Production of 5–15 μm Diameter Alginate-Polylysine Microcapsules by an Air-Atomization Technique

K. Keith Kwok,[1] Michael J. Groves,[1] and Diane J. Burgess[1,2]

Received May 11, 1990; accepted October 8, 1990

A novel method of preparing small-sized microcapsules using a Turbotak air-atomizer is reported. Alginate-polylysine microcapsules containing Bacillus Calmette Guérin vaccine have been prepared by an adaptation of the method of Lim (1) which allows the manufacture of small-sized microcapsules. A Turbotak is used to spray sodium alginate solution into calcium chloride solution to form temporary calcium alginate microgel capsules. These temporary microgel droplets are subsequently cross-linked with polylysine to form permanent membranes. Microcapules in the size range of 5–15 μm have been produced which can be compared to an average diameter of ≥300 μm obtained by the method reported by Lim. The microcapsule size is dependent on the conditions of operation of the Turbotak and the concentration of the sodium alginate solution. Particles within the size range 5–15 μm can be reproducibly manufactured using the conditions of operation reported here. Other size ranges below the minimum of 300 μm reported by Lim are also feasible using this technique.

KEY WORDS: alginate/polylysine microcapsules; atomization technique; 5–15 μm microcapsules; Bacillus Calmette Guérin (BCG) vaccine.

INTRODUCTION

It is our intention to deliver live Bacillus Calmette Guérin (BCG) vaccine to the lung. Delivery to the lung either by intravenous injection or by inhalation is dependent on the particle size. It is well established that the size and charge of colloidal particles will determine their biodistribution following intravenous injection (2,3). Particles in the size range of 5–15 μm injected intravenously will be passively targeted to the lung as a result of entrapment within the lung capillaries. The size range for delivery by inhalation is 1–5 μm. BCG organisms have an average length of 2.36 μm and width of 0.47 μm (4). To achieve the size range desired for delivery to the lung, 5–15 or 1–5 μm, the organisms can be microencapsulated. Microencapsulation of live BCG organisms for targeted delivery to the lung may also aid retention of the organisms at the target site; have an immune adjuvant effect; and protect against possible side effects of live BCG organisms, which can range from nausea and vomiting to a full-blown Tuberculosis-type syndrome.

BCG is known as a potent immune adjuvant, effective in the treatment of cancer patients who retain a degree of immunocompetency (5–8). The effectiveness of BCG in treating neoplasia is attributed to its ability to stimulate the reticuloendothelial system (RES). RES stimulation results in hyperplasia and increased phagocytic and cytolytic activities of the macrophage. However, this effect is localized to those cells in the area surrounding BCG, indicating that close contact between BCG and the tumor is important (9–11). BCG is very effective in bladder cancer (12), where it can be delivered directly to the tumor site. However, in lung cancer where it is difficult to localize BCG at the tumor site, therapy is less successful. We therefore propose to develop a method for the delivery of BCG directly to the tumor site in lung cancer, in an attempt to increase its efficacy.

In order to deliver live BCG to the lung, a method of microencapsulation which is not harmful to live organisms is required. Most methods of microencapsulation involve one of two harsh conditions which would be toxic to cells: contact with an organic solvent and/or heating during processing. For example, microencapsulation by coacervation of oppositely charged gelatins may involve the use of either formaldehyde or heat to cross-link the gelatin (13). The production of poly(*d,l*-lactide) and poly(glycolic acid) microspheres by solvent removal techniques involves the use of organic solvents such as methylene chloride and hexafluoroacetone sesquihydrate to dissolve the polymers (14,15). Albumin microspheres used to encapsulate steroids involve the use of glutaraldehyde solution and/or heat (which can be as high as 130°C) to stabilize the microspheres (16).

The microencapsulation of functioning islets of Langerhans, in an alginate-polylysine-alginate membrane has been reported by Lim and Sun (1). These authors have shown that the cells remain viable after microencapsulation. The size of the microcapsules produced by this method is larger than 300 μm. In the present paper an adaptation of the method of Lim and Sun, which can produce microcapsules in the size range of 5–15 μm suitable for passive delivery to the lung following intravenous injection, is reported.

