Labelling nanoparticles by nanotracers and oligonucleotides

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Abstract. The rapidly developing field of nanotechnologies presents many opportunities and benefits for new materials with significantly improved properties as well as revolutionary applications in the fields of materials, energy, environment, medicine, etc. Nevertheless, this new industry can rapidly compete with microelectronics or automotive in terms of annual turnover only if the nanoproducts are well accepted by consumers. Labelling the nanoproducts as “nanomaterials” is one of the prerequisite conditions for market acceptance. One approach consists in encouraging manufacturers to insert labels since the nanoparticles synthesis, at the last step of the production reactors, in order to give consumers useful information related to nano-fillers contained in commercial products.

The first level of information could be the specification of the presence of nanoparticles in the product: a kind of tag indicating "nanomaterials inside". The second level of labelling could supply more complex information such as: the type of nanoparticles, the manufacturer name, the batch number, the toxicity class, etc.

In European Nanosafe2 project, two main routes have been investigated: the previous one is using biomarkers based tracers. In that case the information is available thanks to conventional biological methods. The second way requests fluorescent nanotracers which are less powerful in terms of data storage but may be more resistant against chemicals, temperature, UV aggressions and could be read in a few seconds by field operational portable decoding devices.

For economical reasons it is necessary to introduce a very small quantity of tracers in the nanoparticles. Nevertheless, for certain applications for which a high purity level of the nanoparticles is requested (e.g. microelectronics) this small amount can be redhibitory.

1. Labelling with biomarkers

A strategy of labelling comprising biomarkers such as oligonucleotides which can contain a very high density of coding information and also an integrated decoding apparatus has been investigated for nanomaterials traceability.

A multifunctional biomarker labelling scheme has been first designed. The nanoparticles labelling has been realised using very classical chemical grafting techniques for a covalent linkage between oligonucleotides and nanoparticles. This chemical grafting includes a generation and activation of carboxylic groups on the nanoparticle surface followed by a reaction between the biomarker and the activated carboxylic functions. The obtained solution after biomarker grafting has been tested with conventional laboratory equipments used for fluorescence detection in 10 µL tubes. It has been shown that serial dilutions can be positively detected for dilutions ranging from 1/10⁴ to 1/10⁸ corresponding to 10⁻⁴ to 10⁻⁸ mg/mL of biolabelled nanoparticles. At the lower concentration of detected nanoparticles corresponding to 10⁻⁸ mg/mL, only 0.1 pg of nanoparticles are present in the 10 µL test tube, which gives a detection limit much lower than what can be obtained with classical techniques for
nanoparticles detection like microscopic techniques (STM, TEM, AFM for example) or spectroscopic methods like Raman spectroscopy.

ElectroWetting On Dielectric (EWOD) principle is based on the use of electrostatic forces for fluidic actuation. On an appropriate device comprising embedded electrodes, it is possible to move liquids, dispense reagents (Figure 1) and mix several droplets, thus accomplishing all basic fluidic operations necessary for performing a complex protocol by simple electrical actuations. As no moving parts, like valves or pumps, are necessary, EWOD lab on a chip are very well adapted to integration in a complete compact apparatus.

Figure 1. Droplets dispensing from a reservoir with EWOD lab on a chip by successive electrodes actuations.

The obtained droplet sits on the last activated electrode.

EWOD silicon chip manufacturing is based on collective and standard micro technology processes involving successive deposition, photolithography and etching steps on a wafer. After manufacturing, the 13 mm by 13 mm silicon chips are individually packaged on a printed circuit board (Figure 2). The chip is glued on the PCB and electrical connection of the electrical pads of the chip to the PCB is obtained by wire bonding.

Figure 2. EWOD actuated chip and associated instrumentation.

The fluorescence detection of a diluted solution containing biomarkers labelled nanoparticles (at 0.1 pg/µL of nanoparticles) has been successfully performed in a 65 nL droplet on the lab on a chip, proving that this kind of device can be used in portable instruments for nanoparticle detection and traceability.

2. Labelling with fluorescent nanotracers

2.1. Basics of encoding

As shown in Figure 3, codes can be constituted by the relative levels of fluorescence intensities at different wavelengths. Using one of the colours as a reference, the number of possible combinations C is given by the number of colours T and the number of intensity levels N: \( C = N^{T-1} \).
For 5 different intensity levels and 4 colours including one reference, the number of combination is 125. This technique is limited in practice by the pick widths and the availability of low cost dyes spread over the whole UV-IR spectrum. The example of 125 combinations given here is a good technical objective and probably sufficient to store a sufficient number of basic information to characterize nanoparticles.

