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NANOCAPS

Nano-capsules for Targeted Controlled Delivery of Chemicals

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

NANOTECHNOLOGIES AND NANOSCIENCES, KNOWLEDGE-BASED MULTIFUNCTIONAL MATERIALS, AND NEW PRODUCTION PROCESSES AND DEVICES

Publishable Final Activity Report

Period covered: March 2004 to February 2007 **Date of preparation: 15. April 2007**

Start date of project: 1st March 2004 **Duration: 36 Months**

Project coordinator: Christian Simon
Organisation name: SINTEF **Final version**

Publishable executive summary

Contractors involved

Partic. Role*	Partic. no.	Participant name	Participant short name	Country	Date enter project**	Date exit project**
CO	1	SINTEF	*SINTEF*	NO	1	36
CR	2	Max Plank Institute of Colloid and Interfaces	*MPIKG*	DE	1	36
CR	3	University of Franche-Comté	*UFC*	FR	1	36
CR	4	Institute of Catalysis and Surface Chemistry	*ICSC*	PL	1	36
CR	5	Center for Research and Technology Hellas	*CERTH*	GR	1	36
CR	6	PlasmaChem GmbH	*PLASMACHEM*	DE	1	36
CR	7	Coventya SAS	*COVENTYA*	FR	1	36
CR	8	Institut Français du Pétrole	*IFP*	FR	1	36
CR	9	KeraNor AS	*KERANOR*	NO	1	36
CR	10	Coatex SAS	*COATEX*	FR	1	36
CR	11	ICB Pharma	*ICB*	PL	1	36

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Project web site

The project web page is available at: http://www.sintef.no/nanocaps/. A detailed list of publications is available

Summary description of project objectives and work performed

The project NANOCAPS aimed at developing new technologies based on micro-encapsulation to solve industrial problems related to controlled release of chemicals. The technical objectives have been to develop nano-materials and nano-composite coatings for cost-efficient production of nano-capsules and, to validate the technical and economical feasibility of the knowledge acquired in the fields of biomedical (anti-proliferating and anti-allergic agents) and metal plating (self-repair) applications. To achieve the objectives, NANOCAPS was configured in five main activities (WP) addressing specific problems. Two of these were technology oriented (WP1 and WP2), and concerned the production of nano-materials as capsule core and shell. Two others were directed to basic research (WP3 and WP4), and considered fundamental studies on the reactivity of the core materials and the capsules, and the development of new advanced methods for the characterisation of nano-capsules. The fifth activity (WP5, started after mid-term) considered the validation and exploitation of the knowledge in multi-sectorial industrial prototypes. The project started in March 2004 and lasted until March 2007.

During the project period from month 1 to month 36, the following materials were produced:

- New types of nanoparticles (to be applied as core vectors for active molecules) have been prepared:
    - For **anti-proliferating application**, poly(lactide-co-glycolide acid) (**PLGA**) nanoparticles containing naphthylacetic acid (Figure 1) were prepared. The produced particles were perfectly **spherical** in the **size range of 0.2 to 2 µm**. The **loading efficiency was between 5.3 and 2 %**.

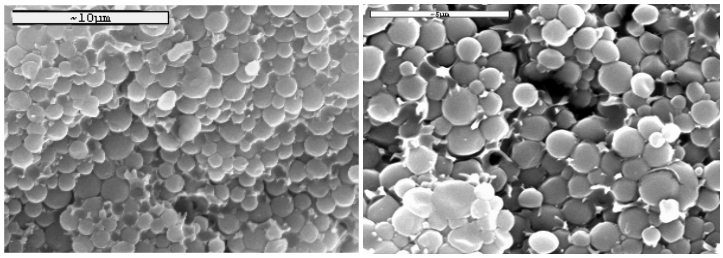

Figure 1. *SEM photomicrographs of PLGA nanoparticles containing napthylacetic acid*

    - A series of **acrylic monodisperse nanoparticle** dispersions was produced by free-radical polymerisation. The main difficulty was to obtain dispersions without aggregates. Acrylic copolymer nanoparticles have **sizes ranged between 13 nm to 371 nm with a dry content of 30%**, a minimum of aggregates and a **stability above 3 months**. These nanoparticles are applied in **anti- proliferating applications and metal plating applications.**
    - For **metal plating application, Polymer/Silica composite** nanoparticles were prepared. The produced particles were **perfectly spherical**, uniform in size, having a **raspberry-like morphology** (Figure 2). Depending on the method of preparation, composite nanoparticles with a **size range of 500 to 800nm** and **silica content 10-15%wt.** and composite nanoparticles with a **size range of 100 to 150nm** and a silica content **~50%wt.** were produced.

