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Pectin–gelatin microglobules: effect of a cross-linking agent (formaldehyde) on in vitro dissolution rate

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Summary

The purpose of this investigation was to evaluate the feasibility of controlling and predicting the dissolution rate of pectin–gelatin microglobules by varying formaldehyde concentration and reaction time. The potential use of this system is as an intravascular biodegradable drug delivery system applied to regional cancer chemotherapy. Spherical pectin–gelatin microglobules were prepared by a microencapsulation technique using a complex coacervation procedure of type A gelatin with pectin. The microglobules had a nominal size of 23 ± 3 μm in aqueous medium and were recovered as water-dispersible powders. Dissolution half-lives, in terms of number of microglobules, were controllable with the use of formaldehyde as a cross-linking agent within a range of about 2 min to more than 12 h with a CV of 10% ($n = 4$). A decrease in dissolution rate of these microglobules was observed with aging of the product stored under ambient conditions. The dissolution rates were fitted by an empirical polynomial equation of the second order. This polynomial equation was used as a tool for the determination of the values of the process variables necessary to meet pre-determined dissolution rates, which would permit rapid dissolution of the carrier once the drug has been released in the target organ. The experimentally determined half-lives correlated well with the predicted values.

Introduction

Pectin–gelatin microglobules are produced by a microencapsulation technique using a complex coacervation procedure (McMullen et al., 1982), which produces spherical microglobules of uniform and controllable size (1–50 μm), recoverable as water-dispersible powders. Water-insoluble liquid and solid substances that do not have any strong ionic or tensio-active properties can be incorporated in these microglobules (McMullen et al., 1984). Certain physicochemical parameters for preparing and recovering medicated and unmedicated microglobules were detailed previously (McMullen et al., 1982; McMullen et al., 1984). It was shown (McMullen et al., 1984) that 25 μm microglobules containing 33% w/w sulfamerazine, released more than 50% of their content in 30 min when introduced in replacement electrolyte solution.

In the past few years, an increasing number of reports indicated that intravascular administration of biodegradable drug carriers or microspheres could be used successfully to achieve localized drug delivery for regional cancer chemotherapy by chemoembolization (Senyei et al., 1981; Kato et al., 1981; Dakhil et al., 1982; Willmott et al., 1985; Aronsen et al., 1979). Detailed investigations regarding hemodynamic effects of in-

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travenously injected microspheres have been reported (Davis et al., 1978; Allen et al., 1978) and demonstrated that the size, size distribution and number of particles injected are the most critical factors in assessing particle toxicity and suggested toxicity guidelines. Senyei et al. (1981) reported that low-dose doxorubicin entrapped in a magnetically targeted carrier, in a rat tail model, yielded approximately twice the local doxorubicin concentration at a preselected target site with no detectable systemic distribution. Temporary capillary bed blood flow reduction induced with starch microspheres were studied in humans and animals to obtain reversible ischemia (Forsberg, 1978; Lote et al., 1980; Lindell et al., 1977) and regional localization of chemotherapeutic agents (Dakhil et al., 1982; Aronsen et al., 1979). The interval of flow reduction, with these microspheres, depends on the degree of cross-linking, the size (Aronsen et al., 1979; Forsberg, 1978), and the number of microspheres injected (Aronsen et al., 1979, Lindell et al., 1977).

In order to evaluate the pectin–gelatin microglobules for potential use in regional cancer chemotherapy as an intravascular biodegradable drug delivery system, an investigation was carried out to evaluate in vitro dissolution rates of these microglobules by varying the concentration and reaction time of a methylene bridging agent (formaldehyde) which is added during the recovery procedure. The dissolution rates obtained from this study were fitted with a polynomial equation of the second order. This allows determination of the values of the process variables necessary to meet pre-determined dissolution rates which would permit rapid dissolution of the carrier once the drug would have been released in the target organ. These dissolution rates must be adjusted on an individual basis depending on the dissolution profile of the microencapsulated drug.

