

N O T I C E

THIS DOCUMENT HAS BEEN REPRODUCED FROM
MICROFICHE. ALTHOUGH IT IS RECOGNIZED THAT
CERTAIN PORTIONS ARE ILLEGIBLE, IT IS BEING RELEASED
IN THE INTEREST OF MAKING AVAILABLE AS MUCH
INFORMATION AS POSSIBLE

HYDROPHOBIC COATING OF SOLID MATERIALS BY
PLASMA-POLYMERIZED THIN FILM USING TETRA-
FLUOROETHYLENE

Keiichiro Hozumi, Keisuke Kitamura
and Tatsuya Kitade

(NASA-TM-75778) HYDROPHOBIC COATING OF SOLID MATERIALS BY PLASMA-POLYMERIZED THIN FILM USING TETRAFLUOROETHYLENE (National Aeronautics and Space Administration) 17 p
HC A02/MF A01 CSCL 07C G3/27 N80-26451
Unclas 27941

Translation of "Tetorafuruoruechiren no purazuma jugomaku ni yoru kotai busshitsu no sosusei kotingu," Yakugaku zasshi, Vol. 99 No. 5, 1979, pp. 521-527.



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. MARCH 1980

STANDARD TITLE PAGE

1. Report No. NASA TM-75778	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle HYDROPHOBIC COATING OF SOLID MATERIALS BY PLASMA-POLYMERIZED THIN FILM USING TETRAFLUOROETHYLENE		5. Report Date March 1980	6. Performing Organization Code
7. Author(s) Keiichiro Hozumi, Keisuke Kitamura and Tatsuya Kitade		8. Performing Organization Report No.	10. Work Unit No.
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, California 94063		11. Contract or Grant No. NASW-3199	13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Adminis- tration, Washington D.C. 20546		14. Sponsoring Agency Code	
15. Supplementary Notes Translation of "Tetorafuruoruechiren no purazuma jūgōmaku ni yoru kotai busshitsu no sosuisei kōtingu," Yakugaku zasshi, Vol. 99, No. 5, 1979, pp. 521-527.			
16. Abstract Glass slides were coated with plasma-polymerized tetra- fluoroethylene films of different thicknesses using the glow discharge technique in a tube-shaped chamber, and the plasma conditions, film growth rates, light permeability of the polymer films, and particle bond strength in the polymer films were studied. Ashed sections of mouse organs and ashed bacillus spores were also coated to give them hydro- phobic treatment without damaging their shapes or appearance. The hydrophobic coating of the specimens was successful, and the fine ash patterns were strongly fixed onto the glass slides, making permanent preparations.			
17. Key Words (Selected by Author(s)) Tetrafluoroethylene; fluoro- carbon polymer; coating; plasma polymerization; plasma ashing		18. Distribution Statement UNLIMITED	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 17	22. Price

HYDROPHOBIC COATING OF SOLID MATERIALS BY
PLASMA-POLYMERIZED THIN FILM USING TETRAFLUOROETHYLENE [1]

Keiichiro Hozumi, Keisuke Kitamura and Tatsuya Kitade
Kyoto College of Pharmacy [2]

Thin films obtained by converting organic monomer gases of 0.1 - 1 /521* Torr into plasma by means of glow discharge and then directly polymerizing the radicals in the vapor phase onto the surfaces of solid substrates are characterized by the uniformity of their film thicknesses, their freeness from pinholes, and their high degree of crosslinking. However, by selecting the monomers it is further possible to give them considerable variety in their surface free energy, optical characteristics and electrical properties [3]. Carbon fluoride type plasma-polymerized films in particular have little surface free energy, are highly hydrophobic and chemically inert, and have abundant durability and superior light permeability [4]. Using tetrafluoroethylene (TFE) as the monomer, the authors have been conducting studies of the properties and applications of the polymerized films. The examples studied here deal with the attainment of hydrophobic properties and the structural reinforcement of ashed biological specimens with powerful hygroscopic properties. The polymerization conditions and effects are reported.

