Review article

Re-design of downstream processing techniques for nanoparticulate bioproducts

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Abstract
There has been much interest generated in the recovery of nanoparticulate (nanoparticle) bioproducts (Second generation of biotechnological products) such as plasmid DNA and viruses as putative gene therapy vectors, macromolecular assemblies as drug delivery vehicles and virus-like particles as vaccine components. Such product must be manufactured in advanced stages of purity, material definition and sophisticated formulation to rival those demanded of the pharmaceutical macromolecules which dominate as the first generation products. Nanoparticulates are characterized by a critical size range (10-300 nm diameter) and complexity of surface chemistry and internal organisation which pose new challenges in separation science and engineering, controlled chemistries of modification and material measurement not readily addressed by extant technologies. Current review article is concerns with structural characterisations of nanoparticulate bioproducts as well as re-design of their downstream processing techniques which are common to all programmes. This focus is upon candidate partition systems which can contribute to the fractionation, recovery and purification of nanoparticulate assemblies from their soluble components (capsid proteins from virus, polynucleotides from plasmid DNA, soluble, agglomerated forms of protein etc.). The mechanistic design of new separation and formulation technologies based upon a sound understanding of quantifiable structural features of these nanoparticle bioproducts is strongly indicated.
Keywords: *Nanoparticle bioproducts, Drug delivery vehicles, Laminated adsorbent, Bioseparation, Downstream processing, Aqueous two-phase system.*

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INTRODUCTION

Many of the *Second generation* of biotechnological products are nanoparticulate in nature and in recent times there has been increased demand for these nanobioparticles such as viral and non-viral gene therapy vectors (Friedmann, 1997; Mhashilkar *et al*., 2001; Schleef, 2001 and Buning *et al*., 2003), gene delivery vectors for non-therapeutical applications (Kost and Condreay, 2002; Maranga *et al*., 2002) and protein vaccines such as core and virus-like particles (Robinson, 1993; Anderson, 1995; Andreadis *et al*., 1999; Casal, 2001 and Maranga *et al*., 2002). Such bioproducts are used directly for specific targets as well as exploited with high technology support (Panatarotto *et al*., 2003; Cao, *et al*., 2003 and Jahanshahi *et al*., 2004). The general large dimensions of these bioproducts (10 to>300 nm) distinguish them from protein macromolecules (commonly>5 nm; Whitesides, 2003).

For instance, adenoviral nanoparticle vectors are exploited for gene therapy applications. They have been used for gene transfer to different cells including liver cells (Li *et al*., 1993), heart cells (Barr *et al*., 1994), the central nervous system cells (Davidson *et al*., 1993), lung cells (Ennist, 1999), skeletal muscle

cells (Tripathy *et al.*, 1994), smooth muscle cells (Channon *et al.*, 1996 and Lu *et al.*, 1996) and to cancer cells (Clarke *et al*., 1995). Based on this, some protocols have been developed for the treatment of diseases such as cystic fibrosis, muscular dystrophy, Parkinson's disease, autoimmune diabetes and different cancers including prostate, colon, cervix and ovary (Yeh and Perricaudet, 1997 and Russell, 2000). By the end of 2002, a total of 26 companies exploited adenoviral vector technology (Adadevoh *et al*., 2002).

In order to meet this demand, production titres can be increased by applying molecular and cellular engineering methods to improve expression systems and cell lines (Strauss, 1997 and Andreadis *et al*., 1999). Downstream processing techniques (DSP) for the recovery of nanoparticles face problems other than those encountered during the recovery of biomolecules such as peptides and proteins. As a consequence of the sensitivity of nanoparticulate products to temperature, pH, chemical reagents or processes that compromise the 3-dimensional structure and conformation of the product, a general approach to the downstream processing of such products has evolved (Fig. 1). However, individual steps during a purification process are product specific. Typical nanoparticulate products that require purification for gene therapy applications (Aubert *et al*., 2003) include viruses, such as adenoviruses, retroviruses and adeno-associated viruses and plasmid DNA (Lorens *et al*., 2001). For such products there are several criteria for the selection of appropriate techniques. The protocols selected must be capable of processing large volumes of viral/plasmid preparations having high yields and it is essential that the stability and activity of the product be preserved.

The number of downstream processing steps required to achieve the desired purity of a product considerably influences the cost of DSP. Datar *et al*., (1993) and Lyddiatt and O'Sullivan (1998) have suggested that DSP can account for 70-80% of the total production costs due to the capital investment and amount of consumables required for each step and the time required for each individual operation. With each additional step there are also inherent handling losses affecting the recovery of the final product and the product activity.