Materials and Methods

Materials

Type A gelatin [1] and pectin [2] NF, were used as 2% w/w solutions containing 1% w/w benzyl alcohol [3] for preservation. Glycerin, formaldehyde solution [4] (36.9% by the USP XX assay) containing 10–15% methanol as a stabilizer and 2-propanol were used for the recovery of microglobules in dry powder form. Polyoxyethylene sorbitan monolaurate [5], monobasic and dibasic sodium phosphate, sodium chloride and phosphorus pentoxide were used for the dissolution runs and drying of microglobules. Acetylacetone reagent [6] was used for the assay of residual formaldehyde. These and other reagents were of analytical reagent grade.

Preparation and recovery of microglobules

The method employed by McMullen et al. (1982) was used for the preparation of the microglobules. Coacervates were prepared at 45°C from 80.0 g batches. Colloids were used as 2% w/w solutions at a 40 : 60 pectin–gelatin ratio and contained 1% w/w benzyl alcohol. These solutions were individually adjusted to pH 10 [7,8] with 1.0 N NaOH at 45°C. The gelatin solution was added to the pectin solution with stirring and the pH was lowered by fast addition (1 ml · min^{-1}) of 0.5 N HCl to pH 5, then by slower addition (0.1 ml · min^{-1}) of 0.5 N HCl until pH 3.8 was reached. The recovery procedure was modified as follows: the batch was stirred for 15 min and cooled at 25 ± 0.2°C, then between 3.00 and 5.00 ± 0.02 ml of 36.9% w/w formaldehyde was added and the batch was stirred over the treatment period in order to prevent aggregation. After a 30.0–60.0 min reaction time, the batch was cooled to 10°C and centrifuged at $1400 \times g$ for 1 min. The sediment was redispersed in 10 ml glycerin with a vortex mixer [9] and 70 ml of 2-propanol was added slowly (30 s) while mixing. The flocculated microglobules were then filtered using a Buchner funnel

[1] Isoelectric pH 8.6, 300 Bloom, Atlantic Gelatin, General Foods, Woburn, MA, lot no. 8121103.

[2] Atlantic Gelatin, Woburn, MA, lot no. H73N-71NF.

[3] Fisher Scientific Co., Fair Lawn, NJ.

[4] American Chemicals, Montreal, Que.

[5] Tween 20, Atlas Chem. Ind., Brantford, Canada.

[6] Ammonium acetate, 150 g dissolved in water, 3 ml of acetic acid and 2 ml of acetylacetone. The solution is diluted with water to 1000 ml.

[7] Fisher Titrimeter II, Automatic Titration systems. Fisher Scientific, Pittsburgh, PA 15219.

[8] Combined electrode, 476182-82, Corning glass works of Canada.

[9] Vortex-Genie mixer, set at 1800 rpm.

and filter paper [10], washed with 400 ml of 2-propanol and dried over phosphorus pentoxide under vacuum (40 mm Hg) for 1.5 h and stored at −44°C.

Experimental design

In many industrial and research applications, it is often desirable to study the effects of process variables on the values of the dependent variables. To achieve this, experiments are carried out and the results are reduced to a regression function by the method of regression analysis. For the experiment, the independent variables are individually set to different levels. The specific values of these levels for each variable depend on the number of variables included in the experiment and the range over which they are to be studied. After a particular function is developed, an analysis of variance is carried out to determine its significance. Once the function is determined and represents the system under study, contour plots are prepared and can be used to estimate the values of the process variables necessary to meet a pre-determined response.

Consequently, in order to evaluate the effect of the two independent variables, formaldehyde concentration and reaction time, on dissolution rate of pectin–gelatin microglobules, a two-factor composite design (Anderson et al., 1974), with three factor levels was constructed to establish the values of the process variables for nine batches and develop a predictor polynomial equation. The dependent variable evaluated for each batch was the dissolution half-life in terms of number of microglobules. The regression coefficients obtained were used to prepare contour plots from which the values of the process variables were estimated from pre-determined dissolution rates and reproduced experimentally to verify the predictive capability of the function. No attempts were made to derive an exact analytical solution for microglobule dissolution.

