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# Characterisation of the aggregation behaviour in a salmeterol and fluticasone propionate inhalation aerosol system

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**Abstract**

The nature of the drug–drug aggregation phenomena between salmeterol xinafoate and fluticasone propionate used in a metered-dose inhaler system has been examined. Interactions between the drugs in the solvents 1,1,2-trichlorotrifluoroethane (CFC-113) and 1,1,1,2-tetrafluoroethane (HFA-134a) have been characterised using a focused beam reflectance measurement probe by measuring the average floc size of the drug particles individually and in combination as a function of stirrer rate. The floc composition in the CFC-113 system, where the drug particles cream, was determined by high-performance liquid chromatography analysis. The aggregation behaviour of the individual drugs was shown to depend on the physical and chemical properties of both the drug substance and the media. Larger flocs were observed for salmeterol xinafoate compared with fluticasone propionate, while both drugs formed larger aggregates in HFA-134a compared with in CFC-113. The floc composition studies demonstrated that, in the combined formulation in CFC-113, salmeterol xinafoate and fluticasone propionate aggregate together to form hetero-flocs. The interaction between the two drugs was such that they did not separate on creaming, despite having different densities. The average floc size of the combined drug suspension was also found to depend on the dispersion medium. 

*Keywords:* Salmeterol xinafoate; Fluticasone propionate; HFA-134a; CFC-113; Aggregation; Focused beam reflectance

## 1. Introduction

Metered-dose inhalers (MDIs) are the most frequently employed dosage forms for delivering active drug substances to the respiratory tract via the inhaled route. MDIs contain fine micronised drugs in a suspension of chlorofluorocarbon

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(CFC) or hydrofluoroalkane (HFA) propellants, which act as both a suspending medium and propellant. Current formulations are predominantly suspensions of single micronised active compound in the range 2–5 μm in liquefied CFCs or HFAs. The physical and chemical properties of the single-drug formulations are, in principle, easier to study. Combined drug formulations are less well understood and, as a result, a significant amount of fundamental scientific research work on the subject is being undertaken. Previous work (Michael et al., 2000) on the combined inhaler formulations of both salmeterol xinafoate and fluticasone propionate in propellant CFC-113 indicates that hetero-aggregation of the two drugs would appear to be taking place. The hetero-aggregation results in reduced drug deposition on to the MDI surfaces as compared with that observed with the individual drug formulations. This phenomenon could be an advantage from a pharmaceutical point of view, i.e. the combination formulation shows a decrease in the total loss of the drugs due to deposition on to the internal surfaces of the MDI. Clinical studies have indicated that drug formulation of salmeterol xinafoate and fluticasone propionate in a single inhaler provides a treatment as efficacious in achieving asthma control and as well tolerated over a 28-week period as the two drugs administered individually (Chapman et al., 1999). However, a clearer understanding of the aggregation behaviour of salmeterol xinafoate and fluticasone propionate will facilitate the design of combination aerosol systems.

A number of methods were considered in an attempt to characterise the aggregation behaviour of suspensions in MDIs. Microscopy is a commonly used technique that allows an optical inspection of the particles and can be used to judge whether a good dispersion has been achieved or if any aggregation is present in the system (Rawle, 1999). Light-diffraction techniques (Sidhu et al., 1993) are also used for the study of flocs in liquid suspensions, and the particles in suspension are measured by re-circulating the sample in front of the laser beam. This technique was, however, found to be unsuitable due to the high optical absorbance of the concentrated suspensions, which made scattering measurements difficult due to low signals and multiple scattering. Rheological methods (Sidhu et al., 1993) have also been used to study the extent of flocculation in inhalation systems. However, these methods normally make use of high solid-phase concentrations of the dispersed materials as compared with those normally encountered in commercial metered-dose inhalers. In addition, small-angle light-scattering techniques have also been used to study the size and structural dynamics of flocs (Spicer et al., 1998); however, flocs in MDIs would be too large for this techniques.

The objective of the work reported herein was to gain a better understanding of the physical and chemical factors involved in the combined formulation of salmeterol and fluticasone propionate in a single MDI aerosol system. The aggregation of salmeterol xinafoate and fluticasone propionate in HFA-134a and CFC-113 was studied using a focused beam reflectance measurement (FBRM), which enables floc size to be determined as a function of stirrer rate. In addition, a floc composition study of the CFC-based combined drug formulation was performed to monitor the migration behaviour of the individual drugs within the suspension.