The traditional techniques employed for the purification of nanoparticulates are not necessarily the ideal DSP operations for such products. The initial clarification step is often carried out using centrifugation or cross-flow filtration (Kahn *et al*., 2000). Their relative

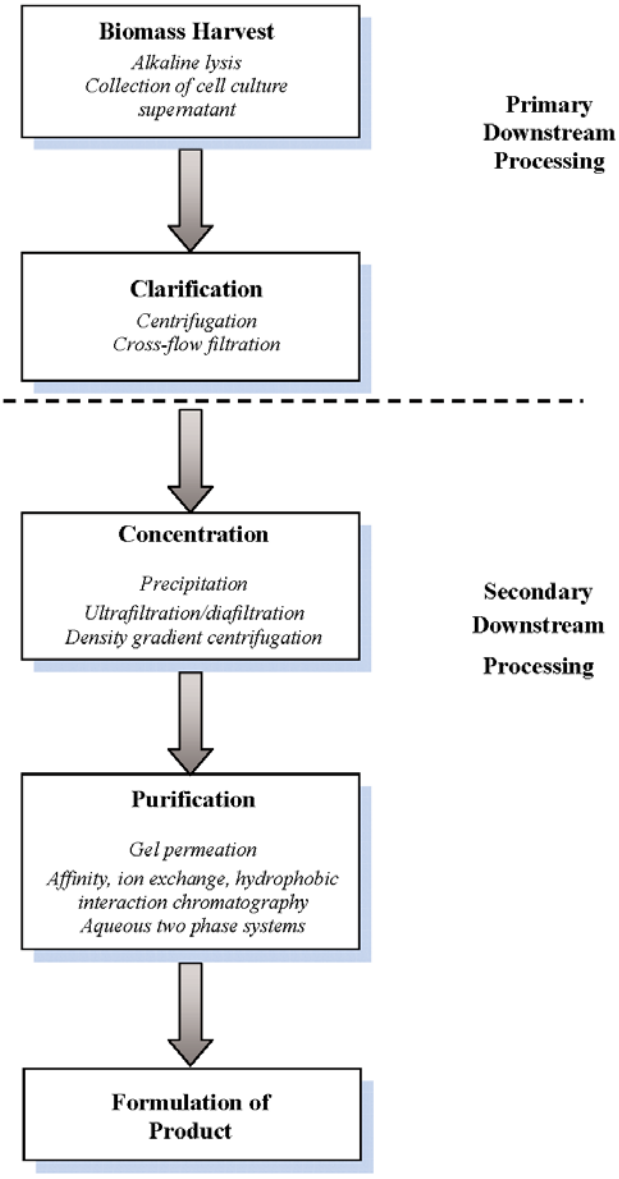

Figure 1: Conventional downstream processing for nanoparticulate bioproducts. This figure represents the conventional downstream processing steps required for the purification of nanoparticulate bioproducts such as plasmid DNA and viruses (Lyddiatt and O'Sullivan, 1998).

success in terms of the resolution of fractionation is dependent upon the differences in size and density between the product, product components and the cell debris and impurities. Also the potential for batch to batch variation of nucleic acid content influences the viscosity of the feedstock (Braas *et al*., 1996). If centrifugation is chosen as the desired operation for the clarification of the feedstock the hydrodynamic shear effects upon the nanoparticulates must be considered when using an industrial centrifuge (Brunner and Hemfort, 1988). Cross-flow filtration has also demon-

