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RESEARCH PAPER

Use of a Novel Modified TSI for the Evaluation of Controlled-Release Aerosol Formulations. I

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ABSTRACT

When considering the development of potential controlled-release pulmonary drug delivery systems, there is at present no standard method available for the assessment of in vitro drug release profiles necessary to understand how the drug might release following deposition in the lungs. For this purpose, the twin-stage impinger (TSI), apparatus A of the BP, has been redesigned and tested. This modified TSI was found capable of discriminating between drug release rates from conventional and different dry powder formulations consisting of model controlled-release excipients, providing information related to (a) *drug diffusion properties of controlled-release dry powder blends with different excipient components and* (b) *the effect of varying drug concentration within a given formulation.*

Key Words: *Aerosol; Controlled release; Dry powder; Inhalation; In vitro.*

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INTRODUCTION

Sustaining the release of drugs in the lung, thereby prolonging drug action, is an attractive prospect for many local and systemic therapies (1). Presently, many medications in aerosol form require inhalation at least three to four times a day because of the relatively short duration of subsequent clinical effects (2). Controlled release of inhaled therapeutics has the potential to reduce the frequency of dosing and so increase patient compliance (1,3).

Pulmonary deposition and clearance of such novel controlled-release pulmonary drug delivery systems can be assessed by looking at in vivo performance, which may, for example, involve studying the pattern of deposition and clearance of radiolabeled particles from the lungs of healthy human volunteers using gamma-scintigraphy (4). Animal studies with rats and guinea pigs have been used to assess in vivo performance (5). However, such in vivo studies are expensive, time consuming, and limiting due to a lack of suitable animal models for studying pulmonary delivery systems (1).

Despite the diversity of in vitro aerosol characterization methods that exist, there is no standard method presently available to assess drug release from potential controlled-release inhalation delivery systems.

The current BP and EP advocate the use of any one of four specified methods for the aerodynamic assessment of fine particles (6,7). These include the single-stage (metal impinger, apparatus B), twin-stage (glass impinger, apparatus A), and multistage (multistage liquid impinger, apparatus C; multistage cascade impactor, apparatus D) sizing methods. The current USP requires that the aerodynamic size distribution be determined using either apparatus 1 (multistage cascade impactor) or apparatus 2 or apparatus 3 (referred to as single-stage impactors) (8). Apparatus C of the BP and EP is not included in the current USP, and there are considerable revisions of the existing procedures for aerosols being made for the next edition (9).

In vitro deposition patterns of potential sustained-release systems have been evaluated using the twin-stage impinger (TSI) (4,10), but in vitro drug release studies to date are not satisfactory with respect to dry powder formulations. Studies have been performed using calorimetric procedures (10) and in the same manner as drug entrapment is evaluated (4), that is, by agitating a suspension of the drug and carrier in buffer, centrifuging, and sampling the supernatant at timed intervals. Drug release rates from different formulations with respect to metered dose inhaler formulations have also been assessed as to the achievement of controlled release as a result of spontaneous liposome formation following actuation of the device (11). Methods such as these, however, cannot be correlated with the mechanism of drug release following deposition of a controlled-release dry powder in the lungs as the powder typically will be deaggregated, thus with probable differing release characteristics from the bulk formulation.

This paper describes the modification of a standard aerosol testing apparatus (stage 1 of the TSI, apparatus A of the BP) to provide data for both deposition characteristics and drug release kinetics of model controlled-release systems for pulmonary delivery. The aim of this initial work was to develop an in vitro testing method capable of discriminating among different formulations, thus the choice of model excipients (xanthan gum and locust bean gum) reflects their known ability to afford differing release rates from controlled-release tablet formulations (12). The principles established by studying stage 1 deposition of controlled-release powders have since been used to develop an in vitro method for studying deposition and release from fine particles; the results will be presented in a subsequent paper.

