

Sampling of biological particles in the upper layers of the atmosphere and in space

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A design is proposed for a sampling device which decelerates biological particles in the sparing regime (sampling with low particle velocities), separating them from inorganic particles, with subsequent trapping and analysis of the biological component of the aerosol. A biological particle colliding with the wall of a standard sampling device may not only be inactivated, but also valorize. In other words, before trapping the particles, it is necessary to slow them down. The sampler consists of a system of quarter-wave high-frequency resonators, which function as a neutral-particle accelerator. This system is used in the given design to brake biological particles. It is shown that the use of a high-frequency nonuniform variable electric field shows promise for solving problems connected with separating biological particles (bacteria, cells, and viruses).

Introduction

The creation of life on Earth is one of the most intriguing riddles of modern times. Many contemporary theories consider general questions of self-organization of inorganic matter as a whole, linking the conditions of the creation of life to the conditions that existed on the Earth billions of years ago.^{1,2} Another hypothesis about the appearance of life on Earth is that it emerged somewhere else in the Universe and subsequently "drifted in" from outer space.³ Indeed, the data in the literature on dehydration and storage of various biological objects (proteins, genetic material, fragments and even whole micro-organisms) show that they can be preserved under the conditions existing in outer space (low temperatures and pressures) for many years. This pertains not only to macromolecules,^{4,5} but also living micro-organisms: various viruses,⁵⁻⁷ bacteria, and fungi.^{5,8,9}

Of course, since the radiation background is high in the upper layers of the atmosphere and especially in outer space, the viability of biological objects is reduced under such conditions.^{8,10,11} It should be noted, however, that some micro-organisms are quite resistant to the action of radiation,¹¹ and, besides, biological objects themselves are never encountered in "pure" form, only in association with other molecules. These play the role of "protective additives," impeding inactivation or destruction of micro-organisms exposed to various factors.^{12,13} In addition, macromolecules and micro-organisms are often adsorbed onto dust particles,^{14,15} which, to a significant degree, can also screen radiation.

Thus, there are grounds for maintaining that biological objects that have drifted in from space can serve as sources for the creation of life. However, a

direct confirmation of the possibility of "drift" of life from space would be the discovery of living micro-organisms (or biological macromolecules) in the upper reaches of the Earth's atmosphere or directly in outer space.

Toward this end, the problem of sampling biological particles existing under conditions of the strongly rarefied atmosphere or outer space with their subsequent identification becomes highly relevant.

It is natural to assume that "drift" of living micro-organisms or their genetic material onto the Earth takes place continuously (from the point of view of time scales of the Universe). This circumstance has the capability of leading to the most unforeseeable consequences in the development of civilization or even to a biological catastrophe,³ which again underscores the necessity of monitoring the biological component of particles in the upper atmosphere and in space.

It should be noted that to detect "live" biological objects, it is necessary to build a "sparing" sampler (a particle sampler with low inflow velocities relative to the sampler); otherwise, when a particle (biological object) moving at a high velocity in the upper atmosphere or in outer space collides¹⁶ with the wall of the device, the biological object may not only be inactivated, but can also vaporize. In other words, before trapping the particles, it is necessary to slow them down.

At present, a significant body of theoretical and experimental material has accumulated which shows that under conditions of a nonuniform electric field even electrically neutral biological particles (cells, bacteria, fungi, viruses) undergo a directed translational motion relative to the electrodes and settle onto their faces, see Refs. 17-19. This effect lies at the basis of the design of the new sampler, which makes it possible to decelerate and trap biological particles.

Description of device for braking neutral particles

First of all, let us dwell on the problem of a "sparing sampler." To decelerate electrically neutral biological particles, it is advisable to make use of the circumstance that the amplitude–frequency dependence of the polarization of biological and inorganic particles in an external high-frequency electric field is fundamentally different. The electrical component of the electromagnetic standing wave formed in the system of quarter-wave resonators of the neutral particle accelerator induces a polarization current in biological particles. As a result of the interaction of the polarization current of the particle with the magnetic field of the electromagnetic wave, a force arises capable of accelerating them to linear velocities of 10^3 – 10^5 m/s (Ref. 20). Such velocities are comparable with the velocities of particles in the upper layers of the atmosphere and in space.

The use of a neutral particle accelerator to decelerate biological particles enables one to decelerate them smoothly and deposit them on a collector with a low relative velocity. Toward this end, it is sufficient to vary the direction of the acceleration force. However, during such braking the particles heat up due to ohmic losses caused by the polarization current. In order to avoid ohmic losses, additional electrodes are mounted in the device which form an elliptically polarized wave.

