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Investigation on the aerosol performance of dry powder inhalation hypromellose capsules with different lubricant levels

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ABSTRACT

HPMC capsules are made by a dipping process and a surface lubricant for the mould pins is an essential processing aid for removing dried capsules shells. For the purpose of this study, the level was determined by quantifying methyloleate (MO) a component found in the lubricant but not in the hypromellose capsules. Here we investigated the influence of the lubricant, low (10.81 μg/capsule = 60 mg/kg MO), medium (15.97 μg/capsule = 90 mg/kg MO) and high (23.23 μg/capsule = 127 mg/kg MO) content on powder (binary mixture of salbutamol: lactose, 1:50 w/w) aerosolization properties was investigated. Results indicated significantly lower emitted dose from capsules with 60 mg/kg MO. Furthermore, the 90 and 127 mg/kg MO level of lubricant capsules produced almost double the Fine Particle Dose & Fine Particle Fraction compared with the low level of lubricant. The data indicates that lubricant level within capsules has an influence on deposition profiles and amount of drug remaining in capsule and inhaler device after actuation. It is suggested lubricant levels greater than 60 mg/kg MO per capsule are required to minimise powder retention within capsules and maximise deposition profiles. AFM (atomic force microscopy) data suggest that internal surface roughness may be related with this phenomena.

1. Introduction

Delivery of therapeutic agents *via* the pulmonary route has gained increasing applications for lung diseases such as asthma and COPD. Pulmonary delivery has many advantages including delivery of medication directly to site of action, bypassing first pass metabolism in the liver (Geller, 2009; Labiris and Dolovich, 2003), it is non-invasive and can achieve therapeutic outcome at lower doses than administration *via* the oral route (Smith and Parry-Billings, 2003).

Dry powder inhaler (DPI) are able to deliver low and high doses within the range of 5–500 μg, do not require co-ordination between actuation and inspiration as with pMDI (Kaialy et al., 2012). They have been developed since the 1960's for a range of conditions such as asthma and COPD using short and long acting beta agonists, anti-cholinergic agents and corticosteroids drugs in order to facilitate drug administration to the lungs *via* the inhalation route (Atkins, 2005). Today there are currently more than twenty commercially available DPI, both active and passive (Chan et al., 2014). New active DPI incorporate additional mechanisms within the device to aid the fluidization of the powder from the device and reduce the reliance on the patient's inspiratory force. These mechanisms include vibration mesh which oscillates upon the patient's inhalation, others include release of the powder formulation only when the patient has achieved the correct inspiratory force (Chan et al., 2014). Passive DPI have unit doses of drug in either blister packs or capsules, which contain the drug and a carrier, *e.g.* lactose, and drug deposition relies on the patient's inspiratory force to de-aggregate the drug from the carrier (Chan et al., 2014; Kaialy et al., 2012; Zhou and Morton, 2012).

The powder mass in the capsules allows flexibility for the administration of low and high dose drugs within the range of 5 to 500 μg. Examples of capsule based devices include the single unit HandiHaler® (Boehringer-Ingelheim) (Islam and Gladki, 2008), TOBI® Podhaler™ (tobramycin) and Colobreathe® Turbospin® for delivery of large doses (Claus et al., 2014), Breezhaler® (Novartis), (Young et al., 2014) and novel multiple pre-metered unit-dose Flowcaps® (Hovione) that contains up to 20 capsules (Friebel and Steckel, 2010). These devices are simple to use, cost-effective and can administer low and high doses. In addition, the capsule based devices improve patient compliance, as they can provide feedback to the patient in the form of a rattling sound, indicating correct

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inhalation flow rate was achieved and passed through the device to deliver the correct dose (Behara et al., 2014; Smith et al., 2010). Moreover, the patient can visually check the capsule to determine if the dose has been administered (Smith et al., 2010).