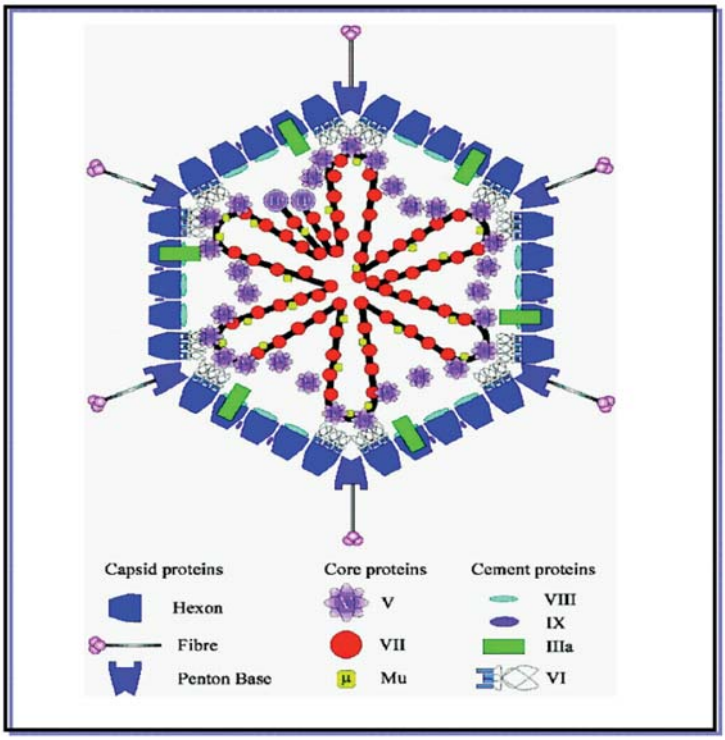

Figure 2: Structure of a nanoparticle bioproduct (e.g. adenovirus structure). The locations of capsid and cement components in adenovirus structure are defined. In contrast, the disposition of the core components and the virus DNA is largely conjecture (Russell, 2000). The main characteristics of the structural proteins of the adenovirus are summarised in Table 1.

strated process advantages, although Aimar *et al*., (1989) highlighted that the pore size distribution of a clean membrane is constantly modified due to fouling. Consequently the resolution of the nanoparticle fraction will be compromised. This is also a factor influencing the use of ultra/diafiltration for the product concentration operational step (Prazeres *et al*., 1999). Other DSP operations employed for product concentration have also demonstrated practical limitations (Andreadis *et al*., 1999; Levy *et al*., 2000). Precipitation using reagents such as polyethylene glycol (PEG) or ammonium sulphate and density gradient centrifugation methods employing sucrose or cesium chloride co-concentrate impurities such as inhibitors resulting in a loss of product activity and integrity. As a result, downstream process routes that are currently in use for biomolecules have to be re-designed (Negrete *et al*., 2001 and Grimm *et al*., 2003).

In addition to characterization of nanoparticles, they are of colloidal size and are generally sophisticated biological structures composed of one or more types of proteins, lipids and/or nucleic acids (Fig. 2 and Table 1). Subsequently, it is also expected various problems are associated with their recovery and purification. Firstly, due to their complex structure, the initial product concentration achievable in production schemes is generally low, being between 10^6 to 10^{12} viruses ml^{-1} for viral particles (Table 2). Given an estimated number between 10^{11} to 10^{14} particles per single dose (Braas *et al*., 1996) for the treatment of a genetic disorder, the supply could be seriously jeopardised by the low product concentration in the broth. It can therefore be concluded that large culture volumes have to be processed to meet such demand. By comparing the location of the product during the production cycle (i.e. intercellular versus extracellular product; Table 2), it is apparent that prior to recovery process volumes are larger for extracellular products.

A feedstock which has a low product concentration may cause an increase in the number of steps required for downstream processing. For example, the adsorption isotherm in column chromatography is generally a function of the product concentration and the adsorbent capacity decreases with decreasing concentration of the latter (Ladisch, 2001; Jahanshahi and Lyddiatt 2002b). As a consequence, the product has to be concentrated prior to more refined purification steps in order to exploit full capacities of adsorbents.

Table 1: Structural proteins of adenovirus

Classification	Protein[1]	No. of Aminoacids[2]	M W (kDa)[3,4,5]	Characteristics[4,6]
Capsid	Hexon (II)	967	110	Major constituent of capsid. Trimeric protein.
	Penton base (III)	571	70	Apical capsomer.
	Fibre (IV)	582	62	Knobbed fibre attached to penton base. Trimeric protein responsible for initial attachment to receptors.
Core	V	368	44	Loosely bound to DNA in a core complex with VII.
	VII	174	22	Tightly bound to DNA.
	μ	19	3	Tightly bound to DNA.
Cement	VIII	134	16	Located in the inner surface of the capsid.
	IX	139	15	Bound to hexon to form group of nine hexons to stabilize the capsid.
	IIIa	566	63	Associated with hexons that surround the penton.Links adjacent facets of the capsid.
	VI	217	24	Bridge between capsid and core.

Table assembled from the following references: (1) Russell, 2000; (2) van Oostrum and Burnett, (1985; (3) Maizel *et al.*, 1968; (4) Russell and Kemp, 1995; (5) Stewart and Burnett, 1995 and (6) Blanche *et al.*, 2001.