## 2. Materials and methods

### 2.1. Reagents

The test samples of salmeterol xinafoate and fluticasone propionate were donated by GlaxoSmithKline Research and Development (Ware, UK), and were used as received. The propellants 1,1,2-trichlorotrifloroethane (CFC-113) and 1,1,1,2-tetrafluoroethane (HFA-134a), with a water content of less than 10 p.p.m., were obtained from ICI Chemicals and Polymers Ltd (Runcorn, Cheshire, UK). Poly(vinyl chloride) (PVC)-coated glass aerosol bottles were supplied by Wheaton Ltd (NJ, USA) and 63 μl metering valves were purchased from Valois S.A. (Le Neubourg, France).

### 2.2. Focused beam reflectance measurement

The aggregation behaviour of the suspensions was studied by FBRM, using a Lasentec Labtec 1000 instrument (Lasentec Inc, Redmond, USA) (Allen and Davies, 1998). The apparatus uses a focused laser diode beam source that is projected on to the sample at a fixed velocity and rapidly scans across the particle structures. As the focused beam transcribes a particle or floc structure, light from the incident beam is reflected back until the beam reaches the opposite edge of the particle. The back-scattered light is collected by a pair of stereoscopic photodiode detectors and converted into an electronic signal. The chord length of the particle or aggregate can then be determined from the product of the pulse duration and the velocity of the scanning laser. The chord lengths measured over a specific time period are sorted into a 38-chord distribution that covers the size range 0.4–250 μm. Because the beam does not intersect the particle in the same way every time, the so-called 'random chord length distribution' of the particles is measured (Fig. 1). During one measurement cycle, adjusted to 8 s, thousands of particles are identified. To produce robust statistics, 2000 or more particles need to be sampled per second. An advantage of FBRM over forward scattering or transmission sizing techniques is that opaque dispersions can be analysed. Hence, experiments can be performed at realistic concentrations, without the need for sample dilution.

Unlike many other particle sizing techniques, pressurised systems can be readily studied using the Lasentec Labtec 100 without the need for building a complex pressure cell. Instead, samples may be presented in cylindrical glass-coated aerosol bottles. Nevertheless, an important aspect of the Lasentec system is that the hydrodynamic conditions must be such that a representative sample flows past the probe window. This cannot always be guaranteed in practice, causing systematic errors or noisy signals (Dijkstra et al., 1996). Measurement of the floc size of the suspensions can be hindered by either gravitational sedimentation or creaming, if the densities of the solid and liquid phase are unequal. To avoid this problem, the suspensions were agitated by means of a magnetic stirrer. By varying the rotational velocity of the magnetic stirrer, it is possible to control the shear conditions within the sample. The shear stress in the aerosol bottle filled with HFA-134a at a stirrer speed of 1000 rev $min^{-1}$, was estimated by assuming laminar flow and was found to be of the order of 0.025 N $m^{-2}$.

Data from the experiments were collected by analysing the samples at three equidistant positions on the aerosol bottle. At each position, two particle size determinations were performed, with each determination being the mean of five measurements, averaged by the instrument software. The floc size measurement of one sample was, therefore, the average of salmeterol xinafoate and fluticasone propionate six particle size determinations from three different positions on the bottle. In this way, errors due to imperfections in the plastic-coated aerosol bottles could be minimised.

### 2.3. Floc size analysis

Model suspensions in HFA-134a were prepared by pressure filling propellant through a valve attached to a pressure-resistant 15 ml round clear PVC-coated glass aerosol bottle, to which known

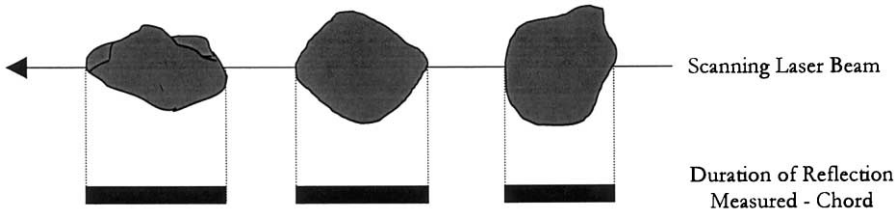


Fig. 1. An illustration of the normalised chord length distribution of a particulate species.

