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**BIODEGRADATION OF CHEMICALLY MODIFIED EGG ALBUMIN  
MICROPREPARATION FOR CONTROLLED RELEASE OF BOUND *VIGNA MUNGO*  
AMYLASE AND THEIR APPLICATION IN FABRIC DESIZING AS COST  
EFFECTIVE BIO-ACTIVE PREPARATION**

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**ABSTRACT**

In our present work, purified and thermally stable *Vigna mungo* amylase was entrapped into toluene modified egg albumin microparticles through covalent coupling by glutaraldehyde. Biodegradation of chemically modified egg albumin microparticles was carried out by using various units of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U) for the controlled and sustained release of entrapped enzymes from prepared microparticles. Enzyme storage stability was found to be increased up to 6 months as well as thermal stability, enhanced up to 72°C as compared to free enzyme (24 hours only and 40°C respectively). Further, this chemically modified enzyme loaded egg albumin microparticles along with studied effective units of alkaline protease was used with different samples of market available detergents powder (Aerial, Surf Excel, Wheel and Tide) for washing of tough and dry lipstick stained cloths pieces having strains of well known brands (Revlon, Maybelline, Lakme and Dior). These lipstick strains are, sometimes, used to be very difficult to leach out from fabrics and, usually, not to vanish in one wash while they were used to be soaked in lukewarm tap water too when washed with well known brands of commercially available washing powders. But, this cost effective enzyme loaded bio-active micropreparation with alkaline protease were used with chosen detergents powder for washing of these dry, tough lipstick stains lead to vanishing those strains very fast with absolute clear visible results when, their results were compared to results of washing of stained cloths pieces with detergents only.

**KEYWORDS:** *Vigna mungo* amylase, egg albumin, glutaraldehyde, microparticles, micropreparation, entrapment



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## INTRODUCTION

Various commercially available enzymes are bio-catalysts that play a vital role in most of the chemical processes<sup>1</sup>. Although, these enzymes are used in a wide range of industrial applications apart their high cost and encountered high labor expense during their handling and preservation<sup>2,3</sup>. The main problem is found their sensitive operational prevailing environmental and instrumentation thresholds which limits their shelf life and limited reusability with least expected reproducibility upon its reuses<sup>4, 5</sup>. Hence, enzyme entrapment is the most common and successful method to get rid out of these problems<sup>6</sup>. Entrapment, itself, enhances change in the inherent properties of free enzyme after their immobilization on chemically viable matrices which widens their industrial applications after chemically modification by increasing their thermal stability and operational threshold<sup>7</sup>. Among these enzymes, amylase has a potential industrial applications, especially in fermentation, textile, food, detergent and paper industries<sup>8,9,10</sup>. Enzyme microentrapment has been used as carriers for the delivery of a wide range of drugs as anticancer, antifertility, antihistamines, antituberculosis chemical preparation as excellent therapeutic clinical robotic chemical signals<sup>11,12</sup>. Albumin is biodegradable and non toxic in its chemical nature and cost effective biological compound due to its exploitable characteristics, it has been previously used for the preparation of drug bound and enzyme bound microspheres<sup>13,14</sup>. In our work, egg albumin was used as a biomatrix for entrapment of *Vigna mungo* amylase in which egg albumin was chemically modified with toluene and glutaraldehyde. This micropreparation was lead to increased storage stability and thermal stability of entrapped enzyme as compared to free enzyme. The kinetic parameters for free and entrapped enzyme such as pH, incubation time, temperature, substrate concentration and calcium chloride concentration were studied by carrying out the enzyme activity by using dinitrosalicylic acid method<sup>15,16,17,18</sup>. The biodegradation of chemically modified egg albumin micropreparation was performed with

different units of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U) to study controlled and sustained release of entrapped enzyme from the chemically modified chosen matrices which was subjected to washing of the stained clothes with detergents<sup>16,18,19,20</sup>. Hence, the fabric desizing applications of chemically modified *Vigna mungo* amylase entrapped egg albumin micropreparation was studied with chosen samples of washing powder (Ariel, Surf excel, Wheel and Tide) for washing of dry, tough lipstick stained clothes pieces with cosmetics lipstick stains of well known brands (Revlon, Maybelline, Lakme and Dior)<sup>19,20</sup>.