Under the action of an additional electric field perpendicular to the direction of the electrical component of the electromagnetic standing wave, the polarized bio-particles rotate about their own axis, in synchrony with the variation of the electric and magnetic fields. As a result of the indicated interactions, the bio-particle does not heat up and a braking force acts on it which is always directed opposite the direction of motion of the particle. Neutral particles of an inorganic nature interact weakly with the electromagnetic field and fly straight through the chamber of the quarter-wave resonator. A diagram of the device for decelerating biological particles is shown in Fig. 1.

To decelerate (and deposit) bio-particles in the resonator 1 with the help of the high-frequency generator 6 and the connection element 7, an electromagnetic standing wave is excited, the electric and magnetic components of which near the longitudinal axis of the resonator passing through the center of windows 2 and 3 are perpendicular to the direction of deceleration of the bio-particles. Along the braking axis, passing through the centers of windows 2 and 3, a rotating electric field is excited with the help of additional electrodes 8 and 9. The bio-particles subject to deposition (collection), e.g., spores, viruses, bacteria, fall (together with other particles) into the empty space of resonator 1 through window 2 orthogonal to the electric and magnetic fields of the

resonator and decelerate thanks to interaction of the induced dipole moment with the magnetic field.

The laboratory setup uses a biological-particle injector 4 and collector 5. The microwave generator works in the pulsed regime with pulse durations of $(1 - 2) \times 10^{-6}$ s. The pulse repetition rate is 50 Hz. The pulse power is $500 \cdot 10^3$ W. The power is 50 W. As a result, the device achieves selective braking of biological particles and concentrates them on the central current-carrying electrode while the remaining particles with small dipole moments pass through. The rate of deceleration is 5×10^5 m/s² for biological particles with diameter 10^{-7} m, which is sufficient to decelerate bio-particles from a velocity of 10^3 m/s to zero within a length of 1 m.

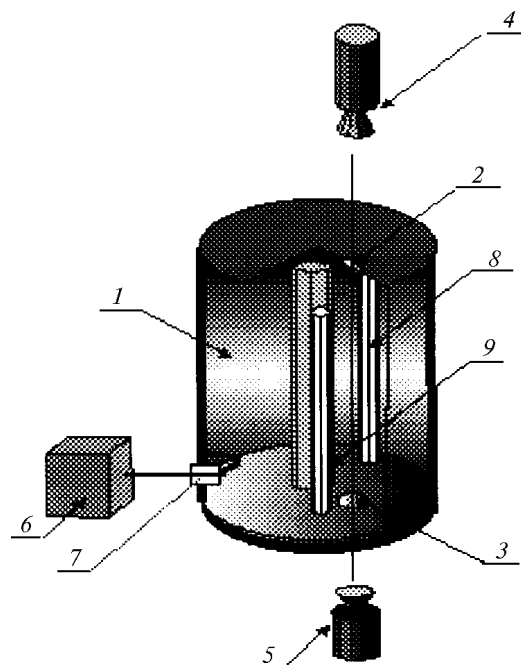


Fig. 1. Diagram of a device for braking biological particles.

The proposed design has not yet been tested experimentally. However, on the basis of experience in the development and operation of neutral-particle accelerators we believe that the technical implementation of the proposed design is in essence analogous (although with a minus sign) to the particle accelerator described by us in Ref. 20. These circumstances convince us of the functionality of the proposed design for a biological-particle sampler intended for use in the upper layers of the atmosphere and in space.

Problem of identification of living micro-organisms

At present there are a number of physical (in the sense of instrumentation) and microbiological methods that are well recommended and in wide use for

identifying micro-organisms. These methods, as a rule, complement one another. The combined set of data obtained with the help of such methods enables a more complete description of the properties of micro-organisms.

An analysis of existing methods²¹ shows that detection of micro-organisms is effected, as a rule, by making use of a distinguishing physical-chemical or biological property. Such an approach is validated by detection and identification of known micro-organisms and their metabolic products.

Physical methods enable one to characterize micro-organisms according to a number of their parameters (size, weight, density, concentration, electric charge, polarizability, light-scattering properties, etc.). These methods are easily automated (the indicated information is collected and analyzed by computer), require an insignificant amount of time to carry out the analysis and generate results, and can be implemented without a human in the loop.

Biological methods possess a high selectivity but they impose significant constraints on their implementation: they require, as a rule, the participation of a human, they require the use of expendable materials which, in addition, must be monitored continuously for quality control, they are insufficiently automated, and the time required to implement biological methods often amounts to hours or even more than a day.

The enumerated virtues and shortcomings indicate that physical methods are preferable.

