spray freeze drying (SFD) and spray drying (SD) techniques were adopted to develop aerosols. The results revealed that aerosols from SFD had low density and free-flowing behavior; whereas, SD samples were cohesive. SFD aerosols were porous and SD samples had smooth structures, with sizes of 8.3 to 9.3 and 9.3 to 9.6 µm, respectively. All formulations exhibited good mass median aerodynamic diameter ($MMAD_t$) of 3.75 to 6.96 µm and % emitted dosage was found higher in SFD aerosols (around 57 to 60%). Release of β-carotene through the *in-vitro* study was found higher in SFD samples and controlled release was observed in 1:50 formulation. In 12 h release, around 64% and 74% contents were released from SD and SFD aerosol samples, respectively. Particle density, size, and morphology strongly affect particle deposition in the lungs' and this approach can be conveniently scale-up for pulmonary supplementation of food bioactive compounds.

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Keywords: Spray freeze drying (SFD); spray drying (SD); mass median aerodynamic diameter; In-vitro release.

1. INTRODUCTION

β-carotene is a red-orange colored carotenoid and a major source of vitamin A. β-carotene 15, 15′-monooxygenase acts on vitamin A precursor, gives two retinol molecules in the presence of O_2 [1]. It also exhibits antioxidant activities that protect against chronic diseases including cardiovascular, cancer, and eye diseases [2]. Although β-carotene has proven beneficial activity, maximum absorption of β-carotene from plant sources is less than 65% and posing a challenge in absorption [3]. β-carotene is sensitive to oxygen, light, and heat; poor solubility and chemical instability further limit its applications [4]. Though encapsulation is a popular technique to overcome these hurdles, the bioavailability of β-carotene supplemented through the oral route is low [5]. Also, a significant amount of β-carotene loses its activity in the gastrointestinal tract caused by the continual action of numerous enzymes and variations in pH conditions, amongst other factors [6].

To avoid these limitations, inhalation therapy (i.e. pulmonary delivery) is an alternative route to the oral, nasal, rectal, sublingual and transdermal route. This route can help to supplement β-carotene for sensitive targeted tissues and the respiratory mucosa that otherwise undergo severe morphological and functional modifications as a consequence of vitamin A deficiency [7]. Lungs have good blood supply with an enormous surface area around ∼100 m^2 along with ∼0.1-0.5 μm thickness of alveolar epithelium [8], making nutrient absorption rapid as compared to other conventional routes [9]. Though metabolic enzymes are present in the lungs [10], the metabolic activity and pathways are different from those with gastro-intestinal enzymes [11]. Also, the pH in the lungs (∼ 7.42) makes the pulmonary system as a very suitable route for inhalation [12].

Alveoli region is targeted for effective nutrient delivery as it is surrounded by a rich blood vessel network. It avoids first-pass metabolism and allows nutrients to be delivered directly to the blood for absorption in different body tissues [9]. For effective pulmonary delivery particle size, density, and porosity are important, with mass median aerodynamic diameter ($MMAD_t$) should be in the range of 1 to 5μm. However, the bioavailability and absorption of nutrients through pulmonary route is also depends on health of an individual and breathing cycle [13]. It may also cause dry mouth and toxicity upon deposition and henceforth pharmacokinetics studies were essential [14]. A research confirmed that by improving particle size by lowering particle mass density, large porous particles with porosity and $MMAD_t$ lesser than the geometric diameter can facilitate deep lung deposition (i.e. in the alveoli region) [15].

The pulmonary route is a well-known delivery system for pharmaceutical drugs, to treat lung diseases and certain infections. Proteins and peptides (insulin, human growth hormone, calcitonin) [16] and other nutrients like retinyl palmitate [17], mannitol [18], β-carotene [7], and lactose (Alhajj et al., 2020) have been delivered successfully through the pulmonary route. Spray drying (SD) is the most commonly used technique for the fabrication of dry powder-based inhalation formulations [19]. SD has many advantages of producing powders with narrow particle size distribution and spherical shape; and offers convenience in terms of scale-up [19]. Nevertheless, low product yield and possible degradation of heat-sensitive compounds are concerns [15]. Over the years, alternative methods to produce aerosols, such as co-spray drying, solvent evaporation, and spray-freeze drying (SFD) techniques were evolved. SFD is an amalgamation of spray freezing and freeze-drying process [20]. The advantage with spray dry, i.e. uniform distribution of tiny spherical particles and, the advantage with freeze dryer i.e. retention of bioactive and heat-sensitive materials co-exist with the SFD approach, without affecting product yield [21].

Cyclodextrin is a well-known molecule used in pharmaceutical formulations, due to its chemical structure that makes it compatible for lipophilic compounds. Hydroxypropyl-β-cyclodextrin was used to produce inhalable insulin and results proved its advantages in the delivery of specific compounds. The objective of this research is to prepare inhalable β-carotene supplementation for deep lung deposition, using 2-hydroxypropyl-β-cyclodextrin (HPβCD). In this study, both SD and SFD were used for the development of β-carotene aerosols.

2. MATERIALS AND METHODS

2.1 Materials

β-carotene powder was purchased from MP Biomedicals, LLC, and Dipalmitoylphosphatidylcholine (DPPC) from Avanti Polar Lipids, Inc. Tween 80, HPβCD and all salts used in simulating lung fluid are of analytical grade and acetonitrile was procured from Himedia, India.

