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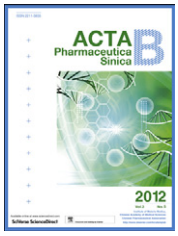

ORIGINAL ARTICLE

Mucoadhesive microparticles as potential carriers in inhalation delivery of doxycycline hyclate: a comparative study

Madhusmita Mishra, Brahmeshwar Mishra*

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221005, India

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KEY WORDS

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Carbopol;
Doxycycline hyclate;
Spray drying;
Aerosolization performance;
Inhalation microparticles

Abstract The present work compares and evaluates the suitability of different polymer-based microparticles for inhalation delivery of doxycycline hyclate. Mucoadhesive polymers, such as sodium carboxymethyl cellulose, sodium alginate, polyvinyl alcohol, polyvinylpyrrolidone, starch, and carbopol were selected as carriers for inhalation delivery. Microparticles were prepared by spray drying and evaluated in terms of yield, moisture content, morphology, tapped density, encapsulation efficiency, *in vitro* mucoadhesion, thermal properties and *in vitro* aerosolization performance. Additionally, the cytotoxicity of the microparticles on H1299 human alveolar cell line was examined. Smooth spherical to collapsed doughnut shaped particles were formed. They exhibited tap densities of 0.202–0.502 g/cm^3 and mass median aerodynamic diameter of 3.74–6.54 μm. Mucoadhesion was highest in case of carbopol-based microparticles. Drug release from these microparticles exhibited biphasic Fickian type of diffusion. Only at the highest concentration of microparticles (1 mg/mL) less than 90% cell viability was seen in DX loaded sodium alginate microparticles (DXSA, 87.2%), starch microparticles (DXST, 85.1%) and carbopol microparticles (DXCP, 82.7%) preparations after 48 h of exposure to alveolar cells. The results clearly indicate that sodium carboxymethyl cellulose-based microparticles may serve as an ideal carrier for inhalation delivery of doxycycline hyclate.

*Corresponding author. Tel.: +91 5422307049.
E-mail address: bmishrabhu@rediffmail.com (Brahmeshwar Mishra).

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1. Introduction

Doxycycline hyclate (DX) is a tetracycline derivative effective against a wide variety of respiratory pathogens such as *Staphylococcus aureus*, *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Mycoplasma pneumonia* by inhibition of the translation step in protein synthesis[1]. A significant advantage of DX is its activity against tetracycline-resistant *S. aureus*. At present, DX is administered orally and parenterally and is associated with dose-dependent side effects[2], such as oesophageal ulceration, photosensitivity, anaemia, teratogenecity, hepatotoxicity and gastro-oesophageal reflux disease on chronic use. DX is readily absorbed, widely distributed, and eliminated in urine. Recently, its activities against matrix metalloproteinase[3] have been applied to the reduction of inflammatory changes associated with asthma, cystic fibrosis and chronic lung diseases. However, one of the major drawbacks in the treatment of lung infections by conventional routes of administration is that only 5–10% of the antibacterial reaches the target site.

Among the different routes of drug administration, inhalation delivery of antibacterials is of recent interest. Compared to oral or parenteral delivery, much higher pulmonary drug concentration can be achieved *via* inhalation route. This non-invasive route circumvents first-pass metabolism and systemic toxicity. However, the therapeutic benefit of many inhaled drugs currently on the market is often short-lived. It is obvious that there has been a growing desire to prolong the duration of therapeutic action of these drugs. In recent years, there has been considerable interest in the development of polymeric carriers for inhalation drug delivery. One strategy with potential implications for inhalation delivery is the use of microparticulate drug delivery systems that intrinsically incorporate a mucoadhesive agent in the formulation. These mucoadhesive agents are typically high molecular weight polymers which can interact with the mucin layer of the respiratory epithelium through hydrogen bonding, electrostatic, hydrophobic or van der Waals interactions. Mucoadhesive microparticles designed for inhalation delivery should be typically in the size range 1–5 μm. The incorporation of antibacterials into these polymeric matrices modifies the drug pharmacokinetics by increasing lung residence, area under the concentration time curve and decreasing the apparent volume of distribution[4]. It is expected to improve the therapeutic index compared with free antibacterials. Although the concept of mucoadhesion at the upper respiratory tract has been broadly studied[5–7], application to the lower respiratory tract has not been explored. Mucoadhesive polymers such as hydroxypropyl cellulose[8], chitosan[9], poly(lactic acid)[10], poly(D,L-lactic-*co*-glycolic acid)[11], carbomer[12] and sodium carboxymethyl cellulose[13] have been studied by various researchers for inhalation drug delivery. While the safety of some carriers has been examined in different routes of drug administration, extensive toxicological studies are required before more mucoadhesive carriers are used in clinical practice for inhalation delivery.