MATERIALS AND METHODS

Sodium alginate (medium viscosity), poly-*l*-lysine (MW 38,500), sodium citrate, calcium chloride, sodium chloride, and bile salts were purchased from Sigma Chemicals. BCG (Tice) were obtained from the Institute for Tuberculosis Research, University of Illinois at Chicago. The Fisher Diagnostic AFB stain kit was purchased from Fisher Scientific. The Millex-GS 0.22 μm filter was purchased from Millipore. Auramine Rhodamine T TB fluorescent stain was purchased from Difco.

Production of Microcapsules

The microencapsulation procedure, optimized to produce microcapsules in the size range 5–15 μm, is as follows. Sodium alginate (1.2%) is infused from a syringe pump (Sage Instrument, Model 355) at 2.2 ml/min into an air-atomizing device, (Turbotak, Turbotak, Inc., Waterloo, Ontario) (Fig. 1) and sprayed into a pan containing 500 ml of 1.5% calcium chloride solution. A Turbotak is a hollow stainless-steel cylinder, 1 in. wide × 1.5 in. long. Liquid is fed into the Tur-

[1] Department of Pharmaceutics, M/C 880, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612.

[2] To whom correspondence should be addressed.

0724-8741/91/0300-0341$06.50/0

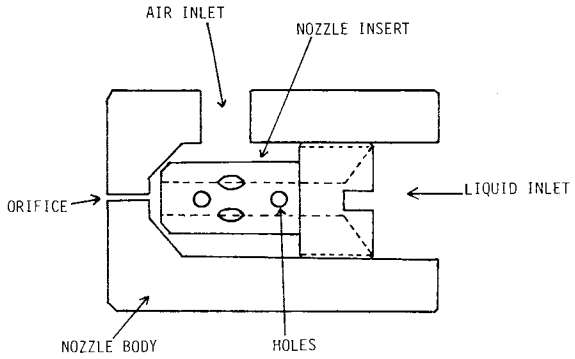

Fig. 1. Schematic diagram of Turbotak.

botak from the top and pressurized air is fed in from the side. The pressurized air mixes with the liquid, forcing tiny liquid droplets out through the orifice of the nozzle. The air pressure is set at 40 psi. The distance between the orifice of the Turbotak and the pan is fixed at 18 in. The size of the orifice is 1 mm in diameter. The divalent calcium ions cross-link the droplets of sodium alginate on contact to form temporary microgel droplets. The temporary capsules are crosslinked by poly-*l*-lysine (0.035%, w/v) for 6 min to form a permanent membrane (initial-stage permanent capsules) (Fig. 2). The resultant permanent capsules are collected by centrifugation at 3000 rpm, washed in 30 ml 0.9% (w/v) sodium chloride, and then suspended for 4 min in 0.03% (w/v) sodium alginate to form an alginate outer layer. The alginate-poly-*l*-lysine-alginate microcapsules are washed twice with 0.9% sodium chloride. These microcapsules are added to 30 ml of 55 m*M* sodium citrate, pH 7.4, for 6 min to liquify the calcium alginate gel inside (final-stage permanent capsules). The resulting capsules are washed twice in 0.9% (w/v) sodium chloride. All references to permanent capsules imply final-stage permanent capsules in the following text.