Hypromellose (HPMC) is used to make inhalation grade capsules (Quali-V®-I) for use in DPI, as it is unaffected by moisture content changes (Jones, 2008). Hence, it does not become brittle as it loses moisture, a common phenomenon with gelatin capsules as patients do not store them as directed resulting in broken capsules and poor performance in their DPI (Nagata, 2002; Ogura et al., 1998; Renswouw et al., 2010). In addition, it has also been shown that HPMC capsules are less influenced by triboelectrification which is common with gelatin capsules (Nakate et al., 2005). Inhalation grade HPMC capsules are made from different grades of raw material chosen for their puncturing properties (Torrisi et al., 2013) and as a result have a slightly higher moisture content; 4.5–6.5% compared to 4.0–6.0% in oral pharmaceutical grade capsules. The HPMC Capsules are manufactured by dipping stainless steel mould pins at room temperature into a warm solution of hypromellose containing carrageenan as a network former and potassium chloride as a network promoter (Jones, 2004). The change in temperature causes the HPMC solution to gel and form a film on the surface of the mould pins. The films are dried by passing groups of pins through a series of drying kilns in which large volumes of air at controlled temperature and humidity is blown over them. As the films dry they shrink on to the pins. To remove them without damage it is essential for the mould pins to be coated with a surface lubricant to act as a release aid. Capsules cannot be manufactured without this lubricant (Jones, 2008). However, a search of the literature only shows one study investigating the influence of the amount of mould lubricant on the internal surfaces of capsules in relation to the aerosolization properties of powders from a capsule based DPI (Saim and Horhota, 2002). Furthermore, this study relates to gelatin capsules and not HPMC.

The lubricant is a mixture of food and pharmaceutical grade materials registered with regulatory authorities and the composition is proprietary for each capsule manufacturer. Hence for quantitative analysis it is necessary to choose a component of the lubricant which is not found in the HPMC capsules. In this study we chose methyloleate (MO) as a marker for lubricant content consisting of free fatty acids together with their esters. A number of sample preparation methods have been proposed in the literature to convert free fatty acids into their esters, such as silylation (Woo and Kim, 1999) or reaction with alkyl chloroformates (Gimeno-Adelantado et al., 2001) as well as for transesterification of the triglycerides (Mason and Waller, 1964).

The aim of this study was to investigate the aerosolization properties of dry powder formulations composed of inhalation grade lactose and micronized salbutamol, filled in to size 3HPMC inhalation grade capsules manufactured with 3 different lubricant levels *via* an 8-pin inhaler device. Size 3HPMC capsules was chosen because this is the size used in the pharmaceutical industry for development of capsule-based DPI. For example the last significant developments in inhalation capsule-based devices, Ultibro and Seebri Breezhaler, incorporate their respective dry powder formulation into a size 3 capsule. This size capsule (0.8 mg/mL) has a powder fill weight of 225 mg. Furthermore, to the best of our knowledge, the capsule inner surface lubricant content has not been determined by GCMS or its distribution in HPMC capsules by AFM. Hence, we describe a new technique with results obtained using these methods in this study.

2. Material and methods

2.1. Materials

8-pin monodose inhaler was provided by Plastiape S.p.a Italy. Hypromellose (HPMC) inhalation grade capsules, size 3 (Quali-V®-I) for this inhaler, manufactured using three different lubricant levels (satisfactory physical quality capsules were made at each level); low (10.81 μg/20 mg of blended powder within capsule = 60 mg/kg MO), medium (15.97 μg/20 mg of blended powder within capsule = 90 mg/kg MO) and high (23.23 μg/20 mg of blended powder within capsule = 127 mg/kg MO) were obtained from Qualicaps® Europe, S.A.U, Spain. Inhalation grade lactose (Respitose) was supplied by DFE Pharma, The Netherlands. Micronized salbutamol was obtained from Lusochimica, Spain. Methanol and 1-heptane sulphonic acid sodium salt were purchased from Sigma, UK. Methyloleate analytical standard, hexane and chloroform were from Sigma–Aldrich (St. Louis USA). 1,2,3 Trichlorobenzene was purchased from Fluka and was used as internal standard. Trimethylsulfonium hydroxide solution, (TMSH), 0.25 M in methanol was used for GC derivatization.