In addition, a problem that is associated with the size of nanoparticles is their greatly reduced rate of diffusion in comparison with smaller biomolecules (i.e. compare the diffusion coefficient for bovine serum albumin (BSA) at 6.81×10^{-11} m^2s^{-1} (Geankoplis, 1978) with that for a tobacco mosaic virus at 0.3×10^{-11} m^2s^{-1} as reported by Juckes, 1971). Such reduced diffusion rates can cause mass transfer limitations during conventional chromatographic adsorption processes (Lyddiatt and O'Sullivan, 1998; Ladisch, 2001). Most matrices applied for nanoparticles adsorption were originally designed for protein products and they have geometries unfavourable for the former (Zhang *et al*., 2001 and Thwaites *et al*., 2002). Such incompatibilities have let to quote working capacities for gene therapy vectors in the range of 10^9 to 10^{12} particles per ml settled adsorbent (O'Neil and Balkovic, 1993). It can be concluded that the relatively high cost of adsorbents and the apparent high amounts required to meet the supply of vectors still challenge the process economy.

However, since nanoparticles can be expected to be within the size range of cell debris, conventional centrifugation methods to remove the cell debris may co-precipitate nanoparticles and hence are inapplicable (Hart *et al*., 1994; Middelberg, 2000 and Zhang *et al*., 2002). Nanoparticles can also be lost within the filter cake during conventional dead-end or cross-flow filtration methods as mentioned above. Another feature creating problems for the production and purification of some nanoparticle is their sensitivity to shear. Some viruses with loosely attached fibres, which are responsible for viral infectivity, may lose their function due to shear forces (Hammar, 2000) generally acting upon the particles during centrifugation or cross-flow filtration. Shear forces can also be detrimental to intercellular nanoparticles if they are released from the producer cells by mechanical disruption methods.

It may be concluded that above mentioned problems and stringent regulations governing the production of nanoparticles highlight the need to consider alternative downstream processing steps. Among the various non-conventional methods which have recently received attention are selective precipitation (Tsoka *et al*., 2000 and Lander *et al*., 2002), filtration with affinity membranes (Warner *et al*., 2001), adsorption

with customised adsorbents (Zhang *et al*., 2001 and Jahanshahi *et al*., 2002), subtractive chromatography (Zhang *et al*., 2002 and Jahanshahi *et al*., 2003) and modified aqueous two-phase extraction (Andrews *et al*., 1995 and Liu *et al*., 1998) with the last three methods being more important and hence will be discussed in this paper. In general, ideal steps should ensure that the product stability is maintained and that the product can be recovered from large culture volumes. Additionally, as for any downstream processing procedure the operation must be easily scalable and have a relatively low cost. Finally, the processing of the end product must meet the Food and Drug Administration (FDA) standards for biological therapeutics (Andreadis *et al*., 1999; Levy *et al*., 2000 and Braas *et al*., 2000).

Adsorptive Recovery of Nanoparticles: In the last three decades, an intense and competitive campaign has been pursued by various manufacturing companies to establish a staggering array of solid phase designs (Thömmes *et al*., 1995; Chang and Chase, 1996 and Hjorth, 1997) suited to the selective recovery of a variety of macromolecules (e.g. protein; McCormick, 1993; Huyghe *et al*., 1995 and Blanche *et al*., 2000). Such constructs are not generally suited to the recovery of nanoparticles by virtue of the related qualities of restricted pore dimensions and limited accessible surface area for adsorption. Performance is additionally compromised by the ability of molecular components of nanoparticles bioproducts (e.g. capsid proteins of adenovirus, oligonucleotide fragments of plasmid DNA etc.) and common system impurities (antigens, endotoxins, host DNA etc.) to penetrate the porous interior of extant adsorbents and compromise the process operation of such materials. Therefore, current chromatographic technology developed primarily for macromolecular products (1-5 nm) is not well suited for the recovery of nanoparticulates due to the large size range of these products (10-300 nm) and their complex surface characteristics.

More recently, an extensive programme has been conducted to evaluate the manufacture of alternative geometries of solid phases for fluidised bed adsorbents to the selective recovery and purification of nanoparticle bioproducts. Fluidised bed adsorption technology (Chase, 1994) is a technique that can be employed for nanoparticulate purification providing practical advantages such as the potential for direct process integration as has been seen with protein products. (Morton and Lyddiatt, 1994; Hamilton *et al*., 1999; Bierau *et al*., 1999; Bierau *et al*., 2001; Jahanshahi *et al*., 2002;

Table 2: Examples of nanoparticulate bioproducts.

