0273-2289/89/2021—0603$03.40

Mass Transfer Effects in Microencapsulated Hybridoma Cells Producing Monoclonal Antibodies

W. W. EDMUNDS,[1] F. KARGI,[1,*] AND C. SORENSON[2]

[1]*Biotechnology Engineering Laboratory, Department of Chemical Engineering, Washington University, St. Louis, MO 63130; and*
[2]*Department of Pathology, Jewish Hospital, Washington University, St. Louis, MO 63130*

ABSTRACT

Rat-mouse and mouse-mouse hybridoma cell lines were used for formation of monoclonal antibodies (MAbs) in microcapsules of different sizes. Microcapsules were made of poly L-lysine-alginate hydrogel membranes. The effects of extracapsule liquid film, intracapsule and transmembrane transfer limitations of nutrients/products on system's performance were investigated. An agitation speed of 45 rpm (4 cm/s tip speed) was found to be optimal in spinner flasks to overcome liquid film resistances around capsules. Capsule sizes need to be reduced to smaller than 350 μ in order to eliminate intracapsule transfer limitations with a typical initial viable cell concentration of 0.5×10^5 viable cells/mL capsule. Double coating of capsules to improve strength of capsules resulted in higher transmembrane transfer resistances.

Index Entries: Mass transfer; microencapsulation; hybridoma cells; monoclonal antibodies.

INTRODUCTION

Various methods have been developed for cultivation of hybridoma cells in different reactor configurations because of potential utilization of these cells for formation of healthcare products such as monoclonal anti-

*Author to whom all correspondence and reprint requests should be addressed.

bodies, lymphokines, and growth hormones (*1–5*). The most widely used cultivation methods for hybridoma/mammalian cells are:

1. Suspension cultures in mechanically agitaged (*6*) or air lift/ bubble column reactors (*7*);
2. Immobilization of cells on microcarriers (*15,16*), in hollow fiber reactors (*8,9*), in a ceramic matrix (*11*), and by encapsulation of cells in polymer membrane capsules (*12*);
3. Use of membrane bioreactors for removal of toxic products and concentration of high MW useful products (e.g., MAbs, lymphokines) (*13*).

Each one of the aforementioned methods has some relative advantages and disadvantages. Mechanically agitated reactors impose high shear stresses on cells and cause cell damage and lysis. However, specially designed sail-type agitators were developed to overcome cell damage problems caused by hydrodynamic shear and collisions (*3*). Airlift and bubble column reactors seem to be more suitable than mechanically agitated vessels for suspension culture of hybridoma/mammalian cells, since cells are exposed to low shear levels. However, shear stress due to direct aeration (i.e., air bubbles) usually causes damage to cells. The use of membrane aeration may require large membrane surface areas that may not be practical.

Immobilization of cells on microcarriers (*14–16*), in hollow fiber reactors (*8,9*), in a ceramic matrix (*11*), in gel beads (*10*), and inside capsules (*12*) were developed to overcome some problems associated with suspension culture of hybridoma cells. Major problems associated with cultivation of hybridoma/mammalian cells are: shear sensitivity of cells, formation of toxic metabolites (i.e., lactic acid and ammonium), and low product concentrations (i.e., MAbs, lymphokines). A good cultivation method should protect the cells against shear, remove toxic metabolites as produced, and concentrate the high MW desirable products as formed. The concept of microencapsulation resolves all of the aforementioned problems and has distinct advantages over other methods. These advantages can be summarized as follows

1. High culture densities may be obtained within the capsule that provides high degree of cell–cell contact and interaction resulting in possibly more favorable microenvironmental conditions.
2. Microencapsulation provides simultaneous product separation and cell cultivation, resulting in concentration of high molecular weight metabolic products within the capsule (e.g., MAbs). Preconcentration of products within the capsule facilitates further purification steps.
3. Toxic metabolic products (e.g., NH_4^+, lactic acid) will continuously diffuse out of intracapsule culture medium, resulting in higher rates of growth and product formation.

4. Cells growing inside capsules will be protected against high shear and other sudden changes in culture medium.
5. Cells and products are separated from nutrient phases during the cultivation, making the downstream processing more efficient.
6. Direct aeration by air bubbles can be used without damaging the cells.

With the use of serum free media and adequate aeration/agitation, microencapsulated cell technology can be further perfected. Encapsulation of cells has been investigated to a certain extent (*12,17–20*). One of the encapsulation methods used for hybridoma cells is the use of alginate-poly-L lysine membrane capsules originally developed by Lim (*20*) and used by Damon Biotech Corporation (Needham, MA) (*12*). Typical MW cutoff of these membranes is 70–80 kd, which allows retention of MAbs inside capsules and removal of low MW toxic metabolic products by diffusion.

The reported studies on encapsulated hybridoma cells (*12*) were conducted with large capsule sizes (~ 800 μ). The linear growth of encapsulated hybridomas in reported studies (*12*) indicates possible transmembrane and intracapsule nutrient transfer limitations. Elimination of possible mass transfer limitations, such as, external liquid film, intracapsule and transmembrane mass transfer resistances would possibly result in improved growth and product formation rates since formation of MAbs are mixed growth associated (*12*).

The objective of this study is to investigate the effects of nutrient/product transfer limitations (i.e., external liquid film, transmembrane, and intracapsule) on the rate and extent of growth and product formation by encapsulated hybridoma cells in batch experiments. External liquid film resistance was reduced by changing the agitation speed (rpm) in the spinner flasks; transmembrane resistance was varied by using single and double coated capsules; and intracapsule resistance was reduced by reducing the capsule size. Double coated capsules were used to improve the capsule strength.

