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Bioaffinity chromatography of yeast-derived recombinant Hepatitis B virus major surface antigen

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Abstract

Chronic Hepatitis B virus infection claims millions of lives across the world every year. Vaccination with recombinant Hepatitis B virus major surface antigen (rHBsAg) is the only key to prevent the spread of virus thereby reducing the occurrence of virus-induced hepatocellular carcinoma. Conventional purification methods for the vaccine antigen follow a laborious and expensive downstream processing. Bioaffinity chromatography using monoclonal antibodies (MAbs) as ligand and NHS-activated Sepharose as support has been optimized in this study for a simple, one-step purification of rHBsAg. With a maximum coupling strength achieved at pH 8.3, an average recovery of $\geq 90\%$ purified rHBsAg protein was eluted at acidic pH followed by a quick neutralization, without affecting its biological activity. The operational binding capacity of the coupled gel was estimated to be $50\mu\text{g}$ of rHBsAg per ml. The purified antigen was demonstrated for its antigen-specificity, protein purity and insignificant levels of leached ligand and host cell proteins. Permissible levels of endotoxin, DNA and carbohydrate contents were recorded, while the lipid content was slightly more than the recommendations for a Hepatitis B vaccine dose. Further improvement of the bioaffinity purification protocol with a delipidation step or detergent treatment can be explored to enhance the quality requirements of the purified rHBsAg as a vaccine. The maximum antigen recovery and the number of purification cycles supported by the chromatographic resin prepared in this study will throw light over the optimized purification method.

Keywords: NHS-activated Sepharose, rHBsAg, bioaffinity, purification, monoclonal antibody

1. Introduction

Murine monoclonal antibodies developed through hybridoma technology ^[1] has paved the way for virologists to study in detail viral antigens, epitope mapping, purification of immunodominant antigens by affinity chromatography, development of *in vitro* diagnostics and therapeutics.

Hepatitis B virus (HBV), a member of the Family: *Hepadnaviridae*, comprises of a circular DNA, partially double-stranded of 3.2Kb size. The genome is organized into overlapped open reading frames encoding seven different viral proteins. Of them, the surface antigenic loop of S-protein (HBs) in the viral envelope, is associated with the “a” determinant region (HBV major surface antigen-Ag) and the primary site of binding for the neutralizing antibodies. Humoral immune response to this surface antigen following natural infection predominantly confers protective immunity ^[2]. This immunogenic potential of the viral surface antigen enabled its identity as a first human recombinant vaccine candidate for protection of humans against Hepatitis B virus infection ^[3]. Most of the current Hepatitis B vaccines available contain yeast-derived recombinant HBsAg (rHBsAg) as the active principle, manufactured using precipitation or adsorption/desorption, coupled with one or more chromatography steps. Several researchers have developed MAbs against rHBsAg for varied applications-namely, in the study of viral antigenic structure, or precisely the group-specific or subtype antigenic determinants, for the development of point-of-care diagnostics, vaccines, therapeutics ^[4, 5] and as efficient ligands for HBsAg purification ^[6]. The inherent properties of antigen-specificity and functional affinity of the MAbs have made them vital for analysing characteristics of proteins and their purification.

One such purification technique based on specific affinity interactions between a biomolecule and biological or synthetic ligand immobilized on a solid support, is referred as Bioaffinity chromatography (BAC). MAb-based BAC, also referred as Immunoaffinity chromatography (IAC) for the production of pure rHBsAg has been explored with variable outputs^[7, 8]. Sepharose has been used as a solid support for binding the MAbs^[9] or plantibody^[10] upon activation by cyanogen bromide (CNBr) or NHS, for the isolation of rHBsAg from crude yeast cell extracts.

The subject of this research work was to study the development and optimization of a Bioaffinity chromatography method using anti-HBs specific mouse MAbs, immobilized on activated Sepharose resin for the isolation of highly pure recombinant HBsAg proteins suitable for vaccination.

2. Materials and Methods

2.1 MAbs and rHBsAg

Well-characterised and affinity-purified anti-HBsAg mouse monoclonal antibody (Mab-HBs06) specific for the HBsAg-‘a’ determinant^[11], with a good reactivity to both recombinant and human plasma-derived HBsAg was used as an immunoabsorbent in this study.

