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In vitro deposition of the respirable fraction of dry powder inhalations determined by laser diffractometry and inertial impaction

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Particle size analysis of drug and excipient is of particular interest for dry powder inhalation (DPI) formulations development and quality control. In this work, the deposition in the upper, the medium and the lower (i.e. the respirable fraction) Twin Impinger compartments of sodium cromoglycate (SCG), lactose (as excipient) and a 1:1 mixture thereof was determined by chemical analysis using a DPI device – the Micro-haler™ – and compared with the results obtained by laser diffractometry for the same fractions. The analytical method for the SCG determination consisted of ultraviolet spectrophotometry and, for the lactose, high performance liquid chromatography with refractive index detection was used. Laser diffractometry as a quickly operating routine method can substitute the chemical analysis in order to evaluate the respirable fraction, under the conditions of the present work and therefore making formulation development easier and quicker.

1. Introduction

Recently, therapeutic aerosols have been changing from Metered Dose Inhalers (MDIs) – which use a propellant gas to transport the micronised drug – to Dry Powder Inhalers (DPIs), which do not use a propellant gas at all: neither CFCs nor more flammable substitutes. By this system, the patient places the device in his mouth and simply inhales thus sucking the dose (in most cases a mixture of drug and excipient) which will eventually deliver the drug into the lungs.

It is very important to measure the particle size distribution from DPIs because this is the determinant factor of lung deposition: generally, particles smaller than 1 μm are inhaled and immediately exhaled and, on the other hand, particles larger than 10 μm impact the patient's mouth and throat and are subsequently swallowed. Particles in the size range of 1.0–6.0 μm usually settle in the lower respiratory tract [1].

A number of instruments have been used to determine the deposition of aerosols having the respiratory tract as a model, all based on the relationship between velocity and mass. According to the European Pharmacopoeia, 4th edition, the *in vitro* deposition can be tested using four different apparatus: A, B, C and D corresponding to the Glass Impinger, also called Twin Impinger (TI) (A), the Metal Impinger (B), the Multi-Stage Liquid Impinger (C) and the Andersen Sizing Sampler (D).

The operation with all these apparatus is time consuming and requires in most cases highly sophisticated analytical methods. In the early stages of galenical development of a new formulation, simple analytical methods are preferred to estimate the galenical progress. The TI is the fastest to operate, but it has a limitation: the total sample is divided into only two size categories, i.e., its cut-off particle diameter at the lower chamber (also called stage II) is 6.4 μm, if operated at a flow rate of 60 l/min, being the retrieved particles of the delivered dose at this part of the TI considered the respirable fraction [2]. However, it has proved to give a good correlation with the clinical performance of therapeutic aerosols, thus this apparatus was used in the present work.

Laser light scattering represents a technique where the particle size analysis is independent of the flow rate generating the aerosol and has been used to characterise aerosols generated by nebulised solutions. Recently the technique has also been employed for dry powder inhalations [3] and it appears to be an useful alternative to inertial impaction in characterising dry powder aerosols by a rapid method useful for development purposes, particularly those containing drug only, generated at different flow rates. It provides an estimate of volume median diameter and particle size distribution but it does not take into consideration the anatomical structure of the human respiratory tract and the aerodynamic behaviour of the particles.

Under the assumption that, in a mixture containing the active compound and an excipient, the distribution of the drug is randomly homogeneous, the particle size analysis will be representative of both the drug and the excipient and would give a result similar to that obtained by the chemical determination of the active compound deposited at different compartments (stages) of the impactor apparatus. In addition, particle size analysis has the advantage of being less time consuming and provides information on the particle size profile of the respirable fraction.

In the present work, the *in vitro* deposition of a pulmonary dry powder inhalation formulation is investigated using the Micro-haler™ inhalation device, the TI apparatus and the Sympatec particle size analyser. The drug used for this investigation is sodium cromoglycate (SCG) (also called disodium chromoglycate or cromolyn sodium) which is the sodium salt of the chromoglycic acid; the excipient used is lactose monohydrate under the trade name of Granulac 220.

