Purification of Glutathione S-transferase (GST) using Mixed Mode Chromatography

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***Abstract*- A simple and small scale laboratory method to compare between ultrasonication, glass bead shaking and chemical lysis were evaluated for the release of recombinant Glutathione *S*-Transferase (GST) from *Escherichia coli*. Since the protein Glutathione *S*-Transferase is expressed intracellularly, cell disruption process is the precursor step for protein recovery.GST was purified using assessment with PPA and HEA HyperCel resin. Optimum release of GST was via ultrasonication, 70% amplitude size with enzyme release of 129.9 U/mL. Purification yields via PPA HyperCel yielded 96% recovery while purification using HEA HyperCel yielded a 93% enzyme recovery.**

Keywords:*Glutathione S-transferase, chromatography, intracellular protein, PPA HyperCel, HEA HyperCel*

1. INTRODUCTION

Protein purified in this study is the Glutathione S-transferase (GST). GST is widely used as a tag system to ease the purification of recombinant protein [1,2].GST was selected based on its established method of separation under different chromatographic modes. Besides, utilization of the GST in a chromatographic process has a number of distinct advantages such as it enables a straight forward detection protocol via the use of an enzyme activity assay, a reproducible purification strategy from lysed cell culture, and an enzyme activity assay facilitates the fast, high throughput assaying [3].

The production and purification of intracellular proteins are difficult because of the inconvenience of cell disruption and other problems associated with protein downstream processing. The mechanical process of ultrasonication is based on liquid shear created by a high frequency sound and transported through a metallic tip to an appropriate concentrated cellular suspension [4]. It is one of the most commonly used methods because it does not require sophisticated equipments and extensive training. However, ultrasonication is said to be unsuitable for small sample volumes where the biochemical integrity of protein is required [5]. The accumulation and generation of heat is a major problem during mechanical cell disintegration [6,7] as the energy required to rupture the cell is retained in the homogenate [7]. Glass bead stirring is also said to be an easy and efficient method for the recovery of recombinant protein, without the need of specialized equipment. This technique can be administered as a preliminary step in both small and larger scale of protein purification [8]. This method of cell disruption is influenced by cell concentration, glass bead size and the ratio of glass bead to suspension volume [9]. Chemical digestion is a process whereby lytic enzymes and buffers degrade the membranes of cells. The mechanism of chemical digestion does not subject the protein of interest to heat or shear and are not energy intensive [10,11].

Purification of GST was assessed using two mixed mode resins, PPA and HEA HyperCel. Advantages of mixed mode chromatography includes; the potential for direct protein capture from unadjusted moderate to high conductivity feeds, thereby minimizing the need for feed pretreatment [12] unique selectivity in a single run and it is said to be capable of reducing endotoxins (contained inside gram negative bacteria) which are clinically undesirable [13]. The aforesaid adsorbent has a particle size distribution of 80-100um on average and is composed of high porous cross-linked cellulose. Its binding is based on hydrophobic interactions and elution on the basis of electrostatic repulsion making capture and recovery of enzyme efficient. Bindings occur typically at physiological pH with no need of high lyotropic salts, unlike conventional hydrophobic interaction chromatography (HIC).

2. MATERIALS AND METHODS

2.1Cultivation and growth of the recombinant E.coli expressing Glutathione-S-Transferase (GST) protein.

E.coli BL21 (DE3) (F-,ompT, hsdS (rB -, mB -,), gal,(GE Bioscience, Uppsala Sweden) was transformed with plasmid pGEX-6P-1 harboring the GST region [14]. Luria-Bertani broth (LB) plus 50 µg/ml ampicillin (CALBIOCHEM, Massachusetts, USA) was used as a culture medium. The strain was incubated overnight at 37 °C and 250 rpm (VISION Scientific, Korea). When the biomass concentration reaches an optical density at 600 nm (OD_{600}) of 0.6 to 0.8, the protein expression was induced by the addition of 0.1 mM IPTG (NOVAGEN, Massachusetts, USA). The *E.coli* cells were harvested by centrifugation (KUBOTA, Osaka, Japan) at 10,000 rpm, 5 min, 4°C. The *E.coli* was stored as a frozen cell pellet at -20 °C until further use. For preparation of *E.coli* an aliquot of the cell pellet was resuspended in running buffer (50 mM Tris-HCl,

pH 6.5) and mixed to homogeneity at 4°C prior cell disruption step.