In the present study, mucoadhesive polymers were selected as potential carriers for inhalation drug delivery. Microparticles were prepared by spray-drying. The aim of the present work was to compare and evaluate the suitability of different polymer-based microparticles for inhalation delivery of DX. The prepared microparticles were evaluated in terms of yield, moisture content, particle morphology, tap density, encapsulation efficiency, *in vitro* mucoadhesion, thermal properties and *in vitro* aerosolization performance. Additionally, the cytotoxicity of the mucoadhesive polymers on H1299 human alveolar cell line was compared with sodium lauryl sulphate (SLS).

2. Materials and methods

DX was obtained as a gift sample from Ranbaxy Research Laboratories Ltd. (Gurgaon, India). Sodium alginate (MW 216 Da), polyvinylpyrrolidone K90 (MW 700,000 Da) and carbopol 974P (MW 300,000 Da) were purchased from ISP Inc. (Canada), BASF AG (Ludwigshafen, Germany) and B.F. Good Rich Ltd. (Cleveland, OH), respectively. Sodium carboxymethylcellulose (1500–3000 cps) and polyvinyl alcohol (80% hydrolyzed, MW 9000–10,000 Da) were obtained from Sigma–Aldrich India Ltd. (New Delhi, India). All other chemicals used were of laboratory reagent grade and obtained from Qualigens Fine Chemicals (Mumbai, India).

2.1. Preparation of spray-dried microparticles

Microparticles were prepared by spray drying (laboratory spray dryer, Jay Instruments and Systems Pvt. Ltd., India) aqueous ethanolic solutions (36%, *v*/*v*) containing drug (10%, *w*/*w*), leucine (33%, *w*/*w*), lactose (37%, *w*/*w*) and polymers (20%, *w*/*w*), such as sodium carboxymethyl cellulose (SC), sodium alginate (SA), polyvinyl alcohol (PA), polyvinylpyrrolidone (PP), starch (ST) or carbopol (CP). A control batch (without polymer) was also prepared using drug (10%, *w*/*w*), leucine (33%, *w*/*w*) and lactose (57%, *w*/*w*) for comparison. The spray dryer operating parameters were as follows: inlet temperature 140 °C, drying air flow rate 28 m^3/h, aspirator pressure −20 bar, feed rate 3 mL/min, outlet temperatures 76 °C. The spray-dried powders were collected from the product collection chamber. Material adhering to the chamber lid as well as lower portion of the cyclone chamber taper was collected using a brush.

2.2. Characterization

2.2.1. Determination of particle morphology and density

Morphology of the microparticles was examined by scanning electron microscopy (JEOL Ltd., Tokyo, Japan). Dry particles were attached to specimen stubs using double-sided adhesive tape and excess particles were blown out using a capillary tube. Microparticles were imaged using a 5 kV accelerating voltage, 10 mm working distance and emission current of 348 μA by scanning fields randomly at several suitable magnifications.

The density of microparticles was assessed by pouring dry microparticles into a 5 mL graduated cylinder under gravity[14]. The ratio of weight to the volume occupied by the powder gives the bulk density (ρ_b). The cylinder was then tapped onto a level surface at a height of about 2 cm until no change in volume was observed. The tap density (ρ_t) and resultant volume was calculated as the ratio of weight to the tapped volume.

2.2.2. Determination of spray-drying yield and drug entrapment efficiency

The yields of the microparticle were calculated as the percentage ratio of weight of recovered spray-dried powder to the total amount of dry solids in the initial feed solution.

An accurately weighed quantity of microparticles (20 mg) was dissolved in 0.1 M HCl. The microparticles were stirred magnetically to promote swelling and complete dissolution of the polymer. The obtained solution was filtered through a 0.45 μm syringe filter. The amount of DX was analysed by ultraviolet spectrophotometry (JASCO 7800, Tokyo, Japan) at 271.6 nm. The drug entrapment efficiency (EE) was calculated using the following equation:

$$EE = A_{\text{actual}}/A_{\text{loading}} \times 100\% \quad (1)$$

where A_{actual} is the actual amount of the drug encapsulated and A_{loading} is the amount of drug loading calculated from the amount of drug added during the manufacturing process. All the experiments were carried out in triplicate.

2.2.3. *Differential scanning calorimetry and thermogravimetric analyses*

The thermal response of each of the powders was analyzed using a differential scanning calorimeter (DSC, Shimadzu, Japan). Samples (about 3–5 mg) were hermetically crimp-sealed in aluminium pans and heated at 30–300 °C at a rate of 10 °C/min.

Thermogravimetric analysis (TGA) was used to determine the water content in the spray-dried powders. For TGA (TG-DTA, Setaram Instrumentation, Caluire, France), each sample (about 10 mg) was loaded in an aluminium crucible and heated under a nitrogen purge at 30–120 °C with a scanning rate of 10 °C/min. The change in weight with temperature was recorded.