The aim of this work was to evaluate if any correlation can be established for the respirable fraction between *in vitro* deposition (TI) and particle size distribution (laser diffraction spectrometer) of the aerosol cloud emitted by an experimental model dry powder delivery device – Micro-haler™, described by Braun *et al*. in 1996 [4] – and thus demonstrate the applicability of laser diffractometry as an alternative to chemical analytical methods using the inertial impaction technique (so as to maintain the human respiratory tract configuration restrictions), for the characterisation of dry powder aerosols for inhalation.

2. Investigations, results and discussion

2.1. Mixture preparation and storage conditions

The dilution of a micronised active substance with carrier material, using a combination of manual sieving to facili-

tate the break up of particle agglomerates and tumble mixing using a Turbula mixer [5] was performed in order to achieve the best flowability and pulmonary delivery of micronised drug particles.
The *in vitro* aerodynamic characteristics of the DPIs are strongly affected by the moisture conditions on storage [6], especially when the drug is very hygroscopic such as SCG. Thus, a 33% RH atmosphere was kept constant to store the SCG, the lactose and the lactose : SCG mixture.

2.2. Particle size distribution using the Sympatec (Rodos experiment)

The Granulac 220 and the SCG particle size profiles showed only one peak at about 20 μm and at 3 to 4 μm, respectively. For Granulac 220 the mean particle diameter was about 14 μm ranging from 2.0 to 47.9 μm for the X_{10} to X_{90} percent range. For the SCG the X_{50} value was 2.7 μm ranging from 0.9 to 6.1 μm for the X_{10} to X_{90} percent range. The mixture profile evidences two populations (two peaks at about 4 and at 12 to 18 μm, not completely separated, plus a shoulder at around 30 μm), varying the particles distribution between 1.0 and 17.9 μm for the X_{10} and X_{90} percent range, respectively.
The profile of the powder mixture is not the result of the "sum" of the two powders'profiles alone. This may be explained by the adsorption effect: the SCG micronised particles tend to distribute uniformly at the surface of the larger lactose particles. The adherence is due to electrostatic forces generated during the mixing process.
In a non-interactive system the particle size profile of the mixture of two components would show the same peaks as the profiles of the individual components. However, it is well known that mixtures of components of different particle size such as used in DPI products can form interactive mixtures [7] and thus give rise to peaks of intermediate particle size such as in the present work.

2.3. Particle size distribution from the dry powder inhaler device (Micro-haler) using the Sympatec (Trimo experiment)

Trimo software gives the particle size distribution of Granulac 220, SCG and the 1:1 mixture under conditions representative for normal inhalation.

The aerosol cloud emitted from capsules loaded into the Micro-haler™ was characterised by both the TI (to determine the deposition of the emitted dose) and the Sympatec Helos laser diffraction spectrometer to determine the particle size distribution of the entire powder discharge, i.e., drug *and* carrier particles, with the aim to establish a correlation between drug deposition (TI) and particle size distribution of the powder discharge (laser diffraction spectrometer).
Depending on the mass, shape, density and speed of the particles, they will impact on a different part of the TI: the upper (U), the medium (M) or the lower (L) compartments.
For convenience of results interpretation, we considered two systems in the present work:
System 1, is the complete TI apparatus, where the quantification of the drug and excipient alone and in the mixture was performed by means of chemical methods.
System 2 (Fig. 1), is the TI apparatus without the L compartment, where the quantification of the drug and excipient alone and in the mixture was performed by means of chemical methods for the U and M compartments and the respirable fraction was analysed by laser diffractometry. To this end, the TI (without the L compartment) was assembled so that the powder leaving the M compartment was passing through the Sympatec laser beam (open system).
Fig. 2 shows the mean values of drug and lactose deposition from TI experiments (System 1) in the U, M and L compartments as well as the drug residue in the capsule and the Micro-haler. The columns for System 2 do not show values for the L compartment of the TI due to the fact that these experiments were done in combination with the Sympatec particle size analysis without that compartment. The deposition of SCG in the L compartment is enhanced in combination with lactose. The results for SCG and lactose including the standard deviations are presented in Figs. 3 and 4, respectively, indicating a good reproducibility.
A more detailed description is presented in Tables 1 to 4, containing results obtained from the TI expressed in mg and as the percentage of drug or excipient dose filled into the capsules i.e. loaded dose (for a better comparison), and from the Sympatec (Trimo experiment). Total is the sum of all chemical results obtained from each com-

