NANOCAPSULES: A NEW TYPE OF LYSOSOMOTROPIC CARRIER

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1. Introduction

The use of endocytizable and lysosomotropic drugs is very promising in the chemotherapy of cancer [1], intracellular infection [2] and many other pathological processes [3]. Work in this direction has resulted in the development of complexes of anti-mitotic drugs with DNA [4], various proteins [5,6] and the entrapment of various pharmacological agents in liposomes [7–10]. This paper describes a new type of lysosomotropic carrier, made of small capsules of plastomers, with a diameter of about 200 nm: nanocapsules. They can be used to entrap a variety of molecules in a stable and reproducible way [11]. In the experiments reported here, we have studied the preparation and the intracellular uptake and localization of nanocapsules made of polyacrylamide and loaded with fluorescein.

2. Experimental and results

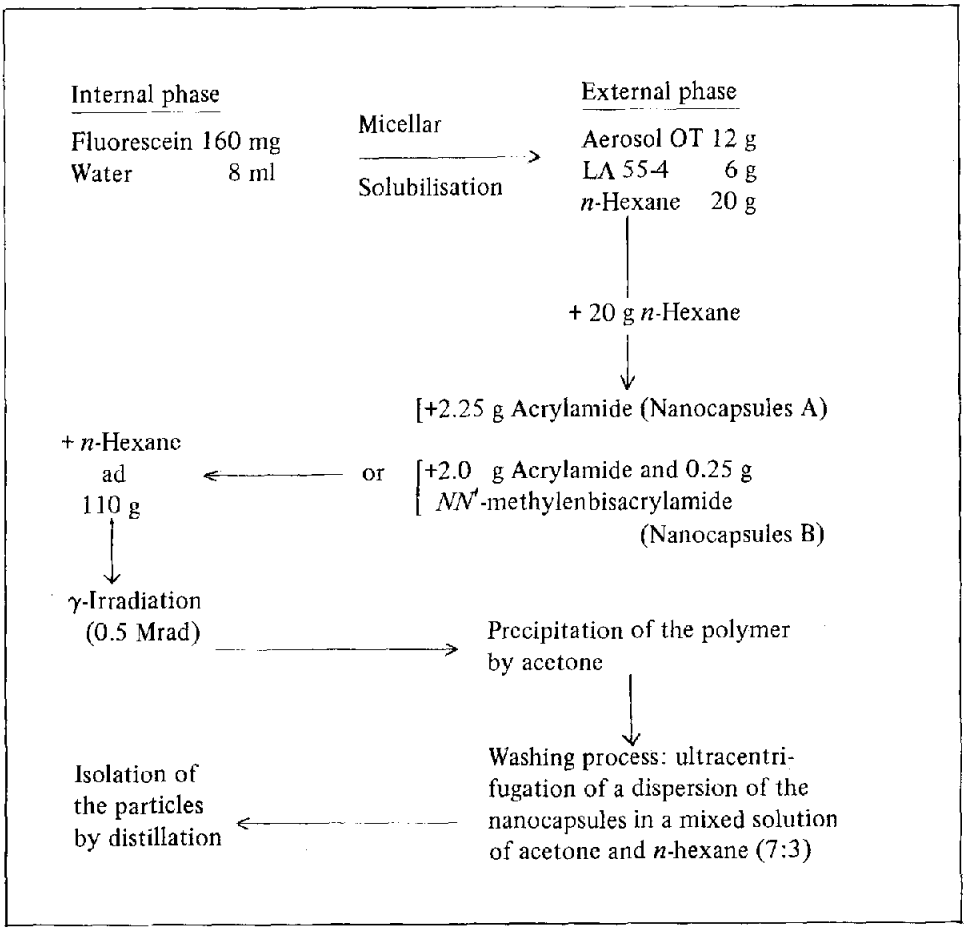
2.1. *Preparation of polyacrylamide nanocapsules*

