行政院國家科學委員會專題研究計畫成果報告

奈米生物性氣膠採樣與分析探討

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執行期間：91 年 8 月 1 日至 92 年 7 月 31 日

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奈米生物性氣膠採樣與分析探討

Sampling and Analysis for Nano Bioaerosol

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一、中文摘要

隨著奈米科技的進步，新技術與新知識提供了大家看問題的新方向與解決問題的新途徑。在氣膠學的研究方面，微粒之粒徑大小一直在微粒特性與健康風險上扮演重要的角色。雖然對於超細微粒的研究已有相當的時間，但對於奈米微粒（<0.1 μm)的研究卻是近年來才開始，因此實在有必要對於奈米微粒作完整的研究。目前已有許多流行病學研究指出許多傳染性疾病是經由氣膠傳播，但一開始生物氣膠的研究並未與物理性氣膠同步，基於人類鑑康危害觀點，感染性的生物氣膠不論在疾病的傳輸與健康風險上都扮演著極重要的角色。

在近 10 年中，已有一些研究針對細菌(1-5 μm)與真菌(2-10 μm)的氣膠特性與環境因子間的關係作討論，但對於粒徑在奈米範圍的病毒微粒（20-300 nm）卻鮮少有研究討論。基於病毒絕對寄生的特性，大部分的病毒被認為是人或動物的致病菌，加上許多病毒性疾病已確定空氣傳染途徑傳播，因此病毒微粒實有必要做進一步的研究，並建立其採樣分析技術，以實際應用於一些高風險環境。本研究藉由監測空氣中病毒濃度，研究病毒微粒濃度與疾病流行狀況的關係，以進一步評估各種控制技術的控制效率，並確實達到公共衛生上預防疾病的目的。

關鍵詞：奈米生物氣膠、病毒氣膠、定量 PCR、流式細胞儀

Abstract

Rencently, nanotechnology draws scientists' eye. The great progresses are in both knowledge and technology. Due to its nano-characteristics, it helps us to see things in different aspect, and help us to find the resolution for those problems we stuck before. In aerosol studies, particle size distribution always plays a very important role in particle's behavior, related to both particle transmission and health issues. As a consequence, it is essential to find out the whole story of smaller particles-nanoparticles.

Although numerous epidemics of infectious diseases have been known to be transmitted by aerosols, widespread interest in study of infectious viable aerosols has not kept pace with that of nonviable aerosol study. Concerning about human health, however, bioaerosol is also a very important part of the whole story. During the last decade, there have been several researches of bacteria (1-5 μm), and fungi (2-10 μm), but fewer studies of virus were performed. Because they are obligate parasites, most viruses must be considered pathogenic to humans or animals. As a part of this nanoparticle study, we will focus on the nano-bioaerosols - virus (20-300nm).

Keywords: nano-bioaerosols, virus aerosols,

real-time quantitative PCR, flwocytometry

二、緣由與目的

In aerosol studies, particle size distribution always plays a very important role in particle's behavior, related to both particle transmission and health issues. As a consequence, it is essential to find out the whole story of smaller particles-nanoparticles (<0.1μm).

Solid or liquid particles that suspended in gas, such as air, are called aerosols and are nearly ubiquitous throughout the earth's biosphere. The significance of aerosols to human beings and human environment depend on their origin, composition, concentration, and physical, chemical, and biological characteristics. Aerosol formation results from both natural and anthropogenic processes, such as wind and wave action or flushing toilet activity, respectively. These aerosols may contain viable and/or nonviable material. Although numerous epidemics of infectious diseases have been known to be transmitted by aerosols, widespread interest in study of infectious viable aerosols has not kept pace with that of nonviable aerosol study. Concerning about human health, however, bioaerosol is also a very important part of the whole story.

