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Preparation and release kinetics of microencapsulated cisplatin with ethylcellulose

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Several kinds of microcapsules containing cisplatin were prepared by the ethylcellulose coacervation process in an attempt to administer cisplatin with chemoembolization. Microcapsules made either with mechanical stirring or with sonication showed similar properties; the chemical structure of cisplatin was not affected by the micro-encapsulation process. The release kinetics of cisplatin from ethylcellulose-walled microcapsules followed different patterns, according to the wall thickness. In each case, the release kinetics did not depend on the stirring rate of the surrounding medium. Only microcapsules with a cisplatin release ratio from 80 to 100% within 24 h were selected for later clinical use.

The antineoplastic compound, cisplatin, is in use in the treatment of several tumour systems (Prestayko et al 1980; Rozencweig et al 1981). Its use is limited by substantial secondary effects, especially nephrotoxicity. In an attempt to reduce these drawbacks and to increase the drug concentration in the tumour, cisplatin was administered by intra-arterial infusion (Stewart et al 1983). The results obtained by this method seem to be encouraging and an interesting suggestion is that cisplatin could be administered by arterial chemoembolization.

Chemoembolization is the combination of intra-arterial infusion of a chemotherapeutic agent, generally micro-encapsulated, and arterial embolization of the vascular supply to a neoplasm (Kato et al 1981a). In addition to the direct increase of the drug concentration in the tumour, the emboli enhance the tumour cell killing by anoxia.

Chemoembolization had previously been used in treatment with mitomycin C in patients with invasive or non-resectable malignancy, especially renal carcinomas (Kato et al 1981b, c). Yet, to our knowledge, this method has never been applied to administer cisplatin. The present work describes the preparation and the properties of cisplatin-containing microcapsules designed for clinical chemoembolization.

The molecule of cisplatin can be easily hydrolysed and as such is much more reactive and probably very toxic (Rosenberg 1979). Hence a microencapsulation technique without any cisplatin hydrolysation was needed. Coacervation with ethylcellulose seemed to be suited to this aim, all the more so as methods for the determination of ethylcellulose microcapsule properties were already well documented (Senjkovic & Jalsenjak 1981, 1982; Donbrow & Benita 1982; Benita & Donbrow 1982a, b; Vidmar & Jalsenjak 1983).

We have attempted to prepare microcapsules with a relatively slow release of cisplatin and, if possible, with cisplatin release independent of the variations of external medium flux, since, in an embolized artery, cisplatin-concentration decrease seems to be determined only by its tissue diffusion, which is not a clearly specified phenomenon.

MATERIALS AND METHODS

Materials

The ethylcellulose (Fluka AG. Suisse) used had a viscosity of 45 mPas for a 5% w/w solution.

Cisplatin (bulk powder) was from 'Laboratoires Roger Bellon' (France). All materials were of reagent grade purity. Low density polyethylene was supplied by Dr Morcellet (University of Science of Lille).

Methods

Microcapsule preparation. The method of microcapsule preparation was similar to that of Kato et al (1980). Cisplatin particles were encapsulated with ethylcellulose by coacervation in the presence of low-density polyethylene. Ethylcellulose, cisplatin and polyethylene were dispersed in cyclohexane at 80 °C and then gradually cooled to room temperature (20 °C), either with mechanical stirring or sonication. The microcapsules were then rinsed with n-hexane, dried and screened.

All preparations were made in a sterile atmosphere.

Assay of total drug content. A known amount of microcapsules (2–3 mg) was dissolved in chloroform

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(1 ml). Platinum was then extracted from the chloroform by 20 ml distilled water and analysed by flameless atomic absorption spectrophotometry (FAAS) as previously described (Hecquet et al 1983).

Assay of encapsulated and released platinum species. Hplc was used to determine the nature of the encapsulated compounds. The microcapsules were dissolved in chloroform as previously described and platinum species were extracted by 2 ml distilled water. An aliquot of the aqueous phase was chromatographed on an ion-exchange SAX-radial compression column (Water Associates). The solvent was 0·2 M acetate buffer pH 3·7–methanol (1 : 4 v/v). The flow rate was 1·5 ml min^{-1}. Fractions were collected and analysed for platinum as previously described.

Microcapsule release kinetics. The microcapsules were incubated in-vitro in plasma at 37 °C and dispersed by stirring. Checks were made to ensure that no physical deterioration occurred due to the mechanical stirring. Aliquots of plasma were sampled at regular time intervals, using a filter-fitted pipette to avoid microcapsule intake. Platinum in plasma was analysed by FAAS.