## MATERIALS AND METHODS

### (i) Extraction of amylase enzyme from *Vigna mungo* germinated seeds

*Vigna mungo* seeds were soaked for 3 days to get their 3 days germinated seedling as sprouts and homogenized in a pestle mortar by adding 4-6ml of 0.05M sodium phosphate buffer (pH 7.0) per gm of germinated seeds. The enzyme extract was filtered in two layers of cloth and centrifuged for 15mins at 4°C at 5000rpm. Supernatant was collected which contained crude amylase extract and stored at 4°C<sup>18</sup>.

### (ii) Amylase Assay

Enzyme assay was done by using 1 % (w/v) starch solution in which 0.5 ml enzyme extract was added. It was incubated at 37°C for 20 minutes. 2 ml of dinitrosalicylic acid was added and the mixture was boiled at 100°C for 5 minutes. Absorbance was taken at 570nm<sup>18, 21, 22, 23</sup>.

### (iii) Purification by ammonium sulphate precipitation and dialysis

80% of ammonium sulphate precipitation was done in ice bath by adding ammonium sulphate salt in to crude enzyme extract and stirred with aglass rod for 25 minutes after each and every addition of salt. After the complete addition of ammonium sulphate salt was stirred for 30 minutes and incubated for 1 hour and centrifuged for 25 minutes at 4°C at 10000rpm.

Supernatant was discarded and the pellet was dissolved in 0.05 M sodium acetate buffer (pH 3) and kept at -20°C. Dialysis was done in partially purified enzyme extract by using dialysis tubing which was rinsed with distilled water whose one end was clutched with a thread and 10 ml of sample was poured into it and the other end was also clutched called dialysis bag which was placed in 10mM Tris HCl buffer. It was incubated for 24 hours at 4°C. The pure sample was poured out after incubation and kept at 4°C<sup>24,25</sup>.

#### **(iv) Study of kinetic properties**

The free enzyme and immobilized enzyme are characterized for their different kinetic properties i.e. effect of pH, effect of temperature, effect of incubation time, effect of CaCl<sub>2</sub> concentration and effect of substrate concentration. The effect of pH on activity of free and immobilized enzymes was studied by performing enzyme assay at different pH varied from 2 to 12. The effect of time on the activity of free and immobilized enzyme was studied by performing the enzyme assay at different time (5minutes-25minutes). Optimal substrate concentration needed for maximal enzyme activity for free and immobilized enzymes, which were estimated by incubating the reaction mixture at different concentrations of starch solution (0.25%-1.50%). The effect of CaCl<sub>2</sub> on activity for free and encapsulated enzymes was studied by performing the enzyme assay at different concentrations (2%-10%). Optimal temperature needed for free and encapsulated enzyme for maximal activity was studied by incubating the reaction mixture for 15minutes at

different temperatures (10°C – 100°C). These kinetic properties of enzyme were determined by performing dinitrosalicylic acid method<sup>15,16,17,18</sup>.

#### **(v) Entrapment of Vigna mungo amylase into Chemically Modified Egg Albumin**

A solution of 0.5 ml of glutaraldehyde and 4.3 ml of toluene was mixed with 20ml with 0.05M sodium acetate buffer (pH 3.0) in beaker as chemically modifier bath. Then, 50U of free enzyme and 8-10 ml of egg albumin was mixed well in test tube and vortex it. Hence, it was taken in 10 gauge syringe and dispersed uniformly in one stroke in prepared chemically modifier bath. It was kept overnight in an incubator shaker at 37°C. Next day, it was centrifuged at 5000 rpm at 4°C for 30 minutes. Supernatant was removed and the pellet was washed with cold diethyl ether and acetone. The prepared bioactive enzyme bound egg albumin micropreparation was dried and stored at 4°C. Enzyme assay was done in supernatant to estimate % of entrapment of enzyme in chemically modified egg albumin micropreparation<sup>15, 19, 27</sup>.

