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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 Executive Summary
Confidential**

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	<i>SINTEF</i>	NO	1	36
CR	2	Max Plank Institute of Colloid and Interfaces	<i>MPIKG</i>	DE	1	36
CR	3	University of Franche-Comté	<i>UFC</i>	FR	1	36
CR	4	Institute of Catalysis and Surface Chemistry	<i>ICSC</i>	PL	1	36
CR	5	Center for Research and Technology Hellas	<i>CERTH</i>	GR	1	36
CR	6	PlasmaChem GmbH	<i>PLASMACHEM</i>	DE	1	36
CR	7	Coventya SAS	<i>COVENTYA</i>	FR	1	36
CR	8	Institut Français du Pétrole	<i>IFP</i>	FR	1	36
CR	9	KeraNor AS	<i>KERANOR</i>	NO	1	36
CR	10	Coatex SAS	<i>COATEX</i>	FR	1	36
CR	11	ICB Pharma	<i>ICB</i>	PL	1	36

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Project web site

The project web page is available at: <http://www.sintef.no/nanocaps/>.

Summary description of project objectives and work performed

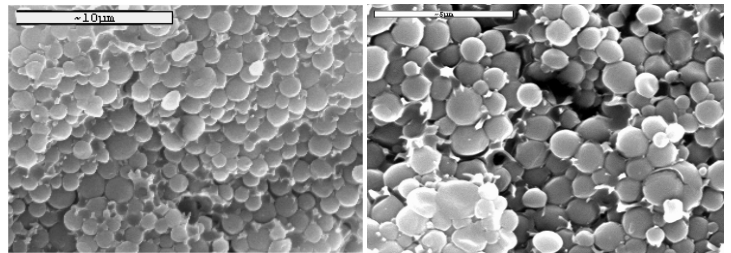
The project NANOCAPS aimed at developing new technologies based on micro-encapsulation to solve industrial problems related to controlled release of chemicals. The technical objectives have been to develop nano-materials and nano-composite coatings for cost-efficient production of nano-capsules and, 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:

In WP1,

- 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 naphthylacetic acid



- A series of **acrylic and styrol-acrylic monodisperse nanoparticle** dispersions was produced by free-radical polymerisation. The main difficulty was to obtain dispersions without aggregates. Acrylic and styrol-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**, **Vinyl Polymer/Silica composite** nanoparticles were prepared. The produced particles were **perfectly spherical**, uniform in size, having a **raspberry-like morphology**. 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.

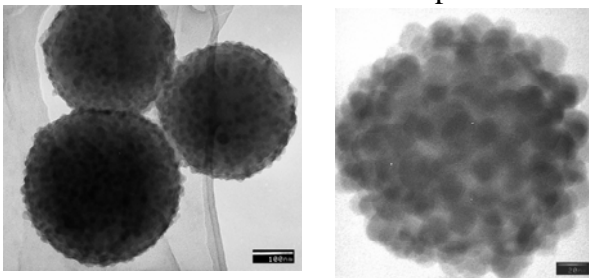


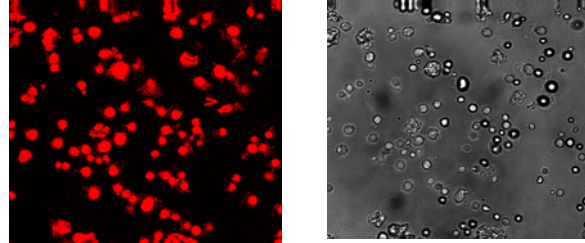
Figure 2. TEM photomicrographs of Vinyl Polymer/Silica composite nanoparticles.

- **Nanosized emulsions for anti-proliferating application** have been prepared by membrane emulsification. The size of **chloroform/water** emulsion droplets is as low as **44 nm**. Fluorinated 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 (e.g. PSS-PAH complex) particles** for multiple applications have been prepared by membrane emulsification. The smallest size is **80 nm**. The stability needs to be improved.