Bioaerosols are those airborne particles that are living or originate from living organisms. Bioaerosols include microorganisms (i.e., culturable, viable but nonculturable, and dead microorganisms) and fragments, toxins, and particulate waste products from all varieties of living things. Individual bioaerosols range in size from submicroscopic particles (<1 μm) to particles greater than 100 μm in diameters. During the last decade, there have been several researches of bacteria (1-5μm), and fungi (2-10μm), but fewer studies of virus were performed. Because they are obligate parasites, all viruses must be considered pathogenic to humans or animals. As a part of this nanoparticle study, we will focus on the nano-bioaerosols - virus (20-300nm).

The first attention drawn by viruses pandemic was in 1950s (Zeterberg et al., 1973). After the influenza pandemic, many epidemiological studies were performed to determinate the relationship between specific virus and disease (Zeterberg et al., 1973). It had also been recognized that aerosol transmission plays a critical role for many viral diseases. Since smaller particles are more likely to achieve deep lung penetration than larger ones, the virus aerosol collection in the assessment of the risk of respiratory infection is obviously of high value.

三、結果與討論

Test Microorganisms

In our study, two coliphages, MS2 phage (BCRC 70106) and T7 phage (BCRC 70058), were selected to serve as surrogate for simulating the human viruses. The 10^5 pfu/ml bacteriophage was mixed with 10^8 *E.coli* and then the infected cultures were incubated for 15 min at 37 ℃ to allow the phage attached to the cells. After the culturvation, 3 ml top agar was added to the sterile tube of infected cells. The contents of the tube were mixed by gentle tapping for a few seconds and poured onto the center of a labeled agar plate. Finally, the plate was incubated for 12 to 16 hr at 37 ℃ and the SM buffer was added after the culturvation. The resulting phage plate stocks was stored at 4 ℃. Moreover, the collecting medium was 10 % solution of gelatin in sterile LB Broth/distilled water and heated at 121 ℃ for about 15 min.

Aerosol Generation and Test system

The test chamber is 29 cm in diameter with a height of 32 cm. A Collison three-jet nebulizer (BGI Inc.,Waltham, MA) was used for nebulization of the microbial MS2 and T7 phage suspension at 3 L/min of dry, filtered, and compressed laboratory air, then passed through a Kr-85 particle charge neutralizer (model 3077, TSI). The aerosolized suspension was then diluted with filtered and compressed air at 57 L/min. SMPS (Model 3071, API Inc) with CPC (Model 3025, API Inc.), and an aerodynamic particle sizer (APS, Model 8000, API Inc., Hadley, MA), were used to determine real-time number concentration and size distribution of viral bioaerosols. The SMPS could measure 15 nm to 710 nm particle size, and APS could measure 0.487 um to 29 um particle size, respectively. Moreover, An Andersen six-stage viable sampler (Andersen Samplers, Inc., Atlanta, GA) was also used for determination of size distributions of generated viral strains with the size classes of 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–7.0, and > 7.0 μm at flow rate of 28.3 L/min .

Test Samplers

Andersen 1-STG sampler is the sixth stage of the Andersen six-stage sampler with 400 0.25-mm holes, drawing air at a flow rate of 28.3 L/min (the corresponding velocity is 24 m/s) by using 20 ml LB Broth plates. The calculated and reported cut-point diameters of this sampler is 0.57 μm and 0.65 μm, respectively .

The AGI-30 (Ace Glass Inc.) is an all-glass impinger with a 30-mm jet-to-plate distance. Generally, 20 ml of sterile deionized water with 0.5% NaCl, 0.5% antifoam A (Sigma Chemical Co., St. Louis, MO) and LB broth was injected into each autoclaved AGI-30 impinger. The antifoam was added to reduce foaming and to prevent excessive fluid loss.(Terzieva et al., 1996)

A Nuclepore filter consists of a polycarbonate membrane with a 0.4-μm pore size and a 37-mm diameter supported by cellulose pads were loaded into open-face and three-piece plastic cassettes. Filters and support pads were autoclaved, and plastic cassettes were sterilized with ethylene oxide before sampling. (Kemp et al., 1995, Palmgren et al., 1986)

