Accepted Manuscript

Title: Particle engineering of materials for oral inhalation by dry powder inhalers. II - sodium cromoglicate.

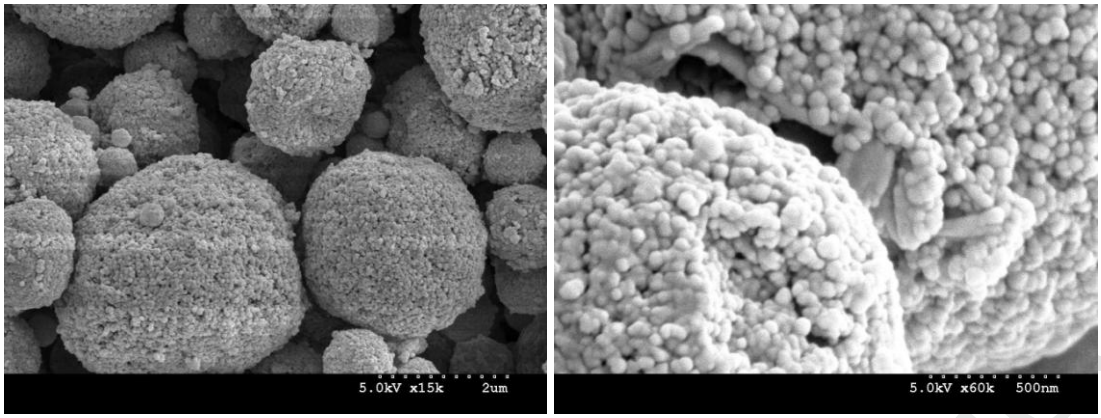
Authors: Lorraine M. Nolan, Jianhe Li, Lidia Tajber, Owen I. Corrigan, Anne Marie Healy

PII: S0378-5173(10)00889-6
DOI: doi:10.1016/j.ijpharm.2010.11.040
Reference: IJP 11560

To appear in: *International Journal of Pharmaceutics*

Received date: 19-8-2010
Revised date: 11-11-2010
Accepted date: 24-11-2010

Please cite this article as: Nolan, L.M., Li, J., Tajber, L., Corrigan, O.I., Healy, A.M., Particle engineering of materials for oral inhalation by dry powder inhalers. II - sodium cromoglicate., *International Journal of Pharmaceutics* (2010), doi:10.1016/j.ijpharm.2010.11.040



Particle engineering of materials for oral inhalation by dry powder inhalers. II - sodium cromoglicate.

Lorraine M. Nolan, Jianhe Li, Lidia Tajber, Owen I. Corrigan and Anne Marie Healy,
School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland.

Suggested running head: Nanoporous microparticles of sodium cromoglicate

Corresponding author:

Anne Marie Healy
School of Pharmacy and Pharmaceutical Sciences,
Trinity College, University of Dublin, Dublin 2, Ireland.
Phone: 00353-1-8961444
Fax: 00353-1-8962783
e-mail: healyam@tcd.ie

ABSTRACT

Sodium cromoglicate is an antiasthmatic and antiallergenic drug used in inhalation therapy and commonly administered by a dry powder inhaler.

In the present study we sought to examine the feasibility of producing nanoporous microparticles (NPMPs) of this hydrophilic material by adaptation of a spray drying process previously applied to hydrophobic drugs, and to examine the physicochemical and *in vitro* deposition properties of the spray dried particles in comparison to a commercial product. The storage stability of successfully prepared NPMPs was assessed under a number of conditions (4 ^{o}C with dessicant, 25 ^{o}C at 60% relative humidity and 25 ^{o}C with dessicant).

Spray dried sodium cromoglicate was amorphous in nature. NPMPs of sodium cromoglicate displayed superior aerodynamic properties resulting in improved *in vitro* drug deposition, as assessed by Andersen Cascade Impactor and twin impinger studies, in comparison to the commercial product, Intal®. Deposition studies indicated that porosity and sphericity were important factors in improving deposition properties. The optimum solvent system for NPMP production was water:methanol:n-butyl acetate, as spherical NPMPs spray dried from this solvent system had a higher respirable fraction than non-spherical NPMPs of sodium cromoglicate (spray dried from methanol:n-butyl acetate), non-porous sodium cromoglicate (spray dried from water) and micronised sodium cromoglicate (Intal®).

While particle morphology was altered by storage at high humidity (60% RH) and *in vitro* deposition performance deteriorated, it was possible to maintain NPMP morphology and aerosolisation performance by storing the powder with dessicant.

1. INTRODUCTION

Sodium cromoglicate (SC), also known as cromolyn sodium and disodium cromoglycate, is an antiasthmatic and antiallergenic drug commonly used in inhalation therapy. When administered as an aerosol, it is effective in preventing or reducing symptoms in patients with mild to moderate allergy and exercise-induced asthma (Laube et al., 1998). It is currently marketed as a single-dose dry powder inhaler (DPI), Spinhaler®, used with a gelatin capsule containing a 20 mg dose of micronised sodium cromoglicate (Intal Spincaps®).

Initial attempts by Vidgrén *et al.* (1987) to use spray drying to produce particles of sodium cromoglicate suitable for inhalation therapy, by spray drying from a water/ethanol solution, produced almost spherical, partially shrunken particles. These researchers compared the physical properties and *in vitro* inhalation behaviour of the spray dried particles with mechanically micronised particles and reported the spray dried particles to be smaller, mainly in the range of 1-5 μm, with improved lung deposition as ascertained from impaction studies. Steckel *et al.*, (2003) also prepared sodium cromoglicate in a respirable particle size, using an in situ micronisation controlled crystallisation technique and compared these particles to the commercial dry powder formulation, Intal®. Delivery of these engineered particles via the Spinhaler® device at a flow rate of 100 l/min resulted in a measured fine particle fraction of 45.5%, a statistically significant increase in the fine particle fraction compared to the commercial product (14.5%). The particles produced by both Vidgren *et al.* (1987) and Steckel *et al.* (2003) were spherical in shape and were non-porous.

Spray drying has also been used to prepare porous microparticles of sodium cromoglicate. Dellamary *et al.* (2002), for example, have formulated hollow porous sodium cromoglicate PulmoSpheres™ from fluorocarbon-based emulsions. Morphologically these particles had a sponge-like appearance, with pores in the order of 50-300 nm and bulk density of 0.06 g/cm^3, significantly less than the 0.5-1.0 g/cm^3 values typically found for micronised powders.

However these particles are not excipient-free as the emulsion-stabilising excipient of the porous PulmoSphere™, phosphatidylcholine, remains in the recovered powder material

We have previously reported on the use of a spray drying method to produce excipient-free nanoporous microparticles (NPMPs) of bendroflumethiazide (Healy et al., 2008) and budesonide (Nolan et al., 2009) with advantageous micromeritic and aerosolisation properties for pulmonary drug delivery. An improvement in the deposition properties of any drug substance indicates a possible commercial advantage, as the same therapeutic effect may be achieved with a smaller device-loaded dose.

The hydrophobic/hydrophilic nature of the compound drug under investigation is an important factor in selecting the solvent system for spray drying using the spray drying method previously described. Previous studies have indicated the requirement to have a solution which consists of a combination of solvent and antisolvent for the material which is to be processed as NPMPs. Spray drying from an alcohol/water solvent system resulted in the production of NPMPs from bendroflumethiazide (Healy et al., 2008) and budesonide (Nolan et al., 2009), both of which are relatively water-insoluble, hydrophobic compounds. In these cases the more volatile component, i.e. ethanol, acted as the solvent and water as the antisolvent for the active pharmaceutical ingredient. Ní Ógáin *et al.* (2010) determined that for the hydrophilic non-reducing sugars trehalose and raffinose, the solvent system used needed to be altered in order to successfully prepare NPMPs. NPMPs of these hydrophilic materials could be prepared by spray drying from a methanol/n-butyl acetate cosolvent system, in which case methanol, again the more volatile component (boiling point 65 ^{o}C), acted as the solvent and n-butyl acetate (boiling point 126 ^{o}C) acted as the antisolvent for the sugar. Due to the hydrophilic nature of sodium cromoglicate, and based on the findings of Ní Ógáin *et al.* (2010) it was postulated that the methanol/n-butyl acetate (MeOH:BA) system would offer most potential in terms of NPMP production of sodium cromoglicate.

In the present study we investigate the feasibility of producing NPMPs of the hydrophilic active material sodium cromoglicate and investigate the physicochemical characteristics and *in vitro* deposition behaviour of the spray dried microparticles produced. This particular API was chosen because it allowed us to compare the *in vitro* deposition characteristics of NPMPs to that of a commercially available product, Intal® which is delivered using a Spinhaler® device and Spincap® capsules.

2. MATERIALS AND METHODS

2.1. Materials

Crystalline sodium cromoglicate raw material was purchased from Sigma-Aldrich, Ireland. Ethanol was obtained from Cooley Distillery (Ireland). Methanol was purchased from Lab Scan Analytical Sciences, Ireland, while n-butyl acetate was purchased from Merck, Germany**.** Deionised water was produced by a Purite Prestige Analyst HP water purification system. All other reagents were analytical grade. Intal® Spincaps and Intal® Inhaler were purchased from a local community pharmacy.

2.1 Spray drying

All solutions prepared were spray dried with a Büchi B-290 Mini spray dryer, using a standard 2-fluid nozzle with a 0.7 mm tip and 1.5 mm cap.

When spray drying aqueous solutions, the spray dryer was operated in the open mode, whereby the drying gas (compressed air) passes through the drying chamber and then is exhausted. When spray drying all other solutions, the Büchi B-290 was operated in the closed mode, whereby the drying gas (nitrogen) is recycled to the drying chamber after precooling in a preheat exchanger and solvent condensation in a refrigerator unit (B-295 inert loop). In

these cases the high performance efficiency cyclone, designed to improve the separation rate and collection efficiency of particles, was also used (Brandenberger, 2003)

In all cases the gas flow rate was 670 Nl/h (based on air, 4 cm on the gas rotameter indicator), the pump setting was 30% (9-10 ml per minute) and the aspirator setting was 100%.