2.2 Sample Preparation

β-carotene samples were prepared in 3 different ratios with HPβCD as wall material and β-carotene as core i.e. 1:10, 1:25, and 1:50. To analyze the effect of active material on the excipient ratio on the formulation and release of active compounds from aerosols, three different formulations were developed. β-carotene was dissolved in 0.3% w/w tween 80 and gently stirred using a magnetic stirrer (800 rpm) until the carotene got dissolved completely without any lumps. HPβCD was added to the dissolved β-carotene with water as the continuous phase and 10% solid content was maintained followed by stirring for 30 min and homogenization (high-speed homogenizer: IKA, Ultra-Turrax) at the speed of 18,000 rpm for 10 min.

2.3 Spray Drying

β-carotene aerosols prepared using lab-scale, single-stage SD (Spray Mate, JISL, Navi Mumbai, India). A two-fluid atomizer was used for atomization at 20 psi compressed air. Ambient air was heated and used for drying of the sprayed particles. Initially, inlet and outlet temperature, and the feed rate were adjusted by feeding distilled water. When inlet temperatures and outlet temperature reached 140°C and 55°C respectively the emulsion was sprayed at 2 ml/min feed rate. The deposited dried aerosols in the cyclone separator were collected and stored airtight.

2.4 Spray-freeze Drying

SFD was a two-stage process, according to Karthik et al. [22] the experiments were carried out. During SFD, the feed solution was sprayed using an atomizer (twin fluid nozzle), into liquid nitrogen, with agitation. The distance between the atomizer and liquid nitrogen was maintained as 15 cm and compressed air was passed at 20 psi and the feed rate was kept at 25 ml/min. Frozen micro-particles were then shifted to a freeze dryer (Borg Scientific, Chennai) for removal of ice at -60°C. After 5 h of drying, particles were collected in an airtight container for further analysis.

2.5 Particle Size and Morphology

The particle size distribution of the powdered sample was measured by Malvern Zetasizer (Malvern Instruments Ltd., UK). A 50 µl (10 mg of sample in 1 ml of ethanol) of the sample was used for the analysis. Geometric particle size was expressed in micrometers. The morphology and shape of the aerosols were analyzed by Scanning Electron Microscope (SEM: TESCAN, VEGA, Czech Republic, EU). Samples were evenly spread and placed on the specimen holder, later gold was coated on the holder (2 min, 2 mbar) under vacuum (9.75×10^{-5} Torr).

2.6 Flow Properties of the Aerosols

2.6.1 Bulk and tapped density

The density of powder was explained as using bulk (ρ_B) and tapped density (ρ_T). 100 mg of samples (M) were taken and noted down the volume (V) for measuring the bulk density of the aerosol sample. By using equation 1, bulk density was calculated. The cylinder was filled with a known amount of aerosols (M) and was tapped for 300 times manually to calculate compacted volume (V_T). Equation 2 was used to calculate tapped density.

$$\rho_B(\mathrm{g/ml}) = \left({}^{M}/_{V}\right) \quad (1)$$

$$\rho_T(\mathrm{g/ml}) = \left({}^{M}/_{V_T}\right) \quad (2)$$

2.6.2 Hausner ratio and Carr's index

Hausner ratio (*H*) and Carr's index (*C*) were calculated to explain aerosol flow characteristics, using ρ_B and ρ_T of the powder. According to Turchiuli et al. [23], the reference values were used in this study for H and C, and the values were calculated using equations (3) and (4), respectively.

$$H = {}^{\rho_T}/_{\rho_B} \quad (3)$$

$$H = \left({}^{\rho_T - \rho_B}/_{\rho_T}\right) X\ 100 \quad (4)$$

2.7 Moisture Content and Water Activity

As per the method given by Wu et al. [15], moisture content (MC) of powder was calculated. One gram of sample was weighed in a moisture dish; dried at 105°C for 12 h in a hot air oven [7]. Moisture content was measured using equation (5).

$$\text{MC (wet basis \%)} = \left[{(W_1 - W_0)}/{W_0}\right] X\ 100 \quad (5)$$

Where; W_0 and W_1 are the initial weight of the sample before drying and the final weight of the sample after drying. The water activity of the samples was measured using a water activity meter (Aqua lab dew point, 4TE DUO) at 25°C.

2.8 Entrapment Efficiency

Entrapment efficiency (EE) was estimated according to Priamo et al. [24], with slight modifications. β-carotene aerosol (10 mg) was washed in acetonitrile (5 ml) and followed by 5 min vortex. Then, add 5 ml of water to disturb the cyclodextrin layer by vortexing the solution for 10 min. The mixture was kept for centrifugation at the speed of 5000 rpm for 10 min and the upper layer was separated and evaluated for β-carotene content. β-carotene content was evaluated against a previously calibrated standard graph (R^2= 0.998) using UV-spectrophotometer (Shimadzu, UV 240V) at 482 nm.

$$EE\ (\%) = [(Total\ \beta - carotene\ content\ in\ encapsulates)\ /\ (Theoretical\ \beta - carotene\ content\ in\ feed\ solution)] \times 100 \quad (6)$$

2.9 Aerosolization Properties of β-carotene Aerosols

2.9.1 Emitted dosage

The emitted dosage was determined by using a reusable revolizer dry powder inhaler (DPI). The hard gelatin capsules were filled with approximately 30 mg of powder aerosols and kept in the powder chamber [25]. DPI was weighed before and after aerosolization to calculate the % emitted dosage.

2.9.2 Mass mean aerodynamic diameter

Theoretical mass mean aerodynamic diameter ($MMAD_t$) of the powder was calculated based on the definition of aerodynamic diameter “a unit density sphere that has the same settling velocity as the particle which will settle/ deposit in the deep lung region” [26,27]. $MMAD_t$ was calculated using equation (7).

$$d_{ae}(\mu m) = d_g\sqrt{\rho} \quad (7)$$

Where; d_{ae} is aerodynamic diameter, aerosol density is ρ and d_g is the aerosol geometric diameter.

