G - 55

**Conjugation of protein to fatty acids and incorporation into liposomes**

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Liposomes, a promising adjuvant for vaccine, can be used to modulate the immune response to an antigen. Entrapped antigens preferentially elicit IgG antibodies production and antigens exposed to the surfaces preferentially elicit the formation of IgM antibodies. Some antigens are soluble and so they must be derivatizated to increase their hydrophobicity so to enhance their partition into liposome membrane. We present here one derivatization precedure by using the activated carboxylic function, N-hydroxysuccinimide ester of palmitic acid in a containing deoxycholate medium , that was incubated for 18 hours with the soluble recombinat protein of 18 kDa from *Mycobacterium leprae*. The derivatizated protein was added to previously prepared multillamelar vesicles of egg phosphatidylcholine: dicetylphosphate: cholesterol (18:2:5), and chromatographied on Sepharose 4B column. The liposomes containing the protein eluted on void volume as followed by turbidity at 400nm, protein, phosphate and ELISA measurenments. Control experiments were done by PAGE-SDS, gel filtration and ELISA. It was observed that the derivatization procedure preserved the protein epitope recognized by a monoclonal antibody.

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G - 56

**Protein microspheres on the *in vitro* study of controlled release system.**

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Many adjuvants such as aluminum oxide, polymethacrylate simply provide depots for antigen, releasing adsorbed antigen over a relatively short period of time, ranging from several hours to a few weeks at most. Recently it was shown that microparticles consisting antigens conjugated to biodegradable polymers or proteins release the antigens in a controlled manner, providing an ideal delivery system for a new generation vaccine. In this study, we have examined the production of microspheres by conjugation of the 18 kDa recombinant protein of *Mycobacterium leprae* with bovinum serum albumin as matrix. Cross-linking was achieved by incubation with 0.25 % glutaraldehyde. Protein suspension containing glutaraldehyde was immediactely dispersed on a vortex and rapidly injected in sunflower oil and petroleum ether (1:4) at room temperature. Chromatography of reaction mixture on Sepharose-2B or Sepharose-4B shown that the proteins were 100% conjugated and produced particles that eluated in the void volumes. The epitope of the 18 kDa leprae protein was not altered in the formed beads as they were recognized by a monoclonal antibody. The liberation of this protein was studied for 23 days using gel filtration, turbidity, ELISA and protein determinations. As time passed it was observed that particles become smaller and migrated to Vi direction.

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G - 57

ANTI-APHTHOSA VACCINE ENCAPSULATED INTO POLY(D,L-LACTIDE) NANOCAPSULES

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Colloidal drug delivery systems aims mainly at parenteral administration. Nanoparticles are colloidal polymeric particles of a size below 1μm used as carriers for drugs as well as for vaccines. Nanocapsules are suitable drug carrier systems due to their biodegradability and good stability (Kreuter,*J. Control. Release*,16:169,1991). In the present work, we have studied the preparation and physicochemical characterization of injectable anti-aphthosa vaccine entrapped into nanocapsules of polyD,L(lactide) polymer. Nanocapsules were prepared using interfacial deposition of a preformed polymer (Ammoury *et al.,J.Pharm.Sci*,79,763,1990). Poly(d,l lactide) and soya phospholipid was dissolved in differents parties of acetone. Purified sunflower oil is added to the acetone solution. The resulting organic solution is dropped into the aqueous phase containing poloxamer and vaccine and moderate stirred magnetically. The aqueous phase immediately turns milky owing to the nanocapsules formation. The acetone, which has rapidly diffused into the aqueous phase, is then totally removed under reduced pressure. The colloid suspension of nanocapsules is concentrated to the desired final volume by removing water under the same conditions. Immediately after fabrication, both accelerated and long-term stability tests are carried out. Nanocapsules suspension was subjected to ultra centrifugation, mechanical stress and cycles freezing/thawing. The physicochemical characterization of nanocapsules is made by determining the mean diameter, the electrical conductivity, the pH variation and the visual and morphological examination. All parameters were measured just after formulation, and then after 7, 15, 30, 45 and 60 days of storage at a temperature 4ºC. The mean diameter was determined with a coulter Nanosizer by Laser light scattering and the morphological examination was performed using a scanning electron microscopy. The nanocapsules of anti-aphthosa vaccine were obtained with 238±86nm mean diameter. They remain stable for at least two months. However it was observed an increase in the diameter of particles while the pH decreases from 8.0 to 6.2 after seven days. In order to preserve the vaccine activity the nanoparticles were lyophilized using standard experimental conditions with no modification in the physicochemical properties.

