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SCIENTIFIC PAPER

577.16:576:539.24-036.8

DESIGN, PRODUCTION AND CHARACTERIZATION OF POLYMERIC MICROPARTICLES CONTAINING VITAMINS FOR IN VITRO CELL TREATMENT ON LAB-ON-A-CHIP

A new lab-on-a-chip prototype, recently described, thanks to the use of integrated circuit technology, can generate dielectrophoretic fields that immobilize and allow the control of single biological objects, such as cells, liposomes, or microspheres immersed in a liquid overhanging and in contact with the same chip.

With the aim to design and produce polymeric microparticles for specific lab-on-a-chip applications, in the present paper the preparation and characterization of microparticles based on cellulose acetate and cellulose acetate plus other cellulosic polymers is described, by a solvent evaporation procedure.

In particular the following aspects were investigated: (a) the polymer solubilities, (b) the experimental parameters used for the solvent evaporation procedure, (c) the effect of dyes on the microparticle preparation and morphology, (d) the entrapment efficiency of the vitamins (D₃ and E) and, finally, (e) the release kinetics of vitamins from cellulosic microparticles.

Key words: Lab-on-a-chip, Cellulose acetate, Microspheres, Vitamins.

The development of advanced analytical and bioseparation methodologies based on micro- or macroarrays and biosensors is one of the strategic objectives of so-called postgenomics, but it also has an impact on strategic application fields, such as predictive oncology, diagnostics in the biomedical field, and drug research [1–3]. A comprehensive list of recently published articles describing studies employing commercial biosensors and emerging applications in the areas of drug discovery, clinical support, food and environment monitoring, and cell membrane biology were reported by Rich and Myszka [4].

Many applications in the biological, pharmaceutical and medical field are characterized by complex experimental protocols which need both microorganism detection and manipulation. The development of the so-called "lab-on-a-chip" needs to integrate functions such as sensing, processing, and actuation to increase their effectiveness and will enable highly complex laboratory testing to move from the central laboratory into non-laboratory settings [5–7].

In particular a new lab-on-a-chip prototype, recently described [8], thanks to the use of integrated circuit technology, can generate dielectrophoretic fields [9–13] that immobilize and allow the control of single biological objects, such as cells, liposomes, or microspheres immersed in a liquid overhanging and in contact with the same chip.

With this system it was possible to (a) entrap tumor cells within dielectrophoretic cages and move them under software control [14] and (b) program the binding of microspheres to target cells [15].

Taking into consideration the above described possibilities to manipulate different cell types and to facilitate the interactions between cells and microparticles, we started a research project aimed to evaluate the effect of vitamin-loaded microparticles on in vitro cultured cells in a lab-on-a-chip experimental model.

The choice to study vitamin-loaded microparticles was done considering the recently proposed use of vitamins as antioxidants for in vitro cell treatment [16].

In fact, it has been demonstrated that vitamin E (d- α -tocopherol) and D₃ (cholecalciferol) can improve the in vitro viability and cell performance, resulting in a beneficial effect on cell apoptosis.

It has been found that these vitamins can protect cell proteins and membranes against oxidative stresses by inhibiting the peroxidative attack of membrane lipids [16–19]. In fact free radicals generated from metabolism intermediates are capable of initiating lipid peroxidation by reaction with polyunsaturated fatty acids, inactivating proteins and enzymes by reacting with amino acids and also damaging RNA and DNA by reacting with guanine. If the cell is insufficiently protected by enzymatic and non-enzymatic antioxidants, free radicals can react with biomolecules thus damaging the cellular structure. In addition, antioxidant vitamins may protect from genetic changes by preventing DNA damage, directly induced by free radical attack.

Recently, vitamin D₃ has also been shown to act on tissues not related to calcium homeostasis such as brain, pancreas, skin, muscle, placenta, immune cells

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Paper received: December 6, 2005

Paper accepted: December 30, 2005

and the parathyroid [20]. The vitamin D₃ receptor complex is localized in the nucleus, and undergoes phosphorylation by reacting with a kinase. This form of the receptor then interacts with the Vitamin D₃ responsive element of the target gene and modifies the transcription of those genes to develop the action. The modulation of gene transcription results in the induction or repression of specific messengers. In vitro and animal studies indicate that vitamin D₃ may have anti-cancer benefits, both on the progression and metastatic processes, against a wide spectrum of cancers. Supporting the anti-cancer effect of vitamin D₃ is the ability of many cells to convert 25(OH)D, the primary circulating form of vitamin D, into 1.25(OH)₂D, the most active form of this vitamin [21].

Summarizing, in the present paper the preparation and characterization of microparticles based on cellulose acetate and cellulose acetate plus other cellulosic polymers is described by a solvent evaporation procedure.

