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A monolith purification process for virus-like particles from yeast homogenate

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ABSTRACT

Monoliths are an alternative stationary phase format to conventional particle based media for large biomolecules. Conventional resins suffer from limited capacities and flow rates when used for viruses, virus-like particles (VLP) and other nanoplex materials. The monolith structure provides a more open pore structure to improve accessibility for these materials and better mass transport from convective flow and reduced pressure drops. To examine the performance of this format for bioprocessing we selected the challenging capture of a VLP from clarified yeast homogenate. Using a recombinant *Saccharomyces cerevisiae* host it was found hydrophobic interaction based separation using a hydroxyl derivatised monolith had the best performance. The monolith was then compared to a known beaded resin method, where the dynamic binding capacity was shown to be three-fold superior for the monolith with equivalent 90% recovery of the VLP. To understand the impact of the crude feed material confocal microscopy was used to visualise lipid contaminants, deriving from the homogenised yeast. It was seen that the lipid formed a layer on top of the column, even after regeneration of the column with isopropanol, resulting in increasing pressure drops with the number of operational cycles. Removal of the lipid pre-column significantly reduces the amount and rate of this fouling process. Using Amberlite/XAD-4 beads around 70% of the lipid was removed, with a loss of VLP around 20%. Applying a reduced lipid feed versus an untreated feed further increased the dynamic binding capacity of the monolith from 0.11 mg/mL column to 0.25 mg/mL column.

1. Introduction

Chromatography is a commonly used method in the purification of biologics, with the market dominated by conventional particle based resins. But with large nanoplexes, such as viruses and DNA, these resins show low capacities and long processing times as titres are typically relatively low. To improve the limited capacity of large particles, companies produced resins with smaller bead sizes, however the consequence is a higher resistance to the flow of the mobile phase [1]. The 1990s saw the development of solid phases designed for nanoplexes based on membranes [2–4] and monoliths for viruses and plasmid DNA [5–9].

Research on the first monoliths appeared around 1967 but was abandoned before it was revived in the late 1980s. The first commercial columns from BIA separations called CIM (Convective Interaction Media) disks were on the market in 1998. The stationary phase of monoliths is formed from a continuous porous material and can be in the shape of disks or columns. The characteristics of monoliths are a high porosity allowing mass transport by convection, low pressure drops and a good capacity for nanoplexes [1,10,11]. Particle based absorbents require the transport of molecules though their pores via diffusion, this can be slow or even prevent large molecules accessing the large internal surface area. The open pore structure in monoliths uses flow to improve the mass transfer to the sites of adsorption [12].

Hence monoliths exhibit flow-independent performance and can run over a range of flow rates, which would be unachievable with a conventional resin column, indicating that the adsorption is not mass transfer limited. The advantages of this can be seen in reduced processing times, although the flow rate becomes limited by the high-pressures formed at very high rates [10,13,14]. Scale up of the monolith based columns is complex as the manufacturing process is highly exothermic, resulting in the possibility of an inhomogeneous structure [11]. Therefore monoliths are changed from axial flow in the disks to radial flow in the larger columns [1]. Columns of up to 8 L have been produced this way for a commercial plasmid DNA process [8].

Virus-like particles (VLP) are a proven alternative to viral vaccines, their success is in part due to the fact that they contain no genetic material but mimic the overall structure of virus particles [15]. This still causes the immune response needed to induce protection in the patient but with fewer health risks over the classic attenuated, or inactivated viral vaccines [16]. Currently two types of VLP vaccines are available on the market against the hepatitis

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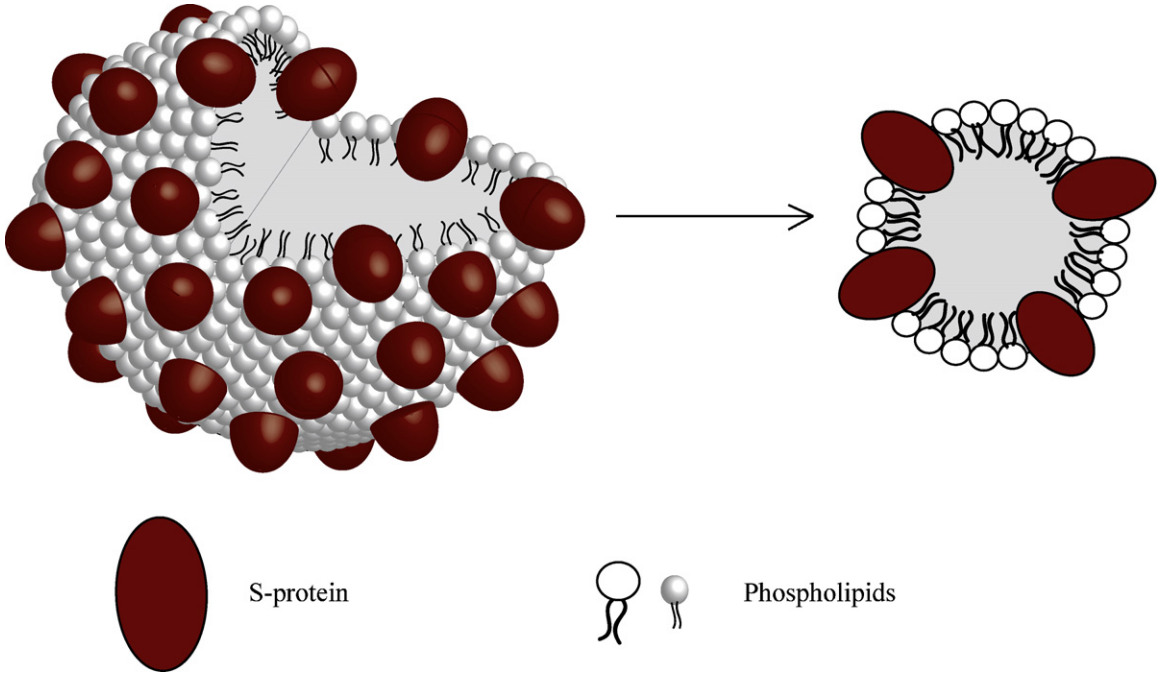

