SYNTHESIS AND CHARACTERIZATION OF CARBOXYL AND ACETAL LATEXES BY EMULSION POLYMERIZATION. APPLICATION TO THE PRODUCTION OF LATEX-PROTEIN COMPLEXES FOR DETECTING CHAGAS DISEASE

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***Abstract* — Monodisperse polymer particles with carboxyl and acetal functionalities were synthesized through a two-step emulsion polymerization process. In the first step, latex particles were synthesized by batch emulsion polymerization of styrene (St); and in the second step, the functional monomers (methacrylic acid or acrolein diethyl acetal) were copolymerized with St onto the previously formed polystyrene particles. The synthesized "core-shell" latexes were used as support for their sensitization (by covalent coupling) with two antigenic recombinant proteins of *Trypanosoma cruzi* (RP1 and CP1). Polymer latexes and latex-protein complexes were characterized by measuring their colloidal stability, average particle size, shell thickness and protein thickness through conductimetric titration, dynamic light scattering, turbidimetry and scanning electron microscopy.**

***Keywords* — Emulsion Polymerization, Functionalized Latex, Latex-Protein Complex, Recombinant Protein, Chagas Disease.**

I. INTRODUCTION

The employment of polymer particles as protein carriers in diagnostic kits dates to the middle of the twentieth century. At that time polystyrene (PS) particles with adsorbed specific antibodies against a particular antigen were used as reagents in the so-called agglutination tests (Bolin *et al.*, 1968; Hipp *et al.*, 1970; Smith and Tsáo, 1973; Heymer *et al.*, 1973).

PS particles were broadly used in the early diagnostic kits, but they exhibited an important deficiency: proteins were attached onto the particles only by physical adsorption. The latex-protein complexes obtained by physical adsorption have a limited applicability because the adsorbed protein could slowly be desorbed during storage or denaturated due to the structural rearrangements that they suffer along the adsorption process (Andrade and Hlady, 1986; 1991; Haynes *et al.*, 1994; Basinka and Slomkowski, 1995; Haynes and Norde, 1995; Han *et al.*, 1996). Also, proteins can be removed from the particle surface by adding an emulsifier to the dispersion medium or by increasing its ionic strength.

Along many years researchers have developed synthetic processes to obtain particles with functional groups suitable for the covalent coupling of proteins, without significantly affecting the colloidal stability of the particles and the biological activity of the proteins. Particles functionalization has motivated many efforts for controlling nature, location, distribution and density of the chemical groups on the particle surface for latex application in diagnostic tests (Gibanel *et al.*, 2001; Slomkowski *et al.*, 2002; Choi *et al.,* 2003; Lambert, 2003, Gonzalez *et al.,* 2008a).

Several heterogeneous polymerization techniques are appropriate for synthesizing latex particles. Emulsion polymerization is the most popular one due to the deep understanding of the involved mechanisms. By controlling the experimental polymerization conditions, it is possible to obtain polymer colloids with uniform particles sizes, and controlled compositions, morphologies, and specific surface characteristics, as required for their use in immunodiagnostic tests. Dispersion polymerization is an alternative when particles larger than those produced by the emulsion process are required.

Functionalized latexes are typically produced by emulsion copolymerization. In the unseeded emulsion process, the main hydrophobic comonomer (e.g., St) is polymerized in the presence of small quantities of a hydrophilic functionalization comonomer. In the seeded (or two-step) process, core-shell morphologies are obtained by polymerizing one or more comonomers in the presence of a uniform latex seed (typically a monodisperse PS latex). In this last case, new polymer particles are not generated along the polymerization, and the final particle diameters are reached by a simple growth of the original seed (Ramos *et al.*, 2003; Sanz Izquierdo *et al.*, 2004; Pichot, 2004; Forcada and Hidalgo-Alvarez, 2005; Gonzalez *et al.* 2008a).

Functionalized particles employed for immunodiagnostic tests should fulfill requirements related to size, surface charge density and colloidal stability. Uniform particle size and surface charge density are useful for increasing the colloidal stability, which must be carefully controlled either during the latex synthesis (by adequately adjusting the recipe) or by a post-stabilization protocol. Electrostatic stabilization can be reached by using initiators (persulfate salts, azo-derivatives), whose decomposition provides ionic charges that are covalently bound at the interface. In addition, the incorporation of ionically charged comonomers usually produces a significant increase in the surface charge density (Fitch, 1997). Combination