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Deposition velocities of airborne microbe-carrying particles

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The deposition velocity of airborne microbe-carrying particles (MCPs) falling towards surfaces was obtained experimentally in operating theatres and cleanrooms. The airborne concentrations of MCPs, and their deposition rate onto surfaces, are related by the deposition velocity, and measurements made by a microbial air sampler and settle plates allowed deposition velocities to be calculated. The deposition velocity of MCPs was found to vary with the airborne concentration, with higher deposition rates occurring at lower airborne concentrations. Knowledge of the deposition velocity allows the deposition on surfaces, such as product or settle plates, by a known airborne concentration of MCPs to be predicted, as well as the airborne concentration that should not be exceeded for a specified product contamination rate. The relationship of airborne concentration and settle plate counts of MCPs used in Annex 1 of the EU Guidelines to Good Manufacturing Practice to specify grades of pharmaceutical cleanrooms was reassessed, and improvements suggested.

Key words: Deposition velocities, airborne micro-organisms, microbe-carrying particles, surface deposition, cleanrooms, operating rooms.

Introduction

In cleanrooms and hospital operating rooms, the high efficiency filtration of the air supply to the room ensures that no microbe-carrying particles (MCPs) enter a room in the supply air. Also, these ventilated rooms are normally positively pressurised with respect to adjacent areas, and this ensures that few MCPs enter from outside. Therefore, airborne MCPs in ventilated rooms are generated within the room and most, if not all, come from personnel.

Micro-organisms grow on the cells and glands of the skin, and are dispersed into the air on skin detritus. Skin cells have been reported by Mackintosh *et al*.[1] to have a top surface area of approximately 33 μm × 44 μm, and 3 to 5 μm thick. They may be found in the air either as whole cells, or fragments of cells. Particles found in room air will vary in size, shape and density, and their size is normally given as an equivalent particle diameter, which is the diameter of a sphere of unit density that settles in air at the same velocity as the particle under consideration. Noble, Lidwell and Kingston[2] have reported that the average equivalent particle diameter of MCPs in hospital air is about 12 μm, and Whyte and Hejab[3] have confirmed this during experiments carried out on people exercising in a dispersal chamber.

MCPs may deposit from the air onto surgical wounds and products manufactured in cleanrooms and, based on results published by Whyte[4], a deposition velocity of 0.46 cm/s has been used to calculate airborne deposition of MCPs onto pharmaceutical products[5]. However, recent research by Whyte, Agricola and Derks[6] carried out on airborne particles in a cleanroom has shown that the deposition velocity of particles increases as the air cleanliness increases. It was, therefore, decided that a fresh investigation of the values of deposition velocities of MCPs was desirable. Sampling results had been gathered over a number of years in pharmaceutical cleanrooms and operating theatres, and were available to calculate the deposition velocity of MCPs, and ascertain if it varied with airborne cleanliness.

Deposition of particles onto surfaces

The number of MCPs that will deposit onto a surface, such as manufactured products, open surgical wounds, or settle plates, can be calculated by use of the following equation[5,6].

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Equation 1

$$\text{Number of MCPs deposited} = c*a*t*v_D$$

Where c = airborne concentration of MCPs, a = area of deposition surface, t = deposition time, and v_D = deposition velocity

If **Equation 1** is rewritten in terms of the airborne concentration of MCPs, the airborne concentration can be calculated that should not be exceeded for a specified and acceptable amount of surface deposition. Both these methods of calculation are described by Whyte, Agricola and Derks[7]. However, to complete these calculations, the deposition velocity of airborne particles must be known.

A useful approach to the investigation of the surface deposition and deposition velocity of MCPs is use of the microbial deposition rate (MDR), which is the deposition rate of MCPs onto a standard surface area in a standard time and can be obtained from counts on settle plates. Settle plates of 9 cm diameter (64 cm^2 surface area) are most commonly used in cleanrooms, but settle plates of 14 cm diameter (154 cm^2 surface area) are also used, as they are more sensitive, as are several 9 cm plates. If the number of MCPs that deposit onto a settle plate of known area, exposed for a known time, is ascertained, then the MDR (no./m^2/s) can be obtained by calculating the number of MCPs that will deposit onto one square metre in one second. This approach allows **Equation 1** to be transformed to the following **Equation 2**.

Equation 2

$$\text{MDR} = c * v_D$$

Rearranging **Equation 2** gives the following **Equation 3**.

Equation 3

$$v_D = \frac{MDR}{c}$$

Use of **Equation 3** allows the deposition velocity of MCPs in a ventilated room to be ascertained by simultaneously measuring, at the same location, the airborne concentration with an air sampler and the MDR with a settle plate.