Transformed *Pichia pastoris* cells expressing rHBsAg were propagated in large scale fermenters, harvested and disrupted with standard procedures. The cell lysate was clarified, filtered, dialysed against the binding buffer chosen for the Bioaffinity chromatographic protocol and stored at 4°C. This was used as the input material for the optimization of the chromatographic method intended in this study.

2.2 Immunocapture ELISA (IC-ELISA) to quantify HBsAg

An IC-ELISA method optimized using anti-HBs MAb, namely Mab-HBs06 for the estimation of HBsAg content in hepatitis B vaccines^[11] was used for the purification analysis in this study.

2.3 Preparation of Bioaffinity resin

2.3.1 Immobilisation of purified MAb on Sepharose

The affinity purified Mab-HBs06 was immobilised onto the NHS-activated Sepharose 4 fast flow TM (GE Healthcare, Sweden) used as the solid support as per manufacturer's instructions with modifications^[12]. The preparations included dialysis of the MAb in the binding buffer (200mM sodium bicarbonate, 500mM sodium chloride, pH 8.3) and wash of the NHS-activated Sepharose resin with ice-cold 1mM HCl at a flow rate of 2 ml/minute. The dialysed Mab was covalently bound with activated Sepharose by gentle stirring at 25°C for 4 hours and loaded onto a XK 16/20 column. The binding efficiency (δ) was calculated using the formula^[8]; $\delta\% = \chi / \lambda \times 100$, where χ is amount of bound protein estimated as difference between the initial concentration of MAb and λ the unbound MAb detected in the wash fractions after coupling. The column containing Mab-coupled resin was maintained at 4°C in 20% (v/v) ethanol until further use.

2.4 Selection of desorption buffer conditions for Bioaffinity chromatography by Immunoassay

The ideal desorption buffer and working conditions were determined by Sandwich ELISA format using Biotin-Avidin system as described earlier^[13] with modifications. A known concentration of Mab-HBs06 was coated onto microtiter plates for overnight at 4°C. Intermittent washing of the ELISA

wells between each step was carried out thrice with PBS containing Tween20. Blocking of non-specific sites was carried out using 1% (w/v) bovine gelatin. Purified rHBsAg added @ 500ng/well was incubated for one hour at 37°C. Different desorption reagents chosen for optimization included; i) 100mM glycine-HCl (pH 2.25); ii) 200mM glycine-HCl (pH 3.5); iii) 100mM sodium bicarbonate (pH 8.0); iv) 8M urea (pH 7.0) and v) Phosphate buffered saline (pH 7.0, control), which were added to the antigen-bound wells for 15 minutes at 37°C. Antigen detection was enabled using primary binding with biotinylated Mab-HBs06 followed by secondary HRPO conjugate of streptavidin (@1:8000). The reaction was developed using a chromogen (TMB-3, 3', 5, 5'-Tetramethylbenzidine)-substrate and following an incubation, stopped with 1.25M sulphuric acid. The A450 values were recorded using an ELISA reader. The respective desorption buffer and conditions producing highest optical density values was identified.

2.5 Standardisation of Bioaffinity chromatography

The equilibrated crude cell lysate (as described in Section 2.1) was applied twice over the immunoabsorbent at a linear flow rate of 1.0 ml/minute followed by residence time of 30 minutes. The total protein and HBsAg concentration of the input feed for the BAC process was assayed by optimised methods^[11] and always maintained less than the maximum binding capacity of the coupled matrix. The washing step was carried out with PBS at a linear flow rate of 2 ml/minute. The bound HBsAg was eluted with 10 column volumes of the optimized desorption buffer (100mM glycine, pH 2.25) at a linear flow rate of 1.0 ml/minute and neutralized with 2M Tris-HCl (pH 8.6). The final wash of the coupled matrix was carried out with PBS and maintained at 4°C.

