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An investigation of the use of UV fluorescence microscopy as a method for quantifying the homogeneity of powder mixes

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Summary

An experimental study was made to assess a potential new method for quantifying the homogeneity of pharmaceutical ordered mixes. A UV fluorescence microscope fitted with a photomultiplier system was used to determine the distribution of fluorescing fine drug particles on the surface of non-fluorescing coarser excipient particles after mixing. The concentration of drug in a given field of view was related to the fluorescence level determined by measuring the current output from the photomultiplier. The change in current levels measured in different sample fields was used to quantify homogeneity. Mechanistic interpretation of data was also possible by comparison of quantitative and qualitative microscopical analyses. Homogeneity data obtained using UV fluorescence microscopic analysis was compared with data obtained from similar experiments using UV spectrophotometric analysis. It was found that the two methods produced quantitatively similar results. It was considered that the fluorescence microscope method offered several potential advantages over other conventionally used analytical methods for assessing powder homogeneity.

Introduction

Assessment of powder homogeneity is important in determining mixer performance and efficiency, degree of mixing and extent of segregation, especially in pharmaceutical systems where relatively small concentrations of drug particles must be mixed with inert excipient particles to a high degree of content uniformity.

Several different methods have been used in assessing the degree of homogeneity of powder systems.

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Crooks and Ho (1976) determined the homogeneity of mixtures of sulphaphenazole in Celutab and Dipac, two different direct tableting sugars, by removing samples of fixed mass which were then dissolved in 0.5% sodium carbonate solution and assayed using UV spectrophotometry. Adaptations of spectrophotometry are the most frequently cited methods of assaying samples for content uniformity (Yip and Hersey, 1977; Hersey et al., 1979; Lai et al., 1981; Malmqvist and Nystroem, 1984). Other methods which have been employed include a conductance method, used by Staniforth and Rees (1982), to determine the homogeneity of potassium chloride in different tableting excipients. The conductance method has the advantage over UV spectrophotometric meth-

ods in that a very wide range of concentrations can be accurately determined without need for dilutions. Harwood (1977) used a non-invasive sampling method to monitor the movement of a radioactive band of sand particles through beds of non-radioactive sand. Although this method was able to follow change in distribution of radioactive tracer sand, no attempt was made to characterise homogeneity per se.

The aim of the present study was to develop an alternative, non-invasive method for quantifying the homogeneity of a pharmaceutical powder mix, using a modified UV fluorescence microscope.

Methods and Materials

Preparation of ordered mixes

Crystalline sucrose (British Sugar, Peterborough, U.K.) was used as a coarse carrier powder for the formation of ordered mixes with a fine drug powder, triamterene (Sigma Chemical Co., Poole, U.K.) having a particle diameter less than 10 μm at a concentration of 0.025% w/w. Mixing was carried out using a drum blender (Pascall Engineering, Crawley, U.K.) which had a capacity of 2.5 dm^3 and was rotated at approximately 50 rpm. Powder mixing was carried out for up to 60 min and during this time the blender was stopped at different intervals to allow samples to be removed for analysis of drug content uniformity.

Mechanical stability testing of ordered mixes

Following blending to an acceptable homogeneity, the powder mixes were filled into a specially constructed perspex container shown in Fig. 1A. A powder-tight perspex lid was fitted to the open side of the container which was then clamped to a vibration table (type VP4, Derritron, Hastings, U.K.) as shown in Fig. 1B. An accelerometer was fixed to the horizontal lower end of the containing clamp and the output was fed to a vibration level monitor (type DCA4, Derritron). This arrangement enabled the R.M.S. acceleration of the powder container to be quantified and the vibration frequency was measured using a digital frequency counter connected to the output of a signal generator and power amplifier (type