When spray drying from aqueous solutions the inlet temperature was set at 130^{o}C and the outlet temperature was 56^{o}C. In the case of solutions comprising methanol:n-butyl acetate or methanol:n-butyl acetate:water, the inlet temperature was set at 100^{o}C, with the outlet temperature varying from 60 to 65^{o}C.

2.2. Characterisation of physicochemical properties of materials

X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC) measurements were made as previously described (Healy et al., 2008). XRD measurements were made on samples in low background silicon mounts, which consisted of cavities 0.5 mm deep and 9 mm in diameter (Bruker AXS, U.K.). The Siemens D500 Diffractometer used consists of a DACO MP wide-range goniometer with a 1.0° dispersion slit, a 1.0° anti-scatter slit and a 0.15° receiving slit. The Cu anode X-ray tube was operated at 40 kV and 30 mA in combination with a Ni filter to give monochromatic Cu Kα X-rays. Measurements were generally taken from 5 to 40° on the two θ scale at a step size of 0.05° per s.

DSC was performed using a Mettler Toledo DSC 821^{e}. Samples were accurately weighed and placed in closed 40 µl aluminium pans with three vent holes. Samples were run at a heating rate of 10 °C/min under nitrogen purge.

Fourier Transform Infrared Spectroscopy (FTIR) was carried out using a Magna – IR 560 Spectrometer E.S.P. (Thermo Electron Corporation, U.S.A.) Fourier transform infrared spectrometer. Potassium bromide (KBr) discs were prepared based on 1% (w/w) sample

loading. Discs were prepared by grinding the sample with KBr in an agate mortar and pestle, placing the sample in an evacuable KBr die and applying 8 tons of pressure, in an IR press. The software used for processing the data was OMNIC E.S.P. software.

Scanning electron micrographs of powder samples were taken using a Hitachi S-4300N (Hitachi Scientific Instruments Ltd., Japan) variable pressure scanning electron microscope. The dry powder samples were fixed on aluminium stubs with double-sided adhesive tabs and a 10 nm thick gold film was sputter coated on the samples before visualisation.

Particle size and density measurements were determined as previously described (Healy et al., 2008). Particle sizing was performed by laser diffraction using a Malvern Mastersizer 2000 particle sizer (Malvern Instruments Ltd., Worcs., U.K.) with Scirocco 2000 accessory. The dispersive air pressure used was 2 bar. Samples were generally run at a vibration feed rate of 50%. The particle size reported is the d(0.5), which is the median particle size of the volume distribution. The values presented are the average of at least two determinations. Mastersizer 2000 software was used for analysis of the particle size.

In some cases particle size was determined from scanning electron micrographs. The mean particle diameter (Feret's diameter) (Carstensen, 2001) of some of the spray dried systems was calculated using SEM photographs. The diameter of at least 20 particles was measured and the mean particle diameter was taken as the average of these measurements.

Theoretical estimates of particle primary aerodynamic diameter (d_{ae}) were derived from the median particle size ($d_{0.5}$) and tapped density ($t\rho$) data, according to the following equation (1) (Bosquillon et al., 2004):

$$d_{ae} = d_{0.5}\sqrt{\frac{t\rho}{\rho}} \quad \text{where } \rho = 1 \text{ g/cm}^3 \qquad (1)$$

Bulk and tapped density measurements were performed as previously described (Healy et al., 2008). Bulk (aerated) density was determined by pouring a known mass of powder under gravity into a 1 cm^3 graduated glass syringe. The bulk density ($b\rho$) was calculated as the ratio of the mass to volume of the sample. The tapped density ($t\rho$) was measured for the same sample and was calculated as the ratio of the mass to the tapped volume of the sample after 100 strokes. The strokes were performed manually, in a vertical manner, from a distance of approximately 5 cm onto a level bench top surface. Measurements were performed in triplicate.

Surface area analysis of both unprocessed and spray dried samples was performed using a Micromeritics Gemini 2370 Surface Area Analyser with nitrogen as the adsorptive gas, as previously described (Nolan et al., 2009). Samples were degassed using a Micromeritics FlowPrep 060 Degasser for 24 hr at 25 °C. Free space measurements (particularly when the measured surface area was < 25 m^2/g) were performed using glass beads of negligible surface area in the reference tube. The number of glass beads used was such that approximate volume balance was achieved, i.e., the measured free space was between -0.5 and + 0.5 cm^3. During each analysis the evacuation rate was 500 mmHg/min, the evacuation time was 1 minute and the equilibration time was 10 seconds. For all samples the volume of nitrogen adsorbed at the relative pressures of 0.05, 0.1, 0.15, and 0.2 0.25 and 0.3 was found. For each sample, the BET multipoint surface area was determined using the relative pressures from 0.05 to 0.3. Where it improved the correlation coefficient for the BET plot, the first or last points were omitted. All analyses were performed at least in duplicate.

2.3. *In vitro* deposition studies using the Andersen cascade impactor (ACI)

ACI studies were carried out, using a Spinhaler® device, at a flow rate of 60 l/min, which allowed sonic flow conditions to be achieved (USP, 2007). Size 2 hard gelatin capsules (Farillon Ltd., U.K.) were filled with 20±1 mg of powder.

The recovered dose (RD) was defined as the total amount of drug recovered from the capsule, device, mouthpiece and all parts of the impactor. The emitted dose (ED) was determined as the total drug recovered from the mouthpiece, induction port and cone, stages and plates, as well as the filter following simulated inhalation (USP, 2008). The percentage emission was calculated as the ratio of the ED to the RD, expressed as a %. The fine particle dose (FPD) or fine particle fraction (FPF) %, were calculated as the portion of the inhaler output (emitted dose) having an aerodynamic diameter less than 5 μm. The FPF was calculated by interpolation from a plot of the inverse distribution of the cumulative % (i.e. probability, calculated from the total drug mass recovered from the apparatus) versus the logarithm of the effective cut-off diameter of the respective stages. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were also derived by interpolation from this plot. Both the MMAD and the GSD were determined from the linear region of the plot (stage 1 to stage 6 corresponding to the best fit of the data points) and the best fit line was used to calculate their values. The experimental MMAD of the particles is defined from this graph as the particle size corresponding to the 50% point of the cumulative distribution and the GSD is determined from the slope of this line. The amount of powder deposited on each stage of the impactor and plates and also in the mouthpiece, induction port and cone and filter was determined by rinsing the drug from these parts (with water), and quantitatively diluting each to a suitable volume before assaying by UV at an absorbance of 326 nm.

In vitro deposition studies were performed in triplicate and the results presented are average results of the replicated analyses.

2.4. *In vitro* deposition studies using the twin stage impinger

The *in vitro* drug deposition was investigated using the twin stage impinger Model TI-2 (Copley Scientific Ltd., U.K.) containing 7 and 30 ml of deionised water, in the upper (stage 1) and lower (stage 2) impingement chambers, respectively. The air flow through the apparatus was adjusted, as measured at the inlet to the throat, to 60±5 l/min. The Spinhaler® was loaded with a size 2 capsule containing 20±1 mg of powder. The powder in stages 1 and 2, mouthpiece, device and capsule was collected by rinsing with fresh solvent. The rinsed solutions were diluted to appropriate volumes and the drug content determined by UV analysis at an absorbance wavelength of 326 nm. Results are expressed in terms of the respirable fraction %, calculated on the basis of drug content captured in stage 2 (less than 6.4 μm), as the recovered dose. The recovered dose (RD) was defined as the total amount of drug recovered from the capsule, device, mouthpiece, Stage 1 and Stage 2 of the impinger. All twin stage impinger studies were carried out in triplicate and the results presented are average results of the replicated analyses.

2.5. Stability studies

Solid state stability studies were conducted using the conditions of temperature and humidity recommended in the ICH protocol for long-term testing (ICH, 2003). Solid samples of spray dried porous sodium cromoglicate were placed in open jars in a glass chamber containing a saturated solution of sodium bromide to maintain a constant relative humidity of 60% (Nyqvist, 1983). The glass chamber was stored at 25˚C in an incubator (Gallenkamp, U.K.).

Samples were also stored at refrigerator temperature of 4 °C in a sealed desiccator containing silica gel desiccant and at 25 °C in a sealed desiccator containing silica gel desiccant.

At appropriate time intervals, samples were removed for subsequent analysis. Physical stability was assessed by SEM, XRD and DSC and aerosolisation behaviour was also determined.

2.6 Statistical analysis

Statistical analysis was carried out using Minitab™ statistical software, version 14 (Minitab Inc, USA). Two sample t-tests were used to compare the means and standard deviations of two independent samples. The test was carried out at a significance level of 0.05, with a p-value less than 0.05 taken as indicating that the observed difference between the means was statistically significant, i.e. rejecting the null hypothesis.

3. RESULTS AND DISCUSSION

3.1. Non-porous sodium cromoglicate microparticles

A 1% (w/v) aqueous solution of sodium cromoglicate was spray dried with an inlet temperature of 130^{o}C, producing almost spherical, crinkled-like non-porous particles as shown in Figure 1a and 1b.

The size of the particles produced was compared with the micronised sodium cromoglicate material obtained from an Intal® Spincap®. Laser diffraction particle size analysis, using the Malvern Mastersizer measured a median particle size of the volume distribution ($d_{0.5}$) of 2.87±0.1μm and 2.93±0.01 μm for micronised sodium cromoglicate (Intal®) and non-porous sodium cromoglicate spray dried from an aqueous solution, respectively. Both systems displayed monomodal size distribution patterns. As is evident in Figure 1 some non-porous sodium cromoglicate particles consisted of a hollow interior with thick outer walls. Sodium cromoglicate in the form of the raw material purchased from Sigma (Figure 1c), was determined to have a median particle size ($d_{0.5}$) of 7.42±0.53 μm, displaying a bi-modal size distribution profile, reflecting the heterogenous nature of the starting material.