2.2.4. *Determination of microparticle aerodynamics*

The aerodynamic properties of the microparticles were determined using an eight stage, nonviable Andersen cascade impactor (ACI) with a preseparator (Graseby-Andersen, Atlanta, GA, USA). A hard gelatin capsule (size No. 2, Universal capsules, Mumbai, India) previously stored in a dessicator for at least two days was manually loaded with the microparticles to approximately 50% of its volume and placed in a monodose inhaler (Miat S.p.a Milan, Italy). For each actuation (4 s), the capsule was pierced and the liberated powder was drawn through the impactor operated at a continuous air flow rate of 60 L/min (produced by a vacuum pump connected to the outlet of the ACI). After 15 actuations per determination, the amount of microparticles deposited in the device, throat, pre-separator and each stage of ACI was collected. Under these conditions, the effective cut off diameters were 8.6, 6.5, 4.4, 3.3, 2.0, 1.1, 0.54 and 0.25 μm for stages 0–7, respectively.

The recovered dose was defined as the total amount of drug recovered per capsule after each actuation. Emitted dose was determined as the percent of total powder mass exiting the capsule (i.e., from throat to filter in the ACI). The fine particle fraction (FPF) was defined as the fraction of drug less than 4.7 μm. The mass median aerodynamic diameter (MMAD) of the microparticles was determined from the plot of inverse cumulative mass percentage undersize in each stage against the log effective cut off diameter of the respective stages. Geometric standard deviation (GSD) is a measure of the spread of an aerodynamic particle size distribution. It was calculated as the ratio of diameters at which 84% and 16% of the aerosol mass are contained.

2.2.5. *In vitro mucoadhesion*

The *in vitro* mucoadhesion studies were carried out in triplicate on rat intestinal mucosa by modification of a previously reported method[15]. Briefly, a piece of intestinal mucosa (2×2 cm^2) was mounted on to a glass slide using cyanoacrylate glue. Microparticles (20 mg) were spread uniformly on the surface of the intestinal mucosa using a monodose insufflator (Miat, Spa, Milan, Italy). This was followed by hydration in a humidity–temperature control cabinet (Narang Scientific works Pvt. Ltd., New Delhi, India) at 80% R.H. and temperature of 25 ± 0.5 °C. After 20 min, the mucosal lumen was washed with physiological saline (pH 7.4) at an angle of 45° using a peristaltic pump. The washings were dried at 60 °C in a hot air oven. The ratio of adhered to the applied microparticles was calculated as percent bioadhesion. The rat intestine was obtained by decapitating mature albino rats (Approved by the central animal ethical committee, B.H.U.).

2.2.6. *Determination of in vitro drug release*

In vitro release of DX from the prepared microparticles was studied in triplicate by diffusion with dialysis bag. The dialysis membrane (Sigma, thickness 0.025 mm, mol. wt. cutoff 6000–8000 Da) was cut into equal pieces (6×2.5 cm^2) and soaked in distilled water for 12 h before use. An accurately weighed quantity of microparticles (equivalent to 10 mg DX) was suspended in 5 mL of phosphate buffer saline (PBS) pH 7.4 and placed in the dialysis pouch with the two ends fixed by thread. The pouch was attached to the paddles of USP type II dissolution tester (Electrolab, TDT 06P model) and put into the flask containing 500 mL of PBS maintained at 37 ± 0.2 °C and stirred at 50 rpm. Aliquots of samples were withdrawn at regular time intervals (and fresh PBS replaced) and analyzed by UV spectrophotometer as described earlier.

2.2.7. *Determination of formulation cytotoxicity*

The *in vitro* cytotoxicity of microparticles[13] was evaluated using MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on H1299 mammalian alveolar cells. The assay is based on the conversion of MTT to formazan (a soluble purple colored compound) through mitochondrial activity. Since, this conversion can only be made by living cells; the quantity of formazan produced is directly correlated to the number of living cells or cell viability.

The H1299 cells were seeded (5000 cells/well) into a 96-well plate, incubated overnight at 37 °C and 5% CO_2 atmosphere, and allowed to recover for approximately 24 h. The culture medium consisted of DMEM, FBS (10%, *v*/*v*) with L-glutamine (2 mM), penicillin (100 units/mL), streptomycin (100 μg/mL) and amphotericin (50 μg/mL). At 90% confluence, the culture medium was removed and replaced with 100 μL of the solution to be tested. The test suspensions were prepared by diluting sterile, aqueous microparticle suspension with DMEM to obtain different concentrations (0.01–1.0 mg/mL). The suspensions were sonicated (4 s) and made sterile by passing through 0.22 μm disposable syringe filters. The cells were then treated with the test suspensions and SLS (1%, as positive control). After alveolar cells were incubated with the test suspension for 24, 48 or 72 h, the reaction medium was removed and the cells were washed twice with DMEM and 100 μL of fresh medium along with 20 μL of MTT (0.5 mg/mL in DMEM) was added to each well. After 3 h of incubation the supernatant was removed and 200 μL of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. Finally, the absorbance was measured using a microplate spectrophotometer (Microplate

reader 680XR, Bio-Rad Laboratories, CA, USA) at 570 nm. The results were expressed as the percentage of absorbance of the treated wells (Abs_{test}) with respect to the untreated wells ($Abs_{control}$). The untreated cells contained a mixture of only medium and MTT without cells.