2.6 Evaluation of ligand leakage by Antibody-mediated ELISA

Despite the covalent bonding between the ligand and resin, presence of any unbound ligand or ligand release during chromatography run was ascertained by an antibody mediated ELISA. Briefly, whole molecule of anti-mouse IgG was coated on the ELISA plate (1000ng/well) and unbound sites were blocked with 1% (w/v) bovine gelatin in PBS. The flow through, wash and eluates of BAC run were added to the blocked wells, aiming to detect any ligand (Mab) released. Detection of the antibody complexes if any, was carried out using secondary HRPO-conjugated anti-mouse IgG (@1:8000). A substrate activated chromogen (TMB) was used to develop the enzymatic reaction, and the colour development stopped after an incubation time, measuring the A450 values.

2.7 Evaluation of BAC-purified rHBsAg

The BAC-purified rHBsAg from the two experimental batches were subjected to estimation of total protein content by Bicinchoninic acid method, HBsAg content by IC-ELISA, antigen purity by silver-stained SDS-PAGE and determination of ligand release by antibody-mediated ELISA, per unit vaccine dose.

The endotoxin content and level of yeast or host cell proteins (HCPs) in all the samples as mentioned above, were determined by Limulus amoebocyte lysate (LAL) test and a commercial immunoassay (Cygnus Technologies, USA) respectively following manufacturer's instructions.

The other evaluations included: an improved Phenol-Sulfuric Acid method^[14] to analyse the carbohydrate content; Sulpho-

phospho-vanillin (SPV) colorimetric method for total lipid content A260/ A280 values ^[16] using Nanovue (GE Healthcare, Sweden) and ethidium bromide-stained agarose gel for DNA levels.

3. Results and Discussion

Murine monoclonal antibodies against Hepatitis B virus have been developed and validated by several researchers in the last decade for development of diagnostics, therapeutics ^[4, 5] and viral antigen purification ^[6, 10, 15-17]. Safe, efficacious and economical Hepatitis B vaccines are manufactured following a laborious downstream processing of rHBsAg molecules which include acid precipitation, ultrafiltration and ion exchange chromatography. The major shortcomings of conventional purification methods include limited self-assembling, formation of protein aggregates and quick degradation of the protein during purification. Bioaffinity chromatography (BAC) using MABs as biological ligands aims at one-step, targetted isolation of rHBsAg biomolecules from the unpurified cell lysates ^[13, 16], effectively removing most of the impurities (namely DNA, host cell proteins). This study involved the development and standardization of a bioaffinity chromatographic procedure for the quick recovery of significant yields of pure rHBsAg, in order to produce improved recombinant hepatitis B vaccines.

3.1 MABs specific for HBV major surface Ag and purification resin

The MAB-HBs06 ^[11] (IgG1 κ isotype) used in this study, reacted specifically with both the native *ad* and *ay*-specific HBsAg subtypes (human plasma) and rHBsAg (subtype *adw*₂). The MAB specifically bound a conformational epitope on the HBV major surface antigen with a high affinity constant. These properties formed the basis for exploring the anti-HBs MAB as an immunoadsorbent for BAC. Among the various support materials available for affinity chromatography, an agarose-based matrix like Sepharose was used in the study due to its inherent merits of high binding capacity, good flow characteristics, high stability to physical and chemical conditions and stable scale-up options ^[16].

3.2 Preparation of bioaffinity chromatography resin

The bioaffinity chromatographic (BAC) resin was prepared using an anti-HBs MAB adsorbed over NHS-activated Sepharose. This formed the major determinant towards the generation of pure rHBsAg with maximum recovery intended in this study ^[18].

3.3 Anti-HBs MAB and chromatographic resin

The intended chromatography in the study relied on the stronger binding ability of the MAB, as higher the affinity constants, better is the purification yields. This binding strength ensures a hold over the bound antigen even under the preliminary harsh acidic desorption conditions, although neutralised later ^[19]. Hence the characterized anti-HBs antibody (MAB-HBs06), with a high affinity constant was chosen as an immunoadsorbent for rHBsAg purification.

The large pores within Sepharose-4B beads enable molecules of varying sizes (60KDa to 20,000 KDa) for efficient gel filtration, coupling of larger molecules and as an ideal matrix for all affinity purification methods. This solid matrix thereby permits the Mouse MAB (MW: 150 KDa) binding the high MW rHBsAg particles (2400 KDa) ^[21] during the BAC process. NHS (N-hydroxysuccinimide) activation of the Sepharose-4B resin helps in the formation of a stable amide

bond with the anti-HBs MAB, facilitating a better coupling chemistry ^[10].