Dissolution studies

Samples of 40 mg of microglobules were weighed, dispersed in 110 mg of polyoxyethylene sorbitan monolaurate and 10 ml of 0.9% NaCl, introduced in a thermostated, flat bottom, cylindrical glass vessel, 6.5 cm in diameter, equipped with a vertically mounted two-bladed glass impeller (2.9 cm in width) set at 1.5 cm from the bottom and containing 210 ml of a 0.07 M isotonic phosphate buffer (pH 7.4) at 37 ± 0.2°C and rotated at 330 rpm. At pre-determined intervals, 2.0 ml samples were taken and introduced in 15 ml 0.9% NaCl solution for counting by a Coulter Counter [11] previously calibrated with microglobules using size distribution obtained by optical microscopy. The dissolution kinetics, 5 for each batch, were monitored by recording microglobule number as a function of time up to 85% decrease in number of microglobules. Buffer and 0.9% NaCl were filtered twice on 0.45 μm filter [12] to remove contaminant particles.

Assay of residual formaldehyde

The remaining amount of hardening agent in the microglobules was determined by the method previously described by Takenaka et al. (1980). One batch of 300 g was prepared and denatured with 19 ml of formaldehyde for 30 min (which corresponds to 5 ml for an 80 g batch). After 1.5 h drying period over phosphorus pentoxide, three samples of 1 g microglobules were each extracted for 1 h in 100 ml of distilled water for residual denaturing agent. The samples were then filtered on millipore filter [12] and 10 ml of the filtrate was introduced in a 50 ml flask containing 10 ml of acetylacetone reagent (prepared just prior to use) and the volume was brought to 50 ml with distilled water. The flasks were introduced in a shaking bath at 37°C for 30 min. A Beer's plot was prepared with the 36.9% w/w formaldehyde solution at a 2–6 $\mu g \cdot cm^{-3}$ concentration range using the same procedure except the filtration step as described for the extracted samples. The absorbance of each extracted sample and standard solutions were determined at 415 nm with a spectrophotometer [13].

[10] 595, 5.5 cm, Schleicher and Schuell, Keene, NH 03431.

[11] Model TA II, Coulter Electronics, Hialeah, FL 33010.

[12] 0.45 μm, Millipore, HA filter, Millipore, Bedford, MA 01730.

[13] Beckman, Model 34.

TABLE 1

VALUES OF THE VARIABLES AT THE INDICATED FACTOR LEVELS AND DISSOLUTION PARAMETERS FOR THE FORMULATIONS USED IN THE STUDY

Formaldehyde volume factor level	Actual [a] formaldehyde added (ml)	Reaction time factor level	Actual reaction time (min)	T_L (min)	k [b] (number/min)	Experimental [c] $T_{50,N}$ [e] (min)	Predicted [d] $T_{50,N}$ (min)
−1	3.00	−1	30.0	0.8	3465.5 ± 1911.0	2.7 ± 0.2	2.7
0	4.00	−1	30.0	5.0	1460.0 ± 771.0	9.5 ± 2.5	10.2
+1	5.00	−1	30.0	8.3	534.9 ± 119.0	20.5 ± 4.7	18.8
−1	3.00	0	45.0	5.0	2420.4 ± 817.6	7.7 ± 1.4	7.1
0	4.00	0	45.0	16.7	264.6 ± 13.1	41.2 ± 5.8	39.0
+1	5.00	0	45.0	33.3	107.7 ± 29.8	93.7 ± 15.6	106.8
−1	3.00	+1	60.0	8.3	527.9 ± 121.1	20.6 ± 2.8	21.9
0	4.00	+1	60.0	66.7	57.3 ± 12.3	180.1 ± 27.2	177.4
+1	5.00	+1	60.0	480.0	23.9 ± 19.7	751.9 ± 159.3	719.0

[a] Used as 36.9% w/w solution at pH 3.8 and 25°C, ranging from 0.0399 (3.00 ml) to 0.0665 (5.00 ml) mol per 0.960 g of type A gelatin in 80.0 g batches.

[b] Represents the 95% confidence interval, r^2 ranged from 0.89 to 0.99 (n = 4–8).

[c] Represents the 95% confidence interval (n = 5).

[d] Computed from Eqn. 2.

[e] Represents the time for a 50% decrease in number of microglobules.