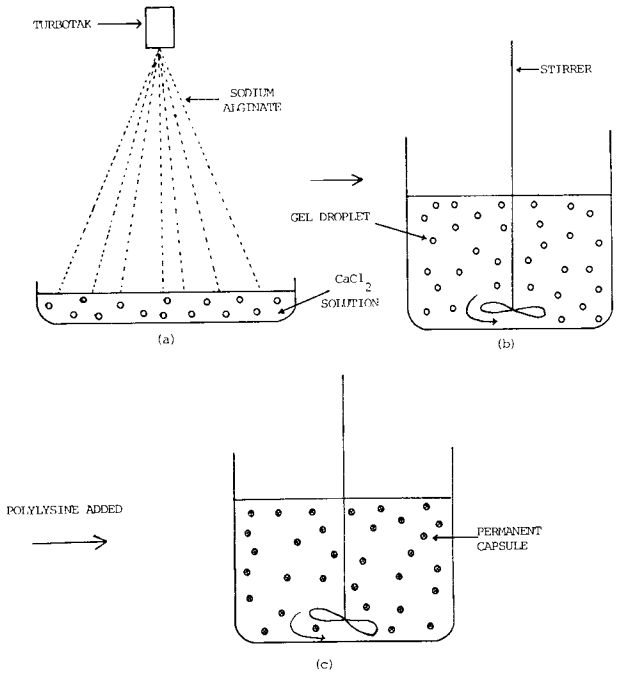

Fig. 2. Schematic diagram of the preparation of alginate-polylysine microcapsules: (a) spraying of sodium alginate solution into calcium chloride solution; (b) temporary gel microcapsules formed; (c) permanent microcapsules (initial stage) formed.

Encapsulation of BCG

The BCG used in the following experiments were heat killed at 80°C for 1 hr, to avoid contact with atomized live BCG during these developmental studies. One ampoule of BCG is reconstituted in 0.5 ml of 0.1% (w/v) bile salt and dispersed in 15 ml of 1.2% (w/v) sodium alginate. This suspension is sprayed into calcium chloride solution and processed to form permanent capsules as described above. The encapsulated BCG are identified using Auramine Rhodamine T TB fluorescent stain under a fluoresence microscope and using a Fisher AFB stain kit under an optical microscope.

Particle Size Analysis

The mean diameter and the size distribution of the capsules, with and without encapsulated BCG, are determined using a HIAC/Royco particle sizer, Model 4100. The temporary capsules are suspended in 1.5% (w/v) calcium chloride solution, to prevent dissolution of the calcium alginate gel, and the permanent capsules are suspended in 0.9% (w/v) sodium chloride.

Freeze-Drying of Microcapsules

Permanent microcapsules are washed twice with distilled water and freeze-dried using a Labconco freeze-dryer. The capsules are frozen at −70°C overnight, and then freeze-dried (−60°C, 10 μm Hg) for 72 hr. Scanning electron micrographs of the freeze-dried microcapsules are taken.

RESULTS AND DISCUSSION

Selection of Method of Air-Atomization

Various methods of air-atomization to produce small droplets were investigated. Intially an air brush (H type, No. 3, Paasche Air Brush Co.), connected to a compressed air source and operated in short bursts of 1–2 sec, was tested and shown capable of producing droplets in our desired size range (5–15 μm) (17). This method was operator dependent and therefore irreproducible. Moreover, this method was inefficient and time-comsuming. Plugging of the air brush nozzle was a major problem. A hospital jet nebulizer and a commercial nasal inhaler were also investigated. The mean droplet size produced by these methods was either too large or too small and the size distributions were too broad.

The Turbotak air-atomizer can produce droplets within the desired size range, with a narrow size distribution, both reproducibly and efficiently (18). The data presented here, obtained using a HIAC/Royco particle sizer, indicate that all the microcapsule preparations—empty temporary, empty permanent, temporary with encapsulated BCG, and permanent with encapsulated BCG—approximate to a log normal (Poisson) size distribution (Fig. 3). The HIAC/Royco instrument is a light blockage device detecting a cross-sectional area of the profile of the microcapules. It detects the size of the particles as the diameter of a circle of equivalent area and determines the surface/number diameter, *dsn* (19). This di-