The kinetic data were analysed using different laws that govern the release mechanisms of drugs from microcapsules:

Fick's law. In the hypothesis where microcapsules can be assumed to be spheres, if the diffusion follows Fick's law, then the permeation rate must be given by the equation:

$$\frac{dm}{dt} = \frac{A.\Delta c}{R}$$

where m is the mass of diffused drug, t the time, A the microcapsule surface area, R the total diffusional resistance, and Δc the difference in drug concentration between external and internal media.

If we assume that Δc can be considered as a constant in the experimental conditions (a substantial volume of solvent in comparison with the amount of microcapsules), the function $m = f(t)$ must be a straight line (Senjkovic & Jalsenjak 1982).

Matrix mechanism. In the case of matrix mechanism, the Higuchi equation can be applied to the diffusion of a drug from microcapsules (Benita & Donbrow 1982a) and has the form:

$$m' = k.t^{\frac{1}{2}}$$

where m′ is the amount of drug liberated per unit surface area of matrix, and k is a complex function taking into account the initial drug concentration in the microscapsules, the solubility, the diffusion coefficient, the porosity and tortuosity of the polymer.

In this case, the function $m = f(t^{\frac{1}{2}})$ must be a straight line; but experience shows that this condition is generally insufficient to discern a matrix mechanism from first order release (see next paragraph). It is therefore necessary to carry out a more elaborate analysis to check if experimental data conform with the equation:

$$\frac{dm}{dt} = \frac{km.S^2}{2m}$$

where S is the microcapsule surface area and km a constant. The function $dm/dt = f(1/m)$ must therefore be a straight line in the case of matrix mechanism.

First order kinetics. The third approach is a first order release of the encapsulated compound (John et al 1979). The diffusion law can be expressed as:

$$-\frac{d(m_0 - m)}{dt} = kc(m_0 - m)$$

Where m_0 is the initial drug concentration in the microcapsules and kc the first order rate constant.

or: $$\log (m_0 - m) = \log (m_0) - kc.t$$

The function $\log (m_0 - m) = f(t)$ must be a straight line, as well as the function $\log (P) = f(t)$ where P is the percentage of drug remaining in the microcapsules at time t. Unlike the matrix mechanism, dm/dt is then proportional to m, and not to 1/m.

For each kind of microcapsule, experimental release data were systematically tested with the functions $m = f(t)$, $m = f(t^{\frac{1}{2}})$, $\log (P) = f(t)$ and, if any ambiguity, with the functions $dm/dt = f(1/m)$ and $dm/dt = f(m)$.

The linearity was evaluated by estimation of the linear regression coefficient for each function.

Wall thickness. For a clinical application, microcapsules must have relatively slow rates of release. Therefore, we attempted to synthesize microcapsules with greater wall thickness. Although the formula can theoretically be only applied to spherical particles with limited thickness, we have used this equation with a view to giving an approximate evaluation of the wall thickness:

$$h = \left\{ \left[\frac{dc}{dec} \left(\frac{1}{F} - 1 \right) + 1 \right]^{\frac{1}{3}} - 1 \right\} .rl$$

(Benita & Donbrow 1982a) where rl is the mean radius of the microcapusle, dc the density of the core material, dec the density of ethylcellulose, and F the fractional drug content. All densities were determined in cyclohexane, using a pycnometer.

RESULTS AND DISCUSSION

To prepare the microcapsules with the above-described method, we used several initial amounts of cisplatin, ethylcellulose and polyethylene. During the cooling stage, two stirring methods were tested: mechanical stirring and sonication. The characteristics of microcapsule fractions obtained are given in Table 1.

Table 1. Effect of initial conditions on the composition of the microcapsules.

Microcapsule type	Initial conditions	Diameter (μ)	Yield* (%)	Cisplatin content (%)
1	E.C. 5%** P.E. 1% C.P. 2% mechanical stirring	75–150	17	32
		150–300	37	31
2	E.C. 5% P.E. 1% C.P. 2% sonication	75–150	18	32
		150–300	39	32
3	E.C. 1% P.E. 1% C.P. 2% mechanical stirring	75–150	18	73
		150–300	49	74
4	E.C. 1% P.E. 1% C.P. 2% sonication	75–150	14	78
		150–300	45	76
5	E.C. 1% P.E. 5% C.P. 2% mechanical stirring	75–150	52	69
		150–300	0	/

* The yields are calculated by the ratios: microcapsule cisplatin content/initial cisplatin amount.
** E.C., ethylcellulose; P.E., polyethylene; C.P., cisplatin.

Two kinds of microcapsules showing very different characteristics were prepared by varying the initial conditions: low-drug-content microcapsules (30% of cisplatin) and high-drug-content microcapsules (75% of cisplatin). In both cases, microcapsules of two different sizes were obtained (mean diameters: 150–300 μM and 75–150 μM). The preparation yield, based on the encapsulated cisplatin, was always greater than 50%.