In WP2,

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

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



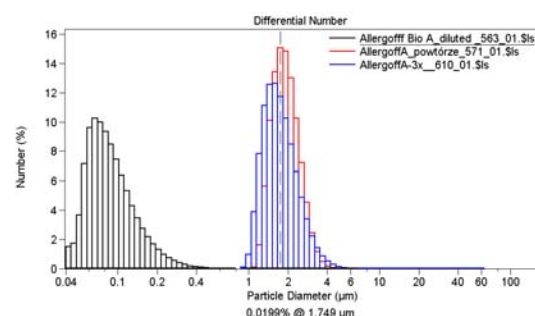
- **Microcapsules for anti-proliferating application** were prepared by **LbL** on chloroform/water emulsions from **membrane emulsification**.
- **Polyelectrolyte capsules with embedded drugs** were prepared from porous calcium carbonate. Encapsulation **efficiency** was found to be **40 and 21%** for Sulforhodamine B 2-acid fluoride (water insoluble dye) and +/- amethopterin (water soluble drug) respectively.
- Encapsulation of nanosized hematite particles was feasible by **complex coacervation** of gelatin and arabic gum. The **shell thickness** was ca. **10 nm** and could be dependent on the core size.
- For **metal plating** application, several **silane 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. Co-deposition of silica encapsulated in PSS/PAH (size 1 μm) with zinc is feasible in acid bath media. Codeposition of silica and zinc showed that nanosized particles (below 50 nm) gave a high silica coverage on the zinc surface. Several types of nanoparticles have been co-deposited with zinc.

Figure 4. Promising results of anti-corrosion properties of MTES/TEOS formulation 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 the **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 CaCO₃ cores.
- **Polymethylcyanoacrylate (PMCA) nanocapsules** (average diameter ca. 100 nm) containing benzyl benzoate/tea-tree oil were prepared for **anti-allergic application**. The capsules had a high loading efficiency (23.5-50%).

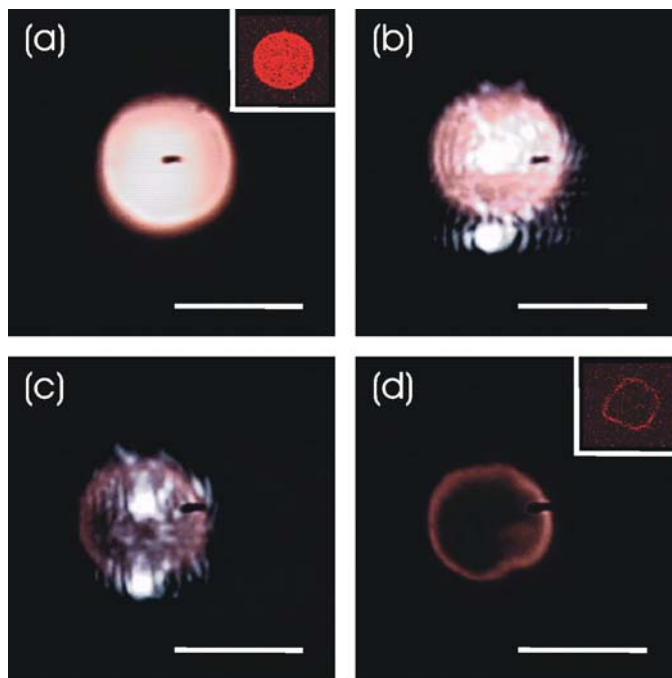
In WP3,

- 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 with Mirapol for **metal plating application**. The alumina particles produced can be used for membrane preparation.
- **Hollow micro- and nanocapsules** with functionalisable silica shells 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.
- **Cationic poly(methyl methacrylate)** nanoparticles (d_{av} : ~300nm) were prepared by emulsifier-free emulsion polymerization for **metal plating applications**.
- 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.

In WP4,

- 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 nanocapsules 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.