MATERIALS AND METHODS

Microcapsule Preparation

Microcapsules are prepared using the procedure developed by Lim and Moss (*21*). The major steps of encapsulation are depicted in Fig. 1. Cell suspension was mixed with 3 wt% Na-alginate solution by using a 50%/50% mixture of concentrated cell suspension and alginate solution until a uniform suspension is formed. This mixture is extruded in form of droplets into a 1.3 wt% $CaCl_2$ solution using a special droplet extrusion de-

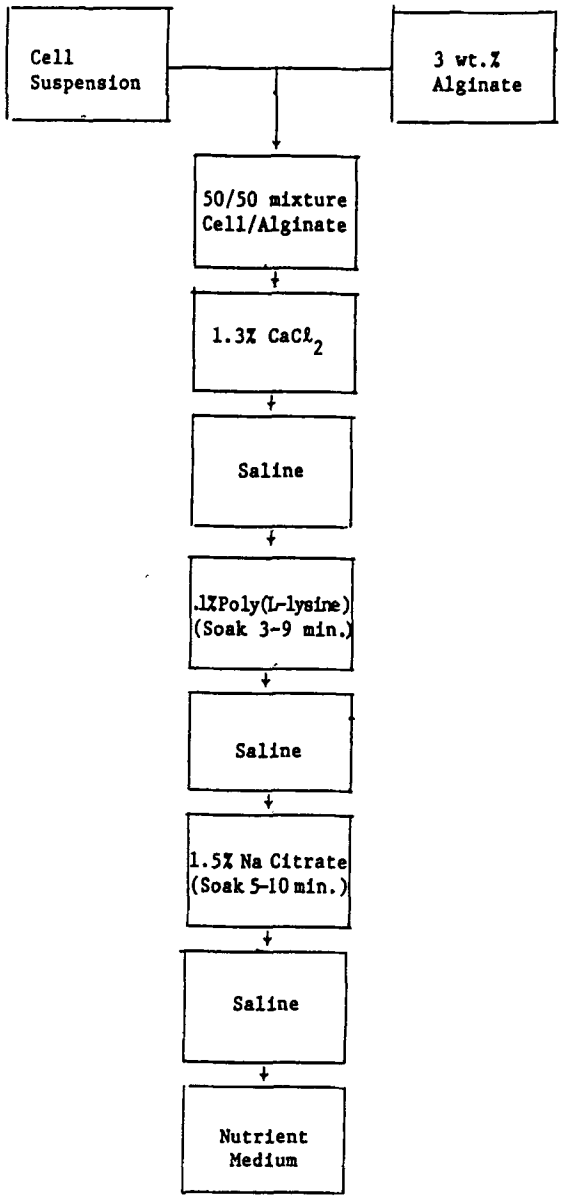

Fig. 1. Encapsulation procedure used (Lim, 1981).

vice to form Ca-alginate beads. After washing the Ca-alginate beads with saline solution, the beads were treated with poly-L-lysine (PLL), a positively charged polyelectrolyte. Gel beads were soaked in a dilute (0.1 wt%) PLL solution for 5–10 min to form the PLL-alginate complex in the outer shell of the beads. The concentration of MW of poly-L-lysine (PLL) and contact time of PLL with the beads determine the thickness of the PLL-alginate outer shell. After washing the PLL-treated beads with saline

solution, the beads were soaked in a 1.5 wt% Na-citrate solution for 5–10 min. Citrate molecules diffuse inside the beads and liquify Ca-alginate unbound to PLL inside the beads by removing Ca^{+2} ions from alginate. Sodium citrate treatment generates hollow spherical capsules with a Na-alginate liquid core surrounded by a PLL-alginate membrane. Cells remain and proliferate inside the liquid core. After Na-citrate treatment, the capsules were washed with saline solution for the last time and were suspended in nutrient medium for further experiments. Microcapsule formation was carried out under aseptic conditions in a laminar flow hood. All solutions and equipment were steam sterilized before use except PLL which was filter sterilized. Culture sterility was checked throughout the experiments.

Cultivation of Hybridoma Cells

A rat–mouse B-cell hybridoma line (984D4.65) obtained from the hybridoma center at the Medical School was used for two sets of experiments on external and internal mass transfer limitations. A mouse–mouse hybridoma cell line was used for experiments on transmembrane mass transfer limitations.

The media used for cultivation of cells had the following composition: RPMI 1640: (a complex media) 90.8%; glutamine: 1%; sodium pyruvate: 1%; 2ME:0.1%; Calf or feotal bovine serum: 5% antibiotics: 1%; Na bicarbonate: 1% (22, 23). Cells were maintained in T-flasks and cultured in spinner flasks (250–500 mL) before encapsulation. T-flasks were placed in a carbon dioxide incubator at 36.5°C. A CO_2-air mixture (5% CO_2) was passed through the CO_2 incubator. Spinner flask experiments were conducted in a clean warm room at 36.5°C.

Encapsulated cells were cultivated in 250 mL spinner flasks with 100 mL total mixture volume. The volume fraction of capsules was nearly 7% of total volume. The agitation speed was varied between 15–60 rpm. The head space of the spinner flasks was purged with a CO_2-enriched, tri-gas mixture with the following composition: 7% O_2, 10% CO_2, 83% N_2. The pH of the media was pH=7.0–7.3 throughout the experiments. The experiments were carried out in a clean, constant temperature room at 36.5°C. No significant evaporation losses were observed.

Analytical Methods

Samples were removed from culture media on a daily basis. The samples were analyzed for total and viable cells, glucose, and MAb concentrations after capsules were disrupted.

Capsules were disrupted using heparin (*24*) before analysis. Capsules removed from culture media were washed with 150 m*M* NaCl two times. The volume of capsules was determined by adding the capsules to a NaCl solution in a test tube and measuring the volume displaced by cap-

sules. Then the capsules were incubated in a Na-heparin solution (1000 u/mL) containing 75 m*M* $CaCl_2$ at 37°C for 10 min. After heparin treatment, the capsules were washed once with 150 m*M* NaCl. Then, a certain volume of 55 m*M* Na-citrate in 150 m*M* NaCl was added to the capsules. The mixture was incubated at 37°C for 5 min, leaving the capsules completely disrupted.

Total cell concentration was determined using a hemocytometer (*25*). Cell viability was determined by using fluorescein diacetate assay (*26*). Viable cells take up fluorescein diacetate and hydrolyze it to fluorescein which fluoresce. Nonviable cells cannot catalyze this conversion and do not fluoresce. Number of viable cells were determined by counting the fluorescing cells using a hemocytometer. Then, the viability was expressed as the fraction of total cells fluorescing.

After counting the cells from lysed capsules, the cells and capsule fragments were removed by centrifugation. The supernatant was analyzed for MAbs. Samples for glucose analysis were removed from liquid nutrient media exterior to capsules.

Glucose concentration was determined using the hexokinase assay (*27*) followed by colorimetric determination at 520 nm.

Monoclonal antibody (MAb) concentration was determined using the enzyme linked immunosorbent assay (ELISA). The assay is based on coupling highly specific antibodies to an enzyme and an ELISA plate. The Ab-enzyme complex retains the immunological specificity of the antibody and catalytic activity of the enzyme (*30*).

RESULTS AND DISCUSSION

The major objective of this study was to investigate the effects of various levels of nutrient/products transfer resistances (external liquid film, transmembrane, intracapsule) on performance of encapsulated hybridoma cells producing monoclonal antibodies.