quantity of the drugs had been added. Individual drug suspensions over the concentration range 0.02–0.50% w/w salmeterol xinafoate and fluticasone propionate were prepared. Suspensions containing 0.05% w/w salmeterol xinafoate together with 0.07% w/w fluticasone propionate were used to study aggregation behaviour in the combination product. These concentrations are equivalent to the strengths of salmeterol and fluticasone propionate combination products that were marketed initially in the United Kingdom from July 2000. A glass-coated magnetic stirrer bar ($13 \times 5$ mm$^2$) was added to each aerosol bottle before a metering valve was crimped into place (Pamasol model type 2002). The bottles were filled with approximately 18 g HFA-134a via a pressure burette, which was pressurised at about 5 bar with HFA-134a at room temperature. Each of the formulations was prepared in triplicate. The aerosol bottles were sonicated (Decon FS300 ultra-sonic bath) for 10 min to remove any entrapped air from the suspension and to promote dispersion. After equilibration, the suspensions were re-weighed to ensure that significant amounts of propellant had not been lost due to evaporation. Drug suspensions in CFC-113 were prepared in the same way, except that the CFC was filled directly into the aerosol bottles prior to being sealed with the metering valve.

The median chord length of the individual drug suspensions in the two propellants was measured as a function of drug concentration using the FBRM system at a constant stirrer rate of 300 rev min$^{-1}$. The robustness of the flocs was investigated by measuring the average floc size of the drug suspension as a function of increasing stirrer speed up to a maximum of 1500 rev min$^{-1}$. The suspension was allowed to equilibrate for 5 min at each speed setting before measurements were made.

The reproducibility of the Lasentec FBRM response was checked by performing repeat measurements of an external standard of aqueous graphite dispersion at a constant stirrer rate of 250 rev min$^{-1}$ and interspersed with the samples throughout the measurements. The median chord length distribution of the graphite standard was repeatedly found to be $6.00 \pm 0.33$ μm. Furthermore, the particle size of the graphite standard was found to be independent of stirrer speed.

### 2.4. Electrophoretic mobility studies

The electrophoretic mobility of the suspended drug particles was determined using a Malvern Zetasizer 3000 (Malvern Instruments, UK) in conjunction with a non-aqueous dip cell that has a narrow electrode gap of 2 mm. Individual dispersions of 0.0025% w/w salmeterol xinafoate and 0.0035% w/w fluticasone propionate were prepared in CFC-113 to give a suspension of suitable conductance. The dispersions were ultrasonicated for 10 min prior to analysis to ensure that the drug was fully dispersed.

### 2.5. Floc composition studies

Floc composition studies were performed on suspensions of the two drugs to examine the settling behaviour of the drug particles in the absence of shear. Individual and combined dispersion concentrations of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate were studied in CFC-113 solvent only, as the high vapour pressure of HFA-134a propellant makes sampling difficult. Suspensions were prepared as described in Section 2.3 except that the aerosol bottles were sealed with a rubber septum. Using a glass syringe, 1 ml suspension was slowly removed from a position approximately one-quarter of the way from the bottom of the aerosol bottle without re-dispersing the sample. The dispersions were sampled at time intervals of 0, 1.5, 5 and 10 min and the 1 ml aliquots transferred to 50 ml volumetric flasks. Fig. 2 shows photographs taken to illustrate the separation behaviour of concentrated drug dispersion after a time interval of 10 min. The collected samples were left open to the atmosphere to allow evaporation of the CFC solvent, and the drug then dissolved in 70/30 (v/v) methanol/water and assayed by high-performance liquid chromatography (HPLC) with UV detection at 228 nm. The details of the HPLC analytical method have previously been described by Michael et al. (2000).

