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Research paper

Preparation and characterisation of controlled release co-spray dried drug–polymer microparticles for inhalation 2: Evaluation of *in vitro* release profiling methodologies for controlled release respiratory aerosols

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Abstract

Three *in vitro* methodologies were evaluated as models for the analysis of drug release from controlled release (CR) microparticulates for inhalation. USP Apparatus 2 (dissolution model), USP Apparatus 4 (flow through model) and a modified Franz cell (diffusion model), were investigated using identical sink volumes and temperatures (1000 ml and 37 °C). Microparticulates containing DSCG and different percentages of PVA (0%, 30%, 50%, 70% and 90%) were used as model CR formulations. Evaluation of the release profiles of DSCG from the modified PVA formulations, suggested that all data fitted a Weibull distribution model with $R^2 \geqslant 0.942$. Statistical analysis of the t_d (time for 63.2% drug release) indicated that all methodologies could distinguish between microparticles that did or did not contain PVA (Students *t*-test, $p < 0.05$). However, only the diffusion model could differentiate between samples containing different PVA percentages. Similar results were observed when analysing the data using similarity and difference factors. Furthermore, analysis of the release kinetic profiles for all samples suggested the data fitted the Higuchi diffusion model ($R^2 \geqslant 0.862$ for the diffusion methodology data set). Due to the relatively low water content in the respiratory tract and the lack of differentiation between formulations for USP Apparatus 2 and 4, it is concluded that the diffusion model is more applicable for the evaluation of CR inhalation medicines.

Keywords: Inhalation; *In vitro* analysis; Controlled release; DPI; Dissolution apparatus; Flow trough cell; Modified Franz cell

1. Introduction

Pulmonary administration of drugs has been employed for many years for the treatment of localized disease states such as asthma. Furthermore, with the high surface area and permeability of the lung, the 21st century has seen a paradigm shift to inhaled therapy for systemic use. Although, many compounds are either in use or under investigation as potential respiratory medicines, little thought has gone into controlling the rate at which drugs are absorbed and metabolized. Local disease states, infectious respiratory pathogens and systemic delivery systems would benefit from control over release rate of the active pharmaceutical ingredient (API). For example, asthma, which in many cases has exacerbated effects overnight, would benefit from a treatment that would result in controlled release delivery, for instance of a β_2-agonist, over an 8-h period. Moreover, the treatment of chronic infections with local antibiotics would benefit from an increased API residence time in the lung, improving the antimicrobial activity whilst potentially reducing the high dosing regime that is commonly required for these rapidly metabolized molecules.

To date, there is no controlled release (CR) product for inhalation on the market, however, recently there has been

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a research focus within the field [1]. One of the potential hurdles in developing CR inhalation formulations is the use of suitable methodologies for *in vitro* evaluation. Particulate systems for inhalation are regularly tested in terms of particle size and aerodynamic diameter, using pharmacopoeia methodologies that are well established. For example, the deposition pattern of a particular formulation in the respiratory tract can be estimated using inertial impactor testing. However, for a CR inhalation system it becomes essential to evaluate the release of an API molecule from the formulation matrix as a function of time. Currently, no pharmacopoeia methodology exists for the evaluation of the *in vitro* release rates from CR respiratory medicines. Subsequently, the evaluation of release from these novel systems has been conducted using a variety of methods, which are based on different principles, rely on certain assumptions and allow little comparison between different CR approaches.