Experimental Methods

Polymerization Apparatus. A low-temperature ashing device [5] with a Pyrex chamber approximately 7 cm in inner diameter and approximately 25 cm in length was used. It was arranged so that the air and TFE /522 could be admitted alternately so that it would be possible to ash and polymerize the specimens continuously (Fig. 1). The high-frequency output (13.56 MHz) of the exciting power source had a maximum of 100 W, and the pressure inside the chamber could be adjusted within a range of 0.2 - 1 Torr. The effective high-frequency input into the chamber was

*Numbers in the margin indicate pagination in the foreign text.

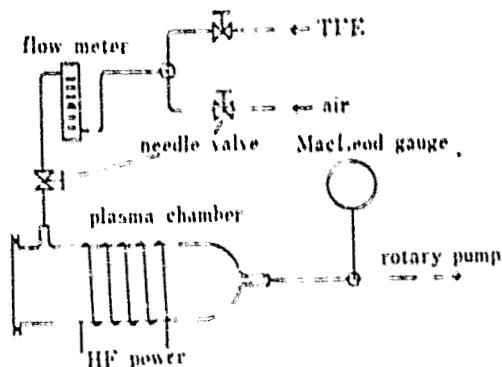


Fig. 1. Schematic Diagram of Plasma Ashing and Coating Apparatus

corrected and measured by means of a standing wave ratio meter (model SWR-200, Oscar Block Co.). The reading of reflected waves given by the standing wave ratio meter was ordinarily 40-50% in this apparatus.

TFE. The TFE was contained in high-pressure cylinders. A gas chromatograph equipped with a Polapac* column was used to confirm that there were no impurities.

Organ Sections. Mouse lung and liver isolates were fixed in formalin, and the specimens used were frozen sections of 25 μm mounted on glass slides.

Bacterial Spores. *Bacillus Megaterium* IFO 3003 was cultured in a bouillon-agar culture medium to which an excessive amount of Mn^{2+} was added. After spores had been produced on the surface, water was added and the spores were suspended. The suspension was centrifugally separated, the spores were collected, water was added again to suspend them, and centrifugal separation was carried out again. This process was repeated. Finally, the suspension was coated onto glass slides and dried.

Scanning Electron Microscope. Model JSM35, JEOL, Ltd. Acceleration voltage 20 kV.

*Conjectural spelling supplied by translator. Original has transcription in Japanese phonetics (*porapakku*). Translator could not determine correct English spelling. - Tr.

Results and Discussion

Plasma Conditions

The velocity energy of electrons is utilized in plasma polymerization to radicalize the monomers. Therefore, the polymerization rate will depend on the high-frequency electric power which is applied. At the same time, however, an unnecessarily high electron energy will also act to destroy the products. Ordinarily a mean electron energy of several eV will be necessary in order to maintain a glow discharge. This can correspond to the dissociation energy of 3-4 eV required for radicalizing organic compounds. Therefore, in terms of polymerization conditions, it is thought that the most suitable electric power would be one slightly greater than the minimum power at which discharge is possible.

A somewhat excessive electric power is necessary at the beginning of discharge. After conversion into plasma has occurred, the electric power is immediately lowered to the target value. The minimum high-frequency power necessary for maintaining the plasma state while varying the flow rate of the monomer supply (STP) and the pressure inside the chamber by this method was plotted, and the results shown in Fig. 2 were obtained. The pressure calibrations enclosed in the parentheses indicate the state before the beginning of discharge, and the calibrations

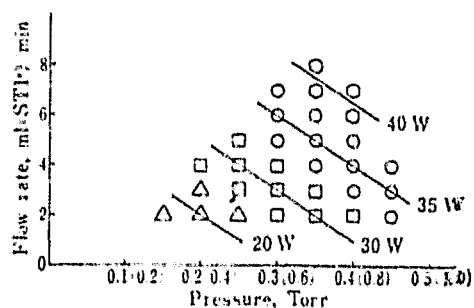


Fig. 2. Appropriate HF Powers for sustaining Mild Plasma Conditions with TFE

Pressures in parentheses indicate values before discharge.

which are not enclosed in parentheses indicate the state during discharge. The latter values indicate the decrease in the molecular count caused by polymerization of the monomer. The electric power contour lines in the figure were drawn visually from a plot of the number of watts within a range of ± 5 W expressing the various lines. The experimental blank part in the upper left corner of the figure was due to

the insufficient capacity of the vacuum pump, which had an evacuation rate of 100 l/min. The blank part in the upper right part was due to

limits on the high-frequency power source necessary to start the discharge.