3.4 Coupling kinetics of MAB-NHS activated Sepharose

The ligand concentration and pH for the adsorption are major determinants of an effective immunoadsorbent for BAC ^[16]. An approximate concentration of 10 mg anti-HBs MAB /ml was immobilized on NHS-activated Sepharose 4 fast flowTM. This ligand (MAB-HBs06) concentration seemed to be ideal for the adsorption with the Sepharose matrix. Lowest rHBsAg recovery with ligand densities less than 10 mg/ml have been observed earlier ^[12].

About two-thirds of the anti-HBs MAB bound to the activated Sepharose in the first 30 minutes at room temperature with a 75% binding efficiency, which improved to 95% in the next 210 minutes (Figure 1). The recommended coupling pH range was 6-9, with NHS ester groups capable of rapid hydrolysis at alkaline pH. The MAB-NHS activated Sepharose coupling efficiency was maximum at a pH of 8.3 with the help of ice-cold buffers, in agreement with the earlier research of MAB coupled with CNBr-activated Sepharose ^[8].

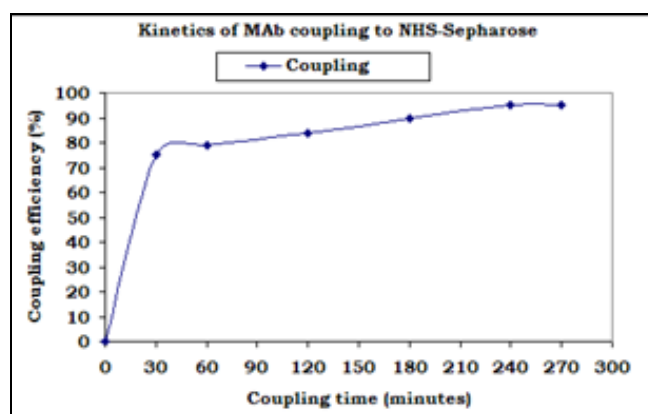


Fig 1: Coupling efficiency of Mab-HBs06 to NHS-activated Sepharose

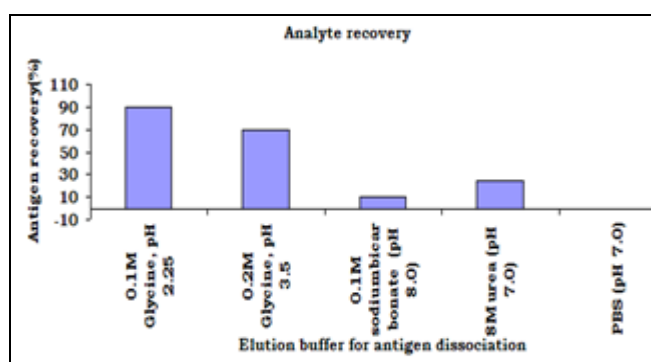


Fig 2: Analyte (rHBsAg) recovery with different elution buffers as demonstrated by in-house based IC-ELISA

3.4 Desorption buffer conditions standardized for Bioaffinity chromatography

Eluant selection is also critical during the standardization of a BAC protocol. The ideal desorption buffer and working condition was chosen such that >95% of pure and stable antigen with the same functionality was retained.

Desorption buffer containing 100mM glycine (pH 2.25) was found to be efficient with more than 90% of HBsAg recovery unlike other buffers used in the study. The other buffers studied-namely, 8M urea (pH 7.0) and 100mM sodium bicarbonate (pH 8.0), exhibited lower recovery of target antigen [25% and 10% respectively] (Figure 2), indicating an

unfavourable antigen desorption. Glycine-based acidic desorptions of HBsAg were neutralized using an alkaline buffer for a stable antigen recovery, further confirmed by ELISA [1, 10].