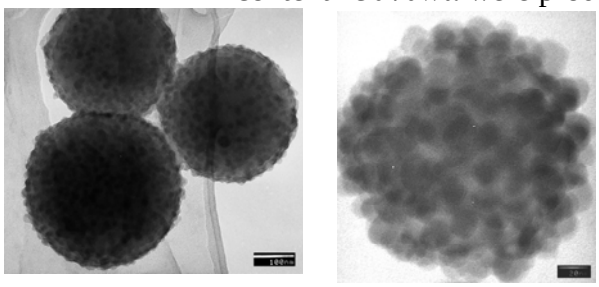

Figure 2. *TEM photomicrographs of Polymer/Silica composite nanoparticles.*

- **Nanosized emulsions** for **anti-proliferating application** have been prepared by membrane emulsification. The size of **chloroform/water** emulsion droplets was as low as **44 nm**. Flurorinated oil emulsions in water were prepared with a **size of ca. 200 nm and a polydispersity index of 0.8**. New ceramic **membranes** have been produced with better **homogeneity** and **surface quality**. **Monodisperse** emulsion droplets could be prepared with **surface modified membranes** in order to reduce the porosity.
- **Nanosized gel complex particles** for multiple applications have been prepared by membrane emulsification. The smallest size is **80 nm**. The stability needs to be improved.

- For **anti-proliferating** application, nano-engineered **shells made by LbL** on nano-emulsion containing a model drug molecule were produced (See Figure 3). About 10% of drug molecule was quickly released in the aqueous phase.

Figure 3. *CLSM fluorescence (left) and transmission (right) channels images of drug in oil solution filled capsules. Fluorescence comes from drug. Capsule average size is ca. 5µm*
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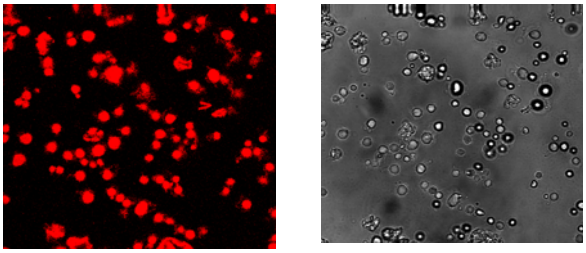
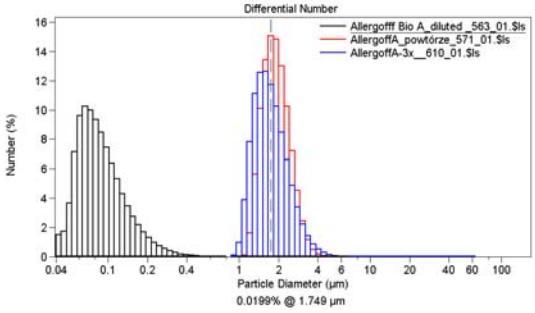


- **Microcapsules** for **anti-proliferating application** were prepared by **LbL** on chloroform/water emulsions from **membrane emulsification**.
- **Polyelectrolyte capsules with embedded drugs** were prepared from porous calcium carbonate. Encapsulation **efficiency** was found to be **40 and 21%** for a water insoluble dye and a water soluble drug, respectively.
- For **metal plating** application, several formulations that are potential candidates as active components for encapsulation have been tested and one formulation (see Figure 4) showed **very promising anti-corrosion properties** on steel.

Figure 4*. Promising results of anti-corrosion properties of formulations applied on electrogalvanised steel. Salt spray test after 336 hours*

- Theoretical **model of capsules**, capsules interactions and capsules-surface interactions were formulated based on the combination of DLVO, hydrodynamic forces and steric interactions.
- For **anti-allergic application**, two types of capsules were focused on: One with **fast release** to obtain immediate effect and the other with **slow release** for longer term effect (up to 6 months). Different **shell materials** have been tried out and the best candidates were identified. Capsules **stable** up to **90 days** under in-vitro conditions could be prepared (See Figure 5).