2.10 Aerodynamic Behavior of Inhaled Aerosols

Theoretically, the aerodynamic behavior of inhaled aerosols was calculated according to Crowder et al. [26]. Some physical principles are related to the fine particles dispersed as aerosols which help to predict and improve the deposition of inhalable particles in the lungs.

2.10.1 Settling velocity

According to Edwards et al. [28], spherical particles fall under gravity (g) with a velocity proportional to particle mass density (ρ_p) and the square of its geometric diameter. This explains that particles settling velocity (V_{TS}) as in equation (8).

$$V_{TS}(m/s) = \left[{\rho_p {d_e}^2 g C_c}/{18\mu X}\right] \quad (8)$$

Where; the dynamic shape factor ‘X’ for a sphere is 1, μ is defined as the viscosity of air. The Cunningham slip correction factor (C_c) is applied to Stroke’s law for particles less than 10 μm [29] as equation (9).

$$C_c = 1 + K_n\left[A1 + A2\ exp\left(-{A_3}/{K_n}\right)\right] \quad (9)$$

Where; The constants A1, A2, and A3 are 1.257, 0.4, and 1.1, respectively and were calculated based on experimental measurements on small particles [26]. Knudsen number is a dimensionless number denoted as K_n. The K_n is calculated by equation (10) and λ can be calculated by equation (11).

$$K_n = {2\lambda}/{d} \quad (10)$$

$$\lambda = v\sqrt{\left({\pi m}/{2kT}\right)} \quad (11)$$

Where; the molecular mass is denoted as m, v is the kinematic viscosity of the gas, absolute temperature is T and k is the Boltzmann's constant.

2.10.2 Relaxation time

Particle deposition mechanisms are impaction, sedimentation, and diffusion mechanisms and depend on particle slip, shape, and density [29]. The main particle deposition mechanisms like inertial impaction and sedimentation were directly depended on the relaxation time of the inhaled particles [26]. By using equation (12), the relaxation time (T) is calculated.

$$T\ (\text{sec}) = \left({d_p}^2 \rho_p \Big/ 18\mu \right) C_c \qquad (12)$$

2.11 *In-vitro* Release of β-carotene Aerosols

The β-carotene release profile studied using membrane dialysis in simulated lung fluid (SLF) according to Ungaro et al. [25]. SLF was prepared according to Marques et al. [30]. 2011 and pH was maintained at 7.4. A 40 ml of an equimolar mixture of chloroform and methanol was used to dissolve 200 mg of DPPC. The solvent was evaporated; 200 ml of water added to the obtained dry film at 55°C and followed by 2 h agitation and then sonication at 55°C for 1 h. The obtained DPPC was stored at 4°C for further use. It was diluted with SLF before use in release studies. Release profiles of bioactive compounds were assessed by suspending around 10 mg of aerosol powder in 37 ml of SLF and kept in an incubator (37°C, 100 rpm). A 2 ml of the sample was collected and 2 ml of fresh release medium was replaced at scheduled time intervals. The collected release medium was centrifuged (5000 rpm for 5 min) to obtain the supernatant to analyze β- carotene. The results were expressed in the % release of β- carotene.

2.12 Statistical Analysis

The experiments conducted in these studies were in triplicates and statistically significant differences were analyzed by Duncan's multiple range test for the core to wall ratios at $p<0.05$ [7]. To know the significant difference among fabrication techniques one-sample t-test was used at $p<0.05$ significance level using statistical software SPSS (ver. 20.0).

3. RESULTS AND DISCUSSION

3.1 Particle Size and Morphology

Particle size strongly affects aerosol properties; the particles were distributed in the range of 8 to 9 µm, for all the particles evenly (Fig. 1a). Particle size depends on % EE and properties of wall materials. The average particle size of SD particles varied from 9.6 µm, 9.8 µm, and 9.3 µm, and for SFD particles 9.31 µm, 9.92 µm and 8.38 µm, for 1:10, 1:25, and 1:50, respectively.

All samples were spherical and SD samples showed a smooth surface, whereas SFD samples showed internal and external porosity (Fig. 1b). During SFD, droplets were frozen in liquid nitrogen and then, later the ice crystals were removed from the particles, leading to the formation of highly porous samples. The porous structure of SFD samples resulted in low particle density, making it suitable for inhalation. Large porous particles having less mass density can aerosolize more efficiently than small smooth surfaces from a DPI [31]. The large particle size of aerosols can also avoid phagocytic clearance from the lungs until the active compounds are delivered [31], particularly helping in the controlled release of β-carotene. Similar *in-vitro* release results were found for inhalable insulin with the sustainable release, desired aerodynamic properties, and deposited in the deep lung [25].

Wu et al. [15] developed lactose inhalation powder using SD and exhibited spherical and amorphous nature, key essential for dry powder inhalation approaches. L-leucine (Leu) aerosol was prepared by SFD and results showed porous spherical aerosols had lower density, making it suitable for inhalation, providing high inhalation performance, reduced adhesion force regardless of inhalation patterns and increased dispersibility [32].