Some of the most common physical characteristics of a biological particle are its dipole moment and polarizability in a variable electric field.²² These characteristics depend on the dimensions of the biological particle, the makeup of its cell membrane and nucleus, the chemical composition and electric charge of the bio-particle and its individual parts, the permittivity of the bio-particle and its various parts, and the spectrum of the external variable electric field. Thus, measurements of the polarizability of biological particles at several frequencies allow one to judge its internal construction and give a good part of the information needed for its identification.

It is well known that in a nonuniform variable electric field an individual biological particle (a cell or bacterium) is polarized, i.e., a dipole moment d is induced in it, proportional to the coefficient of polarizability of the particle α and the intensity of the electric field E

$$D = \alpha E . \quad (1)$$

A force F acts on a particle with a dipole moment d , which is proportional to the gradient of the electric field intensity

$$F = (d \nabla)E = \alpha \text{grad}(E^2)/2. \quad (2)$$

If the particle is immersed in a liquid with viscosity η , a viscous friction force F_{St} , the Stokes

force, acts on a particle with radius r moving with velocity v

$$F_{St} = 6\pi\eta vr. \quad (3)$$

In the steady-state regime, equilibrium of forces leads to motion of the particle with constant velocity

$$v = \alpha E \text{grad}(E^2)/3\pi\eta r . \quad (4)$$

The polarizability of the particle can be determined by measuring the velocity of the particle in suspension in prescribed electric fields

$$\alpha = 3\pi\eta vr/\text{grad}(E^2)/2 . \quad (5)$$

On the basis of the above approach, we developed a method of determining the polarization characteristics of particles in a nonuniform variable electric field (NVEF) and carried out measurements for a number of biological particles.²²⁻²⁴ This method can be used to solve a wide class of problems associated with the identification and separation of live cells, and also electrically conducting and dielectric particles.²⁵

A device which uses a nonuniform variable electric field to separate biological particles on the basis of their polarizability enabling subsequent identification is depicted in Fig. 2. It consists of two parts: a separation part and a counter. The upper part (the separation part) consists of several channels formed by inclined electrodes. They are designed so that the biological particles are repelled by the electrodes and move in a flux along them. The second (measurement) part counts the number of particles passing through each channel of the separation part.

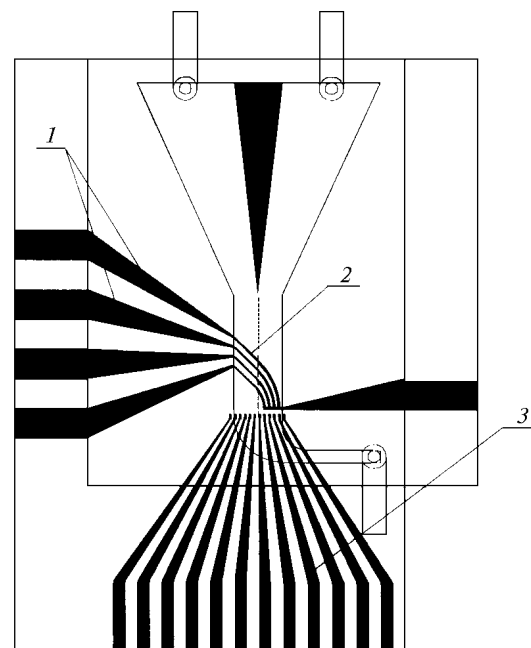


Fig. 2. Diagram of a device for separating biological particles by polarizability and for their identification comprising separation electrodes 1, region of the separation chamber 2, and measuring electrodes 3.

While viable cells are being concentrated in the first electrode, it is possible to concentrate various biological particles in the remaining electrodes by varying the frequency and voltage on the remaining electrodes in steps. In this event, biological particles of different kinds will be concentrated each in its own channel and diverted under the action of the friction force of the flux and the braking action of the electrodes. Electrodes are mounted at the end of each channel to count biological particles of a definite kind, isolated in each channel with the help of an analyzing signal. This enables the device to count a wide spectrum of biological particles.

Our studies have shown that not only cells and bacteria, but also viruses, are polarized in an electric field. Figure 3 shows a nuclear polyhedrosis virus in a nonuniform variable electric field.

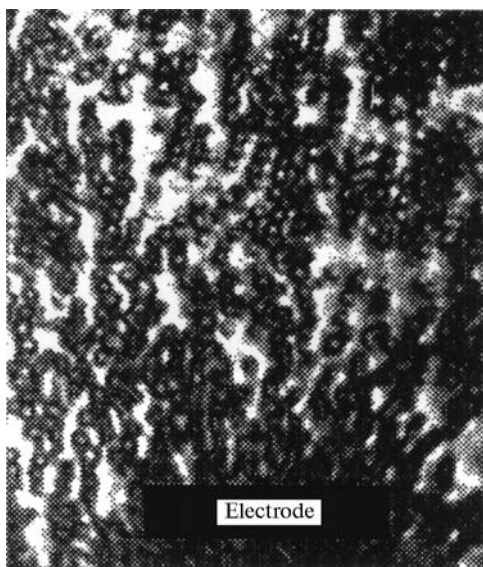


Fig. 3. Nuclear polyhedrosis virus in a nonuniform variable electric field.