Results and Discussion

Preliminary experiments examined dissolution rate by varying one variable at a time to locate the interior of a "square" bounded in each dimension by the limits of experimentation, each dimension representing a single independent variable, to obtain complete dissolution of microglobules within a range of few minutes to 4–5 h. Once a window had been determined for each of the two independent variables, the two-factor composite design with three factor levels was constructed. Table 1 shows the denaturing conditions for the nine formulations used in the study. Actual values of the process variables corresponded exactly to high (+1), medium (0) and low (−1) levels of the composite design because it is possible to adjust precisely the volume of formaldehyde added and the reaction time (25°C) to the values fixed by the design.

Fig. 1 shows optical and scanning electron photomicrographs [14] of typical microglobules. All batches of dried microglobules were dispersed in a 0.07 M isotonic phosphate buffer (pH 7.4) and their size distribution was evaluated by optical microscopy. In all cases, the size distributions were log-normal with geometric mean diameters between 20 and 26 μm and geometric standard deviations (which is defined as σ_g = (16% oversize/50% size) (Martin et al., 1983)) ranged between 1.13 and 1.17. Virtually 100% of the microglobules were distributed between 15 and 32 μm.

The calibration of the Coulter Counter was performed using a sample of microglobules characterized by optical microscopy. Fig. 2 shows the log-probability plots of the microglobule size distributions indicating no significant differences. This calibration procedure was performed to compensate for the high electrical conductivity of the hydrated pectin–gelatin microglobules in aqueous medium. A stirring rate of 330 rpm was chosen for the dissolution runs to prevent settling of the microglobules and provide greater hydrodynamic flow than by conventional dissolution apparatus which may better simulate in vivo conditions.

In preliminary experiments we noted a gradual decrease in the dissolution rate with aging of microglobules stored under ambient conditions over a 15 days period. This phenomenon was

[14] ISI-40, International Scientific Instruments, Santa Clara, CA 95051.

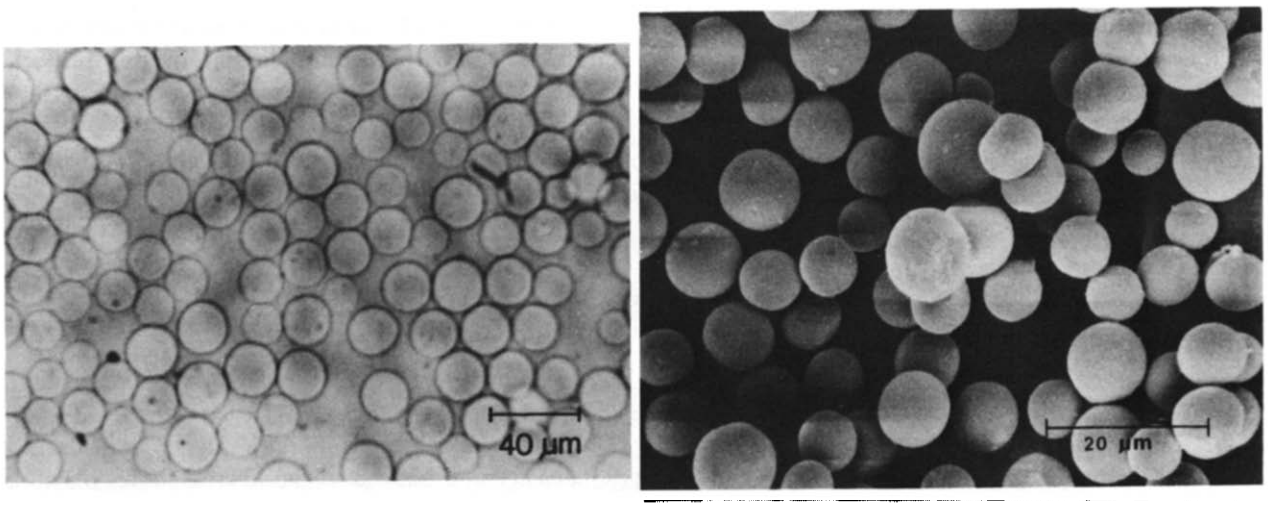

Fig. 1. Photomicrograph (left) of pectin–gelatin microglobules dispersed in an isotonic phosphate buffer, pH 7.4 and scanning electron photomicrograph (right).