External Liquid Film Resistance

Since the culture media was well mixed and surface aerated with CO_2-enriched air, the mass transfer resistances around the gas bubbles and in the bulk liquid were considered negligible. Aeration rate was adjusted such that the dissoved oxygen concentration in liquid media was above the limiting level (>2 ppm). Because of the relatively large size of capsules ($\overline{D}_p = 700\ \mu$), the liquid film resistance around capsules was considered to be significant. The influence of this resistance was investigated by changing the agitation speed in spinner flasks between 15–60 rpm.

Four 250 mL spinner flasks were filled with 100 mL nutrients and capsule mixture and were agitated at 15, 30, 45, and 60 rpm. A 28 mL of uni-

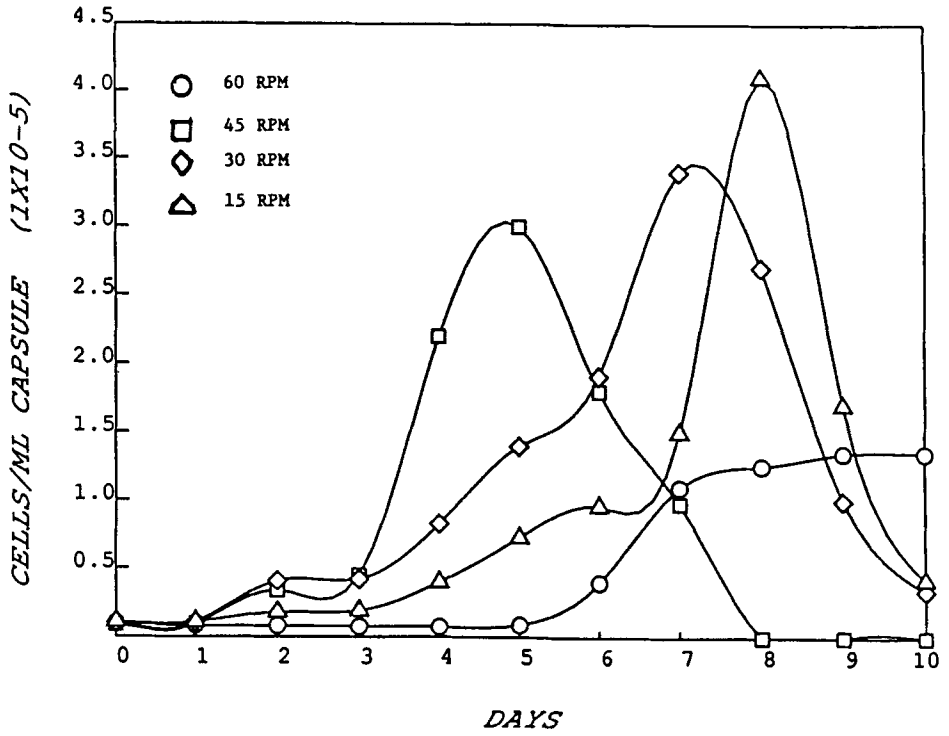

Fig. 2. Time course of variation of viable encapsulated cell concentration at different agitation speeds. Double coated capsule size: 700 μ.
○ 60 RPM, □ 45 RPM, ◇ 30 RPM, △ 15 RPM.

form batch of capsules ($\overline{D}_p = 700\ \mu$) was prepared and divided evenly into four spinner flasks. Initial viable cell concentration in all capsules was nearly 0.1×10^5 cells/mL capsule. The capsules were double coated with PLL. The MW of the PLL used for the inner coat was 41 kd and that of the outer coat was 59 kd. A 93 mL of nutrient media containing 5% calf bovine serum and 7 mL of capsules were mixed in each spinner flask. The gas head space of each flask was purged with a tri-gas mixture (7% O_2; 10% CO_2, 83% N_2). The flasks were incubated in a clean, warm room at 36.5°C. The pH of culture media was controlled between pH = 7.0–7.3 by passing CO_2-enriched air through head space of flasks. Samples were removed from flasks everyday and were analyzed for glucose, total and viable cell concentrations.

Time course of variation of viable cell and glucose concentrations in four different spinner flasks are depicted in Figs. 2 and 3, respectively. The fastest increase in viable cell concentrations was obtained at 45 rpm (4 cm/s tip speed). Similarly, glucose consumption rate was fastest at a 45 rpm agitation speed. The rate of cell growth and glucose consumption increased steadily with increasing agitation speed between 15–45 rpm indicating the presence of external liquid film resistance around capsules at

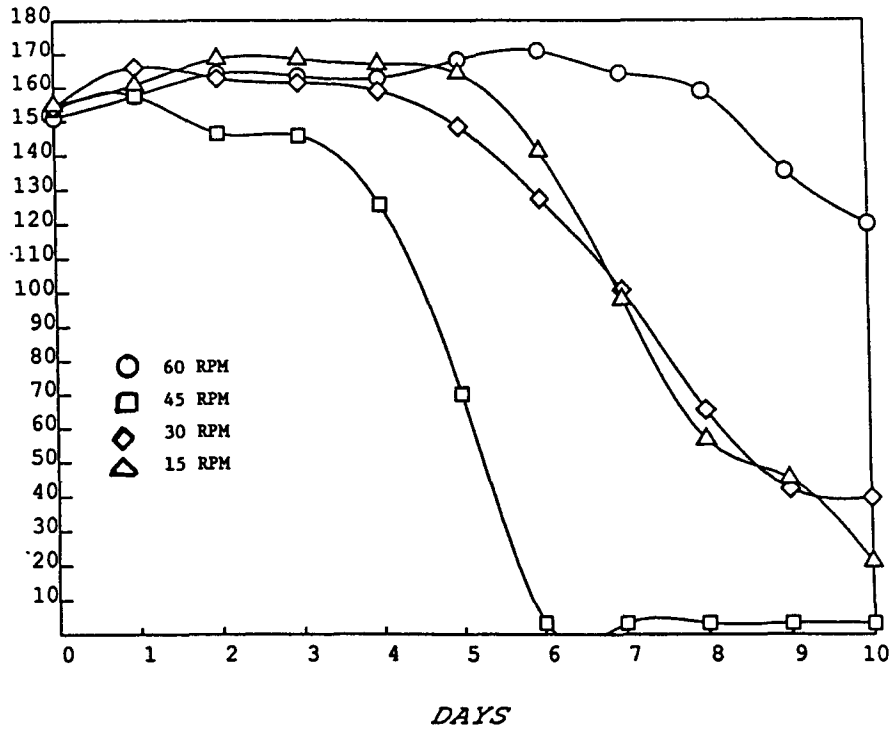

Fig. 3. Glucose consumption profiles by encapsulated cells at different agitation speeds. Double coated capsule size: 700 μ.
○ 60 RPM, □ 45 RPM, ◇ 30 RPM, △ 15 RPM.