2.2. Determination of methyl oleate (MO) in capsules by gas chromatography mass spectrometry

Gas chromatography mass spectrometry, GCMS, is the technique most suitable for its qualitative and quantitative determination after derivatization and extraction into an organic solvent (Driscoll et al., 2009; Sutherland, 2007; Zhanga et al., 2014). Capsules inner lubricant content was evaluated by determining MO which was taken as a marker of the lubricant content using GCMS. Eleven HPMC capsules were weighted in a glass vial and 5 mL of Hexane: chloroform, 60:40 (v:v) extraction solvent containing 10 mg/L of the internal standard was added. The vial was sonicated for 1 h in an ultrasonic bath; then 100 μL of the extract was transferred into a 2 mL vial for derivatization using 50 μL of TMSH. The MO was identified by MS (Mass spectrometry) and was quantified using an internal calibration method with six points in the 0.5–20 mg/kg concentration range. 1 μL of the derivatized MO was injected in split less mode in the GCMS instrument.

2.3. Preparation of inhalation grade lactose & powder mix

Inhalation grade lactose and powder mix were prepared according to previously published method (Saleem et al., 2008) with slight modifications. Inhalation grade lactose was fractionated by sieving with a sieve stack (250, 125, 90, 63, and 45 μm) using vibration amplitude of 40 for 10 min and collected on a 90 μm sieve to be used in all subsequent studies. Micronized salbutamol sulphate and lactose were mixed in a ratio of 1:50 (w/w) *via* geometric dilution to obtain a 2% binary blend. The formulations were blended with a Turbula® orbital mixer (Glen Mills, Clifton, New Jersey) for 30 min at 46 rpm. The blend uniformity was determined by randomly selecting five 20 mg samples, and formulations were considered uniform when the coefficient of variation (% CV) was ≤6%. Samples were analyzed using high-performance liquid chromatography (HPLC) method below (Section 2.4). Once blend uniformity was achieved 20 ± 1 mg of blended powder was manually loaded into HPMC capsules (size 3) with different lubricant levels (low, medium and high) and stored in a humidity chamber (Sanyo Atmos Chamber) at 22 °C and 40% RH for 2 weeks (Nine HPMC capsules were filled for each lubricant level at weeks 1 and 2).

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2.4. In vitro *aerosolization performance*

For each lubricant level, three capsules (20 ± 1 mg of powder loaded into HPMC capsules (size 3)) corresponding to a dose of 408 μg were dispersed through a 8-pin DPI inhaler into a next generation cascade impactor (NGI; MSP Corporation, Shoreview, MN) at a flow rate of 60 L min^{-1} actuated for 4 s, with 15 mL of mobile phase added to the pre-separator. This was repeated three times (n = 3). Drug depositing in the capsule, inhaler, mouthpiece adaptor, induction port, pre-separator and NGI stages were collected by rinsing each component with mobile phase. This was repeated at week 2 and the drug content was assessed *via* HPLC method (Section 2.4).

The emitted dose (ED) was calculated as the total mass of drug depositing in the mouthpiece, induction port, pre-separator, and NGI stages. The fine particle dose (FPD) was determined as the mass of drug deposited in the NGI with aerodynamic diameters ≤ 4.46 μm. The percentage fine particle fraction (% FPF) of each dose was the ratio of the drug mass depositing in the NGI (aerodynamic diameter ≤4.46 μm) over the emitted dose. Mass median aerodynamic diameter (MMAD) was calculated by subjecting the inertial impaction data to log-probability analysis.

2.5. Chemical analysis

Capsules internal lubricant analysis were carried out by gas chromatography coupled to mass spectrometry, GCMS, using a GC 7890 (Agilent Technologies, Palo Alto, CA, USA) and a 5975C quadrupole mass spectrometer (Agilent Technologies, TX, USA). A Supelcowax 10 (30 m × 0.25 mm × 0.25 μm film thickness) fused silica capillary column was used. The injection port temperature was 240 °C and the oven temperature program changed from 40 °C to 240 °C at 10 °C/min.