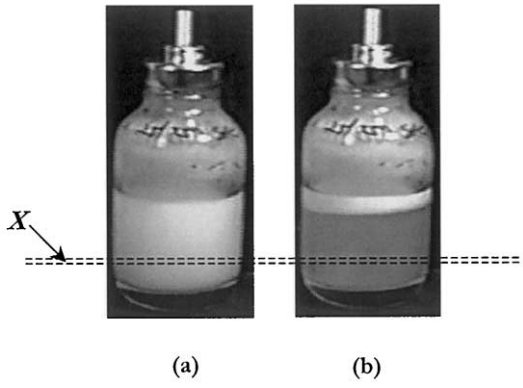


Fig. 2. Photographs illustrating the settling behaviour of concentrated drug dispersion at (a) 0 min and (b) 10 min intervals. Samples for floc composition studies were taken from position $x$ using a syringe.

## 3. Results and discussions

Characterisation of the MDI formulations initially concentrated on understanding the dispersibility of the individual drug suspensions in CFC-113 and HFA-134a aerosol propellants, and was then extended to examine the combined drug system at different drug concentrations and molar ratios of drugs.

Suspension concentration is an important factor in determining the behaviour of suspensions of drugs in liquid suspensions. In the absence of shear, the drug particles tend to flocculate, which leads to a separation of the solid phase due to the relative density difference between the drug and the liquid continuous phase. When the suspension is subjected to stirring, random collisions of drug particles with the walls of the container and with each other can result in breakdown of the floc structure. Plots of the average aggregate size as a function of the suspension concentration of salmeterol xinafoate and fluticasone propionate in HFA-134a and CFC-113, at a constant stirrer speed of 300 rev $min^{-1}$, are shown in Fig. 3. In all cases, the floc size of the suspensions increased with increasing drug concentration. Bower et al. (1996) have reported that in a system of constant shear stress the disruptive force is unchanged and therefore increasing the drug concentration results in the equilibrium being shifted towards aggregate formation.

Fig. 3 illustrates that the average floc size of the salmeterol xinafoate aggregates is larger than that of the fluticasone propionate aggregates, in both CFC and HFA media. This may be partially due to salmeterol xinafoate having a lower density (1.2 g $cm^{-1}$ as determined by helium pycnometry) compared with that of fluticasone propionate (1.3 g $cm^{-1}$), and therefore occupying a larger phase volume at the same w/w concentration. However, the nature of the drug may also influence the aggregation behaviour in the suspensions. The electrophoretic mobilities of salmeterol xinafoate and fluticasone propionate in CFC-113 were found to be $3 \times 10^{-9}$ and $1 \times 10^{-9}$ $m^2$ $s^{-1}$ $V^{-1}$, respectively, at 15°C. This reflects the fact that a higher charge is present on the surface of salmeterol xinafoate compared with that of fluticasone propionate, which is believed to increase the polarity of salmeterol xinafoate relative to fluticasone propionate. It would be expected that the hydrophobic fluticasone propionate particles would be wetted more readily in the non-polar CFC-113 environment than the salmeterol xinafoate particles. This could explain why fluticasone propionate forms more compact aggregates than salmeterol xinafoate.

The results presented in Fig. 3 also illustrate that the suspending medium has a considerable effect on the floc size of the drug suspension. The average floc size of both salmeterol xinafoate and fluticasone propionate was larger in HFA-134a as compared with in CFC-113. For diffusion-limited aggregation, the aggregation kinetics of the suspension will be dependent on the viscosity of the medium. Einstein's law of diffusion, states that the diffusion coefficient of spherical particles is given by (Shaw, 1992): $D = (kT)/(6\pi\eta a)$, where $k$ is Boltzmann's constant, $T$ is the absolute temperature, $\eta$ is the viscosity and $a$ is the particle radius.

Comparing the liquid viscosity of HFA-134a (0.20 mPa s) with that of CFC-113 (0.66 mPa s) at 25°C (Solvay, 1992), it can be seen that the diffusion coefficient of drug particles in HFA-134a is approximately three times faster than in CFC-113. Hence, under equivalent shear conditions, the faster rate of coagulation would be expected to result in the observed floc size increase.