3.5 Standardisation of bioaffinity chromatography

Crude yeast cell lysates containing rHBsAg from two different batches of variable total protein content and target antigen contents (288 µg/ml and 121 µg/ml) were used as input materials for bioaffinity chromatography. The rHBsAg content in the input material for the BAC process averaged to 0.5-1.9% of the total proteins. An operational binding capacity of 50µg of rHBsAg per ml of coupled gel [5] enabled to recover >90% of the target antigen. Despite the inherent nature of Sepharose resin to resist higher flow rates, some diffusion might have restricted the contact of the antigen with the bound MAb [7]. A lesser binding capacity of the target rHBsAg molecule might have resulted due to some structural hindrances of the immobilized ligand.

3.6 Evaluation of ligand leakage in BAC

A ligand leakage at a concentration of < 1ng IgG/µg of rHBsAg was demonstrated by ELISA in this standardized chromatography, which was well below the approved level (< 3ng IgG/µg of rHBsAg) of ligand contamination in Hepatitis B vaccines. The MAb residue in the final vaccine product can induce anti-mouse antibodies in humans (HAMA response)

due to its greater immunogenic nature in humans. Hence the ligand leakage in any affinity purification is a mandatory evaluation. A simple spectrophotometric estimation of the amount of uncoupled MAb at A280 was not possible in this study, which might be attributed to the formation of an amide linkage between the activated ester and reactive amines, as reported earlier [22]. This screening limitation was circumvented by the use of antibody-mediated ELISA to assess the amount of MAb-leak from the coupled resin during BAC of rHBsAg. Earlier research findings of lesser antibody leaching with NHS-activated supports, than CNBr-activated supports were in agreement with this study results [2].

3.7 Evaluation of BAC-derived rHBsAg

The guidelines laid by Indian Pharmacopoeia [23] and European Pharmacopoeia [24] recommend the analysis of the quantity and specificity of HBsAg in the recombinant hepatitis B vaccines by either ELISA or immunoblotting or SDS-PAGE for antigen purity and a standard total protein estimation. These vaccines also need to be tested for impurity traces like lipids, nucleic acids, carbohydrates, host cell and vector-derived DNA, protein expected as part of vaccine quality control tests.

The samples collected during the BAC process-namely, input material, flow through, different eluates, were analysed for the target antigen content and the other impurities and results summarized in Table 1.

Table 1: Analysis of rHBsAg purified by Bioaffinity chromatography

Parameters analysed	Feed		Flow through		Eluate	
	I	II	I	II	I	II
Total protein (mg)	45	37.5	42	33.4	0.31	0.72
HBsAg (µg)	310	720	97.5	-	295	705
DNA (ng)	778	980	853	726	0.91	2.62
Endotoxin (EU)/20 µg	>30	>30	>30	>30	< 30	< 30
IgG (ng/ µg HBsAg)	-	-	-	-	0.11	0.14
HCP (ng/ml)	+	+	+	+	3.4	4.8

I-3 ml of MAb-Sepharose gel – Unpurified cell lysate @ 15 mg/ml Total Protein (310 µg HBsAg)

II-10 ml of MAb-Sepharose gel – Unpurified cell lysate @ 15 mg/ml Total Protein (720 µg HBsAg)

An adult dose of commercial hepatitis B vaccine is expected to contain an approximate level of 20 micrograms of pure recombinant HBV major surface antigen with a relative potency of 1.0. The antigen content of the BAC purified