2.3. Statistical analysis

Significant differences between formulations were determined using ANOVA (GraphPad InStat software v 3.06, CA, USA) followed by Tukey, Kramer, and Bonferroni multiple comparisons, and $P<0.05$ was considered to be significant.

3. Results and discussion

3.1. Particle morphology

Scanning electron microscopy (SEM) images were used to visualize the particle diameter, structural and surface morphology of the spray-dried microparticles (Fig. 1). In general, the micrographs indicate that the dry powders were roughly spherical in shape and approximate 1-5 μm in diameter. There were no visible crystalline particles in the micrographs of the microparticles. The DX loaded microparticles containing SC and SA were found to be moderately dimpled but spherical in shape (Fig. 1a and b). The number of indentations was increased resulting in the formation of corrugated spherical particles in case of PA and PP containing spray dried powders (Fig. 1c and d). The DXST microparticles were found to have a mixture of hollow and collapsed particles resembling a doughnut shape (Fig. 1e). This may be attributed to the outer shell formation due to rapid evaporation of the solvent from the droplet surface during spray drying. But, the solvent evaporates very slowly inside the droplet and gas expands resulting in the build up of internal pressure. Then, the outer shell becomes thinner to allow faster diffusion of solvent droplets. As the shell thickness is low and impermeable, the particles rupture resulting in the formation of hollow particles. In contrast, the micrographs of microparticles obtained from spray drying of viscous solutions of CP indicated aggregates of smooth spherical particles (Fig. 1f). This aggregation may have occurred during storage. The above results indicate that the type of polymer used during the preparation of spray-dried microparticles significantly influenced the particle shape and morphology. This is in agreement with earlier studies[16].

3.2. Spray-dried powder characteristics

The spray-drying yield, powder tapped density, drug encapsulation efficiency and water content of the different polymer-based microparticles are shown in Table 1. The yield of the recovered powder varied considerably. The yield of DXPP formulation was high (63.04%), whereas DXST exhibited the lowest yield (50.79%). The DX encapsulation efficiency was observed to be in the range of 75.23%–92.82% of the total drug loading. TGA results indicated that the moisture content of the powders ranged 2.16–5.03% (*w*/*w*). It was observed that the sodium alginate-based formulations retained more water during spray drying, thus exhibiting significantly greater moisture levels.

Tap density is an important physical attribute of spray-dried microparticles. The tapped density of the powder is directly proportional to the aerodynamic diameter[17]. It was found to vary from 0.202 ± 0.02 to 0.502 ± 0.01 g/cm^3 and was largely affected by the particle morphology. The tap density of hollow, collapsed particles is statistically lower than that of smooth spherical particles. So, the lightest powder (0.202 ± 0.02 g/cm^3) was observed with DXST while tapped densities greater than 0.421 g/cm^3 were observed in DXSC, DXSA and DXCP microparticles.

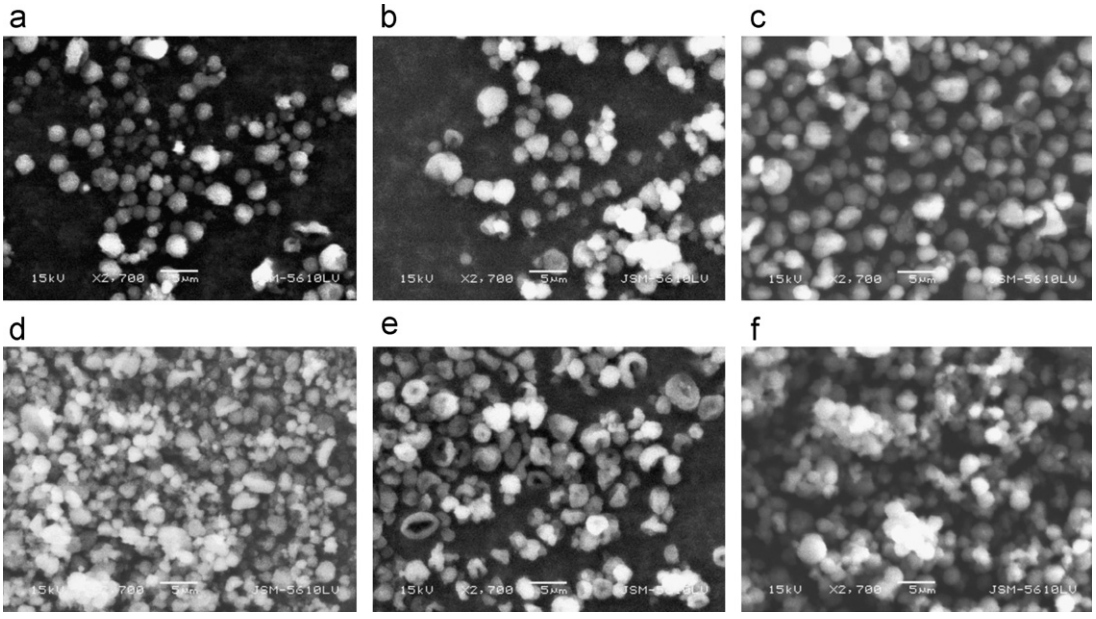

Figure 1 Scanning electron microscopy of spray-dried DX loaded microparticles prepared using different polymers: (a) DXSC; (b) DXSA; (c) DXPA; (d) DXPP; (e) DXST; (f) DXCP. Magnification: × 2700; bar: 5 μm.