2.2Cell disruption of the E.coli homogenate for the release of GST using various lysis buffers

A commercial lysis buffer marketed as BPER (Bacterial Protein Extraction Reagent) (PIERCE, Illinois, USA) was used as a control benchmark for assessing the performance of other cell disruption methods. Twenty mL of bacterial culture was used in all cell disruption experiments. The cells were separated from growth media by centrifuging at 5000 g for 10 minutes. Five mL of lysis buffer was added and vortexed for 10 minutes. Then sample was centrifuged again at 15,000 g for 15 minutes. The supernatant was transferred into a new 50 mL Falcon tube for protein and enzyme assay. These steps were carried out for all lysis buffers identified as A-D (Table 1)

2.3 Static binding capacity of GST onto HEA and PPA HyperCel using an adsorption isotherm model

Clarified *E.coli* homogenate containing the protein GST was resuspended in 200 mM sodium phosphate buffer, pH at a range of 0-100 % buffer to protein ratio. To each tube, 200 µL of resin slurry (50:50 resin to buffer) was added. Tubes were sealed and mixed to its equilibrium for 10 hrsat 24°C. The initial enzyme activity and protein concentrations were analysed. After the appropriate times, adsorbent were settled by centrifugation and supernatant collected and assayed for unbound enzyme and protein concentrations.

2.4 Dynamic binding capacity (DBC) for GST onto mixed mode adsorbents, PPA and HEA HyperCel using frontal analysis

Tricorn 10/50 column was equilibrated with 5 CV (19.6 mL) of 200 mM sodium phosphate buffer pH 7. DBC was determined at 10% with a continuous loading of the clarified feedstock until saturation occurs. Bound protein was then eluted with a gradient elution of 100 mM sodium citrate pH 2.6 for both the resins. Breakthrough points were determined at 10% relative to the initial sample adsorption at $A_{280nm.}$ Column regeneration was done for 15 min with 1 M NaOH and re equilibrated with the respective equilibration buffers for subsequent run.

2.5 Purification of GST via packed bed adsorption chromatography using mixed mode resins.

Clarified feedstock containing GST (20 mL) was loaded onto the column packed with the mixed mode resin (HEA HyperCel or PPA HyperCel) which was pre equilibrated with the equilibration buffer, 200 mM sodium phosphate buffer, pH 7 at a flow rate of 1 mL/min. The column was subsequently washed with the same washing buffer for 6 CV (23.52 mL) after which the elution buffer, 100 mM sodium citrate pH 2.6 and was gradiently applied for 11 CV. Eluted protein were collected throughout the chromatography run and assayed for GST enzyme activity and protein concentration.

2.6 Analytical Procedures

2.61Cell concentration

The cell concentration was analysed by optical density measurement using UV spectrophotometer (THERMO, Wisconsin, UK) at a wavelength of 600 nm.

2.62Total protein determination

Protein concentration was quantified according to the Bradford assay [15] with bovine serum albumin (BSA) as a standard, (PIERCE, Illinois, USA). To calibrate standard curve, 2 mg/mL of BSA was prepared. To 20 µL of sample, 1 mL of dye reagent was added and was incubated at room temperature for 5 mins. Absorbance reading was then taken at 595 nm. Standard curve generated was then used to interpolate the protein concentration in unknown samples.