3.2. Production of NPMPs by spray drying sodium cromoglicate

Sodium cromoglicate was spray dried as a 0.3% (w/v) solution from a methanol:n-butyl acetate (MeOH:BA) solvent system at solvent ratios (MeOH:BA) of 95:5, 90:10, 80:20 and 70:30 (v/v) using an inlet temperature of 100 ^{o}C.

The morphology and porosity of the spray dried particles produced varied considerably depending on the solvent concentrations employed, as shown in the SEM micrographs presented in Figure 2.

At the solvent ratio of 95:5 (v/v) MeOH:BA highly irregular shaped, non-spherical porous particles were produced with visible pores in the range of 50 to 100 nm (Figure 2a and 2b). The median particle size of the volume distribution ($d_{0.5}$) of these particles, as measured by the Malvern Mastersizer was 2.18±0.03 µm, displaying a monomodal size distribution profile. The majority of the particles produced at the solvent ratio of 90:10 (v/v) were porous; however some particles had flattened and non-porous areas (Figure 2c and 2d). At the solvent composition of 80:20 (v/v), the porous morphology was maintained (Figure 2e), however not all particles were uniformly spherical in shape, with some non-porous, irregular shaped particles observed. A further change in the MeOH:BA concentration to 70:30 (v/v) produced non-porous, spherical particles, with a roughened, dimpled-like particle surface, consisting of some smooth surfaced areas (Figure 2f). The particles varied in size from 2.18±0.63 µm to 1.64±0.35 µm and 1.11±0.3 µm for solvent ratios of 90:10, 80:20 and 70:30 (v/v) MeOH:BA respectively, as observed from SEM micrographs.

Sodium cromoglicate was also spray dried from a mixed solvent system of water:MeOH:BA 1:15:15 (v/v). Spray drying of this solution produced uniform, spherical NPMPs of sodium cromoglicate with visible holes in the range of 25-100 nm (Figure 3a). The porous particles produced displayed a monomodal symmetrical size distribution profile and had a median particle size of volume distribution ($d_{0.5}$) of 2.16±0.01 µm. No significant difference in the median particle size ($d_{0.5}$) was measured between non-spherical (95:5 (v/v) MeOH:BA solvent system, Figure 2a and 2b) and these spherical NPMPs of sodium cromoglicate ($p > 0.05$).

3.3. Physicochemical characterisation of micronised sodium cromoglicate and spray dried sodium cromoglicate systems

The physical characterisation of micronised sodium cromoglicate and crystalline sodium cromoglicate raw material was carried out using Intal® and a sample sourced from Sigma, Ireland, respectively. Spray dried sodium cromoglicate systems characterised included non-porous sodium cromoglicate spray dried from aqueous solution (Figure 1c and 1d) non-spherical NPMPs of sodium cromoglicate spray dried from the solvent system 95:5 (v/v) MeOH:BA (Figure 2a and 2b) and spherical NPMPs of sodium cromoglicate produced from the solvent mixture of H_2O:MeOH:BA at 1:15:15 (v/v) (Figure 3a and b). These systems were chosen so that the effect of a change in particle shape, morphology and density could be investigated in the *in vitro* deposition studies (section 3.4).

3.3.1. Solid-state characterisation

Figure 4 shows the X-ray powder diffraction profiles for spray dried, micronised and sodium cromoglicate raw material samples. The presence of sharp diffraction peaks in the X-ray diffractogram obtained from micronised sodium cromoglicate and sodium cromoglicate raw material indicates the crystalline state of the material. In both instances the powder diffraction profiles obtained corresponded with the XRD pattern reported by Chen *et al.* (1999), in the range of 5 to 30 2θ at 23°C and 21.6% relative humidity. Cox *et al.* (1971) reported that sodium cromoglicate crystals can quickly absorb (or lose) water as a continuous series of interstitial solid solutions to give an infinite series of nonstoichiometric hydrates with a limiting composition of about 9 molecules of water per molecule of sodium cromoglicate. The reported continuous changes in crystal lattice parameters of sodium cromoglicate, associated with changes in water stoichiometry, renders the compound physically variable and unstable. Chen *et al.* (1999) reported considerable changes in the XRD pattern of the hydrate phase of sodium cromoglicate as a function of environmental relative humidity.

The XRD diffractograms of the spray dried sodium cromoglicate samples, both porous and non-porous did not show peaks corresponding to micronised sodium cromoglicate or sodium cromoglicate raw material, displaying instead the characteristic "halo" pattern typical of an amorphous state.

A broad endothermic process was detected by DSC, occurring between ~108 to 145°C and ~104 to 125ºC for sodium cromoglicate raw material (Sigma) and micronised crystalline sodium cromoglicate (Intal®) respectively. This broad endotherm was absent in spray dried sodium cromoglicate samples (Figure 5). This endothermic process was assigned as a mesotropic crystalline transition, consistent with the findings of Cox *et al.* (1971). No solid polymorph of sodium cromoglicate has been observed, although there are mesophases (liquid crystalline forms) of the hydrate. A large broad endothermic peak present in the spray dried samples of sodium cromoglicate, over the heating range of 40-120°C, was characteristic of residual solvent content, as confirmed by TGA, measuring ~7 to 9% for NPMPs of sodium cromoglicate and ~10% for non-porous spray dried sodium cromoglicate. TG analysis measured moisture content of ~11% for micronised sodium cromoglicate, equating to approximately 4 molecules of water.

Previous studies have shown that up to nine water molecules can be accommodated in the crystalline structure of one sodium cromoglicate molecule, depending on the relative humidity (Cox et al., 1971). Stephenson and Diseroad (2000) explained the non-uniform dehydration process of sodium cromoglicate was as a result of two types of water molecules: sodium coordinated water and water in the lattice channels. At lower temperatures the water in the lattice channels is evaporated, followed, at higher temperatures, by the evaporation of metal-coordinated water molecules. No glass transition temperature ($T_{g)}$ was detected for the amorphous spray dried sodium cromoglicate systems as it overlapped with the large diffuse

endotherm of the residual solvent liberation. Both quench cooling and rapid heating and cooling in situ proved unsuccessful in T_g detection of the spray dried samples.

DSC thermograms of sodium cromoglicate raw material, micronised sodium cromoglicate and spray dried sodium cromoglicate samples also displayed a second endothermic peak, characteristic of melting, the melting point varying between samples. Similar melting points were determined for sodium cromoglicate raw material and micronised sodium cromoglicate (Intal®) at ~265°C and 263ºC respectively. Najafabadi *et al.* (2004) reported a melting endotherm at 262.5ºC for a commercial micronised sodium cromoglicate sample. Melting endotherms at ~253°C, ~251°C and ~249°C were determined for non-porous spray dried sodium cromoglicate and spherical and non-spherical NPMPs, respectively, lower than that for unprocessed and micronised sodium cromoglicate. A melting endotherm at 253ºC for a sample of sodium cromoglicate spray dried from aqueous solution was reported by Najafabadi *et al.* (2004). Each melting endotherm was followed by an overlapping exotherm-endotherm reflecting product degradation. DSC and TGA curves clearly showed the decomposition process for all samples at temperatures higher than ~275ºC.

The infrared spectrum of the spherical NPMP sample spray dried from H_2O:MeOH:BA was comparable to that of the unprocessed material, indicating the similarity of vibration in bonding energies of the sample and that processing did not alter the chemical structure of the compound.

3.3.2. Micromeritic analysis

A bulk density of 0.242±0.013 g/cm^3 was measured for micronised sodium cromoglicate. A 2-fold decrease in bulk density was measured for NPMPs of sodium cromoglicate, both

spherical and non-spherical, relative to micronised sodium cromoglicate, with calculated densities of 0.114±0.006 g/cm^3 and 0.120±0.004 g/cm^3, respectively (Figure 6).

The lower bulk density of NPMPs of sodium cromoglicate together with a relatively thin porous particle surface, exposing a void interior as shown in Figure 3b, suggests that the particles are hollow, not solid. A bulk density of 0.299±0.008 g/cm^3 was measured for non-porous spray dried sodium cromoglicate, significantly higher ($p < 0.05$) than that measured for NPMPs of sodium cromoglicate. Tapped densities were 0.232±0.011 g/cm^3 and 0.248±0.014 g/cm^3 for non-spherical and spherical NPMPs, respectively.

Theoretical estimates of particle aerodynamic diameter d_{ae} ranged from 1.0 μm for both spherical and non-spherical NPMPs of sodium cromoglicate to 1.7 μm and 2.4 μm for micronised and spray dried non-porous sodium cromoglicate, respectively. These values indicate that the powders are of a suitable size for deposition in the lungs, considering particles with an aerodynamic diameter between 1 and 5 μm are required to maximise deposition in the lower respiratory tract and avoid deposition by inertial impaction in the oropharyngeal cavity (Edwards et al., 1998).

The porous structure of NPMPs of sodium cromoglicate, consisting of roughly spherical formations with irregular surfaces of fused nanoparticulate spherical structures, was substantiated by the high surface area measurements obtained (Figure 6). An 18-fold increase in surface area was measured for spherical NPMPs of sodium cromoglicate (97.66±1.32 m^2/g) relative to micronised sodium cromoglicate (5.53±0.15 m^2/g), and a 59-fold increase relative to non-porous spray dried sodium cromoglicate (1.66±0.20 m^2/g).

Surface area can be used to assess particle morphology such as shape irregularity and surface asperities (Zeng *et al.*, 2001), which can significantly influence interparticulate forces between particles, and thus their aerosolisation performance. A statistically significant 3-fold

increase in the surface area of micronised sodium cromoglicate in comparison to spray dried non-porous sodium cromoglicate was measured. Both particles have a similar particle size, the difference in surface area is possibly attributed to the fairly irregular, elongated shape of the mechanically micronised particles.