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G - 58

**OBTENTION OF POLYURETHANE AS BIOFUNCTIONAL POLYMER**

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Polyurethane (PU) is extensively used in medicine, especially in the manufacture of blood conduits such as artificial arteries, diaphragms and adhesives. Graft copolymers obtained by radiation techniques have been used for the introduction of functional groups onto polymeric matrices. These functional groups may be activated for the chemical coupling of proteins, hormones, drugs or enzymes. In order to prepare materials for biological use we have been studied the bovine serum albumin (BSA) immobilization onto PU films. The graft copolymer with carboxilic groups was obtained by irradiation of PU films (4x4 cm) in aqueous solutions of acrylic acid (AA) (5-50% v/v) with gamma rays of $^{60}$Co source. The PU-g-AA (grafting degree: 12%) was chemically activated for the azide formation on the surfaces. The immobilization of BSA was achieved by immersion of the films into a BSA solution (PBS pH 7.4 and 1.0 mg/ml) at 0$^0$C for 24 h. The coupling yield of BSA was 6%. The introduction of carboxyl groups onto PU surfaces as well BSA alter the hemocompatibility property of the polyurethane elastomer

G - 59

RADIATION MODIFIED POLYMER SUPPORTS FOR THE UREASE IMMOBILIZATION.

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Pellets of low density polyethylene (LDPE) and polypropylene(PP) were used in the immobilization of urease. Acrylic acid (AA) was grafted onto the polymers by simultaneous irradiation technique with $^{60}$Co gamma rays. Hydrogels of LDPE-g-AA and PP-g-AA with various grafting levels were obtained. Hydrogel is a material which posses the capacity of swelling and water retention in its structure, allowing the protein diffusion in the inner nature. That hydrophilicity, measured by the degree of hydration, increased with grafting degree. For the enzyme immobilization the pellets were chemically activated by the method of Coulet et al, where acyl azide radicals are formed from the carboxylic groups grafted on the surface. The relationship between the amount of coupled enzyme and degree of hydration of 0,05, the LDPE-g-AA coupled 10,5% of urease. That amount increased to 14% at the degree of 0,15. Similar results were given by the immobilized PP-g-AA. A better immobilization efficiency was observed in the copolymers with higher grafting level.

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G - 60

**α-AMYLASE IMMOBILIZED ON POLYVINYL ALCOHOL GLUTARALDEHYDE**

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Beads of polyvinyl alcohol (PVA) crosslinked with glutaraldehyde were synthesized under acidic catalysis (0.3 M $H_2SO_4$) using sodium dodecyl sulfate as surfactant in mineral oil. On the other hand, fiber of PVA/glutaraldehyde was produced in 1 M $H_2SO_4$. α-Amylase (Novo; 1.7 % of α-1,4 bond cleavage/min/μg of protein using starch as substrate) was covalently linked onto these two forms of PVA/glutaraldehyde. The enzymatic insoluble water derivatives were incubated with 1 % w/v soluble starch prepared in 50 mM acetate buffer, pH 5.0, in batch reactor (beads) and column (fibers). The total hydrolysis of the starch (5 ml) occurred in about 11 min using beads (1.2 g), as proved by the increase of reducing power and the decrease of the blue value (reading at 660 nm after addition of lugol reagent). The batch reactor using beads showed to be more efficient than the enzymatic column.

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