2.6.3GST enzyme assay

GST activity was measured using 1 mM 1-Chloro-2, 4-dinitrobenzene (CDNB) and 1 mM reduced glutathione in 100 mM phosphate buffer at a pH of 6.5. In a microcentrifuge tube, 880 µL distilled H_2O, 100 µL reaction buffer, 10 µL CDNB and 10 µL glutathione solution was mixed and inverted several times. 500 µL of the above CDNB solution was transferred into UV-transparent cuvettes and 50 µL sample added. To the blank cuvette, 1× reaction buffer equal in volume to that of the sample was added and absorbance measured at 340 nm. Absorbance readings at 340 nm were recorded at 1 min intervals for 5 min. One unit of GST activity is defined as the amount of enzyme required to react with 1 µmole glutathione with CDNB at 340 nm ($\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$) per minute at 20°C.

3. RESULTS AND DISCUSSION

3.1 Cell disruption of rE.coli for the of release intracellular GST

GST is expressed intracellularly within periplasmic space of *E.coli* host. The cell harvested from fermentation was subjected to various cell disruption techniques such as physical, chemical and mechanical methods. In this study, three techniques were assessed for their performance in releasing the intracellular GST which were lysis buffers (chemical), ultrasonication (mechanical) and glass bead shaking (physical).

Four different lysis buffer (coded as: A, B, C and D) based on different mode of cell breakage (detergent, fragmentation and swelling) were tested on 2 mg/mL *E.coli* cells pellets for releasing the GST (Table 1). All digestions experiments were performed in the similar manner at 24°C for 20 min. As a benchmark, the performance of the lysis buffer formulation was compared to the commercially available bacterial protein extraction reagent (BPER) in phosphate buffer saline at pH 6.5. Buffer C resulted n the highest amount of GST release at 130 U/mL followed by Buffer A (94.2 U/mL), Buffer B (93 U/mL) and Buffer D (6.5 U/mL).

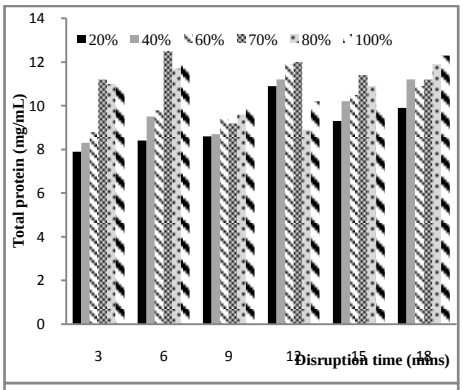
The possible explanation for the poor cell disruption in Buffer D could be due to the addition of dithiothreitol (DTT) which broke the disulfide bonds in the enzyme and denaturing the protein. The amount of enzyme released using lysis buffer C (130 U/mL) was comparable to that of BPER (at 135 U/mL). Since, BPER is not economical to use for large working volume due to its high cost lysis, buffer C may be seen as an alternative.

Table 1.Lysis buffer formulation for the disruption of *E.coli* to release the intracellular protein GST

Mode of lysis	Buffer code and formulation		Total Protein Released (mg/mL)	GST activity released(U/mL)
Detergent	A	1% Triton X100, 10% glycerol, 137 mM NaCl, 20 mM Tris-HCl, 2 mM EDTA, pH 7	10.57	94.2
Fragmentation	B	2% Triton X100, 1% SDS, 100 mM NaCl, 10mM Tris-HCl, 1 mM EDTA, pH 7	10.66	93
Swelling	C	5% glycerol, 20 mM NaCl, 25 mM Tris-HCl, pH 7	13.9	130
Fragmentation	D	150 mM KCl, 1 mM DTT, 1mM EDTA, pH 7.4	9.9	6.5
BPER	E	100 mM sodium phosphate buffer, pH 6.5 Non-ionic detergent	13.5	135