Salbutamol sulphate was analyzed by HPLC (Agilent Technologies) using a Kinetex C18 column (50 × 4.6 mm i.d. packed with 2.6 μm, phenomenex, UK). The mobile phase consisted of methanol and 0.25% (w/v) 1-heptane sulphonic acid sodium salt (45:55 v/v), the flow rate was 1 mL/min, injection volume 10 μL, temperature 25 °C and wavelength was 200 nm. The retention time for salbutamol sulphate was 3 min and the limits of detection and quantification were 0.60 and 1.12 μg/mL respectively.

2.6. Morphology of inner capsule surface

Atomic force microscopy, AFM, is a widely used technique for micro- and nanoscale material characterization creating a three dimensional image of a physical surface (Garcı'a and Pérez, 2002). A commonly employed measurement approach is based on tapping-mode AFM which involves a short and pulsed contact between the tip of an oscillating micro-cantilever and the sample surface. Vibrations of the cantilever tip are induced through dither piezo oscillations from which heights and phases are monitored for imaging purposes (García, 2010).

The AFM (atomic force microscopy) experiments were made using a multimode Nanoscope III A (Bruker) in tapping mode in order to access surface topography. It is equipped with three scanners of 1, 1.5 and 150 μ. Small pieces of 5 × 5 mm were cut and placed onto the Nanoscope probe. The instrument standard sample capsule probe was made of stainless steel but it was not suitable to handle the curved shape of a capsule; so, a special home-made support device was designed. Topographic measurements of the inner capsules surface at a fixed scanning angle equal to zero were made using tapping mode (intermittent contact mode). The cantilever/tip assembly was sinuously vibrated by a piezo device mounted above it, and the oscillating tip slightly taped the surface at the resonant frequency of the cantilever with constant oscillating amplitude introduced in the vertical direction with a feedback loop keeping the average normal force constant. Measurements were made using a silicon probe (Veeco probe) with a spring constant of 5 N/m and a resonance frequency of 150 kHz. All experiments were performed in air at ambient conditions. In order to stabilize thermally the piezo driver, the machine was turned on two hours before use. During each measurement a 15 × 15 μm surface was covered using a FESP tip.

2.7. Statistical analysis

The data obtained were analyzed statistically by one-way analysis of variance (ANOVA) with the Tukey's comparison using