A range of pharmacopoeia methodologies exist for the testing of conventional solid dosage formulations, however, these systems generally are designed to mimic the GI tract and as such are based on 'sink' conditions. The most common apparatus used are United States Pharmacopoeia (USP) apparatus 1 and 2, which monitor the dissolution of a solid dosage form in a 900–1000 ml solution, either suspended in a rotating basket (apparatus 1) or resting below a rotating paddle (Apparatus 2) [2]. This methodology has recently been adopted by many researchers for the assessment of drug release from microparticulate formulations. For example, carbamazepine-loaded enteric microparticles [3] and spray dried CR sodium diclofenac microparticles [4] were recently investigated using USP Apparatus 2. In these cases, however, the microparticulates were not of respiratory size range (e.g. $\leqslant$6 μm [5]) and had mean diameters of the order 9.1–34.8 μm. This method was also recently used to test microparticles intended for pulmonary delivery by Huang et al., to assess the *in vitro* release of betamethasone from spray dried microparticles with different release modifiers [6], and by Asada et al. for the testing of co-spray dried theophylline-chitosan microparticles [7]. Interestingly, in the former study, dissolution measurements were performed in a smaller volume (300 ml). The approach of modifying a conventional dissolution apparatus for evaluating inhalation medicines was further investigated by McConville et al., where a conventional twin stage impinger was modified, to incorporate a dissolution compartment. Subsequently, using this apparatus, the aerosol properties and dissolution rates of CR inhalation formulations could be measured simultaneously [8].

An alternative to USP apparatus 1 and 2 is USP apparatus 4. Based on a continuous flow system published in 1972 [9], the USP apparatus 4 utilizes a reciprocal housing that contains a formulation, housed between two filters. A liquid flow is past through the housing and the output concentration monitored as a function of time [2]. Apparatus 4 has been successfully used to evaluate the dissolution profiles of poorly soluble and extended release powder formulations [10] and has been regarded as the method of choice for novel dosage forms including microspheres, liposome formulations and stents [11]. Recently, this method has been utilized to evaluate the *in vitro* dissolution rate of poorly soluble glucocorticoids, administered as aerosols [12]. In this study, it was concluded that a modified USP Apparatus 4 could be successfully used to examine aerosol formulations, which are intended to have extended residence time in the respiratory tract. Both methods described above are based on the evaluation of particle dissolution, in a media where the formulation is exposed to a volume-flow and sink conditions. Other studies investigating the dissolution of aerosol particles have utilized much smaller volumes. For example, volumes as small as 2 ml have been used to assess release rates of drug from microparticles under continuous stirring (150 rpm) or in a shaking water bath [13,14].

The respiratory tract has a large surface area ($>$100 m^2) [5] and contains approximately $\sim$1 μl cm^2 of liquid (generally endogenous phospholipids and mucus), present as a 10-μm layer [15]. Subsequently, evaluation of the dissolution profile of a CR drug particulate in a large volume may not be representative of the *in vivo* release rate. An alternative approach is to treat the release mechanism of drug from CR inhalation particles as analogous to a diffusion process, where the formulation is merely wetted and will diffuse out into the surrounding media. Recently, this approach was investigated by Cook et al., who used a custom built horizontal diffusion cell to determine variations in release rates of terbutaline sulphate from microparticles prepared by spray-drying the API with different concentrations of hydrophobic excipients [16].

Direct comparison between the various methodological approaches is difficult to make, since current literature is based on different CR formulation methods. Furthermore, the literature in this field is generally very barren and information is very limited about *in vitro* testing of release from particulate systems. Subsequently, the focus of this paper is to evaluate three methods for the measurement of drug release from inhalation CR formulations. The methods studied are (1) the conventional USP Apparatus 2 paddle method [2], (2) the modified USP apparatus 4 flow through system [10] and (3) a diffusion model based on a transdermal diffusion Franz cell [17].

To study these methods, a series of model CR inhalation formulations were prepared. Disodium cromoglycate (DSCG), a prophylaxis drug used for severe bronchial asthma, was chosen as model compound. DSCG is water-soluble and has low molecular weight. The CR component of the formulation was polyvinyl alcohol (PVA). PVA was chosen as a model polymer since it was recently reported that viscous solutions containing only 1% w/w PVA altered the *in vivo* release of 5(6)-carboxyfluorescein in an *in vivo* rat model [18].

2. Materials and methods

2.1. Materials

Disodium cromoglycate (DSCG) was obtained from Sanofi-Aventis (Cheshire, England). Polyvinyl alcohol (PVA) was supplied from BDH Ltd. (Poole, England). The molecular weight of PVA was approximately 22,000 and the minimum degree of hydrolysis was 98%. Water was purified by reverse osmosis (Milli-Q, Millipore, Sydney, Australia).