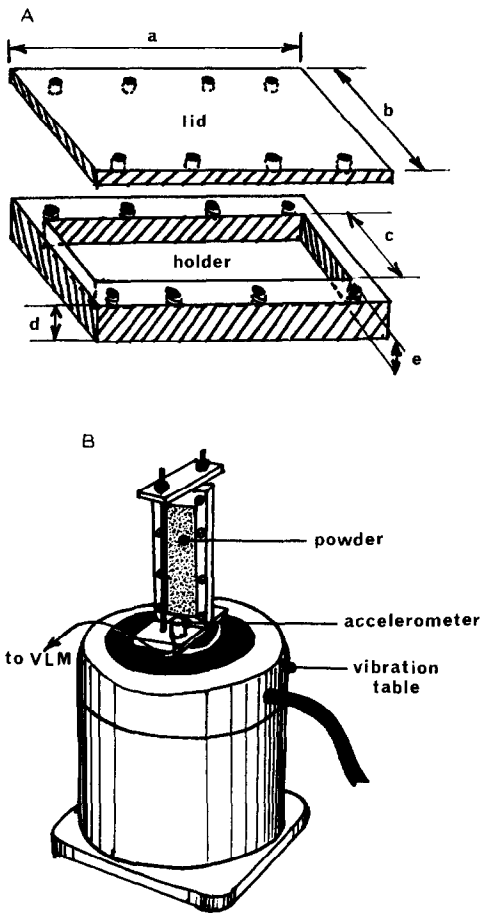

Fig. 1. A: diagram of powder holder showing dimensions used for construction: a = 15.3 cm; b = 6 cm; c = 3.5 cm; d = 1.4 cm; e = 0.5 cm. B: Diagram showing powder holder clamped in position on vibration table.

TA1201, Derritron). The powder mixes were vibrated at a frequency of 50 Hz and an acceleration of 29.43 $m \cdot s^{-2}$ (3 g), for periods up to 1 h. Following vibration, the homogeneity of the powder mixes was analyzed.

Determination of the homogeneity of powder mixes

(a) Fluorescence microscope method

The drug selected for this study, triamterene, is one of a group of compounds including other drugs such as tetracycline, which fluoresces when irradiated with UV light. Using a fluorescence microscope it was possible to identify individual

fluorescing drug particles in a powder whose other constituent particles did not fluoresce. In addition to simple identification of drug particles in a given powder specimen or field of view, the level of fluorescence was also measured using a photomultiplier system. The method described below was designed to allow the level of fluorescence at different locations in powder samples to be quantified so that statistical comparison of fluorescence data could be used to assess the homogeneity of a given powder mix.

The UV fluorescence microscope system (Zeiss, Oberkochen, F.R.G.) was set up as shown in Fig. 2. After mixing for a given time, powder was sampled at random from the drum blender and transferred to a perspex container (Fig. 1A) so that the upper exposed surface of powder was level and smooth. The perspex container was then placed on the microscope stage where it was illuminated from above by a focussed beam of UV light at a wavelength of 290 nm. Any triamterene particles in the field of view fluoresced in the incident UV light. Emitted light in the visible region of the spectrum was focussed and passed to a video camera (ITG Ikegami, Japan) and a photomultiplier tube (Øltronix, Sweden). The current generated by the photomultiplier was measured using a nanoammeter (Keithley 610C, OH, U.S.A.) connected to a chart recorder (type 210, Servogor, Crawley, U.K.). To determine the homogeneity of an ordered mix, 10 readings of current were taken from different areas of the powder bed. Before each current reading was made, the microscope was adjusted to focus on the upper exposed layer of particles. The magnitude of the current produced was found through preliminary calibrations to be proportional to the concentration of triamterene in the field of view. The homogeneity of a given powder system was determined by calculating the coefficient of variation (CV%) of the 10 sample current readings.