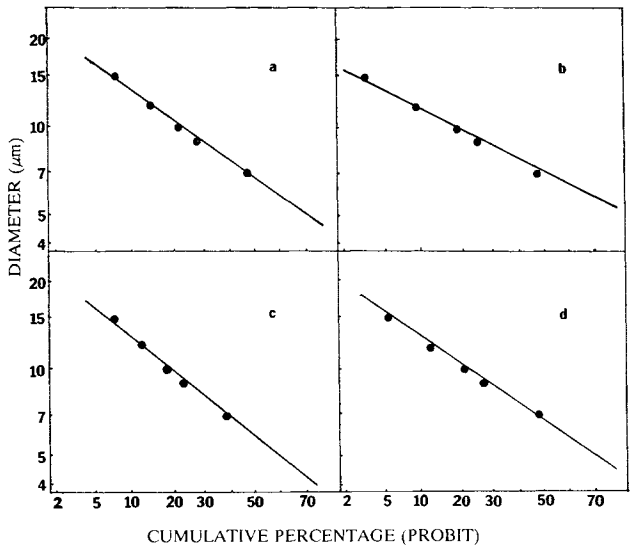

Fig. 3. Cumulative size distribution of microcapsules obtained using HIAC/Royco instrument and plotted as a log-probit graph. (a) Temporary microcapsules; (b) permanent microcapsules; (c) temporary microcapsules with BCG encapsulated; (d) permanent microcapsules with BCG encapsulated.

ameter can be converted to a specific surface area per unit volume (S_v), by using the Kapteyn transformation to derive a surface-weighted mean diameter, *dvs* (20):

$$\ln dvs = \ln dsn + 1.5 \ln^2 \delta g$$

where ln is the natural logarithm and δg is the geometric standard deviation of the number distribution. The size characteristics of different microcapsules are summarized in Table I. There are more permanent microcapsules within the desired size range than the temporary ones, from the data obtained from both *dsn* and *dvs*. For example, from the *dvs* data, there are 86.5%, as compared to 65%, for the empty microcapsules and 67.5%, as compared to 63%, for the microcapsules with BCG encapsulated. This is due to the loss of smaller-sized microcapsules during the production process of permanent microcapsules. The smaller percentage of BCG microcapsules within the desired size range, compared to the empty microcapsules, is probably due to aggregation of the BCG organisms. This method of microencapsulation is quite efficient, as in all cases the percentage of microcapsules over 15 μm is no greater than 26% by surface-weighted mean.

The specific surface area per unit volume can also be calculated using the following equation:

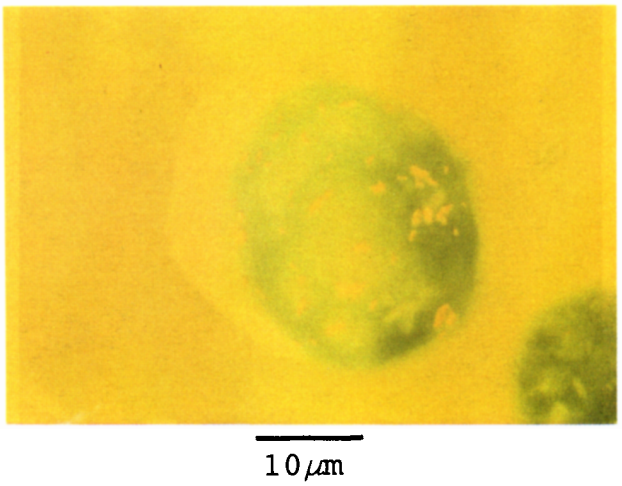

Fig. 4. Photomicrograph of BCG-encapsulated microcapsules under a fluorescence microscope. × 400; reduced 35% for reproduction.

$$S_v = \frac{6}{dvs} \text{ cm}^2 \text{ per cm}^3$$

The specific surface area per unit volume is equivalent to the total surface area in one cm^3 of microcapsules. The total surface area of the microcapsules is a critical factor in terms of the viability of the BCG organisms, as it determines the diffusion of nutrients into the microcapsule matrix and the excretion products out of the microcapsules.