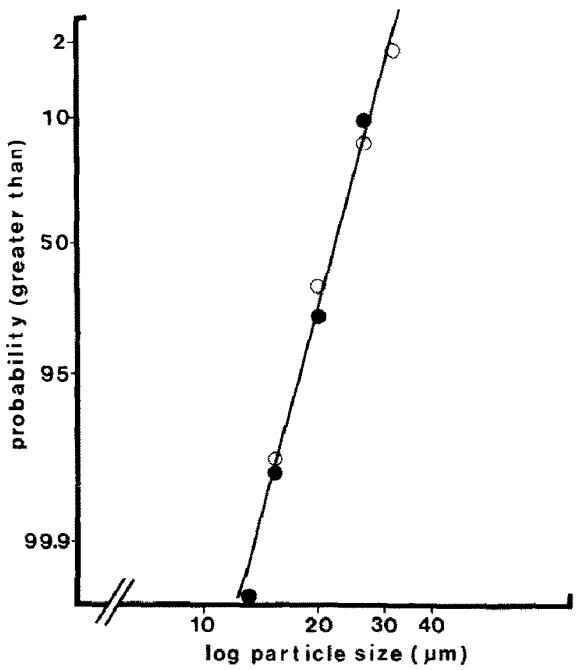

Fig. 2. Log-probability plots of microglobule size distributions denatured with 5 ml of formaldehyde solution for 60 min. Key: (●) optical microscopy, n = 210, geometric mean diameter (d_g) = 21.9 µm, geometric standard deviation (σ_g) = 1.15; ○, Coulter Counter, d_g = 22.3 µm, σ_g = 1.17.

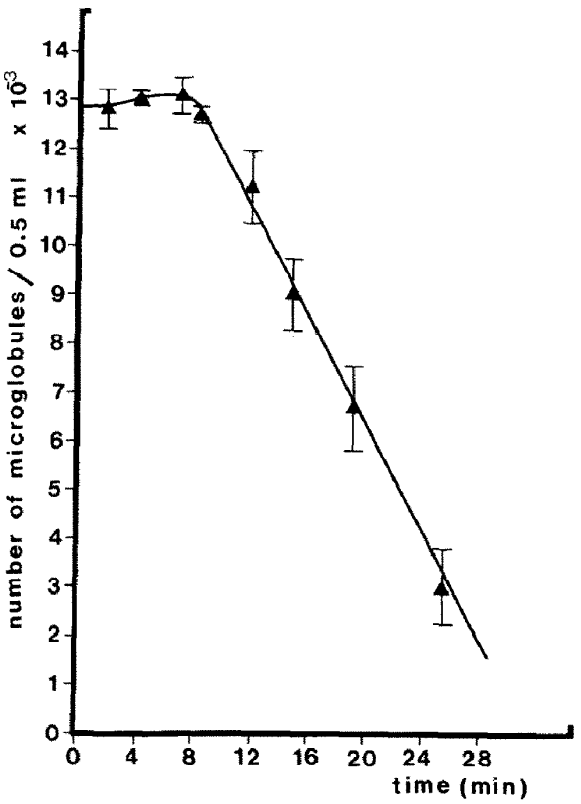

Fig. 3. Typical plot of the total microglobules number versus time for a batch denatured with 3 ml of formaldehyde solution for 60 min. Bars represent S.E.M., n = 5.

minimized over the experimental period by storing the samples at −44°C. This decrease in dissolution rate could be due to residual formaldehyde entrapped in the matrix after denaturation and recovery of the microglobules. Consequently, the residual amount of formaldehyde was assayed and results indicated that 1.96, 1.70 and 1.79 mg formaldehyde/g of pectin–gelatin microglobules remained in each of three samples. It is reasonable to think that such high quantities of residual formaldehyde could explain the decreased dissolution rate with aging of the product due to an after-hardening phenomenon.