In Fig. 2, when the monomer flow rate is constant, the minimum necessary power increases as the pressure rises, and when the pressure is constant, the necessary power increases together with the flow rate. In the former case, this means that the power/pressure ratio, which is a proportional parameter of the electron energy, is maintained more or less constant. In the latter case, this is interpreted as meaning an increase in the energy consumed per unit of time in conversion into a plasma. The points plotted out on the graph are believed to express plasma states having more or less the same energy level.

Film Growth Rates

Microscopic glass slides were cut into half-length pieces and put at the center of the high-frequency coils in the chamber. TFE was admitted, and the film growth rates on the glass substrates were measured. The film thicknesses were found by measuring the weight increases of the substrates by means of a chemical microbalance, and calculating from the substrate area and the density of fluororesin (approximately 2.3).

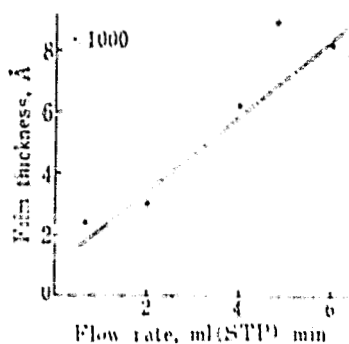


Fig. 3. Rates of Polymer Film Deposition on Glass Substrate for 20 min under Varying Flow Rates of TFE.
HF power, 35 W, pressure, 0.4 Torr.

The relationship between the changes in flow rate of the monomer and growth rate of the polymer film was measured with a fixed pressure of 0.4 Torr and a fixed power of 35 W during discharge. The results obtained are shown in Fig. 3. These /523 plasma conditions corresponding to the region in Fig. 2 where the broadest range of flow rates is available. When the film growth rate for 20

minutes was 2 ml/min, the film thickness was approximately 2,000 Å, and this increased more or less linearly as the flow rate was increased. Since the conditions involve constant

power, it would seem that the radical concentration in the vapor phase decreases together with increases in the flow rate. However, it is believed that the effect of the renewal of the diffusion layers on the surface of the glass substrate is more pronounced than the concentration effect.

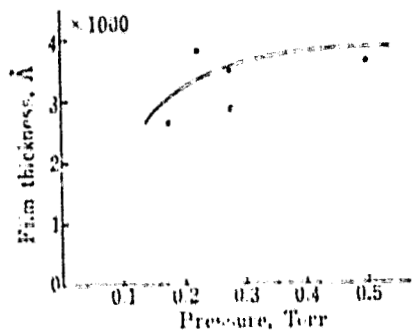


Fig. 4. Rates of Polymer Film Deposition on Glass Substrate for 20 min under Varying Gas Pressure of TFE
HF power, 30 W, Flow rate, 2 ml (STP)/min.

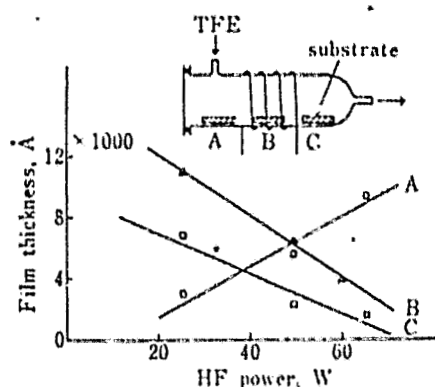


Fig. 5. Rates of Polymer Film Deposition for 20 min at Varying Locations of Substrate in Plasma Chamber
Pressure, 0.3 Torr, flow rate, 3 ml (STP)/min.

Fig. 4 shows the influence of the pressure during discharge when the monomer flow rate was fixed at " " and the power was fixed at the same value as that given above. The polymerization rate decreased as the pressure was lowered, but this can be interpreted as resulting from the fact that scission of the chemical bonds in the monomers and oligomers becomes more and more pronounced as the electron energy of the plasma gas is increased.