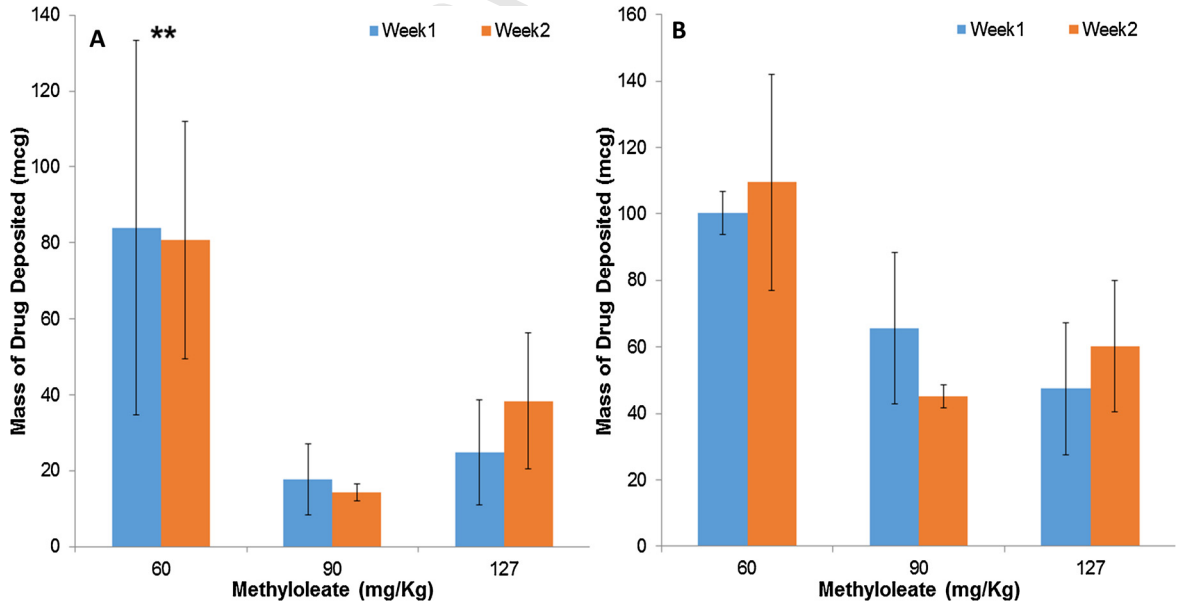

Fig. 1. Deposition of salbutamol sulphate remaining in capsules (A) and device (B) following aerosolisation at 60 L min^{-1} from a 8-pin inhaler (Mean ± SD, n = 3) $^{**}P < 0.05$ (ANOVA/Tukey's) Low (60 mg/kg MO) versus medium (90 mg/kg MO) & high (127 mg/kg MO) lubricant levels at weeks 1 and 2.

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Minitab 17 Statistical Software® (Minitab Inc., PA, USA). Statistical significance were considered when $p < 0.05$. All values are expressed as the mean ± standard deviation.

3. Results and discussion

3.1. In vitro *aerosolization performance*

3.1.1. Comparing capsules and device

Comparing HPMC capsules (Fig. 1A) the results clearly indicate a significantly larger salbutamol retention in the low lubricant capsule (week 1: 84.01 ± 49.23 μg, week 2: 80.76 ± 31.25 μg) compared to the medium (week 1: 17.70 ± 9.34 μg, week 2: 14.37 ± 2.20 μg) and high lubricant capsules (week 1: 24.91 ± 13.79 μg, week 2: 38.36 ± 17.89 μg) ($p < 0.05$, ANOVA/Tukey's). However, there was no significant difference regarding salbutamol retention between medium and high lubricant capsules at week 1 and 2. A similar trend was observed with drug deposition remaining in the 8-pin inhaler (Fig. 1A). It is evident from the data that lubricant level within the capsule is important, with data suggesting lubricant levels between 90 and 127 mg/kg MO per capsule result in significantly lower drug deposition within the capsule and inhaler device. The high salbutamol retention in low lubricant capsules occurred, because during the removal of the capsules from the mould pins there is a high degree of adhesion that causes roughness, which can be seen by the mountain and deep valleys, as shown by AFM (see Section 3.2). Hence during inhalation the salbutamol particles become entrapped or lodged within these peaks and crevices (Saim and Horhota, 2002). However, this effect is reduced significantly as the level of lubricant increases to greater than 90 mg/kg MO lubricant per capsule. This results in a smooth inner surface of the capsule with less mountain and valleys (see Section 3.2) as there is less adhesion during the removal from the moulding pins, and hence, reduction in contact between particles and crevices (Ibrahim et al., 2000; Saim and Horhota, 2002).

3.1.2. Comparing emitted dose, fine particle dose, fine particle fraction & MMAD

Fig. 2 shows the ED, FPD, FPF and MMAD of salbutamol aerosolized from an 8-pin inhaler at 60 L/min. The ED is significantly lower for the low lubricant level capsules (week 1: 223.73 ± 42.72 μg, week 2: 217.69 ± 63.85 μg) compared to the medium (week 1: 324.57 ± 32.06 μg, week 2348.42 ± 1.17 μg) and high lubricant capsules (week 1: 335.65 ± 33.70 μg, week 2: 309.35 ± 37.67 μg) ($p < 0.05$, ANOVA/Tukey's). These results were repeated for fine particle dose (μg) (Fig. 2B) and fine particle fraction (%) (Fig. 2C) where the values are almost twice that obtained using low lubricant capsules ($p < 0.05$, ANOVA/Tukey's). This coincides with the high deposition of salbutamol remaining in the low lubricant capsules (Fig. 1A) and the device (Fig. 1A). Furthermore, the MMAD (μm) (Fig. 2D) is significantly greater from low lubricant capsules (week 1: 3.37 ± 0.78 μm, week 2: 2.71 ± 0.03 μm) compared to medium (week 1: 2.13 ± 0.11 μm, week 2: 1.78 ± 0.57 μm) and high (week 1: 2.12 ± 0.16 μm, week 2: 1.65 ± 0.17 μm) ($p < 0.05$, ANOVA/Tukey's). This also confirms the low FPD and FPF within the lungs, due to large particle size and hence less drug depositing within the deep lungs.