Figure 5. *Bimodal size distribution measured on capsules prepared for anti-allergenic application*



- For **metal plating** application, stable hollow capsules were made using (PSS/PDADMAC) as a shell by LbL deposition. **PSS and PDADMAC** are **promising** materials that can withstand plating solution compositions. A fluorescent substance was encapsulated to follow the release properties. Immediate **deformation** was observed in the plating solution. After 1 hour in the alkaline plating solution all of the fluorescent material was released.
- **Microcapsules** were prepared from **coacervation** of PAH/PSS complex on $CaCO_3$ cores.

- The understanding of the **mechanism of layer deposition** of the shell was greatly improved with synthetic polyelectrolytes, Poly(allylamine, HCl)-Poly(styrene sulfonate) and on natural polymers: chitosan and alginate. **New homopolymers** of MADQUAT and **copolymers** of acrylic acid- MADQUAT aiming to be applied instead of the natural chitosan have been synthesized.
- **Nanodiamonds, nanoalumina and nano-SiC** particles have been successfully produced and the scale-up was realised. Nanodiamonds and nanoalumina particles were coated for **metal plating application**. The alumina particles produced can be used for membrane preparation.
- **Hollow micro- and nanocapsules** were prepared for **metal plating** application. The in-situ formation of the silica shell has been studied.
- **LbL growth mechanism** was explained with help of advanced characterisation methods (reflectometry, streaming potential, imaging ellipsometry, microelectrophoresis), and equipment constructed and developed. **Laser reflectometry** provided a sensitive way to measure polymer adsorption on surfaces and permitted the determination of **in-situ adsorption-desorption** processes, to adsorption rate and possible polymers **rearrangement** in the shell.
- The **transfer** of **active molecules** through capsule shells was characterized by **permeability measurements** using electrochemical techniques and fluorimetry and fluorescent microscopy. The permeability of PAH/PSS shells is lower than alginate/chitosan. **Release** of small molecules (fluorescein) through capsule shells of five polyelectrolyte bilayer is retarded 100 times with respect to bare dye particle. **Temperature and pH** of the solution may be used to control the transfer of small molecules through shells. Both factors may affect the stability of the shell layers as well.
- New **capsules** with **thermo-sensitive** and **pH-responsive** properties were prepared and used to encapsulate model polymers. The encapsulation and release mechanisms were studied.

- A novel method for **remote release** of encapsulated polymers using **laser light illumination** was developed. The release of a fluorescently labelled polymer from a polyelectrolyte multiplayer capsule was observed for the first time (See Figure 6). The composition of the shell was defined.

- Methods of **time dependent streaming potential** and **dynamic contact angle**, **imaging single wave ellipsometry** were developed, extended and applied for studying mono and multilayer polyelectrolyte systems. The methods were implemented to perform the analysis of interfacial properties and stability of polyelectrolyte films.

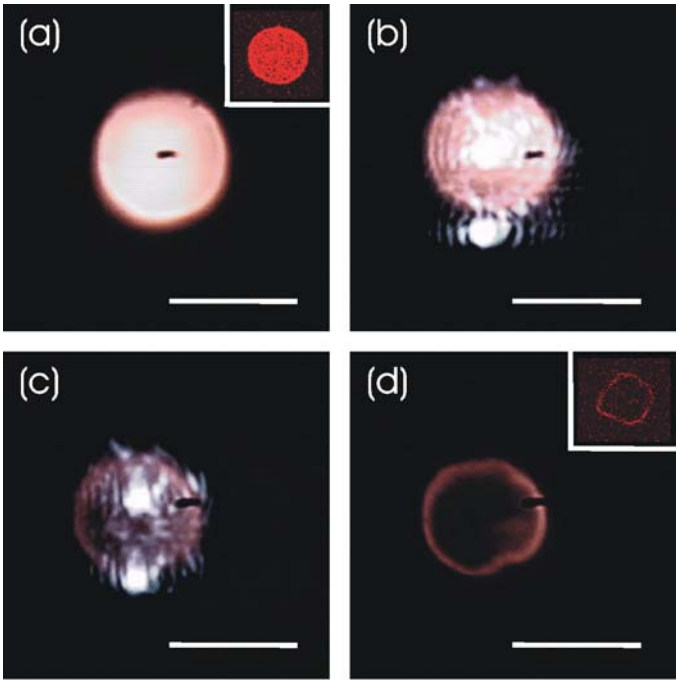


Figure 6. *Sequence of images illustrating the release of rhodamine labelled PSS from PEM capsules by light*