2.2. CR microparticulate preparation

A series of model controlled release (CR) microparticle formulations, containing DSCG, were prepared by spray drying from aqueous PVA solutions. Variation in the potential CR profile was achieved by varying the concentrations of PVA in the aqueous solution prior to spray drying (0%, 30%, 50%, 70% and 90% w/w). All the solutions were spray dried using a laboratory scale spray dryer (Büchi Mini Spray Dryer B-191, Switzerland), with the following conditions: inlet temperature 140 °C, measured outlet temperature 70–75 °C, air flow 700 NL h^{-1} and solution feed rate 4 ml min^{-1}.

All the generated particulates were characterised as previously described by the authors in terms of particle size, crystal structure, density, surface morphology, moisture sorption, surface energy and *in vitro* aerosolisation efficiency. All the particles were found to be amorphous, of spherical geometries and similar particle sizes [19]. In general, the average median volume diameter across all DSCG CR formulations was 3.15 ± 0.375 μm, when measured by laser diffraction. The similarity in particle diameters allowed confident comparison of release profiles between formulations and methodologies.

2.3. Sample analysis

The concentration of DSCG released from the CR microparticles was analysed using spectrophotometry. The concentration of DSCG, released from the CR microparticles, was analysed at $\lambda = 245$ nm in an aqueous solution using a Hitachi U2000 spectrophotometer. Standards of DSCG were prepared in phosphate buffer pH 7.4 (0.05 M KH_2PO_4, Biolab, Australia) and linearity over the range 1.0–20.0 μg ml^{-1} ($R^2 = 0.999$) was observed. Samples spiked with PVA did not interfere with the calibration curve.

2.4. Dissolution studies

As previously discussed, the release profiles for the DSCG CR microparticles were investigated using three methodologies: USP apparatus 2, a flow through cell (modified USP apparatus 4) and modified Franz diffusion cell. To maintain consistency, all tests were conducted on 20 mg samples of the CR microparticles using 1 L of degassed dissolution medium (0.05 M phosphate buffer, pH 7.4) at 37 °C ± 0.5 °C. Each sample was analysed in triplicate. Cumulative percentages of drug release from the tested particles were calculated based on a total drug content for each formulation. This was achieved by dissolving any remaining CR microparticles in solution at 90 °C for a minimum of 5 min to insure dissolution of the PVA and full release of any remaining drug material [20]. Details of each specific methodology are given below.

2.4.1. United State Pharmacopoeia Apparatus 2

A Vanderkamp six spindle dissolution tester (VanKel Industries, Inc., Edison, NJ, USA) was fitted with six 1 L dissolution vessels and Apparatus 2 paddle set. The paddle rotation speed was set at 50 rpm and the vessels, containing the dissolution medium described previously, maintained at 37 °C. Approximately, 20 mg of formulation was added to each of the dissolution vessels and 3 ml samples were drawn at selected time intervals using a filter tip dissolution filter, 2 μm (Varian, Sydney, Australia). All the samples were tested in triplicate and randomized for PVA concentration.

2.4.2. Flow through apparatus

A modified USP apparatus 4, flow through dissolution apparatus, developed by Davies and Feddah [12], was assembled as shown in Fig. 1A. The dissolution media equilibrated at 37 °C in a water bath reservoir were

