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International Journal of Pharmaceutics 242 (2002) 389–391
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Note

Barium alginate cell-delivery systems: correlation between technological parameters

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Received 14 December 2001; received in revised form 27 December 2001; accepted 9 January 2002

Abstract

The most recent trends for the development of several in vitro cell cultures have been oriented towards the cell immobilisation in 3-dimensional scaffolds and cell encapsulation. In fact, an important requirement of cell survival is self-assembly in functional communities, in the presence of an artificial extracellular matrix. In our research, a previously described technique for spermatozoa encapsulation was applied to obtain capsules loaded with an opaque agent as a model, and to perform a formulative study. A process variable, barium ion concentration, was correlated to some capsule properties, such as weight, gel thickness, total and core diameter. Ion concentration can be modified to obtain capsules with predictable characteristics.

Keywords: Alginate; Capsule formulation; Cell delivery systems

The in vitro culture of mammalian cells isolated from tissues is a commonly used technique in the investigation of cell biology and the etiopathogenesis of numerous diseases. However, standard monolayer in vitro cell culture cannot adequately simulate the functions of whole tissues, where cells act together in a specific intercellular matrix. The anomalous behaviour of single cells in culture is attributable to such causes as their adhesion to an atypical support, e.g. culture dishes, an inadequate nutrient supply, the two-dimensional plane in which cell-growth takes place; and the lack of an appropriate extracellular matrix. The distribution of proteins, oligo- and polysaccharides and other molecules in the extracellular matrix, is closely connected to cell differentiation and function within tissue (Mauchamp et al., 1998). The in vivo development of tissue would require both a three-dimensional arrangement of the cells and a physiological intercellular matrix. There have been several successful attempts to use polymeric matrices, or scaffolds, to culture isolated cells in three-dimensional space. Hydrogels seem to mimic the extracellular matrix well (Mauchamp et al., 1998).

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PII: S0378-5173(02)00223-5

Table 1
Ba^{2+} concentrations, number of batches produced and capsule properties

Ba^{2+} (mM)	Batches	*n*	Weight (mg)	Gel thickness (mm)	Capsule diameter (mm)	Core diameter (mm)
5	5	100	48.6 ± 7.5	0.54 ± 0.10	5.8 ± 0.5	4.7 ± 0.5
10	2	40	90.6 ± 15.4	1.07 ± 0.29	6.8 ± 0.4	4.6 ± 0.5
20	2	40	93.8 ± 12.8	1.08 ± 0.16	5.9 ± 0.2	3.8 ± 0.2
30	2	40	92.2 ± 7.6	1.12 ± 0.10	6.3 ± 0.3	4.1 ± 0.3
50	2	40	122.8 ± 11.6	1.30 ± 0.17	6.3 ± 0.2	3.7 ± 0.6
70	2	50	146.6 ± 16.4	1.63 ± 0.15	6.6 ± 0.4	3.4 ± 0.3
100	2	50	145.8 ± 14.5	1.62 ± 0.23	6.5 ± 0.3	3.4 ± 0.3
120	1	20	194.9 ± 19.6	1.83 ± 0.19	7.1 ± 0.2	3.4 ± 0.3
150	1	20	215.7 ± 32.4	1.87 ± 0.35	7.0 ± 0.6	3.1 ± 0.4

Cell encapsulation is a strategy by which three-dimensional cell cultures are obtained by entrapping a pool of live cells within a semi-permeable membrane. The extracellular matrix is essential to the in vitro self-assembly of cells into pseudo tissue structures. The right degree of selectivity and permeability of the membrane to allow metabolite and nutrient diffusion and cell life (Chang, 1998) is the primary challenge in capsule development. Others include mechanical properties of the membrane such as resistance to rupture, elasticity, particle size distribution, surface properties and morphology. All these characteristics combine to determine ease of handling and application (Chang, 1998).

Our proposal, based on these two experimental lines, was to develop a barium alginate cell delivery system using a technique previously described for swine semen encapsulation (Conte et al., 1998); capsules containing an opaque agent were prepared as a model. The correlations between encapsulation process parameters and capsule properties were analysed: the relationships between Ba^{2+} ion concentration and some selected variables were evaluated: weight, gel thickness, capsule diameter and capsule core. Gel thickness was chosen so as to influence membrane permeability and mechanical properties, the other characteristics combining to determine ease of handling and application.

Twenty millilitres of a xanthan gum 1% solution (Satiaxane CX 2, SKW Biosystems, I) and titanium dioxide 0.5% as opaque agent were prepared. A saturated $BaCl_2$ solution was then added to this suspension to obtain nine different Ba^{2+} ions concentrations from 5 to 150 mM; some formulations were produced in replicates (Table 1). The resulting suspension was dropped into a 0.5% w/v sodium alginate solution (Sodium alginate medium viscosity, Sigma–Aldrich, D) using a peristaltic pump through a 25G needle. Capsules were obtained, collected by filtration, rinsed twice and suspended in an extender (aqueous medium containing: glucose 60 g/l; $NaHCO_3$ 1.2 g/l).

The whole capsule diameter, the core diameter and gel capsule thickness were measured by a digital video camera connected to an image analyser (CV 9000 ver. 4.0 image analyser, FKV Srl, Sorisole, BG, I); the weight of capsules was also determined. The determinations were carried out on randomised samples from each production batch; data are reported in Table 1. Linear regression and Pearson correlation coefficients between ion concentration (independent variable) and capsule parameters were evaluated.

Table 2
Regression results and correlation coefficients for Ba^{2+} concentrations and analysed variables

Variable	*n*	Intercept	Slope	*r*
Weight	400	59.8 mg	1.05	+0.92
Gel thickness	381	0.75 mm	0.009	+0.83
Total diameter	381	5.99 mm	0.007	+0.55
Core diameter	381	4.51 mm	−0.010	−0.70

$P < 0.001$ for all parameters.

Linear regression and correlation coefficients are reported in Table 2: a strong positive correlation ($P < 0.001$) between Ba^{2+} concentrations and capsule weight was observed; gel thickness also shows a significant positive correlation with ion concentration. Thus, ion concentration represents a fundamental technological parameter and may be employed to control this membrane characteristic. The effect of the ion concentration employed on capsule size was less evident. This feature can indicate a modification in capsule structure. The core diameter was inversely correlated with ion concentration, therefore an increase of barium results in a smaller core. This indicates a possible interaction between the electrolyte and the nucleus components, and in particular the xanthan gum, as reported by Zatz and Knapp (1984).

In conclusion, there are marked relationships between concentration and the weight/gel thickness of the capsules obtained using this technique: capsules with the desired requirements can be obtained using predictable Ba^{2+} concentrations. Furthermore, these results could represent the starting point for the development of a protocol to analyse other technical properties and many process variables by means of more sophisticated statistical methods, such as, for example, multivariate analysis.

References

Conte, U., Torre, M.L., Maggi, L., Giunchedi, P., Vigo, D., Maffeo, G., Russo V., 1998. European patent application no. 98122633.5-2114.

Mauchamp, J., Mirrione, A., Alquier, C., André, F., 1998. Follicle-like structure and polarized monolayer: role of the extracellular matrix on thyroid cell organization in primary culture. Biol. Cell 90, 369–380.

Chang, T.M.S., 1998. Pharmaceutical and therapeutic applications of artificial cells including microencapsulation. Eur. J. Pharm. Biopharm. 45, 3–8.

Zatz, J.L., Knapp, S., 1984. Viscosity of xanthan gum solutions at low shear rates. J. Pharm. Sci. 73, 468–471.