Annex 1 of the European Union Guidelines to Good Manufacturing Practice (EU GGMP)[8] specifies grades of cleanrooms with maximum concentrations of particles and MCPs. These include maximum airborne concentrations of MCPs/m^3 and settle plate counts of MCPs deposited onto a 90 mm diameter plate in 4 hours. The settle plate counts for a given airborne concentration of MCPs were based on information published in 1989 by the Parenteral Society (now the Pharmaceutical and Healthcare Sciences Society) Technical Monograph No 2[9] that used an equation published by Whyte[4] that is similar to Equation 1 and used a deposition velocity of 0.46 cm/s. A reappraisal of the deposition velocities of MCPs in this article gives an opportunity to reconsider the maximum settle plate counts given in the EU GGMP for different grades of pharmaceutical cleanrooms.

Experimental methods

Cleanrooms and operating rooms studied

Experiments were carried out in three pharmaceutical manufacturing rooms in which the air supply, the number of people present, and clothing worn, were varied to obtain a range of airborne concentrations of MCPs. Two hospital operating theatres were also studied. The rooms studied were as follows.

(1) **Vial-filling cleanroom:** This cleanroom was a non-unidirectional airflow (non-UDAF) cleanroom that was supplied with approximately 20 air changes per hour, and the vial filling carried out in a horizontal UDAF cabinet.

(2) **Diagnostics cleanroom:** This was a non-UDAF cleanroom supplied with 18 air changes per hour. The air quality of the room could be enhanced by use of a free-standing UDAF unit of 1.8 m × 0.7 m surface area with an air supply velocity of 0.3 m/s.

(3) **Ampoule-filling cleanroom:** This cleanroom had a floor area of 175 m^2 and about 25 air changes per hour. Vertical UDAF units were fitted over two ampoule filling areas.

(4) **Non-UDAF operating theatre:** This was used for orthopaedic surgery and was 6.1 m × 6.1 m in floor area, with an air change rate of about 22 per hour.

(5) **UDAF operating theatre enclosure:** Orthopaedic surgery was carried out within a UDAF enclosure located in a non-UDAF operating room. The UDAF enclosure had a high-efficiency particulate air filter ceiling that was 3 m × 4 m in area and had a downward air velocity of 0.3 m/s.

The cleanroom clothing worn in the pharmaceutical cleanrooms was made from a woven polyester fabric and consisted of a coverall, hood and knee-length over-boots, a mask and sterile gloves. When higher airborne microbial counts were required, either cotton garments or normal indoor clothing was worn. Further increases in the airborne concentration of MCPs were obtained by introducing additional personnel into the room.

In the non-UDAF operating theatre, the surgical clothing consisted of sterile cotton gowns worn over cotton shirts and trousers. In the UDAF enclosure, non-woven fabric gowns were worn over cotton shirts and trousers. Hoods, face masks and sterile gloves were worn in both theatres.

Air sampling methods

Microbial sampling in the pharmaceutical cleanrooms was carried out during microbial growth media (broth) experiments for the filling of either vials or ampoules. In the diagnostics preparation cleanroom, microbial monitoring was undertaken during normal production activities, but when the room ventilation was switched off,