The gelatin filter (Sartorius, Gottingen,Germany) (3.0-μm pore size, 80-mm diameter) was placed in a sterile filter holder by carefully letting the filter slide out of the pocket onto the filter support of the aluminum filter holder. The filter could dissolve on the agar surface because of the moisture in the agar culture medium or in sterile liquid at the temperature of 35–40 °C (Jaschhof et al., 1993)

For comparison of samplers, the parameter, Ctest /Csusp (colony survival, CS; Csusp: PFU/m^3 by the evaluated sampler, Csusp: PFU/ml in the suspension), was used as a reference.

Results and Discussion

The aerodynamic particle diameter of the target aerosol is one of the most important physical factors which determine the stage collection efficiencies of inertial devices. Moreover, the particle size of virus-containing aerosol is important in determining their physical stability, dispersion, disposition and retention in the respiratory tract. By using SMPS and CPC, it was found that there was no difference in the size distributions of SM buffer and T7 phage (Figure 1). By APS, average geometric mean aerodynamic diameter of T7 phage phage was observed to be 1.07 μm with geometric standard deviation of 1.47

(Fig 2). Another MS2 phage was observed to be 1.08 μm with geometric standard deviation of 1.4 (Fig 3). In addition, size distributions of T7 and MS2 phage by the Andersen 6-STG sampler indicated that more than 70% of the recovered PFU colonies were ≦ 2.1 μm (Fig 1, Fig 2).

Regarding viability stability in nebulizer suspension, it was found that there was no significant loss in the viability of T7 and MS2 phage (coefficient of concentration variation were 6.9%). Regarding generated airborne virus viability, our experimental results demonstrated that concentrations of the generated T7 and MS2 phage during varied with 20% CV, it was stable during high relative humidity (RH=85%).

For evaluating bioefficiency of the tested samplers (table 1), all of the experiments were performed using T7 phage at RH 85%. For Andersen 1-STG sampler, it was demonstrated that the observed CS of T7 phage was 0.07. For impinger, CS was found to be 0.06. On the other hand, CSs were indicated to be 0.002 and 0.04 for nuclepore and gelatine filters, respectively. Moreover, it was demonstrated that average CSs of MS2 phage was 0.017, 0.01, 0.018 and 0.0007 for one-stage Andersen sampler, AGI-30 impinger, gelatin filter, and nuclepore filter, respectively.

Our findings demonstrated that impactor, impinger, and gelatine filter for virus sampling performed better than nucelpore filter. The biological stress during filtration includes impaction and dehydration effects caused by the large volume of air over the collected bioaerosols.

四、計畫成果自評

本計畫已在實驗室中建立病毒生物氣膠採樣分析技術的評估系統，並已運用此系統評估兩種病毒採樣技術之採集效能，此成果可運用於環境採樣，並提供更完整的病毒生物氣膠暴露危害評估之採樣技術。

五、參考文獻

[1] Zeterberg, J. M. (1973). A review of respiratory virology and the spread of virulentand possibly antigenic viruses via air condition systems. Part I. Ann. Allergy 31:228-234

[2] Terzieva, S., Donnelly, J., Ulevicius, V., Grinshpun, S. A., Willeke, K., Stelma, G. N., and Brenner, K. P. (1996). Comparison of Methods for Detection and Enumeration of Airborne Microorganisms Collected by Liquid Impingement, Appl. Environ. Microbiol. 62:2264 –2272.

[3] Kemp, S. J., T.H.Kuehn, D.Y.H.Pui, D.Vesley and A.J.Streifel (1995). "Filter collection efficiency and growth of microorganisms on filters loaded with outdoor air." ASHRAE Transactions 101(1): 228.