3.2. AFM studies

Topographic plots from three capsule inner surfaces are represented in Fig. 3. They were taken as examples of the twelve capsules analyzed, which were selected at a low, medium and high concentrations that were previously determined by GCMS (Driscoll et al., 2009; Sutherland, 2007; Zhang et al., 2014).

As can be seen, the topographic images show a mountain and deep valley distribution of the lubricant oil in the inner surface of the capsules. Similar results have been obtained using gelatin

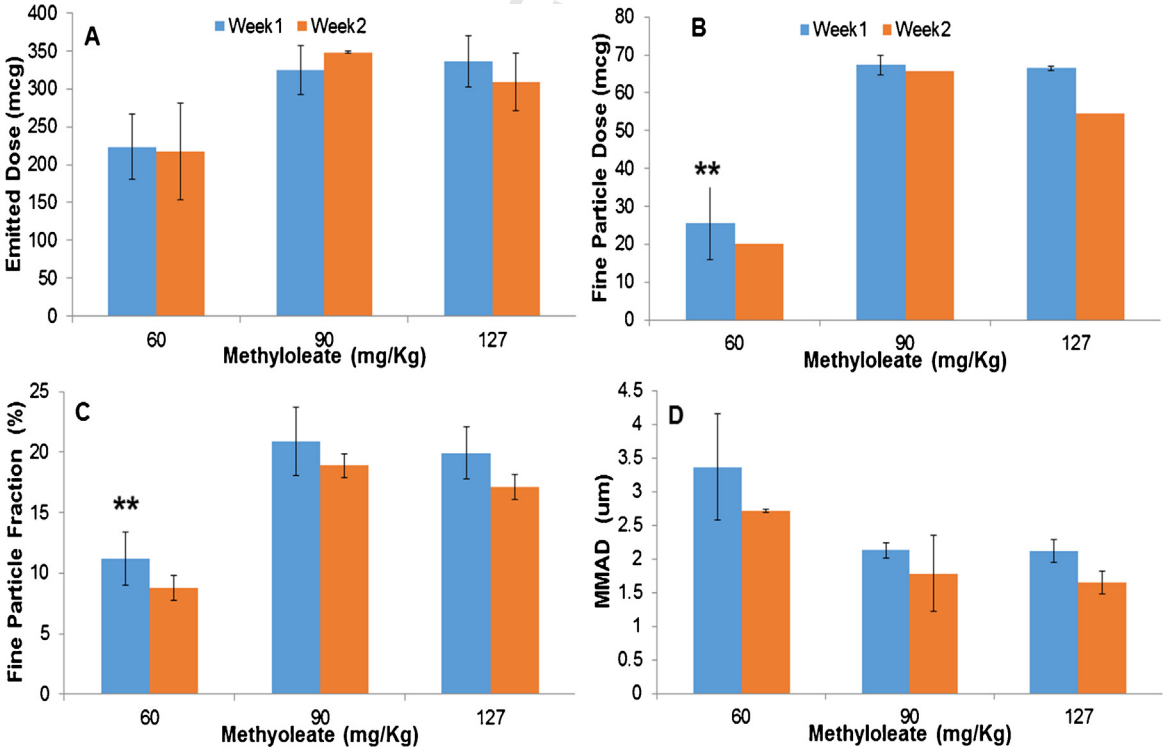

Fig. 2. Emitted dose (μg) (A), Fine particle dose (μg) (B), Fine particle fraction (%) (C), MMAD (μm) (D) of salbutamol sulphate following aerosolisation at 60 L min^{-1} from an 8-pin inhaler (Mean ± SD, n = 3) $^{**}P < 0.05$ (ANOVA/Tukey's) Low (60 mg/kg MO) versus medium (90 mg/kg MO) & high (127 mg/kg MO) lubricant levels at weeks 1 and 2.

