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Interactions of Chinese Hamster Ovary (CHO) cell cultures with second generation expanded bed adsorbents

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ABSTRACT

We have employed extended DLVO colloid theory to understand and explain unfavorable adhesive biomass–adsorbent interactions affecting the performance expanded bed adsorption (EBA) chromatography. These involve thermodynamic as well as electrostatic surface characterizations, adapted and streamlined towards biomass and adsorbent, which significantly improves over previous methods and avoids mechano-chemical alteration of the adsorbent. For the first time, the capillary rise method was used to determine the surface energy components of the chromatographic adsorbents. Predicted biomass–adsorbent interactions between several chromatographic adsorbents (HIC, IEX, and affinity) and different cell types (*Escherichia coli* and CHO) in varying solution conditions were shown to correlate well with biomass deposition experiments.

1. Introduction

Expanded bed adsorption (EBA) is an initial recovery unit operation in protein purification to combine solid–liquid separation within a single step [1]. EBA has been employed for the downstream processing of biotechnological feedstock directly from the bioreactor, where clarification, concentration and purification are performed simultaneously [2]. It therefore offers a major advantage in significantly minimizing costs by reducing process times and consumables [3]. The operating principle of EBA is simple and is shown in Fig. 1. Once the unclarified feedstock is introduced within the column of fluidized adsorbent, cells and cell debris pass unhindered through the interstitial voids created in the expanded bed and ultimately escape through the top of the column. The target adsorption onto the chromatographic media takes place in this stage, and subsequent elution can be accomplished either in the packed bed mode or otherwise in the expanded bed mode at reduced superficial velocity [4,5]. EBA chromatography's higher process and energy efficiency as compared to conventional techniques [6] has led to several applications ranging from the purification of therapeutic and commercial proteins [7,8] to enzymes [9] from bacterial [10], yeast [11], mammalian[12], algal [13], expression systems as well as from plant extracts [14].

Biomass–adsorbent interactions are one of the major drawbacks of the EBA process, where a gentle hydrodynamic environment, high adsorbent surface area, and high residence times make the system highly vulnerable to unfavorable bio-colloidal interactions (both reversible and irreversible) [15,16]. These interactions not only lead to unstable hydrodynamics [17,18] of the system, but also to the elutriation of adsorbent beads, and the deposition of intact cell particles [19], cell debris [20], and suspended materials on the adsorbent phase [21,22] which ultimately results in the reduced dynamic binding capacities. To optimize the EBA process conditions, it is essential to understand and characterize these biomass–adsorbent interactions from a fundamental perspective. Extended Derjaguin, Landau, Verwey and Overbeek (xDLVO) theory, which is based on classical colloid theory, serves as an efficient tool to study the biomass deposition onto chromatographic adsorbents at the particle level [23,24]. This method takes into account several types of interactions, where the net interaction energy between a colloidal particle (biomass) and a solid support (chromatographic adsorbent) is the cumulative sum of Lifshitz–van der Waals (LW), acid–base (AB), as well as electrostatic (EL) energies as shown in Eq. (1).

$$U^{\mathrm{xDLVO}} = U^{\mathrm{LW}} + U^{\mathrm{EL}} + U^{\mathrm{AB}} \quad (1)$$

Each individual energy component is calculated on the basis of several experimentally determined parameters. LW and AB energies of the interacting surfaces are parameterized by their surface energies, which are calculated using the contact angles made by 3

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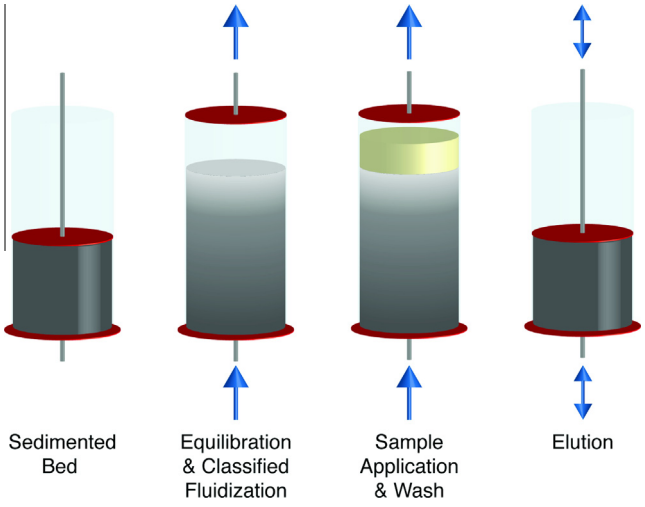

Fig. 1. Principle of expanded bed adsorption chromatography.

probe liquids onto these respective surfaces. EL energies are parameterized by their apparent surface charge in solution or zeta potential by measuring their electrophoretic mobility. The formulae describing these xDLVO calculations have been mentioned abundantly in previous literature [23].