The effects of initial conditions were not systematically studied but several results were noted:

Losses of microcapsule drug content were observed on increasing the ethylcellulose concentration as described by Benita & Donbrow (1982a, b) for salicylamide and theophylline microcapsules.

Mean drug content did not change if the initial amount of polyethylene was increased, but the proportion of small-size microcapsules was enhanced.

Unlike previously published results (Benita & Donbrow 1982c), the microcapsule composition seemed to be independent of the particle size; but the drug content of the microcapsules was higher than in those previously prepared and comparison seems to be unwise.

The evaluation of wall thickness by the commonly used equation is a rough approximation since the conditions of sphericity and wall thinness were not fulfilled by the microcapsules prepared here. We have tried to minimize the errors by expressing ratios of 'wall thickness' between the different capsules rather than calculating the absolute values. Table 2 shows that for the same capsules, the diameter ratio was equal to the wall thickness ratio; consequently the wall thickness seemed to be proportional to the microcapsule size. The wall thickness increased with an increase in ethylcellulose concentration whatever the microcapsule size (type 1/type 3), as shown by Benita & Donbrow (1982c). Conversely, the wall thickness seemed to be independent of the polyethylene content (type 3/type 5).

Table 2. Wall thickness ratios between the several types of microcapsules.

Microcapsule type	Wall thickness ratios
Type 1 (75–150)/Type 1 (150–300)	0·5
Type 3 (75–150)/Type 3 (150–300)	0·52
Type 1 (75–150)/Type 3 (150–300)	3·33
Type 1 (150–300)/Type 3 (150–300)	3·47
Type 3 (75–150)/Type 5 (75–150)	0·88

It was of interest to compare the two stirring methods (mechanical stirring and sonication) during the cooling stage, since mechanical stirring can destroy the microcapsules when large quantities of microcapsules are synthesized. Table 1 shows that no difference in microcapsule characteristics could be detected with either stirring method.

Microencapsulation is an exploitable method for clinical use, only if the encapsulated drug is not altered by the preparation process. Hplc is the most useful technique to detect cisplatin degradation (mainly hydrolysis). The chromatograms of cisplatin before and after microencapsulation showed two peaks, one of them corresponding to the unchanged initial molecule, the other corresponding to the hydrolysed form. As shown in Table 3, the ratio of

Table 3. Composition of cisplatin after microencapsulation. Hplc of cisplatin bulk powder and microencapsulated cisplatin treated with the same conditions of extraction.

	% of platinum in peak 1 retention time : 2,1 min	% of platinum in peak 2 retention time : 5,7 min
Cisplatin bulk powder	5·4	94·6
Cisplatin from microcapsules		
Type 1	4·6	95·4
Type 2	4·2	95·8
Type 5	6·1	93·9

the two peaks is similar before and after the microencapsulation, whatever type of microcapsules we prepared.

Microcapsule release studies. Release of cisplatin was studied in plasma by the above described methods. The rate of drug release was very different according to the microcapsule type (Fig. 1). The low drug-content microcapsules with the highest wall thickness (type 1) released very slowly, less than 20% of the cisplatin content being released after 24 h. When the ethylcellulose amount decreased (increase in drug content), a higher rate of release could be noted, 80% of the drug content being released after 24 h (type 3). For the same amount of ethylcellulose, the release increased with rising amount of polyethylene: 100% of the drug content being released after 24 h (type 5).

For types 3 and 5, the drug release was complete, but for type 1, the total removal of cisplatin from microcapsules was not observed, even after many

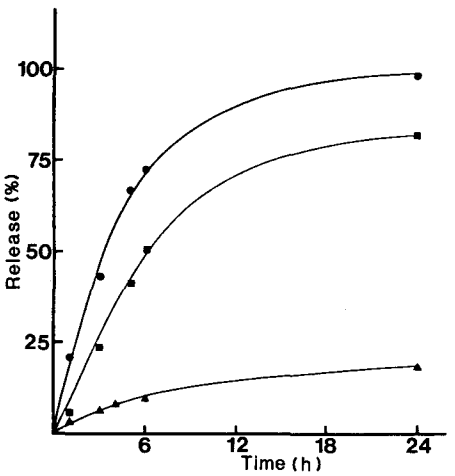

FIG. 1. Plot of % drug diffused through the microcapsule membrane with time. Influence of initial conditions: type 1 (▲), type 3(■), type 5 (●).