Fig. 3 shows a typical dissolution profile by number obtained for a batch of microglobules. In Table 1, the nine dissolutions half-lives, by number ($T_{50,N}$), are shown and represent the time for a 50% decrease in number of microglobules. A zero-order dissolution was assumed based on a significant linear regression performed in the dissolution region. The slopes were computed from the microglobule concentrations for each time interval starting after the lag period and used to compute the time necessary to reach 50% of initial microglobule concentration. $T_{50,N}$ were computed from the following simple relationship:

$$T_{50,N} = T_L + \frac{0.5\, C_0}{k} \qquad (1)$$

where T_L is the last sampling time (lag time) before a drop in microglobule concentration is observed, C_0 is the initial microglobule concentration (number/0.5 ml) and k is the zero-order dissolution rate constant computed from the slope of a linear regression equation on the dissolution region. Table 1 shows the microglobule denaturing conditions studied and their corresponding T_L and k. All half-lives were normalized to 13,000 microglobules/0.5 ml, which corresponds to the initial mean concentration used in the experiments. When a system is to be used as an intravascular drug delivery system, an important parameter to control is the dissolution characteristics of the carrier, because its toxicity greatly depends on the number of trapped particles in the vasculature (Davis et al., 1978; Allen et al., 1978). A carrier which would release the drug in the target area without dissolving itself would not be of great utility because of in vivo accumulation. In this respect, $T_{50,N}$ must be controlled and is a parameter that should be evaluated and taken into consideration in the design of any new particulate drug delivery system for intravascular administration. It is remarkable to see (Table 1) that a small change in denaturing conditions have profound effect on the dissolution rate of the microglobules. $T_{50,N}$ increased from 2.7 to 751.9 min when varying formaldehyde volume from 3 to 5 ml and increasing treatment time from 30 to 60 min. It is reasonable to think that in increasing reaction time and formaldehyde concentration, a higher cross-linked density is produced in the microglobules between gelatin chains which decreases their mobilities and solubilities. As the dissolution proceeds, there may be a gradual hydrolysis of the intermolecular bridges leading to greater water uptake and further swelling until complete dissolution of the matrix is achieved.

The inter-batch reproducibility of the denaturing process has been evaluated on 4 batches of microglobules denatured with 4.20 ml of formaldehyde for 45.0 min. The $T_{50,N}$ observed were 38.9, 40.3, 31.3 and 34.8 min with a 95% confidence interval of 36 ± 6 min. The experimentally determined $T_{50,N}$ (Table 1) were fitted by multiple regression, using the regression sub-program of the SPSS package (Nie et al., 1975), to explain the experimentally determined $T_{50,N}$. Various polynomial equations of first- and second-order were tried (Table 2) but the best fit was obtained with the following second order polynomial equation:

$$\log Y = B_0 + B_1X_1 + B_2X_2 + B_3X_1^2 + B_4X_2^2 + B_5X_1X_2 \qquad (2)$$

where X_1 is the volume of formaldehyde added (ml), X_2 is the reaction time (min), B_0–B_5 are the regression coefficients and Y is the experimental value of $T_{50,N}$ in min. The experimentally determined values correlated well with the predicted values as shown in Table 1. The best equation was chosen from an analysis of mean square residuals (MSR), r^2 and *F*-ratio (regression mean square/MSR). The regression technique used is straight-

TABLE 2

POLYNOMIAL EQUATIONS USED TO FIT THE EXPERIMENTALLY DETERMINED $T_{50,N}$ AND THEIR STATISTICAL MEASURES OF THE FIT

Function used [a]	r^2	Mean square residual	F-ratio
(A) $\log Y = B_0 + B_1X_1X_2$	0.9750	0.0163	272 *[b]
(B) $\log Y = B_0 + B_1X_1 + B_2X_1X_2$	0.9874	0.00958	235 *
(C) $\log Y = B_0 + B_1X_1 + B_2X_1^2 + B_3X_2 + B_4X_1X_2$	0.9976	0.00277	411 *
(D) $\log Y = B_0 + B_1X_1 + B_2X_2 + B_3X_1^2 + B_4X_2^2 + B_5X_1X_2$	0.9982	0.00279	326 *

[a] Regression coefficients for equation D are: $B_0 = -2.6598693$, $B_1 = 1.2830867$, $B_2 = -0.018749777$, $B_3 = 0.15081564$, $B_4 = 0.00016305717$, $B_5 = 0.011365046$.
[b] Significant, $P < 0.05$.

forward, the simplest equation is first tried and at each regression step, another term is added to the function until the highest r^2, F-ratio and lower MSR are obtained. In this way, the best function which represents the system under study, is easily found. Eqn. 2 (D in Table 2) provides an excellent fit between the experimentally observed $T_{50,N}$ and the predicted $T_{50,N}$ values (Table 1).