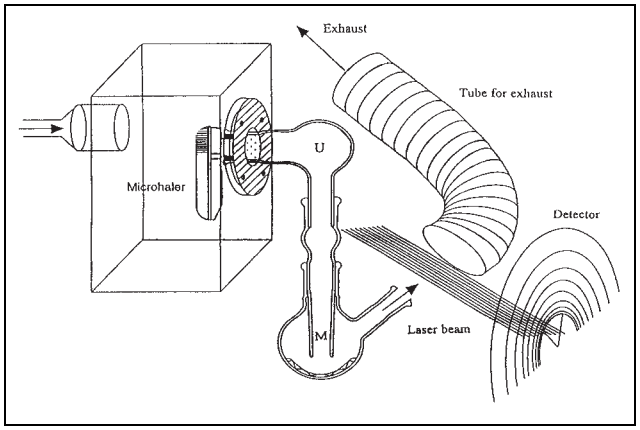

Fig. 1: System 2: Twin impinger (TI) operating without the lower compartment and connected to the Sympatec laser diffractometer. Micro-haler™ is placed inside of a metal box and connected to the TI (according to Braun, 1995). U = upper compartment, M = medium compartment

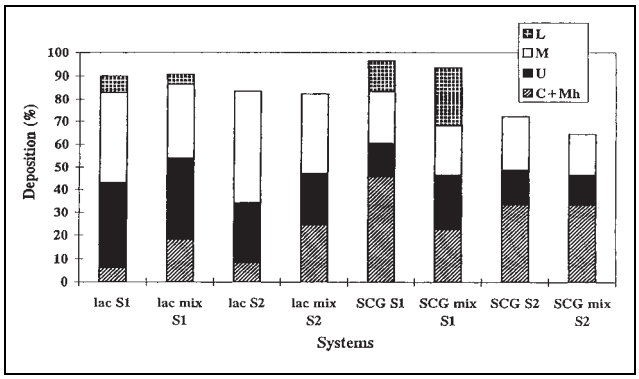

Fig. 2: Deposition pattern of the SCG, the lactose and the SCG : lactose (1:1) mixture from capsules using the Twin Impinger (System 1) and the Twin Impinger without the lower compartment (System 2), on the lower (L), medium (M) and upper (U) compartments and the remaining on the capsule and the device (C + Mh), using chemical analysis. Bars represent the mean of 6 capsules except for lactose on System 1 which represents 9

Table 1: Summary of the average results for the deposition in the sucessive stages of the T.I. (system 1) and for the powder particles passing through the laser diffractometer (system 2) obtained from capsules filled with 40 mg of SCG

	System 1 (n = 6)		System 2 (n = 6)	
	average ± sd	average ± sd	average ± sd	average ± sd
C + Mh	18.32 ± 7.43 mg	45.80 ± 18.58%	13.53 ± 3.62 mg	33.82 ± 9.06%
U	5.89 ± 1.45 mg	14.72 ± 3.64%	6.01 ± 1.22 mg	15.01 ± 3.06%
M	9.16 ± 4.56 mg	22.90 ± 11.40%	9.39 ± 1.55 mg	23.48 ± 3.86%
L	5.22 ± 1.62 mg	13.06 ± 4.04%		
Total	38.59 ± 1.45 mg	96.48 ± 3.62%	28.92 ± 1.97 mg	72.31 ± 4.93%
Sympatec (amount passing through)			11.08 ± 1.97 mg	27.69 ± 4.93%
X 50			5.06 ± 0.49 μm	
X 90			16.21 ± 20.15 μm	
Q (6.4)			72.74 ± 7.45%	

Table 2: Summary of the average results for the deposition in the sucessive stages of the T.I. (system 1) and for the powder particles passing through the laser diffractometer (system 2) obtained from capsules filled with 40 mg of mixture (1:1) Granulac 220 (lactose) : SCG. Assay for SCG (20 mg <> 100%)