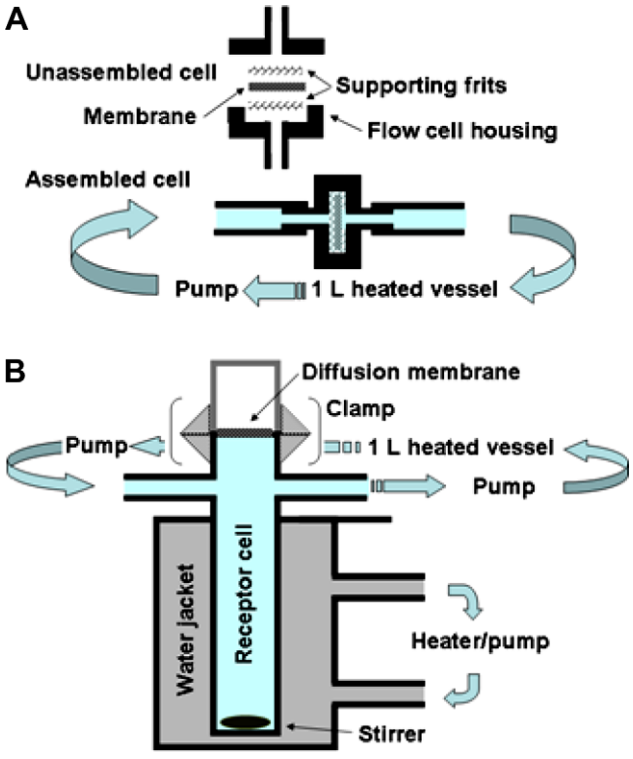

Fig. 1. (A) Schematic of the flow through dissolution apparatus; (B) schematic of the diffusion-Franz cell apparatus.

pumped, using a Tygon® tubing (I.D. 1.59 mm, Saint Gobain Performance Plastics, USA) through the system using an HPLC pump (model 426 Alltech, Illinois, USA), at a constant flow rate of 0.5 ml min^{-1}. Approximately, 20 mg of CR microparticles was accurately weighed onto a 0.45 μm nitrocellulose membrane filter (MF™ Membrane Filters, Millipore, Bedford, MA, USA), which was then sandwiched with a second membrane filter and positioned between the metal mesh screens inside the filter adaptor. The flow exposure area was 2.5 cm in diameter and the flow direction was maintained vertically through the assembly to avoid dead volumes. The dissolution media exiting the flow cell was returned to the 1 L reservoir, where samples (3 ml) were collected at pre-determined intervals for analysis as in the USP method 2, described above. At the end of the experimental procedure, the flow cell was disassembled and remaining particulates dissolved as previously described to attain total mass recovery. All the samples were repeated in triplicate and randomised for PVA concentration.

2.4.3. Diffusion apparatus

The diffusion apparatus was based on a modified Franz diffusion cell [17], which was assembled as shown in Fig. 1B. As with the flow through apparatus, the degassed dissolution medium (1 L) was equilibrated at 37 ± 0.5 °C in a water bath and pumped at 5 ml min^{-1} via a peristaltic pump (Minipuls 3, Gilson, France), to the inlet port of the Franz cell. The sampling port of the cell was connected to the pump that, in turn, returned the sample flow to the 1 L vessel. This set-up ensured the volume in the Franz cell remained constant and the 0.45 μm filter membrane remained constantly and uniformly wetted. The membrane diameter available for diffusion was 2.5 cm, as in the flow through apparatus. The continual renewal of dissolution medium, along with the magnetic stirrer in the bottom of the Franz cell ensured sink conditions. When the system was equilibrated and stable, the pre-weighed powder sample was evenly spread on the previously soaked membrane filter in the Franz cell. As with the flow through apparatus, 3 ml samples were taken from the 1 L reservoir at predetermined time intervals and assayed. At the end of the analysis period, any remaining CR microparticles were dissolved using the method described previously. All the samples were analysed in triplicate and randomised for PVA concentration.

2.5. Data analysis

The release rates from the model CR microparticles systems were analysed using a series of statistical and kinetic models to evaluate both the formulations performances and the validity of testing methodologies.

2.5.1. Similarity and difference factors

The dissolution release data obtained using the three different methodologies were statistically analysed and compared using Fit Factors described by Moore and Flanner [21] adopted by the Food and Drug Administration guidance for dissolution testing [22]. Briefly, Fit Factors are model independent methods that directly compare the difference between percent drug released per unit time for a test and a reference product. The difference factor (f_1) and the similarity factor (f_2) can be calculated to compare different pairs of dissolution profiles using Eqs. (1) and (2), respectively:

Difference factor (f_1)

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| / \sum_{t=1}^{n} R_t \right] \right\} \times 100 \quad (1)$$

Similarity factor (f_2)

$$f_2 = 50 \times \log \left\{ \left[1 + (1/n) \sum (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

Where n is the number of dissolution sample times, R_t and T_t the mean percent drug released at each time point: t, for the reference and the test dissolution profiles, respectively. The difference factor (f_1) calculates the percent difference between the two curves at each time point and is a measure of the relative error between the two curves. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percentage released between curves.