In particular the following aspects were investigated: (a) the polymer solubilities, (b) the experimental parameters used for the solvent evaporation procedure, (c) the effect of dyes on the microparticle preparation and morphology, (d) the entrapment efficiency of the vitamins (D₃ and E) and, finally, (e) the release kinetics of vitamins from cellulosic microparticles.

MATERIALS AND METHODS

Materials

The polymers used for microparticle preparation namely, cellulose acetate (CA), hydroxypropylcellulose (HPC), cellulose acetate phthalate (CAP) and cellulose acetate trimellitate (CAT) were gifts from the Eastman Chemical Company (Kingsport, TN, USA). Vitamin D₃ and Vitamin E were purchased from Fluka (Buchs, Switzerland). The plasticizers used were isostearyl isostearate, corn oil PEG-6 esters (Labrafil M 2125 CS), corn oil PEG-8 esters (Labrafil WL 2609 BS) (Gattefossé, Parc des Barbanniers, France), triethyl citrate, tributyl citrate and acetyl tributyl citrate (Fluka). The dyes used, namely: oil red o, fat red bluish, scarlet red, Nile blue A, and bromophenol blue were from Fluka. Sudan black was from Aldrich (Steinheim, Germany). When not specified, all other chemicals and reagents were of the highest purity grade commercially available.

Production of vitamin-containing cellulose acetate microspheres

Microspheres were prepared by an oil-in-water emulsion (o/w) method. The polymer was dissolved in an appropriate solvent, and after solubilization of the vitamin and/or the dye, the organic solution was emulsified with an aqueous phase containing hydrolysed poly(vinyl alcohol) (PVA) (Celvol 205, Celanese Chemicals Europe GmbH, Frankfurt, Germany) as stabilizer. The obtained emulsion was

maintained under continuous stirring with a three-blade turbine impeller. At different time intervals, samples were observed microscopically throughout complete evaporation of the solvent, usually occurring in 3–5 h.

Microparticle morphology

The microparticle morphology, size and size distribution were evaluated by light, as well as electron microscopy examination, considering at least 300 particles/batch. In order to study the internal morphology, dried microparticles were sectioned under a binocular microscope. The sectioned particles were gold coated (Edwards Sputter coating S150). The internal and external morphology were analysed at 15–20 kV by a scanning electron microscope (Cambridge S 360).

Microparticle recovery

Microparticle recovery efficiencies were calculated as the percentage of the weight of the obtained microparticles taking as the reference the total amount of polymer used for the preparation.

Drug content of the microparticles

In order to determine the amount of vitamin per unit weight of microparticle, 10 mg of microparticles were dissolved in 1 ml of solvent. The vitamin concentration was determined by UV spectrophotometric analysis taking as the reference a previously made vitamin calibration curve.

Kinetics of vitamin release

A determined quantity of vitamin-containing microparticles was dispersed in a releasing buffer constituted of water:ethanol:tween 80 in the proportion 80:20:0.5 (v/v) and maintained under stirring at 37°C. Samples from the receiving buffer, at different time intervals, were withdrawn and the vitamin concentration was determined by UV spectrophotometric analysis taking as the reference a previously made vitamin calibration curve.

RESULTS AND DISCUSSION

Preparation and characterization of CA microspheres

The aim of the first part of the present study was to investigate the experimental parameters controlling the procedure of vitamin encapsulation in CA microparticles.

Namely, the microparticle morphology, size and encapsulation efficiency were investigated. These parameters are particularly important since the small volume of the location site (the lab-on-a-chip microchamber) can only accept a limited amount of microparticles with a very narrow size-distribution.

For microparticle preparation, CA was chosen as a highly lipophilic polymer, due to its very low swellability and solubility in water. CA microspheres were prepared

by an in-liquid drying process (solvent evaporation) as described in the experimental section.

Different experimental variables such as the type of oil and water phases, stirring speed, polymer and plasticizer type, surfactant type and concentration and presence of dyes and drugs were analysed. The effects of these variables on the morphological characteristics and encapsulation efficiency of the microspheres are described in the following sections.

Light and scanning electron microscopy were employed to analyse the external and internal structure of the microparticles since it is well known that the microcapsule morphology can influence both the applicability to lab-on-a-chip and the release of the contained drug and the in vivo general performances of the microparticles. The electron micrographs of the external and internal structure of empty CA microparticles (panel A and B, respectively) are shown in Fig. 1. As clearly evident, the preparation procedure did not lead to aggregation/agglomeration of the microparticles that on the contrary showed regular spherical geometry. Moreover, the internal morphology of the microspheres demonstrated that the particles were compact and homogeneous.