low agitation speeds. No significant damage on capsules (less than 5% capsule lysis) was observed at agitation speeds under 45 rpm. A sudden drop in the system's performance at 60 rpm (i.e., low proliferation and glucose consumption rates) is mainly owing to breakage of more than 20% of capsules by shear. Therefore, an agitation speed of 45 rpm (4 cm/s tip speed) was found to be optimal, resulting in minimal external nutrient transfer limitations and negligible capsule lysis. An agitation speed of 45 rpm corresponds to a maximum shear rate of $\sim 68\ s^{-1}$ at the tip of the paddle impeller used.

Transmembrane Resistance

The poly-L-lysine-alginate hydrogel membrane surrounding capsules constitutes a mass transfer barrier for nutrients and products. The MW cutoff (i.e., pore size) and thickness of the membrane determine the effective permeability of the capsule membrane. The poly-L-lysine (PLL) treatment in preparation of capsules is the key factor determining the MW cutoff of the capsule membrane. The permeability of capsule membrane can be adjusted by changing the MW of PLL used (20-300 kd) and dura-

tion of PLL treatment (*31*). Diffusion of PLL into Ca-alginate gel matrix is the critical step in forming the hydrogel membrane (*32*). Lower MW of PLL, higher PLL concentration, and longer reaction times result in more extensive penetration of PLL and thicker and less permeable membrane since PLL fills the molecular pores in Ca-alginate gel as it penetrates (*33*). However, the charged nature of PLL and alginate and diffusing compounds (i.e., ammonium, lactic acid, serum components) also affect permeability of capsule membranes.

In order to vary the permeability of the capsule membranes, a second coat of PLL was used. Double coated capsules are advantageous from the point of view of improved capsule strength and retention of some lower MW products such as lymphokines produced by T-cell hybridomas. The inner coat of PLL is primarily responsible for the MW cutoff of the membrane (low MW PLL such as 41 kd) and the outer coat usually adds up to the strength of capsules (high MW PLL such as 59 kd). The second coat also affects the permeability of capsule membrane depending on its thickness and the MW of PLL used.

Some T-flask experiments were conducted with single and double coated capsules. A mouse-mouse B-cell hybridoma (cell line 751H1A3.7) was used for this experiment. The same procedure was used as described before. Single coated capsules were prepared by treating Ca-alginate beads with a PLL (0.1 wt% solution) of 41 kd molecular weight for 10 min. Double coated capsules were prepared first by treating Ca-alginate beads with 41 kd MW PLL for 10 min. These capsules were treated with 0.3 wt% Na-alginate for 2 min and then with 59 kd MW PLL solution (0.1 wt%) for five minutes. The capsules ($\overline{D}_p = 700\ \mu$) were cultured in T-flasks in a CO_2-incubator with repeated media changes for 15 d. Time course of variation of viable cells and MAb concentrations are depicted in Figs. 4 and 5. The rates of cell proliferation and MAb formation was higher in single coated capsules as compared to double coated ones indicating possible transmembrane nutrient transfer limitations (i.e., lower membrane permeability) in double coated capsules. However, a second PLL coating improved the strength of capsules.

Intracapsule Transfer Resistance

Nutrients are consumed by encapsulated cells as they diffuse inside capsules and metabolic products diffuse out of capsules as they are produced. Therefore, cell proliferation, product formation and nutrient/product diffusion inside capsules are simultaneous. Nutrients may be consumed within the outer shell of the hollow capsule volume if the capsule size is large, resulting in a nutrient devoid central core. Capsule size should be kept small enough in order to avoid possible intracapsule nutrient transfer limitations resulting in finite nutrient concentrations inside capsules.

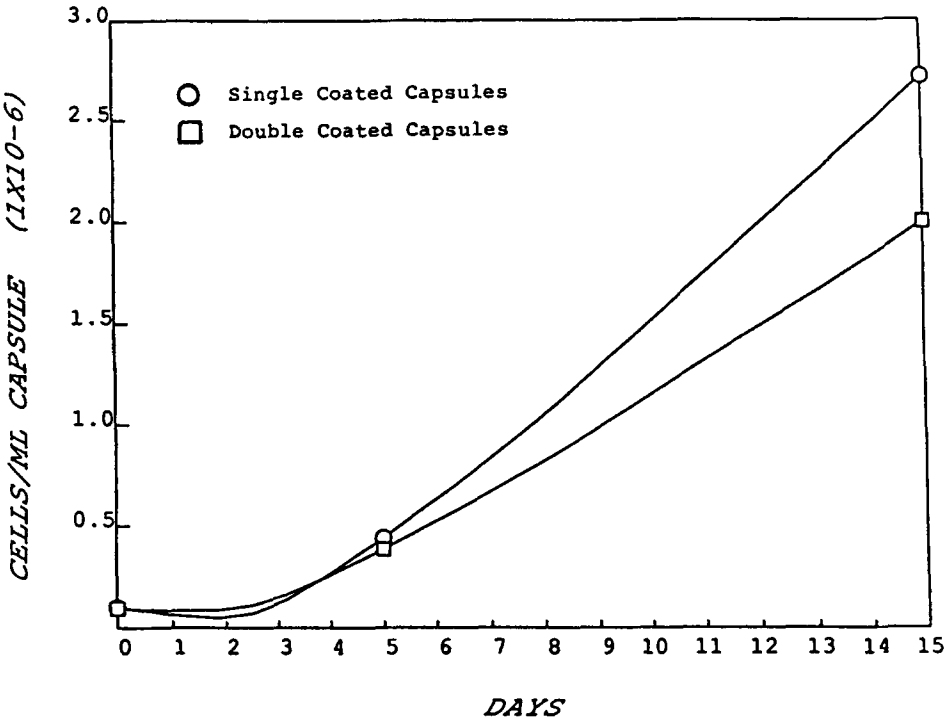

Fig. 4. Variation of viable encapsulated cell concentration with time for single and double coated capsules. Agitation speed: 45 RPM. Capsule size: 700 μ. ○ Single coated capsule, □ Double coated capsule.