Edwards et al. (1997) previously showed that very light particles ($\rho < \sim 0.4 g/cm^3$) with geometric diameters greater than 5 μm could be deposited in the lungs. These authors purported a two-fold advantage for large porous particles. The first proposed advantage was that, as a consequence of their large size and their low mass density, such large porous particles could aerosolize from a DPI more efficiently than smaller nonporous particles, resulting in higher respirable fractions of inhaled therapeutics. Also, large, porous particles inhaled into the lungs could potentially release therapeutic substances for long periods of time by escaping phagocytic clearance from the lung periphery, thus enabling therapeutic action for periods ranging from hours to many days. While, on the basis of their particle size, NPMPs are not expected to be able to avoid phagocytosis, they do possess the required density to support an improved delivery from the DPI. Although the geometric particle size is not in itself expected to result in a reduced fractional surface area (or likelihood) of particle-particle contact in a dry powder and thus in less tendency to aggregate (Edwards et al., 1998), it is expected that the porous surface structure will result in the same effect (i.e. reduced possibility of particle-particle interactions).

3.4. *In vitro* drug deposition of NPMPs of sodium cromoglicate

3.4.1. Cascade impactor analysis studies

The *in vitro* drug deposition of micronised sodium cromoglicate (Intal®) and spray dried sodium cromoglicate samples was determined, using an Andersen cascade impactor (ACI)

after aerosolisation through a Spinhaler® device, at a flow rate of 60 l/min. The deposition profile of the samples after aerosolisation is presented in Figure 7. The amount of sodium cromoglicate deposited on the various stages of the ACI varied considerably for the different samples. Spherical NPMPs of sodium cromoglicate deposit to a greater extent in the lower stages (S2 to F) of the ACI in comparison to non-porous sodium cromoglicate and Intal®, thus measuring a higher fine particle fraction (62.22±2.08%).

NPMPs of sodium cromoglicate, both spherical and non-spherical, gave a statistically significant increase in the fine particle fraction in comparison to Intal® and non-porous sodium cromoglicate ($p < 0.05$) (Table 1).

Non-spherical NPMPs of sodium cromoglicate and micronised sodium cromoglicate (Intal®) display an elongated shape. The favourable aerodynamic behaviour of elongated particles of cromoglycic acid crystals was previously reported (Chan and Gonda, 1989). Elongated particles tend to align along their long axis and interact with each other in a manner that produces a loose pack.

Such a system can be expected to present weak interparticulate forces due to the small contact area and large separation distance between adjacent particles, promoting deaggregation properties (Otsuka *et al.*, 1988). Thus elongated particles of micronised sodium cromoglicate might be expected to display a similar aerosolisation pattern as non-spherical NPMPs of sodium cromoglicate. However, optimum aerodynamic properties such as particle size and density in addition to an elongated shape, may explain the improved aerosolisation behaviour of elongated particles of non-spherical NPMPs of sodium cromoglicate in comparison to micronised sodium cromoglicate.

NPMPs of sodium cromoglicate both spherical and non-spherical in shape share similar particle size and micromeritic properties, however on average a higher ($p < 0.05$) fine particle

fraction (62.22±2.08%) was measured for spherical NPMPs in comparison to non-spherical NPMPs (40.25±0.71%), demonstrating the importance of particle shape on powder dispersion and lung deposition.

The superior aerodynamic properties of NPMPs of sodium cromoglicate relative to non-porous sodium cromoglicate and Intal® are summarised in Table 1. The mass median aerodynamic diameter (MMAD) of both non-porous spray dried sodium cromoglicate and Intal® was significantly larger than that of the NPMPs, reflecting the dependence of the aerodynamic diameter on particle size and density. Spherical NPMPs of sodium cromoglicate measured a 2-fold decrease in comparison to the MMAD of micronised sodium cromoglicate. Numerous studies have confirmed that particles of 2-3 μm, as found with spherical NPMPs of sodium cromoglicate, are optimal for deep lung penetration and clinical benefit for asthmatic patients (Ruffin et al., 1981; Zanen et al., 1995).

The high standard deviation observed for some powders for % emission (Table 1) demonstrated a lack of reproducibility in powder output following aerosolisation which could compromise dosage uniformity. This may be due to a variety of reasons such as differences in powder packing in the gelatin capsules, moisture levels of the gelatin capsule shells or environmental humidity during the tests. Non-porous spray dried sodium cromoglicate measured a % emission of 24.79±3.95 %, significantly lower than that obtained from the other samples. The lower emission of non-porous spray dried sodium cromoglicate after aerosolisation was postulated to be due to the high solvent/moisture content in the sample, leading to solvent/moisture-induced agglomeration of sodium cromoglicate particles and or increased adhesive and cohesive interactions between the capsule wall and non-porous sodium cromoglicate.

3.4.2. Twin impinger studies

This study was carried out in order to investigate any potential correlation between the fine particle fraction % measured using impaction studies (ACI) and impinger studies (twin impinger). Table 2 summarises the % emission and fine particle fraction of both spherical and non-spherical NPMPs of sodium cromglicate and micronised sodium cromoglicate.

No significant difference in the % emission was measured on comparison of the three different systems, and also in comparison to the % emission data obtained from the Andersen cascade studies. This is as expected considering the inhaler device (Spinhaler®) and flow rate (60 l/min) are the same.

Twin impinger studies measured a fine particle fraction of 13.13±3.16% for micronised sodium cromglicate (Intal®). This correlates well with the findings of Steckel and Müller (1997) who reported a fine particle fraction of 10.13±2.51% for the Intal® Spinhaler®, determined using the twin impinger at a flow rate of 60 l/min. Considering the high % deposition (50%) of micronised sodium cromoglicate in stage 1 of the twin impinger, in comparison to stage 2, this being the respirable fraction (less than 6.4 μm), a low fine particle fraction was measured. A fine particle fraction of 38.17±5.53% and 27.34±5.75% was measured for NPMPs of sodium cromoglicate, both spherical and non-spherical respectively. In contrast to the results obtained for micronised sodium cromoglicate, the % drug deposition of NPMPs was significantly higher in stage 2 ($p<0.05$) of the twin impinger, thus resulting in a higher fine particle fraction measured.

No statistically significant difference ($p > 0.05$) was evident between the fine particle fraction measured using twin impinger studies of NPMPs of sodium cromoglicate, both spherical and non-spherical, however on average spherical NPMPs measured a higher fine particle fraction.

This is in contrast to the results obtained using the ACI, where a statistically significant difference between spherical and non-spherical NPMPs of sodium cromoglicate was evident, explained in part by the different porous particle morphology. The higher fine particle of NPMPs of sodium cromoglicate, also evident in the cascade impactor studies, is due to the favourable aerodynamic properties of the spray dried particles, those being particle size and low density. In all cases the fine particle fraction measured using the Twin impinger was significantly lower than that measured for the same system using the ACI.

As shown in Figure 8, a correlation between the fine particle fraction % measured using either impaction studies (ACI) and impinger studies (twin impinger) of micronised sodium cromoglicate and NPMPs of sodium cromoglicate was discerned ($r^2 = 0.8664$). This indicates that the aerosolisation properties of spray dried powders (porous/non-porous) could be initially investigated using the twin impinger, enabling rapid screening of a large number of powders. Powders which demonstrate promising aerosolisation properties in twin impinger studies could be further evaluated using the ACI to obtain more detailed aerodynamic particle size distribution data.

3.5. Stability studies

The hygroscopic nature of sodium cromoglicate is well known, due to its ionic structure and the various polar functional groups: carboxylate, carbonyl, ether and hydroxyl (Chen et al., 1999). Young *et al.* (2003) reported that increasing the water content of sodium cromoglicate powder upon exposure to different relative humidities (15% to 75% RH) decreased the emitted dose of the drug significantly (92.1% to 16.3%, respectively) after aerosolisation through a Turbohaler®. The physical stability of spherical NPMPs of sodium cromoglicate was assessed under the stability conditions of 4°C/desiccant, 25°C/60% relative humidity and 25°C/desiccant.

3.5.1. Stability study of spherical NPMPs at 4 °C/desiccant

Spherical NPMPs of sodium cromoglicate on stability at 4 °C over a 12 week period displayed no significant changes in porous morphology. The amorphous nature of the starting NPMPs was maintained throughout this 12 week period, as confirmed by XRD. No change in chemical structure was evident by FTIR analysis. After 12 weeks on stability, the sample weight loss by TGA was ~7%, which was consistent with the residual moisture/solvent content of the starting material (7%). *In vitro* drug deposition of NPMPs on stability was investigated by impaction studies, using the ACI after 4, 8 and 12 weeks. Table 3 summarises the ACI results. The aerosolisation performance of NPMPs of sodium cromoglicate was not adversely affected, with measured fine particle fractions of 58.03±6.35%, 61.27±3.90% and 57.24±5.08% after 4, 8 and 12 weeks respectively. No statistically significant difference in the fine particle fraction %, MMAD or the GSD of spherical NPMPs was measured after 12 weeks on stability, in comparison to freshly prepared NPMPs.

3.5.2. Stability study of spherical NPMPs of sodium cromoglicate at 25 °C/desiccant and 25 °C/60% relative humidity (RH)

Under the stability conditions of 25°C/desiccant the porous morphology of spherical NPMPs of sodium cromoglicate was maintained over the 12 week stability period (Figure 9). X-ray diffraction analysis confirmed the amorphous nature of the sample after 12 weeks. No change in chemical structure as evidenced from FTIR analysis was detected. After 12 weeks, ~5% of residual solvent was measured by TGA in contrast to ~7.5% for the starting material, this decrease being induced by the hygroscopic nature of the desiccant.

Table 4 summarises the results of ACI studies carried out after 2 wks, 4 wks and 12 wks. Following 2 weeks on stability at 25 °C/desiccant a small but significant decrease in the fine

particle fraction of NPMPs of sodium cromoglicate was evident, measuring 54.34±3.01%. No subsequent change in the fine particle fraction, to that measured after 2 weeks on stability was evident at 4 weeks and 12 weeks. The MMAD values obtained for the samples on stability, showed a small but significant increase in size in comparison to the starting material. No significant difference in the % emission was measured. Despite this small decrease in aerosolisation performance over the stability period of 12 weeks at 25 ºC/desiccant the respirable fraction of spherical NPMPs of sodium cromoglicate was still significantly higher than the respirable fraction of micronised sodium cromoglicate (Intal®).