In order to find the relationship between the positions of the glass substrates in the chamber and the film growth rates, we took measurements while varying the power at positions upstream from the high-frequency coils (A), in the center of the coils (B), and downstream from them (C), as shown in Fig. 5. The

reaction conditions were: monomer flow rate 3 ml/min, pressure 0.3 Torr, and power 25-65 W. At position A, the film growth rate increased with the increases in the power, but at positions B and C it decreased. It is assumed that this phenomenon can be explained in terms of the following. That is, at position A there is a low field intensity outside of the coils, and radicalization of the monomer is intensified as the power is increased. However, at position B the radicalization has approached

saturation, and it is assumed that, as the electron energy increases, there is rather a progressive trend towards lower molecular weights in the vapor phase. At position C, there is the same field intensity as at position A, but the gas passing over the substrate contains low-molecular species which are not suitable for the formation of polymer films at position B. Consequently, the film growth rate patterns will have the same tendency as at position B.

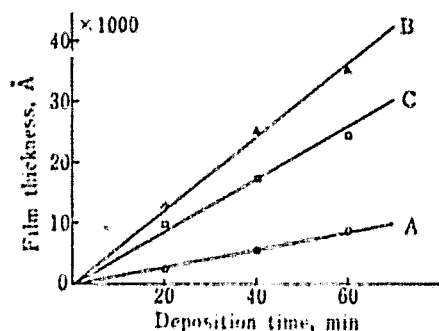


Fig. 6. Polymer Film Thickness vs. Time

Substrate locations A, B, and C are as indicated in Fig. 5. HF power, 25 W, flow rate, 3 ml (STP)/min, pressure, 0.3 Torr.

Time plots of film growth were measured for substrate positions A, B and C. The results were as shown in Fig. 6. Good linearity was attained in all the positions. However, once the apparatus had been shut down, when operations were resumed on another day, differences of about 10% /524 were observed in the growth rate even when the plasma conditions were set in the same way. Measurements of

the weights of the glass plates cannot be applied to measurements of the thicknesses of thin films. However, one can form a polymer film for 20-30 minutes after the conditions have once been set and then calculate the film growth rate per minute from the measurements of the weights. This makes it possible to estimate with considerable accuracy the thickness of the film grown in any short period of time under these conditions.

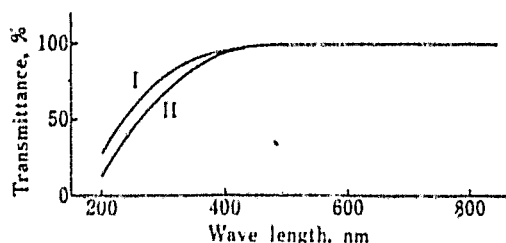


Fig. 7. Absorption Spectra of Plasma-Polymerized TFE

Film thickness, (I) 2000 Å, (II) 5000 Å.

Light Permeability of Polymer Films

The usual purpose of a coated film is to interrupt mass transfer between the solid phase and the vapor phase. However, the added value will be further increased if there is a good light permeability. We formed films of about 2,000 Å and 5,000 Å

on quartz planes and measured the ultraviolet and visible absorption spectra. As is shown in Fig. 7, the films were more or less perfectly transparent in the visible section. In the ultraviolet section, although absorption increased gradually, there was still considerable transmission even at 200 nm.

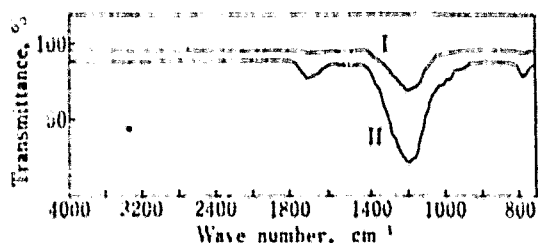


Fig. 8. IR Spectra of Plasma Polymerized TFE
Film thickness, (I) 2000 Å, (II) 10000 Å.