Vector	Titre/ concentration	Location
Viral vectors	-	-
MMLV (retrovirus)[1]	10^6 pfu ml^{-1}	extracellular
HIV (retrovirus)[1]	10^6-10^7 pfu ml^{-1}	extracellular
Adenovirus[1]	10^{12} pfu ml^{-1}	intracellular
Non-viral vector	-	-
Plasmid DNA[2]	4 -220 μg ml^{-1}	intracellular
Protein vaccine	-	-
Recombinant protein[3]	10 -100 μg ml^{-1}	Intra-or extracellular

The table shows a selection of nanoparticle used as gene therapy vectors or vaccines. The location of the product during the production cycle determines the type of initial downstream processing steps (i.e. recovery from large volumes for extracellular products or product release from cells for intracellular products). Titres are given in plaque forming units (pfu) per unit volume of fermentation medium. 1 Data for titres for moloney murine leukaemia virus (MMLV), human immunodeficiency virus (HIV) and adenovirus was taken from hashilkar *et al*., (2001). 2 Plasmid DNA yields per unit volume of fermentation medium rabging between 4 g ml^{-1} (Horn *et al*., 1995) and 220 g ml^{-1} (Lahijani *et al*., 1996) have been reported. 3 For the baculovirus expression system, the above expression levels (polyhedron promoter) for recombinant proteins per unit volume of fermentation medium have been reported (Mannix and Jarman, 2000).

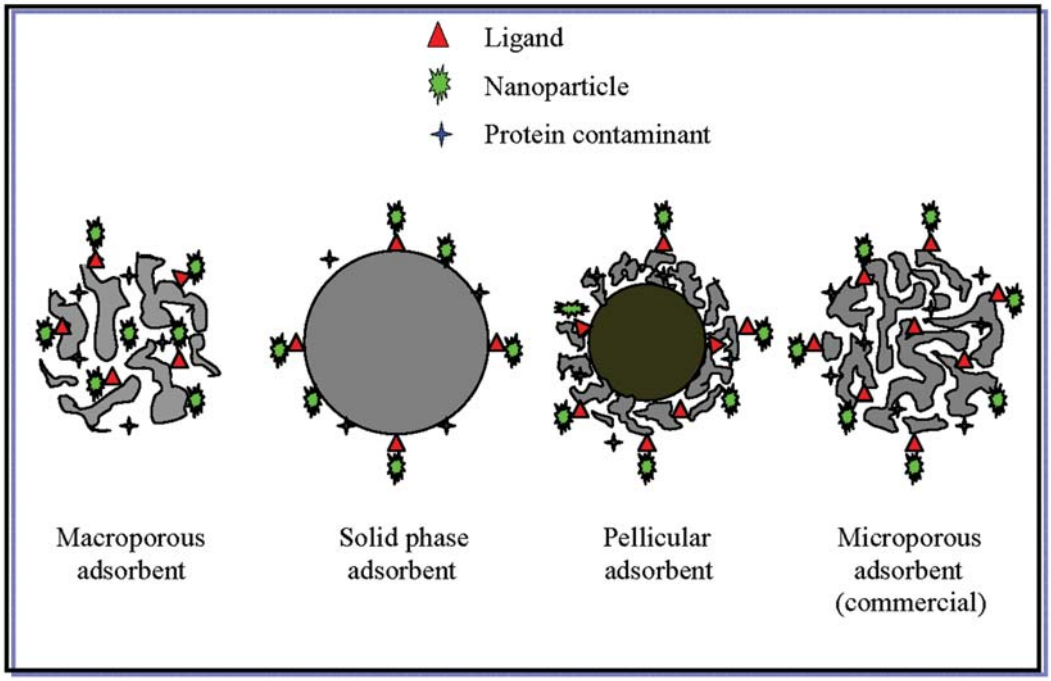

Figure 3: Various adsorbent designs for nanoparticle recovery. This figure is a diagrammatic representation of the adsorbent configurations included in the present paper. Macroporous adsorbents are classified by a pore size of greater than 0.6 μm in diameter. Solid phase are designated as solid or relatively solid adsorbents having either no pores or having a molecular size exclusion of 10,000 Daltons. Pellicular adsorbents have a dense, solid core surrounded by a layer of porous agarose material. Microporous adsorbents are designated and characterised here by a pore size of less than 0.2 μm (commercially available adsorbent in market; Zhang *et al*., 2001; Jahanshahi *et al*., 2002).