Nanocapsules were made by the micellar polymerization method [11], in presence of sodium fluorescein, as shown in scheme I. After washing and isolation of particles, nanocapsules contained 47 μg fluorescein/mg polymer, i.e., 71% of the expected concentration. After ultrafiltration (Membrane UH 100, Schleicher und Shull, Zürich) of a 0.1% suspension of nanocapsules in water, about 30% of the fluorescein remained firmly bound. The appearance of nanocapsules type A in the scanning electron microscope is shown in fig.1. They present a spherical form, indicating a satisfactory isolation procedure; their mean diameter is about 200 nm. Similar pictures were obtained for nanocapsules type B. The aggregation seen in the pictures results from the technique used for the preparation of the sample; when nanocapsules are dispersed in water they form clear solutions and are not sedimented after centrifugation at $1.3 \times 10^6 \times g$ (Beckman J-21 centrifuge; rotor 20-J-A, operated at 20 000 rev/min).

2.2. *Uptake and intracellular fate of entrapped fluorescein*

Cultured rat fibroblasts [12] were incubated with fluorescein-laden nanocapsules type A, at a concentration of 1 mg polymer/ml, i.e., 47 μg encapsulated fluorescein. After 12 h, cells had accumulated 155 ng fluorescent material (expressed in terms of fluorescein)/mg cell protein. In the fluorescence microscope, fluorescence was mainly seen in the form of numerous, discrete granules surrounding the nucleus, and of appearance similar to that of lysosomes [13,14]. After isopycnic centrifugation of a cytoplasmic extract in sucrose gradient (fig.2), 2/3 fluorescent material showed a distribution pattern close to that of the lysosomal enzyme *N*-acetyl-β-glucosaminidase; the remaining 1/3 was found in the top fractions of the gradient; complete dissociation was observed from 5′-nucleotidase, a marker enzyme of plasma membranes and related structures. Since 1 mg cell protein corresponds to approx. 5 μl, of which lysosomes represent only 3% [13], the concentration of fluorescein in lysosomes can be calculated to be 15-fold larger than that in the culture medium, if it is distributed uniformly within these organelles.

Scheme 1
Preparation scheme of polyacrylamide nanocapsules loaded with fluorescein



Type A: linear polymer; Type B: branched polymer. (See [11] for details)

By contrast, cells incubated with 50 μg/ml free fluorescein took up only 25 ng fluorescein/mg protein; under the microscope, the fluorescence was weak and diffuse over the whole cell body; after isopycnic centrifugation, fluorescent material was only collected in the top fractions, where neutral pyrophosphatase, a soluble enzyme of the cytoplasm, is found.

3. Discussion

Our results show that polyacrylamide nanocapsules of a diameter of 200 nm or less can satisfactorily be made to entrap and retain material of low molecular weight. Entrapment of fluorescein in nanocapsules significantly increases its uptake by cultured fibroblasts but more important, allows its association with and accumulation into lysosomes, a cell compartment in which fluorescein does not spontaneously accumulate, probably because it is an organic acid [3]. Not all intracellular fluorescence is however confined to lysosomes; this may result from the partial leakage of free fluorescein from the capsules, before or after uptake by cells. Access of encapsulated fluorescein to lysosomes most probably occurs by endocytosis, since this is the only route clearly described by which non-