In addition, the dipole moment of HFA is known to be greater than that of CFC (Blondino and Byron 1998). The greater polarity of HFA combined with the presence of hydrogen substituents allows for considerable charge separation in this propellant molecule, owing to the electronegativity of the fluorine atoms (Blondino and Byron 1998). The presence of a layer of oriented dipolar molecules at the surface may make a significant contribution to the nature of the electrical double layer (Shaw, 1992). This polarisation effect induces a temporary dipole on the drug molecule without changing the net surface charge of the drug. This may lead to an increase in the van der Waals force of attraction between drug particles and therefore an increased tendency to form larger flocs in the HFA medium compared with in CFC (Fig. 3).

Fig. 4 shows how the average floc size of the drugs varies with increasing stirrer speed in HFA-134a and CFC-113. In all cases, the average floc size of the suspensions initially decreased sharply as the stirrer rate was increased, before becoming less shear dependent at higher stirrer speeds. For example, the floc size of the combined drug dispersion of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate decreased from 101.2 to 39.5 μm in HFA-134a and from 19.8 to 8.2 μm in CFC-113, i.e. an approximately 60% reduction of the original size of the floc over the range 200–1000 rev $min^{-1}$. The shape of these profiles is attributed to the structural breakdown of floc structure as they are subjected to higher shear forces. However, it is noticeable that the flocs persisted even at the highest shear rate studied, suggesting that the aggregates are not broken down to the size of the input drugs (2–5 μm). The average floc size of the combined formulation of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate in CFC-113 was greater than that of the individual drugs. This was expected on the basis of the higher drug concentra-

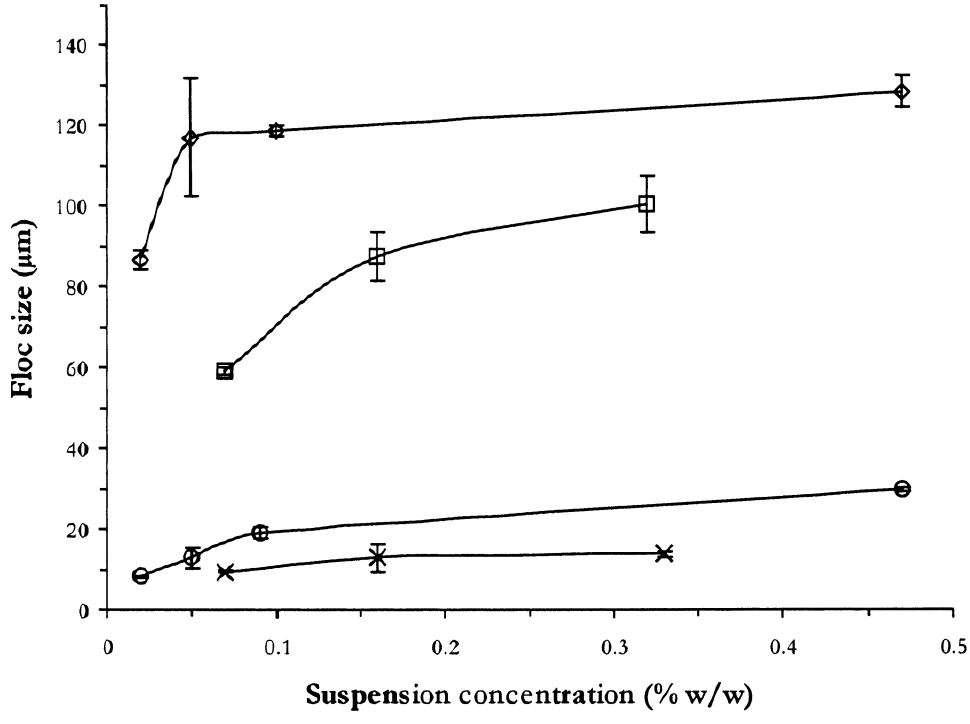


Fig. 3. Effect of increasing suspension concentration on average floc size of salmeterol xinafoate aggregates in (◇) HFA-134a and (○) CFC-113, and fluticasone propionate aggregates in (□) HFA-134a and ( × ) CFC-113. All measurements were made at a stirrer rate of 300 rev $min^{-1}$.