3.2 Flow Properties of the Aerosols

Bulk and tapped density influence aerosolization, packaging, and transportation of aerosols. The lower density of aerosols indicates entrapment of air inside the powder. Bulk and tapped density were found be in ranging from 0.11 to 0.17 g/ml for SD and 0.19 to 0.45 g/ml for SFD; 0.15 to 0.22 g/ml for SD and 0.56 to 0.22 g/ml for SFD, respectively as shown in Table 1. The density of aerosols also affects the flowability of particles; SFD β- carotene aerosols were free-flowing as

compared with SD aerosols that were cohesive and exhibited poor flowability. SD and SFD aerosols showed significant difference at $p<0.05$ and core to wall ratios also showed a significant difference at $p<0.05$. [33], also reported similar results in SFD and SD inhalable nano-composite microcarriers (NCM). The study reported SD microcarriers showed poor flowability, ranging between 22-28 and SFD microspheres had good flowability, with 6-14 as Carr index values. Our results are coincident with Ungaro et al. [25], they investigated inhalable insulin had excellent flowability with good aerosolization properties. According to these researchers, SFD aerosols can be aerosolized faster than SD aerosols from capsules of DPI.

Ungaro et al. [25] reported inhalable insulin loaded with PLGA (poly (lactide-co-glycolide))/cyclodextrin, reporting the large porous spherical particles that are desirable for effective deep lung deposition with desired density (0.134 g/ml) and aerodynamic properties. Inhaled vitamin A proved to be advantageous in treating functional impairments, diseases, and pathological changes in the mucous membrane, particularly in the epithelia of the nose-throat cavity [34].

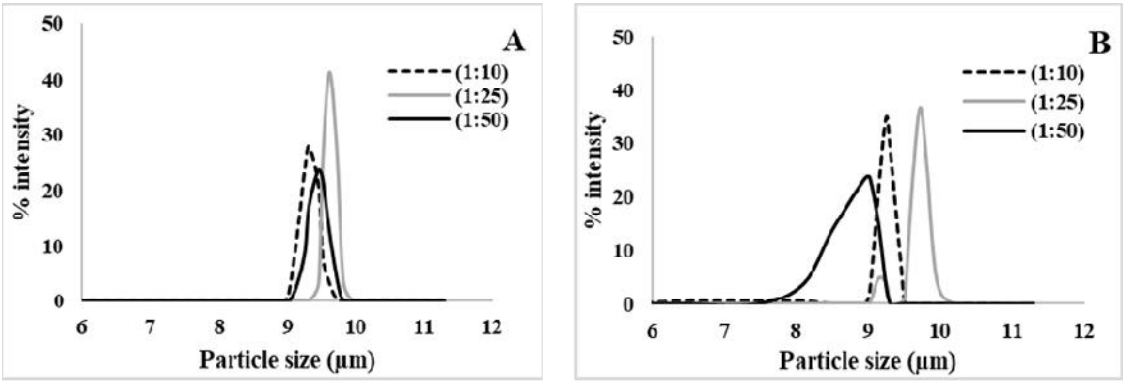


Fig. 1a.

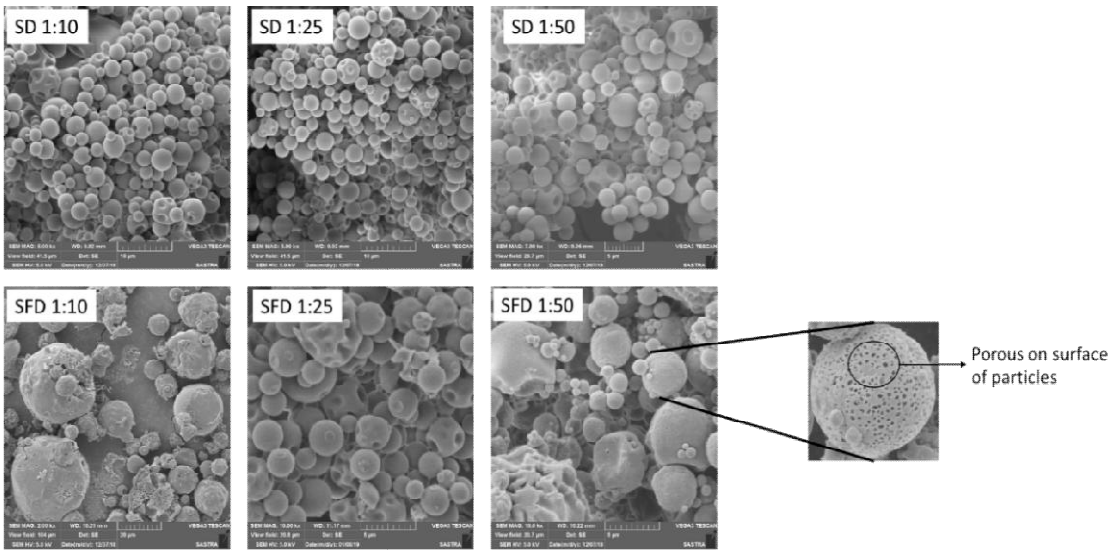


Fig. 1b

Fig. 1. (a) Particle size distribution of developed β-carotene aerosol A. SD B. SFD; (b) SEM micrographs of β-carotene aerosol

3.3 Moisture Content and Water Activity

Moisture content is very crucial in the context of the storage stability of powder samples. Higher moisture content and a_w can affect product quality and favor the growth of microorganisms. In aerosols, higher moisture content can lead to the formation of lumps and may create problems in aerosolization [15]. MC and a_w of β-carotene aerosols were found to be in the range of 3.78% to 6.79% and 0.38 to 0.56, respectively for all three concentrations in both SD and SFD samples as shown in Table 2. In SFD samples, MC and a_w was found to be low as compared to SD. In this context, SD and SFD samples showed a significant difference at $p < 0.05$. Lactose inhalable powders produced from SD showed 6% of MC [15], whereas Karthik & Anandharamakrishnan, [22], showed encapsulation of DHA by SD and SFD, showed moisture content in SD (2.4%) and SFD (3.6%). The variation in moisture content may be due to variation in spraying conditions and formulations. Bromelain aerosols developed by SFD also showed lower moisture content of 3.58 to 3.98% and aw ranged between 0.54 to 0.56 with good flowability [35].