The size of the temporary capsules is determined by the processing variables: size of the nozzle, infusion rate and concentration of sodium alginate solution, and applied air pressure. These variables have been manipulated successfully to obtain microcapsules in the desired size range. Microcapsules can be produced in other size ranges by changing one or more of these variables. Decreasing the air pressure and the viscosity of the sodium alginate solution and increasing the infusion rate and the nozzle size all increase the average particle size.

Figure 4 is a photomicrograph of encapsulated BCG obtained by fluorescence microscopy. Due to the limitations of the microscope and the camera a larger size microcapsule (approximately 30 μm in diameter) was photographed. The microcapsules with encapsulated BCG are processed by Auramine Rhodamine T TB fluorescent stain. The BCG organisms fluoresce under a Zeiss fluorescent microscope. It is evident that the organisms are encapsulated and that the microcapsules are spherical. The encapsulated BCG organisms were also identified using a Fisher Diagnostic AFB stain kit. The BCG organisms stain red.

The microcapsules were freeze-dried to test whether

Table I. The Size Characteristics of Different Microcapsules as Measured by HIAC/Royco Particle Sizer

Microcapsules	*dsn* (μm)	δ	% within range	% >15 μm	*dvs* (μm)	% within range	% >15 μm	S_v (cm^2/cm^3) × 10^{-3}
Temporary	6.7	1.74	62.5	6.5	10.61	65.0	26	5.66
Permanent	7.2	1.44	81.5	2.5	8.79	86.5	7	6.83
Temporary with BCG	5.8	1.81	55	6.0	9.83	63.0	24	6.10
Permanent with BCG	6.7	1.72	65	5.3	10.42	67.5	24	5.76

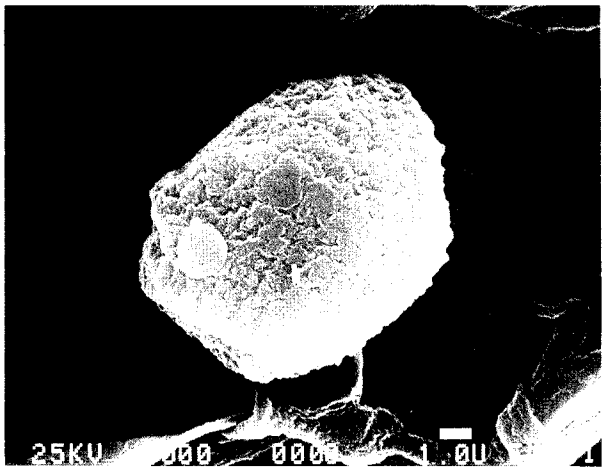

Fig. 5. Scanning electron micrograph of a permanent microcapsule after freeze drying. × 6000; reduced 30% for reproduction.

they could withstand this process, as a freeze-dried product would be ideal for maximum stability during storage. A scanning electron photomicrograph of the permanent microcapsules after freeze-drying is shown in Fig. 5. Different batches of freeze-dried microcapsules were examined by SEM. Apparently the freeze-drying process reported does not destroy these microcapsules, as all the microcapsules were intact. The surface of the microcapsules appears wrinkled when viewed under SEM. This is probably due to the loss of water content during the freeze-drying process.

CONCLUSIONS

The Turbotak air-atomizing method reported here can produce small-sized alginate-polylysine-alginate microcapsules in the size range of 5–15 μm, with about 70% of the microcapsules in this size range. The microcapsules are roughly spherical in shape and are able to withstand freeze-drying. BCG organisms have been successfully encapsulated within these microcapsules.

ACKNOWLEDGMENTS

The authors wish to thank I. Kairys of the EMF Department and L. McDuffy of Virology Section, Hospital Clinical Laboratory, University of Illinois at Chicago, for technical assistance and the Institute for Tuberculosis Research for funding this project.