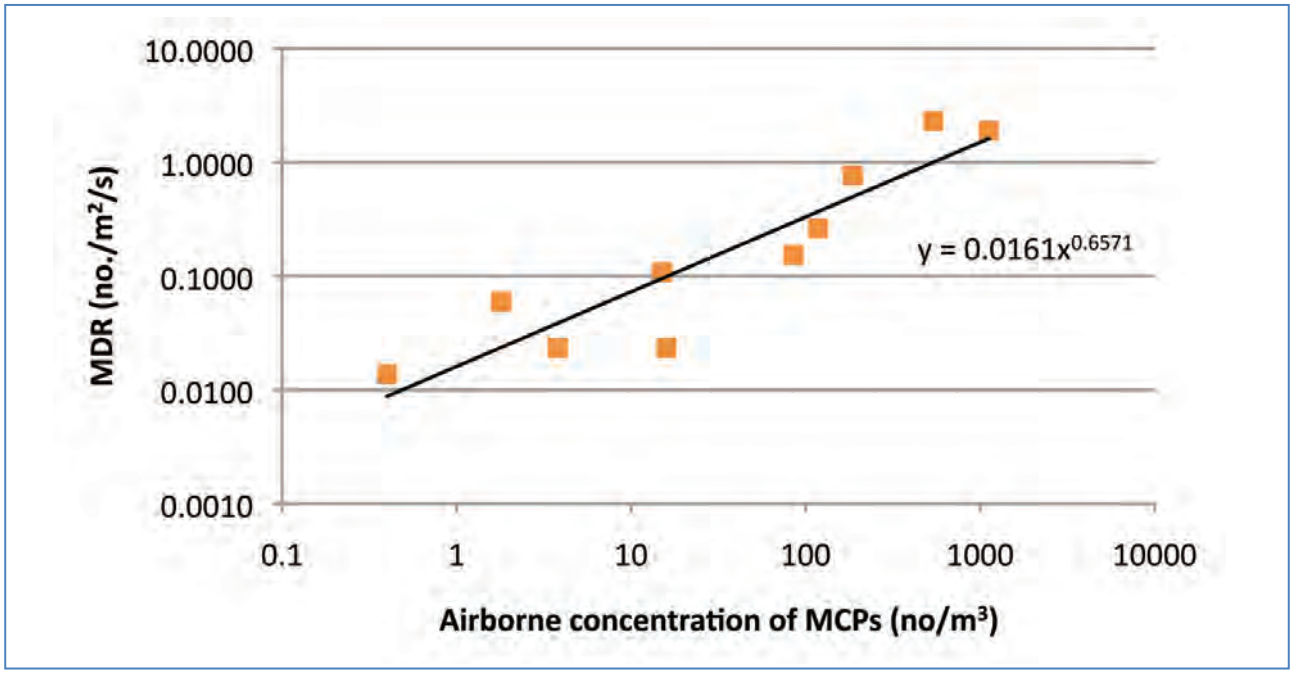

Figure 1. Relationship of MDR and airborne concentration of MCPs.

or additional personnel wearing indoor clothing were introduced, production activities were suspended, and monitoring undertaken with personnel moving about and active within the cleanroom. In the operating theatres, sampling was carried out during routine orthopaedic surgical operations.

Airborne MCPs were sampled by means of a slit sampler (Casella Ltd) that sampled 700 litres of air per minute onto Petri dishes containing tryptone soya agar (Oxoid Ltd). After use, the Petri dishes were incubated for 36 to 48 hours at 37°C and the microbial colonies counted. To the air intake of the sampler, a 60 cm length of 10 cm diameter flexible tubing was added that was bent through 90° to the horizontal, and connected to a sterile metal cone which converged to a 4 cm opening through which air samples were drawn. This allowed airborne MCPs to be efficiently sampled at the locations where containers were filled or close to the surgical wound, but where the sampler was too large to be placed. Sampling times varied from 0.5 minutes to 15 minutes, depending on the expected MCP counts, and sampling was carried out over 2 and 5-hour periods in the same day. In the case of pharmaceutical manufacturing, samples were taken at both the vial and ampoule turntable

Table 1. Experimental conditions and results.

Number	Cleanroom/ operating theatre application	Room ventilation	UDAF	Type of clothing	MCP volumetric count (no./m³)	Settle plate count 14 cm diameter (no./h)	MDR (no./m²/s)	Deposition velocity (cm/s)
1	Vial filling	On	Horizontal – on	Cleanroom	0.4	0.76	0.014	3.43
2	Vial filling	On	Off	Cleanroom	85	8.5	0.15	0.18
3	Vial filling	Off	Off	Cotton	186	42.6	0.77	0.41
4	Diagnostics	On	None	Cleanroom	15.9	1.3	0.023	0.15
5	Diagnostics	On	Free-standing UDAF	Cleanroom	1.8	3.3	0.060	3.31
6	Diagnostics	Off	None	Indoor*	1116	106	1.9	0.17
7	Ampoule filling	Off	Off	Indoor†	118	14.6	0.26	0.22
8	Ampoule filling	On	Off	Indoor	15	6	0.11	0.72
9	Orthopaedic surgery	On	None	Cotton	539	128	2.3	0.43
10	Orthopaedic surgery	On	On	Non-woven	3.8	1.3	0.023	0.62

* 10 people present; † 8 people present.

and filling point, and an average calculated. During surgical operations, samples were taken within 20 cm of the open wound.

Settle plate sampling was carried out at the same time as sampling with an air sampler, using 14 cm diameter Petri dishes containing tryptone soya agar, which were incubated after use for 36 to 48 hours at 37°C and the microbial colonies counted. The settle plates were laid out in the pharmaceutical cleanrooms on the filling machine close to the filling area. Plates were exposed when filling started and the lids replaced when filling stopped; this exposure time varied from between 2 and 5 hours. During the surgical operations, the open settle plate was placed within 20 cm of the wound for the time between incision and closure, and, depending on the operation, exposed for between about 1 and 2 hours.

Experimental results

Given in **Table 1** are the type of cleanrooms and operating theatres studied. Also included are the MCP air concentrations/m^3, the number of MCPs that deposited onto a 14 cm diameter settle plate per hour, and the MDR (number/m^2/s). The average deposition velocity of MCPs calculated by **Equation 3** is also included but given in the more commonly used units of cm/s.