The second technique of cell dirusption investigated was mechanical method via ultrasonication. There were limited studies reported on the optimization of cell disruption conditions such as amplitude size and time for *E.coli* cells [4]. The effect of ultrasonication amplitude on the disruption efficiency of *E.coli* cell (2 mg/mL) was analysed using a laboratory ultrasonicator equipped with 13 mm diameter titanium probe. The sonication temperature was kept constant throughout by immersing the homogenate in salt-ice water bath. Results in Figure 1.0 exhibited an increase in total protein released as the amplitude size increased regardless of the disruption time. Both total protein and GST activity release were fluctuated throughout the course of sonication, depending on the amplitude used. Although the total protein release can be generically predicted to be constant throughout the 18 min disruption time, GST activity was not. Elevated GST activity values were observed when higher amplitude size was used with the highest recorded at 70% amplitude after 6 min of sonication time releasing 129.9 U/mLGST. However, prolonged disruption time beyond 6 mins, resulted in drastic loss of GST activity to below 10 U/mL. This could be due to denaturation of GST by the sonication following its liberation into the reaction mixture.

The reduction on GST activity over a prolonged time may caused by the accumulation and generation of heat which was reported to be a major problem during mechanical cell disintegration by others [16, 7]. [4,17] stated that ultrasonication to be a better method to disrupt *E.coli* compared to the chemical method in terms of protein and enzyme release. Sonication is often carried out in small scale but not common for large scale operation, due to cost and high energy requirement [18, 19] in cases where ease for scale up and faster mass transfer between solvent and host material are required, ultrasonication has been the method of choice [20]. Besides, it is practical to disrupt the cell using techniques that do not required any additional lysis chemicals or enzymes making mechanical methods the preferred choice by many bioprocess practitioners and industries.



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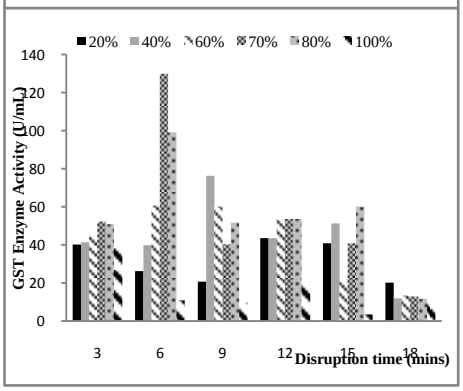

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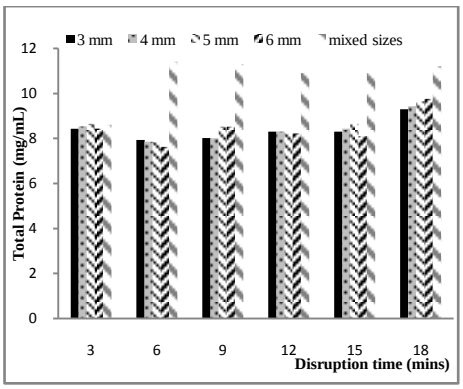

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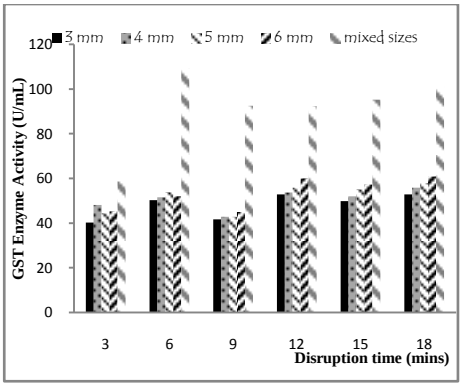

D

Figure 1.0.Column graphs showing total protein (TP) release (mg/mL) and GST enzyme release (A) (U/mL) for disruption methods of glass bead shaking (GB) and ultrasonication (US), A: TP released at different US amplitude size (%); B: A released at different US amplitude size (%); C: TP released using different glass bead sizes; D: A released using different glass bead sizes.

Cell disruption using glass bead shaking is considered to be the most economical method due to lower power input requirement than the ultrasonication and cheaper than the chemical method. Different glass beads sizes (3 mm, 4 mm, 5 mm, 6 mm and a mixture of equal mixed bead sizes) was used with. The amount of glass beads to cell suspension ratio was set at 1.5 g/mL based on previous work by [9]. Total protein released followed the similar trend over time with highest protein concentrations recorded using the mixed bead composition (Figure 1.0 C and Figure 1.0 D). Shaking with the glass beads resulted in sufficient release of GST enzyme activity over time and similar trend was observed regardless the duration of the shaking. All the uniform bead size (3, 4, 5 and 6 mm) resulted in almost uniform GST (50 U/mL) and protein (8 mg/mL) release over period of 18 min. Cell disruption improved when a range of bead sizes (mixture of different sizes) were used. The highest GST enzyme yield was observed at the 6th min with 109.2 U/mL and total protein of 11.4 mg/mL using mixed bead.