In simple terms, for curves to be considered similar, f_1 values should be close to zero (between 0 and 15) and f_2 values should be close to 100 (between 50 and 100). It should be noted that in a study by Polli et al., however, limiting f_2 value of 50 was found to be conservative when comparing two metoprolol tartarate formulations and finding them to be different depending on this scale while they were proven to be bioequivalent [23]. For data analysis, arbitrary descriptors of difference and similarity need to be chosen. Subsequently, arbitrary analysis values where: if $f_1 \geqslant 10$ or $f_2 \leqslant 50$, a curve was considered different.

2.5.2. Analysis of DSCG release kinetics using mathematical modelling

The DSCG release data obtained from the three different methodologies, using the spray-dried DSCG CR microparticles, were fitted to the following release kinetic functions: Zero order, First order, Higuchi, Hixon–Crowell, and the following mathematical curve fitting models: Weibull, Logistic and Gompertz. These functions and related equations are summarized in Table 1 [23–28]. Data were fitted using Origin™ v6.0 (Microcal Software, Inc., MA, USA) and the linearised form of each function evaluated using R^2 regression analysis. The best-fit mathematical model derived parameter was further used to compare the dissolution profiles of the different formulations tested using the students t-test, where results were found to be significantly different based upon 95% probability values.

Table 1
Mathematical functions describing release rate of DSCG from the CR microparticulate systems

Function	Equation
Zero order	$r_{\%} = kt$
First order	$r_{\%} = 100[1 - e^{-kt}]$
Hixon–Crowell	$r_{\%} = 100\left[1 - (1 - \frac{kt}{4.6416})^3\right]$
Higuchi	$r_{\%} = kt^{0.5}$
Weibull	$r_{\%} = 1 - e^{-(\frac{t}{td})^{\beta}}$
Logistic	$r_{\%} = \frac{A}{[1-e^{-k(t-\gamma)}]}$
Gompertz	$r_{\%} = Ae^{-e-k(t-\gamma)}$

$r_{\%}$ = percentage drug released at time t; k = rate constant; t_d = mean dissolution time (i.e. time at which 63.2% of material had been released); β = shape factor; γ = scale factor.

3. Results and discussion

As discussed, the release rates of DSCG from each of the CR microparticle formulations were assessed using three methodologies. Drug concentrations throughout each experiment were measured and expressed in terms of percent total recovery. Data were subsequently plotted as mean cumulative percentages (±standard deviation) of drug released versus time. The release profiles of DSCG microparticulate formulations containing different percentages of PVA using USP Apparatus 2, USP Apparatus 4 and the modified Franz diffusion cell are shown in Fig. 2. In general, variations in the release profiles between methodologies and formulations were observed. Such observations suggest that the CR profile of simple micro-particle systems could be modified with the addition of polymers such as PVA. Furthermore, variations in the release profiles between each technique suggest that the experimental approach to CR measurement is sensitive to the methodology chosen. The release profiles with respect to PVA concentration and methodologies are discussed below.

3.1. Analysis of similarity and difference factors between CR formulations

Analysis of the similarity and difference factors (Table 2) for the release rate of DSCG CR microparticles using USP Apparatus 2 suggested all the curves to be similar (where $f_1 < 10$ and $f_2 > 50$). As seen in Fig. 2A, the cumulative release curves followed a similar trend with the concentration of DSCG being released, at any time point, being independent of PVA concentration. The DSCG CR microparticles have a high surface area to volume ratio, and DSCG is highly soluble. Subsequently, in a large volume dissolution bath, the diffusion of DSCG through the PVA matrix into the sink is likely to be high. Additional analysis of the similarity and difference factors between curves containing different paired concentrations of PVA (i.e. 30 and 50% PVA; 50 and 70% PVA; 70 and 90% PVA), suggested all the curves to be similar. Undoubtedly, the USP Apparatus 2 dissolution method is the easiest and the most convenient methodology to study release profiles