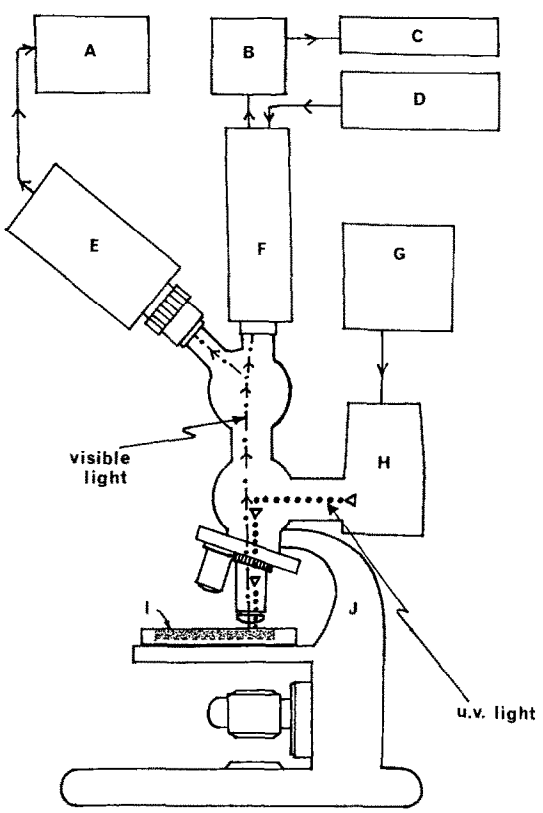

Fig. 2. Schematic diagram of UV fluorescence microscope system used to quantify homogeneity of powder mixes. Key: A = video monitor; B = nanoammeter; C = chart recorder; D = H.T. power supply; E = video camera; F = photomultiplier; G = power supply; H = super pressure mercury lamp (UV light source); I = powder sample; J = microscope body.

(b) UV spectrophotometer method

Samples each weighing 500 mg were removed from 10 randomly selected points in the powder bed and dissolved in 50 ml of distilled water. The concentration of triamterene in solution was determined using a UV spectrophotometric assay at a wavelength of 365 nm. As with the fluorescence microscope method described above, coefficients of variation of the 10 spot sample concentrations were calculated as a method of quantifying the homogeneity of a given powder system.

Results and Discussion

The processes of formation and segregation of a binary powder containing triamterene in crystalline sucrose, were monitored using both UV fluorescence microscopy and UV spectrophotometry (Figs. 3 and 4). In both methods, the powders were mixed in a drum blender for 60 min and

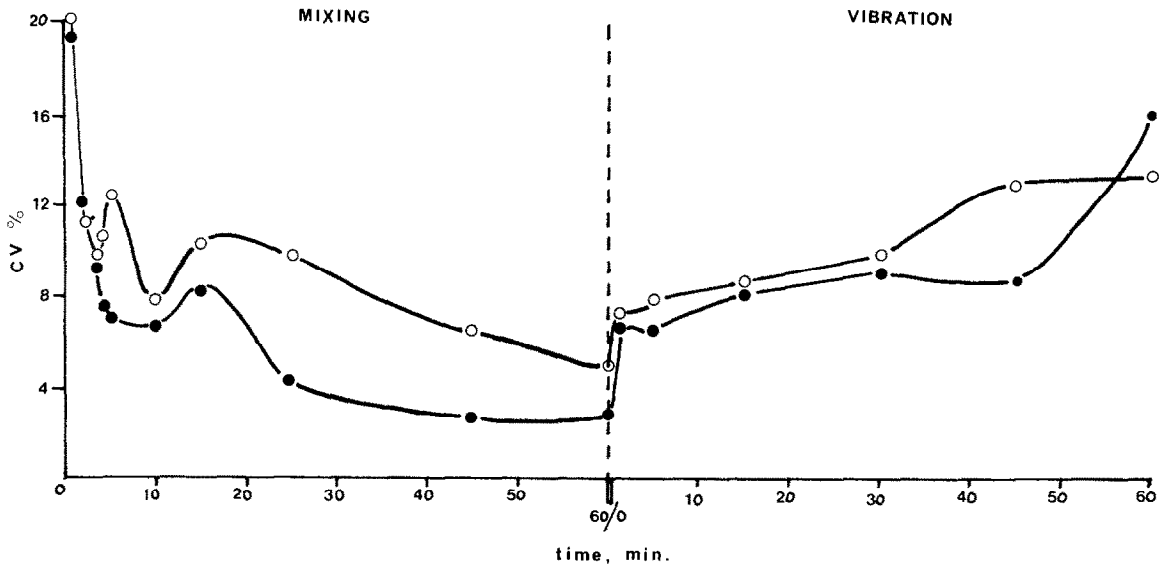

Fig. 3. Change in homogeneity of two replicate powder systems containing triamterene and sucrose particles during 60 min mixing and vibration, quantified using a UV fluorescence microscope system. Key: ○ = (a); ● = (b).

during this period the homogeneity of powder samples improved from a CV > 20% to < 6%.