We also formed 2,000 and 10,000 Å films on NaCl plates and measured their infrared spectra. The results are shown in Fig. 8. At 2,000 Å there was almost no absorption except for a broad multiple absorption band of ν_{C-F} observed at 1,400 - 1,000 cm^{-1} . At a thickness of 10,000 Å, $\nu_{C=O}$ appeared at 1,720 cm^{-1} , but there was no

pronounced absorption for $\nu_{C=C}$ which adjoins this on the low-frequency side. Generally speaking, the chemical structure was estimated to be one with a high degree of saturation. As for the formation of a slight amount of the carbonyl group, it is believed that this resulted when the free radical trapped in the polymer film was oxidized by the diffusion of oxygen in the film when the substrate was taken out into the air. This is a phenomenon which is constantly experienced in plasma polymerization. A thickness of 100 Å is sufficient for the surface of a hydrophilic solid to be rendered hydrophobic, and TFE films are believed to be materials which excellent infrared permeability suitable for coatings for rendering hydrophobic optical crystals which have hygroscopic properties.

Particle Bond Strength in Polymer Films

Since polymer films grow on the solid phase directly from the vapor phase, they can be applied also to strengthen the bond construction between powder particles. As a familiar example, we took up the preservation of ash structure in ashed biological specimens. As a preliminary experiment, we first measured the bond strength of particles on glass surfaces.

Slide glass was washed with a neutral detergent, and silica gel (particle diameter about 10 μm), Polapac* (particle diameter about 20 μm), kaolin (particle diameter about 150 μm) and Amberlite (particle diameter about 150 μm) were scattered on the glass, which was then coated with TFE. Then the pieces of glass were mounted, with their coated sides facing downwards, on the upper end of the tube case of a centrifugal separator so that the particles would come off the glass surfaces in a rectangular direction when subjected to centrifugal force. The centrifugal force required to remove the particles was calculated as the shearing force per particle from the revolution speed and the turning radius.

When the particles were scattered directly onto the washed glass surface, they had considerable bonding strength with the glass surface even without a coating. This was especially true of the silica gel, no doubt largely because of the small particle diameter. It did not come off the glass even at the maximum revolution speed of the centrifuge (4,000 rpm). Since it seemed likely that the polarity on the glass surface and the intermolecular force of the adsorbed moisture was strengthening the bonding properties with the particles, the glass surface was given one coating of TFE at a thickness of approximately 5,000 \AA , and the particles were then scattered over it. The silica gel, Polapac and Amberlite still displayed some bonding force and did not fall off easily, but the kaolin fell off even at slow speeds.

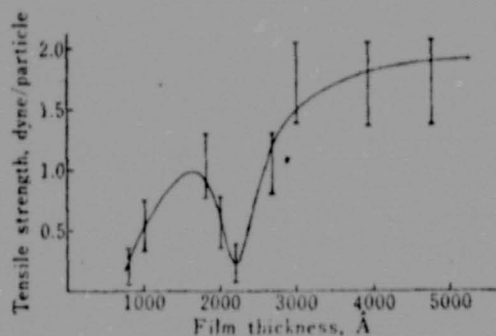


Fig. 9. Bond Strength of Kaolin Particles to Glass Surface after Polymer Film Deposition
Average diameter of kaolin, 150 μm .

Kaolin was scattered on coated glass surfaces, and these were again coated to have various film thicknesses. These specimens were put into a centrifuge, and the bonding strength per particle was measured. The results obtained are shown in Fig. 9. On the whole, the bonding strength increased together with the

*See translator's note on p. 2. - Tr.

film thickness. At a thickness of 1,000 Å it was 1 dyne/particle, and at 5,000 Å it reached 2 dyne/particle. A bonding strength of 1 dyne/particle means that a kaolin particle (particle diameter 150 μm) can withstand a weight about 200 times its own weight.

In Fig. 9, there is seen a pronounced decrease in the bonding strength in the vicinity of 2,000 Å. This decrease is reproducible. The reason is not clearly understood, but it is believed that particles which have an irregular surface structure are at first in contact with the substrate at a number of points. The polymer film at the beginning operates to strengthen the junctions at the points of contact, but the TFE is coated on the polar base on the bottom surfaces of the particles at a later time than on the upper surfaces or sides. When the coating on the bottom surfaces of the particles is completed, the bonding strength of the particles will decrease apparently if there is an electrostatic repulsion caused by opposition with the TFE film on the substrate. Not only the neutral radicals, but also some cationic radicals are present in the plasma space. Consequently, the accumulation of a positive charge on the opposing surface would not be inconceivable. One explanation of the pattern in Fig. 9 could be furnished if it were assumed that, when the polymer film grew even thicker, the state of contact between the particles and the substrate changed to a state of plane contact and the increases in the bonding strength gradually came closer to saturation.