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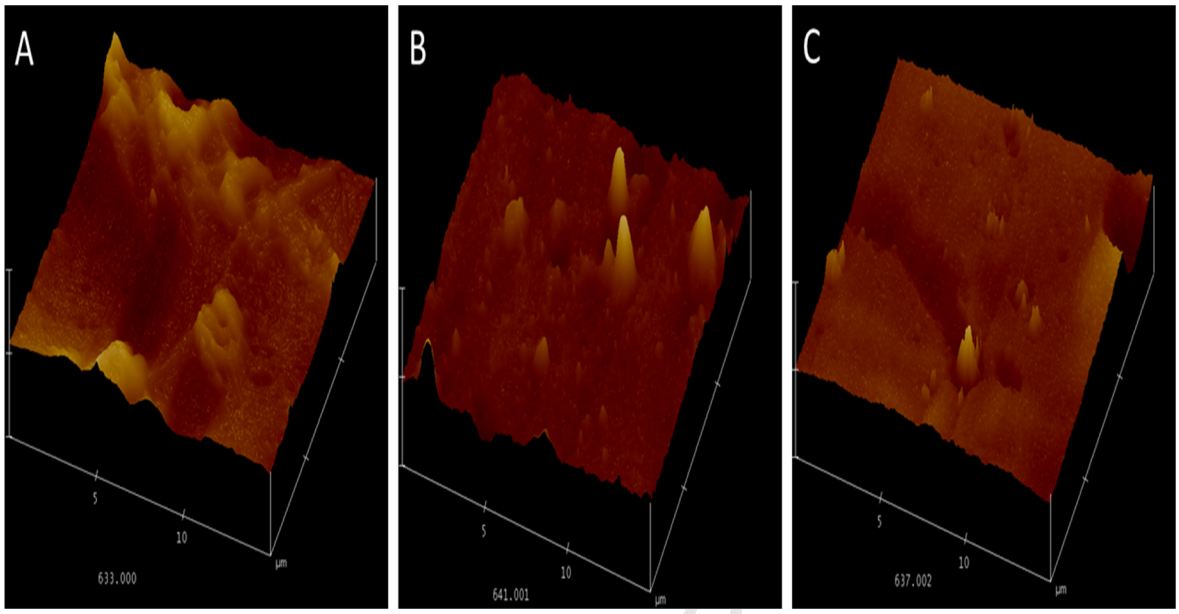

Fig. 3. Topographical image of capsules inner surface containing (A) low (26 mg/kg MO), (B) medium (63 mg/kg MO) and (C) high (137 mg/kg MO) levels of methyloleate.

capsules (Ibrahim et al., 2005). When the MO amount increased, the inner surface appeared to be more homogenous (*i.e.* reduced mountain and deep valley distribution) (Fig. 3A and C).

Four parameters derived from these plots (roughness, depth, particle height and grain height) (Fig. 4) were used to obtain better knowledge regarding inner capsule lubricant distribution. The average from three different points on each capsule surface was used to plot the different parameters versus MO concentration. It is apparent (Fig. 4a) when the MO concentration increases, the roughness, represented by Ra, decreased, indicating that homogeneity of capsule surface is higher when it is covered more completely with the lubricant.

Reproducibility of the capsule inner surface can be evaluated by paying attention to two capsules groups containing three capsules with similar MO concentration, approximately 59 and 104 mg/kg respectively as indicated by the red circle in Fig. 4A. The reproducibility of the Ra parameter increases when MO concentration is higher. This seems to indicate that homogeneity of the lubricant inner surface is also enhanced when its concentration is higher.