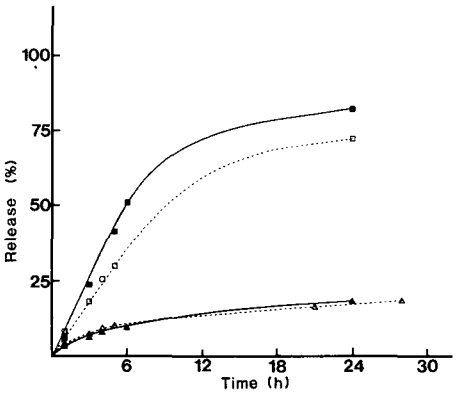

FIG. 2. Plot of % drug diffused through the microcapsule membrane with time. Influence of size: type 1, 75–150 μM (▲); type 1, 150–300 μM (△); type 3, 75–150 μM (■); type 3, 150–300 μM (□).

weeks. This fact is a major drawback for the usefulness of type 1 microcapsules in clinical use. Increase in the microcapsule size did not modify the release of type 1 but it resulted in a decrease of the release rate for type 3 (Fig. 2).

The discrepancy between type 1 and type 3 properties could result from a difference in the release mechanism according to the microcapsule type. Thus we treated the data on the basis of the three hypothetical mechanisms described in materials and methods. Table 4 shows the most significant linear correlation coefficients obtained for the different equations.

Type 1. Treatment yielded a linear relation between drug amount in the sink solution and square root of time. However the data also fitted the first order

Table 4. Mechanism of cisplatin release from prepared microcapsules. Most significant linear correlation coefficients for data analysis with several functions.

Microcapsule type (μM)	$m = f(t^{\frac{1}{2}})$	$\log P = f(t)$	$\frac{dm}{dt} = f(m)$	$\frac{dm}{dt} = f(1/m)$
1 (75–150)	0·996	−0·981		0·993
1 (150–300)	0·986			0·986
3 (75–150)	0·998	−0·997	−0·996	
3 (150–300)		−0·981	−0·984	
5 (75–300)		−0·997	−0·992	

release pattern (log (P) = f(t)) for the smaller microcapsules. A more stringent test was hence needed to distinguish between the matrix mechanism and the first order mechanism. The two mechanisms were clearly differentiated by the dm/dt = f(1/m) function which was linear whereas the dm/dt = f(m) was not. The release process of the type 1 microcapsules follows the matrix mechanism.

Type 3 and 5. A linear relation between the log of the retained amount in the microcapsules and time was obtained. The treatment previously applied showed that only the dm/dt = f(m) function was linear which confirmed the expected first order mechanism. Even though the overall kinetics pattern was first order, a zero-order equation could nevertheless be fitted to the initial phase of release, as noted by many authors for several drugs (Donbrow & Benita 1982; Senjkovic & Jalsenjak 1981, 1982).

Therefore two different release mechanisms could be observed for the same drug similarly encapsulated, according to the initial conditions of the preparation procedure. It may well be that the two mechanisms are present in all cases but that one would predominate according to the wall thickness. Matrix mechanism could be observed with thick-wall microcapsules while there was a first order mechanism with thinner-wall microcapsules.

The influence of the surrounding medium stirring rate on the drug release is also critical. We studied the release rate of each preparation of microcapsules in plasma stirred with three different speeds (1, 2 and 4 rev s^{-1}). No significant influence of the stirring speed was observed. This result does not agree with the results of Benita & Donbrow (1982c) or Senjkovic & Jalsenjak (1982). Independence between release and external flux could be explained by the unusual wall thickness of the prepared microcapsules (15–30 μm for types 3 and 5). Whatever flux we could apply to the microcapsule boundary layer, the solvent diffusion inside the microcapsule remained constant. On the other hand, the ratio plasma volume/microcapsule weight was great enough to minimize the difference of gradient between the inside and outside concentrations of dissolved cisplatin.

Finally the release was measured as a function of the stirring type in the cooling stage of the microcapsule preparation. The values of rates indicated that there was no dependence either on the mechanical stirring or on the sonication methods.

Conclusion

Cisplatin-containing microcapsules (types 3, 4, 5) were prepared with appropriate properties for chemoembolization use. Their drug content release was relatively slow (80–100% released after 24 h) and did not depend on the surrounding medium flux. The microcapsules can be prepared either by mechanical stirring or sonication during the cooling phase. No alteration of the cisplatin chemical structure was observed after microencapsulation by ethylcellulose. Throughout the release period, the process followed a first order release pattern. When we tried to decrease the release rate by increasing the microcapsule wall thickness (types 1 and 2), the kinetics of the release were modified and followed the matrix mechanism process, but microcapsules of this type were not useful for clinical application because a part of the drug content was not released even after several weeks.

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