	System 1 (n = 6)		System 2 (n = 6)	
	average ± sd	average ± sd	average ± sd	average ± sd
C + Mh	5.73 ± 0.70 mg	22.66 ± 3.52%	6.72 ± 2.55 mg	33.58 ± 12.75%
U	4.74 ± 0.61 mg	23.71 ± 3.06%	2.55 ± 0.78 mg	12.75 ± 3.90%
M	4.39 ± 0.37 mg	21.95 ± 1.86%	3.63 ± 0.85 mg	18.16 ± 4.23%
L	5.05 ± 0.37 mg	25.26 ± 1.85%		
Total	19.92 ± 1.39 mg	99.58 ± 6.94%	12.90 ± 1.08 mg	64.49 ± 5.39%
Sympatec (amount passing through)			7.10 ± 1.08 mg	35.51 ± 5.39%
X 50			4.25 ± 0.18 μm	
X 90			8.50 ± 0.30 μm	
Q (6.4)			76.52 ± 1.79%	

Table 3: Summary of the average results for the deposition in the sucessive stages of the T.I. (system 1) and for the powder particles passing through the laser diffractometer (system 2) obtained from capsules filled with 40 mg of Granulac 220 (lactose)

	System 1 (n = 9)		System 2 (n = 6)	
	average ± sd	average ± sd	average ± sd	average ± sd
C + Mh	2.44 ± 0.91 mg	6.09 ± 2.28%	3.41 ± 1.74 mg	8.52 ± 4.35%
U	14.77 ± 2.09 mg	36.92 ± 5.22%	10.33 ± 1.59 mg	25.83 ± 3.97%
M	15.96 ± 3.52 mg	39.90 ± 8.81%	19.69 ± 2.24 mg	49.21 ± 5.61%
L	2.86 ± 0.39 mg	7.15 ± 0.98%		
Total	36.02 ± 4.60 mg	90.06 ± 11.51%	33.43 ± 2.01 mg	83.56 ± 5.03%
Sympatec (amount passing through)			6.57 ± 2.01 mg	16.44 ± 5.03%
X 50			5.35 ± 0.21 μm	
X 90			10.37 ± 0.54 μm	
Q (6.4)			62.93 ± 1.98%	

Table 4: Summary of the average results for the deposition in the sucessive stages of the T.I. (system 1) and for the powder particles passing through the laser diffractometer (system 2) obtained from capsules filled with 40 mg of mixture (1:1) Granulac 220 (lactose) : SCG. Assay for lactose (20 mg <> 100%)

	System 1 (n = 6)		System 2 (n = 6)	
	average ± sd	average ± sd	average ± sd	average ± sd
C + Mh	3.65 ± 0.21 mg	18.26 ± 1.05%	4.91 ± 2.41 mg	24.57 ± 12.06%
U	7.15 ± 0.84 mg	35.73 ± 4.21%	4.52 ± 1.56 mg	22.61 ± 7.78%
M	6.52 ± 0.65 mg	32.58 ± 3.26%	7.06 ± 1.48 mg	35.31 ± 7.41%
L	0.86 ± 0.02 mg	4.28 ± 0.09%		
Total	18.17 ± 0.54 mg	90.85 ± 2.72%	16.50 ± 1.07 mg	82.48 ± 5.35%
Sympatec (amount passing through)			3.50 ± 1.07 mg	17.52 ± 5.35%
X 50			4.25 ± 0.18 μm	
X 90			8.50 ± 0.30 μm	
Q (6.4)			76.52 ± 1.79%	

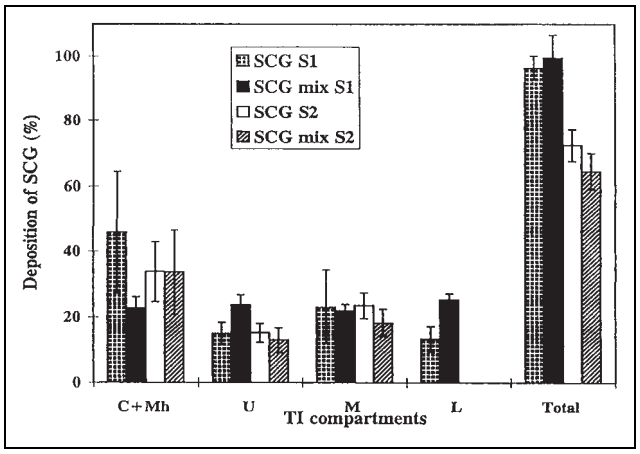

Fig. 3: Deposition pattern of the SCG pure and the SCG in the SCG : lactose (1 : 1) mixture from capsules using the Twin Impinger (System 1) and the Twin Impinger without the lower compartment (System 2), on the lower (L), medium (M) and upper (U) compartments and the remaining on the capsule and the device (C + Mh), using chemical analysis. The sum of all compartments and C + Mh is also represented. Bars represent the mean of 6 capsules, error bars = ± standard deviation