#### **(vi) % of entrapment of enzyme into chemically modified egg albumin micropreparation**

The % of entrapped enzyme was calculated by determining the residual enzyme activity from the reaction mixture in which encapsulation of enzyme was done into chemically modified egg albumin was done. Amylase assay was performed by using dinitrosalicylic acid<sup>14, 15, 19, 27</sup>.

$$\% \text{ of entrapment} = \frac{\text{Specific activity of entrapment enzyme}}{\text{Specific activity of free enzyme}} \times 100$$

#### **(VII) Characterization of Vigna mungo amylase entrapped chemically modified egg albumin micropreparation**

The prepared bio-active micropreparation were subjected to Scanning Electron Microscope (SEM) for determination of the microparticle size of enzyme bound egg albumin micropreparation.

#### **(VIII) Biodegradation of chemically modified egg albumin micropreparation for controlled release of entrapped Vigna mungo amylase by using alkaline protease**

2 mg chemically modified egg albumin micropreparation were taken in test tubes with reaction solution of different units of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U,

40U). The reaction tubes were incubated at 4°C for overnight. Next day enzyme assay was done at 570 nm using dinitrosalicylic acid method. This study of enzyme assay for different units of alkaline protease was done from 1<sup>st</sup> day till 10<sup>th</sup> day<sup>20, 25, 34</sup>.

**(IX) To study application of *Vigna mungo* amylase bound chemically modified egg albumin micropreparation in fabric desizing**

*Vigna mungo* amylase bound chemically modified egg albumin micropreparation was used in removal of tough, dry cosmetics long lasting lipsticks stains of different international brands from cotton cloth by various market available detergents such as Ariel, Tide, Surf and Wheel only and with the prepared bioactive mixture of *Vigna mungo* amylase bound chemically modified egg albumin micropreparation and effective unit of alkaline protease with the chosen detergent powders. Stained cloths pieces having dry strains of lipstick brands (Revlon, Maybelline, Lakme and Dior) were chosen and dipped in reaction solution of 2 mg of prepared ssbioactive enzyme bound egg albumin preparation with 1ml of different chosen washing powder solution in petri plates. Each sample of stained cloths

was tested with all the four chosen detergents only and with the combination mixture of bioactive enzyme bound chemically modified egg albumin micropreparation for carrying out its comparative washing study for desizing of fabrics<sup>19, 20</sup>.

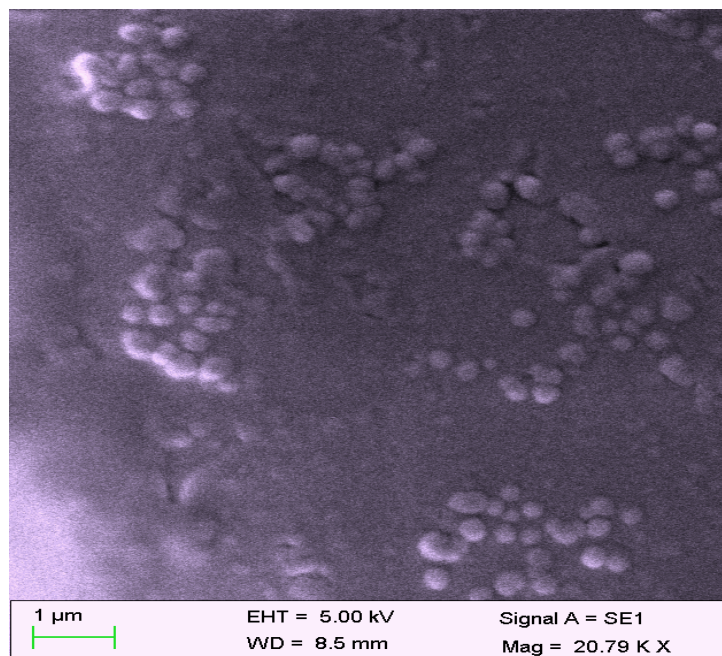
## RESULTS AND DISCUSSION

### (i) % of Entrapment

*Vigna mungo* amylase bound chemically modified egg albumin micropreparation has 72% retention of enzyme activity whose result was similar to previous studies in which *Vigna mungo* amylase had 72%-84% retention activity of enzyme into chemically modified bovine serum albumin<sup>15, 26</sup>.

### (ii) Characterization of *Vigna mungo* amylase entrapped chemically modified egg albumin micropreparation

The prepared bio-active micropreparation were subjected to Scanning Electron Microscope (SEM) (AMITY ZEISS) for determination of the microparticle size of enzyme bound egg albumin micropreparation (Fig 1)<sup>34</sup>.