/525

Hydrophobic Coating of Ashed Organ Sections

The low-temperature ashing method using oxygen plasma has been utilized for observations of the distribution patterns of inorganic matter in biological tissues. The method is used because of its good preservation of the original shapes [6]. However, ashed vegetable tissues are relatively stable even in the atmosphere and can be observed microscopically without any further modifications as long as they are not subjected to impacts. Nevertheless, large amounts of free phosphoric acid are present in ashed organs of animals. When they are removed from the chamber after ashing, they will rapidly absorb moisture, fuse and turn into drops of water.

If it were possible to perform both ashing and hydrophobic coating of the ashed patterns one after the other in the same chamber, the above-mentioned drawback ought to be eliminated, and at the same time it ought to be possible to strengthen the structure of the ash by means of a transparent polymer film and to obtain perfect, permanent preparations. For this reason, we mounted 25 μm frozen sections of mouse lungs and livers on slide glass. We then vacuum-dried the specimens in a chamber in which the pressure was adjusted at 1 Torr while admitting air at a rate of several ml/min . After this, a high-frequency power of about 20 W was applied, and the specimens were ashed at a low temperature for 7 hours. Next the gas being admitted was changed to TFE, and coating was performed for about 30 minutes by applying a high-frequency power of about 40 W while admitting the TFE at a rate of 2 ml/min (the pressure during discharge was 0.5 Torr).

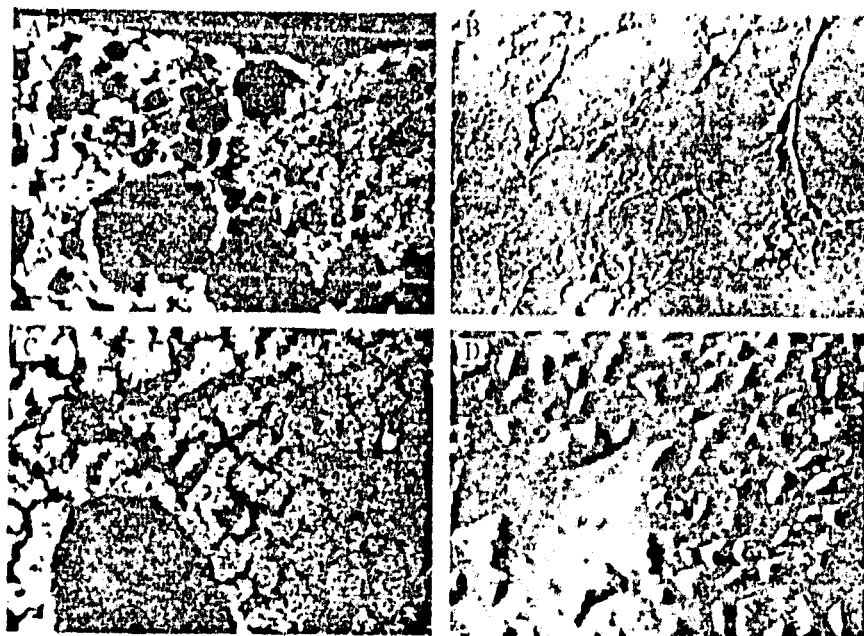


Fig. 10. Optical Micrographs of Coated and Uncoated Ash Patterns derived from Thin Sections of Mouse Organs

A; lung, coated (5000 \AA film thickness), B; lung, uncoated, C; liver, coated (*ditto*), D; liver, uncoated. Lens combination; 10×10 . All specimens were sectioned in 25 μm thickness.

ORIGINAL PAGE IS
OF POOR QUALITY

Fig. 10 shows a comparison of the ash patterns when TFE coating was performed and when it was not. In the former case, the thickness of the coating film was approximately 5,000 Å. This amounts to about 2% with respect to the section thickness of 25 µm. At the magnification of a light microscope, the effects of this film thickness will not interfere with observation of the microstructure of the ash. In addition, the strengthening of the ash structure is extremely effective, and the shapes of the ash will not be damaged even if the slide glass is tapped against the table top. The photographs were taken after the specimens had been stored for 6 months, and no changes were observed at all with the passage of this time. On the other hand, in the latter case [i.e., when coating was not provided - Tr.], the ash patterns began to deteriorate immediately, and in a few hours they turned into an aggregate of drops of water. The photographs were taken within one hour after the ashed specimens had been taken out of the chamber.