A number of spinner flask experiments were conducted with the same initial intracapsule viable cell concentration (0.5×10^5 viable cells/mL capsule and different capsule sizes. Four different capsule sizes 350μ, 450μ, 750μ, and 900 μ were used. Extrusion conditions were varied (extrusion rate, N_2 flow rate and needle size) in order to obtain various capsule sizes. Double coated capsules (first 41 kd MW then 59 kd MW PLL) and a rat-mouse B-cell hybridoma cell line 984D4.6.5 were used in these experiments. Seven mL of capsules of different sizes were added to 93 mL of nutrient media containing 5% CBS to result in a 100 mL of total mixture volume in spinner flasks. The flasks were stirred at 45 rpm and the gas head space of each flask was purged with filter sterilized tri-gas mixture (7% O_2, 10% CO_2, 83% N_2). The flasks were incubated in a constant temperature clean room at 36.5°C. The pH of culture media was between pH=7.0-7.3 through-out experiments.

Samples were withdrawn everyday and were analyzed for total and viable cell, MAb and glucose concentrations. Time course of variation of viable cell, MAb and glucose concentrations obtained with different sizes of capsules are depicted in Figs. 6, 7, and 8, respectively. The highest rate of cell growth, glucose consumption and MAb formation were obtained with 350 μ capsules, which was the smallest capsule size tested. The rates

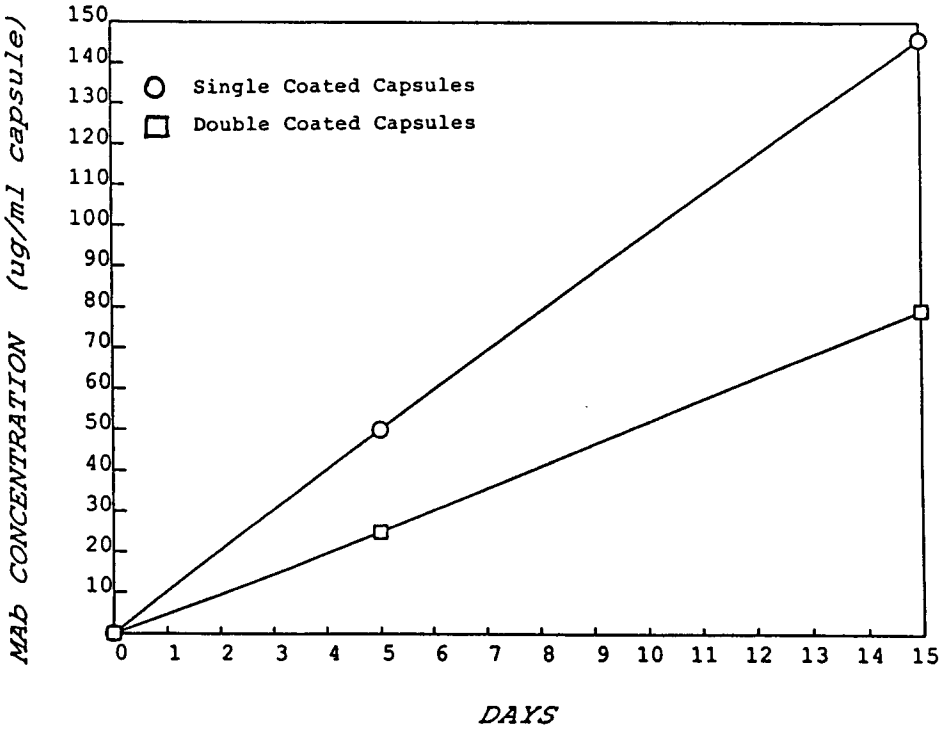

Fig. 5. Monoclonal antibody formation profiles by encapsulated cells in single and double coated capsules. Agitation speed: 45 RPM. Capsule size: 700 μ. ○ Single coated capsule, □ Double coated capsule.

of cell proliferation, glucose consumption, and MAb formation increased steadily with decreasing capsule size from 900 to 350 μ indicating intracapsule mass transfer limitations. Calculations indicated the presence of nutrient transfer limitations even with 350 μ capsule size. Capsule sizes of about 180–200 μ are required to overcome intracapsule diffusion limitations according to calculations based on the data presented (23). However, it was quite difficult to obtain capsule sizes as small at 200 μ with the extrusion device used. For this reason, we were unable to obtain experimental data with 200 μ size capsules.

Maximum rates of MAb formation and glucose consumption were plotted against reciprocal capsule diameter in Figs. 9 and 10 since reciprocal capsule diameter is a measure of surface to volume ratio of capsules. As expected, the maximum rates increased with decreasing capsule diameter. Further reduction in capsule diameter below 350 μ would still result in higher rates of MAb formation and cell proliferation. The calculated effectiveness factor with 350 μ capsule size was nearly $\eta = 0.7$ with respect to dissolved oxygen.

In order to determine if reduction in capsule size would improve performance of individual cells, a plot of maximum specific rate of MAb formation vs reciprocal capsule diameter was made. As depicted in Fig. 11,