The TSI was considered a favorable analytical tool that could be modified for the purpose of this study. It has been shown that the TSI is a valuable tool for routine quality assessment of aerosols during product development, for stability testing, and for the quality assurance and comparison of commercial products (13). It is true that the TSI is considered to have limitations in that the total sample is only divided into two size categories, and that the separation between these two categories is not perfectly sharp (14); however, multistage devices are slow and tedious as chemical and physical quantification of collected particles is very time consuming (14). The TSI was developed originally to overcome this problem as a simpler, but reproducible, method for routine use (13,15), as was required for this study.

Stage 1 deposition represents delivery to the bronchiolar regions of the respiratory tract. Though this is not the conventional approach for pulmonary delivery (which is generally aimed at the large surface area of the alveolar region), there is scope for drug deposition to these parts for local therapy, for example, with bronchodilators and anti-inflammatory steroids, as it is the smooth muscle in this region that is the primary target site for these drugs (16). The obvious concern with such an approach is that particulate material deposited in the upper airways will be subjected to rapid mucociliary transport, resulting in a short duration of residence of both drug and carrier (1,17), whereas for a controlled-release carrier to be ef-

fective, it needs to be able to reside in the lung for a prolonged period of time so that the drug can be released and then absorbed. To overcome this, deposition should be directed to the deeper ciliated regions of the lung, the respiratory bronchiole region, as described by Weibel (18), where the mucociliary escalator moves slower (17). In this instance, as the dose is carried up the mucociliary tract, the slow release of drug should occur within the vicinity of its site of action (17).

EXPERIMENTAL

Materials

Salbutamol base (batch 73263501) was obtained from Leiras OY, Turku, Finland. Xanthan gum (lot 97801.EXP) and locust bean gum (LBG) (lot 97802.EXP) were supplied by Penwest Pharmaceuticals Company (Patterson, NY). Pharmaceutical-grade lactose was purchased from Lactochem, Borculo Whey Products Limited (Saltney, UK). Other chemicals were high-performance liquid chromatography (HPLC) grade.

Modification of the Twin-Stage Impinger (BP Apparatus A)

Initial experiments were conducted using an unmodified TSI in which potential controlled-release dry powder formulations were fired into the TSI, and the solvent from stage 1 was removed and diluted to a specific volume. From these studies, the amount of drug appearing in the solvent could be calculated and a release profile constructed based on the time elapsed between firing of the powder and removal of the solvent from stage 1. This technique proved unsatisfactory as the amount of drug detected was generally determined by the amount of powder impinging in stage 1, with no indication of how the drug was releasing from the gel that had formed.

To construct more reliable release profiles of given powder blends, aspects that are known to influence apparent release rates in standard tablet dissolution procedures were investigated, namely, the dissolution volume and stirring rate. The round-bottom flask of the stage 1 assembly was modified as shown in Fig. 1. A 300-ml reservoir was added to its base, providing a large enough volume for sink conditions to be present (19).

A 1-mm brass mesh was fused between the reservoir and the round-bottom flask. This size was chosen so that drug could diffuse easily across it without being restricted by the mesh size. Also, the gel that formed was able to rest on the grid and not fall onto the stirrer situated at the bottom of the reservoir.

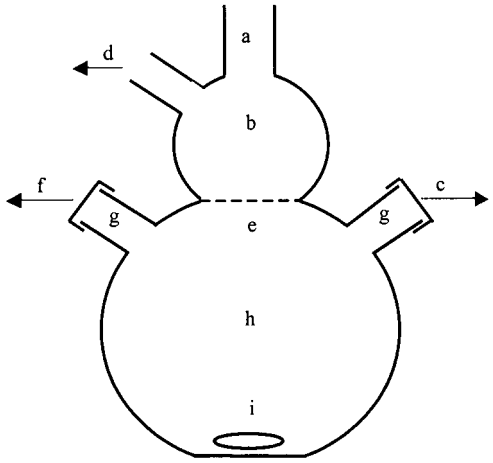

Figure 1. Modified stage 1 of the TSI: a, connection for stage 1 jet; b, stage 1; c, to spectrophotofluorimeter; d, to stage 2; e, 1-mm brass mesh; f, from peristaltic pump; g, sampling ports; h, 300-ml water reservoir; i, magnetic follower. Specifications: a, B24 joint; b, height 65.6 mm × width 65.3 mm; d, B14 joint plus 22-mm side arm; e, diameter 50 mm; h, height 74 mm × width 83 mm; i, length 20 mm × width 7 mm.

