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Physics and Nuclear Engineering**

Process for preparing of the silicon dioxide-aminopropyltriethoxysilane-glutaraldehyde-ovalalbumin-trenbolone nanoimmunosorbent nanoparticle to be employed in trenbolone ELISA dosing technique

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Abstract

The invention relates to a process for preparing an immunosorbent as nanoparticle, to be employed in trenbolone ELISA dosing techniques, by treating the SiO₂ nanoparticles with a 10% HNO₃ solution, for one hour, followed by incubation with a 10% alpha-aminopropyltriethoxysilane aqueous solution, for 3 hours, at 70°C, washing with distilled water and ethyl alcohol and removing the supernatant by centrifugation, activating the nanoparticles by treating them with a 0.1% glutaraldehyde solution, while stirring, at 35°C, removing the supernatant by centrifugation, suspending the activated nanoparticles in a phosphate buffer, at a pH value of 8.6 and conducting the reaction with trenbolone-ovalbumin immunogenic conjugate obtained by trenbolone activation during the reaction with N-hydroxysuccinimide and carbodiimide in dimethyl formamide and coupling the ovalbumin to the activated steroid, stirring the reaction mixture for 2 hours and centrifuging it to remove the supernatant, after which the resulting immunosorbent nanoparticles precipitate is washed in phosphate buffer, centrifuged and stored at 4°C.

ELISA (Enzyme Linked Immunoasorbent Assay) technique is an immunoassay that use antibodies to measure the concentration of an analyte. Is based on the ability of non-labeled antigen (e.g. hormone) in a specific volume of standard solution or in an unknown sample to complete with a fixed amount of enzymatic labelled antigen for a limited number of binding sites of a specific binding antibody protein.

Trenbolone (17β-Hydroxyestra-4,9-11-trien-3-one) is a steroid used by veterinarians on livestock to increase muscle growth and appetite. Trenbolone compounds have a binding affinity for the androgen

receptor five times as high as that of testosterone. Once metabolized, the drugs have the effect of increasing nitrogen uptake by muscles, leading to an increase in the rate of protein synthesis. It also has the secondary effects of stimulating appetite, reducing the amount of fat being deposited in the body and decreasing the rate of catabolism. Short-term side effects on human include high blood pressure, insomnia, night sweats, increased aggression.

Technology stage

The obtained product can be used in ELISA immunochemical technique for dosing of the trenbolone steroid from biological samples; it was validated in ELISA dosing technique.

Applications

- ELISA kits for detection of androgenic growth promoter, trenbolone from food samples which leads to increased quality of life by using uncontaminated food;
- Human and veterinary endocrinology: quantitative determination of concentrations of anabolic substance;
- Biochemical industry.

Advantages

-covalent coupling of the antigen: immunogenic conjugate ovalbumin-trenbolone to the silica nanoparticles eliminates desorption from the conventional method;
-large specific surface area (> 200 m²/g) as compared to the traditional method (cm²/g);
-decreased assay time in the ELISA of the homogeneous phase to the conventional technique in which the antigen-antibody reaction is heterogeneous (place at the surface of the reaction tube);
-reduced the incubation time necessary to achieve chemical equilibrium due to the number of nanoparticles in suspension (homogeneous phase) with the test substance;
-smaller diffusion distances;
-rapid kinetics of the immune reaction;
-high stability of the nanoimmunosorbent is given by covalently linking of the antigen to the surface of nanoparticles with large surface area (eg. 1 g nanoimmunosorbent is used in the analysis of 10⁵ samples).

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