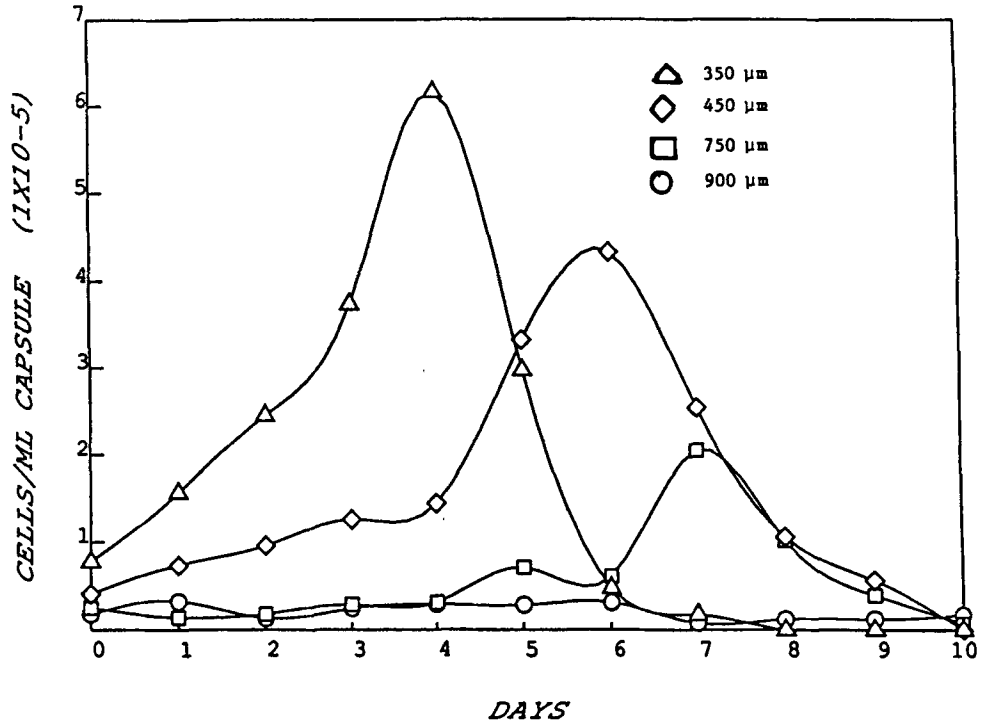

Fig. 6. Viable cell growth profiles with different sizes of capsules. Agitation speed: 45 RPM. Double coated capsules.
△ 350 μ, ◇ 450 μ, □ 750 μ, ○ 900 μ.

maximum specific rate of MAb formation increased with decreasing capsule size. The specific MAb formation rate was maximum with 350 μ capsule size, indicating possible favorable effects of small capsule sizes due to improved cell-cell interaction and reduction of mass transfer limitations.

CONCLUSIONS

Effects of extracapsule liquid film, transmembrane, and intracapsule nutrient/product transfer limitations on performance of microencapsulated hybridoma cells were investigated. System performance (rate of MAb formation and cell proliferation) was improved by increasing the agitation speed up to 45 rpm (~4 cm/s impeller tip speed). At agitation speeds lower than 45 rpm external liquid film resistance was a limiting factor. At an agitation speed of 60 rpm capsule disruption became a problem. Double coating of capsules to improve capsule strength caused more pronounced transmembrane mass transfer limitations and a reduction in MAb productivity. System's performance was improved by reducing the

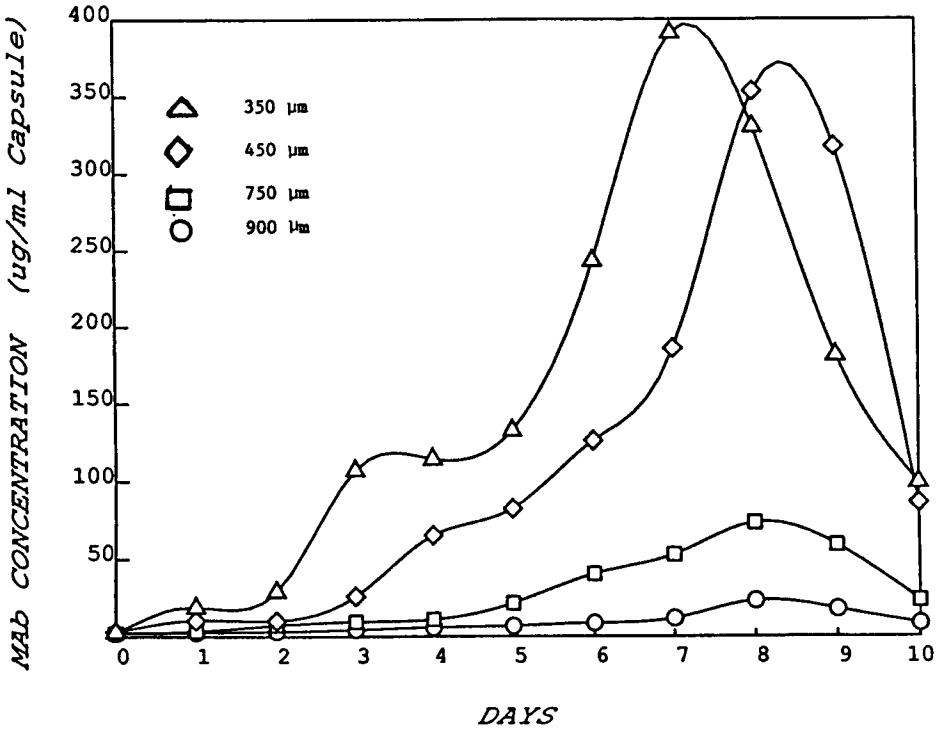

Fig. 7. Monoclonal antibody (MAb) formation profiles by encapsulated cells in different sizes of capsules. Agitation speed: 45 RPM. Double coated capsules.
△ 350 μ, ◇ 450 μ, □ 750 μ, ○ 900 μ.

capsule size from 900 to 350 μ. Capsule size needs to be reduced to lower than 350 μ in order to eliminate intracapsule mass transfer limitations.

ACKNOWLEDGMENT

This study was supported in part by NIH-BRSG funds at Washington University. Expert advice of C. Thies on encapsulation procedures and of C. Pierce on handling hybridoma cultures are greatly appreciated.

REFERENCES

1. Bullock, D. E. (1983), *Biotechnol Advs.* **1,** 81–93.
2. Kohler, G. and Milstein, C. C. (1975), *Nature* **256,** 495–497.
3. Feder, J. and Tolbert, W. R. (1983), *Sci. Am.* **248,** 24–32.
4. Hu, W. S. and Dodge, T. C. (1986), *Biotechnol. Prog.* **1**(4), 209–216.