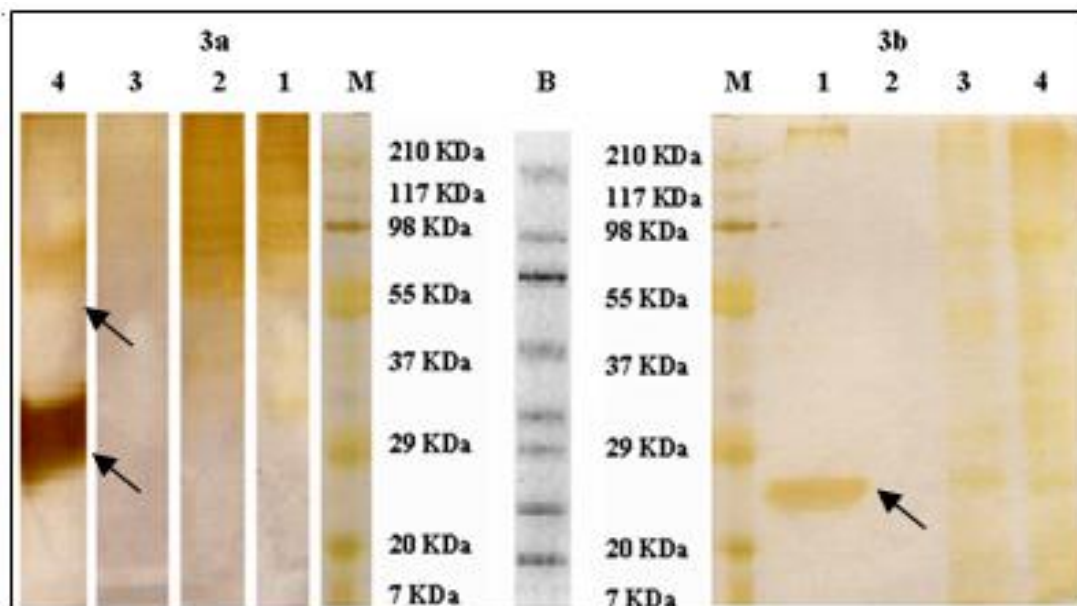
rHBsAg incorporated per unit vaccine dose was demonstrated by IC-ELISA in addition to the other essential parameters post purification. The estimations made per adult vaccine dose are summarized in Table 2.

Table 2: Analysis of final bulk antigen (per unit vaccine dose) purified by Bioaffinity chromatograph

Parameters analysed	Recommended levels / 20 µg dose	Estimations in final bulk antigen	
		I(A)	II(A)
Description	Clear, colourless	Clear, colourless	Clear, colourless
pH	6.4-7.4	6.4-7.2	6.4-7.2
Identification by SDS-PAGE	≥ 95%	≥ 95%	≥ 95%
Total protein (mg/ml)	Not less than 0.080	0.26	0.72
HBsAg (µg/ml)	Not less than 20	20.5	21.3
DNA (pg)	< 100	70	74.5
Endotoxin (EU)	< 30	< 30	< 30
Total lipids (µg)	6-10	25	29.5
Carbohydrates (µg)	≤ 3	2.05	2.25
IgG (ng)	≤ 60	44	56

Silver-stained reducing SDS-PAGE analysis of the BAC purified rHBsAg revealed the major 24 kDa band and its dimer (48 kDa band) (Figure 3a, 3b), in accordance with earlier results [16]. The antigen purity of >90% as demonstrated by SDS-PAGE is crucial in the analysis of the standardized bioaffinity chromatography process [7, 9, 10, 15, 21,

25]. Disulfide bond formation between the antigen subunits observed in this study has been reported in the past [26], wherein the monomeric antigenic units form dimers especially during *in vitro* purification of the yeast-derived rHBsAg.



Lane M-Marker, 1-Sample before purification, 2-Flowthrough, 3-Wash, 4-Concentrated eluate, 5-BSA, B – Bio Rad Pre-stained protein marker

Fig 3a: Purification of rHBsAg by immunoaffinity chromatography (12% reducing SDS-PAGE). The results show the dimer bands of rHBsAg post purification

Fig 3b: Purification of rHBsAg by immunoaffinity chromatography (12% reducing SDS-PAGE). The results show the monomeric band of rHBsAg post purification.

Lane1-Marker, 1-Concentrated eluate, 2-Wash, 3-Flowthrough, 4-Crude cell lysate containing rHBsAg. Storage studies of the BAC-purified rHBsAg at 4°C for 3-4 weeks, revealed a variation in the size of the monomer, approximately equal to a molecular weight of 26 kDa (higher than that of S-protein). This may be a result of a probable damage of some of the domains [25] or an improper cross-linking of cysteine residues in the disulfide bonds after purification which might continue through storage. Hence, a stable low temperature storage of the BAC-purified antigen at temperatures <4°C would be the ideal solution.