**Figure 1**  
**SEM of chemically modified egg albumin micropreparation**

**(iii) Studied kinetic properties**

The optimal pH was found to be 4.0 for entrapped *Vigna mungo* amylase same as that of free enzyme. The optimum incubation time was found to be 25 minutes for maximal enzyme activity for entrapped enzyme which was higher than that of free present study the optimum incubation time for an entrapped enzyme was 25 minutes which is higher than the free enzyme (20 minutes) which are pretty similar to the previous results<sup>17, 25</sup>. There was no change in optimal substrate concentration for free and entrapped enzyme (1%-1.25%) whose observations was similar to the previous observations findings and optimal CaCl<sub>2</sub> concentration was found to be 2% for entrapped enzyme which was less than that for free enzyme (8%) whose results was similar to previous results<sup>17, 25</sup>. Optimal temperature was

found 50°C for encapsulated enzyme as compared to free enzyme which was 40°C at standard optimal kinetic parameters and thermal stability was found to be up to 72°C after the entrapment which was happened due to change in hydrophobic and other secondary interactions of immobilized enzyme to have changed conformational flexibility needed higher temperature for enzyme molecule to recognize and attain a proper conformation in order to keep its maximal reactivity to get changed temperature optima as compared to unbound enzyme<sup>16, 28</sup>. Hence, It was confirmed that after chemically modified egg albumin entrapment, thermal stability of entrapped enzyme was enhanced as compared to free enzyme and pretty comparable to previous data (Table 1)<sup>19, 24, 28, 29, 30</sup>.

**Table 1**  
**Kinetic Parameters on free and entrapped *Vigna mungo* amylase into chemically modified egg albumin micropreparation**

Kinetic Parameters	Free enzyme	Entrapped enzyme
pH optima	4.0	4.0
Optimal Time of incubation	20 minutes	25 minutes
Optimal Substrate concentration	1.00%	1.00%
Optimal CaCl <sub>2</sub> concentration	8%	2%
Temperature Optima	40°C	50°C
Thermal Stability	Up to 40°C	Up to 72°C
Storage Stability	Up to one week	Up to 6 months

**(IV) Biodegradation Study**

Biodegradation of chemically modified egg albumin micropreparation was performed by incubating 2mg of encapsulated enzyme egg albumin micropreparation with different units of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U) overnight at 4°C to study controlled and sustained release of bound enzyme. The study was carried out for consecutive 10 days. It is also well known that a alkaline protease is more suitable for detergent industry because its pl coincides with the pH of the detergent solution.<sup>35</sup> We were observed that

35U of alkaline protease are more effective bio-activator to achieve controlled and sustained release of entrapped *Vigna mungo* amylase form chemically modified egg albumin micropreparation (Fig 2) and biodegradation pattern for each bio-active incubation mixture showed an increase from 1<sup>st</sup> to 5<sup>th</sup> day and remain constant till 10<sup>th</sup> day whose results were comparable with previous study in which release of entrapped enzyme was studied from chemically modified bovine serum albumin with 30U of alkaline protease solution<sup>15, 19, 25, 26, 28, 34</sup>.