Fig. 1. A schematic diagram of the virus-like particle HBsAg from yeast. Each VLP is around 22 nm in size and 3.5 mDa. It is made of approximately 75% protein (S-protein) and 25% lipid. The core of the VLP contains free host lipids.

B virus (HBV) and the human papilloma virus (HPV). The hepatitis vaccine is composed of a VLP based on the hepatitis B surface antigen (HBsAg). It can be produced recombinantly in yeast, such as *Saccharomyces cerevisiae* [17–21] or mammalian cells [22]. It buds from the endoplasmic reticulum as a 22 nm lipoprotein (Fig. 1) composed of 25% protein and 75% lipid [23].

Purification of the VLP is possible using chromatographic separation with a weak hydrophobic interaction media, such as Butyl-S Sepharose 6 Fast flow [24,25]. The use of complex homogenised feeds can cause fouling during consecutive chromatography runs resulting in reduced dynamic binding capacities and earlier breakthroughs [26–28]. Using a homogenised and clarified crude *S. cerevisiae* feed, containing the HBsAg VLP, Jin et al [24] saw fouling effects from the lipid released during the detergent VLP liberation step during purification. Pretreatment of the feed before loading on to the column can greatly reduce the effect of fouling [26,29] which can be visualised using confocal microscopy [24,30] to confirm the position and degree of fouling.

In this paper the chromatographic process was successfully transferred to a hydrophobic interaction monolith. A lipid removal step was then introduced into the current purification process for the VLP (see Fig. 2), after screening for a suitable lipid removal method. The dynamic binding capacity of the monolith was compared with crude and reduced lipid feeds to highlight the effect of clarification on the feed.

2. Materials and methods

All chemicals were purchased from Sigma–Aldrich (Poole, UK) unless stated.

2.1. Fermentation

The virus-like particle (HBsAg) was produced using a recombinant *S. cerevisiae*. Cultures were grown in three stages; stages 1 and 2 were in shake flasks on a rotary shaker for 24 h and stage 3 was in a 75 L fermenter (Inceltech High containment fermenter, Maidenhead, UK) for 72 h. The full process and media components are

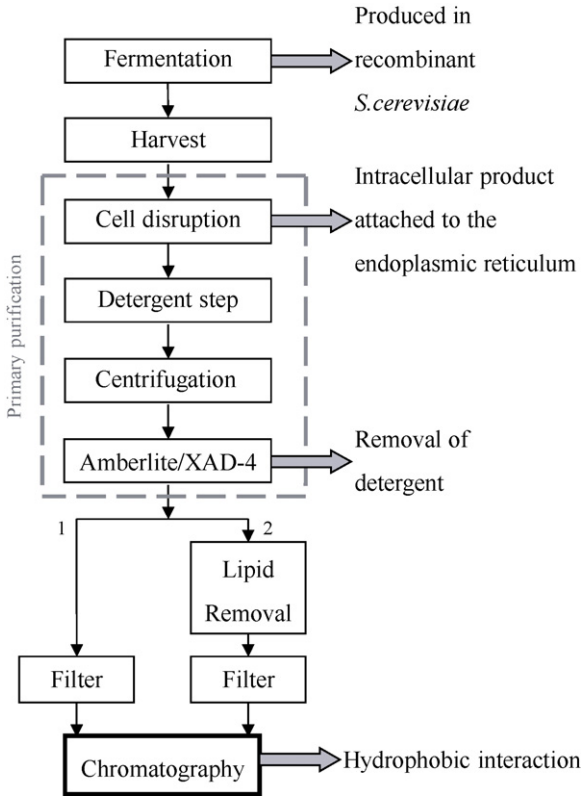

Fig. 2. Flow sheet of primary purification and pre-chromatography preparation steps adapted from [32]. The VLP is produced as intracellular product in recombinant *Saccharomyces cerevisiae* and the detergent Triton X-100 cleaves it off the endoplasmic reticulum. Removal of the detergent is with Amberlite (XAD-4) before filtration prior to being placed on the column. Material can then follow two different routes; route one is for crude material and is filtered before the column, route two is for a reduced lipid feed where a lipid removal method is applied before filtration.

detailed in [31]. Glucose and galactose was monitored off-line (YSI UK, Hampshire, UK) along with OD_{600} and dry cell weight. Cells were harvested at 72 h and centrifuged at 15,000 rpm at a flow rate of 1 mL/min using a tubular bowl centrifuge (CARR Powerfuge P6, Pneumatic Scale Corporation, FL, USA). The cell paste was stored at −80 °C.