Table 1 Physicochemical characteristics of the spray-dried microparticles (mean ± SD, $n=3$).

Batch[a]	Yield (%)	Tapped density (g/cm^{-3})	Encapsulation efficiency (%)	Water content (%)
DXSC	56.27	0.471 ± 0.03	83.74 ± 2.31	4.16 ± 0.52
DXSA	57.68	0.502 ± 0.01	85.46 ± 1.11	5.03 ± 0.17
DXPA	51.98	0.310 ± 0.02	80.10 ± 4.53	2.65 ± 0.05
DXPP	63.04	0.488 ± 0.01	88.71 ± 2.01	2.16 ± 0.48
DXST	50.79	0.202 ± 0.02	75.23 ± 1.53	3.17 ± 0.86
DXCP	62.34	0.421 ± 0.04	92.82 ± 3.56	3.86 ± 0.52

Data are expressed as mean ± SD (standard deviation), $n=3$.
[a]Each batch contains a theoretical drug loading of 10% (w/w).

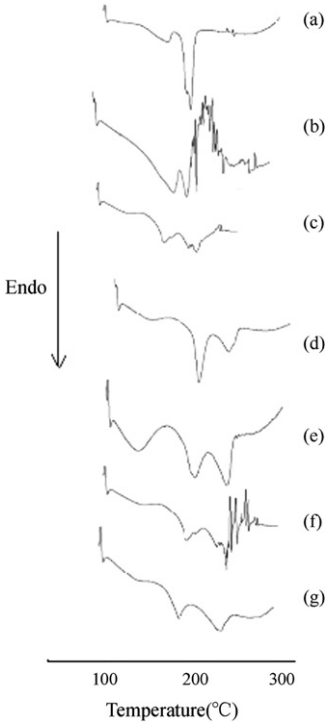

Figure 2 DSC thermograms of (a) DX; (b) DXSC; (c) DXSA; (d) DXPA; (e) DXPP; (f) DXST; (g) DXCP.

3.3. *Differential scanning calorimetry*

The physico-chemical characterisation of microparticles was performed by DSC analysis. The DSC thermograms of pure drug (DX) and DX-loaded microparticles are shown in Fig. 2. Only DX (Fig. 2a) showed a complex transition and a large endothermic peak due to the melting of the drug at 168.3 °C, followed by a series of two small exothermic peaks at around 220 °C. Two broad endothermic peaks were observed at approximately 150 and 220 °C in batches of DXSC and DXCP (Fig. 2b and g). This may be attributed to glass transition followed by a peak of crystallisation[18]. These results suggest presence of a solid dispersion of drug in the polymer matrix. A very small and broad endothermic peak around 168 °C was seen in batches of DXPP and DXST (Fig. 2e and f) while it shifted to approximately 162.3 °C in the calorimetric curve for DXPA (Fig. 2d). Polymer decomposition peaks were observed beyond 187 and 220 °C for DXSA (Fig. 2c) and DXST (Fig. 2f) containing spray-dried microparticles.

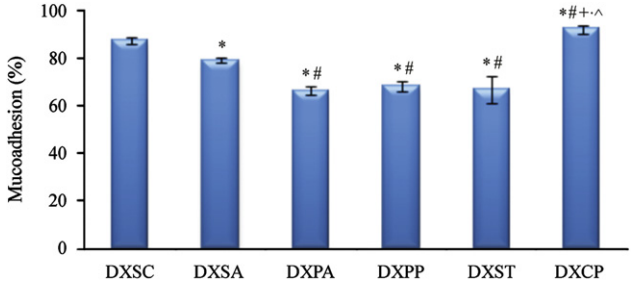

Figure 3 Percentage mucoadhesion of different polymer-based microparticles on intestinal mucosa (error bars represent standard deviation, $n=3$). $^{*}P<0.05$ compared with DXSC; $^{\#}P<0.05$ compared with DXSA; $^{+}P<0.05$ compared with DXPA; $^{+}P<0.05$ compared with DXPP; $^{\hat{}}P<0.05$ compared with DXST.