Many previous works reported that a higher degree of bacterial cell disruption could be achieved with the smaller beads. [21] used 1.2 g of glass bead per mL of *E.coli* suspension with a uniform size of 0.3 mm in diameter and found that at 6 minutes the highest level of enzyme activity was found. This observation contradicts the present work where mixture of sizes of glass beads ranging from 3 mm to 6 mm produced the highest amount of enzyme released. Possible explanation could be the size of beads used by [21] was 10x fold smaller than the smallest bead size used in this study, which may provide a higher collision frequency per unit volume of cell mass. Another observation of cell disruption by glass bead shaking is the loss of enzyme activity after prolonged homogenization time [22]. However, this phenomenon was not observed in this study.

3.1 Static binding capacity of GST from E.coli homogenate using an adsorption isotherm analysis

Adsorption isotherms values for Langmuir on mixed mode are not widely available [12, 23] limitation of this mechanism impedes its successful applications. This is due to the dual mechanism property of the mixed mode resin. However, most studies done in the past incorporated the use of Langmuir to derivative values such as the maximum binding capacity (q_m) and dissociation constant (K_d) which has been seen in the work of [23, 24, 25].Parameters for isotherm were said by these authors to be merely empirical with no physical meaning and focused mainly on BSA as the model protein [23, 26, 27, 24, 28].

Tubes containing clarified enzyme was diluted with appropriate buffers from a concentration of 0-100%. Mixed mode resins were added with 50:50 resin to slurry ratio with rotational agitation for a period of time.

Maximum binding capacity, q_m for GST from *E.coli* clarified feedstock onto PPA HyperCel and HEA HyperCel in 200 mM sodium phosphate buffer, pH 7 was 123.46 and 126.6 U/mL/resin, respectively and dissociation constant values were at 4.9 x 10^{-1} M and 5.9 x 10^{-1} Mrespectively. At high q values, experimental data deviates from the Langmuirian equation for both the resins tested as depicted in Figure 1.1

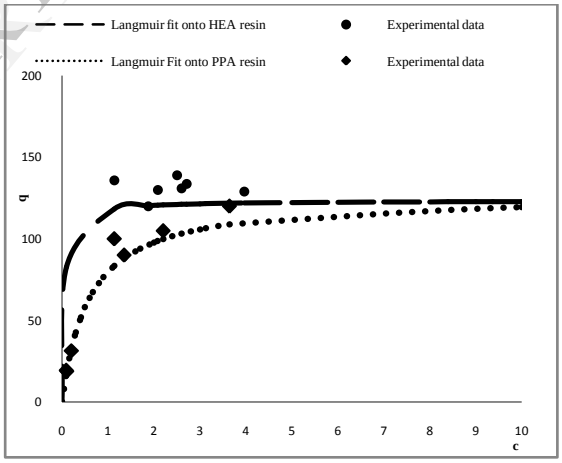

Figure 1.1.Graph of experimental data of equilibrium isotherm of GST onto PPA HyperCel and HEA HyperCel and the least square fit to the Langmuir equation