The porous morphology of NPMPs of sodium cromoglicate was significantly altered following stability studies at 25 °C/60% RH (Figure 9). While the particles maintained their spherical structure, the visible holes on each particle surface significantly increased in size; within the range of 100 to 300 nm, as assessed from SEM micrographs, after only 24 hr. After 4 weeks on stability these larger pores were still evident and subsequently, after 12 weeks under the same storage conditions, the number of pores visible had decreased as a result of surface fusion. The particles also displayed smooth surfaces in comparison to the original irregular surfaces of the NPMPs which consisted of fused nanoparticulate structures. At this increased humidity level a significant increase in moisture sorption by the particles was apparent from DSC analysis, which saw a substantial increase in the size of the broad endothermic peak between 40 to 120 °C. This was confirmed by TGA analysis which measured an increase in residual solvent content from ~7% for the starting material to ~12.5% after just 24 hr on stability and ~14.5% after 12 weeks. X-ray diffraction analysis confirmed that the amorphous nature of NPMPs of sodium cromoglicate was maintained over the 12 week stability period.

A highly significant 3.5-fold decrease in the fine particle fraction was measured (17.39±1.72%) by ACI following 1 week on stability at 25 °C/60% RH (Table 4). Moisture

content is one of the main factors influencing the cohesiveness of a powder. The increase in moisture content of the NPMPs probably resulted in an increase in the cohesiveness of the powder particles, leading to agglomeration. The mass median aerodynamic diameter (MMAD) of the particles was increased from 3.59±0.09 μm to 12.59±1.11 μm. The larger MMAD obtained reflects the cohesion of individual particles to form larger aggregates that failed to disperse during the impaction studies. This variability in the particle diameter, due to aggregate formation explains the significant increase in the GSD value. FTIR analysis confirmed no changes in the chemical structure of the compound.

4. CONCLUSIONS

Spray drying sodium cromoglicate from the mixed solvent systems of methanol:n-butyl acetate or water:methanol:n-butyl acetate results in nanoporous microparticles.

The micromeritic properties of NPMPs of sodium cromoglicate were characteristic of a low density powder, of high specific surface area, suggesting that NPMPs of sodium cromoglicate may be attractive for inhalation therapy providing optimum lung deposition. NPMPs of sodium cromoglicate displayed superior aerodynamic properties resulting in improved *in vitro* drug deposition in comparison to the commercial product, Intal®, following aerosolisation through the ACI using the Spinhaler® device. Deposition studies suggested that the optimum solvent system for NPMP production was water:methanol:n-butyl acetate as spherical NPMPs of sodium cromoglicate spray dried from water:methanol:n-butyl acetate exhibited the highest deposition profile in comparison to non-spherical NPMPs of sodium cromoglicate (spray dried from methanol:n-butyl acetate), non-porous sodium cromoglicate (spray dried from water) and micronised sodium cromoglicate (Intal®).

A correlation between the fine particle fraction measured using impaction studies (ACI) and impinger studies (twin impinger) of micronised sodium cromoglicate and NPMPs of sodium cromoglicate was determined, indicating that twin impinger analysis may be used as an initial screen of *in vitro* deposition characteristics before undertaking more detailed but time-consuming cascade impactor studies.

Stability studies indicated that the most favourable condition for storage of sodium cormoglicate NPMPs is 4 °C/desiccant, since after 12 weeks of storage at these conditions, the morphology of spherical NPMPs of sodium cromoglicate remained the same, in addition to there being no changes in the solid-state behaviour of the material (amorphous as confirmed by XRD) and no observable change in *in vitro* aerodynamic properties. However, storage at 4 ^{o}C may not be practicable. It would appear that storage with dessicant is the

crucial factor in maintaining stability. Under conditions of 25 °C/60% RH significant changes in the porous morphology and aerosolisation performance of NPMPs was measured. Controlling the humidity at 25 °C with the use of a desiccant assisted in maintaining the porous morphology of NPMPs at 25 °C. A small but statistically significant decrease in the fine particle fraction was measured at these storage conditions but after 12 weeks of storage at 25 ^{o}C/dessicant, *in vitro* performance was still superior to micronised sodium cromoglicate from the commercial product.

ACKNOWLEDGEMENTS

This work was supported by Enterprise Ireland (grant CFTD/06/119) and by Science Foundation Ireland (grant 07/SRC/B1158) under the National Development Plan, co-funded by EU Structural Funds.

The authors would like to thank Stephen Doran for help with the *in vitro* deposition studies.

REFERENCES

Bosquillon, C., Rouxhet, P.G., Ahimou, F., Simon, D., Culot, C., Préat V., Vanbever, R., 2004. Aerosolization properties, surface composition and physical state of spray-dried protein powders. *J. Control. Release* 99, 357-367

Brandenberger, H., 2003. Development of a novel high-performance cyclone to increase the yield in a mini spray dryer. Best@buchi Evaporation Inf. Bull. 27.

Carstensen, J. T., 2001. Micromeritics, in: Advanced Pharmaceutical Solids, Marcel Dekker, New York, pp. 61-88.

United States Pharmacopeia (USP), United States Pharmacopeial Convention Inc., USA (2007).

Chen., L.R., Young, V.G., Lechuga-Ballesteros D., Grant, D.J.W., 1999. Solid-state behaviour of cromolyn sodium hydrates. *J. Pharm. Sci.* 88, 1191-1200.

Cox, J.S.G., Woodard, G.D., McCrone, W.C., 1971. Solid-state chemistry of cromolyn sodium (disodium cromoglycate). *J. Pharm. Sci.* 60, 1458-1465.

Dellamary, L.A., Tarara, T.E., Smith, D.J., Woelk, C.H., Adractas, A., Costello, M.L., Gill, H., Weers, J., 2002. Hollow Porous Particles in Metered Dose Inhalers. *Pharm. Res.* 17, 168-174.

Edwards, D., Hanes, J., Caponetti, G., Hrkach, J., Ben-Jebria, A., Eskew, M., Mintzes, J., Deaver, D., Lotan, N., Langer, R., 1997. Large porous particles for pulmonary drug delivery. *Science* 276 1868-1871.

Edwards, D.A., Ben-Jebria A., Langer, R., 1998. Recent advances in pulmonary delivery using large, porous inhaled particles. *J. Appl. Physiol.* 85, 379-385.

Healy, A.M., McDonald, B.F., Tajber L., Corrigan, O.I., 2008. Characterisation of excipient-free nanoporous microparticles (NPMPs) of bendroflumethiazide. *Eur. J. Pharm. Biopharm.* 69, 1182 – 1186.

ICH, (International Conference on Harmonisation) Guideline Specification Q1A (R2), Step 4. Draft, Stability testing of new drug substances and products, 2003.

Laube, B. L., Edwards, A. M., Dalby, R. N., Creticos P. S., Norman P. S., 1998. Respiratory pathophysiologic responses. The efficacy of slow versus faster inhalation of cromolyn sodium in protecting against allergen challenge in patients with asthma. *J. Allergy Clin. Immunol.* 101, 475-483.

Najafabadi, A.R., Gilani, K., Barghi M., Rafiee-Tehrani, M., 2004. The effect of vehicle on physical properties and aerosolisation behaviour of disodium cromoglicate microparticles spray dried alone or with *L*-leucine. *Int. J. Pharm.* 285, 97-108.

Ní Ógáin, O., Li, J., Tajber, L., Corrigan O.I., Healy, A.M., Particle engineering of materials for oral inhalation by dry powder inhalers. I - Particles of sugar excipients (trehalose and raffinose) incorporating a model protein. Submitted to *Int. J. Pharm.*

Nolan, L.M., Tajber, L., McDonald, B.F., Barham, A.S., Corrigan O.I., Healy, A.M., 2009. Excipient-free nanoporous microparticles of budesonide for pulmonary delivery. *Eur. J. Pharm. Sci.* 37, 593-602.

Nyqvist, H. , 1983. Saturated salt solutions for maintaining specified relative humidities. Int. J. Pharm. Tech. & Prod. Mfr. 4, 47-48.

Otsuka, A., Lida, K., Danjo, K., Sunada, H., 1988. Measurement of the adhesive force between particles of powdered materials and a glass substrate by means of the impact separation method. III. Effect of particle shape and surface asperity. *Chem. Pharm. Bull.*, 36, 741-749.

Ruffin, R.E., Dolovich, M.B., Oldenburg F.A., Newhouse, M.T., 1981. The preferential deposition of isoproterenol and propranolol in asthmatic patients, *Chest* 80, 904-907.

Steckel, H., Müller, B.W., 1997. In vitro evaluation of dry powder inhalers I: drug deposition of commonly used devices, *Int. J. Pharm.* 154, 19-29.

Steckel, H., Rasenack N., Müller, B.W., 2003. In-situ-micronisation of disodium cromoglycate for pulmonary delivery. *Eur. J. Pharm. Biopharm.* 55, 173-180.

Stephenson, G.A., Diseraod, B.A., 2000. Structural relationship and desolvation behaviour of cromolyn, cefazoline and fenoprofen sodium salts, *Int. J. Pharm.* 198, 167-177.

United States Pharmacopoeia (USP), United States Pharmacopoeial Convention, Inc., USA, 2008.

Vidgrén, M. T., Vidgrén P. A., Paronen, T. P., 1987. Comparison of physical and inhalation properties of spray-dried and mechanically micronized disodium cromoglycate. *Int. J. Pharm.* 35, 139-144.

Young, P.M., Price, R., Tobyn, M.J., Buttrum M., Dey, F., 2003. Effect of humidity on aerosolisation of micronised drugs. *Drug Dev. Ind. Pharm.* 29, 959-966.

