INSTANT

Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products

Project no. NMP4-SE-2012-280550

Final publishable summary report

CONTENT

Executive summary

Nanotechnology has been a hot topic in the science community due to the specific properties in nano-scale and has become an enabling technology for numerous applications. Especially engineered nanoparticles (ENPs) have shown various beneficial properties. In many fields of application, these ENPs have left the scientific laboratories and made their way to consumer products. Besides their advantages, ENPs are under discussion in the scientific community due to possible unforeseen hazards and an unknown disposition in living organisms and the environment. Accordingly, nanoparticles have drawn vast public attention due to their application in many consumer products. Therefore, the public demonstrates a rising interest in detecting, identifying, and quantifying ENPs. Thus, within the nanocluster projects, INSTANT (Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products) had the objective of a holistic approach to combine different and complementary techniques into a single analytical tool.

The newly developed INSTANT device had to be capable of transferring ENPs from literally any matrix into a defined one and detecting these ENPs from there afterwards. INSTANT developed a generalized extraction protocol for isolation and pre-concentration of different ENP species (e.g. silver, gold, TiO_2) from food and cosmetic samples. Next, INSTANT developed an innovative, cheap and robust sensor device to detect ENPs after the extraction and pre-concentration process. By using specific recognition structures, the resulting sensor system combined two complementary transduction principles, an optical and an electrochemical one. The electrochemical sensing principle was sensitive to ENP speciation, including conductivity, surface properties and chemical composition. The optical transduction principle provided information on ENP size and refractive index. The approach furthermore allows to adapt this system to a sensor array, which allows for simultaneous detection of various ENPs. In addition, this idea facilitates an increase of selectivity and parallelization from the present molecularly imprinted polymers and tailored carbon-nanotubes (tCNTs) to a wide range of recognition elements that are chemically modified in order to generate a material for enhancing sorption properties towards the targeted ENP species. The developed device requires adjustable process control and advanced chemometric methods.

Extraction, pre-concentration, both detection methods, and microfluidics are assembled in a rack, which is connected to a computer system where process software and chemometric evaluation are running. Extraction procedures, pre-concentration approaches, detection principles and software have proven the possibility to classify and measure ENPs such as gold, silver and titanium dioxide not only under lab conditions, but also in a round robin approach with real matrices in competition with other projects within the nanocluster such as Nanodetector and SMART-NANO.

Summary description of project context and objectives

INSTANT Consortium

Project reference: 280550
Funded under: FP7-NMP

Topic(s):NMP.2011.1.3-1 (FP7-NMP-2011-SME-5) - New methods for measuring, detection and identification of nanoparticles in products and/or in the environment

Funding scheme: CP-TP - Collaborative Project targeted to a special group (such as SMEs)

The cooperation started in March, 2012. The project INSTANT will face the challenge of the detection, identification and quantification of engineered nanoparticles (ENPs) in complex matrices such as cosmetic products and engineered food and drinks. Therefore, new detection methods and technologies are mandatory. This is completely in line with the Call FP7-NMP.2011.1.3-1, which deals especially with innovative, practically implementable and cost effective measurement approaches for ENPs in complex matrices.

Universities

- EBERHARD KARLS UNIVERSITAET TUEBINGEN (EKUT)
- UMEA UNIVERSITET (UMU)
- UNIVERSITAET WIEN (UNIVIE)
- UNIVERSIDAD DE CORDOBA (UCO)

Research Institutes

- BUNDESANSTALT FUER MATERIALFORSCHUNG UND -PRUEFUNG (BAM)

SMEs

- BIAMETRICS GMBH (BM)
- SOCIEDAD DE INVESTIGACION EN NANOESTRUCTURAS SL (SINATEC)
- CORPUS DATA MINING HANDELSBOLAG AB since May 2013 CORPUS DATA & IMAGE ANALYSIS AB (CD)
- SITEX 45 SRL (SITEX)
- NANORDIC OY (NANO)

These 10 partners from 10 institutions in 6 different countries formed a Europe-wide consortium (see Figure 1) to follow this challenging approach. The idea to include five different SMEs in the project turned out to be extremely valuable to define needs and intentions representing the present status in Europe and taking into account different social economic structures and a variety of different demands to such instrumentation.

SMEs together with universities can combine their intentions to develop new devices bringing together the needs of industry and academia. Thus, the INSTANT consortium provides a large variety of expertise in different areas of research and application, different countries and various socio-economic understanding, realizing present and future needs in nanoparticle analysis. Within the duration of the project, co-workers in nanotechnology, analytics, electrochemistry, optics, electronics, and data treatment have succeeded in developing a variety of new recognition elements (REs), setting-up assay procedures and fabricating an tailor-made device to researchers dealing with nanoparticle characterization or to the legislative organs of the EU.

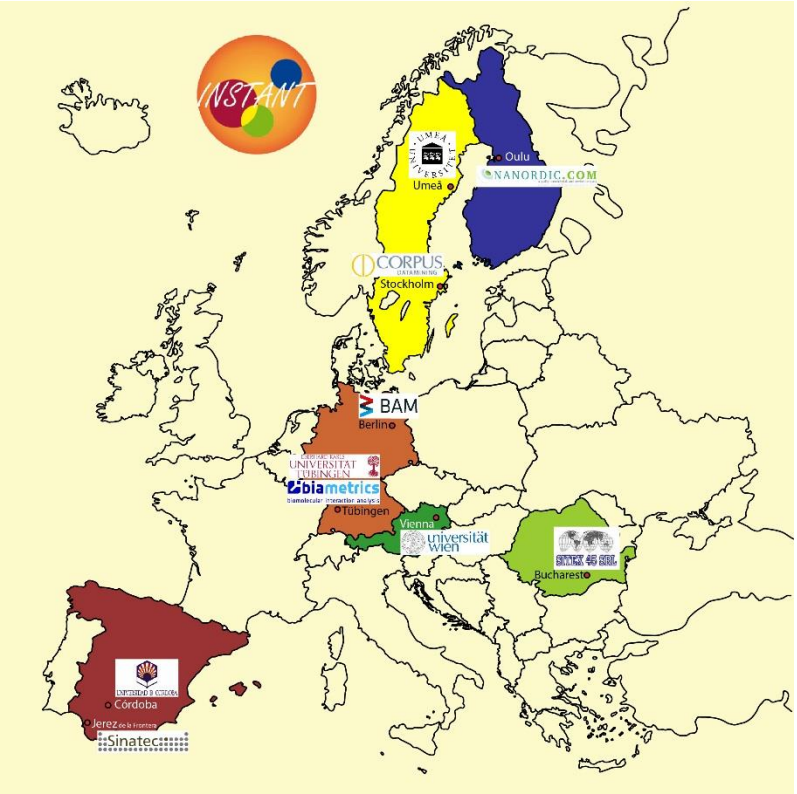


Figure 1: INSTANT Consortium allocated all over Europe

They can be delivered with such an instrumentation to help the decision-making process on nanoparticles within the EU. The purpose was to develop a simple and fully integrated (**SITEX**) sensing device (see Figure 2), combining sample preparation and microfluidics together with a suitable number of sensor elements for the detection and identification of ENPs in one device. From the beginning the consortium focused on the combination of two complementary label-free detection techniques (an optical [**EKUT, BM**] and an electrochemical [**UMU**] one) to create a robust sensor system providing a high information value. For detecting different properties of ENPs (size, size distribution, material, and composition) a multi spot array of different REs (**UNIVIE, NANO**) was intended.

Innovative recognition elements (REs) were implemented into electrochemical/optical transduction components using sieve effects of tailored Carbon Nanotubes (tCNTs) as well as affinity interaction properties of molecularly imprinted polymers (MIPs). Furthermore, the improvement of sampling and separation techniques (**UCO**) with regard to complex matrices like sunscreen or fruit juice using ionic liquids and sieve effects of tCNTs was a big topic within the project. To facilitate complex data handling, the development of advanced chemometric methods (**CD**) to extract information from the complex data sets received from the combined transduction readout was essential. Logically, also the need of standardized and well characterized ENPs was satisfied by BAM and the SME SINATEC to meet requirements for the permanent supply of such ENPs. For the development of a first stage analytical tool with an upstream sample preparation, four different metallic (Ag, Au) and metal oxidic (TiO2, ZnO) nanoparticles were chosen. These nanoparticles were proposed from the consortium.

The advisory board members agreed on the proposal and recommended further to exchange the focused samples with other projects that were also involved in nanoparticle characterization. This called the round robin into existence, whose aim was to test and compare the feasibility of the

different projects in validating their detection methods and materials. Different types of nanoparticles were analyzed, whilst the test included two rounds of sample measurements. Each project suggested one sample per round. The first round started in winter 2014 with basic and commercially available samples in a water matrix:

- Au (BBI Solutions, particle size: 50 nm, water)
- Ag (Sigma Aldrich, particle size: 60 nm, aqueous buffer)
- TiO_2 (Sigma Aldrich, particle size: <150 nm, water)

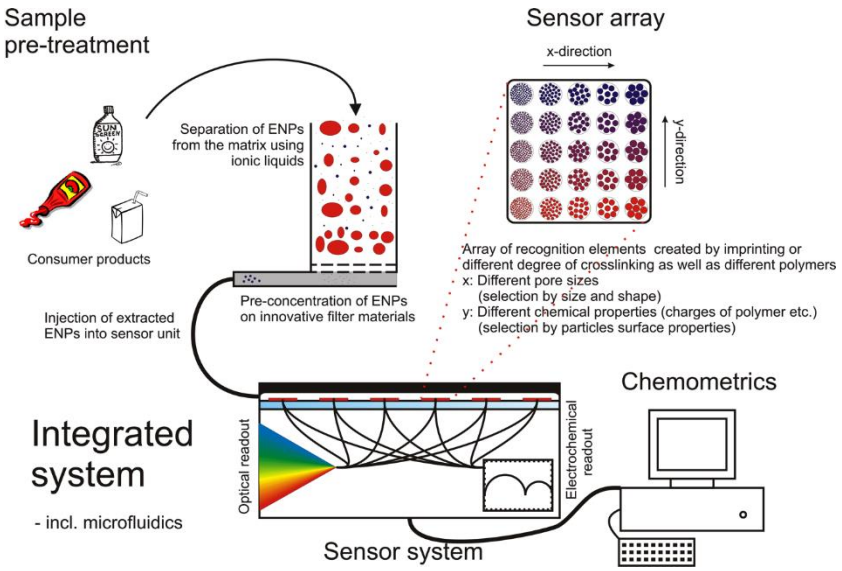


Figure 2: Overview of the integrated INSTANT device, containing sample preparation, measurement and data evaluation.

The second round consisted of the same three NP species but in complex matrices to simulate the detection of NPs in real samples, which is the final aim of all the three participating projects. It was suggested to use fruit juice with gold nanoparticles, wine with silver nanoparticles and sunscreen with TiO2 -nanoparticles. The particular results were presented at a joint public workshop of the three FP7 projects (INSTANT, SMART-NANO, NANODETECTOR) working with nanoparticle detection named “Nanoparticle analysis: present and future”. This workshop was held in the Vienna House in Brussels on the 17th of November 2015. Thereby, a final conclusion was inter alia that the detection and identification of ENPs in cosmetic products, food and/or drinks require an efficient sample preparation. This part of the INSTANT device was well developed by the University of Cordoba (UCO), evolving a generalized extraction protocol (same stabilizer and eluent for all types of chosen ENPs) in the project and the round robin. This helped the detection modules of the INSTANT project to focus only on one stabilizer matrix, which facilitates the preparation of transducers and their shielding to avoid unspecific binding signals. Accordingly, the consortium concentrated on testing the devices, optimizing the detection modules, and selecting the best conditions for appropriate applications. Thus, during the last phase of the project the validation of the INSTANT instrument was performed with real samples and compared with the results of the other projects of the NanoSafetyCluster.

Each module of the INSTANT device and its methods (sample preparation and detection) were combined and assembled to a final unique prototype, which showed valuable results at the end.Final

in-house tests have been performed in Bucharest and Tübingen. The results of in-lab experimental testing were in accordance to initial requirements. The suitability of the organization on the casing as well as the timing of all the steps during the analysis, which are controlled with a user-friendly software interface, demonstrated the usefulness of the device for determining nanoparticles in environmental samples, food/drinks and consumer products. The application of the device in a variety of ENP samples with simple and complex matrices like sunscreen or orange juice confirmed the usability of the proposed analytical method for screening NPs in commercial products. Future works could focus on a miniaturization of the sample preparation module and also the detection cell for simplification and for lowering the assembling costs.

Within the project, training sessions for the INSTANT partners have been performed. Especially, a workshop on chemometrics and data evaluation was held in Tuebingen for a better planning of experiments and valuable data extraction. On top of these internal activities, the consortium started to present and disseminate the results in different exhibitions such as the 'Sensor+Test' in Nuremberg 2015, the 'Anakon' in Graz 2015, the Analytica in Munich 2014, the EuroNanoForum 2013 in Dublin and in many talks at several conferences such as the 'SPIE Conference' in Prague, the 'Sensor Symposium' in Dresden or many other national and international meetings. After an accomplished assembly and successful tests at the end, a further public workshop was organized.