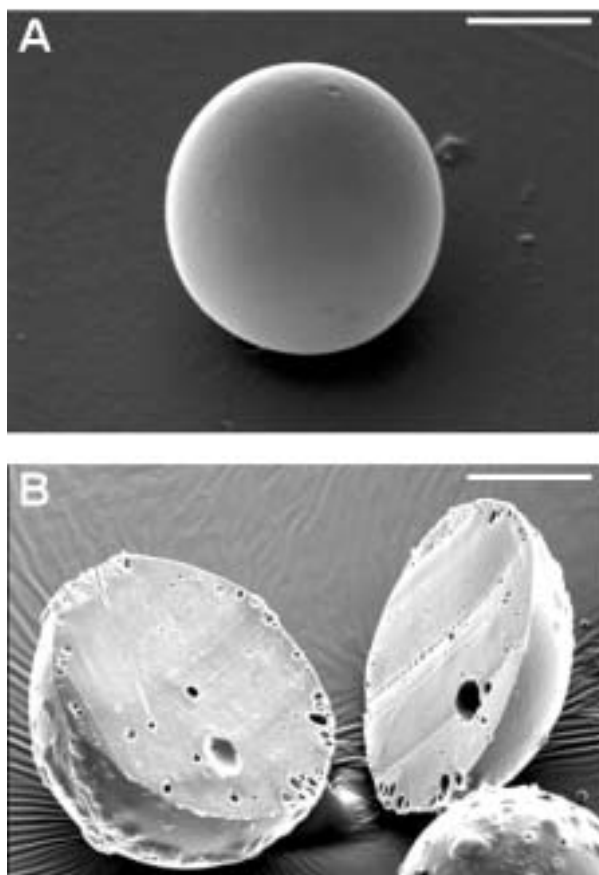


Figure 1. Scanning electron microscopy photographs of cellulose acetate microspheres produced by the solvent evaporation method. The bar corresponds to 20 μm .

Effect of the polymer solvent on the microparticle characteristics

The choice of the organic solvent to dissolve the polymer was found to play a key role either to obtain microparticles or to regulate their general morphological characteristics, recovery and aggregate formation. As a preliminary experiment, the solubility of CA, HPC, CA/HPC blends, CAP, CA/CAP blends, CAT and CA/CAT blends, in different organic solvents or solvent mixtures (at different ratios) were investigated (including: chloroform, chloroform/acetone, chloroform/acetonitrile, chloroform/propanol, ethyl acetate, diethyl ether, dichloromethane, dichloromethane/chloroform). The results of the solubility experiments are reported in Tables 1–7. Special attention was paid to finding solvent mixtures resulting in low viscosity solutions, since it was observed that highly viscous polymeric solutions, invariably failed to yield satisfactory microparticles, always resulting in large clusters or fused material in the form of blobs or filaments. A complete series of

Table 1. Solubility of cellulose acetate

Solvent (v/v)	Solubility
chloroform	insoluble
chloroform/acetone (90/10)	soluble up to 110 mg/ml
chloroform/methyl cyanide (90/10)	soluble up to 30 mg/ml
chloroform/ethanol (90/10)	soluble up to 190 mg/ml
chloroform/iso-propyl-alcohol (95/5)	soluble up to 100 mg/ml
ethyl acetate	insoluble
ethyl acetate/acetone (85/15)	soluble up to 100 mg/ml
ethyl acetate/acetone (90/10)	soluble up to 90 mg/ml
ethyl acetate/methyl cyanide (90/10)	insoluble
ethyl acetate/ethanol (90/10)	soluble up to 67 mg/ml
ethyl acetate/ethanol (70/30)	soluble up to 100 mg/ml
ethyl acetate/methanol(90/10)	soluble up to 100 mg/ml
ethyl acetate/iso-propyl-alcohol (90/10)	soluble up to 100 mg/ml
diethyl ether	insoluble
diethyl ether/acetone (90/10)	insoluble
diethyl ether/methyl cyanide (90/10)	insoluble
diethyl ether/ethanol (90/10)	insoluble
methylene chloride	insoluble
methylene-chloride/acetone (95/5)	soluble up to 100 mg/ml
methylene-chloride/ethanol (90/10)	soluble up to 100 mg/ml
methylene-chloride/iso-propyl-alcohol (95/5)	soluble up to 100 mg/ml
methylene-chloride/chloroform (95/5)	soluble up to 100 mg/ml
methylene-chloride/chloroform (50/50)	soluble up to 100 mg/ml
acetone	soluble up to 100 mg/ml

Table 2. Solubility of hydroxypropyl cellulose

Solvent (v/v)	Solubility
chloroform	insoluble
chloroform/acetone (90/10)	insoluble
chloroform/methyl cyanide (90/10)	insoluble
ethyl acetate	insoluble
ethyl acetate/acetone (90/10)	insoluble
ethyl acetate /methyl cyanide (90/10)	insoluble
diethyl ether	insoluble
diethyl ether/acetone (90/10)	insoluble
diethyl ether/methyl cyanide (90/10)	insoluble
methylene-chloride	insoluble
methylene-chloride/chloroform (50/50)	soluble up to 100 mg/ml
methylene-chloride/chloroform/acetone (45/45/10)	soluble up to 100 mg/ml
methylene-chloride/chloroform/iso-propyl-alcohol (45/45/10)	soluble up to 100 mg/ml

