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Surface modification of chromatography adsorbents by low temperature low pressure plasma

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ABSTRACT

In this study we show how low temperature glow discharge plasma can be used to prepare bi-layered chromatography adsorbents with non-adsorptive exteriors. The commercial strong anion exchange expanded bed chromatography matrix, Q HyperZ, was treated with plasmas in one of two general ways. Using a purpose-designed rotating reactor, plasmas were employed to either: (i) remove anion exchange ligands at or close to the exterior surface of Q HyperZ, and replace them with polar oxygen containing functions ('plasma etching and oxidation'); or (ii) bury the same surface exposed ligands beneath thin polymer coatings ('plasma polymerization coating') using appropriate monomers (vinyl acetate, vinyl pyrrolidone, safrole) and argon as the carrier gas. X-ray photoelectron spectroscopy analysis (first ~10 nm depth) of Q HyperZ before and after the various plasma treatments confirmed that substantial changes to the elemental composition of Q HyperZ's exterior had been inflicted in all cases. The atomic percent changes in carbon, nitrogen, oxygen, yttrium and zirconium observed after being exposed to air plasma etching were entirely consistent with: the removal of pendant Q (trimethylammonium) functions; increased exposure of the underlying yttrium-stabilised zirconia shell; and introduction of hydroxyl and carbonyl functions. Following plasma polymerization treatments (with all three monomers tested), the increased atomic percent levels of carbon and parallel drops in nitrogen, yttrium and zirconium provided clear evidence that thin polymer coats had been created at the exteriors of Q HyperZ adsorbent particles. No changes in adsorbent size and surface morphology, nor any evidence of plasma-induced damage could be discerned from scanning electron micrographs, light micrographs and measurements of particle size distributions following 3 h exposure to air (220 V; 35.8 W L^{-1}) or 'vinyl acetate/argon' (170 V; 16.5 W L^{-1}) plasmas. Losses in bulk chloride exchange capacity before and after exposure to plasmas enabled effective modification depths within hydrated Q HyperZ adsorbent particles to be calculated as 0.2–1.2 μm, depending on the conditions applied. The depth of plasma induced alteration was strongly influenced by the power input and size of the treated batch, i.e. dropping the power or increasing the batch size resulted in reduced plasma penetration and therefore shallower modification. The selectivity of 'surface vs. core' modification imparted to Q HyperZ by the various plasma treatments was evaluated in static and dynamic binding studies employing appropriate probes, i.e. plasmid DNA, sonicated calf thymus DNA and bovine serum albumin. In static binding studies performed with adsorbents that had been exposed to plasmas at the 5 g scale (25 g L^{-1} of plasma reactor), the highest 'surface/core' modification selectivity was observed for Q HyperZ that had been subjected to 3 h of air plasma etching at 220 V (35.8 W L^{-1}). This treatment removed ~53% of 'surface' DNA binding at the expense of a 9.3% loss in 'core' protein binding. Even more impressive results were obtained in dynamic expanded bed adsorption studies conducted with Q HyperZ adsorbents that had been treated with air (220 V, 3 h) and 'vinyl acetate/argon' (170 V, 3 h) plasmas at 10.5 g scale (52.5 g L^{-1} of plasma reactor). Following both plasma treatments:

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the 10% breakthrough capacities of the modified Q HyperZ adsorbents towards 'surface' binding DNA probes dropped very significantly (30–85%); the DNA induced inter-particle cross-linking and contraction of expanded beds observed during application of sonicated DNA on native Q HyperZ was completely eradicated; but the 'core' protein binding performance remained unchanged *cf.* that of the native Q HyperZ starting material.

1. Introduction

Preparative chromatography has been a fundamental unit operation for the pharmaceutical sector for many decades and plays an absolutely pivotal role within biopharmaceutical downstream processing [1–3]. Since Peterson and Sobers prepared cellulose based ion exchangers for the separation of proteins in the mid-1950s [1], chromatographic packing materials for preparative protein separation have been continuously improved with respect to large scale operation, resolving power, separation efficiency, selectivity, and sorption capacity [1–4]. However, though today's chromatography materials are unquestionably superior to their forerunners, in some ways their development has stood still. For example, the basic design and expected tasks have changed surprising little over the past 50 years, with most still performing just a single function. The explosion in new high-level expression systems for the production of recombinant proteins has reduced upstream processing costs to the point where concentration and purification operations, i.e. downstream processing, now dominates the overall manufacturing cost for many protein therapeutics [3–6]. Yet, though this situation creates a great incentive to advance more efficient downstream processing technologies and processes, especially for future products, the reality is that advances in downstream processing over the past decade lag far behind those made in upstream processing over the same period, and further, that process chromatography is increasingly viewed as a serious bottleneck within biopharmaceutical manufacturing [3–6].