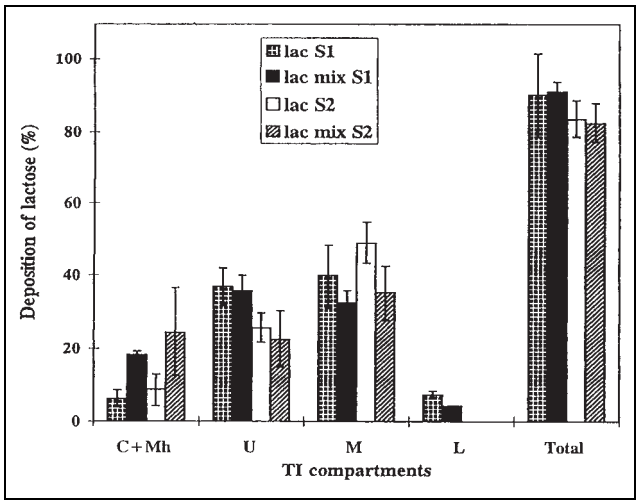

Fig. 4: Deposition pattern of the lactose pure and the lactose in the SCG : lactose (1 : 1) mixture from capsules using the Twin Impinger (System 1) and the Twin Impinger without the lower compartment (System 2), on the lower (L), medium (M) and upper (U) compartments and the remaining on the capsule and the device (C + Mh), using chemical analysis. The sum of all compartments and C + Mh is also represented. Bars represent the mean of 6 capsules except for lactose on System 1 which represents 9 capsules, error bars = ± standard deviation

partment and capsule plus device. The amount of particles passing through the Sympatec is calculated by the difference between the total and the dose loaded in the capsule.

For SCG alone (Table 1), the respirable fraction was 13.06 ± 4.04% and the M and U compartments retained 22.90 ± 11.40% and 14.72 ± 3.64%, respectively. Almost half the dose was retained in the capsule and the device. This might be attributed to the very fine particle size and hygroscopicity of the powder causing adherence to the device and capsule. In the mixture (Table 2) the respirable fraction was 25.25 ± 1.85% and the M and U compartments retained 21.95 ± 1.86% and 23.71 ± 3.06%, respectively. The value for the L compartment shows to be about twice the value for SCG alone. This fact evidences the role of the carrier, which adsorbs the micronised SCG at its particle surface during the mixing of the two components and then releases the drug in the air stream generated when the pump is actuated.

For lactose alone (Table 3), the respirable fraction was 7.15 ± 0.98% and the M and U compartments retained 39.90 ± 8.81% and 36.92 ± 5.22%, respectively. In the mixture (Table 4) the respirable fraction of lactose was 4.28 ± 0.09% and the M and U compartments retained 32.58 ± 3.26% and 35.73 ± 4.21%, respectively. This suggests that, for the respirable fraction, the contribution of the carrier chosen for the formulation is kept constant and shows to be proportional to the amount present in the capsule. The percentage value of lactose in the mixture, at the L compartment, is negligible (2.14% in a 40 mg of mixture) from the lungs deposition point of view.

A comparison between the deposition results for the respirable fraction shows the following: For SCG alone 13.06 ± 4.04 and 27.69 ± 4.93% were obtained for System 1 and System 2, respectively (Table 1). In the case of lactose alone (Table 3) 7.15 ± 0.98 and 16.44 ± 5.03% were obtained using the System 1 and the System 2 respectively. Respirable fraction results for System 1 are obtained by chemical assay, but for System 2 they are obtained by the difference calculation from the total load of the capsule, i.e. 100%.

For lactose alone and for SCG alone, the percentage value obtained for the L compartment with System 2 is about twice the value of the TI. The reasons for that difference can be due to the experimental conditions used: while with the TI experiment a negative pressure was used and the apparatus was complete, for the Sympatec System 2 experiment a positive pressure was used and the L compartment of the apparatus was removed in order to allow the reading by the Sympatec apparatus (open system; see Fig. 1). The missing L compartment of the TI in System 2 would be responsible for a change of pressure within the apparatus and therefore a higher mass stream at the end of the TI equipment.