In the first 20 min of mixing, a rapid improvement in homogeneity occurred, although there were periods of de-mixing which were found to occur reproducibly after approximately 5 and 15 min blending. Both methods of quantifying homogeneity yielded similar curves in this region and both

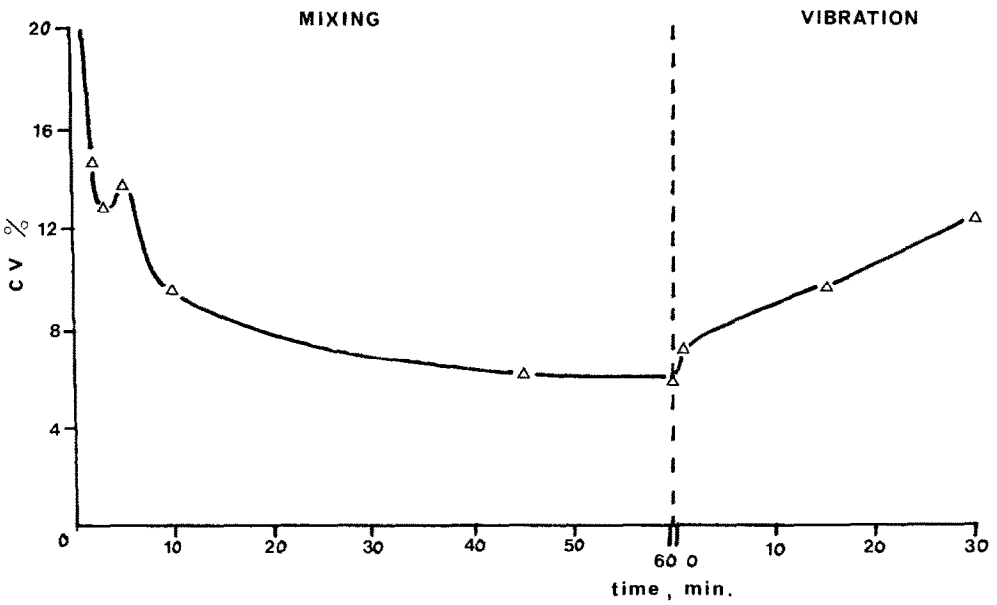

Fig. 4. Change in homogeneity of a powder system containing triamterene and sucrose particles during 60 min mixing and 30 min vibration; quantified using a UV spectrophotometric method.

sets of results were also comparable with those obtained by Yeung and Hersey (1979) for mixing of microfine salicylic acid with crystalline sucrose in a tumbling blender. The phenomenon of demixing found here is not uncommon but is usually associated with random mixing where the non-interacting particles can move relatively freely, sometimes causing a reduction or loss of homogeneity. In the present study, the apparent loss of homogeneity may occur in the initial stages of mixing as a result of triamterene particles being partially agglomerated and before complete adhesion to sucrose carrier particles. After 20 min mixing, both methods of analysis showed that the triamterene was fairly uniformly dispersed, although further improvements to homogeneity continued until the end of mixing after 60 min. Microscope analysis showed that powder samples removed after 60 min contained ordered units with a uniform coating of fine triamterene particles on the coarse crystalline sucrose carrier particles (Fig. 5).

Following mixing, the powders were vibrated for a further 60 min. Both analysis methods showed an initial rapid increase in the coefficient of variation to approximately 8%. The homogeneity of the powders continued to fall steadily as vibration time increased and after 60 min vibration samples from both methods were found to have CV between 12 and 16%.

Visual inspection of the powder bed at different vibration times was possible using the UV fluorescence method and it was noted that no free triamterene particle or agglomerates were found at

Fig. 5. Scanning electron photomicrograph of a single coarse sucrose carrier particle with a uniform coating of finer adherent triamterene particles.