Table 3. Solubility of cellulose acetate/hydroxypropyl cellulose blends

Solvent (v/v)	Polymer bland		
	CA/HPC (95/5)	CA/HPC (90/10)	CA/HPC (85/15)
methylene-chloride/chloroform/acetone (45/45/10)	soluble up to 100 mg/ml	soluble up to 100 mg/ml	soluble up to 100 mg/ml
methylene-chloride/chloroform/iso-propyl-alcohol (45/45/10)	soluble up to 100 mg/ml	soluble up to 100 mg/ml	soluble up to 100 mg/ml

Table 4. Solubility of cellulose acetate phthalate

Solvent (v/v)	Solubility
chloroform	insoluble
chloroform/acetone (90/10)	insoluble
chloroform/methyl cyanide (90/10)	insoluble
chloroform/ethanol (90/10)	soluble up to 140 mg/ml
ethyl acetate	insoluble
ethyl acetate/acetone (90/10)	insoluble
ethyl acetate/methyl cyanide (90/10)	insoluble
ethyl acetate/ethanol (90/10)	insoluble
ethyl acetate/ethanol (80/20)	soluble up to 100 mg/ml
diethyl ether	insoluble
diethyl ether/acetone (90/10)	insoluble
diethyl ether/methyl cyanide (90/10)	insoluble
diethyl ether/ethanol (90/10)	insoluble
methylene-chloride	insoluble
methylene-chloride/ethanol (80/20)	soluble up to 100 mg/ml
methylene-chloride/iso-propyl-alcohol (95/5)	soluble up to 100 mg/ml
methylene-chloride/chloroform/ethanol (40/40/20)	soluble up to 100 mg/ml

Table 5. Solubility of cellulose acetate/cellulose acetate phthalate blends

Solvent (v/v)	Polymer bland		
	CA/CAP (95/5)	CA/CAP (90/10)	CA/CAP (85/15)
methylene-chloride /iso-propyl-alcohol (95/5)	soluble up to 100 mg/ml	soluble up to 100 mg/ml	soluble up to 100 mg/ml
methylene-chloride /chloroform/ethanol (40/40/20)	soluble up to 100 mg/ml	soluble up to 100 mg/ml	soluble up to 100 mg/ml

Table 6. Solubility of cellulose acetate trimellitate





Solvent (v/v)	Solubility
chloroform	insoluble
chloroform/acetone (90/10)	insoluble
chloroform/methyl cyanide (90/10)	insoluble
chloroform/ethanol (90/10)	soluble up to 140 mg/ml
ethyl acetate	insoluble
ethyl acetate/acetone (90/10)	insoluble
ethyl acetate/methyl cyanide (90/10)	insoluble
ethyl acetate/ethanol (90/10)	insoluble
ethyl acetate/ethanol (70/30)	soluble up to 100 mg/ml
ethyl ether	insoluble
diethyl ether/acetone (90/10)	insoluble
diethyl ether/methyl cyanide (90/10)	insoluble
diethyl ether/ethanol (90/10)	insoluble
methylene-chloride	insoluble
methylene-chloride/ethanol (80/20)	soluble up to 100 mg/ml
methylene-chloride/iso-propyl-alcohol/ethanol (85/5/10)	soluble up to 100 mg/ml
methylene-chloride/chloroform/ethanol (40/40/20)	soluble up to 100 mg/ml

Table 7. Solubility of cellulose acetate/cellulose acetate trimellitate blends

Solvent (v/v)	Polymer bland		
	CA/CAT (95/5)	CA/CAT (90/10)	CA/CAT (85/15)
methylene-chloride/iso-propyl-alcohol (95/5)	insoluble	insoluble	insoluble
methylene chloride/chloroform/ethanol (40/40/20)	soluble up to 100 mg/ml	soluble up to 100 mg/ml	soluble up to 100 mg/ml

experiments was performed using pure solvents (i.e. chloroform, methylene chloride, acetone, ethyl acetate and isopropanol), as well as their binary mixture in different proportions. In a few cases (see the list in Table 8), we succeeded in obtaining particles with acceptable morphological characteristics, while with many solvent mixtures it was impossible to produce particles of acceptable quality.