The standard ''swan neck'' section of the stage 2 jet, TSI apparatus A, was lengthened by 20 cm. This enabled the magnetic stirrer and a laboratory jack to be placed under the modified stage 1 assembly above the 250-ml conical flask of the stage 2 assembly. The flow rate, for the Rotahaler™ device (GlaxoWellcome, Hertfordshire, UK) used, was set to 60 $Lmin^{-1}$ to maintain a particle size cutoff of 6.4 μm.

Beneath the modified stage 1 assembly, a magnetic stirrer (MR 3002, Heidolf Elektro GmbH and Co. KG, Kelheim, Germany) was set to a constant stir rate of 100 rpm. It was important that this speed was not set too high as a vortex would have disturbed the formation of a gel above.

General Method of Preparation for Excipient Blends

For preparation of the excipient blends, 100.0 g of a given excipient was weighed into the mixing bowl of a high-shear mixer (Magimix™ Cuisine System 2000™, Montceau-Enbourgogne, Cedex, France). Then, 1.0 g of salbutamol base was dissolved into 30 ml of 95% ethanol. While mixing, the solution was added by 1-ml increments to the bowl, and 30 ml of distilled water was then added

to the mixing powder in 1-ml increments. During addition of the liquids, mixing was stopped, and the walls of the Magimix bowl were scraped with a flexible polyethylene spatula for every 10 ml of liquid added. The granules were then transferred to a metal tray for drying overnight in an oven (model IH-150, Gallenkemp, UK) set at 60°C.

Granules were premilled using a Retsch ZM1000 ultracentrifugal mill (Glen-Creston Instruments, Middlesex, UK) set at 14,000 rpm with a 24-tooth rotor, no retaining mesh, and an internal collecting pan.

Preparation of Variable Drug Concentration Blends

A series of different drug concentration blends was prepared by weighing 1.0, 0.8, and 0.5 g of salbutamol separately and dissolving into a 30-ml volume of 95% ethanol. Xanthan gum (100.0 g) was weighed and added to the Magimix bowl. The ethanolic solution was added in 1-ml increments while mixing occurred until all of the solution had been added. Next, 30 ml of water was also added in 1-ml increments, causing granulation of the blend. Scraping of the sides of the Magimix was performed as in the above method. The granules were oven dried at 60°C overnight.

The free-flowing, off-white powders produced, with a particle size of approximately 100 μm, were then milled using a Gem-T air pulverizer (Glen-Creston). Opposing jet pressures were set to 90 psi at the O inlet and 65 psi at the P inlet. The resulting fine powder was analyzed using low-angle laser light scattering (LALLS) on a Malvern Mastersizer X (Malvern Instruments Limited, Malvern, Worcestershire, UK). The fine powder was weighed into size 3 gelatin capsules to a weight of 20 ± 1 mg. Filled capsules were analyzed for content uniformity.

Diffusion Experiment Method

An F-2000 spectrophotofluorimeter (Hitachi Instruments, Tokyo, Japan), fitted with a 90-μl flow-through cell, was calibrated; excitation and emission wavelengths for salbutamol base were set to 279 nm and 305 nm, respectively. Polyethylene tubing was fitted to the Neoprene™ tubing, and the flow-through cell. The F-2000 was fitted with a contact closure timing device. This enabled measurements to be taken using the apparatus at varying intervals, which were set depending on the composition of the blend prepared.

The modified TSI apparatus was set up as in Fig. 2 (shown schematically in Fig. 3) using the specifications set out in the British Pharmacopoeia. The reservoir beneath the modified stage 1 assembly was filled with 300 ml of distilled water; a magnetic follower was placed in the reservoir, and the stirring rate was constant at 100 rpm using a Heidolf magnetic stirrer to ensure mixing throughout. A continuous loop of water was set up with the 300-ml reservoir of the modified stage 1 and the flow-through cell using the peristaltic pump (Watson-Marlow, Cornwall, UK) fitted with 0.5-mm bore Neoprene tubing and polyethylene tubing (1.5 mm internal diameter). The F-2000 spectrophotofluorimeter was blanked, and the magnetic stirrer was switched off.