Beyond these activities the list of accepted publications in conference proceedings and peer-reviewed journals was getting longer and longer. The project has been acknowledged, and the project logo was used in presentations and dissemination activities. As a consequence, discussions started with potential users and practitioners, who could be interested in acquiring an INSTANT device. In addition, the coordinator looked for upcoming topics in real life applications where certainly the monitoring of nanoparticles in consumer products and the environment have been told to be of high interest.

The project and the management were coordinated at the University of Tübingen. The INSTANT web site at http://www.instant-nps.eu/ provides both public and internal access-controlled information. This homepage comprises objectives, concept, news and results of the INSTANT project. The project in progress was supervised by an Advisory Board with members from industry and institutions (Prof. Lampen [BfR], Dr. Wohlleben [BASF] and Prof. Lang Tran [IOM]).

The 4-year-project (extended by 3 months) has a total budget of nearly 5 million Euro with approximate 4 Million Euro EU-Contribution. The project will generate lasting benefit for European Food and Consumer Safety in general, as the device will be tailored to be used as a cost-effective monitoring tool. It allows for analytics of food and cosmetics close to the point of need (Point-of-Product Testing POPT and Point-of-Food Testing POFT).

As nanoparticles are used even more frequently in consumer products and the issue of consumer safety becomes further reaching, INSTANT will contribute to meet this major societal challenge. The merger of partners from academia, industry, and institutions in the INSTANT project to become an interdisciplinary coordinated alliance was essential for further research in nanotechnology, nanoscience and information technology and therefore beneficial for Europe.

Thus, INSTANT was a step towards the visionary intention of characterization and identification of NPs in consumer products.

Description of main S&T results/foregrounds

Main Achievements

The aim of INSTANT was to develop a device for the detection, identification and quantification of engineered NPs in everyday products, such as cosmetics and food. These two groups of products represent very complex matrices and can be seen as model systems to demonstrate the performance of the innovative ENP detecting device.

In order to obtain trustworthy outcomes, to set up standard procedures and standard conditions, and to detect ENPs within these products and matrices, it is necessary to extract the ENPs in a first step. Therefore, ionic liquids (ILs) were applied to solve the ENPs and to remove matrix compounds like stabilizer or proteins from the ENP surfaces.

Following novel filter membranes based on tCNTs were used for the retention of the ENPs in the IL. Several liquids like Methanol or Chloroform elute the ENPs, according to their chemical nature. A new stabilizer for all ENP types is added during the elution step. Subsequently, the microfluidic system carries the eluted ENPs to the combined detection unit consisting of a two-dimensional sensor array based on the described REs, including a highly innovative sensitive layer completed by an optical and electrochemical detection system. On the one hand, the optical detection provides information on size and refractive index of the ENPs. On the other hand, the electrochemical transduction complements information on chemical nature and shape. The combination of these two complementary transduction principles in one sensor device is the basis for a rapid and selective detection of ENPs in targeted matrices. Due to the complexness of the sensor output, powerful chemometric techniques are needed. These techniques can separate noise and signal and will allow for an integrated multivariate analysis of combined signals from optical and electrochemical sensors, distinguishing between different ENP specifications, since neither MIP nor tCNTs can show 100 % specifity. INSTANT has integrated these different tasks for the detection of ENPs into one user-friendly device. Therefore, all device units were built in a housing, including sample preparation, detection and electronical supply units. The work package structure reflects the highly interlinked aspects of the multidisciplinary approach within the project. WP 1 developed the sensor unit by combining electrochemical and optical transduction principles with innovative recognition elements, which were provided by WP 3. WP 2 prepared reference ENPs, prepared spiked samples with reference ENPs and developed a general protocol for the extraction of these ENPs from complex matrices, applying ILs and CNTs as innovative filter materials provided by WP3. After establishing a generalized extraction protocol, the extracted ENPs will be used to optimize and calibrate the combined sensors for the characterization of ENP specifications. Reference ENPs provided by WP 2 were used in WP 3 for the design of innovative recognition elements (REs), based on molecular imprinted polymers (MIPs) and different functionalized tCNTs. These REs are mandatory for the selectivity of the sensor and the detection of the ENPs. WP 4 dealt with data mining for extracting minute signals from the complex and probably noisy measurement data obtained by the sensor system(s) and contributes feedback to all other WPs on experimental design. For data evaluation, different chemometric techniques were evaluated and applied in order to get the maximum signal from the sensor output. Based on the requirements of the different components developed in WP 1-4, WP 5 has set up a fully integrated device, which combines all components and can be operated by a user-friendly software interface. WP 6 guaranteed a well-coordinated management of the overall activities and monitored the workflow within the different WPs. WP 7 took care of efficient dissemination and commercial exploitation of the obtained results from all other INSTANT work packages to follow the idea of FP7 and intentions of the SMEs

Work package 1 – Sensor Development

Highlights:

- Combination of two label-free complementary detection methods
- Advanced fluidics allowing for flow-through measurements without stopped-flow periods.
- Modular and easy to use flow-cell. Easily adaptable towards different requirement (flow path, dead volume, channel geometry, …)
- (to our knowledge) first use of CNTs as sensing material using 1l-RIfS
- Smart combination of detection and sample preparation

Potential Impact:

The potential impact was and is strongly depending on the actions of the EU because the market is related with EU regulations.
The INSTANT project is a joint venture of two groups of partners: universities and SMEs. As the main task of the universities might be generation of knowledge, the driving force for SMEs to participate in such projects is to generate products, IP, and technologies, enabling them to earn money out of this. In the case of INSTANT, the open question is, if new technologies for a cheaper and faster analytical instrumentation to detect nanoparticles are needed a) to safe money during production; b) to fulfill EU regulations (frequent monitoring of nanoparticles in products like food and cosmetics). These regulations are made either by national instances, or the EU.
In short: changed regulations, which would make technologies like the ones developed in INSTANT mandatory would open a huge market and further promote the development and continuation of the consortium (in whichever way). Without this, the developments will be more or less futile.
Biametrics will at least exploit the own developments in the area of transduction, liquid handling and flow-cell design in commercial products for other applications. Up to now, this led to one new permanent position at Biametrics.

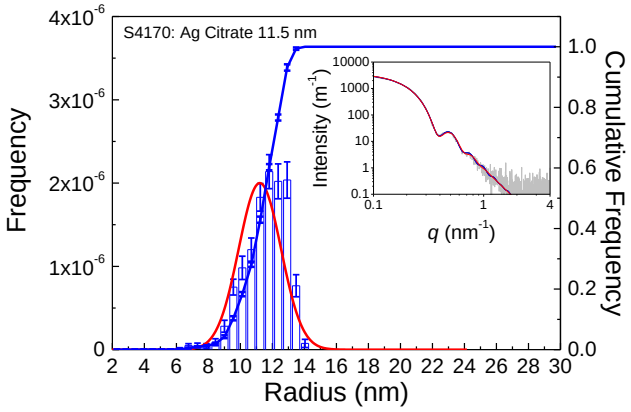
Work package 2 – Sample Preparation and standard materials

Main S&T results/foregrounds

In this workpackage, two main purposes were intended. On the one hand, WP2 synthesizes and characterizes standard ENPs, which will be provided to WP 1 and WP 3. The decision was taken for metallic NPs on one side(Ag-NP and Au-NP) and oxidic nanoparticles on the other side(TiO2-NP, ZnO-NP) as good representatives for ENPs used as food colorants or as additive in food packaging or crèmes in commercial products. The preparation of samples containing well-established amounts of ENPs was also a key aspect to be performed within the project. For this purpose, three different types of spiked samples were chosen (liquid samples, semi-liquid samples, and solid samples) depending on the nature matrix. As test liquid samples, orange juice spiked with Ag Nanoparticles were selected. Meat containing Au nanoparticles was used as a reference material for solid samples, whereby two different ways to spiked the meat samples were tested. TiO2, ZnO, and TiO2+ZnO in moisturizing creams were prepared as spiked samples for semi-liquid samples. These spiked samples were evaluated and measured using different techniques such as capillary electrophoresis,

ICP-MASS, TEM, UV-vis and fluorescence spectroscopy. On the other hand, WP 2 had to develop a generalized and efficient extraction protocol to isolate and pre-concentrate ENPs from food and cosmetic samples. The goal was to achieve a procedure as general as possible, to extend the applicability of the measuring device to a wide range of samples. This generalized extraction protocols were defined to isolate, pre-concentrate and elute the NPs selected in the project. To achieve this goal for all selected types of ENPs a combination of ionic liquids and novel filter membranes (modified with carbon nanotubes) was chosen. The sample preparation process was completed an new defined elution agent which acts as stabilizer to assure that there is no change of chemical and physical properties of eluted ENPs after the sample treatment and taking into account the requirements of the detector system. This was proven by standard analytic measurements showing that there is no significant change in size distribution of NPs after sample preparation process (see Deliverable D2.7). Another prove for the successful elution of NPs are the measurements with the combined readout showing the interaction of NPs with the recognition elements.
A summary of the activities performed in WP2 are listed below:

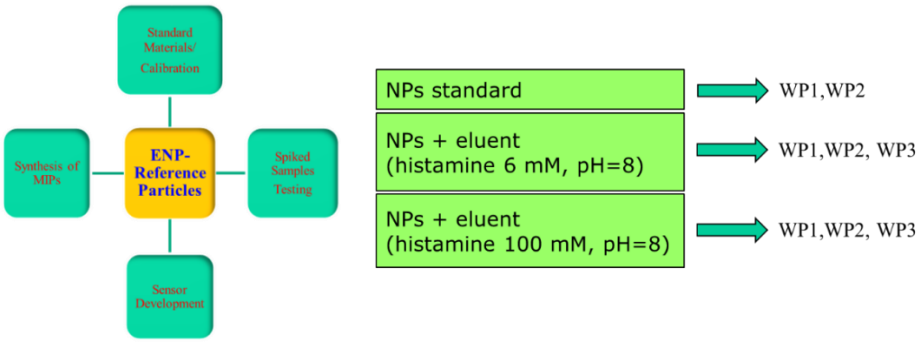
- **SINATEC** and **BAM** synthesized and characterized nanoparticles:
  - TiO2, ZnO and SiO_2 nanoparticles were synthesized at different particle sizes by **SINATEC**. Synthesis conditions (above all pH) were optimized in order to control the particle size of the NPs in the synthesis. TEM was used to verify particle sizes. With this information, the conditions of the synthesis were changed in order to obtain the desirable particle size. For fractioning of NPs, density gradient centrifugation was developed. The characterization of NPs was carried out using TEM, SEM, FITR and XRD
  - Ag and Au nanoparticles were prepared by **BAM** using different coatings (PVP, citrate). Latest SAXS data evaluation procedures were applied for determination of number-weighted and volume-weighted size distributions. DLS was also used for characterization of NPs



- **SINATEC** and **BAM** manufactured standard materials from ENPs synthesized :
  - Different standard materials of NPs at different concentrations and different matrices were prepared and delivered to other partners.

NANOPARTICLE TYPE	AVERAGE DIAMETER (nm)	MATRIX
TiO_2	15-30	SDS
TiO_2	15-30	Histamine
TiO_2	45-55	SDS
TiO_2	45-55	Histamine
ZnO	10-30	SDS
ZnO	10-30	Histamine
ZnO	35-45	SDS
ZnO	35-45	Histamine

- The standard materials prepared were used internally for other tasks in WP2 in order to optimize sample preparation system and additionally were delivered to other WPs



- **UCO** and **SINATEC** have established different spiking procedures for preparing spiked samples, depending on the nature matrix (solid, semi-liquid and liquid samples) selecting representative matrices in the industries. In the case of Liquid samples, orange juice, in the case of solid samples, meat and for the semi-liquid samples was decided to prepare samples of moisturizers. For each one of these sample, the spiked samples were prepared using procedures adapted to the nature of the matrix. All types of samples were prepared at different ranges of concentrations and were evaluated by measuring the samples by ICP-MS, TEM, UV-vis and fluorescence

Table 1: Used nanoparticle, their concentration and stability

Code	Nanoparticle	Concentration	Stability
A1	TiO2	1000 ppm	5 days
A2	ZnO	1000 ppm	5 days
A3	TiO2+ZnO	1000/1000 ppm	3 days
A4	TiO2	100 ppm	10 days
A5	ZnO	100 ppm	10 days
A6	TiO2+ZnO	100/100 ppm	7 days
A7	TiO2	10 ppm	2 month
A8	ZnO	10 ppm	2 month
A9	TiO2+ZnO	10/10 ppm	45 days

- UCO had performed the characterization of ENPs using different techniques (capillary electrophoresis, ICP-MASS, TEM, UV-vis and fluorescence spectroscopy), providing a chemical, morphological and size characterization in close collaboration with SINATEC and BAM.

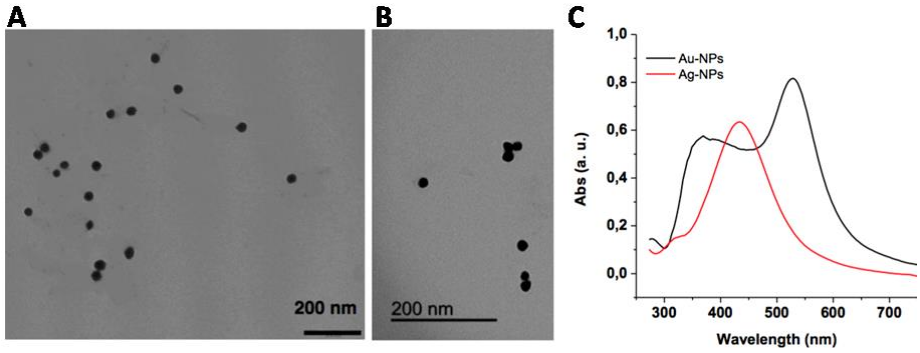


Figure 3: TEM images of citrate-coated AuNPs (35 nm) (A) and AgNPs (20 nm) (B) and their UV-visible spectra. (C).