3.4. *In vitro mucoadhesion*

Mucoadhesion refers to the attachment of polymers to the mucus coat on the surface of the epithelium. The pH of mucus in the lung[19] is close to neutral or slightly acidic (pH = 5.5–6.5). Mucous secreted by the respiratory epithelium is similar to the intestinal mucosa[20]. Thus, *in vitro* mucoadhesion studies were carried out using rat intestinal mucosa. The results from the *in vitro* mucoadhesion studies are shown in Fig. 3. The mechanism of mucoadhesion can be explained in two steps, namely, the initial contact stage followed by the establishment of the adhesive interactions[21]. All the mucoadhesive polymers chosen in this study are hydrophilic and swellable. When spray dried microparticles are spread over the hydrated intestinal mucosa, they attract water from the mucosal surface and swell (wetting theory). This results in polymer-mucus interaction, increased viscosity of this mixture and reduced mucociliary clearance. Among the spray dried microparticles, the mucoadhesivity decreased in the following order: DXCP > DXSA > DXSC > DXPA > DXST > DXPP. In general, the anionic polymers exhibited greater mucoadhesion than the non-ionic ones. At similar concentrations (20%, w/w), CP-based microparticles showed the highest mucoadhesive property (92.09%). CP swells and forms colloidal gel dispersion by hydration on the mucosa layer but does not dissolve. The mucoadhesivity of other anionic polymers (sodium carboxymethyl cellulose and sodium alginate) was comparatively low (Fig. 2). Batches of DXST and DXPA showed significantly lower mucoadhesion. This may be attributed to the overwetting of these microparticles resulting in the wash-induced loss of the less viscous gels. The mucoadhesion of

these polymers is due to the hydrogen bonding between the hydroxyl and carboxyl groups of the polymer and mucin layers.

3.5. *In vitro powder aerosolization*

Spray dried microparticles based on different polymers were evaluated for their aerosolization performance. The results of the *in vitro* aerodynamic properties of the microparticles analyzed in an ACI are summarised in Table 2. Fig. 4 shows the deposition pattern of different polymer based microparticles on the various stages of the ACI following aerosolization.

The recovered dose ranged 92.1–97.9% of the total loaded powder weight. The emitted doses varied between 77.9% (DXST) and 86.8% (DXSA), indicating that a majority of the spray dried particles were dispersed from the device. As shown in Fig. 4, less than 15% of the microparticles were deposited in the preseparator, except with DXST. The deposition of microparticles was primarily in stages 2, 3 and 4. The FPF consisted of microparticles less than 5 μm in size. Although the SEM images revealed that the majority of the particles were in the respirable size range (1–5 μm), the FPF was found to be less than 60%. This trend in FPF may be attributed to the strong cohesive and adhesive interparticulate forces between the particles during aerosolization[22,23]. There was more than a 2-fold difference in the FPF of the microparticles depending on polymer content. Significantly, a high FPF value of 55.5% in DXSC suggests that deep lung penetration of the microparticles is possible. The FPF was also high for collapsed and hollow particles in DXST (50.3%). However, only 24.7% FPF was seen in DXCP due to the considerable aggregation as seen from the SEM images (Fig. 1f). The MMAD values were within the respirable range, i.e., from 3.74 to 5.02 μm except with DXCP. In general, spherical particles exhibited larger MMAD values. For DXCP containing microparticles, the mean MMAD was 6.54 μm and GSD was 1.75. It was found to be unsuitable for deep lung deposition.

3.6. *In vitro dissolution*

There are currently no official methods for determination of *in-vitro* drug release for inhalation products. Researchers have previously carried out dissolution studies in beakers, in flow-through cells, and by directly placing the microparticles in the dissolution medium. These methods often encounter problems like improper stirring, loss of formulation on sampling and lack of differentiation between formulations[24]. In the absence of an appropriate method, *in-vitro* dissolution studies were carried out by dialysis in phosphate buffer in a USP type II dissolution apparatus. Fig. 5 shows a comparison between the

Table 2 Aerodynamic properties of the microparticles.

Batch	Recovered dose (%)	Emitted dose (%)	FPF (%)	MMAD (%)	GSD
DXSC	92.1±1.5	80.2±0.4	55.5±4.2	4.56±0.10	1.54±0.12
DXSA	93.6±0.7	86.8±0.5	27.3±2.3	5.02±0.10	1.68±0.10
DXPA	97.9±0.6	83.4±0.2	41.6±3.1	4.04±0.10	1.51±0.21
DXPP	93.2±1.1	88.3±0.6	47.1±4.6	3.74±0.10	1.45±0.07
DXST	94.7±1.3	77.9±0.1	50.3±1.1	4.86±0.10	2.01±0.15
DXCP	96.1±2.1	79.1±0.5	24.7±3.9	6.54±0.10	1.75±0.02

Data are expressed as mean±SD, $n=3$.

