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Immobilized Enzyme Column Combined with HPLC and Column Switching Method for the Analysis of Complicated Matrix Such As Body Fluids

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Enzymes are bio-catalysts which affect transformation of substrates to products with high specificity. The usage of enzymes in domestic and industrial applications is well known and has been well documented since the early history of civilization. With the advances in understanding of enzymology, usage of enzymes in industrial and biotechnological processes and molecular medicine has proliferated.

One of the key factors in the widespread application of enzymes in modern technologies is the development of enzyme immobilization techniques (Figure 1), which overcome certain practica1, functional and economic constraints. Many natural enzymes can be stabilized by immobilization on solid matrices, with most of the activity retained for a variety of applications. An important application of immobilized enzymes is in liquid chromatography. In the last decade, post-column enzyme detection has become established as an important discipline in liquid chromatography. The new detection approach offers more sensitive and specific ways for measuring major classes of biomolecules (Figure 2).

Reactors are fabricated by packing the immobilized enzymes into small columns, which can be placed immediately after or prior to an HPLC column. When an analyte, which is a natural substrate of the enzyme, passes through the reactor, it will be transformed into a product in the presence of a coenzyme. The enzymatic reaction can be designed in such a way that one of the reaction products will be amenable to optical (UV or visible) or electro-chemical detection (Figure 3). Hence、compounds that are otherwise difficult to detect by conventional approaches are rendered readily detectable with improved sensitivity. Alternatively, certain reactors can be placed immediately before the injector to transform an analyte into a product that can be chromatographed for subsequent detection. The specificity of the reactor, in general, is dependent on the nature of the immobilized enzymes. Some enzymes have broad specificity, catalysing the transformation of a specific functionality of an entire family of compounds、while other enzymes have absolute specificity, reacting with a single substrate (Figure 4).

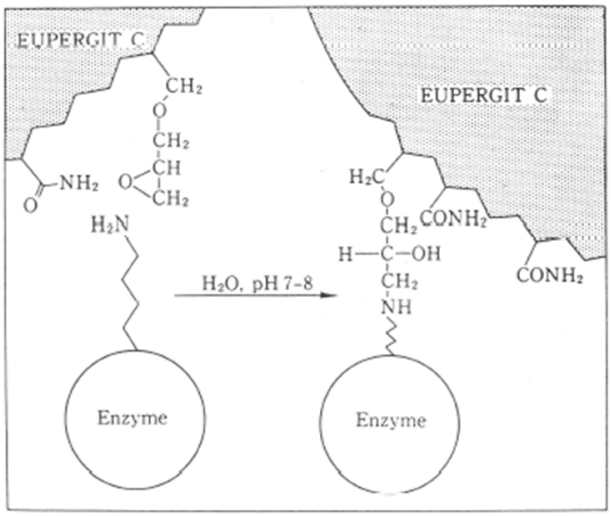

Figure 1: Immobilized urease on commercially available oxirane support.

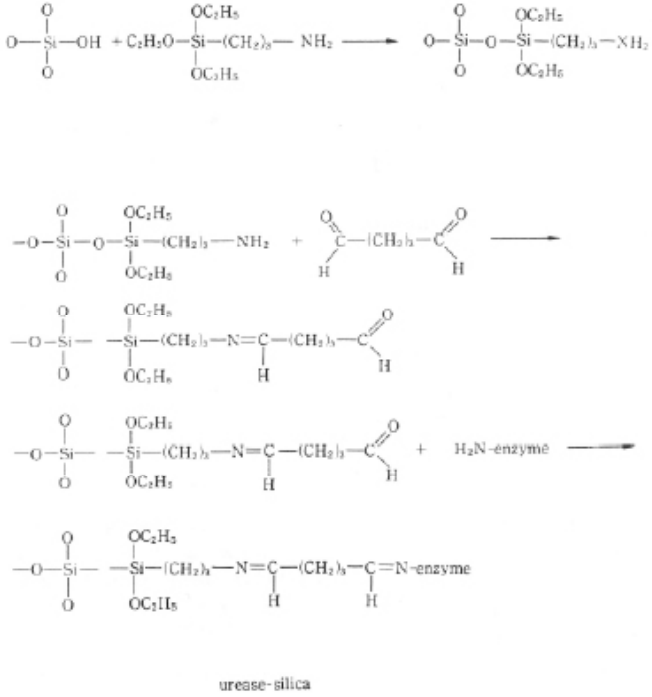

Figure 2: Conventional immobilization of urease on silica base via Schiff base linkage.

The enzyme reaction detection liquid chromatography systems offer new bio-analytical techniques to pharmaceutical scientists and biochemists for effective and sensitive measurement of drugs and conjugated metabolites in pharmaceutical formulations and physiological metabolites. They also offer the broader analytical community a new technique with great potential [1] (Figure 5).

This editorial will describe the analytical application of immobilized enzyme reactors posterior to [1] or prior to HPLC [2] for selective analysis of specific compound in the complicated matrix. The integration of the reactors into detection systems in liquid chromatography provides new ways of measuring biomolecules with very high sensitivities. In this editorial one example of urea analysis combined with immobilized urease and indophenol or OPA detection

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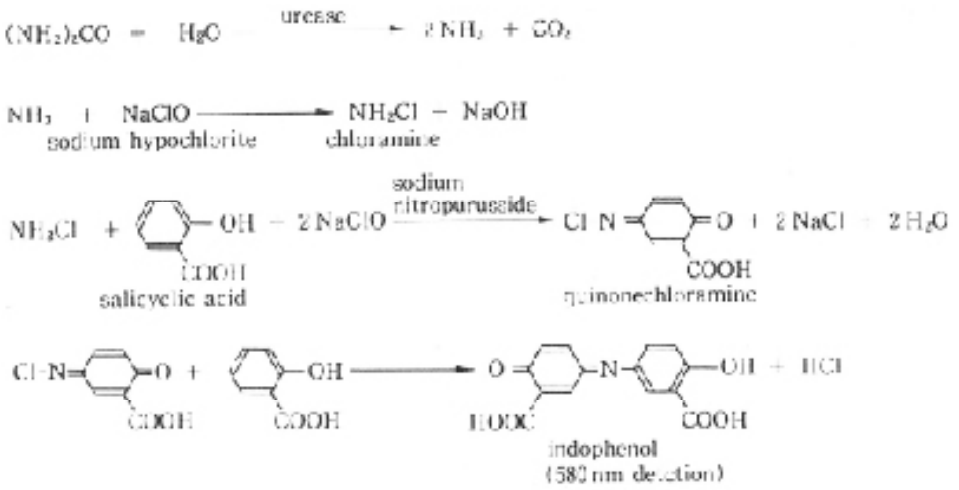

Figure 3: Indophenol colorimetry detection of urea.

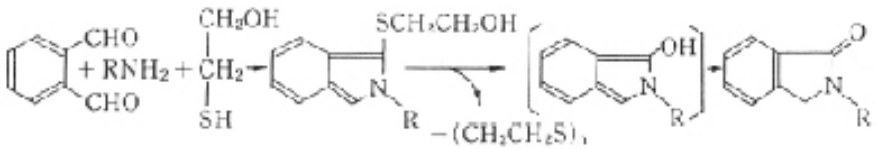

Figure 4: Orthphthalaldehyde (OPA) fluorescence detection of urea.

are provided [1,2]. Immobilized urease can be available commercially and this procedure is also presented as Figure 1. This information may help to prepare immobilized enzymes to the researchers who are studying for the analysis in the complicated matrix.

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