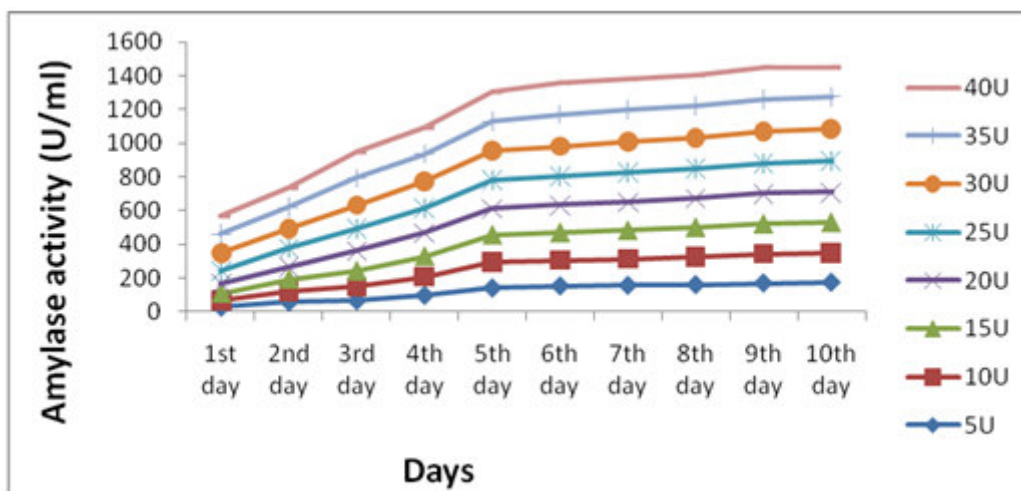


Figure 2

**Biodegradation of chemically modified egg albumin micropreparation with different concentration of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U) for controlled release of bound *Vigna mungo* amylase**

**(V) Applications of chemically modified egg albumin micropreparation of encapsulated *Vigna mungo* amylase in desizing of fabric**

The application of prepared egg albumin micropreparation of entrapped enzyme was studied with four chosen different samples of detergent solutions (Ariel, Tide, Surf excel and Wheel) to remove long lasting lipstick stains of different popular international brands (Revlon, Maybelline, Lakme and Dior) (Fig 3) from stained cloth pieces. The bio-active enzyme bound chemically modified egg albumin preparation with alkaline protease solution with chosen detergents was used for washing to vanish dry tough lipstick strains from stained cloth pieces during its fabric desizing study to enhanced the washing efficiency of detergent powder. Because these kinds of tough stains of cosmetics were usually not to be vanishing completely if we washed with the chosen detergent samples only in one wash even when soaked in lukewarm water too. Among the four

chosen samples of commercially available detergent powder solutions, Ariel detergent with the bioactive enzyme bound egg albumin micropreparation mixture was showed best and fairly well visible washing results as compared to others followed by Surf excel, Wheel and Tide respectively (Fig 4, 5, 6 & 7 respectively) which were fairly comparable to previous washing analysis<sup>20,22,34</sup>. Hence, the use of this eco-friendly, cost effective bio-active enzyme bound chemically modified egg albumin micropreparation with detergents has advantages in desizing of stained fabrics to enhance washing efficiency, to reduce labour cost and time due to nil requirement of expertise or zero involvement of any functional washing step. Even after the entrapment, storage and thermal stability of enzyme was also increased which made it more washing efficient bio-agent as desizing catalyst to vanish the dried and tough cosmetics stains completely form cloths pieces when used with chosen detergents<sup>19, 21, 22, 31</sup>.



**Figure 3**  
**Chosen Lipstick samples used for staining the dried and tough cosmetics stains on cloths pieces**



**Figure 4**  
**Washing results of stained cloth pieces with Ariel only and Ariel with Vigna mungo amylase bound chemically modified egg albumin bio-active micropreparation**