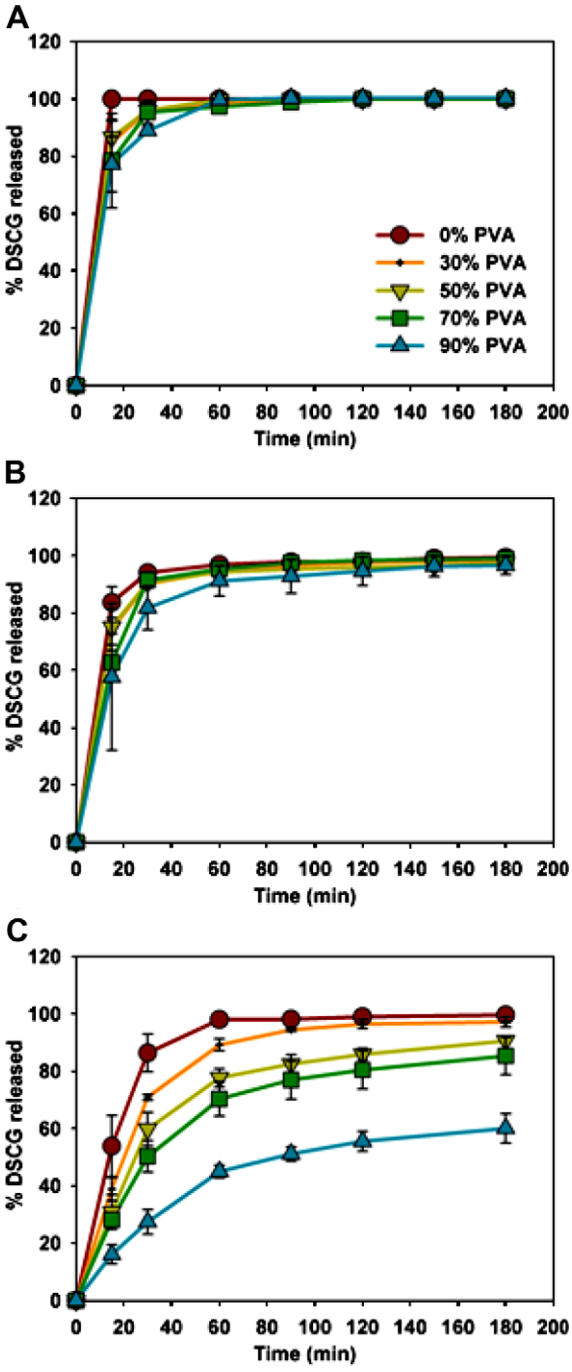

Fig. 2. Release profiles for DSCG CR microparticles using: (A) USP Apparatus 2; (B) flow through apparatus and (C) diffusion-Franz cell apparatus.

of CR formulations, however, for particles of respiratory size range, it is unlikely to be suitable, since it becomes difficult to differentiate between different CR microparticulate formulations.

The release profiles of DSCG CR microparticles using the USP Apparatus 4, flow through model, is shown in Fig. 2B. As with USP Apparatus 2, the release profiles of DSCG from CR microparticles appeared similar. Analysis of the difference and similarity factors (Table 2), suggested that all DSCG microparticles containing different

Table 2
Similarity factors (f_2) and difference factors (f_1) for the DSCG formulations

Reference formulation	Test formulation	f_2	f_1
USP Apparatus 2			
0%	30%	62.42	2.96
0%	50%	64.97	2.52
0%	70%	55.16	4.22
0%	90%	52.34	5.01
30%	50%	NA	NA
50%	70%	NA	NA
70%	90%	NA	NA
Flow through model			
0%	30%	70.82	2.89
0%	50%	70.21	3.63
0%	70%	56	3.98
0%	90%	48	8.74
30%	50%	NA	NA
50%	70%	NA	NA
70%	90%	NA	NA
Diffusion model			
0%	30%	52.08	8.97
0%	50%	37.77	20.13
0%	70%	31.77	26.87
0%	90%	17.94	52.32
30%	50%	51.21	12.26
50%	70%	61.17	9.21
70%	90%	33.36	34.81

concentrations of PVA (with the exception of 90% PVA) had similar release curves. As can be seen from Table 2, the f_1 value increases (and consequently f_2 decreases) as the PVA concentration is increased relative to the 0% PVA control, resulting in a difference in curve profile when comparing 90% PVA to the control DSCG microparticles.