The absence of yeast HCPs, permissible levels of endotoxin and DNA contents in the bioaffinity purified rHBsAg product was well demonstrated in accordance with earlier reports [25, 27]. On the other hand, a slightly higher lipid content was observed in the BAC-purified product. In general, a lipid composition of approximately 25-30% is representative of the total protein mass. When the self-folding of rHBsAg occurs inside the host cells, the lipid gets into association with rHBsAg. This binding might mask the exposed specific epitopes of the multimeric antigen and result in a lesser antigen binding capacity with the immunoabsorbent. The findings of a mild excess of lipids in this study, is quite similar to those of the earlier researchers, where a similar inhibitory element was identified and the antigen recovery was found to improve following the use of a detergent like sodium deoxycholate without affecting the biological activity of the target protein. A 0.1% solution of this mild detergent was capable of dissolving the lipids leaving the rHBsAg functionally-active [9]. The number of multiple purification cycles of BAC resin, enabling desorption of several rHBsAg batches with an average recovery more than fifty percent, would improve the economy of the standardized process. An additional treatment of the input samples with a suitable detergent like 0.1% sodium deoxycholate before applying to BAC may also help to ensure a pure and stable purified rHBsAg product.

4. Conclusion

Conventional purification of rHBsAg involves the preliminary precipitation of the target protein from the cell lysates under acidic conditions, purifying and concentrating proteins based on size and molecular weight by one or more chromatographic methods [27]. Monoclonal antibody ligands immobilized on suitable supports, have gained importance in the recent past for the separation of biomolecules like rHBsAg, owing to their high specificity. Bioaffinity chromatography utilizes immobilized MAbs to bind and recover specific, functional proteins from complex biological materials under suitable physical and chemical conditions [15]. In this study, an immunosorbent was prepared using the ligand (MAb-HBs06) and NHS-activated Sepharose CL-4B with a 95% binding efficiency and an operational binding capacity of 50µg rHBsAg/ml of coupled gel. A laboratory scale BAC method was standardized for the purification of rHBsAg from yeast cell lysates recovering > 90% of target protein. The recombinant yeast-derived HBV major surface antigen with permissible levels of leached ligand, yeast HCPs, DNA, endotoxin, carbohydrates and slightly higher total lipids was obtained by BAC based purification. Further optimization of the process can be explored by delipidation of the crude lysate with a mild detergent before BAC. Also, multiple experimental purifications with the standardized BAC resin would be required for reproducibility of a good quality rHBsAg at an economical cost.