3.4 Entrapment Efficiency

EE is very important to know how much β-carotene is entrapped, and it depends on the properties of wall material and method of sample preparation. EE of aerosols showed significant difference ($p<0.05$) for all core to wall ratios and showed differences between SD and SFD samples (Fig. 2). SD and SFD powders showed % EE in the range of 69.1 to 72.9% and 85.4 to 72.3% respectively. Comparatively, SFD showed higher EE in all core to wall ratio samples. This is because, in SD, the higher temperature was used to dry the microparticles; this causes thermal degradation of β-carotene.

Generally, as the core to wall ratio increases, EE should also increase, because, with an increase in the core to wall distance, wall materials cover the core completely. Comparatively, SFD showed higher EE in all core to wall ratio samples. EE explains that cyclodextrin has entrapped β-carotene efficiently and the approaches suitable for lipophilic compounds. As the core to wall ratio was increased from 1:10 to 1:50, EE also increased due to an increase in wall content, facilitating complete protection of the core material. β-cyclodextrin is an approved pharmaceutical compound and is the most studied cyclodextrin in humans [36]. Carotenoids (such as lycopene, lutein, or zeaxanthin) encapsulated as soft-gels show higher bioavailability in the human intestine, since they have maximum holding capacity of lipophilic compounds in it [37].

Table 1. Powder characteristics of β-carotene aerosols

	Core to wall ratio	Bulk density (g/ml)	True density (g/ml)	Hausner ratio (H)	Carr index (C)	Remarks
SD	1:10	0.11 ± 0.053^{aA}	0.15 ± 0.033^{aA}	1.39 ± 0.66^{cA}	28.2 ± 1.30^{cA}	Poor, cohesive
	1:25	0.17 ± 0.061^{cA}	0.22 ± 0.062^{cA}	1.30 ± 0.28^{aA}	23.0 ± 0.76^{aA}	Poor, fluid
	1:50	0.15 ± 0.015^{bA}	0.21 ± 0.057^{bA}	1.34 ± 0.71^{bA}	25.6 ± 1.53^{bA}	Poor, fluid
SFD	1:10	0.45 ± 0.038^{cB}	0.56 ± 0.029^{cB}	1.21 ± 0.085^{cB}	17.5 ± 2.84^{cB}	Good
	1:25	0.19 ± 0.075^{aB}	0.22 ± 0.033^{aB}	1.17 ± 0.33^{aB}	15.1 ± 0.57^{aB}	Good
	1:50	0.31 ± 0.014^{bB}	0.38 ± 0.063^{bB}	1.19 ± 0.27^{bB}	16.6 ± 2.8^{bB}	Good

**small letters denote significant difference of core to wall ratios at p <0.05 and capital letters denote significant difference between SD and SFD at p<0.05*

Table 2. The physical properties of β-carotene aerosols

	Core to wall ratio	Moisture content (%) % w.b.	Water activity
SD	1:10	5.38 ± 0.50^{aA}	0.56 ± 0.029^{bA}
	1:25	6.59 ± 0.43^{bA}	0.47 ± 0.065^{aA}
	1:50	6.79 ± 0.84^{cA}	0.56 ± 0.017^{bA}
SFD	1:10	3.78 ± 0.63^{aB}	0.38 ± 0.090^{aB}
	1:25	4.09 ± 0.12^{bB}	0.52 ± 0.020^{cB}
	1:50	4.63 ± 0.13^{cB}	0.44 ± 0.067^{bB}

**small letters denote significant difference of core to wall ratios at p <0.05 and capital letters denote significant difference between SD and SFD at p<0.05*

3.5 Aerosolization Properties of β-Carotene Aerosols

3.5.1 Emitted dosage

The emitted dosage of developed β-carotene aerosols is shown in Fig. 3. SFD samples showed maximum aerosolization compared to SD samples with lower tapped density, porous structure and free-flowing behavior, SFD samples favor aerosolization. Different core to wall ratios showed a significant difference ($p<0.05$); as core to wall ratio increased, the amount of aerosols emitted also increased, revealing that cyclodextrin has a significant effect on aerosolization.

Similar results were found in the aerosolization of insulin using HPβCD containing PLGA with large porous particles showed the potential delivery of insulin [38]. SD samples showed a lower % emitted dosage, possibly due to the cohesive nature of particles, a smooth surface that can create denser particles. Large porous particles possess a smaller surface to volume ratio leads to higher efficiency [28].

3.5.2 Mass mean aerodynamic diameter

The performance of any inhalation therapy through dry powder formulation depends on the aerodynamic diameter of aerosols. $MMAD_t$ is affected by bulk and tapped density, particle diameter, handling and dispensability [38]. The effect of tapped density and $MMAD_t$ is shown in Fig. 4. As discussed, tapped density values significantly differed as $MMAD_t$ also showing significant differences among core to wall ratios and between SD and SFD $p<0.05$. Higher tapped density and high geometric diameter results in higher $MMAD_t$, clearly indicating the effect of density and diameter. Edwards et al. [28], proved that a minimum of 3 µm of $MMAD_t$ is suitable for deep lung deposition. This also permits escape from the lungs' natural clearance mechanism.