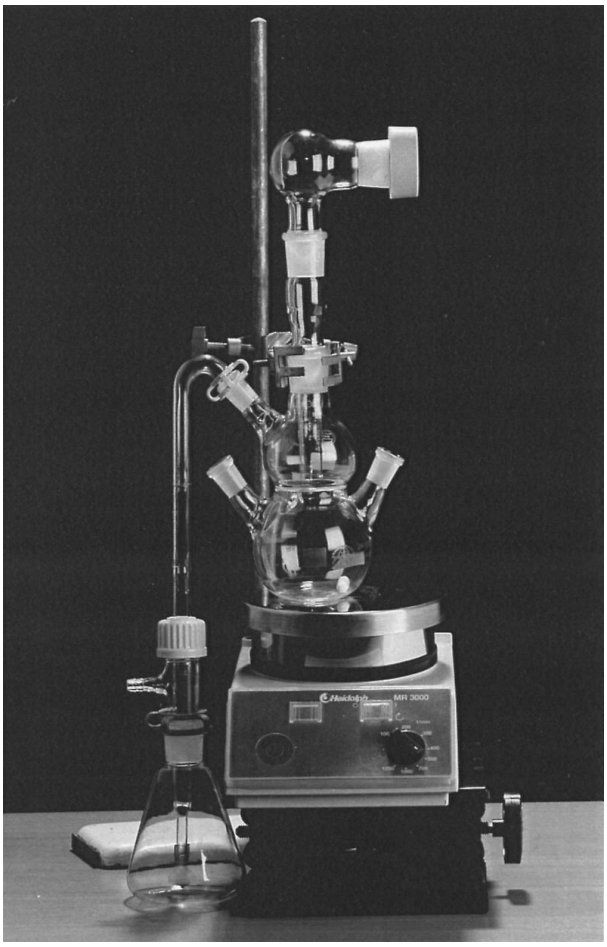

Figure 2. Full assembly of the modified TSI apparatus.

A Rotahaler device was used to fire five gelatin capsules individually into the modified TSI at a rate of 60 $Lmin^{-1}$ using an oil-less rotary vane pump (model 1475, Gast Manufacturing Company Limited, Buckinghamshire, UK). Each capsule was fired for 4 s to achieve an inhaled volume of 4 L of air per capsule, resulting in a total dose weight of 100 mg with a total drug content of 1000 μg. After firing, the magnetic stirrer was switched

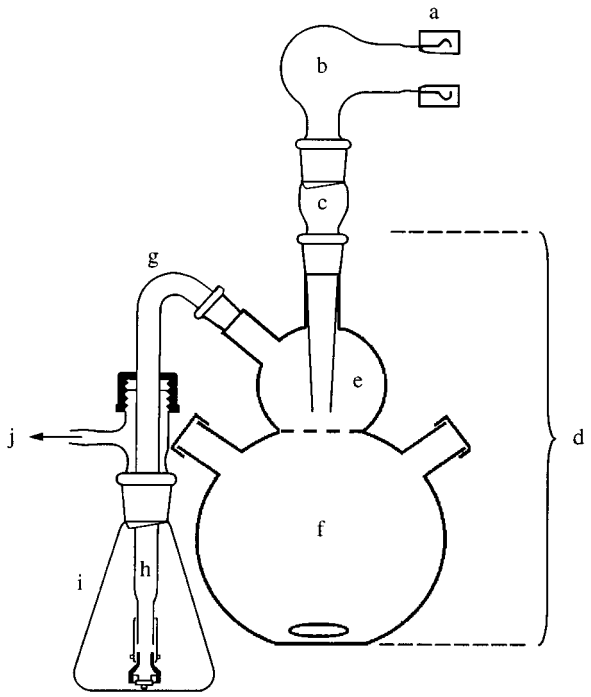

Figure 3. Modified TSI apparatus diagram: a, silicone mouthpiece adapter; b, mouth and throat adapter; c, stage 1 jet; d, modified stage 1 assembly; e, stage 1; f, 300-ml reservoir; g, stage 2 adapter; h, stage 2 jet; i, 250-ml Quickfit conical flask; j, to vacuum pump.

on, and the timing device was activated. Data points were collected at the set interval previously programmed into the timing device.