Jahanshahi and Lyddiatt, 2002a). Small pore size of adsorbents used in fluidised bed (5-400 nm) as mentioned above limits the diffusion of the nanoparticulates into the adsorbent solid phase and thus only the external surface area is available (Jahanshahi *et al*., 2004). In addition low molecular weight contaminants will enter the pores resulting in a process requirement for excessive washing steps to purge the internal volume of the adsorbent solid phase (Fig. 3). Solid phases characterised by large macropores facilitating free diffusive or convective transport of nanoparticles into the internal volume of adsorbents would facilitate enhanced adsorptive performance (Fig. 3). However, the significant processing times required eliminating impurities or process reagents from the internal volumes and surfaces of such particles still remains an important practical issue. Macroporous adsorbents would be expected to shorten processing times, but at the expense of bulk product capacities (Thwaites *et al*., 2001; Tong and Sun, 2002). Pellicular adsorbents have also been demonstrated to impart unique product selectivity for product alone in the recovery of nanoparticles such as plasmid DNA from crude bacterial lysates (Sun *et al*., 2001 and Jahanshahi *et al*., 2003). For example, Thwaites *et al*., (2001) showed the recovery of plasmid DNA from *Escherichia coli* lysates by exploiting macroporous and pellicular adsorbents. Such solid phases should show shorter processing times due to the limited diffusion distances, but the loss of bulk capacity must be considered and the solid adsorbent (Fig. 3) circumvents diffusional limitations and the washing steps required due to the surface limited binding (Jahanshahi *et al*., 2002). Therefore the design and assembly of adsorbent particles is a critical factor to be considered to enable the successful operation of the recovery of nanoparticles.

In addition, it should be mentioned that a feature-creating problem for the purification of some nanoparticles is their sensitivity. Some viruses with loosely attached fibres, which are responsible for viral infectivity, may lose their fibres due to the adsorption/desorption within the adsorbents. However, macroporous, pellicular and solid phase adsorbents (Fig. 3) will bind the contaminant soluble components of nanoparticle by virtue of the possession of common chemical ligands of association and accessible internal geometries. In contrast, laminated adsorbents potentially impart a size exclusion influence upon nanoparticulates in the

fractionation process without compromise to the adsorption of soluble components (see below).

Novel Laminated Adsorbents (Subtractive Chromatography): A novel solid phase design (laminated adsorbent) has been proposed for the recovery of nanoparticle bioproducts and shown schematically in figure 4. The core principle is to coat inert macroporous polymers upon adsorbent beads of varied ligands e.g. anion exchangers, cation exchangers or affinity ligands. With careful manipulation of the pore sizes of polymer coat, only molecules with certain dimensions are allowed diffusing in to contact with the ligands. Therefore, it is possible to separate target from contaminants based upon their size differences as well as the surface charges (Dainiak *et al*., 2002). This construct has potential advantages over the common adsorbents as mentioned above when purifying nanoparticulate bioproducts.

Fabrication of novel laminated adsorbent is carried out with a unique emulsification technique which facilitates the controlled coating of commercial chromatographic adsorbents (100-300 μm) with a thin pellicle of neutral polymer (e.g. electrophoresis grade agarose; Kim *et al*., 1999 and Jahanshahi *et al*., 2003). Such a pellicle acts as a barrier to nanoparticle penetration without restricting the free diffusive penetration of soluble macromolecular components or system impurities to the adsorptive chemistries deployed in the adsorbent core (Fig. 4). Nanoparticulate bioproducts can be recovered in the effluent from adsorbent columns in a purified state. Such constructs, comprising a neutral agarose pellicle laminated upon commercial adsorbents have been tested for the fractionation of nanoparticles and soluble components (Negrete *et al*., 2001).

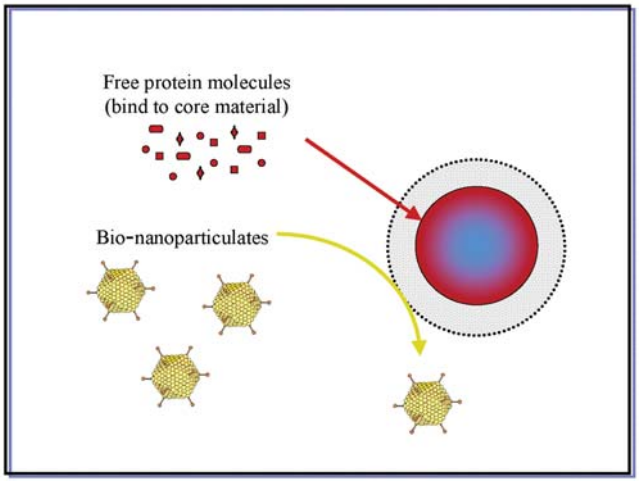

Figure 4: A laminated adsorbent for the subtractive recovery of nanoparticle bioproducts. The ideal adsorbent for nanoparticle recovery would separate free protein molecules from nanoparticles. Product and contaminants share chemistry but different size. Binding of the free soluble protein is possible within the core material after diffusion through the exclusion membrane, which concomitantly limits penetration of the nanoparticles assembly (Negrete *et al*., 2001; Jahanshahi *et al*., 2003).

