

## Synthesis and activation of Immobilized beads by natural dye extracts

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### Abstract

Immobilization technique is used for preservation of enzymes. Here we are using different colors of natural dye in this technique. Our major emphasis would be to create beads of different colors which will act as different carriers for essential enzymes. The need for using colorful dye is because when we need to preserve more than one enzyme then it is helpful in identification of that which enzyme is to be preserved and in which color. Here we used natural dye because most of the chemical dyes are carcinogenic in nature and may alter the nature of preserved enzyme. For this different plant products like mint leaf, rose petal, beat root and Carrot are used for the extraction of dye. In our research studies, we have identified, extracted, characterized, optimized and standardized the natural dyes from plant and microbial sources and we did a comparative study between natural dyes and artificial dyes with respect to different solvent systems like petroleum ether, diethyl ether, acetone, chloroform, ethanol and water systems. The extraction methodologies, characterization, MIC (minimum inhibitory concentration), and solubility studies will be discussed. These immobilization studies will help us to use this application in a variety of fields like in wine stabilization, in modifying the shelf life of food and other natural products which degrade quickly and are difficult to preserve under natural conditions. Here sodium Alginate beads are being used so that there is good number of beads formation and that will help for the proper entrapment of the essential enzymes required for an important reaction in Bio-systems.

### Key words:

characterization, MIC, immobilization, Dye, extraction, Sodium Alginate, beads

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### Introduction

Immobilization is a very useful technique to preserve enzyme. In this technique enzyme can be attached to an inert, insoluble material which is the result of the reaction between sodium alginate and calcium chloride. After the reaction an inert

layer of calcium alginate is formed which is called Bead. Immobilization onto micro-beads via adsorption by the multi-modal ligand could also provide strong electrostatic and hydrophobic interaction between the enzyme and support (Yavuzet al., 2009; Yang et al., 2007). The multi-dentate ligand could hold certain advantages as a multi-modal ligand for various large-scale industrial applications (Johansson et al., 2003; Chang et al., 2007). For example, these molecules are resistant to harsh chemicals, temperature and high salt concentrations. In addition, this kind of support can be recycled after inactivation of immobilized enzyme and offers cost effective processes (Bayramoglu et al., 2008; Torres et al., 2005). This can provide maximum resistance to change in surrounding environment like pH, temperature etc. By this method enzyme can be preserved and use after a long time. In some cases, immobilization of enzymes on hydrophilic supports leads to a pronounced loss of enzymatic activity. This decrease in the enzymatic activity can be due to a partial unfolding of the protein resulting from the adsorption of proteins on solid surfaces (Arica and Bayramoglu, 2006; Lahari et al., 2010). Enzymes can be immobilized using either the isolated enzymes or the whole cells or cellular organelles. Immobilization of whole cells has been shown to be a better alternative to immobilization of isolated enzymes<sup>2-4</sup>. Doing so avoids the lengthy and expensive operations of enzyme purification, preserves the enzyme in its natural environment thus protecting it from inactivation either during immobilization or its subsequent use in continuous system. It may also provide a multipurpose catalyst, especially when the process requires the participation of number of enzymes in sequence. Compared with chemical methods for immobilization of enzymes onto carriers (Huang et al., 2008), physical methods, especially adsorption, may have a higher

commercial potential because it is simpler, less expensive and can retain high catalytic activity. Many protocols for enzyme immobilization involve irreversible binding to a functionalized support. In the reversible enzyme immobilization, the supports could be regenerated using a suitable desorption agent, and they be recharged again with a fresh enzyme. On the other hand, when the covalently immobilized enzyme becomes inactivated upon use both the enzyme and the support should be eliminated as wastes (Bayramoglu et al., 2010a, 2008; Zhou, 2010). The reversible immobilization of enzyme on the ligated support is based on the non-covalent interactions between enzyme and support. In the case of multi-modal ligand, such interactions increase by electrostatic forces and hydrogen bonding in addition to hydrophobic interactions (Arica and Bayramoglu, 2006; Wang et al., 2010; Bolivar et al., 2009; Torres et al., 2005). The target protein itself is also a multimodal molecule.

## Materials and Methods

### Extraction of Dye

Here beat root (Fig 3), rose (Fig 1), carrot (Fig 4) and mint (Fig 2) are used. First all these things are washed and then cut into small pieces. All these small pieces are kept separately in different bowls. After then electronic grinder is used for grinding the small pieces. By doing this four different colours of dyes from all the materials are extracted. Then dyes are collected in four different test tubes differently.