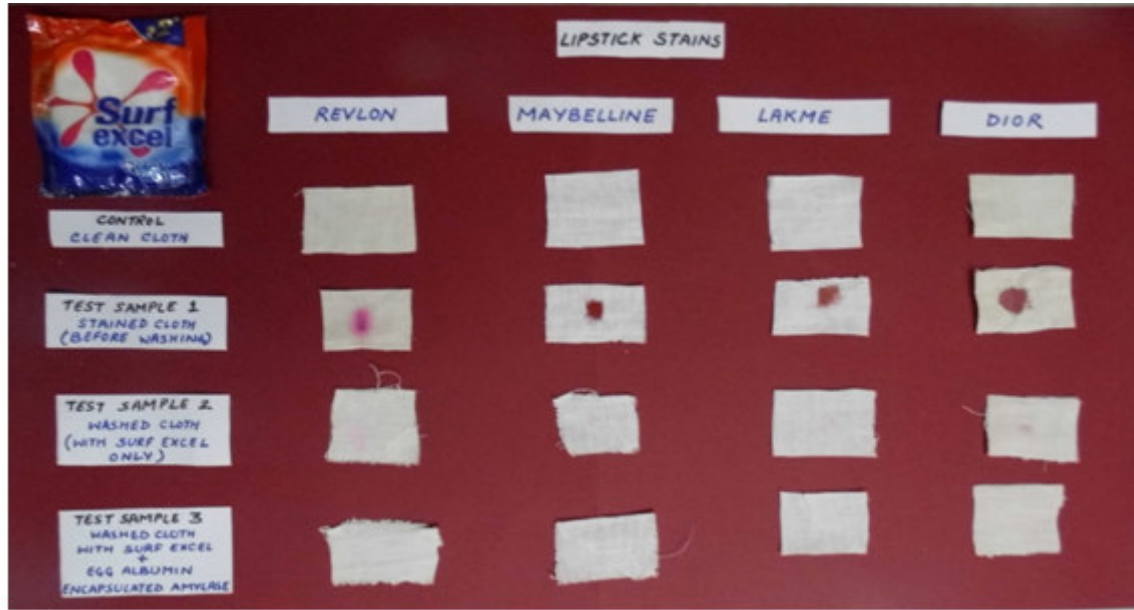


Figure 5

*Washing results of stained cloths with Surf excel only and Surf excel with with Vigna mungo amylase bound chemically modified egg albumin bio-active micropreparation*

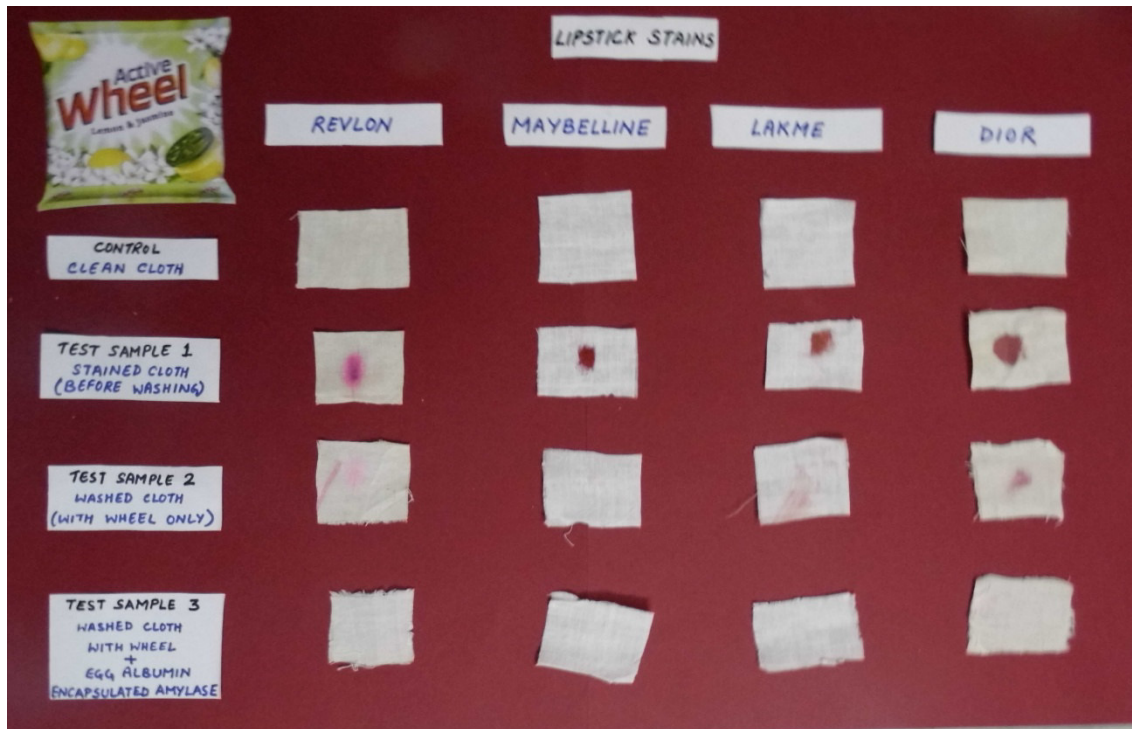


Figure 6

*Washing results of stained cloths with Wheel only and Wheel with with Vigna mungo amylase bound chemically modified egg albumin bio-active micropreparation*