Therefore, rather than directly adsorptive recovery of nanoparticulate bioproducts, polymer-coated adsorbents (laminated adsorbent) can be employed to adsorb protein and/or nucleic acids contaminants from cell lysates. As an indirect result, it is possible to produce nanoproducts free of contaminant molecules. For a generic demonstration of the processing feasibility of this idea, the manufacture of protein nanoparticles as novel drug delivery carriers was chosen as a model system which required the removal of free protein molecules. Adsorption of a mixture of labelled BSA solution and BSA nanoparticle was evaluated in a batch binding as well as fluidised bed experiments and visualised by laser scanning confocal microscopy (Negrete *et al*., 2001 and Jahanshahi *et al*., 2004). In addition, proof of the principle has been demonstrated in the direct recovery and purification of infective adenovirus from crude lysates of host packaging cell lines. Crude virus lysate was run in fluidised bed with the laminated adsorbents and adenovirus purified from its free protein contaminants (Fig. 2, Table 1; Zhang *et al*., 2002). The detailed application of this novel method with a full dissection of the impact of nanoparticle feedstocks, is the subject of a joint paper publication and/or patent (Jahanshahi and the BRG group, Birmingham University, UK).

Aqueous Two-Phase Systems and Interfacial Partitioning: Aqueous two-phase systems (ATPS) form when two polymers or one polymer and a salt are prepared in an aqueous solution above their critical concentrations (Fig. 5). ATPS has been studied in the field of liquid-liquid extraction for molecular purification and reported initially by Albertsson (1958). But the use of aqueous two-phase systems for the separation and purification of nanoparticles is still a niche area. Cells and cell organelles have been partitioned in two-polymer systems (Walter *et al*., 1985). It has been possible to separate different subgroups of cells, cells harvested at different stages during the growth cycle as well as identical cells grown in different media. All of the above observation made during the partition of cells and cell organelles have led to the conclusion that their partition behaviour is mainly a surface-dependent

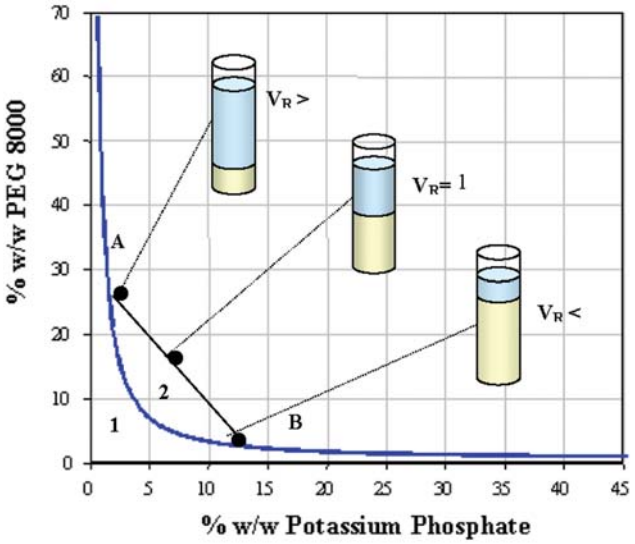

Figure 5: Schematic representation of the phase diagram of a PEG-salt aqueous two-phase system determined by cloud-point methodologies. This figure shows the phase diagram representing the chemical composition of ATPS. The phase diagram is constructed by plotting the concentrations of the chemical components of the ATPS including two polymers or one polymer and a salt. The concentrations are obtained by the cloud point approach method. Biphasic and monophasic systems are separated in two regions (1 and 2, respectively) by the binodal curve. The line AB (tie line) ties all systems with identical compositions in top and bottom phases. The volume ratio varies along the tie line. These systems have identical composition of the phases (Zaslavsky, 1995).

phenomenon (Albertsson, 1986). Such knowledge is important when studying the partition behaviour of other types of nanoparticles.

With regard to the partition of nanoparticles, it has been found that they distribute between one of the bulk phases and the interface so that the use of partition coefficient *K* (ratio of the product concentrations in the top to bottom phase) is insufficient to describe partition equilibria. Instead, it can be shown that relating the number of particles between one of the bulk phases and the interface is a more accurate way of describing the partition equilibrium:

$$G = N_1/N_2$$

Where *G* is the partition ratio and N_1 is the number of particles in one of the phases (top or bottom) while N_2 represents the number of particles in the interface. For certain soluble biomolecules, the partition coefficient *K* has been shown to be independent of the initial solute concentration (Zaslavsky, 1995). Similarly, *G* has been shown to be independent of the initial number of particles in an ATPS (Kimura and Kobayashi, 1996).