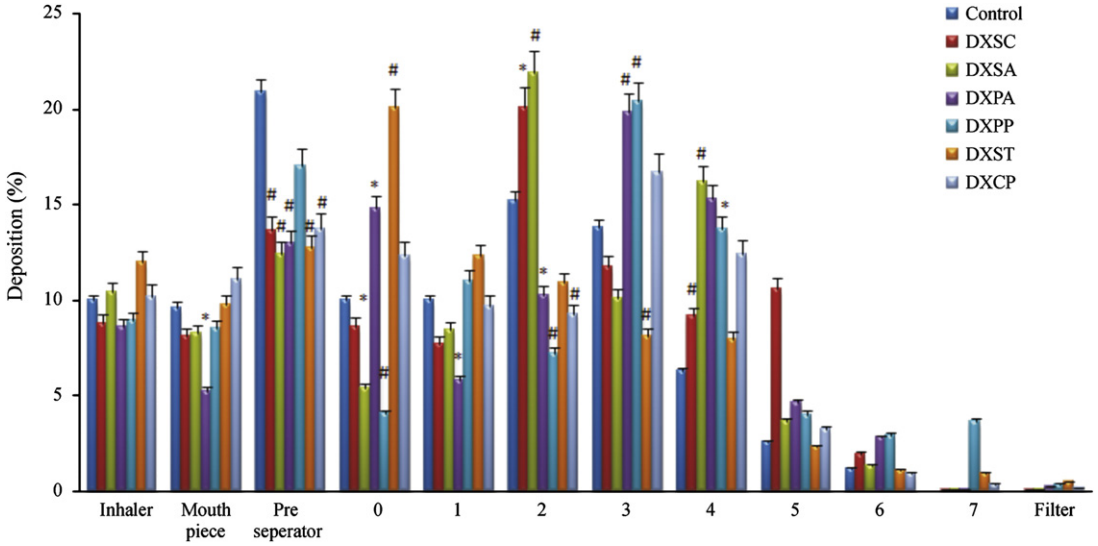

Figure 4 Deposition profiles for spray-dried microparticles showing microparticles deposited (as percentage of total emitted dose) on each stage of the ACI (effective cut-off diameters are 8.6, 6.5, 4.4, 3.3, 2.0, 1.1, 0.54 and 0.25 μm for stages 0–7, respectively). $^{*}P<0.05$, $^{\#}P<0.01$.

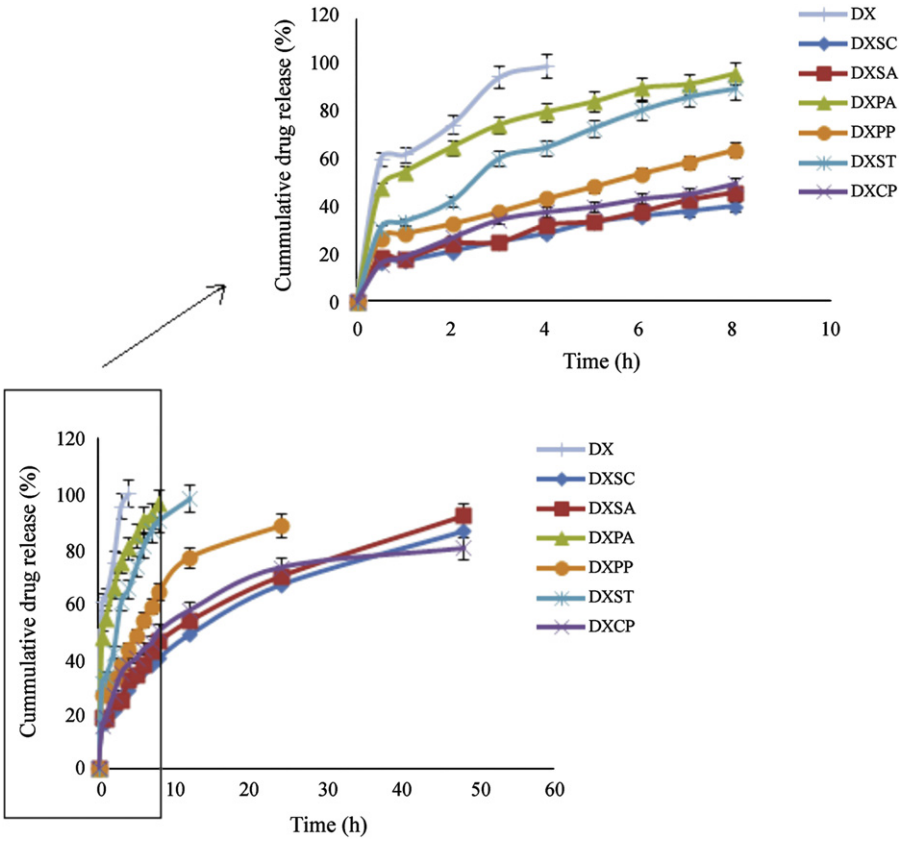

Figure 5 Comparison of *in vitro* release profiles of different polymer-based DX-loaded microparticles.

release profiles of DX from different polymer-based spray-dried microparticles. As expected, the release of pure drug was rapid and resulted in nearly 100% DX release in approximately 4 h. The polymer-based spray-dried microparticles exhibited sustained release characteristics. Previously, researchers have reported that when dry hydrophilic polymers contact physiological fluid the polymer swells to produce a gel diffusion layer as a barrier to drug release. The DX release from microparticles was biphasic. Initially, a burst release of nearly 31.05% and 47.86% was seen in batches of DXST and DXPA, respectively. And at 8 h, greater than 90% of drug was released in these two batches, indicating that the polymer gel layers were not sufficiently viscous to control the drug release profile. However, in case of other polymers, the extent of drug release was statistically slower in the range of 15.61–26.82% within 30 min of release study, indicating no burst release. DXPP was not able to sustain the drug release beyond 24 h. Less than 50% of the drug was released in 12.8, 10.6 and 12.2 h in batches of DXSC, DXSA and DXCP, respectively. At the end of 48 h, greater than 80% of drug was released in the above mentioned batches.