In WP5, Task 5.1,

- For **anti-proliferating application**, biodegradable (**PLGA**) nanoparticles containing Naphylacetic acid (model drug) as well as Rapamycin 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 Rapamycine. The capsule size was 40 nm.
- For **metal plating application**, new capsules (bimodal size distribution 80 nm and 500 nm) with TEOS in oil to prevent hydrolysis of the silane were synthesized.
- For **metal plating application** chloroform/APS miniemulsions and microemulsion stabilized by TWEEN 80 with the average droplet size 40 nm were prepared. The capsules silica shells were obtained by gradual hydrolysis of APS. The codeposition of capsules followed by corrosion tests indicated increased corrosion resistance (around 72 hours before first red rust spot). The same methodology will be continued with MTES/TEOS mixtures of high corrosion protection.
- For **anti-proliferating application**, Rapamycine encapsulated LPA 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 Rapamycine/LPA 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.

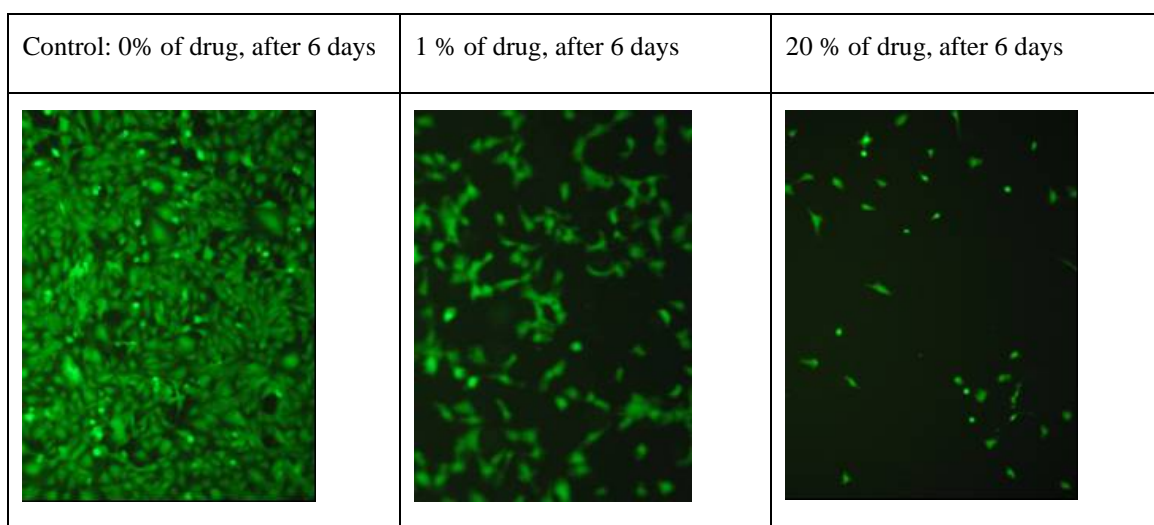


Figure 7. Microscope observation of the results of in-vitro cytotoxicity tests after 6 days on non-coated metal (left side) and on rapamycine-encapsulated PLA nanoparticles coated on metal (right side)

- For **metal plating application**, organophosphorated nanoparticles have been designed and synthesized. The mean particle size was from 88 nm to 590 nm.
- 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**, a new silane formulation in dichloromethane was prepared and tested for anti-corrosion properties on electrogalvanised steel. Coatings made from this formulation give a protection for more than 670 hours in salt spray test.

- For **metal plating application**, **Vinyl Polymer/Silica composite** nanoparticles with an average diameter of 110 nm and a silica content of ~ 40 wt%. were prepared by emulsifier-free emulsion copolymerization of Styrene and 1-Vinylimidazole. The produced particles were successfully synthesized on a larger scale (1 litre).