Table 8. Effect of solvent on the microsphere characteristics

Batch	Polymer	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Observations	Optical stereo micrograph
Mc1	CA	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h R. T. ^b	40	Smooth surface and irregular shape, filaments are present.	
Mc2	CA	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	50	Smooth surface and irregular shape, filaments are present.	
Mc3	CA	methylene-chloride/iso-propyl-alcohol (90/10)	2	750	3h 50°C ^c	80	Smooth surface and irregular shape, filaments are present.	
Mc4	CA	methylene-chloride/ethanol (90/10)	2	750	3h R. T. ^b	20	Irregular shape	
Mc5	CA	ethyl acetate/acetone (85/15)	2	750	3h R. T. ^b	50	Irregular shape	
Mc6	CA	ethyl acetate/acetone (85/15)	2	750	3h 50°C ^c	70	Irregular shape	
Mc7	CA	ethyl acetate/ethanol (90/10)	2	750	3h R. T. ^b	70	Irregular shape, filaments are present.	
Mc8	CA	ethyl acetate/ethanol (90/10)	2	750	3h 50°C ^c	30	Irregular shape	
Mc9	CA	chloroform/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	80	Smooth surface and spherical shape, aggregates are present.	
Mc10	CA	chloroform/acetone (90/10)	2	750	3h 50°C ^c	80	Smooth surface and irregular shape, filaments are present.	
Mc11	CA	chloroform/acetone (95/5)	2	750	3h 50°C ^c	75	Smooth surface and irregular shape, filaments are present.	
Mc12	CA	methylene-chloride/acetone (95/5)	2	750	3h R. T. ^b	65	Smooth surface and quite spherical shape	
Mc13	CA	methylene-chloride/chloroform (95/5)	2	750	3h R. T. ^b	30	Smooth surface and quite spherical shape	
Mc14	CA	methylene-chloride/chloroform (50/50)	2	750	3h R. T. ^b	65	Smooth surface and quite spherical shape	
Mc15	CA	methylene-chloride/chloroform (50/50)	2	750	3h 50°C ^c	80	Smooth surface and spherical shape	
Mc16	CA	methylene-chloride/chloroform (50/50) (7.5 ml)	2	750	3h 50°C ^c	20	Irregular shape, aggregates and filaments are present (the polymeric solution was very viscous).	

^aPercentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.

^bThe preparation was left at room temperature.

^cThe temperature was increased to 50°C after 1 h from the beginning of the preparation.

For instance, a 50:50 (% v/v) mixture of CH₂Cl₂/CHCl₃ was found to yield the best results in terms of microsphere quality (see the comments in Table 8), as compared to the other tested pure solvents or mixtures in different proportions of chlorinated solvents, ethyl acetate, acetone and isopropanol.

Effect of stirring speed on the microparticle characteristics

Since our aim was to obtain microspheres to be loaded in a lab-on-a-chip prototype, we modulated the

stirring speed, and succeeded in obtaining microspheres within a suitable dimensional range by using 1.0 g of polymer and a stirring speed of 750 rpm in the presence of 2% PVA solution as an emulsion stabilizer. In fact, these conditions resulted in the formation of microspheres with an average diameter of about 80 μm. Nevertheless, we found that by varying the stirring speed (from 500 to 1000 rpm), particles with diameters ranging from 120 to 60 μm were obtained (data not shown).

Table 9. Effect of emulsifier concentration on the microsphere characteristics

Batch	Polymer	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Mean diameter (μm) ^d	Observations
Mc23	CA	methylene-chloride/chloroform (50/50)	1	750	3h 50°C ^c	80	84.25 \pm 43.31	Smooth surface and spherical shape
Mc24	CA	methylene-chloride/chloroform (50/50)	3	750	3h 50°C ^c	80	78.99 \pm 27.73	Smooth surface and spherical shape
Mc25	CA	methylene-chloride/chloroform (50/50)	5	750	3h 50°C ^c	80	49.25 \pm 47.90	Smooth surface and spherical shape

^aPercentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.
^dData represent the average of 3 independent experiments \pm standard deviation.
^cThe temperature was increased to 50°C after 1 h from the beginning of the preparation.

Table 10. Effect of plasticizer on the microsphere characteristics

Batch	Polymer	Plasticizer ^e	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Observations
Mc17	CA	isostearyl isostearate	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	70	Smooth surface and irregular shape, filaments are present.
Mc18	CA	labrafil WL 2609 BS	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	65	Irregular shape, filaments are present.
Mc19	CA	labrafil M 2125 CS	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	58	Smooth surface and irregular shape, filaments are present.
Mc20	CA	triethyl citrate	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	83	Smooth surface regular shape
Mc21	CA	tributyl citrate	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	72	Smooth surface and regular shape.
Mc22	CA	acetyl tributyl citrate	methylene-chloride/iso-propyl-alcohol (95/5)	2	750	3h 50°C ^c	75	Irregular shape, aggregates and filaments are present.