3.6 Aerodynamic Behavior of Inhaled Aerosols

The deposition of aerosols depends on physical principles; these principles predict the factors responsible for the deposition of respirable particles. The settling velocity and relaxation time of aerosols were considered as a remarkable parameter for their deposition. These generally represent airflow and inhaled micron aerosol in the tracheobronchial airway and near the bifurcation region [39]. Particle deposition varies according to individuals and mainly depends on the breathing cycle, frequency, and health status of the individual. Particles less than 1 µm in size will get exhaled from the lung; particle size

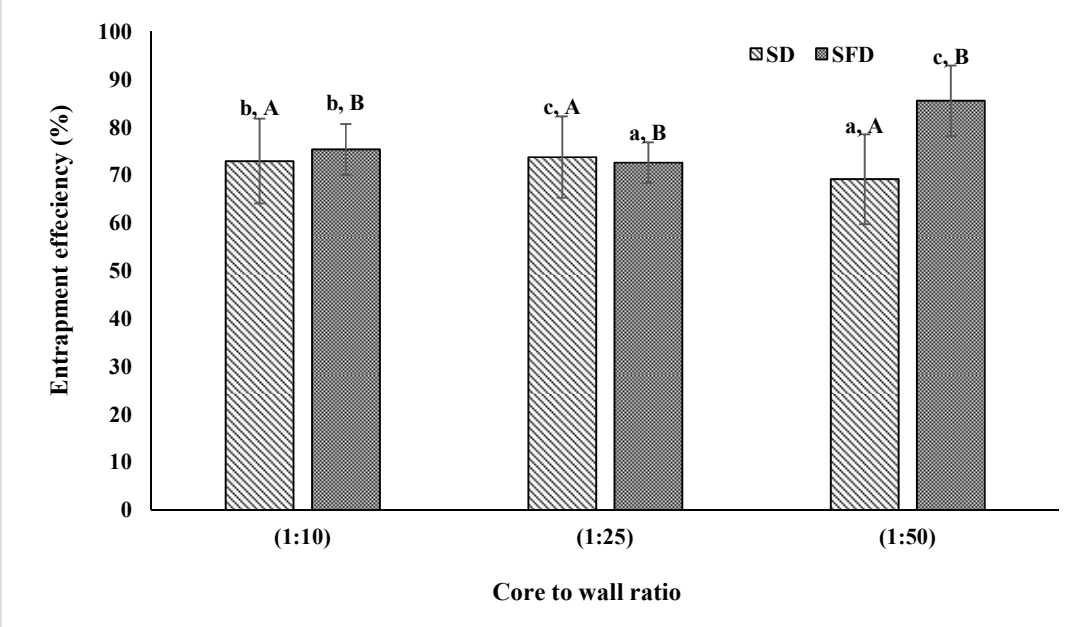


Fig. 2. Entrapment efficiency of β-carotene aerosols

**small letters denote significant difference of core to wall ratios at p <0.05 and capital letters denote significant difference between SD and SFD at p<0.05*

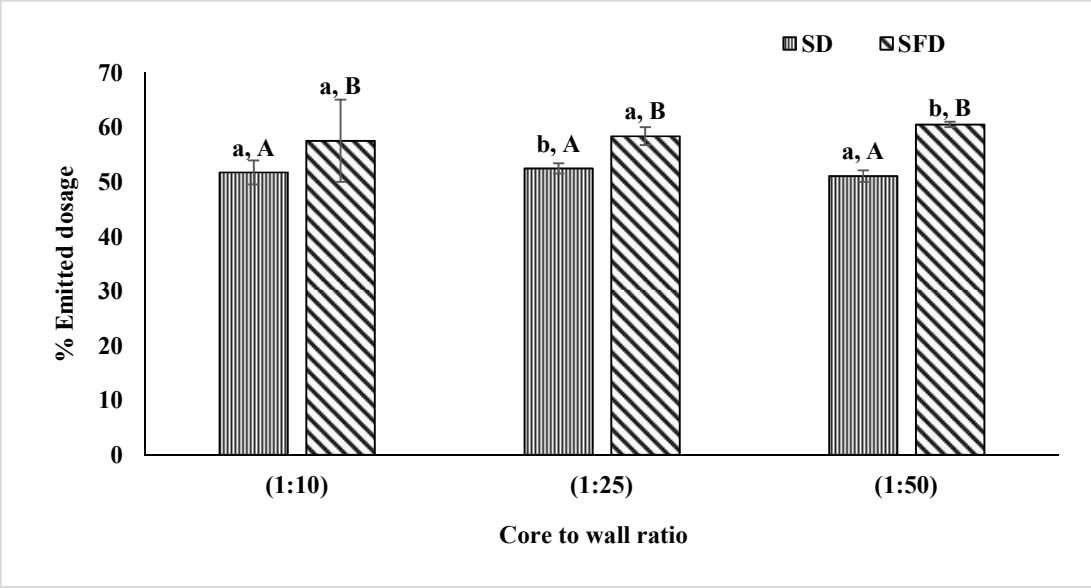


Fig. 3. The emitted dosage of β-carotene aerosols

**small letters denote significant difference of core to wall ratios at p <0.05 and capital letters denote significant difference between SD and SFD at p<0.05*