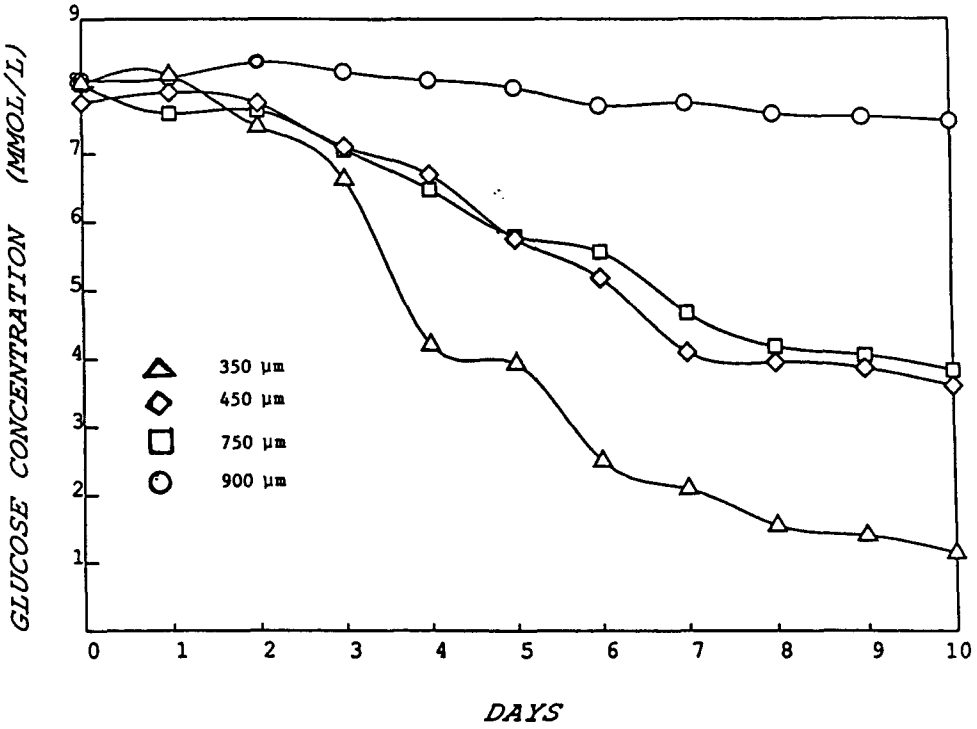

Fig. 8. Glucose consumption profiles with different sizes of capsules. Agitation speed: 45 RPM. Double coated capsules.
△ 350 μ, ◇ 450 μ, □ 750 μ, ○ 900 μ.

5. Spier, R. E. and Fowler, M. W. (1985), *Comprehensive Biotechnology*, Moo-Young, M. ed., vol. 1, pp. 301-330.
6. Tolbert, W. R., Lewis, C., Jr., White, P. J., and Feder, J. (1985), *Large Scale Mammalian Cell Culture*, Feder, J. and Tolbert, W. R., eds., Academic, NY, pp. 97-123.
7. Birch, J. R., Thompson, P. W., and Lambert, K. (1985), *Large Scale Mammalian Cell Culture*, Feder, J. and Tolbert, W. R., eds., Academic, NY, pp. 1-15.
8. Ku, K., Kuo, M. J., Delente, J., Wildi, B. S., and Feder, J. (1981), *Biotechnol. Bioeng.* **23**, 79.
9. Altschuler, G. L., Dziewulski, D. M., Sowek, J. A., and Belfort, G. (1986), *Biotechnol. Bioeng.* **28**, 646.
10. Karkane, S. B., Phillips, P. G., Burke, D. H., and Dean, R. C., Jr. (1985), *Large Scale Mammalian Cell Culture*, Feder, J. and Tolbert, W. R., eds., Academic, NY, pp. 127-149.
11. Lydersen, B. K., Putman, J., Bognar, E., Patterson, M., Pugh, G. G., and Noll, L. A. (1985), *Large Scale Mammalian Cell Culture*, Feder, J. and Tolbert, W. R., eds., Academic, NY, pp. 39-58.
12. Rupp, R. (1985), *Large Scale Mammalian Cell Culture*, Feder, J. and Tolbert, W. R., eds., Academic, NY, pp. 19-36.
13. Brown, P. C., Costello, M. A. C., Oakley, R., and Lewis, J. L. (1985), *Large Scale Mammalian Cell Culture*, Feder, J. and Tolbert, W. R., eds., Academic, NY, pp. 59-71.

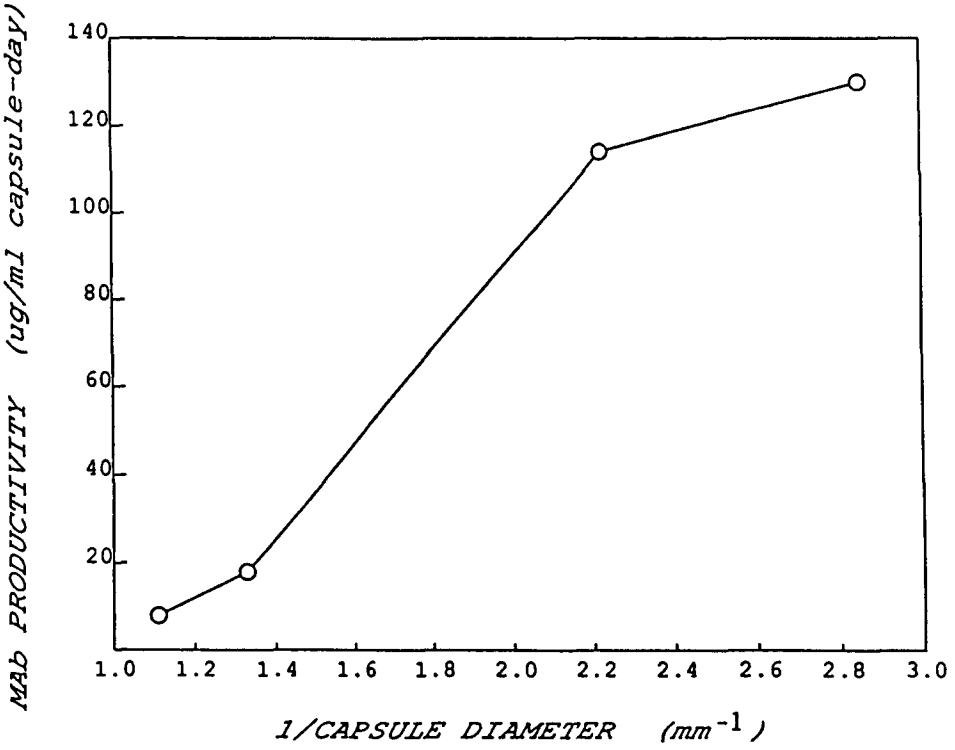

Fig. 9. Rate of MAb formation vs reciprocal capsule diameter. Agitation speed: 45 RPM. Double coated capsules.