Scanning Electron Microscopic Observations of Ash Patterns of Bacillus Spores

In order to determine at the electron microscopic level the effects in preserving the microstructure of ashes, a suspension of *Bacillus Megaterium* spores was dropped a drop at a time onto slide glass, dried, then ashed and coated. Since the spores had outer coats with a relatively strengthened structure, they were quite convenient to use because they had little mechanical collapse after ashing [7]. Fig. 11 A is a scanning electron micrograph of the spores before ashing, and B shows spores which were ashed but not coated. These were observed one week after they had been taken out of the chamber. In the latter case, drops of water were formed once on account of the large amounts of phosphoric acid in the nuclei, and the ash patterns were destroyed. They were then observed again in the electron microscope, where the dried-up carcasses were visible.

C shows the spores which were coated with a film of about 100 Å after ashing (TFE 2 ml/min, power 40 W, pressure 0.5 Torr, time 60 sec). They were observed one week later. There was a slight increase in wrinkles in comparison with the state before ashing, but shapes quite close to the original shapes were retained. It is unclear whether the

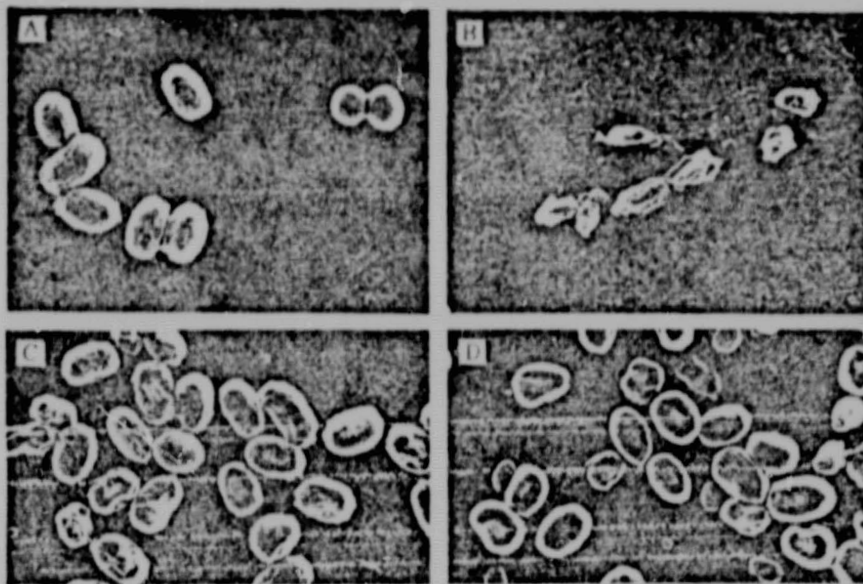


Fig. 11. Scanning Electron Micrographs of Native and Ashed *Bacillus Megaterium* Spores

A; native spores, B; ashed, but uncoated spores, C; ashed and coated spores (100 Å film thickness), D; *ditto* (500 Å film thickness). Magnification; $\times 10000$.

wrinkles were produced during the ashing or during the coating. However, it is believed that morphological changes may occur easily during ashing, and it is therefore supposed that the formation of the coating film will proceed without concomitant destruction of the ash structure. There were also no changes in the shapes in specimens which had been kept for two months. D shows the results with a 500 Å film. The details on the surface of the ash patterns have already been lost. However, when films with the thickness of D are oxidized once again with plasma in air, they will revert to the same shapes as C. Thus it was learned that additions to and deductions from the film thickness can be made freely.