- A new **optical impinging cell** was developed to study capsule **attachment and detachment** or **codeposition** for metal plating – allowing in-situ observation of microcapsules deposition or fluorescently labelled nano-capsules at non-transparent surfaces. For **anti-proliferating** application, a **high affinity** of model capsules to the surface of **surgical steel** was observed.
- For **anti-proliferating** application, **adhesion** tests of capsules on surgery steel have started.
- The **feasibility** of observing nanocapsules and multilayer films by several **near field techniques**: AFM (air and liquid), R-SNOM, STOM and Transmission Fluorescence Near Field Microscopy (emitting probe) was tested. A **transmission fluorescence microscope** is now fully operational.
- For **anti-proliferating application**, biodegradable nanoparticles containing a model drug and real drug with sizes in the range of 200-350 nm were synthesized.
- For **anti-proliferating application** and **metal plating application**, new types of homopolymers of styrene sulfonate with various polydispersity indexes and copolymers were designed and synthesized.
- For **anti-proliferating application** and **metal plating application**, the composition of chloroform/water emulsions prepared by membrane emulsification was optimised as well as the conditions to form a shell layer around the emulsion droplets.
- For **anti-proliferating application**, homopolymers of styrene sulfonate and of MADQUAT have been applied in the preparation of various types of nanosized capsules (97-350 nm) and with various zeta potential (- 40 mV to + 50 mV) by a membrane process. Fluorescein and Red 8 dyes could be encapsulated and released at high temperature.
- For **anti-proliferating application**, a copolymer that precipitates at low pH was synthesized and applied to prepare capsules containing fluorescein with a mean size of 260 nm by a membrane process. Fluorescein could be released at high pH.
- For **anti-proliferating application**, a copolymer that forms spontaneously micelles in presence of water was synthesized and applied to encapsulate drug. The capsule size was 40 nm.
- For **metal plating application** chloroform/APS miniemulsions and microemulsion stabilized by surfactants with an average droplet size 40 nm were prepared.
- For **anti-proliferating application**, drug encapsulated nanoparticles (248 nm) were prepared with a loading of 10% compared to the dry mass.

- For **anti-proliferating application**, In-vitro tests were performed to prove the cytotoxicity of drug loaded capsules coated on metallic model surfaces (Fig. 7). The cell multiplication was reduced by a factor of 100 after 6 days. Consequently, production and marketing of new capsules coated stents have been initiated.

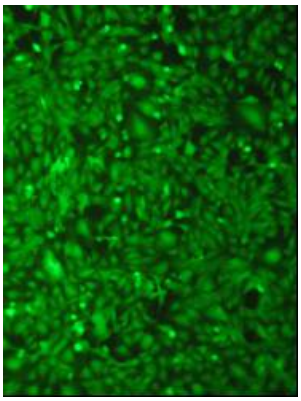
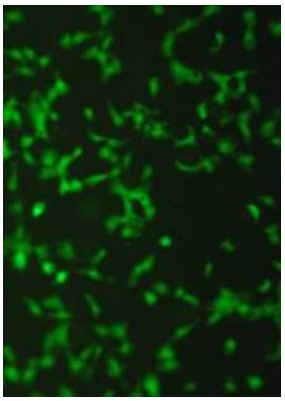
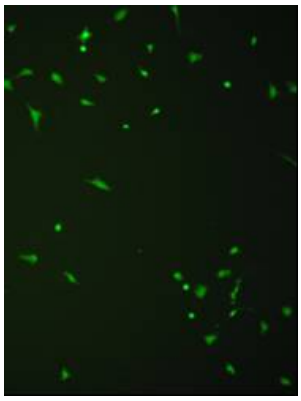
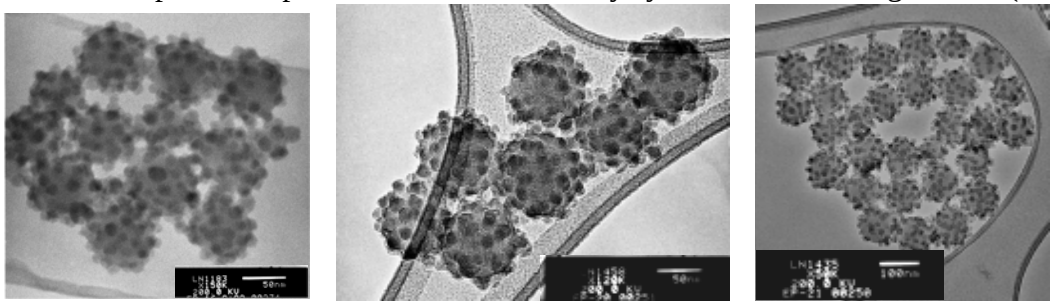
Control: 0% of drug, after 6 days	1 % of drug, after 6 days	20 % of drug, after 6 days
		

