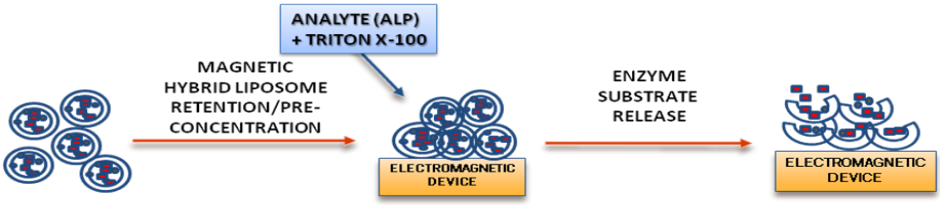
Fluorimetric determination of alkaline phosphatase activity in foods using magnetic-gold nanoparticles liposomes hybrids as useful on-flow micro-container devices

Vanessa Román-Pizarro, Juan Manuel Fernández-Romero and Agustina Gómez-Hens

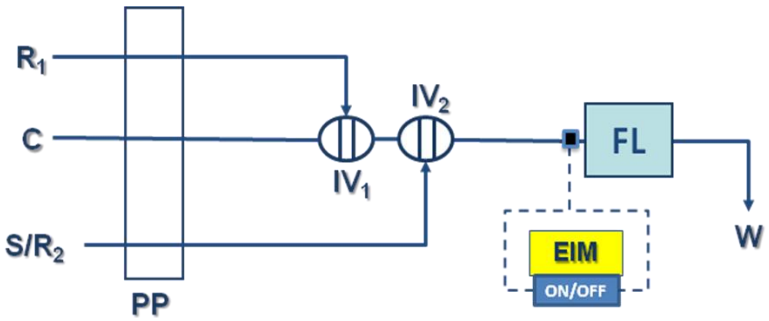
Department of Analytical Chemistry. Institute of Fine Chemistry and Nanochemistry (IUQFN UCO)
Campus of Rabanales. Marie Curie Building (Annex) University of Córdoba
E-14071-Córdoba, Spain. Web site: http://www.uco.es/investiga/grupos/FQM-303
q52ropiv@uco.es

Abstract

The analytical usefulness of hybrid nanostructures formed by magnetic-gold nanoparticles (Fe_2O_3-DT-AuNPs) into liposomes as on-flow micro-containers for the improvement of the reagent preconcentration and the in-situ development of the analytical reaction/detection is presented. The system supposes the use of an electromagnetic device incorporated into a flow injection system for the development the preconcentration analytical reagents, followed by the liposome release and the "in-situ" development of the analytical reaction/detection. The useful of the system was tested by its application to determination of alkaline phosphatase (ALP) activity in foods. A previous synthesis of the hybrid liposomes containing the magnetic-gold nanoparticles and an appropriate target enzyme substrate was also required.



For the production of the hybrid liposomes a step-by-step synthesis process which including the following stages: (1) Synthesis of Fe_2O_3-DT-AuNPs, (2) Covering the magnetic nucleus with a gold layer to form magnetic-gold NPs, which were also modified to produce non-polar Fe_3O_4-AuNPs using 1-dodecanethiol. Finally, these Fe_2O_3-DT-AuNPs were also encapsulated into liposomes with an appropriate surfactant during the liposome formation using the rapid solvent evaporation method. Finally, the hybrid liposomes were slighting resized using mechanical shaking and separated from the un-entrapped NPs using sucrose density gradient centrifugation (SDGC).



A flow injection system equipped with an electromagnetic device was used for develop the automatic preconcentration and enzymatic determination method. The calibration graph of the method was defined in a linear range which covered concentration values from 6.4 – 250 $mU \cdot L^{-1}$, (r^2=0.993, n=7, r=3), with a detection limit of 1.1 $mU \cdot L^{-1}$. The precision, expressed as relative standard deviation (RSD %) was lower than 2.4 %. The overall method shows a sampling frequency of 10 h^{-1}. The features of the method was also compared with those features obtained using two more conventional system developed in batch and flow-injection ways. The method was applied to the determination of the enzymatic activity of ALP in milk samples with recovery values ranging between 87.5 and 104.6 %. The method also have been used for the determination of residual ALP activity in milks subjected to temperature treatments with excellent agreement with the conventional method and also provided acceptable recoveries in all instances.