2.2. Purification process

The primary purification process carried out is detailed in Fig. 2 and was adapted from Kee et al. [32], Frozen cell paste was resuspended at 25% (w/v) in 0.1 M sodium phosphate, 0.5 M sodium chloride and 2 mM phenylmethylsulfonyl fluoride (dissolved in isopropanol). Cell disruption was carried out in a homogeniser at 1200 bar for three passes (Gaulin Micron Lab 40, APV Gaulin GmbH, Germany) or 400 bar for eight passes (Lab 60). The detergent Triton X-100 was added to the homogenate to a final volume of 0.4% (v/v) and incubated for 4 h at 20 °C. Centrifugation was carried out at 3000 × *g* for 5 min to remove cell debris. Removal of the Triton X-100 was by XAD-4 beads in a batch mode at 0.5 g Triton per g XAD-4 (in accordance to values specified on Sigma product information sheet) for 2 h at 20 °C. This was followed by filtration to remove the beads at 1.0 μm and 0.7 μm (Whatman, Kent, UK).

2.3. Lipid removal protocol

Lipid removal was carried out in a batch process using material which was prepared from the purification process. For the ammonium sulphate precipitation saturated solutions were added to the sample in equal volume with the crude yeast material. Samples were mixed for 20 min and then spun at 14 K rpm for 10 min to remove any precipitate. Both lipid removal absorbent (LRA) (Advance Mineral Corp, CA, USA) and XAD-4 (Amberlite) were added at the relevant (w/v) concentrations to the sample and mixed for 30 min. The sample was then filtered with a 1.0 μm filter (Whatman, Kent, UK) to remove the LRA and XAD-4. Cuno Zeta Plus® BC25 capsule filters (Cuno 3 M, Bracknell, UK) were run at varying flow rates using an AKTA Crossflow system controlled by Unicorn version 4.0 (GE Healthcare, Bucks, UK). Filters were equilibrated with a solution of equal parts homogenisation buffer and 0.01 M sodium phosphate, pH 7.0 at a 1:1 ratio. A new filter was used at each flow rate. All filtrates and the supernatants were collected and analysed for VLP and lipid levels.

2.4. Hydrophobic interaction chromatography (HIC)

The monolithic chromatographic process was carried out using CIM® 0.34 mL disk or 1 mL column monoliths (BIA Separations, Slovenia) with either C4 or OH ligands. Flow of the mobile phase and sample in the disks is axial, whereas the 1 mL column is tube shape and the flow is radial. The method was adapted from work by Jin et al. [24]. Buffer conditions were (unless stated) as follows; Buffer A, 20 $mmol^{-1}$ sodium phosphate, 1.0 M ammonium sulphate, pH 7.0; Buffer B, 20 $mmol^{-1}$ sodium phosphate, pH 7.0; Buffer C, 30% isopropanol in Buffer B. Cleaning-in-place was with 1 M sodium hydroxide. The samples were filtered with a 0.45 μm filter (Millipore, UK) and then equilibrated to the salt level in Buffer A by adding ammonium sulphate to the feed. Feed volumes were adjusted depending on column size and material type, with 2 mL for the 0.34 mL disks and 5 or 10 mL for the 1 mL columns for the lipid and reduced lipid feed, respectively. The 1 mL columns were equilibrated with 20CV of Buffer A followed by loading and a wash step totalling 20 or 25CV. Elution was carried out with 10CV of Buffer B, with regeneration of the columns using Buffer C for 10CV to remove tightly bound material. A CIP was carried out after every run, which was followed by a water wash. The 0.34 mL columns were loaded and washed with Buffer A for 10CV, elution with buffer B was for 7CV and regeneration with Buffer C for 10CV. All chromatography was carried out on an AKTA Explorer 100 system controlled with Unicorn Version 4.0 (GE Healthcare, Bucks, UK) and monitored using 280 nm.

The Butyl-S HiTrap 1 mL columns were run on the same system using the method from Jin et al. [24]. Columns were run at 1 mL/min with 0.6 M ammonium sulphate in Buffer A. Buffers B and C were the same as above, and the columns were cleaned with 0.5 M NaOH.

2.5. Enzyme linked immunosorbent assay (ELISA) for VLP

Quantification of the VLP was carried out using the Abbott-Murex HBsAg Version 3 96-well plate ELISA kit (Dartford, UK). An HBsAg standard was obtained from Aldevron GENOVAC GmbH (Freiburg, Germany). Samples were pre-diluted 1000 fold before use to lie within the calibration range.

2.6. Lipid analysis by HPLC

To identify and quantify the type and amounts of lipids present a Jordi Gel Glucose-DVB 500 Å column (Grace, Lancashire, UK) was used as stated in Jin et al. [24]. Samples were applied to the column using a mobile phase of chloroform, methanol and 0.15% TFA (in water) in the ration 50:43:7. Samples were prepared by adding 70 μL of the required sample to 930 μL of extraction solution, which is the same composition as the mobile phase without water. Mixtures was centrifuged 30 min after extraction solution addition to removal any solids. The column was operated at 1 mL/min on an Agilent 1100 HPLC system (Wokingham, UK) with an injection volume of 50 μL. After the column samples were passed through an Evaporative Light Scattering detector (ELSD, Grace) operating at 39.8 °C, a gas flow of 1.4 mL^{-1} and gain 1.