Shown in **Figure 1** is the relationship of the airborne concentration to the MDR, with a regression line that best fits the results. The equation of this line is as follows.

Equation 4

$$y = 0.0161\, x^{0.6571}$$

Where y = MDR (no/m^2/s), and x = airborne MCP concentration/m^3

Equation 4 was used to calculate the expected MDR for a range of airborne MCP concentrations, and from these results the deposition velocity (cm/s) was calculated by means of **Equation 3**. These results are given in **Table 2**.

Shown in the first two columns of **Table 3** are the maximum airborne concentrations and settle plate counts given in Annex 1 of the most recent edition of the EU GGMP (2008) for different grades of cleanrooms. Using the deposition velocities given in **Table 2**, the number of MCPs expected to deposit onto a settle plate of 9 cm diameter (area = 0.0064 m^2) during an exposure time of 4 hours, was calculated for the airborne concentrations given in **Table 3**. Finally, in the last column of **Table 3** are the required settle plate results, given as the MDR, using the base units of the International System of Units (SI units), i.e. no./m^2/s. The MDR allows the number of MCPs deposited onto any size of settle plate, in any time of exposure, to be calculated.

Table 2. Deposition velocities for airborne concentrations of MCPs.

Airborne concentration/m^3	Deposition velocity (cm/s)
0.1	3.55
0.5	2.04
1	1.61
5	0.93
10	0.73
50	0.42
100	0.33
200	0.26
500	0.19

Discussion and conclusions

Knowledge of the deposition velocity of MCPs as they fall through the air onto cleanroom surfaces allows the calculation of the amount of deposition on a surface such as a product, as well as the airborne concentration that should not be exceeded for a specified amount of product contamination. These two calculation methods are described by Whyte, Agricola and Derks[7].

The deposition velocity of a range of particle sizes in a cleanroom was ascertained by Whyte, Agricola and Derks[6] who reported that as the concentration of airborne particles in the cleanroom decreased, the deposition velocity increased. The reason for this was not clear but it was suggested that as lower particle concentrations were associated with higher air supply rates, smaller particles would be quickly swept from the cleanroom with little time to deposit, but larger particles would still be deposited by gravity. This effect was

Table 3. EU GGMP recommended limits for air concentrations and settle plate counts. Present and corrected information.

Grade	Air sample cfu/m^3	Settle plates (diameter 90 mm) cfu/4 hours Current requirements	Settle plates (diameter 90 mm) cfu/4 hours Corrected requirements	Corrected settle plate counts as MDR (cfu/m^2/s)
A	<1	<1	<1.5	0.016
B	10	5	7	0.073
C	100	50	30	0.33
D	200	100	48	0.52

expected to increase as the average residence time of the air reduces owing to an increase in air supply rates. It was also thought that an increase in the turbulent intensity of the air, caused by an increase in the air supply, would also increase the amount of surface deposition.

It is known that MCPs in cleanroom air do not normally exist as single-celled organisms but are commonly found on skin cells dispersed by personnel and have an equivalent particle size of about 12 μm. The deposition velocity of these MCPs had been reported by Whyte[4] to be about 0.46 cm/s, and this velocity has been used in several articles, such as that published by Whyte and Eaton[5] to calculate the contamination rate of products in cleanrooms. However, the results of the investigation carried out by Whyte, Agricola and Derks[6] has questioned the accuracy of the use of a single deposition velocity over a range of the cleanliness conditions found in a cleanroom.

The measurement of airborne concentrations and settle plate counts was carried out in a wide variety of cleanrooms and operating theatres. This data was analysed and the deposition velocities of MCPs obtained. These results showed that the deposition velocity increased as the airborne concentration in the rooms decreased, and the use of these deposition velocities should improve the accuracy of the calculation of surface contamination, as well as the calculation of the concentration of airborne MCPs required to ensure that microbial deposition of surfaces is not greater than specified.

The EU GGMP specifies grades of cleanroom for use in pharmaceutical manufacturing, and gives suggested limits of the airborne concentrations and settle plate counts of MCPs. The settle plate counts that correspond to the airborne concentrations were derived from data originally published in the Parenteral Society[9] and based on the equation published by Whyte[4], which used a single deposition velocity of 0.46 cm/s. Using the set of more accurate deposition velocities, the EU GGMP maximum concentrations can be revised to provide more accurate settle plate counts. **Table 3** shows the settle plate counts currently specified by the EU GGMP and those based on the information obtained in this article. It is suggested that the EU GGMP should be revised to take account of these findings.

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