Zanen, P.L., Go L.T., Lammers, J-H.J., 1995. The optimal particle size for parasympathicolytic aerosols in mild asthmatics, *Int J. Pharm.* 114, 111-115.

Zeng, X. M., Martin, G. P., Marriott, C., 2001. Particulate interactions in dry powder formulations for inhalation, London, Taylor & Francis.

Table 1 The emission %, the fine particle fraction %, Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) of spherical, non-spherical, non-porous and micronised sodium cromoglicate. (mean±sd, n=3)

Sample	**Spherical NPMPs SC**	**Non-spherical NPMPs SC**	**Non-porous Spray dried SC**	**Intal® micronised SC**
Emission (%)	63.87±10.22	42.47±12.60	24.79±3.95	63.59±7.73
Fine particle fraction (%)	62.22±2.08	40.25±0.71	27.29±1.77	28.06±3.72
MMAD (µm)	3.588±0.094	5.035±0.314	7.991±1.345	7.617±1.345
GSD	2.030±0.053	2.262±0.167	3.009±0.137	2.705±0.221

Table 2. The % emission and fine particle fraction (as % of recovered dose) of spherical, non spherical and micronised sodium cromoglicate, Intal® from twin impinger studies (mean±sd , n=3).

Sample	Spherical NPMPs SC	Non-spherical NPMPs SC	Intal® micronised SC
Emission (%)	61.90±5.30	54.67±8.88	65.62±1.41
Fine particle fraction (%)	38.17±5.53	27.34±0.5.75	13.12±3.16

Table 3. The emission %, the fine particle fraction %, Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) of spherical NPMPs of sodium cromoglicate on stability at 4 °C/desiccant. (mean±sd, n=3), *(mean [range], n=2).

Sample	**Spherical NPMPs SC Wk 0**	**Spherical NPMPs SC Wk 4***	**Spherical NPMPs SC Wk 8***	**Spherical NPMPs SC Wk 12***
Emission (%)	63.87±10.22	69.7 [70.54, 68.86]	62.52 [60.28, 64.75]	75.36 [77.08, 73.63]
Fine particle fraction (%)	62.22±2.08	58.03 [62.52, 53.54]	61.27 [58.51, 64.02]	57.24 [53.65, 60.83]
MMAD (µm)	3.59±0.09	3.78 [3.563, 3.994]	3.64 [3.57, 3.72]	3.82 [3.53, 4.11]
GSD	2.03±0.05	2.01 [1.952, 2.07]	1.98 [1.92, 2.04]	2.03 [1.97, 2.09]

Table 4. The emission %, the fine particle fraction %, Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) of spherical NPMPs of sodium cromoglicate on stability at 25°C/60% RH and 25°C/desiccant analysed over a 12 week period. (mean±sd, n=3) *(mean [range], n=2).

Sample	**Spherical NPMPs SC** **Wk 0**	**Spherical NPMPs SC** **Wk 1*** **25°C/60%RH**	**Spherical NPMPs SC** **Wk 2*** **25°C/desiccant**	**Spherical NPMPs SC** **Wk 4*** **25°C/desiccant**	**Spherical NPMPs SC** **Wk 12*** **25°C/desiccant**
Emission (%)	63.87±10.22	52.85 [40.53, 65.16]	68.47 [69.76, 67.18]	69.91 [70.3, 69.52]	49.27 [43.14, 55.4]
Fine particle fraction (%)	62.22±2.08	17.39 [16.17, 18.60]	54.34 [52.21, 56.47]	51.51 [47.94, 55.07]	52.83 [49.85, 55.80]
MMAD (µm)	3.59±0.09	12.59 [11.80, 13.37]	4.11 [3.91, 4.30]	4.37 [4.06, 4.69]	4.12 [3.96, 4.28]
GSD	2.03±0.05	3.28 [3.28, 3.29]	2.02 [2.02, 2.03]	2.08 [2.07, 2.09]	2.15 [2.13, 2.17]

Figure 1 SE micrographs of (a and b) spray dried non-porous sodium cromoglicate from an aqueous solution, (c) sodium cromoglicate raw material (Sigma sample) and (d) micronised sodium cromoglicate (Intal® Spincaps). Note different magnifications.

Figure 2 SE micrographs of spray dried sodium cromoglicate from different cosolvent systems of methanol:n-butyl acetate (v/v) (a and b) 95:5, (c and d) 90:10, (e) 80:20 and (f) 70:30.

Figure 3 SE micrographs of spray dried sodium cromoglicate from different cosolvent systems of water:methanol:n-butyl acetate 1:15:15 (v/v). Note the different magnification of SEM micrographs.

Figure 4 XRD diffractograms of (a) sodium cromoglicate raw material (Sigma) (b) micronised sodium cromglicate (Intal®), (c) spherical NPMPs of sodium cromoglicate (spray dried from water:methanol:n-butyl acetate (v/v) 1:15:15), (d) non-spherical NPMPs of sodium cromoglicate (spray dried from methanol:n-butyl acetate (v/v) 95:5) and (e) non-porous spray dried sodium cromoglicate (spray dried from water).

Figure 5. DSC scans of (a) sodium cromoglicate raw material (Sigma) (b) micronised sodium cromglicate (Intal®), (c) spherical NPMPs of sodium cromoglicate (spray dried from water:methanol:n-butyl acetate (v/v) 1:15:15), (d) non-spherical NPMPs of sodium cromoglicate (spray dried from methanol:n-butyl acetate (v/v) 95:5) and (e) non-porous spray dried sodium cromoglicate (spray dried from water).

Figure 6. Surface area and bulk density measurements of micronised sodium cromoglicate, non-porous spray dried sodium cromoglicate, spherical NPMPs of sodium cromoglicate (spray dried from water:methanol:n-butyl acetate (v/v) 1:15:15) and non-spherical NPMPs of sodium cromoglicate (spray dried from methanol:n-butyl acetate (v/v) 95:5). The left bar refers to surface area (mean±sd, n=4) and the right bar refers to bulk density (mean±sd, n=3).

Figure 7. Drug deposition profiles in the Andersen cascade impactor as % of the recovered dose of Intal®, non-porous sodium cromoglicate and NPMPs of sodium cromoglicate both spherical and non-spherical. (MP = mouthpiece, IP = induction port, S = stage, F = filter) (mean±sd , n=3).

Figure 8. Correlation between the fine particle fraction, %, of (black squares) micronised sodium cromoglicate, (white squares) non-spherical NPMPs of sodium cromoglicate and (crossed squares) spherical NPMPs of sodium cromoglicate measured after aersolisation through the Andersen cascade Impactor (ACI) and twin impinger (TI) using the Spinhaler® device at a flow rate of 60 l/min.

Figure 9. SE micrographs of spray dried spherical NPMPs of sodium cromoglicate on stability at 25°C/60% RH (a) starting NPMPs wk 0, (b) NPMPs post 24 hrs on stability at 25°C/60% RH, (c) NPMPs post 4 wks on stability at 25°C/60% RH (d) NPMPs post 12 wks on stability at 25°C/60% RH, (e) NPMPs post 4 wks on stability at 25°C/desiccant and (f) NPMPs post 12 wks on stability at 25°C/desiccant.

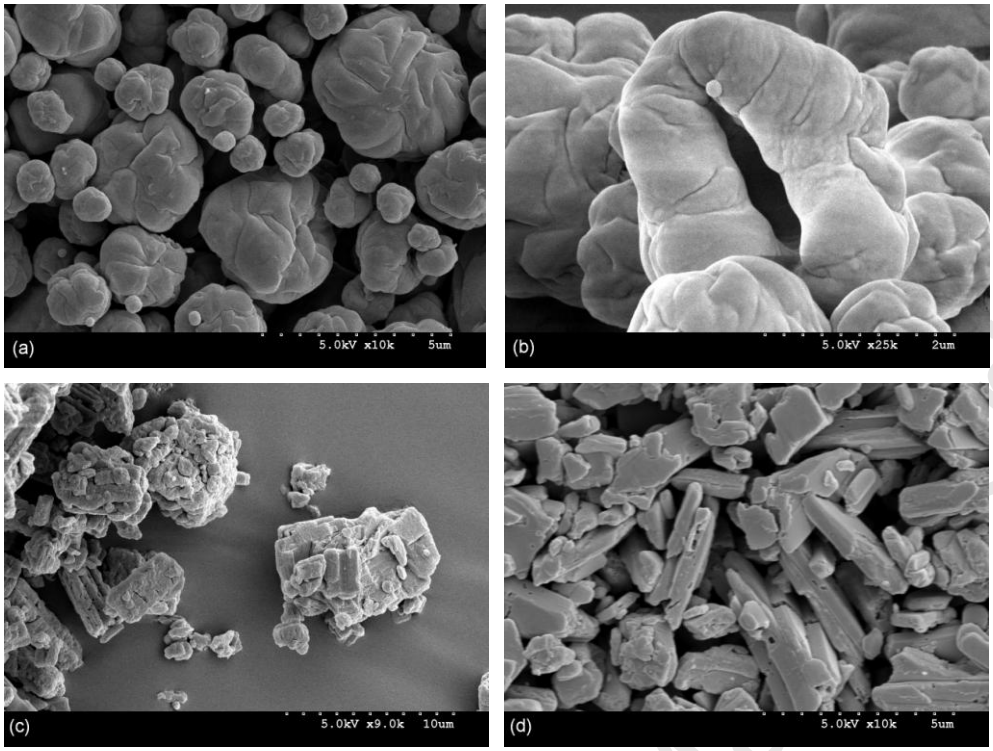


Figure 1.