2.7. Electron microscopy

Chromatography elution samples were concentrated and diafiltered with 0.01 M PBS pH 7 using 7 mL Pierce protein concentrators, MWCO 150,000 (Thermo Scientific, Loughbough, UK). Electron microscopy was carried out at UCL department of Cell and Developmental biology, using a JEOL 1010 transmission electron microscope (Jeol, UK). Samples were placed onto a carbon grid and the sample was left to dry. The carbon grid was stained with 2% uranyl acetate for approximately 1 min. The grid was viewed at 300K×.

2.8. Confocal microscopy

Neutral lipids were labelled with BODIPY 493/503 dye solution (Invitrogen, Paisley, UK) to give a final ratio of 8 μmol L^{-1} g^{-1} lipids. The solution was incubated at 20 °C overnight before use. The C4 monolith disks (0.34 mL) were challenged for 3 chromatography runs with a crude feed containing the fluorescently labelled lipids. Images were acquired using a confocal microscope (Pseemore upright SP1, Leica Microsystem GmbH, Mannheim, Germany) with a 10× objective lens.

2.9. Atomic force microscopy

Glass slides were cleaned with ethanol and silanised with trichloro (1H, 1H, 2H, 2H-perfluoroctyl) silane vapours to make it hydrophobic and encourage the VLP to bind to the surface. The silanised glass slides were attached to magnetic holders (Agar Scientific, UK) using a protocol from Müller and Engel [33]. The glass slide with the VLP was prepared in a similar method to that stated by Milheit et al. [34], with the VLP samples concentrated and diafiltered into Tris–HCl 20 mM, NaCl 150 mM, pH

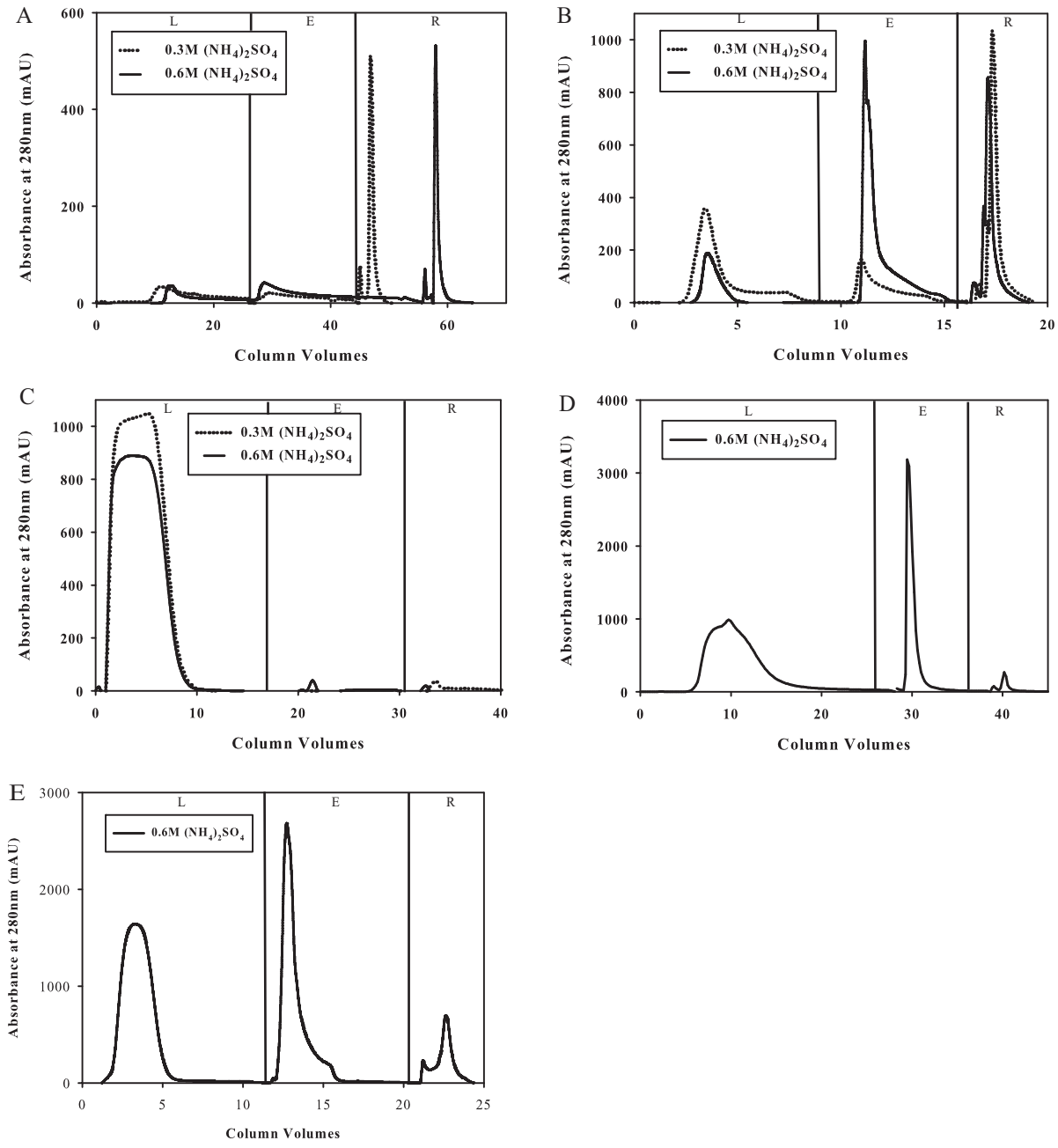

Fig. 3. Chromatograms obtained during screening for a suitable monolith column ligand with post-hydrophobic interaction chromatography material. Material loaded on to C4 and OH column at 0.6 M $(NH_4)_2SO_4$ and 0.3 M $(NH_4)_2SO_4$ to adjust binding affinities within in the column. Absorbance profiles at 280 nm are shown with peaks marked out for (L) Loading Zone; (E) Elution Zone and (R) Regeneration. Graphs correspond to (A) high ligand density (HLD) C4 1 mL monolith columns, (B) low ligand density (LLD) C4 disks, (C) 50% C4 and 50% OH 1 mL monolith columns and (D) OH 1 mL monolith columns. Columns A/B/D are commercially available. Column C was a gift from BIA Separations.