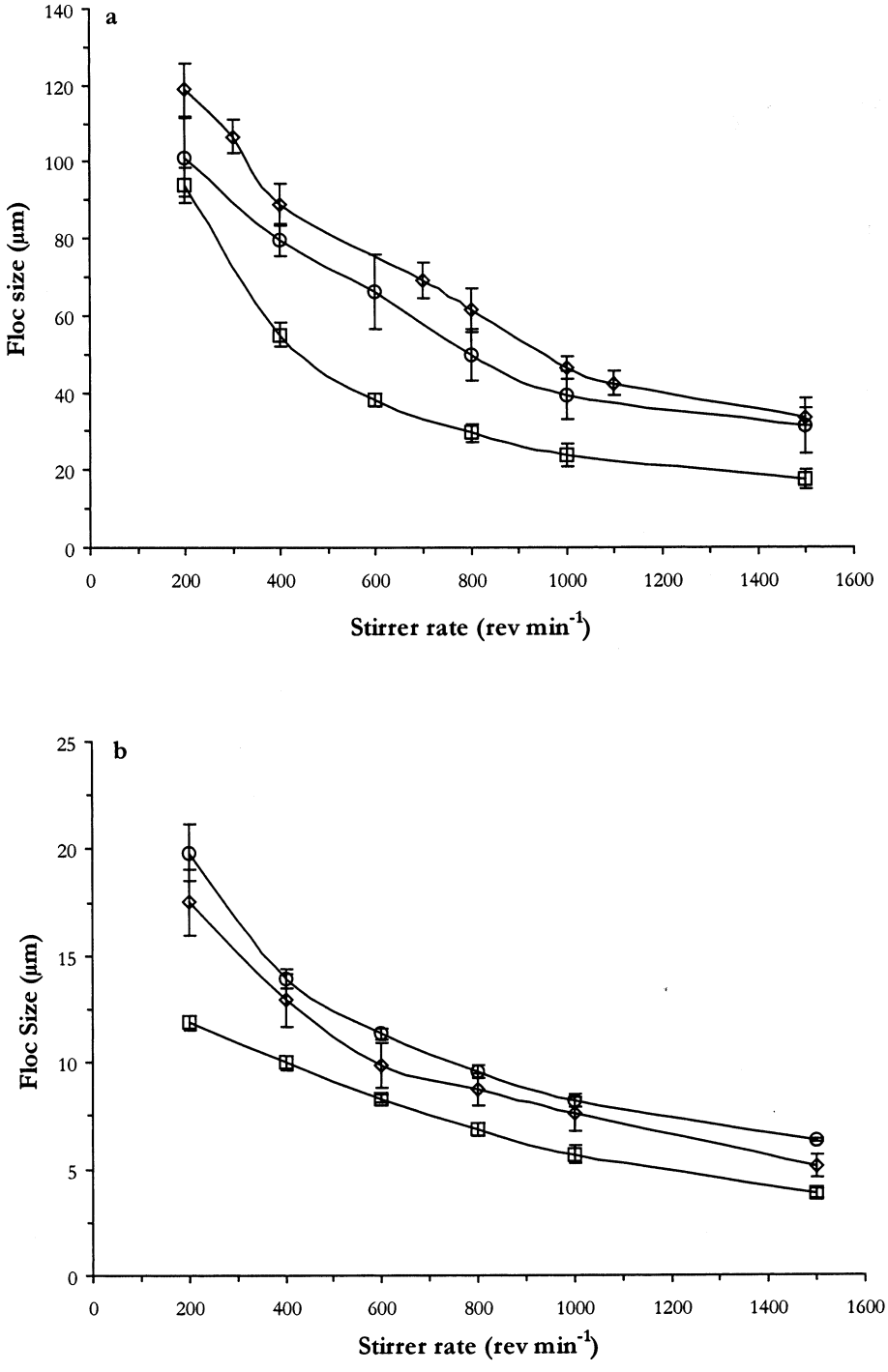


Fig. 4. Effect of increasing stirrer rate on the average floc size of (◇) 0.05% w/w salmeterol xinafoate, (□) 0.07% w/w fluticasone propionate, and (○) 0.05% w/w salmeterol xinafoate/0.07% w/w fluticasone propionate aggregates in (a) HFA-134a and (b) CFC-113.