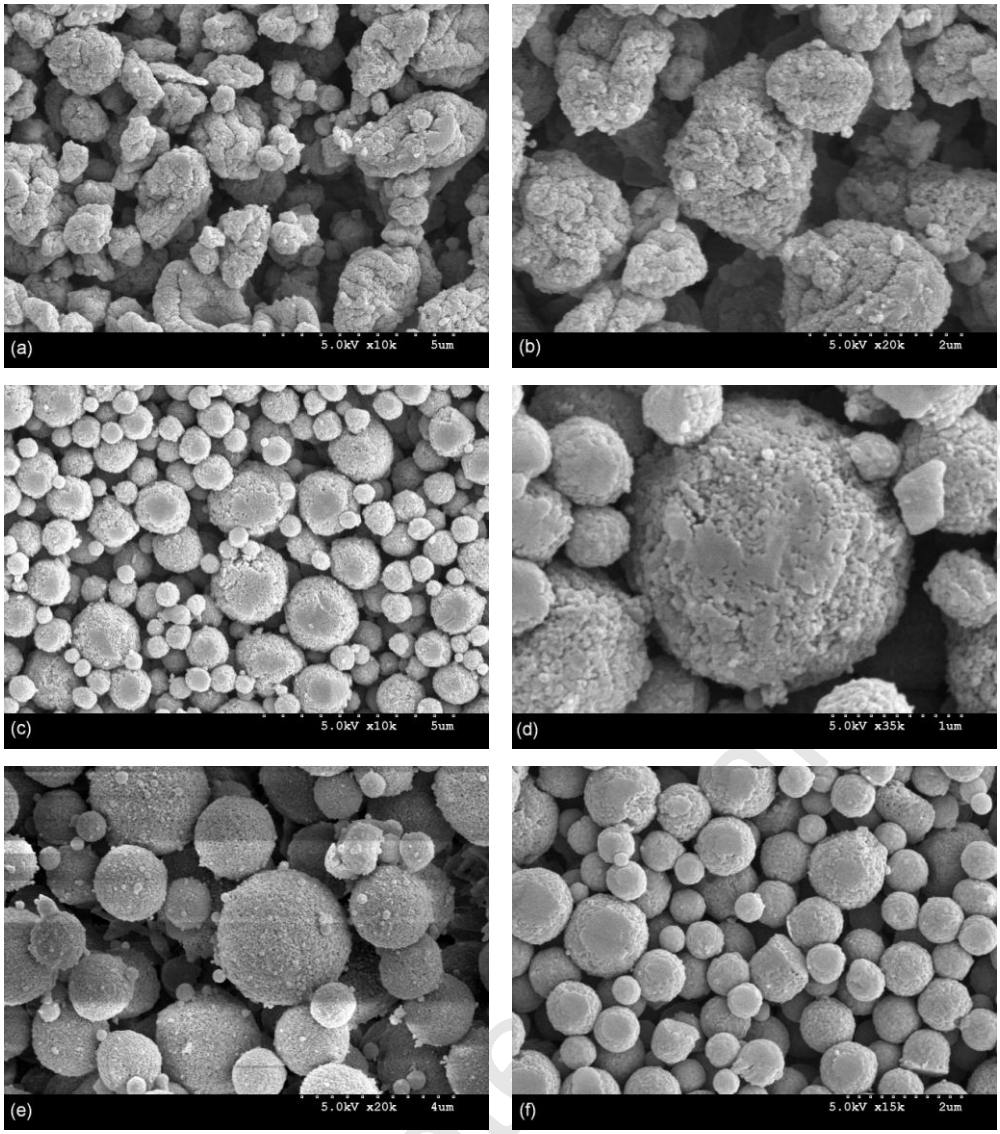


Figure 2.

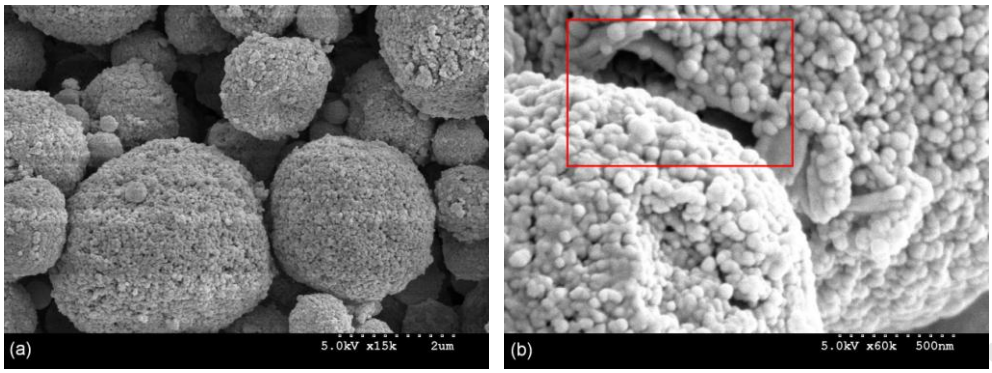


Figure 3.

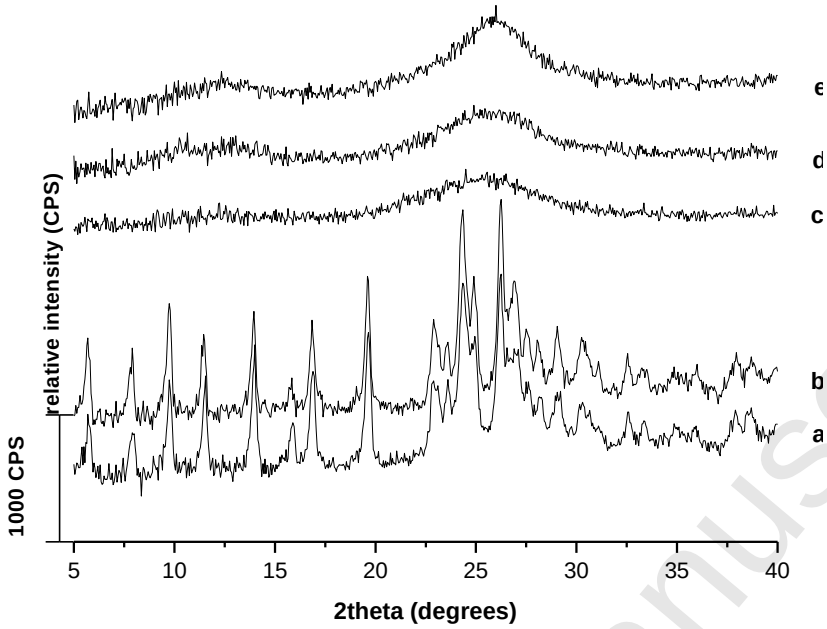


Figure 4.

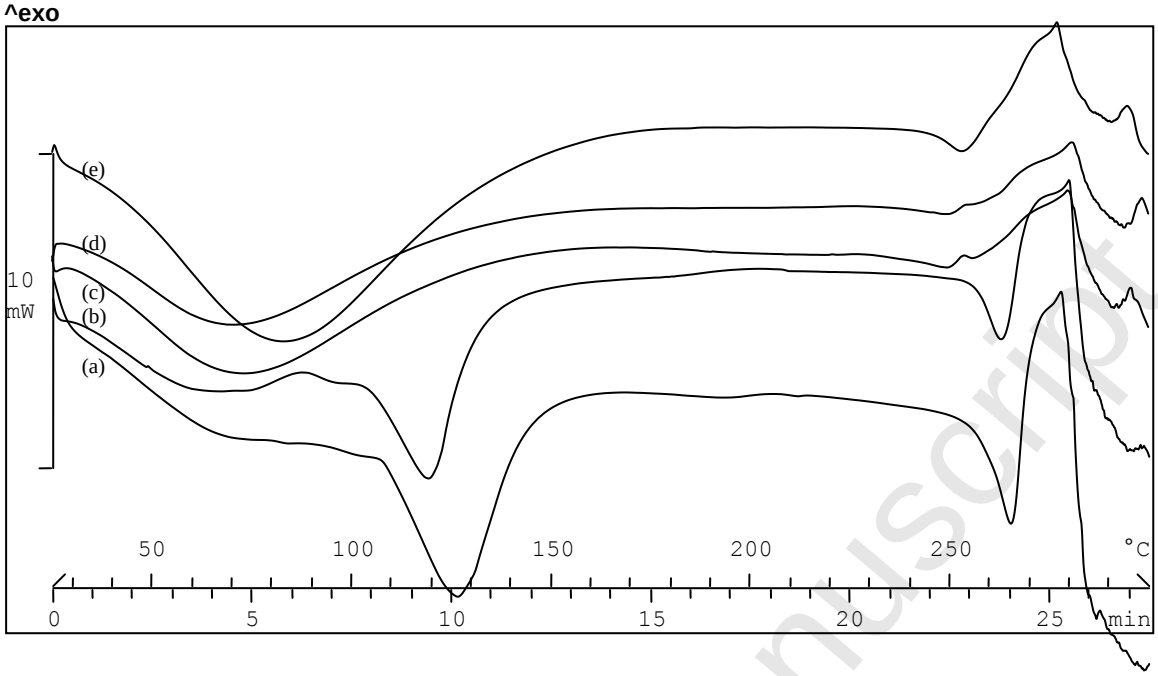


Figure 5.