Other types of nanoparticles such as viruses have also been partitioned in two-polymer phase systems. Such systems have proven to be suitable for purifying extracellular viruses from large culture volumes (Hammar and Gilljam, 1990) and for labile viruses which may otherwise have been denatured in conventional density gradient centrifugation or ultracentrifugation procedures (Hammar, 2000). Depending on system parameters, a large proportion of viruses has partitioned to the interface and Hammar has taken this behaviour further in exploiting interfacial partition as a recovery step for viruses.

The use of ATPS composed of a polymer and salt to recover intermediate-sized nanoparticles has increased. Andrews *et al*., (1995) have studied the removal of cell debris from virus-like particle preparations by multistage PEG-salt extraction. Inclusion bodies have been successfully separated from cell debris in PEG-salt systems (Walker and Lyddiatt, 1998 and 1999). Viral gene therapy vectors such as retroviruses and adenoviruses and artificial protein particles, produced as surrogate particles for potential vaccine applications, have been separated in PEG-salt systems (Braas *et al*., 2000). Luechau and Lyddiatt (2002) have purified B19 parvovirus capsid (produced in a baculovirus expression system which, in its viral state, is pathogenic to humans; Kajigaya *et al*., 1991) by ATPS interfacial partitioning. The success for nanoparticle partition in the mentioned application has been measured with respect to how many nanoparticles could be recovered from one of the bulk phases. However, during the studies, a large proportion of nanoparticles have partitioned to the interface.

The partition behaviour of nanoparticles is influenced by many factors and two of them deserve special attention. Firstly, it appears that the interface is the preferred location for nanoparticles. Secondly, it has been shown that non-thermodynamic factors such as the speed and mode of phase separation strongly influence the partition behaviour of nanoparticles. In order to effectively extract nanoparticles from a crude mobile phase, two prerequisites have to be fulfilled. Firstly, the two phases have to be mixed efficiently until (near) equilibrium to facilitate the mass transfer of the product. Additionally, the dispersion volume must remain constant so that the process is at steady state. Centrifugal devices do not seem appropriate for this task as the two phases enter and leave the device simultaneously. Continuous contact equipment com-

prises the many kinds of extraction columns that have been used recently for interfacial partitioning of nanoparticulate bioproducts. The Kühni-type extraction column is an example of such continuous equipments which has been successfully used for plasmid DNA and virus-like particle purification (Perry and Green, 1997; Luechau and Lyddiatt, 2002).

CONCLUSION

Downstream processing techniques for the recovery of nanoparticle bioproducts need to be re-deigned. The advanced extraction methods are needed to potentially deal with the process. Macroporous, pellicular and solid phase adsorbents (Fig. 3) having suitable designs applicable to the generic recovery of nanoparticles have been demonstrated in this paper. The laminated adsorbents (Fig. 4), subtractive chromatography, were also suitable for the recovery and purification of nanoparticles. It is expected that this method will win the competition among candidate downstream processing techniques for nanoparticle bioproducts in the near future.

ATPS application to the processing of nanoparticles in most cases remains the subject of much experimental speculation. A particular constraint arises due to the fact that the partition behaviour of nanoparticulates commonly departs from that of soluble macromolecules such that under equilibrium conditions the particles locate at the interface a third inter-phase between top and bottom liquid phase. This may be attributed to particulate-induced reduction of the interfacial tension such that interface/inter-phase is the energetically favoured location for nanoparticle bioproducts. In fact, not only partition equilibria but also the phase separation processes have to be taken into account when designing extraction processes. It cannot be expected that scouting experiments for the extraction of nanoparticles can simply be performed on a small scale with centrifugal phase separation as reported in the bulk of publications. In contrast, the speed at which phases separate, the mode of phase separation and the geometry of how the two-phase extraction step is carried out may play an important role as partition equilibria.

In summary, there is clearly much scope for the development of custom designed solid phases for fluidised bed recovery of nanoparticulate bioproducts and application of continuous equipments for ATPS interfacial partitioning of such products. However, it can be anticipated that if large scale fabrication of laminated adsorbents is successful, the application of such adsorbents in the recovery of nanoparticles, will contribute to the de-bottlenecking of current biopharmaceutical manufacture.

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