REFERENCES

1. F. Lim and A. M. Sun. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 210:908–910 (1980).
2. L. Illum and S. S. Davis. Targeting of drugs parenterally by use of microspheres. *J. Parent. Sci. Tech.* 36:242–248 (1982).
3. L. Illum, P. D. E. Jones, and S. S. Davis. Active targeting in-vivo and in-vitro—homing with antibodies and magnetic particles. In S. S. Davis, L. Illum, J. G. McVie, and E. Tomlinson (eds.), *Microspheres and Drug Therapy*, Elsevier, Amsterdam, 1984, pp. 353–363.
4. M. J. Groves, M. E. Klegerman, P. Devadoss, O. N. Singh, and Y. Zong. Characterization of BCG vaccine by Coulter counter and scanning electron microscopic techniques. *Part. Part. Sys. Character.* (in press).
5. M. F. McKneally, C. Maver, H. W. Kausel, and R. D. Alby. Regional immunotherapy with intrapleural BCG for lung cancer: Surgical consideration. *J. Thorac. Cardio. Surg.* 72:333–338 (1976).
6. M. F. McKneally, C. Maver, and H. W. Kausel. Regional immunotherapy of lung cancer with intrapleural BCG. *Lancet* 1:377–379 (1976).
7. M. F. McKneally, C. Maver, and H. W. Kausel. Intrapleural BCG immunostimulation in lung cancer. *Lancet* 1:1003 (1977).
8. E. M. Hersh, J. U. Gutterman, and G. M. Mavligit. BCG as adjuvant immunotherapy for neoplasia. *Annu. Rev. Med.* 28:489–515 (1977).
9. B. Zbar, I. D. Berstein, and H. J. Rapp. Suppression of tumor growth at the site of injection with living BCG. *J. Natl. Cancer Inst.* 46:831–839 (1971).
10. C. Pinsky, Y. Hirshaut, and H. Oettgen. Treatment of malignant melanoma by intratumoral injection of BCG. *Natl. Cancer Inst. Monogr.* 39:225–228 (1973).
11. L. Nathanson. Regression of intradermal malignant melanoma after intralesional injection of BCG. *Cancer Chemother. Rep.* 56:659–665 (1972).
12. D. R. Kelley and W. J. Catalona. BCG therapy for superficial bladder cancer. In W. J. Catalona and T. L. Ratliff (eds.), *Urologic Oncology*, Martinus Nijhoff, Boston, 1984, pp. 169–184.
13. D. J. Burgess and J. E. Carless. Manufacture of gelatin/gelatin coacervate microcapsules. *Int. J. Pharm.* 27:61–70 (1987).
14. S. Benita, J. P. Benoit, F. Puisieux, and C. Thies. Characterization of drug-loaded poly (d,l-lactide) microspheres. *J. Pharm. Sci.* 73:1721–1724 (1984).
15. T. Sato, M. Kanke, H. Schroeder, and P. DeLuca. Porous biodegradable microspheres for controlled drug delivery. I. Assessment of processing conditions and solvent removal techniques. *Pharm. Res.* 5:21–30 (1988).
16. D. J. Burgess, S. S. Davis, and E. Tomlinson. Potential use of albumin microspheres as a drug delivery system. I. Preparation and in vitro release of steroids. *Int. J. Pharm.* 39:129–136 (1987).
17. D. J. Burgess, H. S. Kas, J. M. Kuk, and M. J. Groves. Alginate-polylysine microspheres containing living BCG vaccine for site specific delivery. Presented at the AAPS First National Meeting of the Biotechnology Section, Reno, Nevada, January 1988.
18. K. K. Kwok, M. J. Groves, and D. J. Burgess. Sterile microencapsulation of BCG in alginate-poly-l-lysine by an air spraying technique. *Proceedings of the 16th International Symposium on Controlled Release of Bioactive Materials,* Chicago, August 1989, pp. 342–343.
19. T. Allen, *Particle Size Measurement,* 3rd ed., Chapman and Hall, New York, 1981.
20. G. Herdan. *Small Particle Statistics,* 2nd ed., Butterworths, London, 1960.