7.4, using 7 mL Pierce protein concentrators (Thermo Scientific, Loughbough, UK). The VLP was incubated onto the glass slides, for two hours and fixed with 5% glutaraldehyde. AFM was performed in a tapping/intermittent mode using a Multimode SPM (Veeco, Mannheim, Germany), with a J scanner and NSC15 cantilevers (Mikromasch, Estonia) and Nanoscope 5.3 software.

3. Results and discussion

3.1. Use of monoliths for VLP separation

The current chromatographic method of VLP separation uses a weak hydrophobic ligand, as the VLP is strongly hydrophobic. The purification process was transferred to a 1 mL poly (butyl methacrylate-co-ethylene diamethacrylate) monolith chromatography column and subsequently the salt levels in binding were then studied to maximise binding and recovery. Three commercially available columns with high density C4, low density C4 and hydroxyl (OH) ligands were selected as well as a prototype column, with 50% C4 butyl and 50% OH ligands. Crude yeast containing the VLP was prepared using the purification process (Fig. 2) and passed through a Butyl-S 6 Sepharose FF column. The elution fraction containing the VLP from the column was loaded on to the monoliths at 0.6 M or 0.3 M ammonium sulphate. If the elution peak was smaller than the peak for the regeneration step this was an indication that the VLP had bound too tightly to the column and was only being

Table 1
Recoveries of VLP obtained during initial screening experiments with C4 and OH columns using purified VLP and 0.6–0.3 M ammonium sulphate.

Column type	0.6 M $(NH_4)_2SO_4$ Recovery of VLP (%)	0.3 M $(NH_4)_2SO_4$ Recovery of VLP (%)
High ligand density C4	2–5	1–3
Low ligand density C4	40–50	20–23
50% C4 and 50% OH ligands	0–1	0–1
Hydroxyl ligands	85	–
Butyl-S Sepharose 6 FF [24]	90	–

removed using isopropanol in the regeneration buffer. The isopropanol renders the VLP unusable and so the amount of VLP lost to this fraction must be minimised. Reducing the amount of salt in the loading buffer decreases the binding affinity of the VLP to the column which helps to reduce the VLP being lost in the regeneration step.

The three columns with the C4 ligands proved to be unsuitable for the purification of the VLP (Fig. 3A–C). The reduction in the ligand density from high Fig. 3A to low Fig. 3B did result in an increased amount of VLP eluted from the column but recovery was only around 45% (see Table 1) with most VLP was removed from the column in the regeneration step. Reducing the salt level to 0.3 M resulted in a lower amount of VLP eluted, possibly as the low salt did not promote sufficiently strong binding. The column from Fig. 3C was the prototype column using 50% C4 ligands with 50% OH ligands. Although this column theoretically should have the lowest hydrophobicity of the three columns with C4 ligands it was evident during use that it still retained high hydrophobic properties.

The only column to perform the purification effectively, with recoveries around 85% was the OH column (Fig. 3D), which is comparable to the Butyl-S 6 Sepharose FF column (Fig. 3E and Table 1). This column has the weakest hydrophobicity and exhibits some hydrophilic properties. By increasing the amount of salt in the loading buffer from the initial level of 0.6 M to 1.0 M recoveries of around 90% were obtained. Compared to the current bead based process the monolith had a dynamic binding capacity for the VLP that was approximately three to four times larger when challenged with untreated yeast homogenate (see Fig. 4).

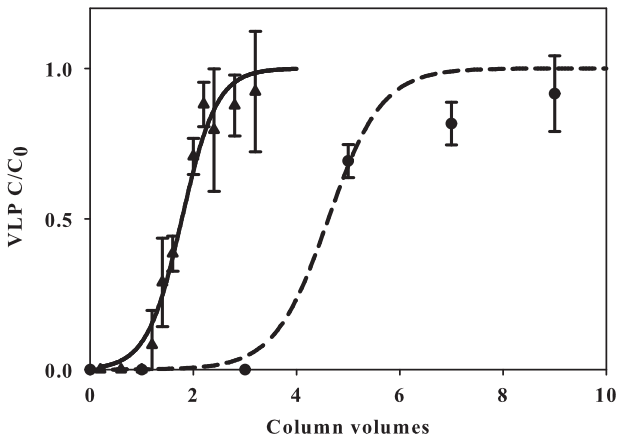

Fig. 4. Comparison of dynamic binding capacity of butyl-S Sepharose 6 FF (GE Healthcare) 1 mL HiTrap column (▲) and a monolith OH 1 mL column (BIA Separations) (●). The columns were loaded with untreated homogenised yeast and the VLP breakthrough was monitored using ELISA ($n = 2$ for butyl-S column and $n = 3$ for OH monolith. Error bars are 1 S.D).