Fig. 4 shows contour plots prepared from Eqn. 2 of treatment time as a function of formaldehyde volume added for constant $T_{50,N}$ from 2 to 960 min. The equation is valid only within the experimental constraints which are 30–60 min treatment time and 3–5 ml formaldehyde added. Eqn. 2 was used as a predictive tool to determine the levels of the process variables for four different batches which meet pre-established $T_{50,N}$ within the constraints mentioned above. These batches were experimentally reproduced (in triplicate) and values of $T_{50,N}$ determined. Table 3 shows the four predicted $T_{50,N}$ of the batches, their corresponding denaturing conditions and the experimentally determined values. Experimental values correlated well with predicted values ($r^2 = 0.9596$, slope = 1.0215, intercept = -0.4715).

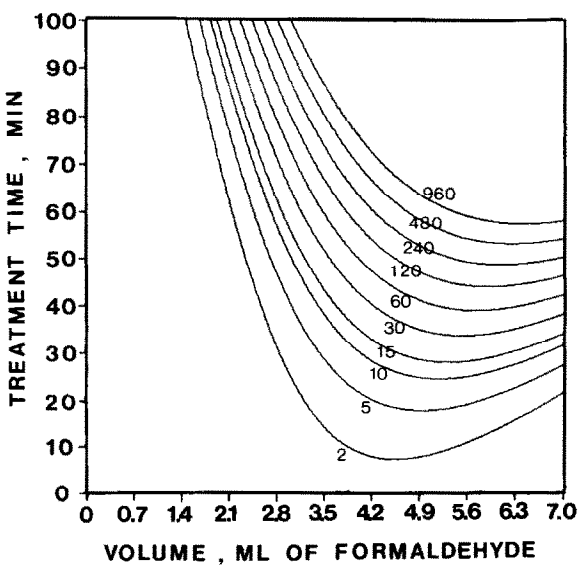

Fig. 4. Contour plots of treatment time as a function of formaldehyde volume added at constant $T_{50,N}$ from 2 to 960 min. Numbers on the curves represent $T_{50,N}$.

Dissolution half-lives of pectin–gelatin microglobules based on number of microglobules is controllable within a range of about 2 min to

TABLE 3

LEVELS OF PROCESS VARIABLES OF FOUR BATCHES WITH THEIR PREDICTED AND EXPERIMENTALLY DETERMINED $T_{50,N}$

Formaldehyde [a] added (ml)	Reaction time (min)	$T_{50,N}$ [b]	
		Predicted (min)	Experimental [c] (min)
4.20	45.0	50.5	36.3 ± 5.2
4.70	45.0	85.0	120.1 ± 26.2
4.30	55.0	165.4	142.9 ± 57.2
4.40	60.0	337.5	327.5 ± 98.0

Predicted-observed linear correlation: $r^2 = 0.9596$, slope = 1.0215, intercept = −0.4715.
[a] Used as 36.9% solution at pH 3.8 and 25°C, ranging from 0.0559 (4.20 ml) to 0.0625 (4.70 ml) mol per 0.960 g of type A gelatin in 80.0 g batches.
[b] Represents the time for a 50% decrease in number of microglobules.
[c] 95% confidence interval (n = 3).