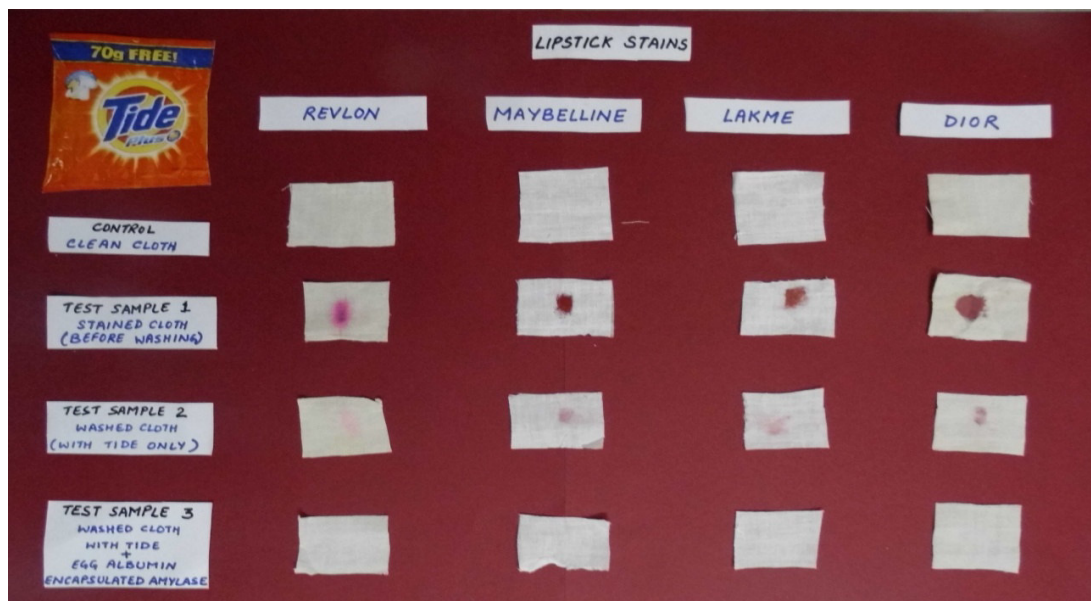


Figure 7

**Washing results of stained cloths with Tide only and Tide with with *Vigna mungo* amylase bound chemically modified egg albumin bio-active micropreparation**

## CONCLUSION

*Vigna mungo* amylase is entrapped into chemically modified egg albumin micropreparation by using toluene and glutaraldehyde via covalent coupling with 72% of its entrapment. There were some changes observed in the studied kinetic properties e.g. pH, incubation time, substrate concentration,  $\text{CaCl}_2$  concentration and temperature of entrapped enzyme Vs free enzyme in which optimum incubation time for the immobilized enzyme (20 minutes) was found to be lower than the free enzyme (25 minutes),  $\text{CaCl}_2$  concentration was higher for free enzyme (8%) as compared to entrapped enzyme (2%) and temperature was to be found higher for entrapped free enzyme ( $50^\circ\text{C}$ ) as compared to free enzyme ( $40^\circ\text{C}$ ). Thermal stability of entrapped enzyme was observed to be  $72^\circ\text{C}$  and its storage stability was also increased up to 6 months with excellent reproducibility as compared to free enzyme ( $50^\circ\text{C}$  and 24 hours respectively). The biodegradation study was showed that 35U of alkaline protease was more effective for controlled and sustained release of entrapped *Vigna mungo* amylase from

chemically modified egg albumin micropreparation as more effective bio-activator when used for the removal of long lasting dry and tough stains from cloths having lipsticks strains of well known brands of lipsticks (Revlon, Maybelline, Lakme and Dior) when used with chosen detergents (Ariel, Surf Excel, Wheel and Tide). Ariel was the most efficient detergent when used with bio-active mixture of enzyme bound egg albumin micropreparation. Thus, this *Vigna mungo* amylase bound chemically modified egg albumin micropreparation may have fairly good industrial application in preparation of fructose syrups and maltose syrups, in paper industries for the treatment of cellulose/ starch, in leather industries for desizing of leather/ various types of natural and synthetic fabrics too as well as may have excellent application in cosmetic, alcoholic/ non-alcoholic/ fizzy drinks/ canned juices beverages, food, pharmaceutical industries, in detergents industries too as a eco-friendly and cost effective bio-active saccharification agent.

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