3.2. Lipid fouling in monoliths

Fouling and column regeneration is a major issue in chromatography often resulting in a reduction in dynamic binding capacities, increasing pressure drops and rapidly decreasing the life span of the column. Lipids are a particularly difficult foulant to control as they often bind irreversibly and cannot be removed easily though cleaning methods. Confocal microscopy is a useful tool to visualise the degree and position of fouling on chromatography resins [24,30]. Using a method adapted from Jin et al. [24], a C4 monolith disk was run in a bind-elute mode with a feed containing fluorescently labelled neutral lipids. The use of a C4 ligand on the monolith and not the OH ligand allowed the formation of the fouling to occur

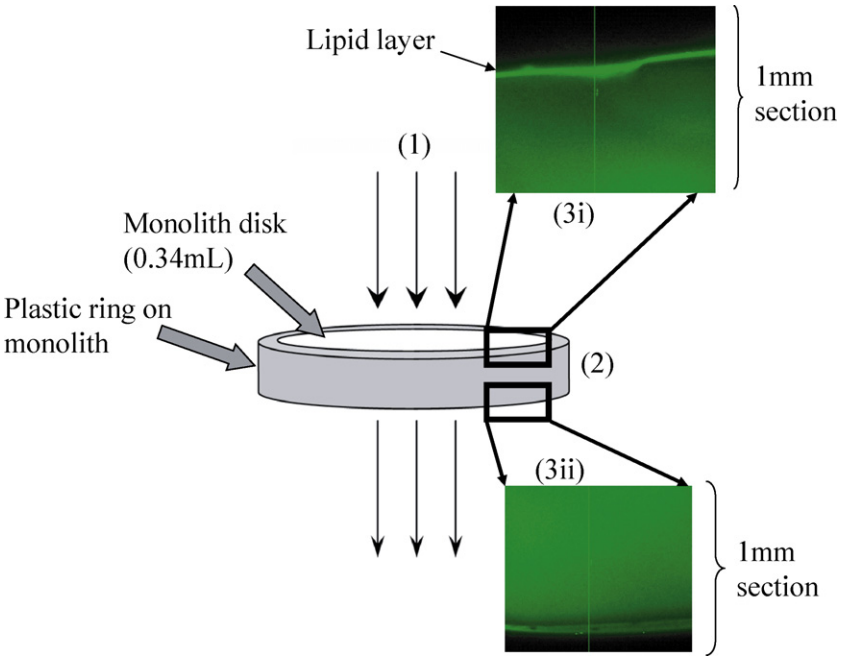

Fig. 5. Confocal microscopy showing the fouling of lipids in a C4 disk monolith. The lipids were fluorescently labelled with BODIPY 493/503 dye (Invitrogen, Paisley, UK). (1) The column was challenged with the labelled crude VLP material; (2) the column was cut into sections and placed under the confocal microscope; (3i) the lipids form a layer on top of the column, causing an increase in pressure and reducing the recovery and (3ii) the lipids move throughout the column too, affecting the VLP recovery.