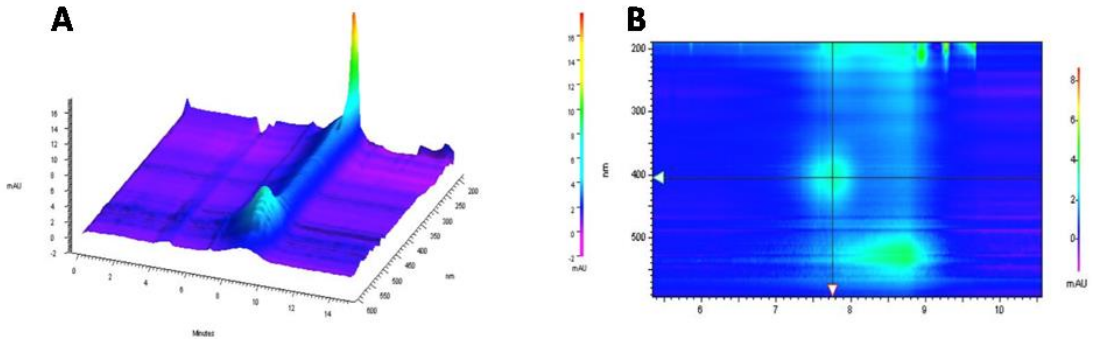


Figure 4: 3D electropherogram image of AuNPs (35 nm) (A) and the contour electropherogram image of samples containing both AuNPs and AgNPs (B) after extraction and pre-concentration.

- UCO had developed two methodologies to determine ENPs in complex matrices, being the possibility of only isolate the ENPs from the matrix (which involved the isolation and pre-concentration of ENPs from the matrix) or by destroying the matrix (which involved acid digestion).

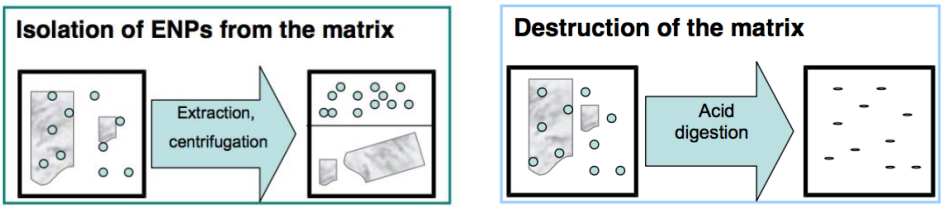


Figure 5: Different methodologies for the determination of ENPs

- UCO had compared the affinity of different membranes (of different materials and pore-sizes) and sorbents to retain ENPs of different sizes and coatings.

Table 2: Retention capability of metallic nanoparticles in different filter membranes.

	Au NPs	Ag NPs
POLYCARBONATE 5 µm	NO	NO
POLYCARBONATE 0,2 µm	YES (T)[a]	NO
PVDF 5 µm	NO	NO
PVDF 0,45 µm	YES (P+)	YES (P)
CELLULOSE 5 µm	NO	NO
CELLULOSE ACETATE 0,2 µm	YES (P+)	NO
POLYAMIDE 0,2 µm	YES (T)	YES (P)
NYLON 5 µm	NO	NO
NYLON 0,45 µm	YES (P)	YES (P)
NYLON 0,22 µm	YES (P+)	YES (P+)
POLYESTER 0,2 µm	NO	NO

T: total retention; P+: Partial retention (≥80%); P: poor retention (<80%); [a]: agglomeration onto the membrane.

- UCO prepared filter membranes with CNTs, supplied by NANORDIC, in close collaboration of NANORDIC.

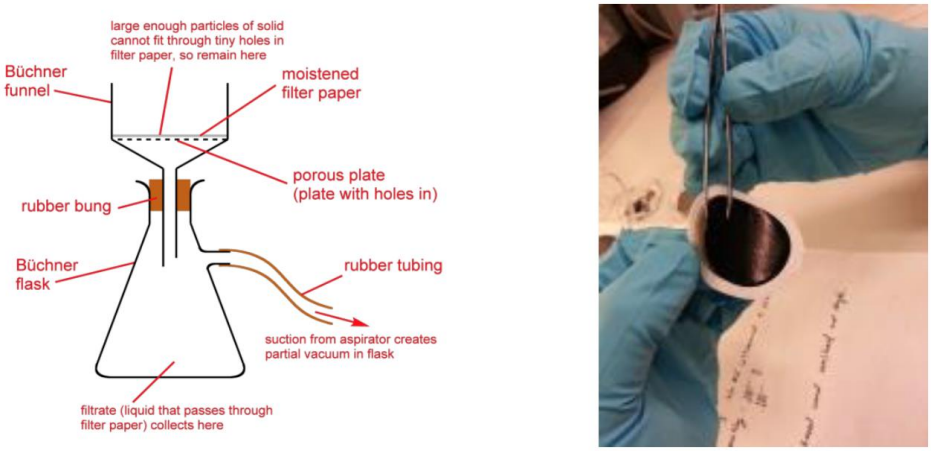


Figure 6: Buchner flask for the filtering of CNTs onto a membrane (on the left) and the final CNT membrane (on the right).

- UCO has investigated the tendency of different ionic liquids (IL) to extract ENPs in close collaboration with SINATEC. Thus, the pre-concentration of ENPs by selecting HMIM-PF_6 as ionic liquid was successfully achieved in the first period, but after the decatalogation of HMIM-PF_6 in the third period of the project, UCO have investigated the selected IL of other suppliers finding many impurities that hamper the ENP extraction. For instance, the selected IL from Merck was characterized by its colourless, whereas the same IL from Sigma Aldrich exhibits a brown colour as a result of the impurities.

  Stability and pre-concentration studies were performed with some different ILs, finding the best results with BMIM-PF_6. All the experiments using different ENPs and different matrices were performed using BMIM-PF_6 with success.

  1. Furthermore, the selected IL extraction capability was evaluated for different ENPs finding similar result to those obtained with the decatalogued HMIM-PF_6.

2. Investigations of the capability of such novel membrane-ionic liquid system towards ENPs was also carried out. The absence of CNTs (from the CNTs-membranes) in the eluted solutions was confirmed using Raman spectroscopy.

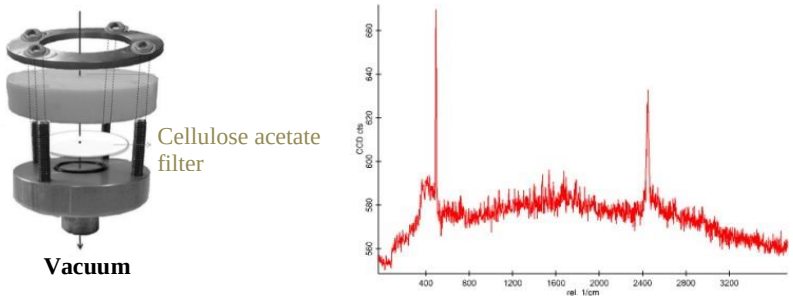


Figure 7: Filter membrane used (on the left) and Raman spectrum obtained (on the right).

- The elution conditions of ENPs from filter were established and optimized accordingly to the requirements of the detection unit. Results showed the stability of the eluted NPs in terms of size and shape.

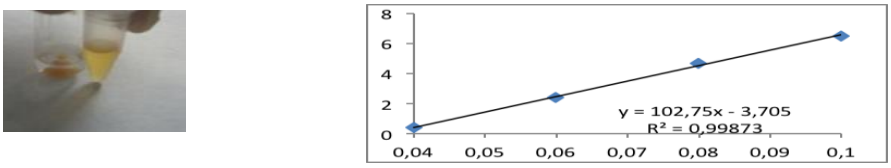


Figure 8: Image of the extracted and eluted AgNPs (on the left) and calibration curve of the eluted AgNPs using capillary electrophoresis (on the right).

Work package 3 – Recognition elements

Main S&T results/foregrounds

Starting point

Within the framework of INSTANT, WP3 has been the core work packaged to deliver selectivity. This is a key point of any sensor development, but even more so in INSTANT: the two transducer principles used in the project have broadband sensitivity. WP3 proposed two different approaches for achieving selectivity, namely:

- (Functionalized) Carbon Nanotubes, and
- Molecularly Imprinted Polymers.

By the end of the project period, both approaches proved successful. One of them was carried forward into the devices used for round robin tests.

Main outcomes

Carbon nanotubes

INSTANT partner NANORDIC developed a range of functionalized carbon nanotube (CNT) inks suitable for surface immobilization on the respective transducer devices. Those tubes contain different functionalities on their respective surfaces, mainly -NH2 and –COOH, respectively. Such nanostructures on the one hand have diameters in the range of the analyte nanoparticles tested – namely several to several ten nm – and thus provide binding cavities. Furthermore, their electronic properties facilitate particle binding.

The CNT approach proved highly successful (see also Figure 9 showing some typical characterization experiments): the inks could indeed be utilized to functionalize both optical and electrochemical INSTANT transducers. Furthermore, they showed strong binding of target nanoparticles, especially so for Ag and Au. In both optical and electrochemical measurements, INSTANT could demonstrate concentration-dependent sensor responses. Binding proved appreciably strong in this case leading to sensitivities in the low ppm range. Therefore, these matrices were also carried forward into round robin testing of inter-project samples.

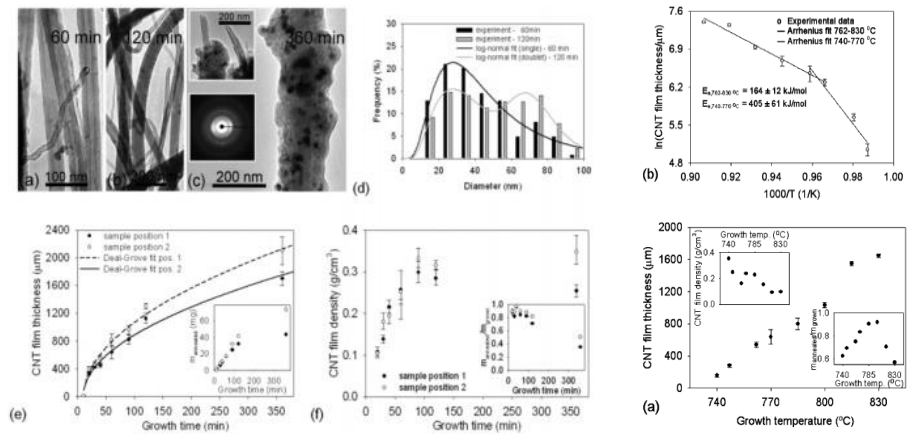


Figure 9: Examples of Characterization measurements for CNT receptors.

Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIP) were the second area of receptor research in INSTANT. The strategy behind this approach is to generate cavities in polymers via a template-directed approach. Those cavities are optimized to fit the target analyte both in terms of their shape and functionality. Before INSTANT, no approach had been published for using nanoparticles as templates during imprinting. Partner UNIVIE developed three different imprinting strategies, namely one-step, two-step and sedimentation imprinting respectively (see Figure 10).

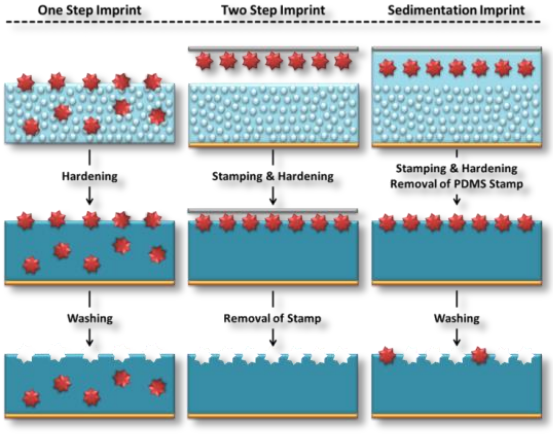


Figure 10: Imprinting strategies developed during INSTANT.

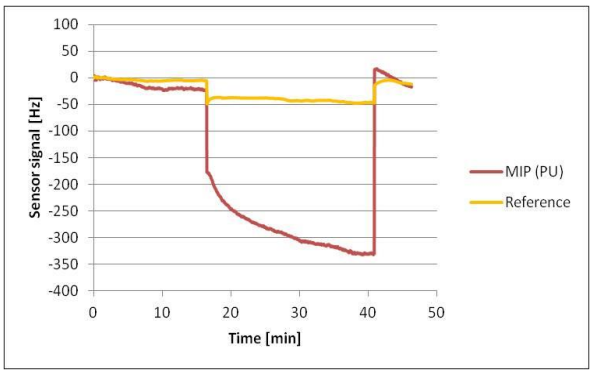


Figure 11: Example of MIP-sensor response to NP solution

All three approaches lead to appreciable sensor responses toward the INSTANT analyte NP. Generally speaking the stamping approach proved best compatible to the transducers developed by partners EKUT and UMU, so that approach was carried forward. By the end of the project the layers were successfully integrated into the devices. During screening, very appreciable sensitivity and selectivity could be achieved as can be seen from the example response in Figure 11.