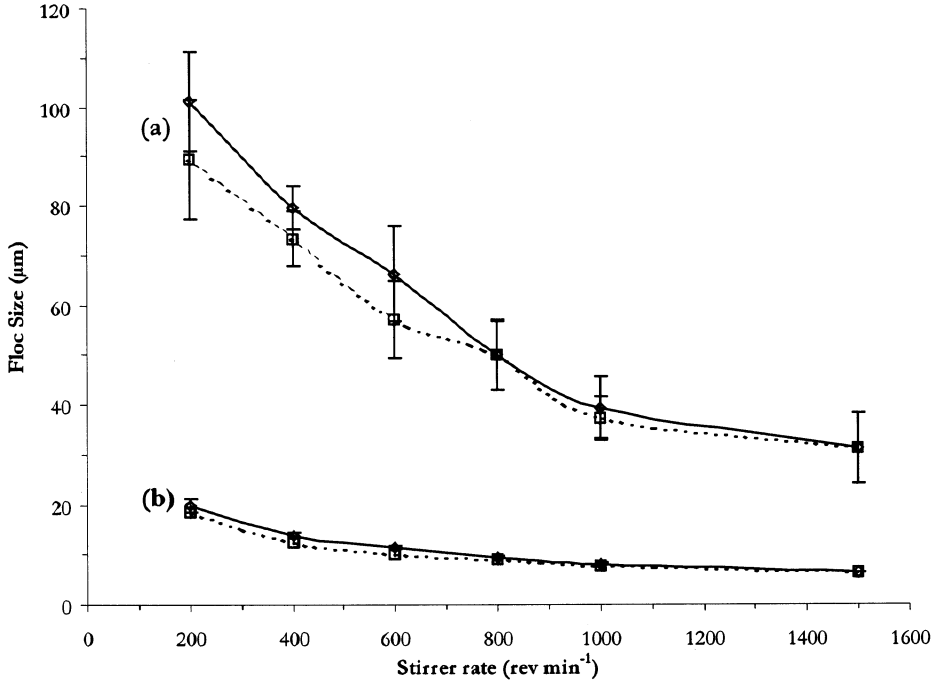


Fig. 5. Average floc size measurement of the combined drug formulation of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate as a function of increasing and decreasing stirrer rate in (a) HFA-134a and (b) CFC-113: ◇, increasing rate; □, decreasing rate.

tion of the combined formulation. In contrast, the floc size of the combined formulation in HFA-134a appeared to be an average of that of the individual drugs, over the entire stirrer speed range. Hence, it would appear that the interaction between salmeterol xinafoate and fluticasone propionate in the combined formulation is dependent on the physical properties of the solvent. This in turn leads to differences in the aggregation behaviour of the two drugs. Wang (1992) studied the hetero-flocculation of particles of different sizes in an apatite–hematite–phlogopite dispersion system. The studies showed that particles in mixed suspensions were affected by each other's stability in a way that the size growth of the less stable particles increased the hetero-flocculation rate constant and caused the loss of flocculation selectivity, especially between the middle and large size particles. This indicates that a difference in floc size has an effect on the mutual interaction between the particles in the dispersion, which can promote hetero-flocculation in preference to homo-flocculation.

In order to determine whether the breakdown and re-formulation of the hetero-floc structures is a reversible process, the effect of increasing and decreasing the stirrer speed on the average floc size was investigated. Fig. 5 shows that the average floc size of the combined drug formulation of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate in CFC-113 was the same in both increasing and decreasing shear experiments. In the case of the combined formulation in HFA-134a, there was a slight hysteresis effect, but most of the floc structure recovered. The interparticulate binding between salmeterol xinafoate and fluticasone propionate is a reversible process. However, it is apparent that the drug particles had not completely returned to their original state after an equilibration period of 5 min. Further work performing additional measurements after different equilibration times could therefore

provide an insight into the flocculation kinetics of these systems.