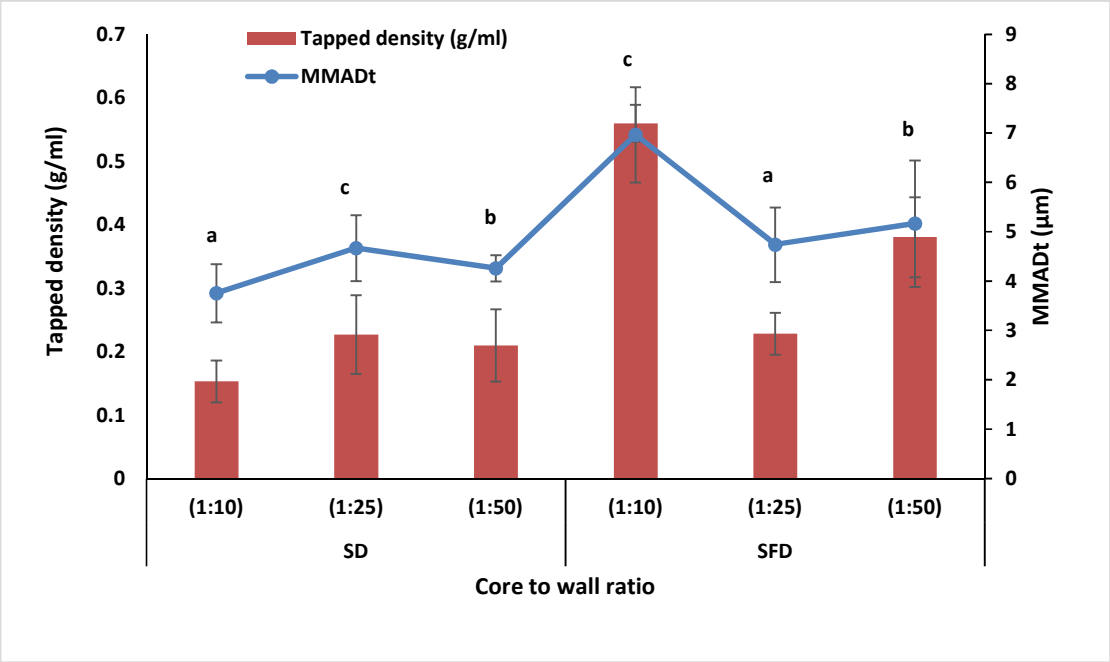


Fig. 4. The relationship between aerosol tapped density and theoretical mass mean aerodynamic diameter

**small letters denote significant difference at p <0.05*

distribution affects the spatial distribution of particle deposition in lungs' [26]. Hence, in settling velocity and relaxation time a correction factor was added. The aerodynamic behavior of inhaled aerosols is shown in Table 3. The settling velocity of β-carotene aerosols ranges between 1.480×10^{-4} to 8.161×10^{-4} m/s, depending on the size and density of particles. Results confirmed

that particles settle at minimum velocity. In the trachea, turbulent airflow occurs but in the deep lungs, velocity decreases. In the alveoli, minimum velocity or no velocity zones are evident [40]. In the human lungs, particles get deposited by sedimentation, impaction, and diffusion. Particles that are deposited by inertial impaction and sedimentation used to depend upon relation time directly. In this study, relaxation time was found to range between 1.508×10^{-5} to 8.319×10^{-5} sec.

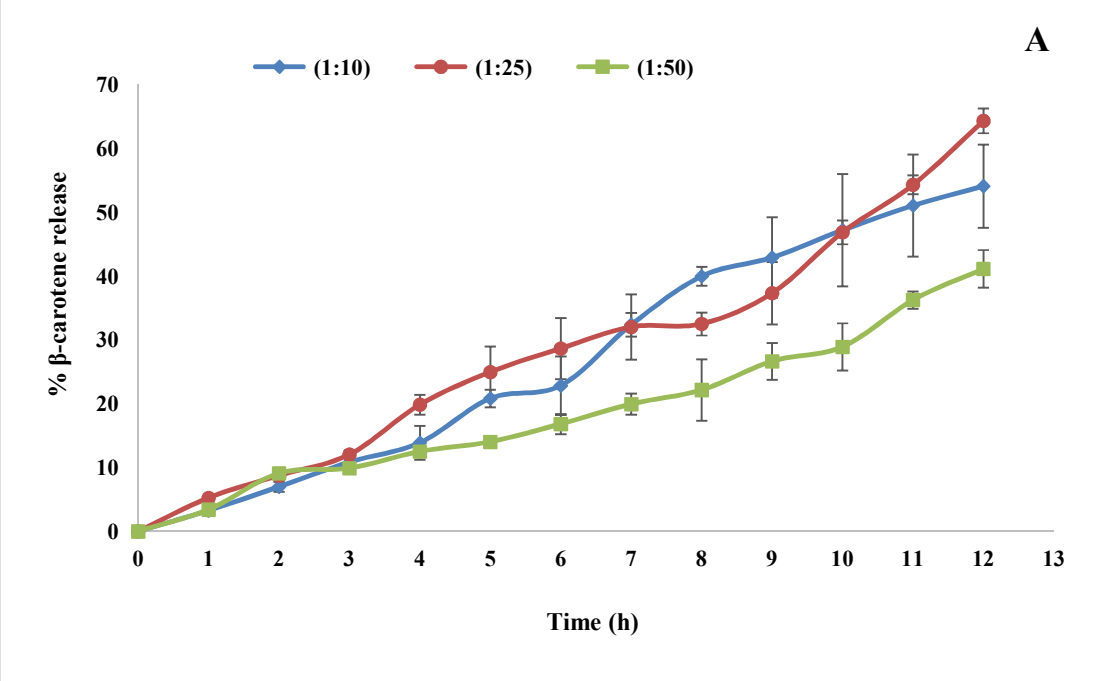
3.7 *In-vitro* Release of β-carotene Aerosols

The *in-vitro* release study of β-carotene aerosols is shown in Fig. 5. The release was strongly affected by the core to excipient ratio and the method obtained for preparation. Percent release increased as time proceeded and results explained that release was through diffusion and in a sustained manner. As the core to wall ratio increased, to 1:50, the release was found to be slow. This may be due to higher distances between core and wall as compared to 1:10 and 1:25 formulations. During the initial stages of release, molecules get hydrated and active compounds were diffused through the microporous structure of the particles, followed by the polymeric membrane.