However, unforeseeable technical challenges made integration of MIP into the INSTANT devices much more laborious than initially planned thus preventing their use during round robin testing.

Overall conclusions

Overall, WP3 achieved its main goals. It:

- Developed CNT-based selective receptors and proved their suitability for NP detection,
- Integrated CNT-based selectivity into the devices and demonstrated NP analysis on real-life samples,
- Designed completely novel MIP receptors for nanoparticles thus resulting in groundbreaking novelty and innovation,
- Obtained sensor characteristics on said MIP layers showing selectivity based on size, dimensions and chemistry of those nanoparticles, and
- Demonstrated that those layers can in principle be integrated into other sensor platforms.

Impact of the results

At the starting point of instant, no selective receptors for the detection of nanoparticles at all were available. Hence, INSTANT opened two possible doors for further development of measuring apparatus for this class of analytes. Analyzing both natural and artificial nanoparticles is one of the main challenges that analytical sciences face in the near future, because science just begins to unveil the ecological and toxicological properties of nanoparticles. As such, receptor development substantially – but of course not exclusively – contributed to this are that is of potential concern for literally everybody.

Work package 4 – Chemometrics and Experimental Design

Main S&T results/foregrounds

Main Outcome of chemometrics and experimental design workpackage by CD

- Use of experimental design used in many other WPs which improved their experiments
- Collaboration with mainly WP2 for testing multivariate data analysis and data filtering
- Electrochemical data analysis made an impact on working with electrochemistry data for UmU (used in a number of publications and master theses)
- Useful working methods transferred to WP5 for programming the prototype
- A better understanding of LabVIEW for a number of partners

Summary written by EKUT

WP 4 deals with data mining for extracting signals from the complex measurement data obtained by the INSTANT sensor systems. For this purpose, the WP will first provide support and educate the other WPs in experimental design in order to obtain optimal information with minimal expense in time and work force. Thus, the organization of a centralized course was part of this WP and carried out in Tübingen.

Figure 12: Workshop on "Chemometrics and Data evaluation" held in Tuebingen, 2012.

In cooperation with other WPs, mainly WP 1, strategies for multivariate data analysis of provided data were tested and optimized. The exploration, classification and calibration of data was thereby a main task. A number of experimental designs were discussed and supplied to the other WPs. Data cleaning and preprocessing was tested on the results gained with MIPs and CNT transducer from WP1. Besides, some calibration methods were suggested and tested. Accordingly, the data of round robin measurements was evaluated (Figure 13). The acquired information was transferred to WP 5 for the development of a prototype software for automated data extraction and analysis.

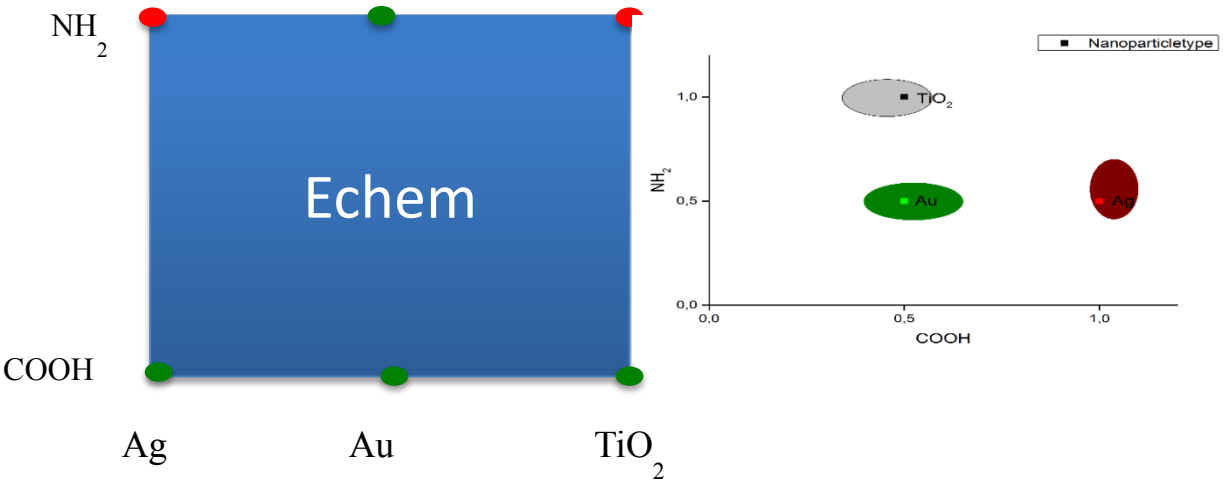


Figure 13: Electrochemical (left) and optical (right) round robin data after chemometrical analysis of complex number vectors and optical time profiles

Work package 5 – System integration

Main S&T results/foregrounds

The aim of WP 5 was to setup a fully integrated analytical tool based on the different components provided by the other workpackages. Therefore, an integration concept for all device units (sample preparation, array based sensor system, chemometric data evaluation) was developed. This included the design of interfaces, the supply and display units and the housing. A CAD-based design and the manufacturing of integrated system hardware including all necessary modules was achieved. Furthermore, standardization and integration of electronics and OEM components could be implemented. The validation of the full setup containing e.g. electric powering, assembling and cooling was carried out. A modular system concept allows the adaption of the system to possible changing needs. The integrated INSTANT device passed all in-house testing procedures related to reliability, robustness and friendly operation by a user generated by the concept design of the housing. This device can be controlled via a user-friendly software interface comprising device operation, data analysis and Graphical User Interfaces (GUI), which allows the user comfortable measurements with the established first stage analytical tool. Based on this previous developments and results including in-lab experiments, calculations and in-house tests, a final device assembly was drafted, constructed, and delivered to the partners for the final testing. This INSTANT device will be capable for the detection of ENPs in complex matrices such as food, drinks and cosmetics. For this reason the round robin measurements, where three consortia had to measure the same samples (three basic ENP samples and three complex samples), were also part of this workpackage. The measurements were carried out together with the assistance of other workpackages and presented additionally on a joint workshop with public attendance, which will be discussed later on in this deliverable. Finally

can be summarized that the demonstrator setup of a full analysis system for NPs in food and cosmetics including each step from sampling to data evaluation could be manufactured (

Figure 14). This concept is in principle transferable to other applications and hence has inherent potential for commercial manufacturing. The testing of the device including GUI application can be seen in a movie, which was presented in Brussels and is available in the internal area of the INSTANT website.

A summary of the activities performed in WP5 are listed below:

- The planned work and tasks of the DOW for WP5 focused on housing concepts, fluidic and microfluidic tests, planning of electrochemical and optical devices and the integration of components as well as the assembly of the devices with an optimized concept for the best workability and friendly operation conditions.
- Design, development and manufacturing of an intensity stabilizer module for the Power LED supply for an image background stability.
- The development of a sample preparation subunit for minimum sample treatment and sample manipulation to achieve good pre-concentration and posterior elution of ENPs for consequent analysis of the detector sub-units.

Figure 14: INSTANT assembly fully integrated with all fluidics components connected electronics and tubings.

- SITEX designed, assembled and delivered a final version of two full integrated devices with hw/sw capabilities of mobile analytical instrumentation approaches for different operation conditions like on-site and in-lab operation.
- The design and manufacturing of several electronic modules for optical imaging system with a programming application for the monitoring of microcontrollers and temperature sensors using overheating protection was achieved.
- The fabrication of integrated demonstrator of fully integrated Instant device, based on the valuable inputs from WP partners has been successfully finished.
- The manufacturing, in-house testing and validation for two complete devices (prototype of Instant and full integrated system) was conducted
- In-house design, development and manufacturing of five different versions of masks and transducers with interdigitated electrode patterns on glass/ITO substrates has been performed
- Design and implementation of up-graded solutions of transducers by SiO_2 layer deposition and related micro-technological processing with masks design and manufacturing has been carried out
- Thickness uniformity of ITO layers with high accuracy measurement was achieved

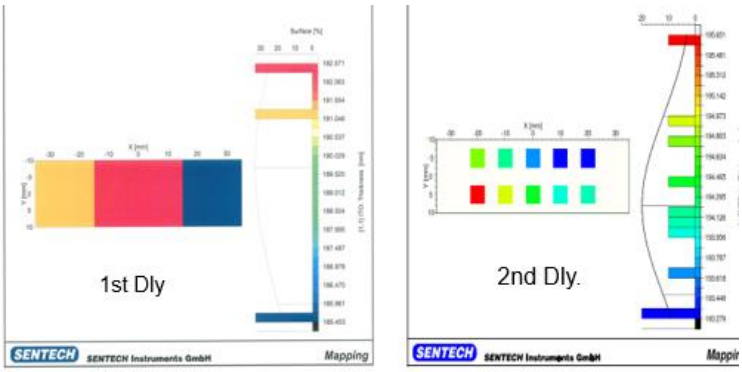


Figure 15: ITO thickness uniformity measurment diagram.

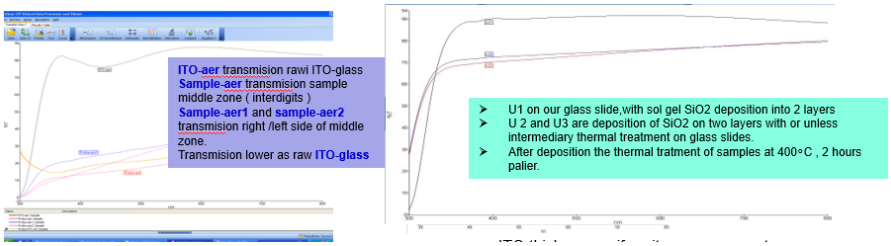


Figure 16: Optical parameters measurement.

- Full technical documentation leading to an operation manual for end-user was approached by SITEX
- Integration concept for all device units (sample preparation, optics and electrochemical sensors array based readout combined system, chemometric data evaluation) was set up
- Implementation of two USB oscilloscopes in each INSTANT device for simultaneous measurement of the impedance between the interdigitated electrodes and two sensor spots was carried out
- The integration and performing of detection modules, sample preparation module, recognition elements and transducers combined into one demonstrator after their definition and availability.
- The fabrication of integrated modules for all main units of the INSTANT device (optics and electrochemical sensors array based readout combined system, sample preparation, chemometric data evaluation and all internal parts) was performed.
- Design and manufacturing of communications and the development of all interfaces and units for the final version of the housing integration.
- Development, CAD design and manufacturing of most important specific parts with original concept of tailored properties like the transducer support of nonmagnetic materials, camera parts settlement and anti-vibration protection, power LED heatsink by OEM mechanical coupling parts, support trays concept, chassis enclosure partitioning by simulation of internal parts and modules optimized placement.
- The testing and upgrading of the components after in-house testing by an OEM software was conducted. The different parts like: pumps, valves, camera, power LED, current stabilizer module, and many more need different voltage levels. Therefore power supply modules for different voltages level were manufactured.

- The fabrication of the integrated demonstrator as fully integrated Instant device, based on the valuable inputs from WP partners and software as well as validation, testing and all final upgrading was successfully finished

Microfluidic Sequence

- With the fluidic schemes of BM, EKUT and UCO, the fluidics assembly in the INSTANT casing could be finalized. By choosing correct tubings with the ideal diameters (which was evaluated in the first stage analytical tool), the necessary connections between all the pumps and valves could be optimized, with the consequent coupling it to flow cell and transducer with respect to small dead volumes
- The INSTANT fluidics is split into two parts, consisting of two different fluidic circuits that are interconnected with a sample vial to collect the extracted sample after sample preparation. First part (the sample preparation), is responsible for extracting nanoparticles from different matrices; second part is responsible to inject the extracted sample from the sample vial to the flow cell for the detection of optical and electrochemical properties
- Fluidics assembly for sample preparation was realized in close cooperation between partner EKUT, BM and partner UCO having been present at the University of Tübingen to participate in experimental modifications and optimization at place. In Figure 17 the integrated INSTANT system can be found with all fluidic components shown, with a special focus on the sample preparation on the right. It can be seen devices in the final arrangement, all tubing interconnections, vessels with different solvents, and the sample injection vessel
- Detection units are placed in the middle of the casing to protect the very sensitive optics and the electrochemical assembly from vibrations generated by the moving pumps and valves. Sample is pumped with high precision using the concept of positive displacement that guarantees a reproducible sample movement over the transducer to gain optimal optical and electrochemical results (Figure 17)

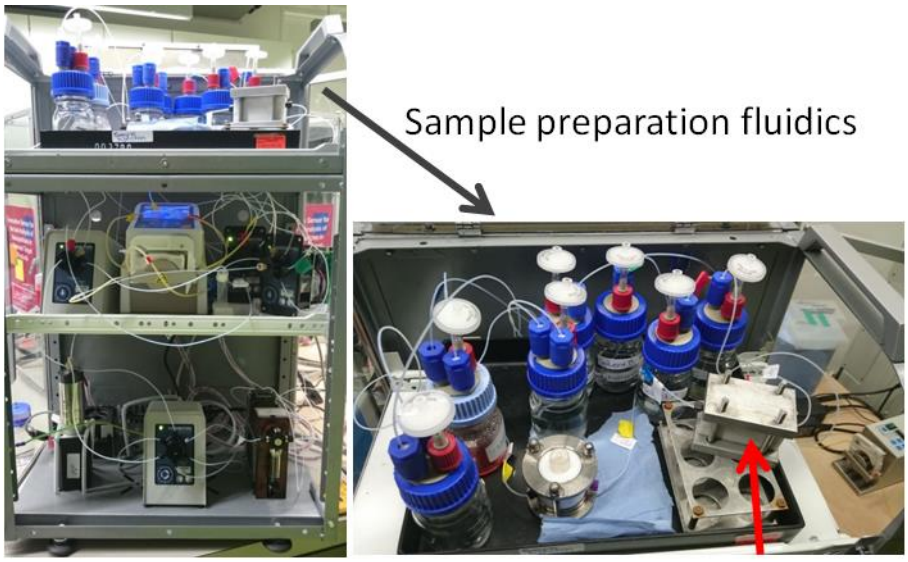


Figure 17: The fully integrated INSTANT assembly with all fluidics components, connected to electronics and tubings (Left). Sample preparation can be found on the upper part with all solutions found on top (Right). Hereunder, also filter and mixing chamber (red arrow) for sample

preparation can be found. On the middle part (Left) all pumps and valves necessary for sample preparation are placed. Finally, on the lowest level (Left) all pumps and valves needed for sample injection are organized. All tubings are optimized in regard to short distances between the single devices to keep the death volume as low as possible.