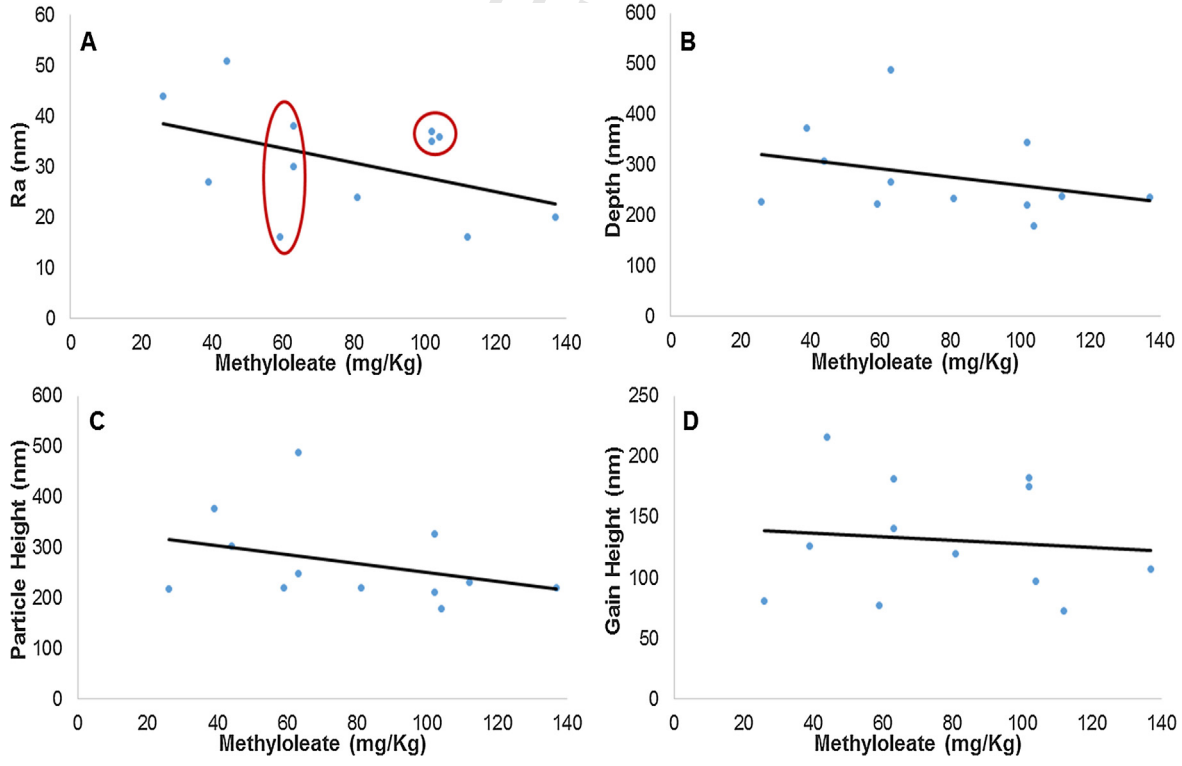

Fig. 4. Methyloleate concentration effect on (A) roughness, (B) Depth, (C) particle height and (D) grain height.

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Similarly, the average depth, particle height and height gain also decreased when MO concentration increased (Fig. 4B–D), which confirmed the surface decreased when MO concentration increases. In fact this tendency is also apparent in Fig. 4A–D, in which dispersion of the respective parameters through an average straight line decreases when the inner lubricant concentration increases.

4. Conclusion

The study clearly indicates that the lubricant level inside capsules has an influence on deposition profiles, amount of drug remaining in capsule and inhaler device after actuation. The results obtained suggest lubricant levels greater than 10.81 μg per capsule (60 mg/kg MO) are beneficial in decreasing drug deposition from capsules in an 8-pin inhaler device, while more than doubling the fine particle dose and fraction. It seems this effect is related to the capsule internal surface roughness. Measurements with AFM indicated that homogeneity of the internal capsule surface is higher when the inner lubricant concentration increases.

Conflict of interest and role of funding source

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