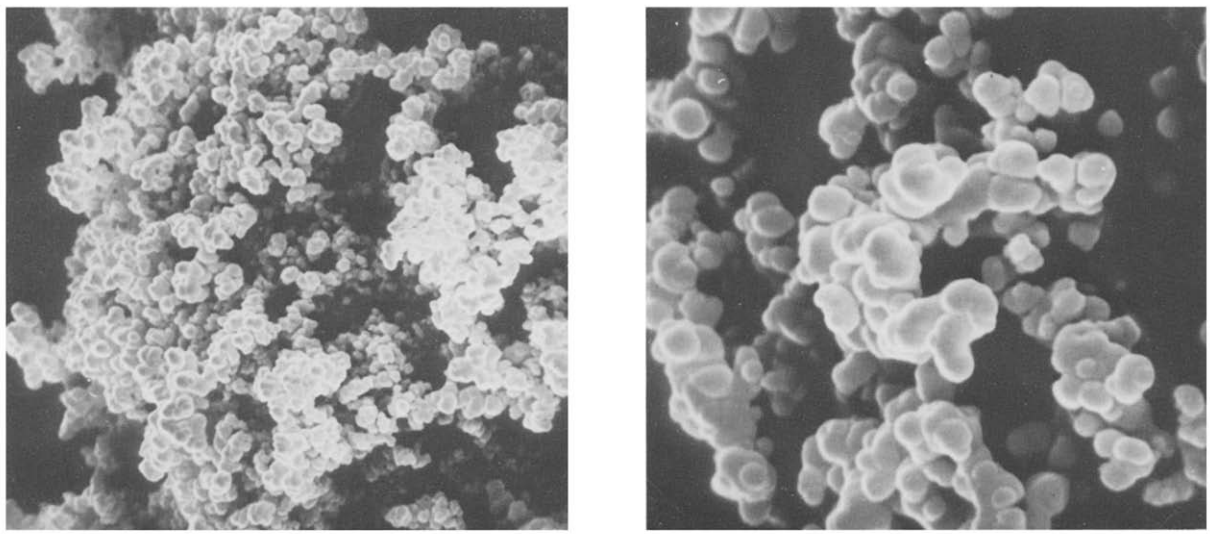


Fig.1. Morphological appearance of nanocapsules type A (linear polyacrylamide) in the scanning electron microscope. Left: magnification 7055 X; right: magnification 14 450 X.

infectious particulate material can center mammalian cells to this extent.

As such, polyacrylamide nanocapsules may represent an important step ahead into the development of lysosomotropic carriers for compounds that do not gain access to lysosomes [3]. They may also be useful in promoting the cellular uptake via endocytosis of compounds that would normally diffuse through biological membranes [14]. In this respect, it is noteworthy that our results were obtained with cells that are not professional phagocytes. However, the polymer used in this preparation of nanocapsules is unlikely to

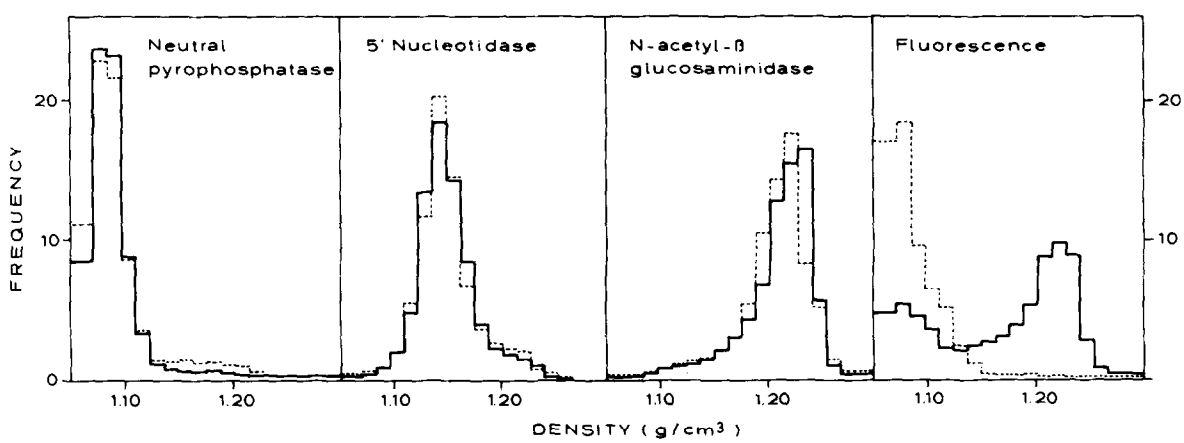


Fig.2. Isopycnic centrifugation of cytoplasmic extracts of fibroblasts cultured 12 h in presence of free (broken line) or encapsulated (solid line) fluorescein. Results are expressed as normalized histograms, the total areas of which are equal to 1 (for methods see [12]). As stated in text, encapsulation resulted into a 6.1-fold increase in the cellular concentration of fluorescent material. In a control experiment, where fluorescein-laden nanocapsules were added to a cytoplasmic extract, after collection and homogenization of the cell, all fluorescent material was recovered above a density of 1.07 g/cm^3.

be digested by lysosomal enzymes and this might restrict its use, since long-lasting overloading of lysosomes could ensue. The design of digestible nanocapsules will therefore be investigated.

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