[4]Palmgren, U., Strom, G., Malmberg, P., and Blomquist, G. (1986b). The Nuclepore Filter Method: A Technique for Enumeration of Viable and Nonviable Airborne Microorganisms, Am. J. Ind. Med. 10:325–327.

[5]Jaschhof, H. (1993) Sampling Virus Aerosols using the Gelatin Membrane Filter. Microbiology.

[6] Wallis, C., J. L. Melnick, and T. E. Sox. (1985). Methods for detecting viruses in aerosols. Appl. Environ. Microbiol. 50:1181-1186.

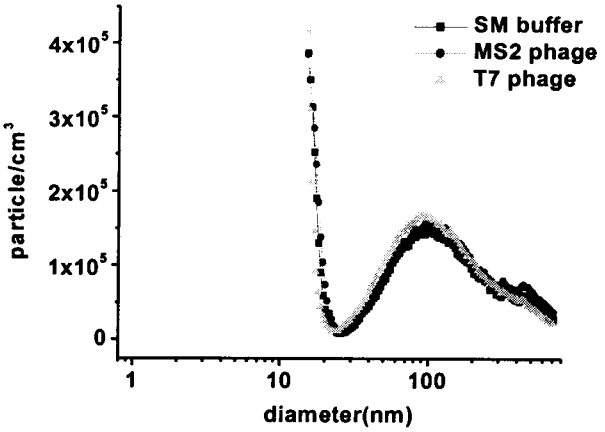

Figure 1. The particle size distributions of MS2、T7 phage in the test chamber measured by SMPS

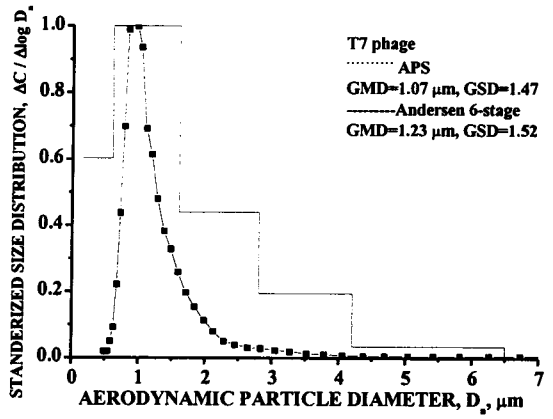

Figure 2. The particle size distributions of T7 phage in the test chamber measured by APS and Andersen 6 –stage sampler.

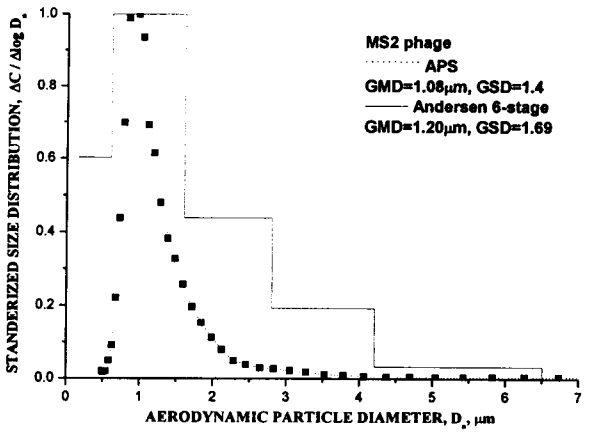

Figure 3. The particle size distributions of MS2 phage in the test chamber measured by APS and Andersen 6 –stage sampler.

Table 1 Colony survival efficiencies (CS, CFU/m3/CFU/ml) of Andersen one-stage impactor, AGI-30 impinger, Gelatin filter and nuclepore filter for T7 and MS2 phage.

Sampling Device	T7	MS2
AMS one stage sampler	0.077±0.006	0.017±0.005
AGI-30	0.06±0.002	0.01±0.001
Gelatin filter	0.049±0.005	0.018±0.0005
Nuclepore filter	0.002±0.0004	0.0007±7.65E-5