- Development of a graphical user interface and communication with the multitude of devices such as pumps, valves, camera, and two oscilloscopes
- A data evaluation module was developed by CD that is responsible to evaluate result data written by camera and oscilloscopes. It contains the multivariate model on the measured RIfS and impedance data
- Software integration for INSTANT device, controlling the INSTANT fluidics and definition of process sequence with optical readout and data storage was performed and integrated by EKUT. The INSTANT software consists of three parts developed as follows:
    1. A hardware communication module used to control all hardware components used. This executable existed already at EKUT before, and it was used to configure on a flexible way the communication with the INSTANT devices
    2. A plugin container as user interface for allowing the user to communicate with the hardware communication module. This executable also existed already at EKUT before, and it was used to allow the user to edit the measurement sequence and observe the measurement state
    3. A plugin library that can be loaded with the above mentioned plugin container to allow controlling all the components of the INSTANT assembly. This library also contains the INSTANT measurement sequence. The plugin library was especially designed and implemented for INSTANT with the following plugins contained:
        - Optical detection plugin to control the camera and save camera data
        - Measurement sequences to control INSTANT fluidics
        - Plugin for illustrating the INSTANT assembly and observe (see Figure 18) measurement state; also used to administrate INSTANT measurement
        - Impedance control plugin to control the two oscilloscopes for simultaneous impedance measurements and saving oscilloscope data (developed by CD)

Software implementation

- Two software packages (see above) were used to implement process software consisting of a highly intelligible graphical user interface (GUI, see Figure 18), different control sequences and an individual hardware control. The GUI allows both to monitor the current state of the measurement and to take control of the individual devices.

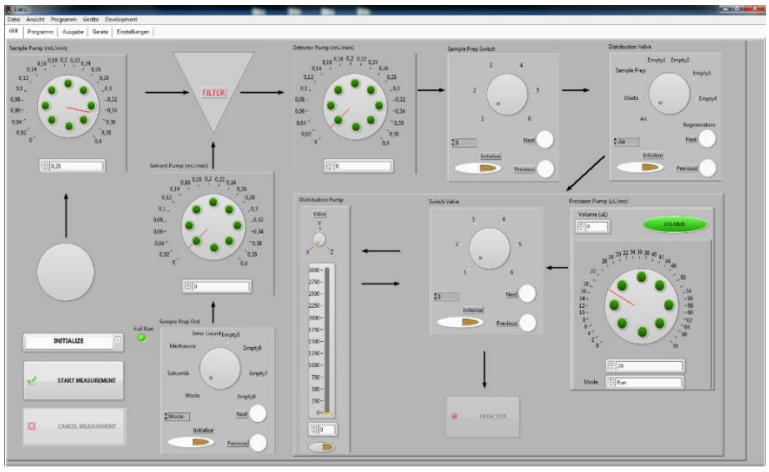


Figure 18: Graphical User Interface (GUI) to monitor measurement sequence of the INSTANT device.

- The GUI can furthermore control each of the existing devices in the assembly individually (plugin based). For each type of device an own graphical representation was chosen so to create an intelligible GUI to represent the INSTANT device. Furthermore, the dependencies and flow directions between the single devices are reflected by the arrows in between. Each graphical representation is able to switch between inactive and active to show the user the current state of work in the currently running measurement sequence. The two main cycles, sample preparation and sample detection, are also discriminated (left and top right: sample prep, bottom right: sample detection).
- To discriminate between control and output, two different GUIs were generated, one responsible for controlling and observing the fluidic system, the other one for observing data sent by the different detection units. Due to the fact that the fluidic system consists of different devices with different functionalities, different graphical representations were chosen for the individual device units. Also, the two different main cycles of an INSTANT measurement, sample preparation and sample detection, were graphically separated in the GUI (see Figure 18).
- The sample detection graphical user interface consists also of two parts, one responsible for loading optical images, the second one for impedance data (Figure 19).

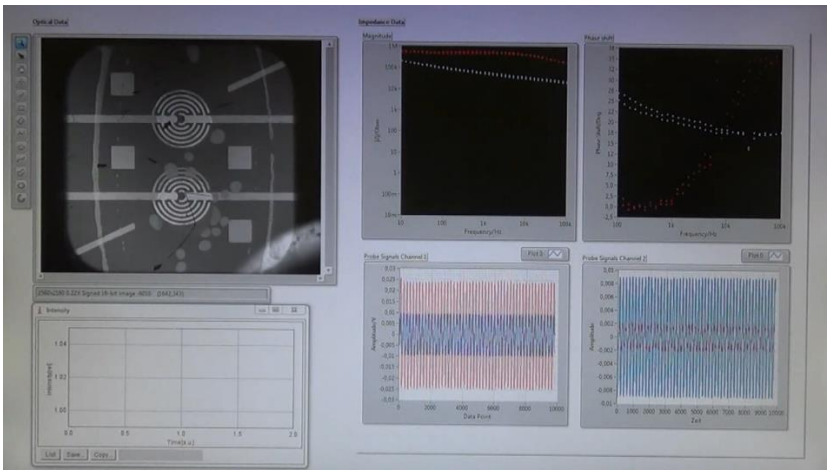


Figure 19: Graphical user interface for sample detection, consisting of an area to monitor camera images (left) including several tools for live imaging operations (e.g. zooming, cutting ROIs, etc.) and an area for observing impedance data from oscilloscopes (right). All the data are recorded continuously and saved on the local hard disk, and sequent read from the chemometric tools that extract the information needed to calculate concentration and species of nanoparticles.

- Final testing and benchmarking of the INSTANT device with the contributions of partners majority like UCO, BAM, SITEX, BM, EKUT, UmU, SINATEC, CD. The focus of this task has been shifted and extended towards the round robin in which spiked food and cosmetic samples will be tested, as well as the final performance testing of device by possible using of different samples including from other EU projects and real world. The further works focused on the testing performed to ensure highest reliability and the optimized system integration details establishment. The technical requirement considerations covered all aspects of interests, i.e. technical features as well as workability conditions for a portable device with capabilities for in-situ operation. Final performance tests on the devices were successfully performed by EKUT and UCO. Three different nanoparticles in three different matrices were measured, silver in wine, gold in orange juice, and titanium dioxide in sunscreen. These measurements were both performed for doing the final tests on the devices and to compare the results of the INSTANT project with the consortia SMARTNANO and NANODETECTOR.
- Each of the samples was treated in the same way, passing sample extraction and pre-concentration, with afterwards measuring on the optical and electrochemical sensor. As already reported before, transducers are used, modified with two different recognition elements on two different spots, carbon nanotubes (CNTs) modified with carboxylic groups and others modified with amino groups; each of these show different behavior towards different nanoparticles.

Work package 6 – Project Management

Main S&T results/foregrounds

This work package focused on the coordination of the scientific activities as well as the overall financial, administrative, legal and contractual management of INSTANT interfacing between the project and the Commission.

It collected information, reports, and financial information for submission to the Commission as specified in the Grant Agreement and ensures that tasks are carried out by the participants in a timely and correct manner. Another task was to manage project meetings, distribution of meeting minutes and follow-up actions, technical meetings, phone conferences and researcher exchanges.

Activities

The intra-project actions were extensively performed e.g. the following summary of researcher exchanges over the project duration:

- EKUT: 2 persons to UMU
- EKUT: 1 person to UNIVIE
- EKUT: 2 persons to BAM
- UMU: 1 person to UNIVIE
- UNIVIE: 1 person to EKUT
- UNIVIE: 1 person to UMU (2x)
- CD: 2 persons to EKUT
- UMU: 1 person to EKUT (2x)
- EKUT, BM, UMU, UNIVIE to UCO

Furthermore, workshops and public presentations were held to inform a wider public about INSTANT.

Figure 20: Poster session of the public midterm seminar at BAM, September 2013.

Besides different panels, also an accompanying advisory board belong to the management workpackage. Overseeing gender equality and science & society issues was an additional task here. The supporting organization of round robin and the organization of the joint workshop of the three FP7 projects INSTANT, NANODETECTOR and SMART-NANO was a main objective in the last period of the project.

Panels and Task forces

Task force public relations (EKUT)

The task force public relations took care of spreading INSTANT news and dissemination materials including press releases, interviews, flyers as well as a project website, which was set up and maintained by EKUT (see for further Details section Dissemination and Exploitation). Furthermore, an interactive DVD was produced aiming to general things:

- engage directly and personally with a specific audience to communicate the motivation for the research and the findings
- show context of research, its professional use in practice and its impacts

DVD`s specific topics include project concept, achievements and project testing results e.g.:

- Instant Logo
- Resume of public summary
- General poster
- Instant flyer
- Movie of casing and transducer
- Movie of the binding of nanoparticles to a CNT`s spot
- Movie of hardware integration and operation modules

Task force risk assessment (BAM)

Inherently, the project did not perform any risk studies of engineered nanoparticles, but supplied a prototype instrument to do risk studies. Accordingly, the task force risk assessment was in contact to other EU projects such as NanoDefine, NanoMag etc. In addition, the members of the advisory board, Prof. Lampen from the Federal Institute of Risk Assessment of Germany and Dr. Wohlleben from the BASF chemical company gave their input during the project and joined several meetings of the project. During the project, silver and gold nanoparticles with low molar mass and high molar mass stabilizer were synthesized, characterized, packed and shipped to the consortium members as standards. The concentration of the particles was low in the range of 1 to 100 micrograms per liter. Their stability in aqueous solution at ambient conditions was regularly monitored with small-angle X-ray scattering and dynamic light scattering. No changes of the mean sizes and the size distribution could be detected. This indicates that the particles are long-term stable at ambient conditions at least for six month, probably for years. Provisional material data sheets were produced for the packed silver and gold nanomaterials. These sheets contain information on the synthesis, composition, labelling and handling. Additionally, detailed information on radius of gyration, hydrodynamic radius, particles concentration is provided together with original measurement data. Based on this information, possible change of the physicochemical properties that affect the analytical results of the INSTANT nanoparticle detection instrument can be identified later on. In summary, the above mentioned points minimized the risk that the analytical results produced by the INSTANT detection instrument are ambiguous.

SME Panel (BM)

In order to optimize the project progress, SME Panel meeting was regularly scheduled during all project meetings and virtually held on occasion between the individual SME partners. The most important decisions, which were extremely important to implement within a short time, were

directly transferred to WP7 and specific action can be found in the corresponding deliverables and minutes. This includes highlight like, but is not restricted to:

- an IPR protection plan
- creation and distribution of dissemination material (virtual and physical)

The evaluation of the SME Panel of the INSTANT project with respect to dissemination activities is rated quite successful (see Exploitation Committee report for details). The identified market opportunities vary and the commercial benefit of the SMEs will depend on the legislation of the EU (see report of WP1).

According to the diffuse market situation, the SME panel discussed different marketing strategies and related business models for the INSTANT device. Beside the traditional device and consumables market for analytical equipment the option based on licence fees per measurements in cooperation with one or max. two lead customers could be beneficial in terms of time to marked and reduced initial investments. Furthermore, this strategy would help to create the basis of the second marketing step following the traditional model described above.

Apart from the commercial perspective, the knowledge transfer from SME to SME, SME to university, and university to SME turned out to be fruitful and promises a good outlook for future cooperation.

Impact

The overall aim of this workpackage was to ensure a good cooperation within the WPs and the project partners and to give organizatorial and logistic support for the preparation of meetings and scientific reports like Deliverables and periodic reports. This target has been successfully achieved. Finally, a joint meeting with two other projects of the NanoSafetyCluster (SMART-NANO, NANODETECTOR) took place in the Vienne House in Brussels with the participation of interested public attendees, organized by EKUT with help of UNIVIE.