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Conflict of Interest

Not available

Financial Support

Not available

6. Reference

- Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;256(5517):495-497.
- Wampler DE, Lehman ED, Boger J, McAleer WJ, Scolnick EM. Multiple chemical forms of hepatitis B surface antigen produced in yeast. *Proc Natl Acad Sci U S A*. 1985;82:6830-6834.
- Ellis RW, Kniskern PJ. In: McLachlan A, editor. *Molecular biology of the hepatitis B virus*. CRC Press, 1991, p. 3077.
- Zhang H, Itoh Y, Suzuki T, Ihara KI, *et al*. Establishment of monoclonal antibodies broadly neutralize infection of hepatitis B virus. *Microbiol Immunol*. 2022;66(4):179-192.
- Paul SS, Patwa SM, Tan YJ. Development of monoclonal antibodies to target the large surface protein of hepatitis B virus and their use in therapeutic and diagnostic applications. *J Viral Hepat*. 2023;30:870-878.
- Fernández de Cossío ME, Díaz T, Galván A, Valdéz R, González E, *et al*. Antigen recognition characteristics and comparative performance in immunoaffinity purification of two monoclonal antibodies specific for the hepatitis B virus surface antigen. *J Biotechnol*. 1997;56(2):69-80.
- Agraz A, Duarte CA, Costa L, Pérez L, Páez R, *et al*. Immunoaffinity purification of recombinant hepatitis B surface antigen from yeast using a monoclonal antibody. *J Chromatogr A*. 1994;672:25-33.
- Hernandez RH, Bayolo LG, Nunez GF, Simon JG, Perez M, *et al*. Optimization of CB. Hep-1 MAb coupling to Sepharose. *Biotechnol Appl*. 2001;18:207-10.
- Leyva A, Sánchez JC, Valdes R, Font M, López L, *et al*. A combinatorial strategy of a new monoclonal ELISA and immunoaffinity chromatography using sodium deoxycholate to increase the recovery of multimeric proteins like r-HBsAg. *Talanta*. 2010;8:314-9.
- Valdés R, Reyes B, Alvarez T, García J, Montero JA, *et al*. Hepatitis B surface antigen immunopurification using a plant-derived specific antibody produced in large scale. *Biochem Biophys Res Commun*. 2003;310:742-7.
- Shanmugham R, Thirumeni N, Sankarashetty Rao V, Pitta V, Kasthuri S, *et al*. Immunocapture enzyme-linked immunosorbent assay for assessment of in vitro potency of recombinant hepatitis B vaccines. *Clin Vaccine Immunol*. 2010;17:1252-60.
- Gallant SR, Koppaka V, Zecherle N. Immunoaffinity chromatography. *Methods Mol Biol*. 2008;421:53-60.
- Ibarra N, Caballero A, Gonzalez E, Valdes R. Comparison of different elution conditions for the immunopurification of recombinant hepatitis B surface antigen. *J Chromatogr B*. 1999;735:271-7.
- Taylor KACC. A modification of the phenol/sulfuric acid assay for total carbohydrates giving more comparable absorbances. *Appl Biochem Biotechnol*. 1995;53(3):207-214.
- Sheng S, Kong F. Separation of antigens and antibodies by immunoaffinity chromatography. *Pharm Biol*. 2012;50(8):1038-1044.
- Quintana M, Ferro W, Llamas A, Gonzalez T, Castillo. Impact of ligand density on hepatitis B virus surface antigen immunoaffinity chromatography efficiency and ligand leakage. *J Validation Tech*. 2020. Accessed on March 21, 2023.
- Jajan LHG, Hosseini SN, Abolhassani M, Ghorbani M. Progress in affinity ligand-functionalized bacterial magnetosome nanoparticles for bio-immunomagnetic separation of HBsAg protein. *PLOS One*. 2022;17(7):e0267206.
- Sánchez-Romeu J, País-Chanfrau JM, Pestana-Vila Y, López-Larraburo I, Masso-Rodríguez Y, *et al*. Statistical optimization of immunoaffinity purification of hepatitis B surface antigen using response surface methodology. *Biochem Eng J*. 2008;38(1):1-8.
- Pepper DS. Selection of antibodies for immunoaffinity chromatography. *Methods Mol Biol*. 1992;11:135-71.
- Chase HL. Affinity separations utilizing immobilized monoclonal antibodies. *Chem Eng Sci*. 1983;39:1099-1125.
- Hernández R, Plana L, Gómez L, Expósito N, Valdés J, *et al*. Optimisation of the coupled monoclonal antibody density for recombinant hepatitis B virus surface antigen immunopurification. *J Chromatogr B*. 2005;816:1-6.
- Ryu CJ, Kim YK, Hur H, Kim HS, Oh JM, *et al*. Mouse monoclonal antibodies to hepatitis B virus preS1 produced after immunization with recombinant preS1 peptide. *Hybridoma*. 2000;19:185-9.
- Indian Pharmacopeia. Hepatitis B vaccine (rDNA). Indian Pharmacopeia Commission, India. 2007;3:1949-1952.
- European Pharmacopeia. Hepatitis B vaccine (rDNA). Directorate for the Quality of Medicines and Healthcare of the Council of Europe (EDQM), France, 2008, p. 800-801.
- Ottone S, Nguyen X, Bazin J, Bérard C, Jimenez S, *et al*. Expression of hepatitis B surface antigen major subtypes in *Pichia pastoris* and purification for in vitro diagnosis. *Protein Expr Purif*. 2007;56:177-188.
- Valdés R, Medina Y, Ferro W, Reyes B, Geada D, *et al*. Comparison of different ligand densities in immunoaffinity chromatography of the plantibody HB-01 coupled to Sepharose CL-4B to purify the rHBsAg. *J Chromatogr B*. 2007;852:1-7.
- Hardy E, Martínez E, Diago D, Díaz R, González D, *et al*. Large-scale production of recombinant hepatitis B surface antigen from *Pichia pastoris*. *J Biotechnol*. 2000;77:157-67.

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