The release rates of DSCG from CR microparticles using the Franz diffusion cell are shown in Fig. 2C. As can be seen from Fig. 2C, the release of DSCG from microparticles, containing different quantities of PVA, suggested different release rates and profiles. Analysis of the similarity and difference factors (Table 2), confirmed all DSCG CR microparticle formulations, with the exception of the 30% PVA formulation, had different release curves than the control DSCG particles. In addition, analysis of paired data sets indicated that all the curves were different with the exception of the 50% and 70% PVA paired formulation, which had 'borderline' f_1 and f_2 values of 9.21 and 61.17, respectively. Interestingly, this can be seen when comparing 50% and 70% PVA curves in Fig. 2C, where although clear variations in the percentage released at any time point exist, the profile of the curve is similar. In comparison to USP Apparatus 2 and 4, it can be concluded that the use of a diffusion model allows greater differentiation between the release rates of soluble drug molecules, such as DSCG, from CR microparticulate formulations, containing simple polymers such as PVA.

3.2. Analysis of release kinetics and mathematical models for the CR formulations

Based on the correlation coefficient criterion, R^2, the best overall mathematical function, to describe the release profile (as outlined in Table 1) was the Weibull distribution. Regression analysis of Weibull, First order and Higuchi models is shown in Table 3. As seen from Table 3, the Weibull model best described the data fit for all testing methodologies where $R^2 \geqslant 0.942$ were observed.

However, the Weibull distribution has been reported to satisfactorily describe all common types of regular dissolution curves, in particular exponential and sigmoidal forms [25].

To further evaluate the differences between the release rate methodologies and the formulations, the percent drug released at 60 min and the Weibull time parameter (t_d), which corresponds to the time to release 63.2% of the drug, was calculated and is shown in Table 4. Both the percent released after 60 min and the t_d was statistically analysed using the Students t-test, where $p < 0.05$ was classed as significant.

Statistical analysis of drug released from DSCG microparticles containing different quantities of PVA suggested that the t_d was significantly different, when comparing samples that contained no PVA for all the methodologies. Such observations are interesting, since similarity and difference factors indicated that the curves produced using USP Apparatus 2 and 4 were similar. However, it is important to note that the analysis of release curves using similarity and difference factors is subjective, since arbitrary values of f_1 and f_2 have to be chosen to assess difference and similarity. Furthermore, paired analysis of the t_d for samples containing different amounts of PVA (i.e. 30–50%, 30–70% etc.) suggested no significant differences using either USP Apparatus 2 or 4 methodologies, suggesting that although these methods could potentially distinguish between pure DSCG and PVA containing samples, it was not sensitive to differentiate between samples containing

Table 3
Correlation coefficient of Weibull, First order and Higuchi models for DSCG dissolution profiles

Methodology	% w/w PVA	R^2		
		Weibull	First order	Higuchi
USP Apparatus 2	30	0.998	0.659	0.767
	50	0.998	0.664	0.758
	70	0.997	0.533	0.794
	90	0.998	0.742	0.836
Flow through model	30	0.991	0.529	0.672
	50	0.995	0.483	0.657
	70	0.991	0.405	0.723
	90	0.980	0.509	0.779
Diffusion model	30	0.942	0.527	0.862
	50	0.996	0.581	0.903
	70	0.999	0.641	0.931
	90	0.999	0.711	0.964

Table 4
Percentage of DSCG drug released at 60 min and the Weibull function derived mean dissolution time (t_d) for the different formulations tested