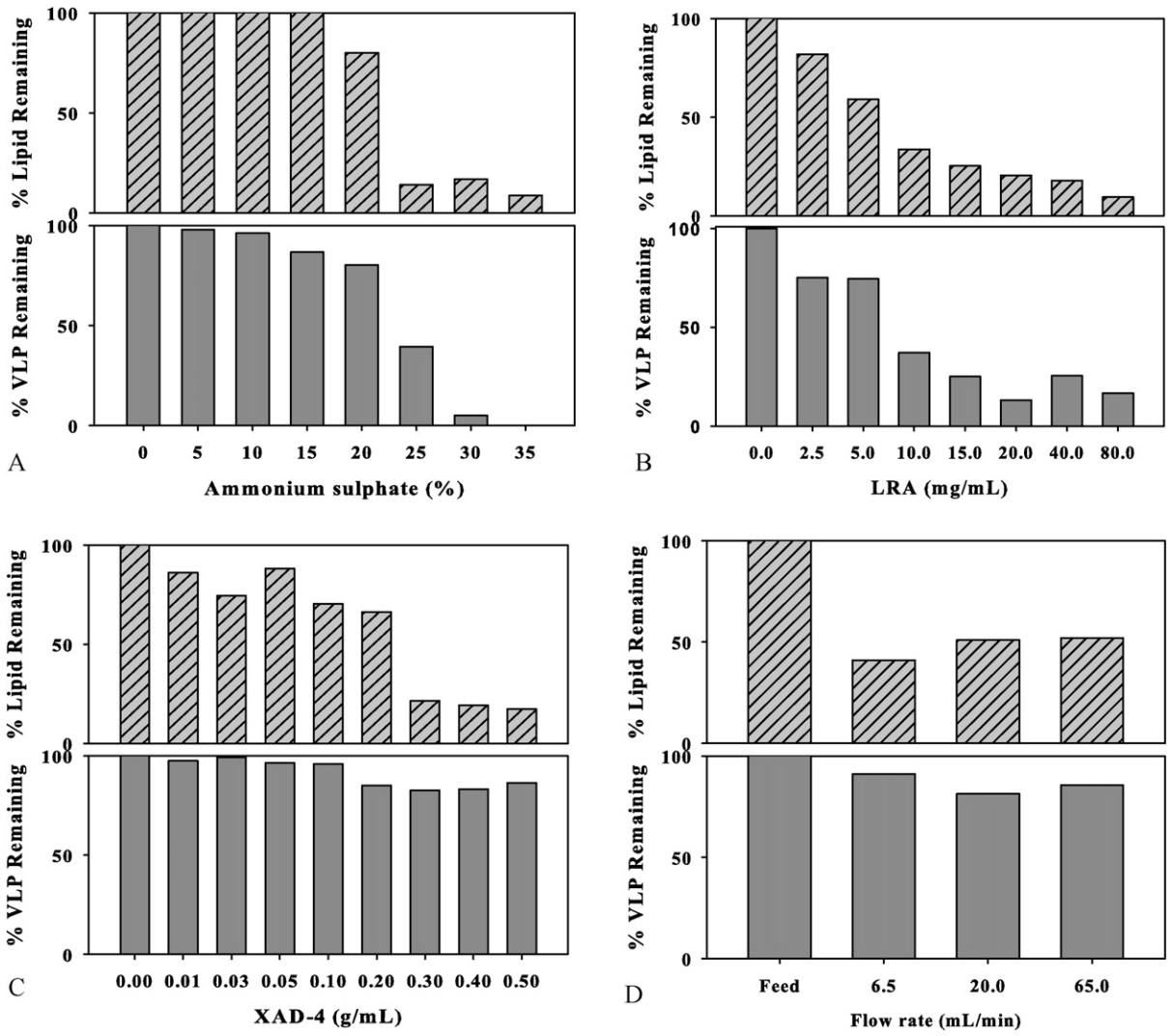

Fig. 6. Comparison of the four lipid removal methods tested on post primary purification material to determine the amount of depletion and the VLP retained. The level of lipid was determined by evaporative light scattering detection and the VLP retained analysed by ELISA. The four methods were (a) ammonium sulphate precipitation; (b) lipid removal absorbent (LRA); (c) Amberlite/XAD-4 and (d) Cuno filters.

at a faster rate and therefore enable a more defined distinction between fouled areas under the confocal microscope. The column was removed from the supporting ring around it and sliced into thin sections with a razor blade. A vertical cross-section was placed on a microscope slide for visualisation under the confocal microscope and 1 mm sections were captured across the column. A distinct layer of lipids was seen on the top of all the sections analysed, compared with the middle and lower sections which had a lower degree of fluorescence (Fig. 5). The build up of lipids suggests that the top of the disk acts to capture a portion of the lipids and lipid micelles while still allowing some to deposit in the main body of the disk. This build up causes an increase in the pressure drop experienced by the column over successive runs (results not shown) which reduces the number of runs the column can be used for as the maximum pressure is quickly reached. Consecutive runs with the OH monolith sees a slower but still highly significant increase in the pressure drop over the column.

3.3. Lipid removal methods from pre-chromatography feed

The lipids in the feed are released during homogenisation of the yeast cells at high pressure and the detergent step to liberate the VLPs from the membranes of the endoplasmic reticulum,

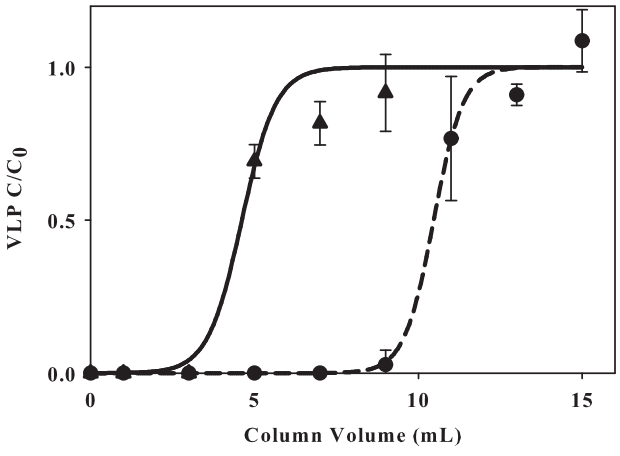

Fig. 7. Comparison of the VLP breakthrough curves of 1 mL hydroxyl monoliths with a crude (▲) and reduced lipid material (●). The reduced lipid feed was prepared by adding 0.3 g/mL of Amberlite/XAD-4 for 30 min at 20 °C to the crude material. Binding was under 1 M $(NH_4)_2SO_4$ pH 7 at 3 mL/min. Points represent triplicate runs.

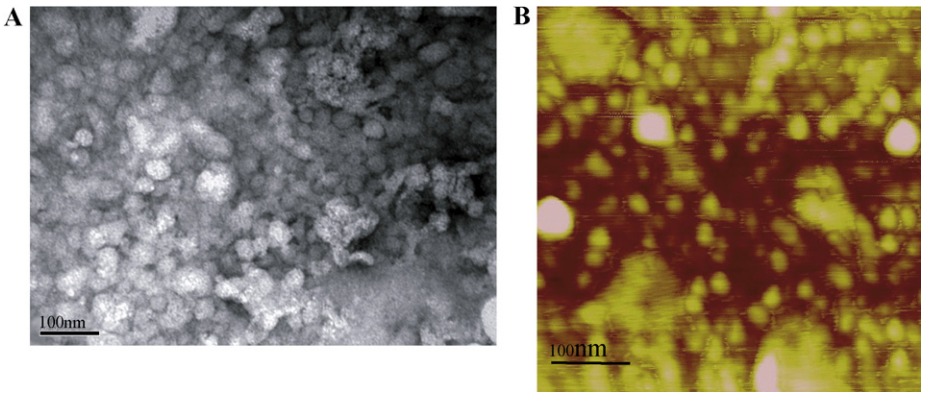

Fig. 8. Pictures of HBsAg from elution samples after hydrophobic interaction chromatography using an OH monolith. The black bar is equal to 100 nm on both pictures. (A) Transmission electron microscope (TEM). Samples were negatively stained with 2% uranyl acetate on carbon grid. (B) Atomic force microscopy picture (AFM). Samples were adhered to a silanised glass slide. AFM was in tapping mode.