^a Percentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.
^c The temperature was raised at 50°C after 1 h from the beginning of the preparation.
^e 1 ml
 Labrafil M 2125 CS: Corn oil PEG-6 esters;
 Labrafil WL 2609 BS: Corn oil PEG-8 esters;

Effect of stabilizer and plasticizer on the microparticle characteristics

We tested the effects of different PVA concentrations, namely 1, 3 and 5% (w/v) (see Table 9), on the size and size distribution of the microspheres prepared with 1.0 g of CA and a stirring speed of 750 rpm. The addition of progressively larger amounts of the polymeric emulsifier, led to stabilization of the emulsion and to a reduction in the size of the polymer solution droplets, during the emulsification step. Both these effects resulted in a significant improvement of the final microsphere quality and monodispersity, together with a size decrease. The results on the effect of different plasticizers on the general characteristic of the produced microparticles are reported in Table 10. Generally, the use of plasticizers had a detrimental effect on the characteristic of the microparticles that resulted in a less regular shape, also with a large amount of aggregates present.

Dye encapsulation

With the aim to produce coloured microparticles to improve their detectability under optical microscopes, when loaded in the lab-on-a-chip chamber, we investigated the possibility to load dyes in the CA microparticles. Initially we selected a number of lipophilic dyes (see the list and chemical structure in Table 11) that were subsequently tested for their ability to be encapsulated in CA microparticles.

For all the tested dyes, we were able to produce and isolate microparticles with optimal morphological characteristics and a homogeneous distribution of the dye as documented by the microphotographs included in Table 12.

Drug encapsulation

With respect to the encapsulation efficiency exploited by CA microspheres, the crucial role of the chemical-physical characteristics of the employed drug

Table 11. Dyes used for microsphere production

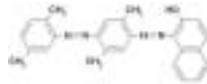
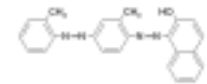
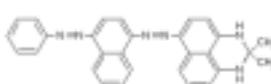
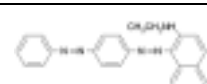
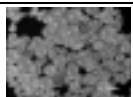

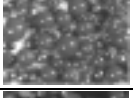
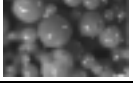
Dye	Molecular formula	Molecular weight (Da)	Absorption λ_{MAX} (nm)	Structure	Solubility in water	Manufacturer
oil red o	C ₂₆ H ₂₄ N ₄ O	408.49	518 359 (2nd)		insoluble	Fluka Chemika
scarlet red	C ₂₄ H ₂₀ N ₄ O	380.44	520 357 (2nd)		insoluble	Fluka Chemika
sudan black	C ₂₉ H ₂₄ N ₆	456.54	598 415 (2nd)		insoluble	Aldrich
fat red bluish	C ₂₄ H ₂₁ N ₅	379.46	533 364 (2nd)		insoluble	Fluka Chemika

Table 12. Effect of dye on the microsphere characteristics

Batch	Polymer	Dye	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Observations	Optical stereo-micro-graph
Mcol-1	CA	oil red o	methylene-chloride /chloroform (50/50)	5	750	3h R.T. ^b	83.3 ± 1.4	Smooth surface and spherical shape	
Mcol-2	CA	scarlet red	methylene-chloride /chloroform (50/50)	5	750	3h R.T. ^b	85.4 ± 1.5	Smooth surface and spherical shape	
Mcol-3	CA	sudan black	methylene-chloride /chloroform (50/50)	5	750	3h R.T. ^b	84.2 ± 1.4	Smooth surface and spherical shape	
Mcol-4	CA	fat red bluish	methylene-chloride /chloroform (50/50)	5	750	3h R.T. ^b	86.7 ± 1.4	Smooth surface and spherical shape	

Data represent the average of 3 independent experiments ± standard deviation.
^a Percentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.
^b The preparation was left at room temperature.

should be stressed. Indeed, hydrophobic drugs can be quantitatively incorporated in microspheres, whilst molecules with hydrophilic portions display a much reduced trapping efficiency (data not shown). For instance, vitamin E and vitamin D₃ that are both highly lipophilic and almost water insoluble, displayed a high encapsulation efficiency (>95%) in all the CA microsphere formulations tested. Fig. 2 shows the release kinetics of both vitamin E and vitamin D₃, in panels A and B, respectively. In both cases, it is clear that the release kinetic occur in two different phases: (a) an initial period (the first 9–10 hours) that is characterized by a relatively rapid release, during which about 65% of the total entrapped drug is released (this behaviour is probably due to the release of the drug entrapped in the peripheral domain of the microparticles); and (b) a second period that is

characterized by a much slower rate, in which the vitamin release is approximately linear.