more than 12 h with an inter-batch reproducibility of 10% (n = 4). Decreased dissolution rate of these microglobules with time was attributed to the residual amount of the denaturing agent entrapped in the matrix after recovery. It is expected that a recovery procedure such as lyophilization would reduce the level of the cross-linking agent in the final product and consequently eliminate the problem of after-hardening. More studies however, are needed to further elucidate this phenomenon. The values of the denaturing conditions necessary to meet pre-determined $T_{50,N}$ can be estimated with a polynomial function. Experimentally determined $T_{50,N}$ were found to correlate well with the predicted values, therefore eliminating the need for unneccessary experiments when a $T_{50,N}$ is needed to meet a particular application. Dissolution rate of the carrier should be characterized for the successful design of any chemoembolization system and the procedure described in this paper is a simple and rapid technique to achieve this goal. In conclusion, the microencapsulation technique of complex coacervation using pectin and gelatin has the potential to meet the requirements for a biodegradable drug delivery system applied to regional chemotherapy.

References

Allen, D.R., Ferens, J.M., Cheney, F.W. and Nelp, W.B., Critical evaluation of acute cardiopulmonary toxicity of microspheres. J. Nucl. Med., 19 (1978) 1204–1208.

Anderson, V.L. and McLean, R.A., Design of Experiments, a Realistic Approach, Marcel Dekker, New York, 1974, pp. 353–360.

Aronsen, K.F., Hellekant, C., Holmberg, J., Rothman, U. and Teder, H., Controlled blocking of hepatic artery flow with enzymatically degradable microspheres combined with oncolytic drugs. Eur. Surg. Res., 11 (1979) 99–106.

Dakhil, S., Ensminger, W., Cho, K., Niederhuber, J., Doan, K. and Wheeler, R., Improved regional selectivity of hepatic arterial BCNU with degradable microspheres. Cancer, 50 (1982) 631–635.

Davis, M.A. and Taube, R.A., Pulmonary perfusion imaging: acute toxicity and safety factors as a function of particle size. J. Nucl. Med., 19 (1978) 1209–1213.

Forsberg, J.O., Transient blood flow reduction induced by intra-arterial injection of degradable starch microspheres. Acta Chir. Scand., 144 (1978) 275–281.

Kato, T., Nemoto, R., Mori, H., Takahashi, M., Tamakawa, Y. and Harada, M., Arterial chemoembolization with microencapsulated anticancer drug. J. Am. Med. Ass., 245 (1981) 1123–1127.

Lindell, B., Aronsen, K.F. and Rothman, U., Repeated arterial embolization of rat livers by degradable microspheres. Eur. Surg. Res., 9 (1977) 347–356.

Lote, K., Folling, M., Rosengren, B., Svanes, K. and Lekven, J., Transient intestinal ischaemia induced by degradable starch microspheres. J. Acta Rad. Onc., 19, Fasc. 5 (1980) 343–351.

Martin, A. Swarbrick, J. and Cammarata, A., Physical Pharmacy, 3rd ed., Lea and Febiger, Philadelphia, 1983, p. 500.

McMullen, J.N., Newton, D.W. and Becker, C.H., Pectin–gelatin complex coacervates I: Determinants of microglobules size, morphology, and recovery as water-dispersible powders. J. Pharm. Sci., 71 (1982) 628–633.

McMullen, J.N., Newton, D.W. and Becker, C.H., Pectin–gelatin complex coacervates II: Effect of microencapsulated sulfamerazine on size, morphology, recovery, and extraction of water-dispersible microglobules. J. Pharm. Sci., 73 (1984) 1799–1803.

Nie, N.H., Hull, H.C., Jenkins, J.G., Steinbrenner, K. and Bent, D.H., SPSS, Statistical Package for the Social Sciences, McGraw-Hill, New York, 1975, pp. 320–373.

Senyei, A.E., Reich, S.D., Gonczy, C. and Widder, K.J., In vivo kinetics of magnetically targeted low-dose doxorubicin. J. Pharm. Sci., 70 (1981) 389–391.

Takenaka, H., Kawashima Y. and Lin, S.Y., Micromeritic properties of sulfamethoxazole microcapsules prepared by gelatin–acacia coacervation J. Pharm. Sci., 69 (1980) 513–516.

Willmott, N., Cummings, J., Stuart, J.F.B. and Florence, A.T., Adriamycin-loaded albumin microspheres: preparation, in vivo distribution and release in the rat. Biopharm. Drug. Dis., 6 (1985) 91–104.