Figure 7. *Microscope observation of the results of in-vitro cytoxicity tests after 6 days on non-coated metal (left side) and on drug-encapsulated nanoparticles coated on metal (right side)*

- For **metal plating application**, silica and **functional silica hollow capsules** (250 nm) have been prepared by membrane emulsification. The formation of the shell layer has greatly progressed with help of NMR techniques.
- For **metal plating application, composite** nanoparticles with an average diameter of 110 nm and a solid content of ~ 40 wt%. were prepared by emulsifier-free emulsion copolymerization. The produced particles were successfully synthesized on a larger scale (1 litre).



(a) **(b)** **(c)**

Figure 8. *TEM photomicrographs of composite capsules. Scaling-up (a) 100 ml reactor, (b)500 ml reactor and (c) 1 litre reactor.*

- For **metal plating application**, Functionalised **polymer nanoparticles** were found to be **compatible** in acid plating bath.
- For **metal plating application**, **composite** nanoparticles were successfully **co-deposited** with zinc on steel (Fig. 9 and 10). A promising protecting effect is observed in salt spray test after temperature treatment to 200ºC for 1 hour.

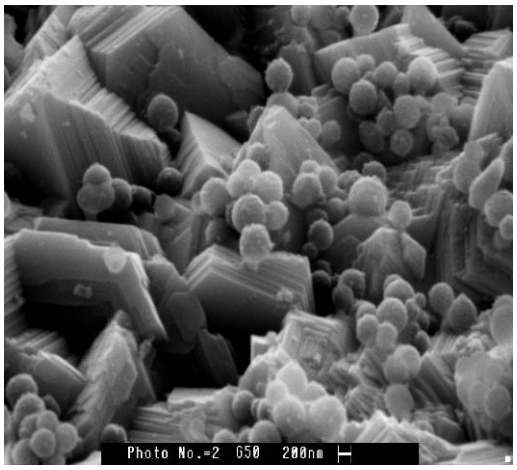


Figure 9. *Composite capsules type 1*

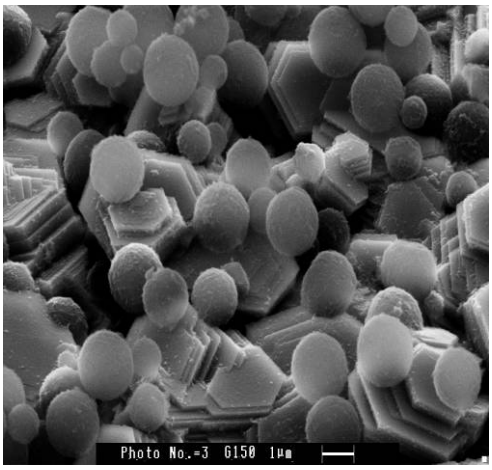


Figure 10. *Composite capsules type 2*

- For **metal plating application**, composite nanoparticles were applied as **organo-mineral formulations after Cr(III) passivation** on galvanised steel and tested in salt spray test for 450 hours. Promising results are observed compared with a standard organo-mineral formulation.
- For **anti-proliferating application**, the conditions to form PE multilayer shells using various combinations of strong and weak polyelectrolyte layers by spraying were optimised.
- For **metal plating application**, the conditions of shell formation by coacervation of polyelectrolytes on nanosized silica particles have been further studied.
- For **anti-allergic application** – good adhesion of antiaccaricide containing capsules to modified surfaces (mimicking mite skin) was confirmed.
- **Consistent** and **reliable** information of the successive and alternate deposition of two polyelectrolytes could be obtained by reflectometry. This was applied in understanding the growth of the LbL film.
- For **anti-proliferating application**, nanosized capsules (200 nm and 500 nm) made of latex core coated by 11 PE bilayers could be successfully deposited on stainless steel.
- The **permeability** of PE shells was characterised in solution and when attached on a metal surface. The influence of the shell thickness on release of fluorescein in solution was great. The release was also slowed down when the shell was attached on the metal surface.
- The characterisation of the **molecular weight distribution** on the polyelectrolytes synthesized in the project was realised by **GPC**.
- A **new method** based on sedimentation gravity was developed to characterise the **stability** of nanosized particles, emulsions and capsules. The efficiency of the method was demonstrated on systems applied in the project.
- The shell **roughness** (AFM) and the shell **thickness** (FIB and Reflectometry) of LbL polyelectrolyte films have been characterised. Very spectacular increases of the roughness with the number of bilayers are observed in Fig. 11.

