A general approach to the encapsulation of glycoenzymes chains inside calcium alginate gel beads.

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In this work an enzyme encapsulation general approach, based on the use of calcium alginate hydrogels, is reported. Alginate gels are biodegradable and low cost and have been found to provide a good matrix for the entrapment of sensitive biomolecules. Alginate is an anionic polymer whose gelation occurs by an exchange of sodium ions from the polymer chains with multivalent cations, resulting in the formation of a three dimensional gel network. For gelation alginate is dripped into a calcium chloride solution. The cations diffuse from the continuous phase to the interior of the alginate droplets and form a gelled matrix. By means of this "external gelation method" beads with a diameter of few millimeters can be obtained (see figure 1).

The entrapment of enzymes in alginate beads suffers some disadvantages, like as low enzyme loading efficiency with reduction of the immobilization yields and reusability, related to the enzyme leakage from the large beads pores (cut off of about 100 kDa).

We used alginate beads to entrap, as model system, three glycoenzymes (trehalase, glucose oxidase and horseradish peroxidase) acting in series. Leakage of entrapped enzymes after gelation has been avoided using the biospecific complexation between concanavalin A and sugar residues of glycoenzymes to form large enzyme networks, that cannot go through beads pores. The aggregation process has been monitored by dynamic light scattering technique, while both enzyme encapsulation efficiency and leakage have spectrophotometrically been quantified. Moreover, operational stability of "as prepared" beads has been largely improved by a coating of alternated shells of polycation poly(diallyldimethylammonium chloride) and of alginate.

As a test for the effectiveness of the overall procedure, analytical bio-assays exploiting the enzyme containing beads have been developed for the optical determination of glucose and trehalose [1].

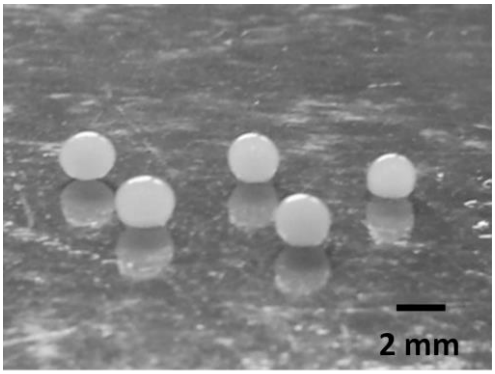

Figure 1 Calcium alginate gel beads.

[1] A. Mallardi, V. Angarano, M. Magliulo, L. Torsi and G. Palazzo, *Anal. Chem.*, 2015, **87**, 11337.