To analyse the mechanism and kinetics of drug release[25], the release profiles were modelled for correlation to Peppas equation for the first 8 h only. The Peppas equation can be described as follows:

$$M_t/M_\infty = kt^n \quad (2)$$

where M_t/M_∞ is the fraction of drug release. Using the least squares procedure, the values of n (diffusion exponent), k (kinetic constant) and r^2 (correlation coefficient) were estimated (Table 3). In spherical matrices, if $n \leq 0.43$, a Fickian diffusion (case-I); $0.4 \leq n < 0.85$, anomalous or non-Fickian transport; $n \geq 0.85$, a case-II transport (zero order) drug release mechanism dominates. The n value for all the batches ranges 0.274–0.451 except DXST ($n = 0.530$). This indicates Fickian-type of drug diffusion.

Table 3 Kinetic parameters of drug release for different polymer-based microparticles.

Batch	k (min^{-n})	n	r^2
DXSC	0.162	0.433	0.988
DXSA	0.172	0.451	0.962
DXPA	0.546	0.274	0.997
DXPP	0.242	0.397	0.845
DXST	0.290	0.530	0.940
DXCP	0.200	0.324	0.966

k, kinetic constant; n, diffusion exponent; r^2, correlation coefficient.

3.7. *In vitro cytotoxicity*

The cytotoxicity results of different polymer-based microparticles as determined with H1229 cells by incubating with various concentrations of formulations for 24 and 48 h are shown in Fig. 6. In general, high cell viability in the range of 123.7–98.8% was observed at concentration of 0.01 mg/mL

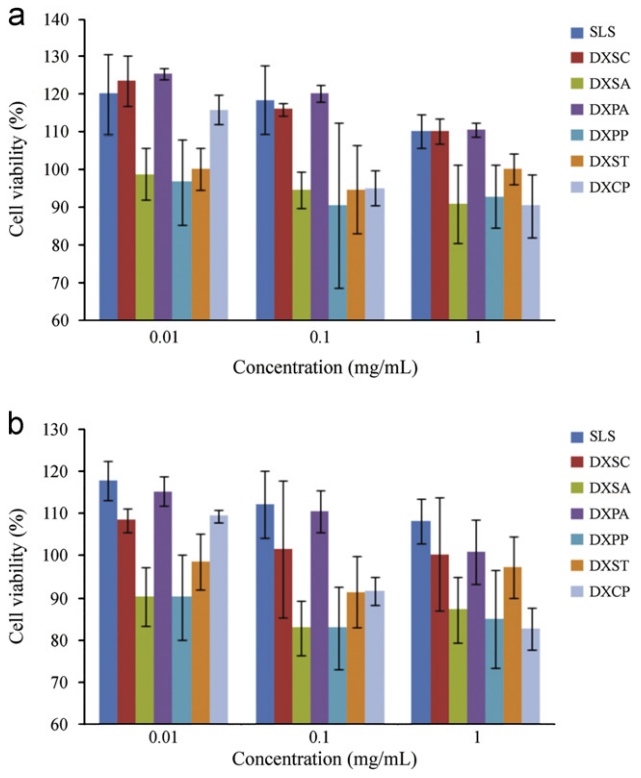

Figure 6 H1299 cell viability after (a) 24 and (b) 48 h exposure to different concentrations of spray-dried microparticles (error bars represent standard deviation, $n=3$).

irrespective of the type of polymer in the microparticles. No significant difference ($P>0.05$) in percent cell viability was observed in batches of DXSC and DXPA on the increasing concentration by 10- and 100-fold as compared to similar concentrations of SLS treated cells. Only with the highest concentration of microparticle (1 mg/mL) was less than 90% cell viability obtained in DXSA (87.2%), DXST (85.1%) and DXCP (82.7%) after 48 h of exposure to alveolar cells.

4. Conclusions

In this study mucoadhesive microparticles of respirable size were prepared by using spray drying. The yield of the DXPP formulation was high (63.04%), whereas DXST exhibited the lowest yield (50.79%). In order to deposit DX effectively within the lung, microparticles containing appropriate mucoadhesive polymers were selected based on their properties. In general, the dry powders were 1–5 μm in diameter and varied from roughly spherical (DXSC, DXSA and DXCP), corrugated (DXPA and DXPP) to hollow particles (DXST). DXSC, DXSA and DXCP exhibited high encapsulation efficiency and mucoadhesion; however; the water content in DXSA was very high. The aerosolization property of carbopol-containing microparticles was least due to considerable aggregation of microparticles. Drug release from these microparticles exhibited biphasic Fickian type of diffusion. Only at the highest concentration of microparticle (1 mg/mL) was less than 90% cell viability seen with DXSA (87.2%), DXST (85.1%) and DXCP (82.7%) after 48 h of exposure to alveolar cells. Thus, sodium carboxymethyl cellulose-based microparticles can serve as an ideal carrier for inhalation delivery of DX.

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