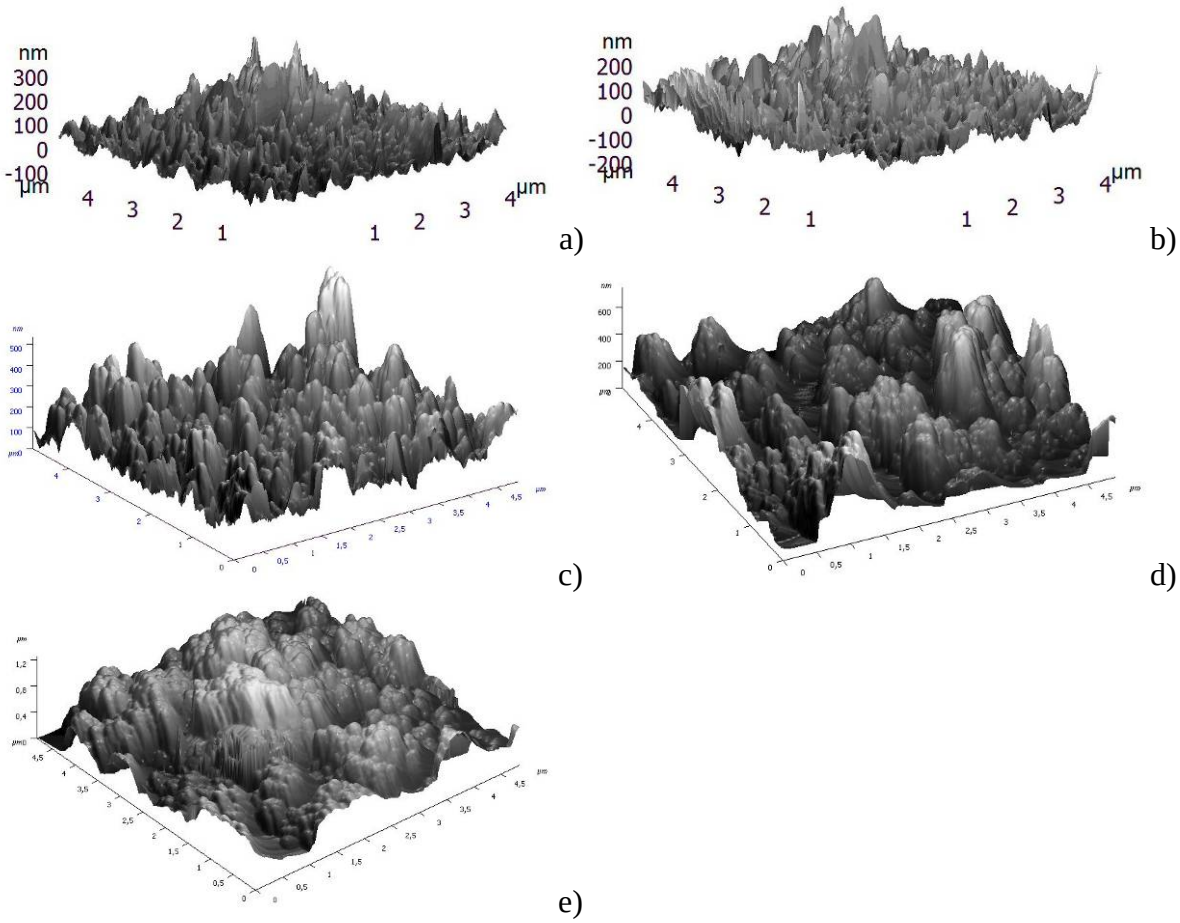


Figure 11: *AFM investigation of the very thick film morphology: a) 30 layers, b) 40 layers, c) 80 layers, d) 160 layers, e) 320 layers*

- **GDOS** showed to be an appropriate tool for **quality contro**l during capsule production by LbL technique.

- For **metal plating application** and **anti-proliferating application**, the **membrane emulsification** technique applied to capsule production was scaled up to a production of 3-5 litres/day.