In general, both the adsorption isotherms show a fast approach towards equilibrium and displayed the typical saturation behaviour of single protein adsorption on mixed mode adsorption. The density of the ligand plays an important role in the binding of the protein onto the surface of the ligand, whereby higher ligand density leads to higher binding capacities, which is seen in the case of the HEA resin. High ligand densities offer stronger driving forces for mass transfer between the protein and resin. HEA showed a substantially higher binding capacity compared to PPA although the latter is more hydrophobic for both the protein tested. This could be due to the higher ligand density of HEA (67 mmol/L gel) and better accessibility of the

hexylamine group on HEA comparing to the phenylpropylamine group on the PPA (65 mmol/L gel) resin. [29], also mentions that the increase in chain length of the HEA resin concurrently increased the strength of protein binding from retardation to reversible binding to very tight binding hence the higher binding strength reported in this study. Another factor could be due to pore sizes of the adsorbents. [30] mentions that the surface area with respect to pore size generally is the primary determinant of adsorption capacity and that too large a pore diameter could render part of the inner adsorbent surface wasted, and the molecules initially adsorbed, prone to desorption at the same time. However an important area to note is the behavior of the initial slope of the curve. Curves with rapid initial slopes indicate a high selectivity of the resin towards the enzyme. In this paper, the resin PPA did depict such behaviour and hence facilitated a better chromatographic separation.

3.2 Dynamic binding capacity for GST onto mixed mode resins via column adsorption chromatography

The dynamic binding capacity (DBC) was determined using both adsorbents at 10% breakthrough. This was performed by determining the difference between initial and the final concentration of the solution and the measuring the protein quantity of the eluted component. The cell lysate was loaded onto the resin to 10% breakthrough point at absorbance 280 nm (A_{280}). Same procedures were adhered to test the DBC performances of both the mixed mode adsorbents, HEA and PPA HyperCel. DBC values were 22.32 mg/mL for GST onto HEA resin and 22 mg/mL for the PPA resin (Figure 1.2).

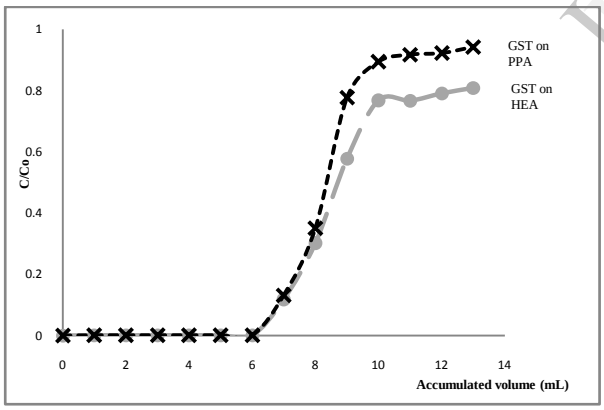

Figure 1.2. DBC of GST onto PPA and HEA HyperCel in column adsorption chromatography

Both experiments were performed at a 1 mL/min flow rate as the slower the flow rate is, the better the contact between the resin and the enzyme. This is due to the mass transfer resistance experienced by the enzymes At higher flow rates, less time is allowed for pore diffusion and hence resulting in poor utilization of the packed matrix. [29] obtained DBC values for PPA and HEA on monoclonal antibodies at 60.3 mg and 39.8 mg with HEA having lower values. In another study by [31], DBC values of PPA using maltose binding proteins were in the range of 24-26 mg/mL and for HEA at 22-24 mg/mL at 10% breakthrough. This correlates with the results using GST as the model protein which also deemed lower DBC values using HEA. DBC of a certain protein depends on many factors such as the flow rate, nature of the gel matrix, column dimension, matrix pore size, adsorption isotherm values, and diffusion constant of the protein (32, 33].

3.3Purification of GST from E.coli homogenate using HEA and PPA HyperCel resin loaded in a packed bed adsorption column chromatography.

In this study, the purification of GST was used as a model study to access the efficiency of a chromatographic separation using both HEA and PPA HyperCel resins. The PPA resin has an aromatic side chain which promotes stronger hydrophobicity. The benzene ring provides two selectivity options and rendering the enzyme to be more retentive compared to its counterpart HEA. The HEA resin however is aliphatic and is less hydrophobic. More selective adsorption and desorption of protein and higher yields can be obtained by optimizing suitable washing and elution buffers, which were conducted prior to column adsorption experiment.