### Preparation of Bead formation

Sodium alginate and calcium chloride are used for preparation of 4% CaCl<sub>2</sub> and 0.1N NaCl sodium solution. For preparation of 4% CaCl<sub>2</sub> by weight 4 gm of CaCl<sub>2</sub> is mixed into 100ml water. Then by using magnetic stirrer the solution is mixed properly. After that the solution is kept at 4 degree centigrade for 2 hours. After then 0.1N NaCl sodium alginate solution

is prepared. For this preparation first 0.1N NaCl solution is prepared. In this preparation 0.685gm of NaCl is mixed into 100ml water. Hence the 0.1N NaCl solution is prepared. In this solution 3.5gm of Sodium Alginate is mixed. So the 0.1N NaCl Sodium Alginate solution is prepared. Then the solution is kept for incubation.

#### **Immobilization Technique**

In four separate beakers, 20 ml of Sodium Alginate is taken in each. Four different coloured dyes prepared earlier are now added separately to four beakers containing Sodium Alginate. Hence four different coloured Sodium Alginate solutions are prepared. Now CaCl<sub>2</sub> is taken into four different beakers. Then using dropper Sodium Alginate solution of different colour is added drop by drop into different beakers containing CaCl<sub>2</sub> so that there are four different colours of beads formation.

#### **Entrapment of Enzyme**

Alkaline phosphatase is brought from Sigma Aldrich and was be used for entrapment after being cross linked with glutaraldehyde. The entire Composition of 5 ml has been kept in with 4 different tubes at 0°C and again 3ml of glutaraldehyde in phosphate buffer was added. This was followed by vacuum rotary evaporation at 60°C, grinding, and washing.

#### **Studies on Degradation of casein in Agar**

Casein plated agar was taken as control. The chemicals required were 50mM potassium phosphate buffer, pH7.5 at 37°C, 0.65 % (w/v) Casein Solution, 110mM TCA, 500mM Na<sub>2</sub>CO<sub>3</sub> and 10mM Sodium Acetate Buffer. In another 4 petridishes, all four types of beads were added and the degradation of casein protein was studied, observed and their diameter of degradation was calculated.

#### **Results and Discussions**

All the natural materials Mint, Carrot, Rose, Beet produce different types of dyes having different colors like dark green, orange, dark red, chocolate green respectively. When different coloured Sodium Alginate solutions is added drop by drop into different beakers containing CaCl<sub>2</sub> then four different colours of beads are formed. Here enzymes can be safely preserved by natural dyes. Using the chemical dye for enzyme preservation technique may affect the chemical nature of the preserving enzyme which leads to some changes in enzyme activity. But if we use dye extracted from natural materials like Mint, Rose, Carrot, etc. it doesn't affect the nature of enzyme. It is beneficial to use different natural material for extraction of dye because we obtain the dye of different colours and it is helpful to identify that which dye is used for preservation of which enzyme. Using chemical dye may harmful for experimenter because dye may have carcinogenic in nature and it may leads to cancer. But natural dye has no such harmful effect on human health.

#### **Bead Formation**

4 different colours of beads are formed as shown in (Fig 5), (Fig 6), (Fig 7), (Fig 8).

#### **Studies on Degradation of casein in Agar**

All four types of beads were added and the degradation of casein protein was studied, observed and their diameter of degradation was calculated as shown in (Table 1). By characterising the peptide sequence, we defined the pathway of casein hydrolysis which leads to the formation of small peptides through intermediate oligopeptides. It was found that the action of enzyme acting on specific lysine residues is the primary step in casein degradation. In this process, a series of potentially bioactive peptides and their precursors are produced.

Casein consists of a fairly high number of proline peptides, which do not interact. There are