Work package 7 – Dissemination and Exploitation

Main S&T results/foregrounds

The key objectives of this workpackage WP7 were e.g. the dissemination activities beyond the consortium to the scientific community and towards a wider international audience through specialized events (like conferences, fairs and exhibitions), the exploitation of project results including workshops, trainings and researcher exchanges and the protection of intellectual property generated during the project. These objectives were successfully fulfilled. Especially the dissemination activities were increased towards the end of the project and further beyond the implementation phase and are still ongoing. Moreover, an INSTANT website, a flyer and a logo were set up for the distribution of the INSTANT project. The website was established and maintained for public and project partners (internal area), informing public about project content and developments and partners about latest dissemination activities, submitted deliverables meeting minutes, meeting presentations. For this purpose also a RSS, feed service was established to inform project partners and

public about latest developments. Publishable material about the project like newsletters, interactive DVD, electronic bulletins, posters, and open discussion forums were periodically edited. Several marketing actions and business contacts with leading industry-OEM's have been made during the period with very good feedback reaction. A possible service-provider model for NP analysis was discussed with some potential end-users, which can provide an interesting business model. Market studies and exploitation activities were carried out and several patent surveys were performed. Dealing with the business case can start now as now investment costs can be estimated more easily than during the project. There is still the demand to companies to take a clear position if instruments are needed or not. The novelty of the INSTANT concept and the innovation solutions of project results will further perform more patent request documentations by the consortium. Wider market surveys of analytical instrumentation with specific requirements of the market for like Nano Particle Size Analyzers have to be carried out in order to keep a permanent up-dated vision towards the potential competition on the field of this specialized market.

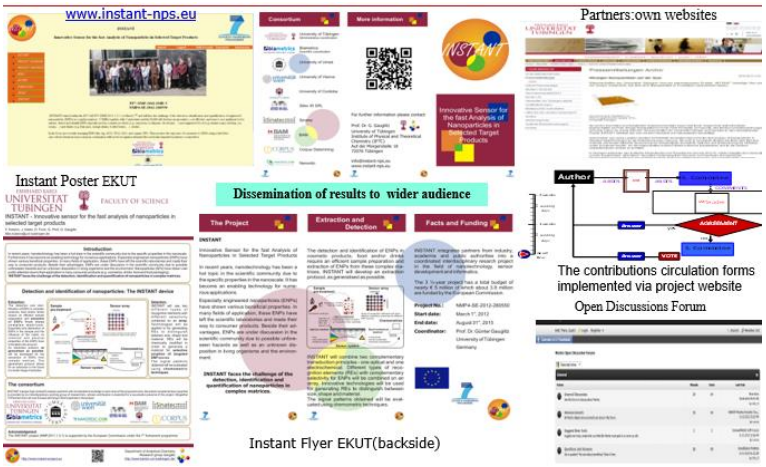


Figure 21: INSTANT Dissemination materials.

The resume of main results

- Dissemination, exploitation and IPR protection were the objectives of WP7 of great interest for all consortium partners, who actively contributed to this workpackage as DoW foreseen.
- The INSTANT website www.instant-nps.eu is functional since a very early stage of the project. It was set up and maintained for public and project partners (internal area), informing public about project content and developments and partners about latest dissemination activities, submitted deliverables meeting presentations and meeting minutes. A RSS feed service was established to inform project partners and public about latest developments of INSTANT by EKUT
- Design of INSTANT Logo and flyer was made accessible to the consortium, both in printed and digital form by EKUT distributing at INSTANT presentation towards the scientific and technical-commercial audience

- Electronic dissemination instruments and implementation such as interactive DVD, Electronic newsletter and Open Discussion Forum, were set up and are available at SITEX.
- The main project results were continuously disseminated to a wider public by the participation at national and international conferences and specialized events like fairs and exhibitions.
- Publications and contributions at conferences, symposiums and congresses were spreaded during the project duration
- The dissemination activities are still continuing after the end of the project and further beyond the implementation phase.
- The IPR strategy was implemented at an earlier project state continued to follow-up the patent situation and to support partners in possible patent requests and technology exploitation
- Exploitation Agreement

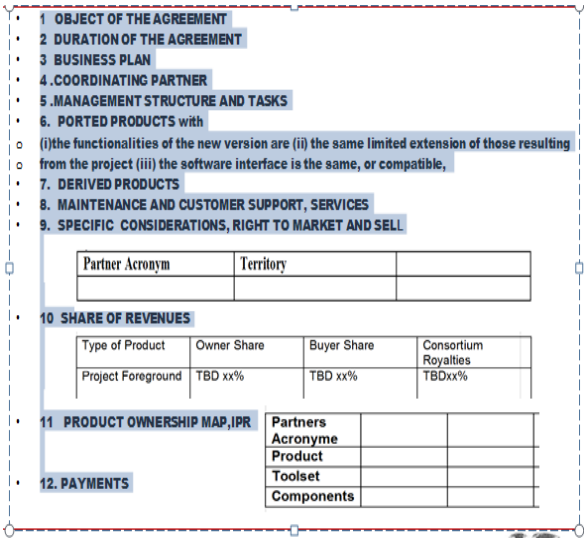


Figure 22: Exploitation agreement.

- A SWOT analysis was written up for the best competition to understand how to develop the marketing and product applications
- After the establishment of an almost complete mailing list, based on the inputs coming from all partners, this list was used to send periodically updated information about INSTANT results, news and achievements press releases, press conferences and interviews. The mailing list fulfilled also with the NCP representatives at international and European level on food quality.
- Studies and comparative analysis of regulations for quality and safety of foods in Europe was documented for a further development on this field.
- The main actors on food safety and quality assurance , driving forces for nanoparticles and control implementation in European or National Authorities were added on the project mailing list
- A wider market survey of analytical instrumentation with specific requirements of the market for Nano Particle Size Analyzers in order to keep a permanent up-dated vision towards the potential competition on the field of specialized market was performed

- The continue publishing of non-confidential materials about the project progress, knowledges and results by different dissemination instruments like: newsletter, electronic bulletins, leaflets, posters and open discussion forum has been conducted.

- Dissemination materials issued and distributed by EKUT, BAM and CD by both printed and electronic ways.
- The audio video dissemination materials were chosen due to a faster public penetration and a shorter circulation time towards a wider areas of audience of potential users community. This way is recommended for the consortium partners. UCO already started with „Informative note in Historias de Luz" http://www.youtube.com/watch?v=Z_nnbAlgJXc.
- Results dissemination and visibility was achieved with the partners corporate websites. EKUT, BM, UCO, NANO, SINATEC and SITEX published project-related information on their own organization website, through either a dedicated page or news about project progress and activities (events, workshops announcements, etc.)
- UCO has disseminated the success of the project by the webpage of the university with the following link: http://www.uco.es/servicios/comunicacion/actualidad/noticias/item/111838-un-consorcio-europeo-con-participación-de-la-uco-desarrolla-un-sensor-para-detectar-nanopart%C3%ADculas-en-alimentos-y-cosméticos
- In addition, UCO shows the most relevant information of the project to a wide audience by using different communication media: Different press issues on the University of Córdoba and local and national media disseminated the INSTANT project are shown in Figure 2 below.

Figure 23: ***2-UCO News of the INSTANT project in press.***

- The Interactive DVDs issued by SITEX was endowed tos all partners and EC at the 6th Semiannual Meeting of May 2015, including a movie of binding signal, AFM pictures, 3D model and animation of flowcell casing, posters provided by EKUT,
- The Exploitation Committee with participation from all SMEs brought a strong contribution at the exploitation planning and implementation that carried out by all the partners for best results exploitation and concerning documentations further produced.
- Intellectual Property (IPR protection strategy) with guidance of the partners through the IPR topics and related CA rules refer to: (1) PR engineering, (2) responsibilities / joint ownerships, (3) Importance of IPR for commercialization linking to SME panel discussions / exploitation managed by BM.

Socio-economic impact and the wider societal implications of the project

The project INSTANT targets three key issues of impact on nanomaterials, their safety, monitoring, and risk assessment:

- INSTANT provided a powerful tool to researchers dealing with nanoparticle detection and characterization.
- The INSTANT device makes it possible to ensure consumer to feel more safe and that both product is either free of nanoparticles (if desired) or contains amounts and speciation of nanoparticles that are considered as safe by the EU.
- With the development of the INSTANT device, the consortium delivers a tailor-made instrument to the legislative organs of the EU on nanosafety.

This device choice is to gather enough data about nanoparticles in different products and exposure of the consumer to these nanoparticles to help the decision-making process within the EU. In addition to these three points, the developed device enabled the partners SMEs to take a leading role in the production and distribution of the next generation device for the detection of nanoparticles in all fields of applications.

What can be expected from the INSTANT device by the customer?

Usually the food and consumer products industries apply several additives, supplements, contact materials (for packaging) and nanotechnology derived ingredients to their products. Hereby, the application and addition of nanotechnology derived additives to the products covering food and beverage is distributed as shown in Food regulation is now largely determined at EU level, and national food laws in EU Member States generally implement decisions taken by EU

This trend is growing rapidly and currently, there are over 400 companies worldwide that conduct R&D into the use of nanotechnology in agriculture, engineering, processing, packaging or delivery of food and nutritional supplements already used in food supplements and food packaging (e.g. nano-clays and nanosilver). However, it is widely anticipated that they will appear on the EU market in the next few years.

The INSTANT device can become a very important analytical tool for European and international actors involved into ENP`s and for nanotechnology applications implementation policy in food technologies, food safety, feed chains and related domains. According to the publication of *Journal of Colloid and Interface Science by Timothy V. Duncan* (US Food and Drug Administration, National Center for Food Safety and Technology, 6502 South Archer Road, Bedford Park, IL 60501, United States) of article entitled „*Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors*" could be shown on the Figure 24 how wider is the involvement and interferences of nanotechnology and related nanomaterials with the food safety and packaging under generic name „nanofood".

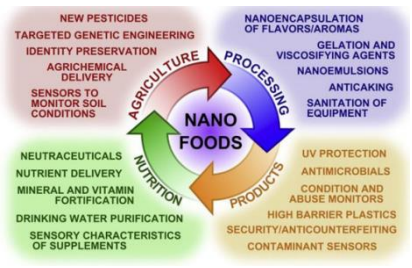


Figure 24: Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors, *Source*: Science Direct, Journal of Colloid and Interface Science, 2011

The tremendous potential of nanofood will attract more and more competitors into this still un-ploughed field. It is almost inevitable that humans will be exposed to engineered nanoparticles, e.g. due to migration of nanoparticles from the packaging into the food products.

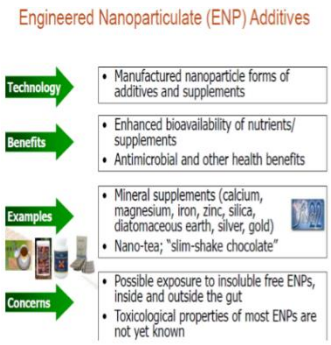


Figure 25: ENPs as additive technology, benefits and applications

Furthermore, nanoparticles have entered just about every personal care product on the market, including deodorant, soap, toothpaste, shampoo, hair conditioner, sunscreen, and many more. The application of these products containing nanomaterials (e.g. TiO_2 and ZnO nanoparticles) directly to the skin is a direct source of exposure. The effects of nano-TiO_2 comparatively with conventional applications, shown a great consummation of resources are used on production of TiO_2 at nanometer scale. (see *Untersuchungen des Einsatzes von Nanomaterialien im Umweltschutz*, Martens, Sonja, et al. (Golder Associates Gmbh), 2010, Umweltbundesamt, no. 34/2010, June 2010, Dessau-Roßlau: Umweltbundesamt). The topics of nanomaterials for foods is a very actual topic and of interest at European and international level, being analyzed also by the specialized organization of France on nanomaterials and related nanotechnologies *AVICENN*. http://avicenn.fr *as editor of act also as editor of sites* http://veillenanos.fr *and* http://wikinanos.fr with the shown logos below

Figure 26: Logos of specialized organization of France on nanomaterials and related nanotechnologies AVICENN

AVICENN issued the monthly published newsletter called **VeilleNanos** national, European and international the main range of nanomaterials and nanotechnology topics related to toxicity, health and environmental impact as well as new results on the fields as the part of **Dossier Nano et Alimentation**. It was published the article entitled as below: « Quels ingrédients nano dans notre alimentation ? (Fr)» « *Which nano ingredients are contrained in our food? (En)* »

The great interest of nanofood as on "The sommaire of Dossier Nano and Alimentation" shown below:

- the approaches of high frequently food packaging solution and materials on contact with food stuff which applications of nano in nano food stuff could be applied
- Other indirect sources of contamination of our foods due of nanomaterials manufacturers.
- Influence of pesticides
- The animals alimentation and the veterinary drugs
- About the environment and food chain of nanomaterials

As for the economic impact of nanotechnology in the food sector, Cientifica's reports predicted that the value of products containing nanotechnologies in the food sector worldwide would grow

permanently. Therefore, the need of reliable testing and explicit labeling of products containing nanomaterials will become more and more mandatory. Regulatory authorities already imposed and will further intensify the obligation of such testing and labeling of products by the respective producing companies. With the INSTANT device, we propose a professional way to meet the consumers demand for "point of food testing" (POFT).

What can be expected from the INSTANT device by researchers?