which also causes the release of lipids from the yeast debris. Removal of lipids before the chromatography step can help increase the binding capacity and lifetime of a column [24]. Four methods were chosen to evaluate the amount of lipid removed and VLP retained; (1) ammonium sulphate precipitation [29], (2) lipid removal absorbent (LRA), (3) Cuno Zeta Plus filters [29] and (4) XAD-4 (Amberlite). The methods were applied in batch mode to material which had completed the primary purification process detailed in Fig. 2.

Solutions of varying ammonium sulphate saturations were added to crude feed material, with levels up to 35% (w/v) (Fig. 6a). Lipid reduction does not occur until the saturation level of ammonium sulphate is at or above 20% (w/v), with around 75% lipid removal achievable. But the reduction in lipid also coincides with a loss of VLP, with complete removal occurring at 35% (w/v), suggesting that the VLP precipitates in a similar way to the lipids. Lipid removal absorbent (LRA) media is a synthetic calcium silicate hydrate adsorbent which is effective for lipids, lipopolysaccarides and lipoproteins. Reduction in lipid levels corresponded to a similar reduction in the amount of VLP which remained (Fig. 6b), suggesting that co-removal occurred. As the VLP is essentially a lipoprotein, this reduction is understandable.

The other two methods were more effective at removing lipids while retaining high levels of VLP. Levels of the VLP recovery were at 80% or greater, showing a distinct advantage over the other two methods. Cuno Zeta Plus Filters are depth filter capsules using diatomaceous earth as an adsorbent. Three different flow rates, 6.5/20/65 mL/min were chosen to study their performance (Fig. 6d). At 6.5 mL/min the greatest reduction in lipid was seen, as expected due to the longer residence time, although the highest amount of VLP was also retained. An increase in flow rate 10 fold to 65 mL/min only decreased the amount of lipid removed and VLP retained by approximately 10%, showing that this method is robust. The use of XAD-4 (Amberlite) adsorbent was the most effective method, with a VLP to lipid ratio of 4 compared to the filters which had a ratio of 2 (Fig. 6c). Hence XAD-4 was selected to produce a reduced-lipid feed for the purification protocol. XAD-4 is a polyaromatic adsorbent for small hydrophobic compounds and is currently used in our primary purification protocol to removed Triton X-100, enabling it to be easily integrated into the process. The results indicate that the adsorbent has a good specificity for lipids in the feed. At 0.3 g/mL the amount of lipids in the feed was significantly reduced by around 70% but had little impact on the VLP remaining, ensuring that the loss of VLP was less than 20%.

3.4. Effect of lipid removal on chromatography process

To determine the effect of the lipid on the performance of the monolith two 1 mL OH columns were challenged with either an untreated or reduced lipid crude feed (Fig. 2), using the XAD-4 procedure. The runs were carried out with fresh OH columns using 1 M $(NH_4)_2SO_4$ at 3 mL/min and 1 mL samples were taken to determine the binding capacity of the column. The lipid feed had a 10% breakthrough point of 4CV, whereas the reduced lipid feed had a 10% breakthrough of 10CV (Fig. 7). These results show that the reduced lipid feed increases the capacity of the monolith column to bind the VLP. This implies that the lipid competitively binds with VLP to the OH ligand resulting in the much reduced capacity when an untreated feed is used; the same effect will happen with conventional resins using butyl-S resins [24].

Elution samples were examined under electron microscopy and atomic force microscopy to investigate the composition of the VLP after it has been though an OH monolith. Both techniques show a majority of circular VLP particles and a few larger masses (Fig. 8A and B). The VLP was found to be around 30–35 nm under TEM and around 25–30 nm with AFM. These differences in size may be due to the different techniques used to capture the VLP onto the sampling surface and it is unknown what effects they have on the structure of the VLP. The TEM sample was dried onto the carbon grid and resulted in the VLP being condensed together, whereas AFM involved a hydrophobic surface. Both methods indicate that most of the VLP is in monomeric form, although no distinct topography can be seen, and the sizes of the particles correlate to that in the literature [34].

4. Conclusion

The above study shows that the VLP (HBsAg) can be successfully purified by hydrophobic interaction using a monolith format. The use of a weakly hydrophobic interaction OH ligand gave the best performance. This is due to the highly hydrophobic nature of the VLP which binds too strongly to more hydrophobic chemistries. Lipids, a common contaminant in biological material can cause serious column fouling issues, proving hard to remove during regeneration. Within the monolith lipids can cause pressure issues due to deposition and build up on the surface of the monoliths, and reduces the column life of the monolith. Reduction of the lipid levels results in slower pressure increases indicating a reduced rate of fouling. The capacity of the monolith is also improved by removing around 70% of the lipids using XAD-4 beads in a simple batch

mode, doubling the dynamic capacity for the VLP (0.25 mg VLP/mL column).

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