14. Hu, W. S. and Dodge, T. C. (1985), *Biotechnol. Progr.* **1,** 209.
15. Hu, W. S. Meier, J., and Wang, D. I. C. (1985), *Biotechnol. Bioeng.* **27,** 585–595.
16. Himes, V. B. and Hu, W. S. (1987), *Biotechnol. Bioeng.* **29,** 1155–1163.
17. Jarvis, A. P., Jr., and Gardina, T. A. (1983), *Biotechniques.* **1,** 22.
18. Gardina, T. A. and Jarvis, A. P., Jr. (1984), *The World Biotech. Report,* vol. 2, USA, Online, Pinner, UK, p. A235.
19. Lim, F. (1983), US Patent 4409331.
20. Lim, F. (1983), US Patent 4391909.
21. Lim, F. and Moss, R. D. (1981), *J. Pharm. Sci.* **70,** 351.
22. Ham, R. G. (1985), *Exp. Cell Res.* **29,** 515.
23. Edmunds, W. W. (1987), MS Thesis, Washington University.
24. Jarvis, A. P., Gardina, T. A., and Sullivan, M. F. (1986), *in vitro Cell. Develop. Biol.* **22,** 589.
25. Freshney, R. I. (1983), *Culture of Animal Cells: A Manual of Basic Technique.* Liss, NY.
26. Rotman, B. and Papermaster, B. W. (1966), *Proc. Natl. Acad. Sci. USA* **55,** 134–141.
27. Sigma Glucose Diagnostic Kit, Procedure No. 115. Sigma Chemical Company, St. Louis, MO.
28. Sigma Pryuvate/Lactate Diagnostic Kit, Procedure No. 726-UV/826-UV. Sigma Chemical Company, St. Louis, MO.

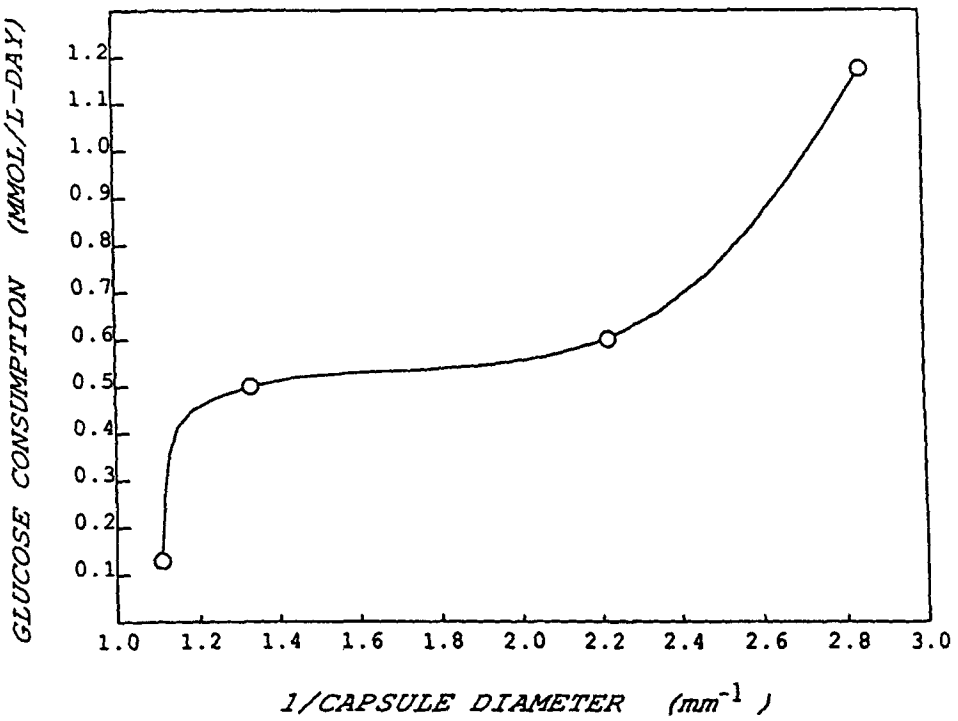

Fig. 10. Rate of glucose consumption vs reciprocal capsule diameter. Agitation speed: 45 RPM. Double coated capsules.

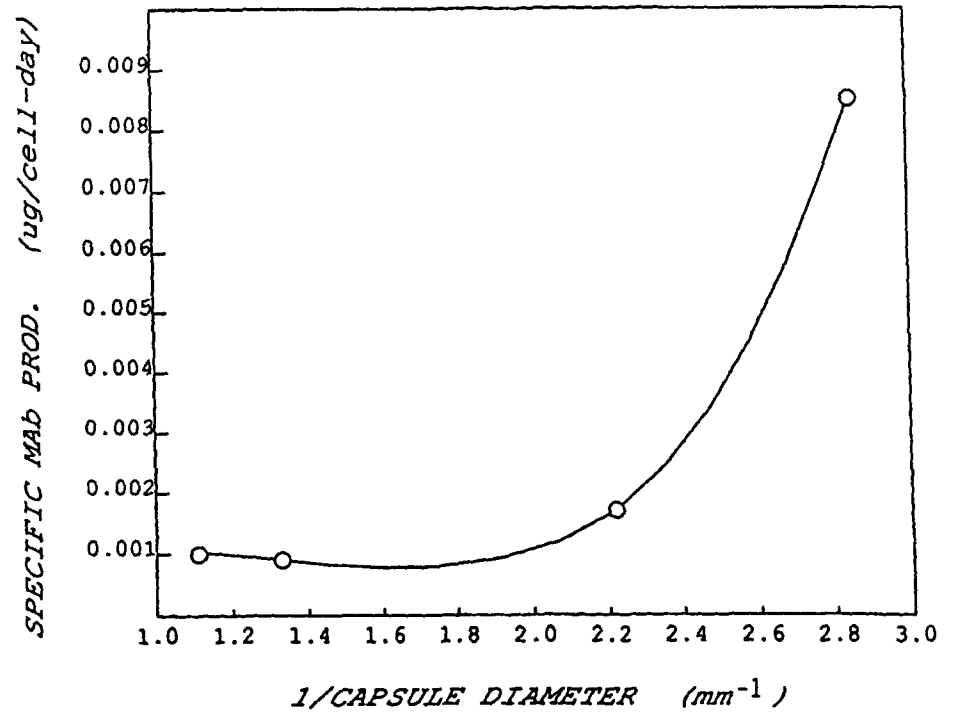

Fig. 11. Variation of maximum specific MAb formation rate with reciprocal capsule size. Agitation speed: 45 RPM. Double coated capsules.

29. Sigma Ammonia Diagnostic Kit, Procedure No. 170-UV. Sigma Chemical Company, St. Louis, MO.
30. Vectastain ABC ELISA Kit. Vector Labs, Burlingame, CA.
31. King, G. A., Daugulis, A. J., Faulkner, P., and Goosen, M. F. A. (1987), *Biotechnol. Progress* **3,** 231.
32. Goosen, M. F. A., O'Shea, G. M., Gharapetian, H. M., Chou, Sheng, Sun, A. M. (1985), *Biotechnol. Bioeng.* **27,** 146.
33. Goosen, M. F. A. (1987), *CRC Crit. Rev. in Biocompat.* **3,** 1–24.