GST purification was performed by loading 20 mL clarified feedstock into column packed with 3.92 mL HEA HyperCel resin, pre-equilibrated with 200 mM sodium phosphate buffer pH 7. Washing of the loosely and unbound proteins from the column was conducted using 6 CV of the same equilibration buffer. Then a gradient elution of 100 mM sodium citrate was applied at pH 2.6. Chromatogram (Figure 1.3) depicts the enzyme activity and protein concentration throughout the purification stages FT (Flowthrough), W (Washing) and E (Elution). Fractions were collected throughout and assayed for enzyme activity and total protein. Elutions achieved 3x purification with regards to specific activity (Table 1.1) with 93% enzyme yield. Average amount GST was lost in the washing step (24%), indicating a better binding specificity compared to the conventional ion exchanger.

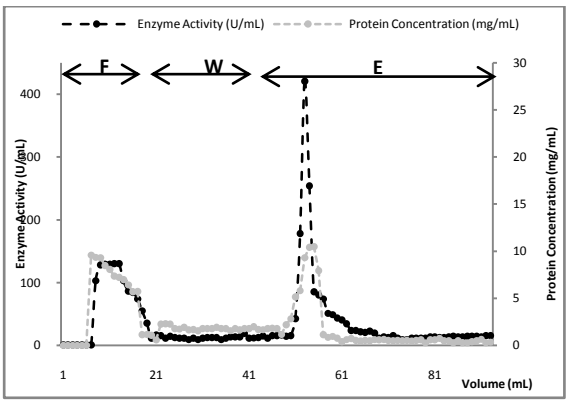

Figure 1.3.Chromatogram of GST separation via HEA HyperCel at 1mL/min. Buffers used: equilibration buffer: 200 mM sodium phosphate buffer pH 7, washing buffer 200 mM sodium phosphate buffer pH 7, gradient elution 100 mM sodium citrate pH 2.6. Column regeneration with 1 M NaOH.

Table 1.1.Purification table for GST recovery using HEA HyperCel

Purification stage	Volume	Total Activity (U)	Total protein (mg)	Specific activity (U/mg)	Fold	Yield
Feedstock	20	2420	250	9.68	1	100
Flowthrough	20	501.6	90.	5.57	0.6	21
Washing	25	762.5	47.5	16.05	1.7	32
Elution	40	2245.5	75.6	29.7	3.1	93

Table 1.2.Purification table for GST recovery using PPA HyperCel

Purification stage	Volume	Total Activity (U)	Total protein (mg)	Specific activity (U/mg)	Fold	Yield
Feedstock	20	2440	250	9.76	1	100
Flowthrough	20	552	73.20	7.54	0.8	23
Washing	25	735.50	26.25	28.02	2.9	30
Elution	40	2331.45	73.35	31.79	3.3	96

For the purification of GST via PPA HyperCel, similar methods were employed using different buffer formulations suited for the resin. Column containing the packed PPA HyperCel resin was equilibrated with 100 mM PBS at pH 8. Unbound protein was then washed using the same buffer until baseline. Then, a gradient elution of 100 mM sodium citrate was applied at pH 2.6. The full chromatogram of GST purification using packed bed PPA HyperCel column is illustrated in Figure 1.4. Fraction were collected throughout and assayed for enzyme activity and total protein. Elution fractions showed a single peak with specific activity fold of almost 4 times (Table 1.2). Efficient recovery of GST at 96% was observed in elution fractions. A similar value of GST lost during flowthrough and washing step was observed at 20 and 24%, respectively as in the PPA HyperCel run.