![Code 2-4-1](image)

*Figure 3. Code 2-4-1 constituted by the 3 colors: orange, green and blue; the red color is used as the reference (left). Schematic of a nanotracer (right).*

As represented above, each nanotracer contains the different fluorescent codes which emit fluorescence under an appropriate excitation radiation.

### 2.2. Tests of different nanotracers

Quantum dots are potentially very interesting tracers for coding application as they present a quite sharp pick. In this case, the fluorescence is given by a quantum confinement effect: when the particle size becomes smaller than the theoretical electron-hole pair dimension. CdSe, CdS and CdZnSe were synthesized. Unfortunately the surface passivation obtained at the laboratory were not strong enough (degradation after one week) to envisage a use for nanotracer application.

Rare earth ions dispersed in oxide matrix can give even sharper picks than quantum dots. As the size is not the intrinsic parameter to obtain different wavelength, one can use bigger particles (10 - 200 nm) which present relatively less surface defects. It is quite easy to obtain phosphors by colloidal engineering. The limitation of nanophosphors for marking application comes from the quite elevated cost of raw materials.

Finally it was found that organic luminescent molecules embedded in mineral matrix represent a good opportunity for marking application as the initial materials are acceptable in terms of cost. It was shown that embedding the active molecules in polysiloxanes or organoclays to constitute the nanotracers, stabilize drastically their optical properties to temperature and UV, leading to acceptable performances for a large field of applications. Nevertheless, the drawback of using organic dyes remains their broad pick width which limits the volume of information.

A synthesis process using a sol-gel route to obtain a 20 nm organoclay with hydrophobic and hydrophilic functions was performed (see Figure 4). The incorporation of organic dye as tracers is made by dissolution in appropriate mixing of organoclays and dye. Dyes are adsorbed at the surface and in the inner porosity of nanoclays by hydrophobic functions.
A three colour nanocode obtained with organic dyes inserted in organoclay (code 4-3, the first pick is used as the reference). The nanotracer size is around 20 nm. Left: TEM picture of organoclay, right: fluorescence spectra of the organoclay containing 3 fluorescent dyes (uranine, rhodamine and porphyrin) in water. Excitation wavelength 410 nm.

A 3 colour nanotracer able to contain about 50 different codes was fabricated. Figure 5 shows the spectrum of one nanotracer obtained with 3 organic dyes (code 2-1) as fabricated and then incorporated in a batch of TiO$_2$ nanoparticles from Degussa (P25). In spite the low concentration of nanotracers incorporated (less than 100 pp), the deconvolution of the peaks on the foot of the diffraction part of the excitation allows to recognize the initial code.

![Figure 4](image.png)

![Figure 5](image.png)

**Figure 4.** A three colour nanocode obtained with organic dyes inserted in organoclay (code 4-3, the first pick is used as the reference). The nanotracer size is around 20 nm. Left: TEM picture of organoclay, right: fluorescence spectra of the organoclay containing 3 fluorescent dyes (uranine, rhodamine and porphyrin) in water. Excitation wavelength 410 nm.

**Figure 5.** Left: optical spectrum of the nanotracer and residual signature when incorporated at trace level in a batch of TiO$_2$ nanoparticles; as measured by a spectrophotometer. Right: example of a portable decoding device able to read the nanotracers based on a micro spectrometer.

2.3. Decoding device

An example of a portable decoding device using a micro spectrometer and a PDA has been designed as a demonstrator (Figure 5). Instantaneous reading of the nanotracers can be performed.

3. Conclusion

Finally both approaches using biomarkers or nanotracers are adapted for labelling nanoparticles. The biomarker method presents the advantage to be able to carry a large number of information. On the other hand, mineral fluorescent tracers are probably more robust against temperature, UV, and chemical aggressions.

Labelling the nanoparticles as described in this work is not adapted when ultra purity is requested for nanoparticles *e.g.* microelectronic, but is suitable for nanoparticles used in a large variety of applications. A standardization initiative at ISO is needed for promoting the labelling of nanoparticles.
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