The composition of the floc structures of single and combined formulations of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate in CFC-113 was determined as a function of time (see Fig. 6). Initially, post agitation, the suspensions are well dispersed. This is followed by macroscopic flocculation, which in turn leads to phase separation between the drug particles and the dispersion medium. The drug particles migrate to the air/liquid surface of the sample (creaming phenomena) and, as a result, the drug concentration at the bottom of the sample decreases over a period of time. Although all the plots in Fig. 6 show a similar characteristic decrease in drug concentration with time, the data demonstrate that there are significant differences between the individual and combined suspension formulations. The densities of salmeterol xinafoate and fluticasone propionate are approximately 1.2 and 1.3 g $cm^{-3}$, respectively. Hence, both the drugs cream in CFC-113, which has a density of 1.57 g $cm^{-3}$ at 25°C. Since fluticasone propionate is denser than salmeterol xinafoate, it creams less rapidly as shown in Fig. 6. It is also interesting to note that, in the combined formulation, salmeterol xinafoate and fluticasone propionate creamed at approximately the same rate. If there were no interparticulate interactions between the two drugs, then they would be expected to separate on creaming as a consequence of their different densities. Hence, this data suggests that salmeterol xinafoate and fluticasone propionate must be inextricably bound in a hetero-flocculated state. The hetero-flocs cream faster than either of the individual drugs. This may be attributed to the higher solids concentration of the combined formulation, giving rise to a larger floc size, as shown in Fig. 4.

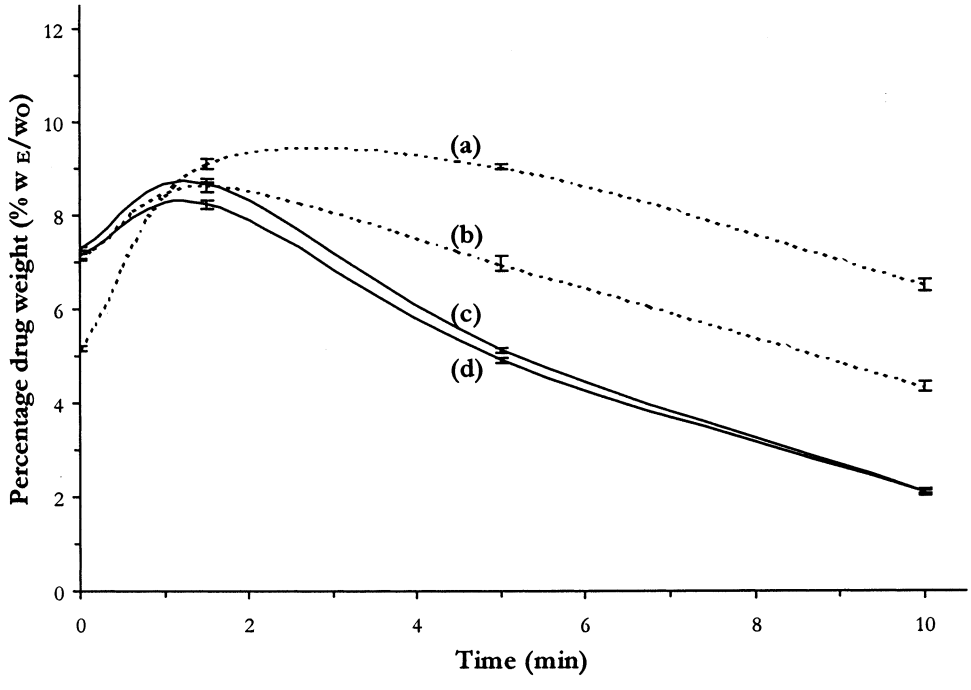


Fig. 6. Percentage drug weight measurement from a flocculated suspension at different time intervals for single and combined drug formulations in CFC-113: (a) 0.07% w/w fluticasone propionate, (b) 0.05% w/w salmeterol xinafoate, (c) fluticasone propionate from a combined formulation of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate, and (d) salmeterol xinafoate from a combined formulation of 0.05% w/w salmeterol xinafoate and 0.07% w/w fluticasone propionate. $w_E/w_O$, Percentage ratio of experimental weight relative to the original weight of the drug.

## 4. Conclusions

The focus of this paper has been to gain an understanding of the interaction between salmeterol xinafoate and fluticasone propionate in single and combined formulations. The study demonstrates that the formulation behaviour of the individual drugs is dependent on the physical and chemical properties of both the media and the drug substance itself. In CFC-113, the two drugs hetero-flocculate in the combination product and consequently do not separate under the influence of gravity. Furthermore, the interaction between the two drugs leads flocculation behaviour that differs from that of the individual drugs. This interaction again appears to depend on the physical properties of the medium.

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