also no disulfide bridges. As a result, it has relatively little tertiary structure. It is relatively hydrophobic, making it poorly soluble in water. It is found in milk as a suspension of particles called casein micelles which show some resemblance with surfactant-type micelle in a sense that the hydrophilic parts reside at the surface. The caseins in the micelles are held together by calcium ions and hydrophobic interactions. Several models account for the special conformation of casein in the micelles (Dalglish, 1998). One of them proposes the micellar nucleus is formed by several sub micelles, the periphery consisting of microvellosities of  $\kappa$ -casein (Walstra, 1979; Lucey, 2002). Another model suggests the nucleus is formed by casein-interlinked fibrils (Holt, 1992). Finally, the most recent model (Horne, 1998) proposes a double link among the caseins for gelling to take place. All three models consider micelles as colloidal particles formed by casein aggregates wrapped up in soluble  $\kappa$ -casein molecules. Casein has been documented to break down to produce the peptide casomorphin, an opioid that appears to act primarily as a histamine releaser.<sup>[15]</sup> Some research indicates that this casomorphine aggravates the symptoms of autism.<sup>[16]</sup> A 2006 review of seven studies indicated that, although benefits were seen in all studies from the introduction of elimination diets (e.g., casein-free or gluten-free) in the treatment of autism spectrum disorders, none of the studies were performed in a manner to create an unbiased scientific opinion.

### **Conclusion**

This process is also helpful during the reaction as the enzyme is held in place throughout the reaction and hence enzyme can be easily separated from the

products and may be used again. Immobilization often stabilizes structure of the enzymes, thereby allowing their applications even under harsh environmental conditions of pH, temperature and organic solvents, and thus enables their uses at high temperatures in non-aqueous enzymology and in the fabrication of biosensor probes.

Dyes have been the subject of much interest in recent years. Many industries (plastics, paper, textile and cosmetics) use dyes in order to colour their products. Over 100,000 commercially available dyes exist and more than 7£105 tonnes per year are produced annually (Pearce et al., 2003; McMullan et al., 2001). Here the use of natural dye is helpful in preserving more than one enzyme at the same time in different colours for its easy identification. Natural dyes/colorants have been used historically throughout the world. The use of natural dyes/colorants has decreased to a large extent due to the advent of synthetic dyes. Recently, dyes derived from natural sources have emerged as important alternatives to synthetic dyes, which have been reported to have carcinogenic effects (Sewekow, 1988). With the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has once again gained interest (Eom et al., 2001; Padhy and Rathi, 1990; Garg et al., 1991). The plant kingdom offers a vast source of natural dyes/colorants which can be obtained from many plant parts e.g., leaves, fruits, seeds, flowers, barks and roots. The sub-Himalayan region of north-eastern India has an abundance of plant species with dye-yielding properties. Conventionally, some of the rural folks of the region extract dyes from leaves, roots, flowers or bark of some plant species mostly by boiling, scrapping, powdering and mixing with other materials to get the desired colour. However, growing environmental concern with regard to synthetic dyes, natural dyes offer scope for eco-friendly way of dyeing of fibrous materials such as textiles or leather and for food coloration. Natural dyes are non toxic



and non allergic which are very important for some sensitive applications. For example red colour can be obtained from rose flower, beat root etc., green colour from mint leaves or any green leaves. These plant product can be easily obtained and at a very low cost. Dyes are also very easy to obtain from these products. It also consumes a very little time. These dyes are also safe to use as they do not cause any bad effects on human health.

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**Table 1:** Showing diameter of degradation by each type of beads

Beads	Diameter of Degradation (mm)
Control	--
Rose	11
Mint	07
Beetroot	15
Carrot	19



**Figure 1:** Rose Collected from the garden



**Figure 2:** Mint leaves brought from Nilgiri



**Figure 3:** Beet root from Vellore Market



**Figure 4:** Carrot from Vegetarian Mess.



Figure 5: Formation of Red Rose Beads

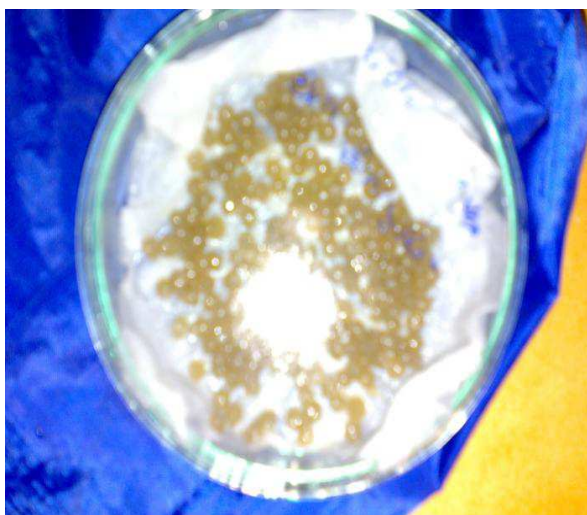


Figure 6: Formation of Orange Carrot Beads



Figure 7: Formation of Reddish Pink Beetroot Beads



Figure 8: Formation of Faded Green Mint Beads

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