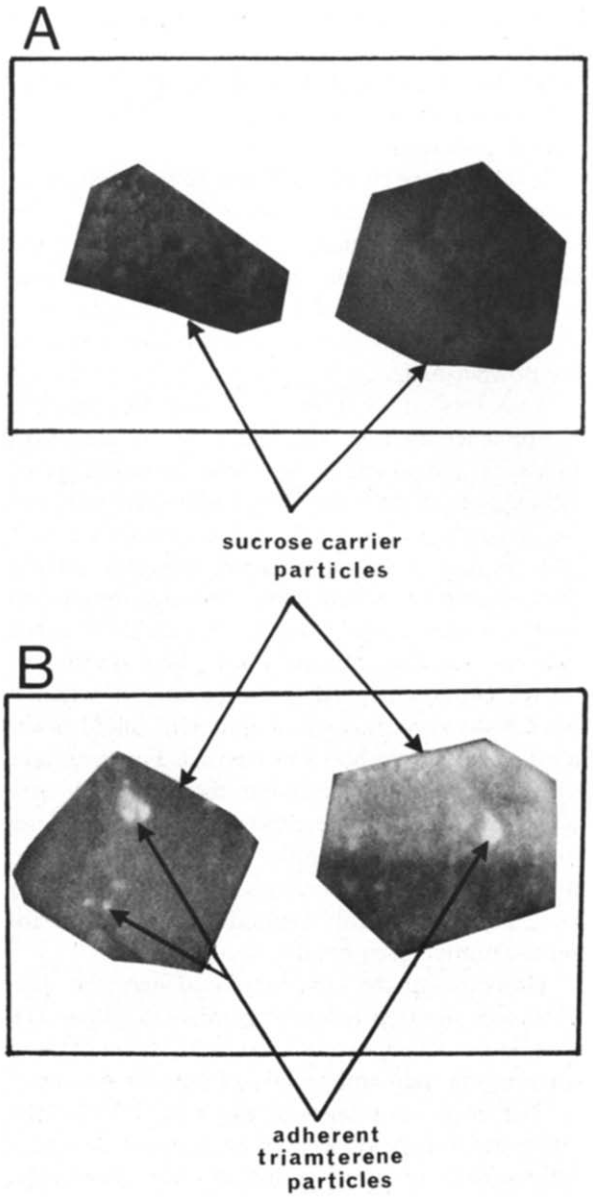

Fig. 6. Fluorescence photomicrographs of specimen articles from (A), the upper part of the powder bed and (B) the lower part of the powder bed. Only triamterene particles fluoresced and there was no other illumination.

any vibration time, and it was therefore considered likely that the major segregation mechanism in this powder system was ordered unit segregation. After 60 min vibration it was also noted that the amount of fluorescence measured in the lower part of the bed was less than that in the upper part of the powder bed. This measurement also coincided with findings from observations of ordered units which suggested that carrier particles from the base of the powder bed were drug-rich whereas those from the upper exposed surface were drug-lean (Fig. 6).

It is therefore considered that size separation of ordered units occurred during vibration with slightly finer units percolating to the base of the powder bed where the greater surface-to-volume ratio carried more triamterene per unit mass or volume than the coarser units located at the top of the powder bed.

Two sets of data are provided for the UV fluorescence method; the results for duplicate (b) follow a similar trend to those in (a) (Fig. 3) although a slightly more homogeneous mix was formed in (b). The difference in absolute values may be due to variation in the sensitivity of the photomultiplier system, since the determinations were not made consecutively. It should be noted that the absolute current values were dependent on the conditions used to set up the photomultiplier system and this was a source of initial problems when the method was devised. Further, since the fluorescence microscope method used here only sampled from the exposed layer of particles immediately adjacent to the wall of the container, it is possible that in conditions where wall effects were pronounced, this method could produce unrepresentative or anomalous results.

However, in the cases reported here the data obtained were sufficiently similar to allow UV fluorescence microscopy to be used as a method of quantifying the homogeneity of powder systems.

The main advantages of using the UV fluorescence microscope method of analysis are: firstly, it allows data to be obtained quickly and easily; secondly, errors due to physical sampling and chemical analysis are removed; thirdly, the method causes no physical disturbance of the powder bed during sample analysis, so that the same powder can be re-sampled following subsequent processing, such as vibration testing and fourthly, because the powder being sampled is also visualized using UV fluorescence microscopy, it is possible to carry out an inspection of the powder and the location of individual particles can be recorded photographically.

Conclusions

The results obtained for the homogeneity of a powder system containing 0.025% triamterene in crystalline sucrose were similar using either UV fluorescence microscopic or UV spectrophotometric methods. The results were also comparable with work carried out by Yeung and Hersey (1979), also using crystalline sucrose.

The method of quantitative UV fluorescence microscopy can be used as an alternative to conventional assay procedures and has several advantages, including: speed of measurement; non-invasive sampling; and the ability to produce a permanent photomicrographic record of the particles analyzed in each sample.

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