The mouth and throat sections (a, b) of the TSI were removed carefully and washed with distilled water into a 250-ml volume; the neck section (c) was also removed and washed into a 100-ml volume, and the stage 2 assembly was washed into a 250-ml volume. From these washings, the total emitted dose, the total recovered dose, and the respirable fraction were obtained for each experiment in addition to the release profile of the blend in terms of the percentage of drug diffusing across the 1-mm mesh over a given period of time. Each experiment was repeated at least three times to obtain concordance.

RESULTS

Average Particle Size Distributions

Particle size analysis of the dry powder blends (Malvern Mastersizer X) indicated that an average particle size range of approximately 10 μm was achieved. It was not necessary to produce particle sizes much smaller than this as all the diffusion profiles were obtained from stage 1 of the modified TSI. The stage 1 jet had not been modified and so had the standard particle size cutoff of 6.4 μm.

Modified Twin-Stage Impinger Diffusion Analysis of Variable Excipient Blends

After firing the 100 mg of the control blend into the modified stage 1, the powder was seen to pass through the 1-mm wire mesh and into the 300-ml reservoir as large aggregated particles. These quickly dispersed and began to dissolve. The modified TSI diffusion data for the 1:100 salbutamol:lactose control blend indicated a quick diffusion rate. With no polymer-based excipient in this particular formulation, total release of the salbutamol occurred in 6 min (Fig. 4).

After firing the 1:100 salbutamol:xanthan blend, the powder could be seen on the surface of the 300-ml reservoir. Over time, this expanded and formed a visible hydrogel structure that gently rested on top of the wire mesh above the reservoir. The average time of diffusion for this formulation was 600 min (Fig. 5), confirming that the xanthan gum had been able to retard considerably the release rate of the drug from this aerosolized dry powder blend.

Initially, on impact with the surface of the water in stage 1 of the modified TSI, the 1:100 salbutamol:LBG blend appeared to stay on the surface as loosely associated agglomerates. After a few minutes, these were seen

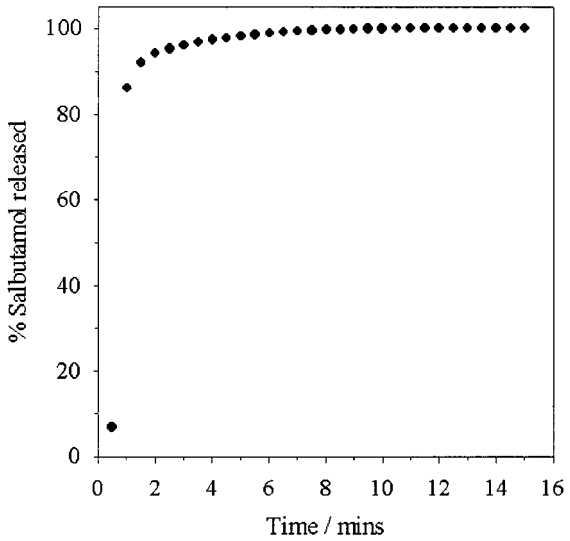

Figure 4. Average release profile for 1:100 salbutamol:lactose capsules ($n = 3$).