The L compartment deposition results, on System 1, related to SCG in the mixture are 25.26 ± 1.85% (Table 2) and to lactose in the mixture, 4.28 ± 0.09% (Table 4).

Concerning System 2 related to the lactose : SCG mixture values can be calculated in two ways:

a) by subtracting from 100% the sum of the C + Mh, U and M mass values of lactose (16.50 mg) (Table 4) and of SCG (12.90 mg) (Table 2) determined in the mixture from the content of the capsule (40 mg), and than converting in percentage, according to the following expression:

$$100 - ((16.50 + 12.90)/40 \cdot 100) = 26.50 \qquad (1)$$

This gives the percentage value of particles passing through the TI open system (i.e. 26.50%).

b) by multiplying the value above (26.50%) by the percentage value of particles below 6.4 μm (Q (6.4)) given by the Sympatec Trimo software (76.52%) (Tables 2 and 4). This gives the "corrected" percentage value (20.28%) of 6.4 μm particles passing through the TI open system.

The first way of calculation assumes that all particles passing the M compartment are below the cut-off value of the TI (6.4 μm), while the second way of calculation shows that about 1/4 of those particles (passing to the TI lower chamber) (26.50%) is above that value. This difference in value might be attributed either to Sympatec measurement errors or constitutes a deviation from the TI cut-off theoretical value. Although both methods measure equivalent sphere diameter, Sympatec measures the volumetric mean diameter and the TI measures the aerodynamic diameter.

For System 2 the values reflect the presence of both lactose and SCG. For System 1, since the percentage of lactose reaching the L compartment is negligible, only the values of SCG are worth to compare to the System 2 value.
The ANOVA analysis of the individual results obtained for SCG in the mixture for the respirable fraction (by UV spectrophotometry; $n = 6$) and those given by the System 2 (using the eq. 1 for the individual values; $n = 6$) shows that they are not significantly different ($p < 0.05$), allowing to conclude that the Sympatec values for the respirable fraction are equivalent to the chemical assay of the drug in the L compartment of the TI. This is in agreement with the lactose results for the respirable fraction, obtained for lactose alone which are very low and consequently will not interfere with the particle size measurement using System 2.
Further research work is suggested, which should point out towards the association of TI and Sympatec in order to use Sympatec "Trimo" data as a recognised method to be used in the galenical development of DPIs and for controlling the quality/efficiency of marketed DPI devices. Two areas where additional work is necessary in order to have more concluding results are:
a) use of a valve between the pump and the TI in order to ensure a continuous and exact air flow, from the beginning until the end of each experiment, i.e., to keep a 60 l/min constant flow rate during the actuation time;
b) modification of the TI – Sympatec assembly in order to make the laser beam pass through the coupling tube E of the TI apparatus (Eur. Ph., 4th ed.), which would also allow to operate with negative pressure. In this way, the system would operate in the same conditions as those of the TI alone and complete.

2.4. Conclusions

The use of Sympatec "Rodos" data is useful to have a mixture particle size profile, which can be valuable to control the efficacy and reproducibility of the method of preparation and the influence of type and amount of carrier of a DPI.
The influence of the carrier on the amount of SCG reaching the L compartment is evident and reduces the impact of the different experimental conditions. This offers the possibility of using the TI without L compartment associated to Sympatec as an additional alternative to the traditional TI apparatus, which gives only results on drug chemical analysis, for the determination of the respirable fraction.
The use of Sympatec with "Trimo" software shows to be an useful instrumental analytical method in the development of powder mixtures for DPIs as, in association with the TI, it provides faster results for the respirable fraction than working with the TI in association with chemical methods for quantifying the drug.
The Micro-haler™ is found to be a suitable device for testing DPI formulations during, at least, the early galenic development stages of the mixtures and also to have the potential to be marketed as a DPI device.