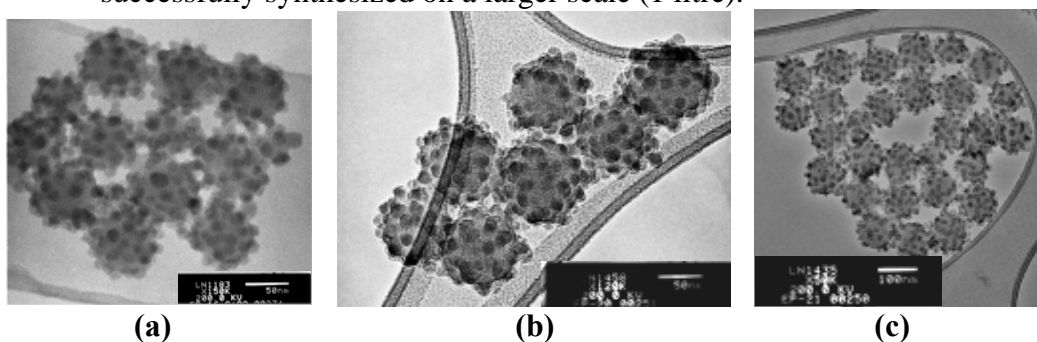


Figure 8. TEM photomicrographs of EP-P(St/1-VID)/Si. Scaling-up (a) 100 ml reactor, (b) 500 ml reactor and (c) 1 litre reactor.

In WP5, task 5.2,

- For **metal plating application**, Functionalised **polymer nanoparticles** were found to be **compatible** in acid plating bath.
- For **metal plating application**, **poly vinyl/silica 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 200°C for 1 hour although the target of 200 hours before apparition of white rust spots could not be reached.

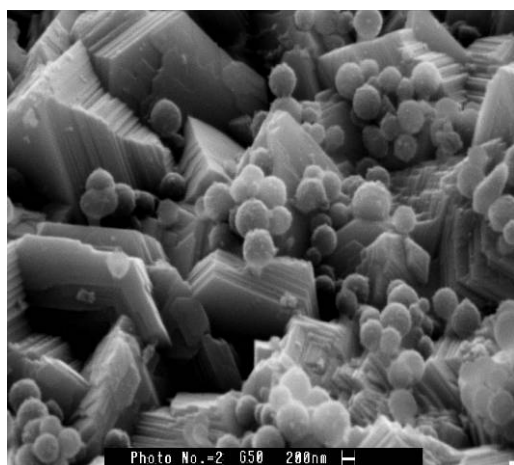


Figure 9. DP-P(St/4VP)/Si-013b latex

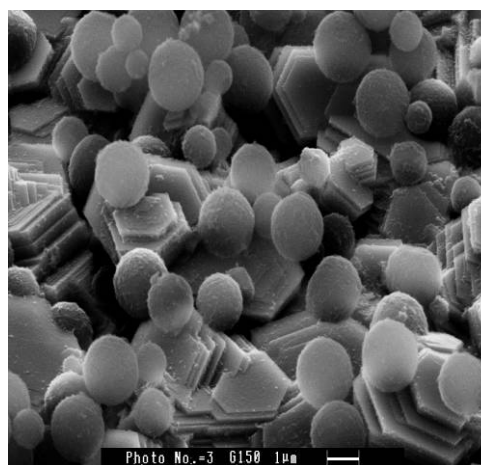


Figure 10. DP-P(St/4VP)/Si-020 latex

- For **metal plating application**, poly vinyl/silica 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 **metal plating application**, functional **silica hollow capsules** without anti-corrosion formulation were successfully **co-deposited** with zinc on steel. No agglomeration was observed on the surface. The coated panels were tested for corrosion in salt spray. Promising results were obtained for co-deposited samples preheated for one hour at 200°C. No real improvement in corrosion resistance could be observed.

- For **metal plating application**, anti-corrosion formulation containing **capsules** were prepared for the first time and were successfully **co-deposited** with zinc on steel. No real improvement in corrosion resistance could be observed.
- 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 antiacarricide containing capsules to the chitosan modified surfaces (mimicking mite skin) was confirmed.

In WP5, task 5.3,

- **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.