Methodology	Formulation % w/w PVA	% Drug released at 60 min ± SE	Weibull t_d (min) ± SE
USP Apparatus 2	0	100.00 ± 0.37	2.55 ± 0.50
	30	98.90 ± 0.37	8.26 ± 1.22
	50	99.70 ± 0.32	8.93 ± 0.79
	70	97.35 ± 1.14	9.37 ± 1.21
	90	99.77 ± 0.19	9.96 ± 0.67
Flow through model	0	96.96 ± 0.52	9.08 ± 0.84
	30	94.51 ± 1.14	12.08 ± 0.39
	50	94.25 ± 1.11	11.53 ± 0.39
	70	95.39 ± 1.3	14.22 ± 0.95
	90	91.17 ± 3.07	19.29 ± 5.05
Diffusion model	0	98.02 ± 0.35	15.75 ± 1.87
	30	89.27 ± 1.19	26.48 ± 0.86
	50	77.65 ± 1.85	38.35 ± 2.72
	70	70.31 ± 3.40	48.23 ± 4.29
	90	44.94 ± 1.29	169.71 ± 13.56

*Not available data due to instantaneous dissolution.

different concentrations of PVA. Statistical analysis of the percentage drug released for different formulations at 60 min showed no significant differences for either USP Apparatus 2 or 4, since in all the cases, the dissolution of DSCG approached 100%.

In comparison, analysis of the t_d and percentage DSCG released after 60 min, using the diffusion model, suggested significant differences between all the samples containing different amounts of PVA. Subsequently, it can be concluded that the diffusion model is a more applicable model for differentiating between similar formulations with high surface area to volume ratios, and that statistical analysis of such a diffusion model can be fitted and statistically modelled using the mathematical Weibull distribution.

Interestingly, amongst the release kinetic determinant models, the Higuchi-diffusion model resulted in the highest R^2 values (Table 3) when compared to the Zero order, First order, and Hixon–Crowell for all the formulations and methodologies. The R^2 values were highest when evaluating the diffusion methodology ($R^2 \geqslant 0.862$), suggesting that the mechanism of release was through wetting and diffusion. In practice, it is common to see both diffusion and dissolution mechanisms operative in a given dosage form, although one of the mechanisms usually predominates over the other. This can present problems in the *in vitro* evaluation of controlled release products [27]. However, when water soluble drugs are dispersed or dissolved in hydrophilic matrices, the release rate is controlled mostly by drug diffusion from a gel barrier rather than polymer dissolution [29,30]. It has been reported that the release of a water soluble drug from a hydrogel system generally involves the simultaneous absorption of water and desorption of drug via a swelling controlled mechanism, which does not follow a Fickian behaviour [31,32]. Furthermore, swelling and solvent penetration mechanisms are not directly determined because hydrogel systems do not have easily defined sample geometry. For example, as water penetrates a glassy hydrogel matrix containing a dissolved drug, the polymer swells, its glass transition temperature is lowered and the dissolved drug diffuses through the swollen rubbery phase into the external releasing medium; consequently, drug release behaviour may range from Fickian to zero order extremes. Furthermore, different drugs alter both the swelling osmotic pressure and the associated time-dependant relaxation of the network. However, this simplified explanation is further complicated when dealing with very small particle size (<5 μm), where each particle behaves solely rather than, as reported in the literature, like the release profiles from much larger beads (>50 μm diameter), or from a whole tablet.

4. Conclusions

A series of methodologies for determining the release rate from CR microparticles, containing DSCG and PVA, have been evaluated. Of these methodologies, a modified Franz diffusion cell provided the greatest differentiation between formulations, showing significant difference between release profiles. Furthermore, the analysis of the release profiles suggested the data fitted a Higuchi diffusion model. Due to the physiology of the lung, this model and methodology is likely to be the most useful for the investigation of release rates in CR microparticulate systems used in inhalation. Since inhalation therapy is becoming a widespread delivery route, the potential for controlled released respiratory formulations is high. However, in order to evaluate such systems, a standard test is required, since this will be utilised, not only to assess *in vitro* formulation performance, but also for the quality control of the final products. Although data collected in this study has shown that the modified Franz cell apparatus was the most promising methodology, further studies are needed to correlate this *in vitro* dissolution test with *in vivo* data.

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