- A **mobile pilot scale unit** for membrane emulsification has been built (Fig. 12 and 13) and nanosized emulsions have been successfully **produced**.

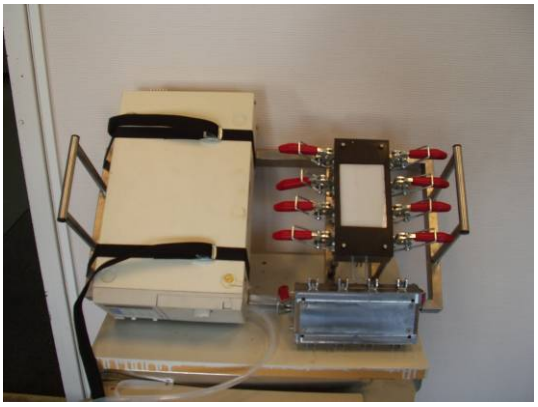


Figure 12 and 13*. Images showing the pilot scale mobile membrane emulsification unit. The membrane is shown on the right figure.*

- For **metal plating application** and **anti-proliferating application**, the **ultrasound** technique applied to capsule production was scaled up to a production of 4 litres/day.
- A **pilot scale unit** for **ultrasound** technique has been built (Fig. 14) and is operative.

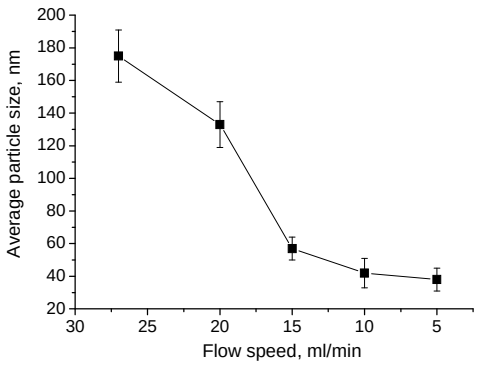


Figure 14*. Particle size vs. flow speed in ultrasonic flow-cell gauge*

- Upscaling capacities for production of composite nanoparticles at the pilot scale level (5 litres) are available (Fig. 15).

a **b**

Figure 15. *Pilot-plant (a) Reactors and (b) Controller.*

- An up-scaling unit for LbL shell formation on capsules was designed (Fig. 16) and the construction was started.

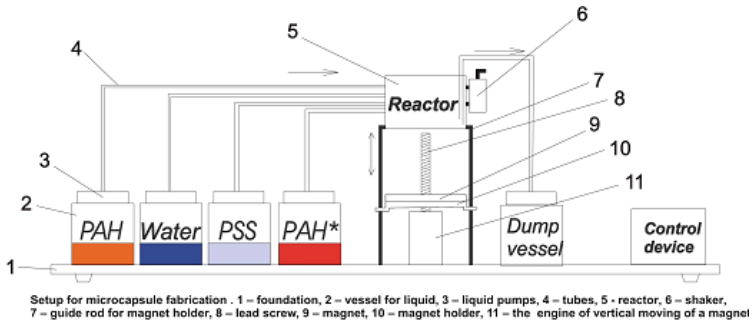


Figure 16. *Sketch of the up-scaling unit for LbL shell formation on capsules*

- Chloroform/water emulsions have been successfully produced from the **mobile membrane emulsification pilot unit**. The rate of production was 3 litres in 30 min. at a transmembrane pressure of 9 bars and a membrane of 80 nm pore size. The mean droplet size was **80 nm** which was comparable to the size obtained with a lab-unit in the same conditions. A batch of **capsules** containing anti-corrosion formulation for **metal plating application** has been produced.
- A large scale production unit based on high speed emulsification was installed for capsule production (Fig. 17).

Figure 17. *Large scale production unit based on high speed emulsification*

In addition:

- **84 publications** have been published in international scientific journals with referees (**29**) and international conferences.
- **5 patents** applications are in progress (1 patent filed).
- **5 PhD Thesis** have been defended.
- **Research scientists, Post Doc. Ph.D. and Master students** have been **exchanged** with stays up to 5 months.
- An **explanatory strategy seminar** was organised in connection to the 24M technical meeting in Berlin on 8th March 2006. The main results and actions from the seminar have been taken over in the Plan for disseminating and using the knowledge for continuous monitoring.
- A **final report** describing the **plans for using and disseminating** the results was updated for 36M reporting.