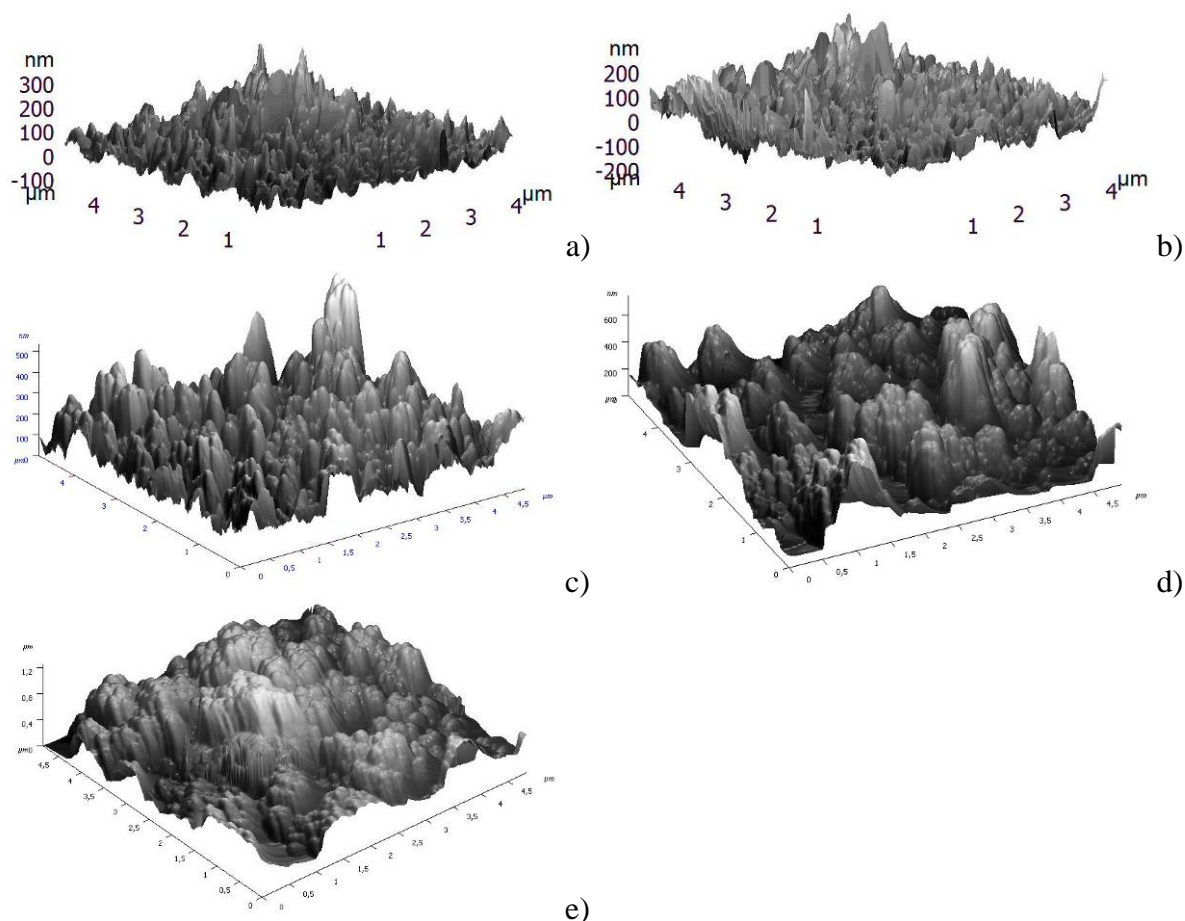


Figure 11: AFM investigation of the very thick film morphology: a) 30 layers, b) 40 layers, c) 80 layers, d) 160 layers, e) 320 layers

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

In WP5, task 5.4,

- 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 by the partner KERANOR (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 at PLASMACHEM.