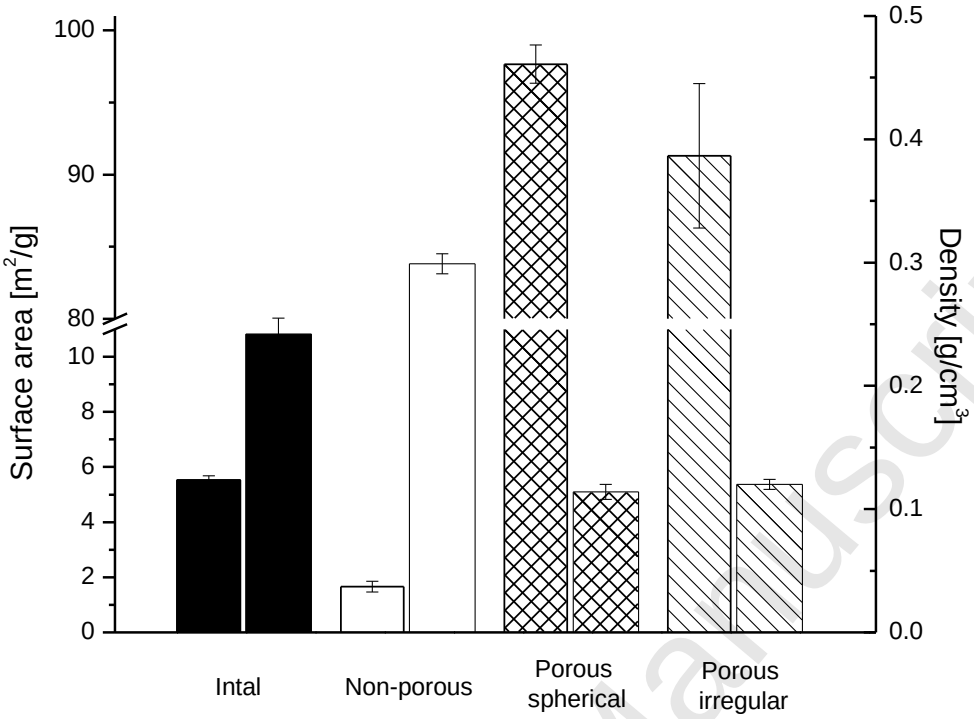


Figure 6.

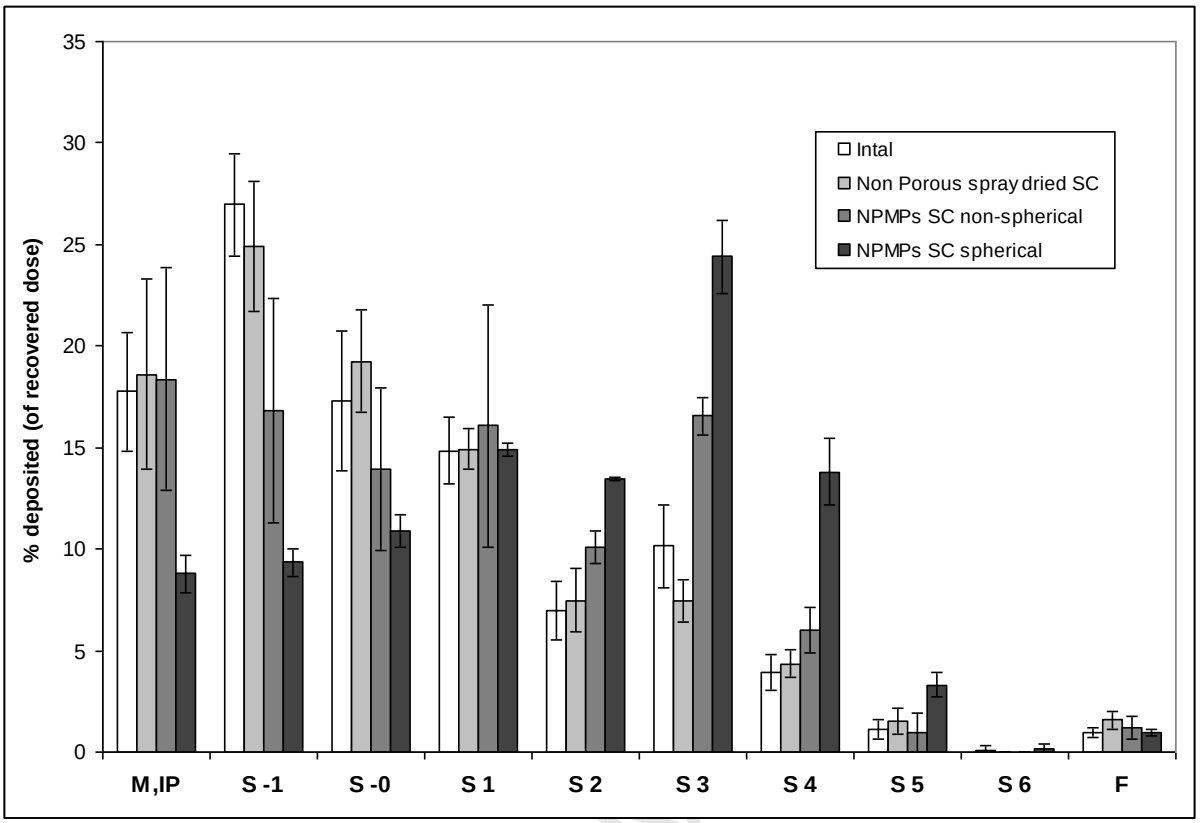


Figure 7.

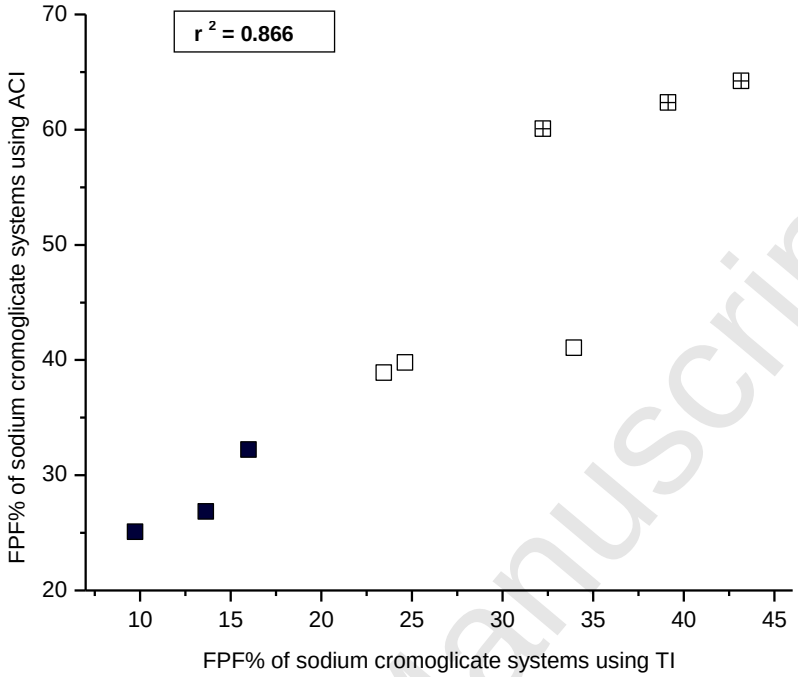


Figure 8.

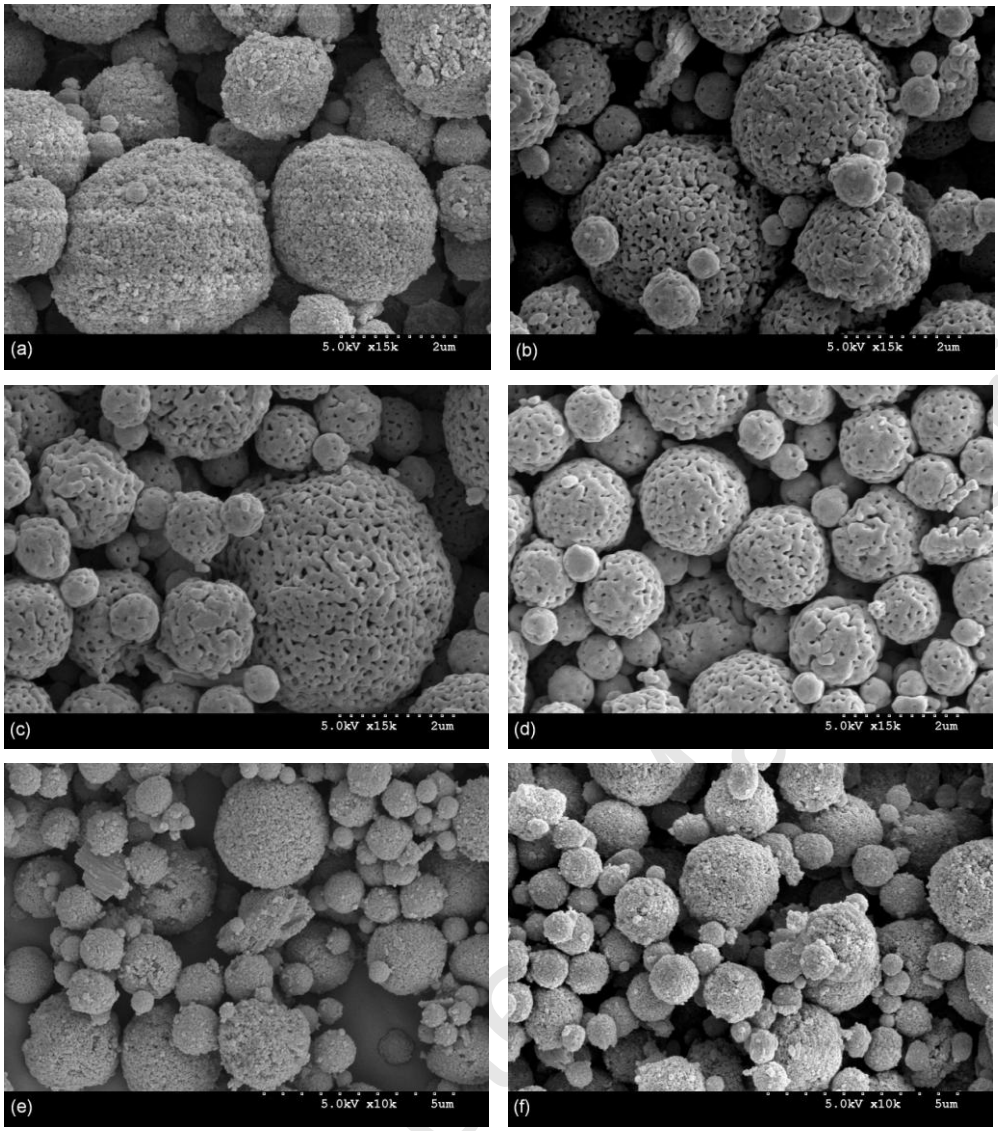


Figure 9.