Among the growing list of challenges facing the development of new improved chromatographic materials for existing and future products are: rocketing product titres; increasing size and complexity of emerging bio-products; escalating cost of goods and waste generation; and increasing competition from alternative techniques/formats. It is difficult to envisage effective solutions to these coming from continued incremental improvement of conventional mono-functional chromatography adsorbent matrices, i.e. materials performing just a single function. Conversely, the concept of multi-functional media featuring two or more distinct functional regions spatially separated from one another within the same support bead, affords attractive solutions, to at least some of these issues, to be envisaged. The present study concerns the simplest multi-layered multi-functional support design one can envisage, namely one featuring just two differently functionalised layers—an inert outer size excluding layer and inner ion exchange functionalised core. The benefits of bi-layered size exclusion chromatography–ion exchange chromatography (SEC–IEC) beaded support designs have been clearly demonstrated in the context of 'nanoplex' purification [7,8], fluidised bed separation of organic acids [9,10] and expanded bed adsorption of proteins [11,12]. The important findings from these studies, inherent flaws in the methods employed thus far to manufacture bi-layered SEC–IEC hybrids, and identification of a simple and effective solution to the future manufacture of multi-layered multi-functional beaded chromatography matrices, are presented immediately below.

Nanoplexes are a rapidly growing and diverse product grouping characterised by large physical size, fragility, complex surfaces plus chemical similarity to smaller contaminating macromolecular components; important examples include non-viral vector components such as naked plasmid DNA, viral vectors, megamolecular vaccines and mega-protein complexes [13–15]. Their properties dictate that their efficient large-scale manufacture must follow a very different 'general' path to that established for therapeutic human proteins of much smaller dimensions [13,16–21]. Current protocols for the purification of plasmid DNA show heavy reliance on packed bed chromatography—centred on capture by anion exchange (AEC) adsorption, followed by polishing of the salt-eluted fraction by size exclusion chromatography [16,17,19,21]. Though ion exchange chromatography has found very widespread use for the large scale purification of antibiotics and protein-based drugs, its application for commercial scale production of plasmid DNA (pDNA) and other nanoplexes is far less attractive [16–19,21].

Recently, it has been demonstrated that much greater productivity could be realised if the SEC and AEC operations are 'passively' combined in a single chromatographic operation, employing a new type of multi-functional chromatography material (known as the lid bead) [7,8]. Starting from an SEC matrix with a nucleic acid exclusion limit of 1000 bp, Gustavsson et al. [7] made a bi-functional restricted access matrix possessing a positively charged core (to adsorb large amounts of RNA and protein) and an inert outer layer to exclude pDNA from accessing the functionalised bead interior. The creation of the two layers within the matrix was achieved in an ingenious multi-step process, which relied on the use of limiting concentrations of reactants and 'diffusion/reaction' balancing in the second step. However, despite showing considerable promise, the bi-functional materials produced did not quite live up to expectations. For example, in tests with plasmid-containing cleared alkaline lysate feedstocks of high ionic strength, the authors noted that in order to prevent pDNA binding, over 30% of the core's RNA binding capacity had to be sacrificed. Though elegant, the methods of manufacture led to three linked problems, namely insufficient control over the: (i) thickness and (ii) inertness of the outer size excluding layer; and (iii) boundary 'definition' between the different zones within the support.

A SEC–IEC design is equally attractive for expanded bed adsorption (EBA), a type of fluidised bed chromatography, originally conceived as a generic solution for combining solid–liquid separation with initial chromatographic capture and purification [22–24]. Despite rapid initial successes, the progress of EBA into industry has been slow, and confidence in the technique is waning [25,26]. Perhaps the greatest technical problem affecting EBA is the physical cross–linking of neighbouring adsorbent particles by biomass or large colloidal molecules (especially nucleic acids) present in crude feedstocks, which leads to gross breakdown/collapse of the structure of the expanded bed and consequent loss of chromatographic performance [18,25–34]. Attempts to relieve problems of inter-adsorbent particle cross-linking in EBA systems by chemically or mechanically conditioning the crude feedstock prior to application have been at best only partially successful [26,32,33]. This is not surprising given that the root of the problem is that the outer surfaces of expanded bed adsorbents, like other beaded chromatographic materials, are populated with functional groups capable of binding both the product of interest and large adsorbent cross-linking contaminants present in crude feedstocks. Commercially available EBA adsorbents are direct descendents of packed bed chromatography matrices employed for the purification of proteins, and since their inception well over a decade ago the design of improved materials has concentrated on improving sorption