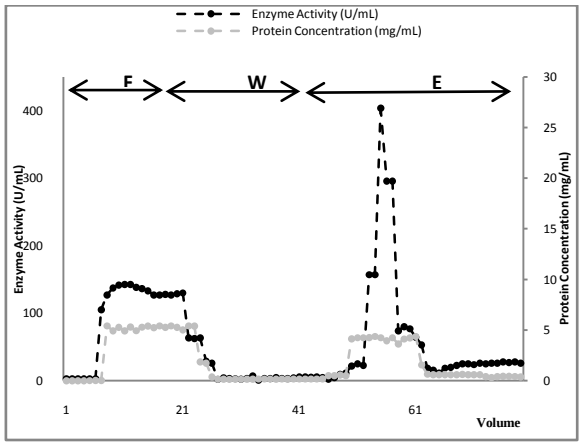

Figure 1.4.Chromatogram of GST separation via PPA HyperCel at 1 mL/min. Buffers used: equilibration buffer: 200 mM sodium phosphate buffer pH 7, washing buffer 200 mM sodium phosphate buffer pH 7, gradient elution 100 mM sodium citrate pH 2.6. Column regeneration with 1 M NaOH.

In the past, the purification of GST was purified using immobilised metal affinity chromatography [34, 35], GSH-affinity [36] and ion exchange chromatography [37]. Knowing that the purification of GST displayed good performance and potential, GST was chosen as a model protein to access the efficiency of mixed mode resins.

MMC eliminates the need of lyotropic or salt additives in binding and elution buffers which in turn reduces the formation of protein aggregation and increases protein stability. Even small reduction in salt amounts during a particular chromatographic run can significantly reduce the cost for commercial production. Although mixed mode resins have been used for recombinant protein purification [38, 39], pre packed columns containing the resin was employed. This is even for the case of other sample types including monoclonal antibodies [38, 40, 41, 29], maltose binding protein [31] and whey protein [42]. Although pre packed columns save time, they lack resolution and is not supported by a dynamic flow [42]. Despite the vast usage of mixed mode resins, an in depth theoretical interaction of the resin and protein is scarce [12] besides screening operating parameters can be resource intensive and time consuming [42]. Screening guides the selection of adsorbents and best operating conditions and in turn narrows the number of chromatography runs to a minimum [42]. As mixed mode resins have two modes of interactions, it is important to play on loading and elution parameters so as to optimize best adsorption and desorption values [41].

Elutions of bound proteins in MMC are accomplished by electrostatic repulsions between the protein and the ionized ligand. This happens when the buffers pH is modulated to mild acidic conditions, where the ligand takes a net positive charge due to the dissociation of the ionisable group. Ionic repulsions in MMC are known to possess an uneven distribution of charge on the resin surface which causes different degrees of tilting of the protein. To achieve a successful elution, the most powerful way to alter selectivity is to change the charge of the solute. For a quantitative binding, residence time of the sample is sufficient. The required residence time is a function of solute pore diffusivity, particle size and solvent viscosity. Since binding is primarily governed by hydrophobic interactions and

elution via electrostatic repulsion, interaction between the enzyme and the resin must be carefully evaluated. The degree of interaction in HIC increases generally at the order of increasing carbon chain length and aromatic content of the ligand. According to a study by [31], flow rates play an important role in purification performances. Flow rates of 100 cm/hr gave best results while increased flow rates led to a lesser efficient binding of proteins to the column due to slow mass-transfer kinetics within the column. During the chromatographic run of GST, a flow rate of 77 cm/hr was employed. Decreased flow rates (50 cm/hr) led to higher desorption values during the washing step leading to target molecule leaking as diluted broad zone.

In both the cases, 20% enzyme loss was observed during the flow through step and about 24% enzyme loss was noticed in the washing step. This is seen often in the case of mixed mode resins as in the case of [42] who used BSA and lysozyme as the study proteins. [31] also noted a substantial amount of the protein of interest was loss during the purification step and was later explained that low adsorption of the protein to the resin or substantial desorption was due to weak interaction as seen in this study.

4. CONCLUSION

GST released was administered via three modes of *E.coli* cell disruption strategies namely the ultrasonication mode, glass bead shaking and chemical lysis. Enzyme was then purified and assessed via two different mixed mode resins, PPA and HEA HyperCel. Purification yields via PPA HyperCel yielded 96% recovery while purification using HEA HyperCel yielded a 93% enzyme recovery.

5. ACKNOWLEDGEMENT

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