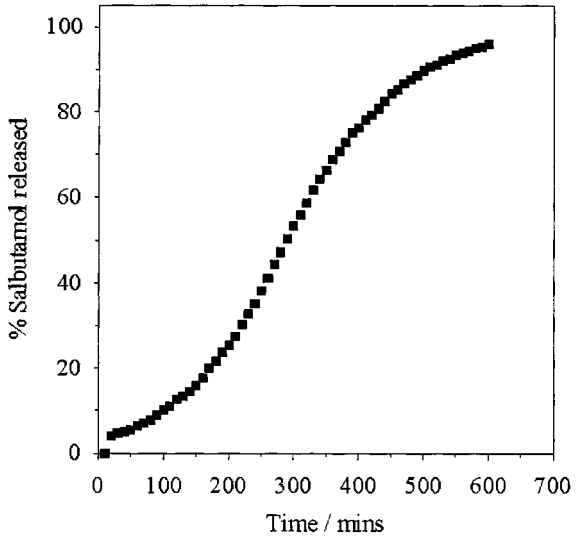

Figure 5. Average release profile of 1:100 salbutamol:xanthan gum capsules ($n = 3$).

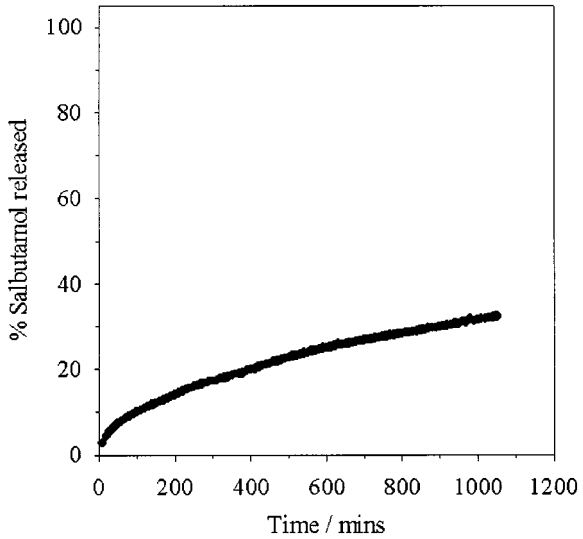

Figure 7. Average release profile of 1:100 salbutamol: (50 xanthan:50 LBG) capsules ($n = 3$).

to disperse gradually through the mesh into the reservoir. Visually, this was not unlike the behavior of the control blend, but the dispersion process was much more gradual. The average time for total drug diffusion into the reservoir was 100 min (Fig. 6), suggesting some retention of the drug had occurred compared to the control.

After the firing of the blend containing both xanthan gum and LBG with salbutamol, the powder settled on the surface of the liquid in the modified stage 1. Over a period of 10 min, the powder became wet throughout and formed the gel structure as seen with the 1:100 salbutamol:xanthan gum blend, which subsequently came to rest on the 1-mm mesh above the 300-ml reservoir. Only 23% of the total salbutamol was able to diffuse into the reservoir over a period of 600 min (Fig. 7).

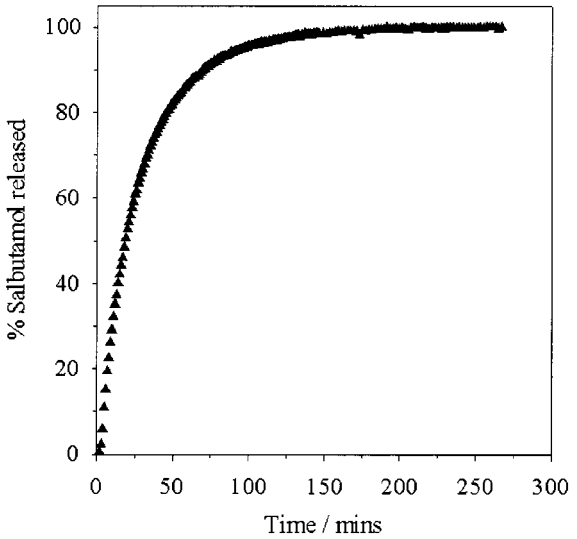

Figure 6. Average release profile of 1:100 salbutamol:LBG capsules ($n = 3$).