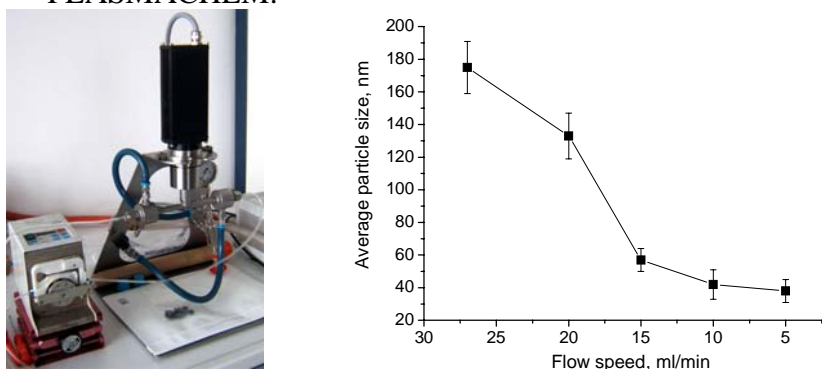


Figure 14. Particle size vs. flow speed in ultrasonic flow-cell gauge

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

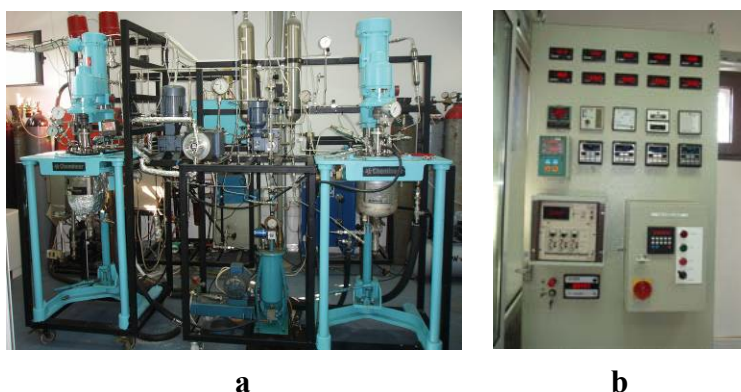


Figure 15. Pilot-plant (a) Reactors and (b) Controller.

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

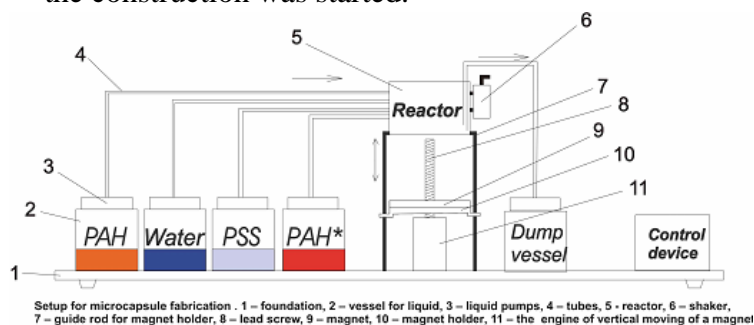


Figure 16. Sketch of the up-scaling unit for LbL shell formation on capsules

In WP 5, task 5.5,

- 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 at ICB for capsule production (Fig. 17).



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

- A complete series of skin sensitization test, efficiency tests, and acute toxicology and ecotoxicology tests have been performed using nanocapsules developed in the project for **anti-allergic applications** using standard EU-tests. It was concluded that Allergoff Bio products did not present any toxicity and kept their efficiency for up to 3 months.
- COATEX disposes of all the capacities for pilot plant and large scale production of the polymers and nanoparticles synthesized in the project (30 litres, 200 litres or 2 m³).
- New exploitation of the materials developed in the project was realised by ICSC in the manufacturing of more efficient catalysts.
- New exploitation in the production of gas capsules by the membrane emulsification has been explored by SINTEF and MPIKG.
- New exploitation of the materials and methods developed in the project were applied by PLASMACHEM in the efficient production of stable alumina and diamond nanosized particles (Fig. 18).



Silver/Nanodiamond

Volume = 400 Ltr.

Content: KCN/Nanoadditive



Silver/Al₂O₃

Volume = 400 Ltr.

Content: KCN/Nanoadditive

Figure 18. Production of alumina and diamond nanosized particles

- PLASMACHEM has started production of commercial additives to galvanic electrolyte (DiamoSilb and AlumoSilb) as well as the additive to engine oils (ADDO) (for end-user oriented product the web site www.addo-oil.com was constructed) – Figures 19, 20.

<p>Figure 19. Commercial product on the basis of coated nano-diamonds – additive to galvanic electrolytes</p>	<p>Figure 20. Commercial product on the basis of coated nano-diamonds – additive to engine oils</p>
	

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 at MPIKG, CERTH, ICSC, UFC and SINTEF.
- 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.