Inhalable insulin, with HPβCD, also showed burst followed by slow release [25,38]. Researchers confirmed that the release pattern of aerosols depends on formulation conditions [25,38]. SD and SFD aerosols showed a significant difference in the release of β-carotene. SFD aerosols are released more as compared to SD aerosols, possibly due to morphological differences. SFD aerosols exhibited porous

Table 3. Aerodynamic behaviour of inhaled β-carotene aerosols

	Core to wall ratio	Cunningham slip correction factor (C_c)	*Settling velocity (VT_s) m/s*	*Relaxation time (T) sec*
SD	1:10	1.017	$4.306X10^{-4}$	$4.390X10^{-5}$
	1:25	1.017	$6.642X10^{-4}$	$6.770\ X10^{-5}$
	1:50	1.018	$5.538X10^{-4}$	$5.646\ X10^{-5}$
SFD	1:10	1.018	$1.480X10^{-4}$	$1.508\ X10^{-5}$
	1:25	1.017	$6.844X10^{-4}$	$6.977\ X10^{-5}$
	1:50	1.020	$8.161\ X10^{-4}$	$8.319\ \ 0^{-5}$



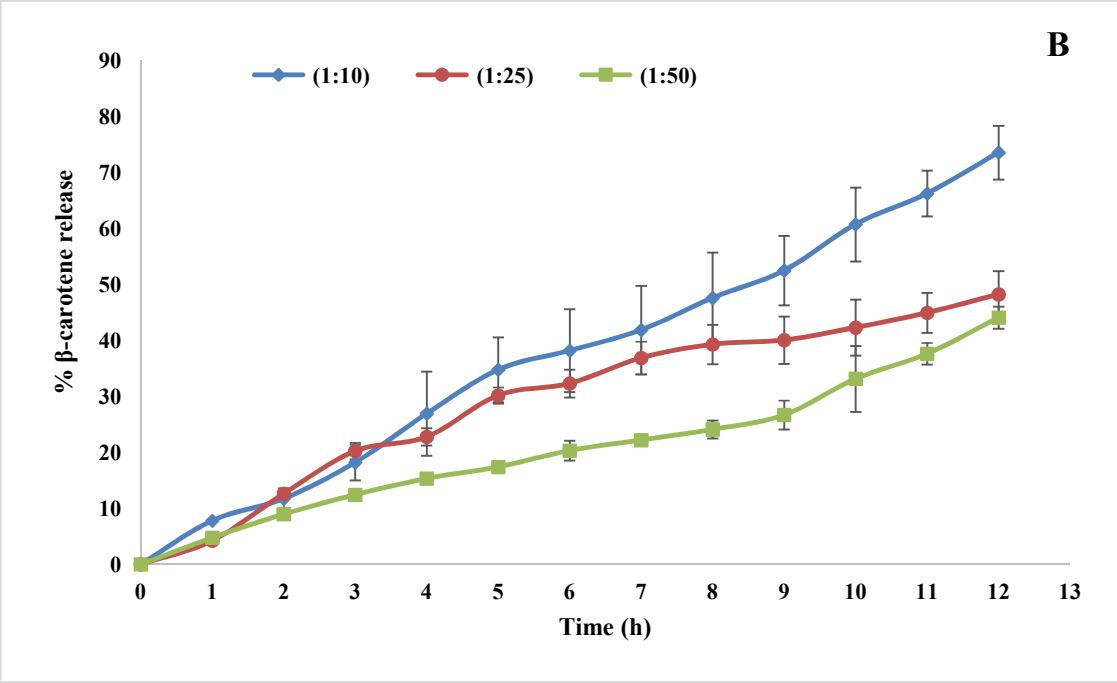


Fig. 5. *In-vitro* release profile of *β*-carotene aerosols (a) SD aerosols (b) SFD aerosols

structure and swelled faster than SD aerosols, which have smooth surfaces. Researchers reported that the hydrophobic nature of wall material builds a strong barrier against the permeation of environmental fluids. However, swelling of molecules in aqueous conditions increases the diffusion path length of bioactive compounds [41]. Since there is no pH change and no enzyme interaction during the release, it is expected that entrapped β-carotene will release completely without any degradation.

4. CONCLUSION

Porous particles containing bioactive compounds and therapeutic compounds can be easily formulated by using SFD. In this study, in this study found that β-carotene aerosols developed by SFD exhibited excellent aerosolization and aerodynamic properties, as compared to SD aerosols. SFD aerosols are large and porous with lower density and better free flow behavior. Results proved that particle size, density, and morphology of particles are very crucial in developing aerosols and these affect depositions in the deep lungs. Variation in the core to wall ratio also affects % release of β-carotene from aerosols; 1:50 showed sustained release as compared to other formulations. Form the present investigation, SFD found to be a promising technology for the development of bioactive compounds having application as a dry powder inhalation. Though, this study has several advantages, yet the pharmacokinetics has to be done to avoid toxicology effect of inhalation of high dosage.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

The first author acknowledges the Council of Scientific & Industrial Research (CSIR), India for the CSIR-SRF fellowship.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61485