The EC implemented the principle "no data, no market" for all applications of nanomaterials in consumer products or in products leading to discharge in environment. Clear labeling of ingredients in form of nanomaterials in consumer products have to be performed by the producers. Also, the development of adequate testing protocols to assess the hazard of, and exposure to, nanomaterials over their life cycle have become mandatory. In conclusion: When food products contain nanomaterials or they are produced using nanotechnology, they can be considered as *novel food*, from what it results the following needs:

- need to undergo pre-market assessment (initial assessment by a Member State)
- need to be labeled accordingly about the dangerous potential

The potential toxicity and behavior of nanoparticles will be affected by a wide range of factors including particle number and mass concentration, surface area, charge, chemistry and reactivity, size and size distribution, state of aggregation, elemental composition, as well as structure and shape. Therefore, when analyzing nanoparticles in different matrices, it is not only the composition and concentration that will be needed to be determined, but also the physical and chemical properties of the engineered nanoparticles within the sample and the chemical characteristics of any capping/functional layer on the particle surface. The analytical tools developed answered at the most important requests as below:

- to be sensitive enough to measure low concentrations
- to minimize sample disturbance to ensure that the analyses reflect the unperturbed environmental state
- not only data on concentrations is generated, but also information about size distribution and properties of nanoparticles
- to be able to analyze samples of diverse elemental compositions and samples containing more than one type of nanoparticle, meaning to measure in heterogeneous samples
- and to be non-destructive or at least do not lead to artifacts due to sample preparation, i.e. being able of measuring in the original state of the sample (the original matrix, e.g. food and consumer products)

The technology developed within the INSTANT project focused to meet all of these requirements. INSTANT developed the first fully integrated and validated sensor system for monitoring ENPs in wider application domains like :cosmetic products, engineered food and drinks. The fully integrated system is simple to operate, reliable, affordable, and will require a minimum of human interaction. The approach will strengthen the European research area (ERA) by developing such techniques in Europe first. The industry will profit by lowering the cost of testing in order to meet future regulations and by adapting the system to production processes. Confidence of the public consuming ENP-supplemented food will grow, if all safety measures are considered. The very demanding topics of INSTANT require leading experts in the fields of separation, imprinting and sensor technologies,

NP production and characterisation, and chemometrics. The consortium members along with the advisory board members combined their outstanding competences, complementing each other in a unique way.

Sampling and separation
So far no standard procedures are available for extracting ENPs from cosmetics and food. INSTANT will close this gap. In addition, knowledge on the fate of ENPs in cosmetics and foodstuff will be gained, i.e. chemical changes/degradation, aggregation and adsorption of matrix components, which is likely to influence hazard characteristics such as stability, bio-availability, organ- and tissue-distribution, cell entry pathways and catalytic activity of ENPs. Characterization of ENPs will be available as reference data. The EFSA's state that metrology is a major issue when dealing with risk assessment of NPs. Even for particles made from the same material the size will determine bio-availability, organ- and tissue-distribution, cell entry pathways, catalytic activity (e.g. generation of reactive oxygen species) and stability of NPs. The classic exposure measurement techniques (mass of toxic compound per kg body weight) therefore need an urgent revision. The EFSA opinion paper concludes that meaningful measurements need to be developed for exposure studies including chemical composition, total mass of nanomaterial, size, size distribution, shape, number of particles, surface area, and surface properties. INSTANT directly addresses these issues by developing and thoroughly characterizing reference particles before and after exposure to food and capturing chemical composition, size and size distribution at once.

Sensor development
The sensors for the detection of nano-materials in cosmetic products, engineered food and drinks developed within INSTANT will be fully-integrated, simple to operate, reliable, affordable, and will require minimum of human interaction. It is possible to extend the system to a broader range of particles or, for the purpose of environmental monitoring, in many cases a broader usability can be achieved with only minor changes in the sample preparation setup. These properties as well as the growing need for monitoring tools make the system very valuable for quality inspections, environmental exposure studies and also very attractive for commercialization. Such a tool will also be very useful for applying the fastest possible countermeasures in the case of contamination due to leakage of dangerous nano-materials from production plants.

Accordingly, three lines of impact of Instant device on the work-programme of the EU can be defined:

1. Developing feasible sampling and separation techniques under defined conditions to standardize the sample as a basis for precise analysis.
2. Characterizing ENPs and their food and packaging environment to obtain reference data under standardized conditions.
3. Setting up a simple fully integrated detection system including sample preparation, sensor array and data evaluation which can be used as a portable system possible to be adapted for environmental monitoring

What can be expected from the INSTANT device by the EU?
INSTANT established synergies with other existing and developing EU projects especially with members of the nano-safety cluster. By the cost-effectiveness of the INSTANT device, it can be assumed to ensure an area-wide coverage in detection of nanoparticles contained in any type of product (and even environment). This will lead to a huge pool of data, which can be used by the EU

to set up exposure scenarios for different groups of consumers in various countries. The generation of data will aid the EU to fulfil the desire of consumers for a strong monitoring and/or banishment of (hazardous) nanoparticles. Ultimately, it will enable the EU to set up or adjust existing regulations and directives targeting nanoparticles, which are used in cosmetic products and foodstuff.

Food regulation: It is now largely determined at EU level, and national food laws in EU Member States generally implement decisions taken by EU. The Commission is considering the EP's request for the systematic labelling of all food containing nanomaterials with a favorable disposition. An amended novel foods regulation may require approval of nano-specific test methods, before foods produced with nanotechnologies can be assessed or authorized for sale. This regulation could hence slow down the commercialization of nano-enabled foods in the EU. The new regulation on a common authorization procedure for food additives, enzymes and food flavorings (EC/1331/2008) and the new food additive regulation (EC/1333/2008) stipulate that enzymes, additives and flavorings "must not be placed on the market or used in foodstuff, unless they are included on a Community list of authorized substances". According to the regulation for food enzymes (EC/1332/2008) food enzymes should only be approved if they are safe and if they fulfil a technological need. A safety assessment has to be carried out before the authorization of a specific enzyme. The Food Contact Material Regulation also establishes special restrictions on "active" and "intelligent" food contact materials. These materials can be subject to an authorization and a safety evaluation under other regulations, such as the Novel Food, Flavoring, or Additive Regulations, if they fall within the scope of those regulations. For example, if a component released by an active material changes the composition of food so that the component is a food additive, that component cannot be used unless it is included on the list of approved additives.

Cosmetics regulation: The adoption of the new Regulation EC/1223/2009 on cosmetic products published in December 2009 changes the regulation of nanomaterials in cosmetics in Europe. In contrast to the former cosmetic directive, the regulatory authority over cosmetics was centralized at the EU level, because the regulation is directly applicable and legally binding in the Member States. The Cosmetics Regulation is the first EU legislation that dedicates an entire Article (16) to nanomaterials. Paragraph 1 of Article 16 explicitly states that for every product that contains nanomaterials, "a high level of protection of human health" shall be ensured. For this purpose, the regulation contains specific guidelines on safety assessments and the cosmetic product safety report, which are obligatory for all manufacturers. For the exposure evaluation of a cosmetic product, the manufacturer must pay particular consideration to "*any possible impacts on exposure due to particle size*". With regard to the toxicological profile of a product, particular consideration must be given to particle sizes and nanomaterials, as well as to the interaction of substances. In addition to these requirements, the regulation stipulates that prior to placing a cosmetic product on the market, the responsible person must notify the EC of "the presence of substances in the form of nanomaterials" and their identification including the chemical name (IUPAC) and other descriptors as specified in paragraph 2 of the Preamble to Annexes II to VI. It also creates a greater legal certainty with regard to the coverage of nanomaterials by explicitly mentioning them. It defines such materials (see also Annex 1). Article 19 establishes a general labelling requirement for nanomaterials in cosmetic products: "All ingredients present in the form of nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word 'nano' in brackets."

These new provisions are expected to strengthen market surveillance. In addition, the regulation stipulates that the European Commission shall make publicly available "a catalogue of all

nanomaterials used in cosmetic products, including those used as colorants, UV filters and others as separate section, placed on the market, indicating the categories of cosmetic products and the reasonably foreseeable exposure conditions". In summary, the cosmetics regulation expands pre-market regulation of products containing nanomaterials including notification, but not the authorization of their use. In addition, post-market tools were established (e.g. good manufacturing practices, labelling, recalls).

What can be expected from the INSTANT device by SMEs?
INSTANT merges experts from different fields such as sensor technology, separation science, polymer chemistry, chemometrics and classical analytical sciences, combining the expertise of 5 SMEs, 4 universities and 1 research institute. The research will result in an innovative next generation tool for the detection of nanoparticles, which is furthermore practically implementable and cost-effective. The INSTANT device will allow for the detection, quantification and identification of ENPs in complex matrices such as food, food packing and cosmetic products. The need of techniques to control food is then clear, because the packaging can be an important source of contamination. The modular concept of the INSTANT device will furthermore allow for an extension to wider market opportunities like the analytics of pharmaceuticals, particle analytics of residential and working environment as well as emission/pollution monitoring.

However, the success of ultimate commercialization of the INSTANT device will also depend on the regulations of the EU that will increase the need for a tool like the INSTANT device, which can provide a cheap and robust analytical tool.

The scientific coordination of INSTANT – led by partner BM, an SME itself –ensured that the success of this project will also be a trailblazing milestone for the participating SMEs. The cooperation of the academia allows the SMEs to get new insights and technologies recently being developed in academia. These insights can be used by the SMEs to develop new products and extend the field of application of their existing techniques and products forming synergies with the other participating SMEs and strengthening their position on the European and world-wide market. Summarized, INSTANT covers the majority of tasks addressed in the work program of NMP.2011.1.3-1 as it aims at developing a platform technology for the selective detection of nanoparticles in matrices focusing on the consumer market, such as in the food area and cosmetics. With its combination of in-line sample preparation and sensing, it therefore targets direct in-situ analysis. The integrative project approach will also ensure that the system is potentially suitable to carry out future screening studies for better assessment of nanoparticle-related risks for European consumers. Such an effort can only be reached on a European level and not on a national one due to the large variety of tasks to be carried out: these include of course the design of novel sensing and analysis modules integrated into a multi-modal tool operating for different environment like *on-lab* and/or *in situ* conditions, but also the development of routines for producing nanoparticle standards suitable for system calibration and validation. Furthermore, the inherent multi-analyte approach makes it imperative to include suitable chemometrics for data analysis and evaluation. As each of these steps requires top-level specialists due to the novelty of the matrix and the approach, the probability of finding all necessary competencies in only one member state is rather low. Furthermore, method design in itself does not make sense unless there are possibilities to potentially market the outcome. Therefore, the consortium of INSTANT had shown a strong industrial impact by including four specialized SMEs as partners, as well as in the advisory board e.g. the BASF representing a global player in chemical industry, having a long-lasting reputation for fostering sustainable efforts.

Dissemination and exploitation of project results, and management of intellectual property

One focus point of this proposal was to use and disseminate the project results by further developing and marketing the INSTANT tool. To this end, during the project a detailed "Plan for the Use and Dissemination of the Foreground (PUDF)" developed. The PUDF make clear how the partners, both as a consortium and as individual organizations, intended to exploit the results of the work. Plan for the use and dissemination of the foreground (PUDF) dissemination presented in the project from the very moment foreground knowledge is produced. Continuous transfer of the technology from RTDs to the rest of the participants was a mandatory action point in the "Dissemination plan". The most important dissemination and transfer of technology took place periodically within the consortium of the project. Dissemination will require real case studies brought by the end-users and in-situ demonstrations. The PUDF collected the individual intentions of each partner and followed the requested format in the contractual agreement with the EC. The final dissemination and Use Plan described the partners achievements in dissemination and plans for the exploitation of their results, including aspects like:

- Results of the project in relation to specific potential
- Assessment of the requirements for other potential collaborators
- "Take-up" activities to promote the early or broad application of state-of-the-art technologies
- To raise awareness within relevant communities and organizations who might benefit from the outcomes of INSTANT (academia, manufacturers, food quality and health protection, lab testing /services providers, industry, etc.).
- To demonstrate to the public authorities and policy makers the benefits of INSTANT proposal.
- To demonstrate the project concept to key stakeholders at the European level, with special emphasis on those events that focus on nanofood, biofood, health montoring and control, etc. The following target group typologies have been defined
    - Policy makers, governmental authorities and NGO`s institutions for implementation rules and documents support for nanofood quality control
    - Research centers, universities and academic communities that have a key role in the development of application procedures and guides. Nanofood companies, service providers and developers that are developing or intend to develop new food processing and solutions using nanotechnology.
    - Associations of enterprises and industries, whose main objective is to represent the interest and needs of their members on the fields of nanotechnologies applications, risks and related decisions.

Dissemination Actions: Work Package WP7 was successfully performed as planned, several dissemination activities mainly within as means for raising public participation and awareness. The dissemination developed by both, the entire consortium and by each partner at a local level. A series of awareness actions took place, such as the following:

Project Website: the project and its progress presented through website where the public deliverables or results of general interest were available. The website address (www.instant-proiect.eu) was issued by EKUT as coordinator and partner as well, maintained and updated regularly. Within the website, also an email service available, RSS feeds and moreover, a visitors' feedback form and detailed visitors' statistics considered for implementation.

Partners' corporate websites and specialized websites: Partners published project-related information on their own organisation website, through either a dedicated page or news about the project progress and activities (such as workshop announcements, etc). Further online information was available e.g. on the official website of Spanish Ministery of Industry Energia and Turism website www.2020-horizon.com*"Website funded by the Plan Avanza 2 as part of Project "OFP - OpenFunding Platform, Where innovation meets Funding Open web-based innovation Platform" number IST-090500-2011-23"*

Interactive DVDs: Interactive DVDs also produced including project concept, achievements and confidential details for Consortium information.