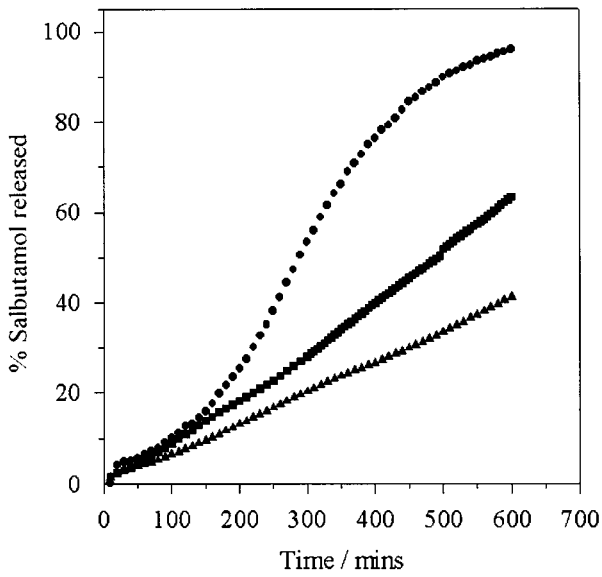

Figure 8. Comparison of average release profiles of variable drug concentration capsules ($n = 3$).

Modified Twin-Stage Impinger Diffusion Analysis of Variable Drug Content Blends

Firing of the various blends onto the surface of the liquid in the modified TSI showed similar characteristics. The 0.5, 0.8, and 1:100 blends of salbutamol: xanthan gum all began to wet on contact with the water. As more powder hit the surface, a ''dry'' area could be seen, caused by powder loading on top of other powder. The powder began to wet through in the first few minutes, and the formation of a gel was observed, which rested on the 1-mm brass mesh above the 300-ml reservoir.

Figure 8 illustrates the release profiles obtained from differing concentrations of salbutamol within the gel matrix.

DISCUSSION

On comparison of results from the different formulations tested as part of this study, we obtained a wide variation in release profiles that were able to be distinguished using the novel modified stage 1 of the TSI.

The control blend of 1:100 salbutamol:lactose, indicating the release rate of the drug with no retardation, took 6 min for 100% detection of salbutamol.

In contrast, for the 1:100 salbutamol:xanthan gum blend, total release of salbutamol occurred after an average of 600 min. Xanthan gum has a high affinity for water and is completely soluble (20). Rapid hydration of individual particles results in extensive swelling, causing them to come into contact and coalesce (20,21). This has thus also been shown to occur following deaggregation of particles prior to deposition in the TSI. The formation of a continuous viscoelastic gel matrix stops the drug from dispersing into the reservoir quickly.

Some retardation of the drug was observed with the 1:100 salbutamol:LBG blend, although to a lesser extent than the other formulations. In this instance, the LBG particles were dispersed quickly throughout the 300-ml reservoir of the modified TSI before they could undergo gelation. LBG is only slowly soluble and cross linking of LBG alone only occurs after prolonged exposure to the dissolution media, allowing time for the molecules to associate (22). This is not possible in the modified TSI due to the constant agitation of the solvent in the reservoir.

As would be expected, the 1:1 xanthan:LBG preparation retarded the release of the salbutamol most, indicating that a stronger gel was formed by the intermolecular interaction between xanthan and LBG (23,24). This prevented the dispersion of the salbutamol to such an extent that ultrasonification was needed to dissolve the hydrogel into solution and to obtain a total release value for the drug into the reservoir.

The comparison of the variable salbutamol concentration formulations indicated a trend showing a quicker release profile for formulations with higher concentrations of drug (Fig. 7), again distinguishable using the novel modified TSI. It is likely that the higher the concentration of salbutamol in the formulation, the greater the ''wetting'' of the xanthan gum will be over the duration of the experiment. This enables the hydrogel to disperse more quickly into the reservoir, and as a consequence, there is an increased release rate.

In conclusion, the modified stage 1 TSI apparatus proved to be capable of discriminating between drug release rates from different model controlled-release formulations for bronchial delivery. As with all in vitro models, this is a simplified representation that cannot take into full account the levels of branching of the airways in the human lung or the tissue surfaces that will influence the impaction of any formulation (24). It can, however, be of valuable use as a noninvasive research tool to compare and optimize drug release characteristics of potential controlled-release inhalation therapeutics. In vivo studies, of course, will be essential to evaluate clinical response (24) and to assess the mode of deposition, the effect of mucociliary clearance, and most important, the safety of potential carriers.

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