3. Experimental

3.1. Materials and equipment

Sodium cromoglycate (SCG) (Francis Chemicals, Italy), Lt. 5700-U-09-02. Granulac 220 (Meggle, Wasserburg, Germany), batch 958. Hard gelatine capsules No. 3 (Snap-fit™, Capsugel, Bornem, Belgium). Phosphate buffer pH = 7.4 was prepared adding 13.63 g KH_2PO_4 and 3.2 g NaOH in a 5000 ml volumetric flask and completing to volume using distilled water; the pH was adjusted if necessary. Solvents were of HPLC grade. The water HPLC grade was obtained by distillation from demineralised water and after passing through a 0.45 μm membrane filter. Reagents were of analytical grade.
Compounds were weighed on a balance, Mettler AT 261, Delta Range. Solutions pH were adjusted with acid or alcali using a pH meter (Metrohm, 638 pH-Meter, Swiss). A Turbula mixer (Bachofen, Basel, Swiss) was used for the mixing process. Mixtures were sieved in a Glass Glove Box (home-made) under a relative humidity of less than 10%. The Micro-haler™ [8] was used as the inhaler device. The TI (Erweka GmbH, Heusenstamm, Germany) and the Laser diffraction spectrometer (Sympatec GmbH, Clausthal-Zellerfeld, Germany) with Helos software packages equipped with Rodos & Trimo dispersing (Sympatec GmbH, Clausthal-Zellerfeld, Germany) were used for particle size analysis. The UV/VIS Spectrophotometer (Perkin-Elmer 550S, Überlingen, Germany) was set at 238 nm.
The HPLC system used was Shimadzu (Shimadzu Corp., Duisburg, Germany) with a refractive index detector. All conditions were set as described previously in the literature [9].

3.2. Methods

3.2.1. Storage conditions of the SCG

SCG was kept inside of a desiccator at 33% RH in an open Petri dish, protected from light. The 33% RH atmosphere was prepared by completely saturating, with stirring at 50 °C, 250 ml of water with $MgCl_2$ until permanent precipitation was observed.

3.2.2. Mixture preparation

In order to prepare a 1:1 mixture SCG : Granulac 220, 5 g of each compound were accurately weighed and premixed in a 50 ml polyethylene screw capped container with a PTFE inlay in the cap, by shaking manually for 5 min. Granulac 220 was first put into the container in order to cover the internal surface and to prevent the SCG adherence. Drug agglomerates were destroyed by passing the mixture through a 500 μm sieve and returned back to the container. This operation took place inside of a glass glove box with a RH < 10%. The container was submitted to a mixing process in a Turbula mixer for 30 min at 42 rpm. In order to achieve an acceptable flowability the powder mixture was passed again through a 500 μm sieve, collected into a 100 ml brown glass vessel (this operation again took place inside of a glass glove box with a RH < 10%) and rotated in a horizontal drum mixer for 12 min at 39–40 rpm; these last sieving and horizontal mixing steps were repeated once more. Applying this procedure, soft round agglomerates of the powder mixture were formed. After the procedure was completed, SCG was assayed in order to validate the mixing process. Thus, five samples of 40 mg of the mixture (1:1 mixture of SCG : Granulac 220, representing 20 mg of SCG) were taken. The results obtained for the uniformity of the mixture were as follows: $n = 5$; average = 20.06 mg (100.3% of the nominal); S.D. = 0.18; rsd = 0.9.
Only one batch was used for the set of experiments performed for the present study, to avoid discrepancies arising from the mixing process.

3.2.3. Filling the capsules

Hard gelatine capsules were filled manually with exactly 40.0 ± 0.1 mg powder load of the mixture SCG : Granulac 220 (1:1), the drug alone and the excipient alone, respectively.
Uniformity of content of capsules was ensured through strict control of powder blend homogeneity (% rsd < 1%; see section 3.2.2.) and capsule fill weight (40 ± 0.1 mg).

3.2.4. Assessment of the in vitro deposition from the dry powder inhaler device (Micro-haler™) using the Twin Impinger

7 and 30 ml of phosphate buffer solution (pH = 7.4) for SCG alone or methanol for lactose (alone or in the mixture) were introduced into the M and L impingement chambers, respectively. The TI was assembled and a capsule was pierced at the top and the bottom, placed in the Micro-haler and the vacuum pump operating for 5 sec. at 60 ± 5 l/min airflow rate was started. The flow rate through the TI was set to the normal operating flow rate of 60 ± 5 l/min using a calibrated rotameter. The flow rate thus set was independent of the presence of the DPI, due to its low flow resistance. The capsule content is discharged by the turbulent air stream generated. At the end of this operation the apparatus was disassembled.
Although the TI was designed to divide the particles in only two main classes (due to a cut-off particles mean mass aerodynamic diameter of 6.4 μm), in this work particles were studied in the three different compartments – U, M, corresponding to Stage I, and L, corresponding to Stage II or respirable fraction. In addition, the remaining in both the Micro-haler (Mh) and the capsule (C) was considered.
The inner surfaces of the three TI compartments were washed separately with the buffer solution (for SCG alone) or methanol (for lactose alone or in the mixture), the rinsings being quantitatively transferred, and added to