To sum up, we have developed and tested a device for separating biological particles on the basis of their polarizability with subsequent identification of the micro-organisms on the basis of this parameter or by other methods²¹ for a narrower spectrum of micro-organisms.

Conclusions

1. We have proposed a design for a biological-particle sampler operating in the sparing regime, which decelerates particles of a biological nature, separates them from inorganic particles with subsequent trapping and analysis of the biological component.

2. The accumulated experimental material obtained from studies of the polarization of biological

particles indicates that the use of a nonuniform variable electric field shows promise for solving problems associated with the separation of biological particles and also with their identification.

3. A combination of these two methods in one sampling device makes it possible to analyze for the presence of biological particles in the upper layers of the atmosphere and in space and to carry out subsequent identification of the sampled biological particles.

References

1. R. Foks, *Energy and Evolution of Life on Earth* [Russian translation] (Mir, Moscow, 1992), 216 pp.
2. R. Shapiro, *Origins: A Skeptic's Guide to the Creation of Life on Earth* (Summit Books, New York, 1986).
3. C.W. Burch, in: *Airborne Microbes. Seventeenth Symposium of the Society for General Microbiology* (Cambridge University Press, 1967), pp. 345–374.
4. C.O. Fagain, *Methods Molec. Biol.* **59**, 339–356 (1996).
5. E.E. Nikitin and N.V. Zvyagin, *Freezing and Drying of Biological Preparations* (Kolos, Moscow, 1971), 343 pp.
6. R.J. Harris, in: *Biological Applications of Freezing and Drying* (Academic Press, New York, 1954), pp. 201–214.
7. D. Greif, in: *Recent Research in Freezing and Drying*, edited by A.A. Parkes and A.U. Smith (Blackwell Scientific Publishers, Oxford, 1960), pp. 167–187.
8. E. Mitscherlich and E.H. Marth, *Microbiological Survival in the Environment: Bacteria and Rickettsiae Important in Human and Animal Health* (Springer-Verlag, Berlin, 1984), 802 pp.
9. R.E. Strange and C.S. Cox, *Symp. Soc. Gen. Microbiol.* **26**, 111–154 (1976).
10. R. Sullivan, A.C. Fassolitis, E.P. Tarkin, R. L. Reed, and J.T. Peeler, *Appl. Microbiol.* **22**, No. 1, 61–65 (1971).
11. A. Nazim and A. James, in: *Microbial Life under Extreme Conditions*, edited by D. Kushner [Russian translation] (Mir, Moscow, 1981), pp. 470–504.
12. J.M. Fournier, *L'Immunitization Collective par Aérosols d'Antigènes Lyophilisés: Technologie, Résultats, Etude des Mécanismes Fondamentaux d'Acquisition de l'Immunité* (Lyon, 1975), 283 pp.
13. D.F. Barlow, *J. Gen. Virol.* **17**, Pt. 3, 281–288 (1972).
14. O.M. Lidwell and E.J. Lowbury, *J. Hyg.* **48**, No. 1, 6–43 (1950).
15. V.G. Frolov, Yu.M. Gusev, A.B. Ryzhikov, and A.S. Safatov, *J. Aerosol Med.* **8**, No. 1, 112.
16. R. Cadle, *Solid Particles in the Atmosphere and in Space* [Russian translation] (Mir, Moscow, 1969), 284 pp.
17. H.A. Pohl, in: *Methods of Cell Separation* (Plenum Press, 1977), Vol. 1, No. V, pp. 167–169.
18. T.S. Bakirov, A.A. Chepurnov, G.I. Tyunnikov, and V.M. Generalov, *Biotekhnologiya*, No. 4, 47–54 (1997).
19. Patent 2105815 6C12Q1/00.
20. Patent 625559 RU MKI. H05H7/00.
21. A.P. Snyder, D.B. Shoff, G.A. Eiceman, D.A. Blyth, and J.A. Parsons, *Anal. Chem.* **63**, 526–529 (1991).
22. T.S. Bakirov, V.M. Generalov, and V.S. Toporkov, *Biotekhnologiya*, No. 2, 73–82 (1998).
23. Patent 1642353 RU MKI. G01N27/22.
24. Patent 1712856 RU MKI. G01N27/26.