Figure 27: INSTANT DVD containing project relevant information

Audio/Video media presentations: Video issued by EKUT with performing tests on the INSTANT device for a wider audience, showing all integrated parts developed. The video is available on the INSTANT project website.

Brochures: Issued leaflets and handouts describing the project activities and results, as well as dissemination material for user groups, using both physical and electronic means in different

Figure 28: INSTANT flyer to be distributed on conferences, symposia and similar events.

Press: Press releases, press conferences and interviews will be organized for relevant European and national newspapers, as well as publications of corresponding papers.

Newsletters: They were distributed through Internet to both, partners and general audience, including EU national representatives.

1. TRUCO newsletter

UCO diffused the INSTANT project using a press issue on the University of Córdoba called "TRUCO". This newsletter has been prepared by the organization OTRI and aims to enhance the dissemination of research results obtained, patents and trademarks, interest activities, technological demands and business ideas generated within our University. The main goal is to promote and disseminate among the university community and the rest of society the research results obtained in the University of Córdoba.

2. ORTI newsletter

Another diffusion was through the OTRI website (OTRI=organization of the transfer of the research results), in this page there is a section that includes all the European research projects, in which universities of Andalusia are involved.

3. Electronic Newsletter Quarterly Bulletin

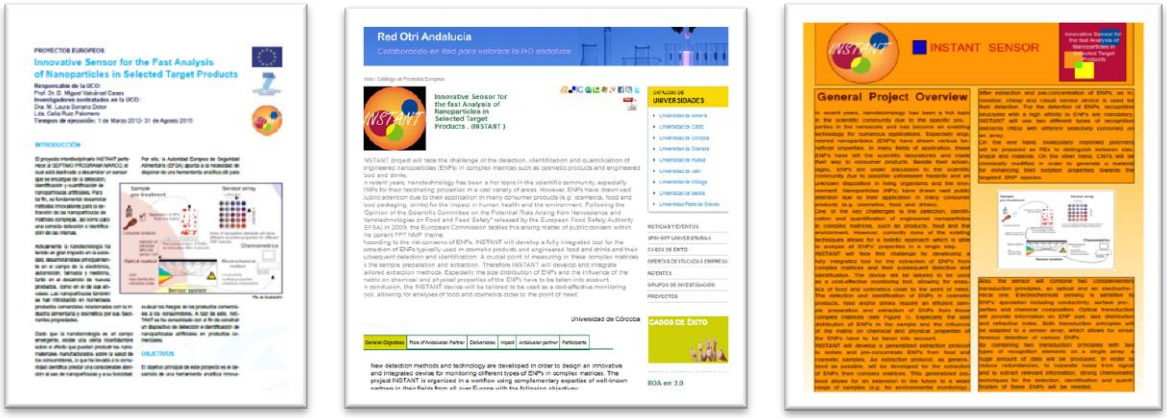


Figure 29: Different newsletters setup by different Spanish distributors (left: TRUCO, center: OTRI). Furthermore, the quarterly electronic bulletin (right).

Workshops: INSTANT managed several workshops where recent development in the detection of NPs was presented to a wider audience. Representatives from QNANO and SMART-NANO were invited as well in order to share knowledge and experiences with other EC funded projects dealing with the preparation of reference materials and the detection of nanoparticles by this will draw the attention from customers to the INSTANT technology and their possibilities.

Figure 30: Workshop on experimental design by CD Tübingen 2013

Workshops were hosted by EKUT, BM, BAM, UNIVIE, SINATEC, UmU, and SITEX

Researchers exchanges were organized for several activities between many partners, presented by EKUT as beneficiary of D7.8 “Final presentation of the results” :

Academic works : PhD thesis of Christoph Jungmann entitled “Chemosensors for the Detection of Engineered Nanoparticles using Molecularly Imprinted Polymers” app. March 2015 ,supervisor Dr. Peter Lieberzeit /UNIVIE.

Public midterm seminar: The midterm seminar took place to disseminate the results of the INSTANT project to an enlarged number of scientists in the field of sensor technology, nanotechnology and separation sciences, including representatives from SMART-NANO and QNANO.

Conferences, symposium, fairs & exhibitions

The INSTANT consortium has shown its interest in publishing several scientific articles in renowned conferences and journals, with international impact and reputable experience. In order to disseminate the results to a wider audience, possible conferences, exhibitions and fairs are: Pittcon, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Annual TNT Series (Trends in nanotechnology), Transducers Conference, Biosensors World Congress, Sensor + Test, Analytica

conference, MEDTEC Europe, International Technical Fair of Bucharest, Europt(r)ode, ANAKON, and many more. During of period between the M19 and M36, dissemination of project results by papers and contributions (See D7.6) were presented at the international specialized events like conferences, symposiums, congresses ,fairs and exhibitions.

A large list of papers, posters and project contributions over the complete INSTANT project term was published on a diverse set of conferences and symposia. An extraction of a specific set is hardly possible. The full list can be looked up on the project website and in deliverable 7.8.

Exploitation

The information gathered by the partners in WP7 was used to prepare an exploitation business plan and periodic market watch. The business plan, discussed under "Exploitation – the Business Case", took into consideration the individual interests of the SME partners as well as the synergies they have created during the choice of the consortium. *The Exploitation Committee* with participation from all SMEs assisted and leaded the works and actions carried out by all the partners.

The Exploitation Agreement (EA) contains the agreements between all the consortium members regarding exploitation issues. It was decided and initialed at M12, with potential revision possibilities at every 6 months. A draft of this Exploitation Agreement took place firstly starting since M12 to identify potential exploitation discrepancies among partners. It reflected procedures for defining and handling the background knowledge and included the conditions and processes by which the consortium will grant further manufacturing and distribution outside the consortium to meet the demand from the end user community. Furthermore, it covers exploitation restrictions, licensing arrangements and protection of the intellectual property generated within the project, and methods for disseminating results and a royalty scheme. Even though the Consortium Agreement (CA) will define the basics and agreed general procedures, the EA complemented and detailed those areas more related to exploitation issues.

The Exploitation Committee consists of representatives from each partner and is chaired by a partner who was responsible for the exploitation management within the project. The responsible partner led the Exploitation Committee due to his years of experience and knowledge in the sensor and analytical instrumentation market in Europe. The exploitation plan gave also the steps for the exploitation of results in future commercial products considering SWOT analysis and market research.

Commercial Exploitation

Manufacturing rights were initially restricted to partners from the consortium, unless the demand exceeds their manufacturing capacity. License fees will be established in such a way that it will not jeopardize the competitiveness of the partners. A brand name developed during the life of the project was selected as INSTANT, implemented by SITEX into manufacturing line and further product promotion on the market under name of product as *„Eleni-1"* given on the memory of company founder. The analytical tool and related technology will reach the market through direct sales or possible licensing the technology. The designated INSTANT device and its related disposables will not be dependent on know-how being restricted to one single company. In the EU there exist potential device manufacturers or companies specialized on the production of disposables. This minimizes the risk that the final outcome of the project would not find its way into market. Project partners developing results in INSTANT will own the Intellectual Property Rights (IPR) for those results and

have rights to exploit them. Other project partners will have favorable conditions to use and exploit the results developed by partners.

Commercialization on the market
The SME panel developed a detailed market survey, market information, market segmentation and market trends studies and patterned the market structure of main industrial end users and competitors which is further shown.

The market of analytical instrumentation as a very dynamic market in permanently changing will need further analysis and research in order to accurate registrate the latest achivements with newest innovative products and technologies based on.

The market demands can be addressed with the individual achievements/results and solutions and the technological components of the applied technologies as follows:

1. Core markets for the INSTANT device: Food Safety (consumers, manufacturers, regulatory bodies, consumer protection agencies), Environmental monitoring, Quality control for NPs production, Quality control for feed production, R&D for basic research on NP
2. Other interesting more markets: Detection of other NPs like natural NPs such as virus particle (Homeland security, POCT, etc.), Manufacturer of complex sensor products in R&D utilizing the novel SW tools, Research market for novel recognition elements, Analytics market using novel extraction technologies

Also it has to be kept in mind that it is necessary not only to protect the overall INSTANT technology for exploitation but also to protect the individual technological components, which are of great value as standalone products in various specialized instrumentation markets.

The commercialization of INSTANT as integrated device
As the results of market research, more specialized companies, acting on the fields of particle characterization, could become a potential user of the INSTANT device. These potential customers could be listed as follows: BASF, GlaxoSmithKline, Merck, Novartis, Pfizer, Unilever, Procter&Gamble, La Roche. The accurate situation of device customers could be finally operated after a specific questionnaire would return with the clear confirmation of expression of interest.

The commercialization of INSTANT device for subsystems a/o sensors parts for implementation as well for instrumentation
The potential users are specified on the separate list for 200 companies (see D7.5) acting worldwide on the instrumentation fields. The main conditions before of wider exploitation actions is compulsory as all device modules and parts for commercialization to have full IPR protection documentations finalized and the agreement between all the partners involved. It has to be mentioned that there is a strong market also for used devices which need spare parts.as could be shown on the list of over 200 instrumentation producers (see D 7.5).

The actual market trends are towards the development of multiparameter analytical instrumentation for particles characterization and measurement in order to fulfil more requirements by the optimization of related investment. It must be kept in mind that costs of accurate analytical instrumentation are very high even if they perform a large volume of measurements. Moreover, the INSTANT device could also be promoted successfully on the pharmaceutical market in accordance with cGMP requirements for Current Good Manufacturing Practice regulators.

IPR exploitable measures taken or intended

Regarding applications for patents, trademarks, registered designs, etc., the IPR policy was initialed in detail in the consortium agreement. If, in case of carrying out work on the project program, employees and/or sub-contractors of more than one partner make joint invention, design of work, and if the features of such a joint invention, design or work are of the sort that there is no possibility to separate them for applying for a relevant patent protection (or other industrial property rights), the partners/contractors involved may jointly apply for the relevant patent or other industrial property rights. The arrangement for assigning and maintaining such a patent or other industrial property right is agreed between the partners concerned on a case by case basis. As long as any suchlike patent or other industrial property right is in force, the partners concerned shall be entitled to use and license such patent or other industrial property right without any financial compensation to or the consent of the other partners concerned, but subject always to the terms of the contract.

Partner NANO	Role in the project	Type of IPR & Exploitation	Product
Nano	Retaining filter & Sensorial material development	Invention	Size selective retaining filter for nanoparticle selection
Nano	Retaining filter & Sensorial material development	Invention	Size and materials selective retaining filter for nanoparticle selection
Nano	Retaining filter & Sensorial material development	Invention	Carbon nanotube sensor for nanoparticle sensing
Nano, all partners	Retaining filter & Sensorial material development	Invention	Final Instant product
SITEX, all partners	System integration & Prototyping	Invention	Final Instant prototype

Conditions for use after the end of project of these underlying patents are described in detail in the Exploitation Plan as well as other access rights e.g. for affiliates and for parties joining or leaving the project. For software development, a “quid pro quo” arrangement was put into the Exploitation Plan. Partners who develop data and models grant the developers of code post-project exploitation rights to their data and models free of charge. Also vice versa, the code developers grant other partners post-project exploitation rights to at least the core components of their software (sufficient to run the “foreground IP modules”) free of charge. Assuming that agreement is reached on this central quid pro quo, there should be no need to make source code available, or to consider any requirement for sub-licensing.

Address of the project website and relevant contact details

http://www.instant-project.eu

Partner	Acronym	Contact person	Address
Eberhard Karls Universität Tübingen	EKUT	Günter Gauglitz	Universität Tübingen Geschwister-Scholl-Platz 72074 Tübingen Germany
Biametrics GmbH	BM	Günther Proll	Biametrics GmbH Auf der Morgenstelle 18 72076 Tübingen Germany
University of Umeå	UmU	Britta Lindholm-Sethson	Umeå universitet SE-901 87 Umeå Sweden
University of Austria	UNIVIE	Peter Lieberzeit	Universität Wien Inst. f. Analytische Chemie und Lebensmittelchemie Währingerstr. 38 1090 Wien Austria
University of Cordoba, Dep. of Analytical Chemistry	UCO	Miguel Valcárcel	University of Córdoba Analytical Chemistry Department Annex "Marie Curie" building, C.U. Rabanales E-14071 Córdoba Spain
Sitex45	SITEX	Dumitru Ulieru	SITEX 45 SRL 114 Ghica Tei Blvd.;BL 40; AP.2;Dept. 2 023709 Bucharest Romania
Sociedad de Investigación en Nanoestructuras S.L.	SINATEC	Bartolome Simonet	Plaza Escultor Ruiz Olmos 12, 5ºB. E-14071 Córdoba Spain **<u>Manufacturing center:</u>** Zona Industrial Aeroportuaria, Aeropuerto de Jerez, 11401- Jerez de la Frontera (Cádiz)

BAM Federal Institute for Materials Research and Testing	BAM	Andreas F. Thünemann	Bundesanstalf für Materialforschung und –prüfung Richard-Willstätter-Straße 11 12489 Berlin Germany
Corpus Datamining	CD	Hans Grahn	Corpus Data & Image Analysis AB Rålambsvägen 66 B , Suite 1408 SE 112 56 Stockholm, Sweden
Nanordic	NANO	Geza Toth	Nanordic Oy TOTH KALIKKAKUJA 2 D 90540 Oulu Finland