the solutions of each chamber already collected in the corresponding volumetric flasks (for SCG alone) or evaporating flasks (for lactose alone or in the mixture). The capsule and the Micro-haler™ were washed and the rinsings collected in one volumetric flask or in one evaporating flask in the same way. After filling up the volumetric flasks, the solutions were, if necessary, diluted and submitted to UV measurement at 238 nm in order to obtain SCG concentrations. For the quantification of lactose (alone and in the mixture), HPLC with refractive index (RI) detection was used [9].

3.2.5. Particle size distribution by laser diffractometry using Rodos and Trimo

Rodos experiments: Particle size distributions of the drug, the excipient and the 1:1 mixture were determined by a Sympatec Helos laser diffraction spectrometer equipped with a Rodos dry powder dispersing system where the sample is fed into the dispersing air stream. Depending on the pressure and speed of the air stream, the dispersing force and the powder concentration in the airstream could be adjusted. Thus, the pressure at which only agglomerates are dispersed without destroying single particles can be determined.
Rodos software operating conditions were: 4 bar, 100 mm lens and the channel 0 [5]. These studies give the particle size distribution of Granulac 220, SCG and the 1:1 mixture.
The working conditions of the apparatus were verified with calibrated glass particles using lenses of 100 and 200 mm.
Trimo experiments: Particle size distributions of the drug, the excipient and the 1:1 mixture from the Micro-haler™ were determined by Sympatec Helos laser diffraction spectrometer equipped with Trimo dry powder dispersing system where the sample is fed from a capsule previously pierced at the top and the bottom, placed into the Micro-haler™ and operated in the TI (Fig. 1).
The particle distributions were recorded every 2.5 s using the Trimo software package. Other operating conditions were: 200 mm lens, number of repetitions = 1, time resolution = 2.5 s, measuring time = 5 s and threshold on/off = 0.1/0% (detection of the L compartment) and 0.2/0.1% (detection of the M and the L compartments) [5]. The channel 20 was selected as reference channel for Granulac 220, the channel 24 for SCG and the channel 29 for the 1:1 mixture. These studies gave the particle size distribution of Granulac 220, SCG and the 1:1 mixture under conditions representative of normal inhalation flow (i.e. 60 l/min.).
Since for System 2 (open system; see Fig. 1), the TI could not be operated using the negative pressure generated by the pump, a special metal box was adapted to allow the application of a positive pressure at the Micro-haler™, using the same pump as for System 1 (60 l/min air flow) and operating the pump for 5 s.

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References

1 Timsina, M. P.; Martin, G. P.; Marriot, C.; Ganderton, D.; Yianneskis, M.: Int. J. Pharm. **101**, 1 (1994)
2 Hallworth, G. W.; Westmoreland, D. G.: J. Pharmacol. **39**, 966 (1987)
3 MacRitchie, H. B.; Martin, G. P.; Marriott, C.; Murphy, L.: Abstracts of The Aerosol Society Conference, London, 118 (1995)
4 Braun, M. A.; Oschmann, R.; Schmidt P. C.: Int. J. Pharm. **135**, 53 (1996)
5 Braun, M.: Ph. D. Thesis, University of Tübingen, Germany (1995)
6 Vidgreen, P.; Vidgreen, M.; Paronen, P.: Acta Pharm. Fenn. **98**, 71 (1989)
7 Hersey, J. A.: Powder Technol. **11**, 41 (1975)
8 Pearce, J. O.: Eur. Pat. Appl. EP 0 333 334 A2 22 Feb. (1989)
9 Cabral Marques, H. M.; Orth, H.; Schmidt, P. C.: Pharmazie, **54**, 785 (1999)

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