Production of CA-blend microparticles

With the aim to possibly modulate the hydrophilic–lipophilic balance of the cellulosic microparticles, we tested the possibility to produce cellulosic microparticles using a blend of CA with different percentages of other cellulosic polymers such as: HPC, CAP and CAT. The results of these experiments are summarized in Tables 13–15. As a general comment the produced microparticles were of poor quality and in some cases (CA/CAT blends, Tables 15) it was impossible to produce microparticles. Better results in term of microparticle morphology were obtained using the blends CA/HPC (75:25, w/w) and CA/CAP (95:5 and 85:15, w/w).

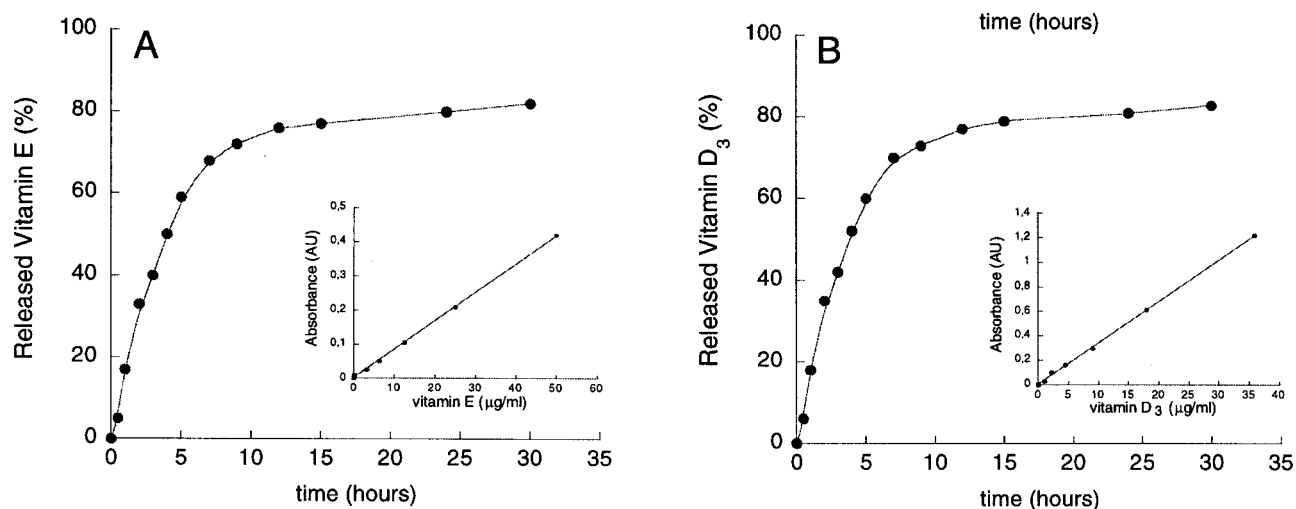


Figure 2. The release kinetics of vitamin E (panel A) and vitamin D₃ (panel B) from cellulose acetate microspheres. The spectrophotometric UV calibration curves in the insets are reported.

Table 13. Effect of HPC percentage on CA/HPC on microsphere characteristics

Batch	CA/HPC ratio (%)	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Observations
Mhpc1	97.5:2.5	methylene chloride/ chloroform/iso-propyl- alcohol (45/45/10)	2	750	3h 50°C ^c	65	Wrinkled surface and irregular shape
Mhpc2	97.5:2.5	methylene chloride/ chloroform/acetone (45/45/10)	2	750	3h 50°C ^c	70	Wrinkled surface and irregular shape
Mhpc3	95:5	methylene chloride/ chloroform/ iso-propyl- alcohol (45/45/10)	2	750	3h 50°C ^c	65	Wrinkled surface and irregular shape, filaments are present.
Mhpc4	95:5	methylene chloride/ chloroform/acetone (45/45/10)	2	750	3h 50°C ^c	72	Wrinkled surface and irregular shape
Mhpc5	95:5	methylene chloride/ chloroform (50/50)	2	750	3h 50°C ^c	65	Wrinkled surface and irregular shape
Mhpc6	90:10	methylene chloride/ chloroform/acetone (45/45/10)	2	750	3h 50°C ^c	65	Wrinkled surface and irregular shape
Mhpc7	90:10	methylene chloride/ chloroform (50/50)	2	750	3h 50°C ^c	70	Wrinkled surface and irregular shape
Mhpc8	80:20	methylene chloride/ chloroform/acetone (40/45/10)	2	750	3h 50°C ^c	57	Wrinkled surface and irregular shape, some microspheres are merged.
Mhpc9	80:20	methylene chloride/ chloroform (50/50)	2	750	3h 50°C ^c	60	Wrinkled surface and irregular shape
Mhpc10	75:25	methylene chloride/ chloroform/acetone (40/45/10)	2	750	3h 50°C ^c	65	Quite smooth surface and quite spherical shape

^a Percentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.

^c The temperature was increased to 50°C after 1 h from the beginning of the preparation.