Conclusion

ORIGINAL PAGE IS
OF POOR QUALITY

TFE in the vapor phase was directly polymerized onto solid surfaces by means of glow discharge in a tube-shaped chamber, and studies were made concerning the plasma conditions under which highly hydrophobic coating films are formed. First a meaningful correspondence was seen between the flow direction of the monomer and the distribution of the field intensity. It was learned that in places where there is a small field intensity the growth rate of the polymer film increases together

with the high-frequency power that is applied, but that in places where there is a great field intensity, as the power is increased, the electron impacts cause a progressive lowering of the molecular weights, and the film growth rate decreases. However, in positions downstream from a powerful field, there is an increase in low-molecular species which do not assist in polymerization, and for this reason the same tendency is displayed in weak fields as well.

When the plasma-polymerized TFE films have a thickness of 2,000 Å, their visible part is completely transparent, and it is possible to give them hydrophobic coatings of solid substances which will not damage their external appearance. In their ultraviolet part, there is a gradual increase in absorption, but there is still considerable transmission at 200 nm. In the infrared region, at the same film thickness, the transparency is almost perfect with the exception of ν_{C-F} absorption in the vicinity of $1,200\text{ cm}^{-1}$. Thus the material is considered to be suitable for coating hygroscopic optical crystals.

As examples of applications to particle aggregates with complicated structures and hygroscopic properties, we attempted to give ashed organ sections and bacillus spores hygroscopic treatment and fixation. When 25 μm sections of mouse lungs and livers were coated with 5,000 Å films, the ash patterns were firmly fixed on the slide glass, and permanent preparations were obtained which would not come off even when the glass was tapped against a table top. In scanning electron microscopic observations of ashed *Bacillus Megaterium* spores, 100 Å films were found to be appropriate. However, it was found that specimens which had films which were too thick so that the details were lost could be restored by plasma oxidation using air, and that the film thickness can be adjusted freely.

Acknowledgments. The authors express their profound gratitude to Daikin Kogyo Co., Ltd., who assisted in obtaining the monomer raw materials in this study, to Mr. Seizo Iwakami of the Osaka Prefectural Institute of Public Health, who kindly prepared the frozen organ sections, to the Imahara Laboratory of Kyoto College of Pharmacy, where the

Bacillus Megaterium spores were cultured, and to Mr. Toru Oku of the Microbiology Department of Kyoto College of Pharmacy, who gave immense collaboration in preparing the scanning electron micrographs.

REFERENCES

- [1] Delivered at the 8th Plasma Chemistry Symposium (Nagoya, November, 1978).
- [2] Location: Misasagi Nakauchi-cho, Yamashina-ku, Kyoto.
- [3] J.R. Hollahan, A.T. Bell, Techniques and applications of plasma chemistry, John Wiley and Sons, Inc., New York, 1974; Hozumi, Keiichiro, ed., Teion purazuma kagaku [Low-temperature plasma chemistry], Nankodo, Tokyo, 1976; H. Kobayashi, A.T. Bell, M. Shen, J. Appl. Polym. Sci., 17, 885 (1973); idem., J. Macromol. Sci. Chem., A9, 373 (1974); idem., Macromolecules, 7, 277 (1974); H. Yasuda, C.E. Lamaze, J. Appl. Poly. Sci., 15, 2277 (1971); idem., 17, 201 (1973); Kitamura, Keisuke, Mori, Yoshio, Horii, Michiyo, Hozumi, Keiichiro, Yakugaku zasshi [Journal of the Pharmaceutical Society of Japan], 96, 880 (1976).
- [4] J.R. Hollahan, T. Wydeven, Applied Optics, 13, 1844 (1974); T. Wydeven, R. Kubacki, ibid., 15, 132 (1976); O. Tsuji, T. Wydeven, K. Hozumi, Microchem. J., 22, 229 (1977).
- [5] C.E. Gleit, W.D. Holland, Anal. Chem., 34, 1454 (1962); J.R. Hollahan, J. Chem. Educ., 5, A401 (1966); Hozumi, Keiichiro, Matsumoto, Mamoru, Bunseki kagaku [Japan analyst], 21, 206 (1972); Hozumi, Keiichiro, Kitamura, Keisuke, Fujita, Tomomi, Mizuno, Kingo, ibid., 24, 410 (1975).
- [6] Hozumi, Keiichirō, Kagaku no ryōiki [The Journal of Japanese Chemistry], 25, 713 (1971).
- [7] R.S. Thomas, J. Cell. Biol., 23, 113 (1964).