^b The preparation was left at room temperature.

Table 14. Effect of CAP percentage on the CA/CAP on microsphere characteristics

Batch	CA/CAP ratio (%)	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Observations
Mcap1	95:5	methylene chloride/ chloroform/ethanol (40/40/20)	2	750	3h R.T. ^b	80	Smooth surface and spherical shape
Mcap2	95:5	methylene chloride/ iso-propyl-alcohol (95/5)	2	750	3h R.T. ^b	75	Wrinkled surface and quite spherical shape, some filaments are present.
Mcap3	90:10	methylene chloride/ chloroform/ethanol (40/40/20)	2	750	3h R.T. ^b	80	Wrinkled surface and irregular shape
Mcap4	90:10	methylene chloride/ iso-propyl-alcohol (95/5)	2	750	3h R.T. ^b	75	Wrinkled surface and quite spherical shape, filaments are present.
Mcap5	85:15	methylene chloride/ chloroform/ethanol (40/40/20)	2	750	3h R.T. ^b	85	Smooth surface and quite spherical shape
Mcap6	85:15	methylene chloride/ iso-propyl-alcohol (95/5)	2	750	3h R.T. ^b	70	Wrinkled surface and irregular shape, filaments are present.
Mcap7	80:20	methylene chloride/ iso-propyl-alcohol (95/5)	2	750	3h R.T. ^b	65	Wrinkled surface and irregular shape, filaments are present.
Mcap8	75:25	methylene chloride/ iso-propyl-alcohol (95/5)	2	750	3h R.T. ^b	60	Wrinkled surface and irregular shape, filaments are present.
Mcap9	70:30	methylene chloride/ iso-propyl-alcohol (95/5)	2	750	3h R.T. ^b	50	Wrinkled surface and irregular shape, filaments are present.

^a Percentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.
^b The preparation was left at room temperature.

Table 15. Effect of CAT percentage on the CA/CAT on microsphere characteristics

Batch	CA/CAT ratio (%)	Solvent (% v/v)	PVA (% w/v)	Stirring speed (rpm)	Process duration and temperature	Recovery (%) ^a	Observations
Mcat1	95:5	methylene chloride/ iso-propyl-alcohol/ethanol (45/45/10)	2	750	3h R.T. ^b	—	No microspheres were obtained
Mcat2	90:10	methylene chloride/ iso-propyl-alcohol/ethanol (45/45/10)	2	750	3h R.T. ^b	—	Microspheres are merged together
Mcat3	85:15	methylene chloride/ iso-propyl-alcohol/ethanol (45/45/10)	2	750	3h R.T. ^b	—	Microspheres are merged together and have a stretched shape

^a Percentage (w/w) of produced microspheres with respect to the total amount of polymer used for the preparation.
^b The preparation was left at room temperature.

Further studies are in progress to select other solvent mixtures of hydrophilic cellulosic polymers to improve the microparticle quality.

Conclusions

This study has provided an understanding of the effects of production parameters on the charac-

teristics of cellulose acetate-based microparticles. The selection of the appropriate preparation procedures and cellulose esters enabled us to obtain microparticles intended for lab-on-a-chip applications. Experiments are in progress to further evaluate the potential of these microparticles on second generation smart slide systems.

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IZVOD

DIZAJN, PROIZVODNJA I KARAKTERIZACIJA POLIMERNIH MIKROKAPSULA KOJE SADRŽE VITAMINE ZA *IN VITRO* TERAPIJU "LABORATORIJSKI ČIP"

(Naučni rad)

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Nedavno opisan nov prototip "laboratorijskog čipa" koji može, zahvaljujući korišćenju integrisane zaokružene tehnologije, da generiše dielektroforetska polja koja imaju sposobnost imobilizacije i kontrole pojedinačnih bioloških materijala, kao što su ćelije, lipozomi, ili mikrosfere uronjene u tečnost iznad i u kontaktu sa samim čipom.

Postupkom isparavanja rastvarača u ovom radu je opisana priprema i karakterizacija celulozno acetatnih mikrokapsula, kao i mikrokapsula načinjenih od celuloznog acetata u kombinaciji sa drugim celuloznim polimerima u cilju dizajniranja i proizvodnje polimernih mikročestica za specifičnu primenu "laboratorijskog čipa".

Posebno su ispitivani sledeći aspekti: (a) rastvorljivost polimera, (b) eksperimentalni parametri korišćeni u postupku isparavanja rastvarača, (c) efekti boja na morfologiju i formiranje čestica, (d) efikasnost inkapsulacije vitamina (D₃ i E) i na kraju (e) kinetika oslobađanja vitamina iz celuloznih mikročestica.

Ključne reči: Laboratorijski čip, Celulozni acetat, Mikrosfere, Vitamini.