Among the various techniques to measure contact angles, the most convenient methods include the sessile drop or goniometric method [25], the Wilhelmy plate method [26], and the capillary rise or 'wicking' method [27]. While the sessile drop and Wilhelmy plate techniques are widely used to measure contact angles on smooth solid surfaces, the wicking method provides a convenient way to obtain the contact angles of particulate or porous materials, which are not accessible by other approaches. In the case of chromatographic beads, whose diameters usually range from 20 to 200 μm, it is not possible to prepare a smooth uniform surface for contact angle measurements without particle fragmentation [28], which may lead to unfavorable mechano-chemical changes in surface properties. Nevertheless, in previous studies, adsorbent surfaces have been prepared with crushed bead particulates of ca. 10 μm diameter to measure contact angles and, in turn, calculate surface energy parameters [29]. The controversial nature of these mechano-chemical alterations in surface properties associated with bead fracture have now been subverted in this study by employing the capillary rise method on beads in their intact form.

We have investigated the applicability of capillary rise method to determine the surface energy components of the chromatographic adsorbents. Subsequently, we have compared the calculated as well as experimental interactions between biomass and chromatographic adsorbents, where xDLVO calculations were correlated well with biomass deposition experiments.

2. Material and methods

2.1. Chemicals and reagents

Toyopearl Phenyl 650C, Toyopearl Butyl 650C, and Toyopearl HW 65C beads were purchased from TOSOH Bioscience (Stuttgart, Germany). Fastline SP, Fastline DEAE, and MabDirect Protein A beads were obtained from DSM biologics (Groningen, The Netherlands). 1-bromonaphtalene (99% purity) was obtained from Fluka (Buchs, Switzerland). All other chemicals used in the buffer preparation were of analytical grade and obtained from Applichem (Darmstadt, Germany). Cell culture media components were obtained from Life Technologies GmbH (Darmstadt, Germany). Milli-Q water was used for all the experiments and buffer preparations.

The goniometric system (OCA 20) for sessile drop experiments was obtained from Data- Physics Instruments (Filderstadt, Germany). Zeta potential measurements were made using a ZetaSizer Nano ZS from Malvern Instruments (Worcestershire, UK). Biomass pulse experiments were performed using Äkta Explorer™ 100 controlled by Unicorn 4.10 software was obtained from GE Amersham Bioscience (Uppsala, Sweden). A Sartorius CPA423S weighing balance connected to computer via RS-232 serial connector and controlled by Sartocollect 1.0 software was obtained from Sartorius AG (Göttingen, Germany).

2.2. Biomass cultivation

Chinese Hamster Ovary (CHO-EBNAL T85) cells were cultivated in the media containing equal amounts of CD CHO medium, 293 SFM II medium, supplemented with 6 mM L-glutamine, 1% of 50× HT supplement and 2% of Puromycin. Yeast (*Saccharomyces cerevisiae*) and *Escherichia coli* cells were produced according to standard methods [30,31], The cell pellets were resuspended to 40% wet weight cell concentration in the respective buffer, and diluted to appropriate concentration before use.

2.3. Contact angle measurements

The cell surfaces were prepared according to Sharma et al. [6] and contact angles were measured by sessile drop method. All cells were washed with phosphate buffered saline (PBS) to remove the media. Cell lawns were prepared by depositing the cells on 1 cm^2 strips of 0.45 μm cellulose acetate filters by employing negative pressure. The filters with cells are then moved on top of a layer of 1% agarose in water containing 10% glycerol for 10 min. This establishes constant and uniform moisture content (*physiological relevant state*) in controlled conditions and to prevent the biomass from drying. The filters are removed from the agarose plate and allowed to air dry for 10 min. Subsequently, contact angles were measured with water, formamide, and 1-bromo naphthalene [32].

The contact angles of the adsorbents were measured by capillary rise method. This method is based on the rate of penetration of a liquid into a bed of solid particles packed in a capillary tube. The contact angles of the liquids are calculated by their dynamic wetting rate and can be described using the Washburn equation (see Eq. (2)) [33], where η is the viscosity of the liquid (mPa s), γ is the surface tension of the liquid (mJ/m^2), r_{eff} is the effective pore radius, h is the height of liquid penetrating the bed in time t, and θ is the advancing contact angle, measured through the liquid phase.

$$h^2 = \frac{r_{eff}\gamma\cos\theta}{2\eta}t \quad (2)$$

The Washburn equation can also be transformed (see Eq. (3)) to calculate contact angles by measuring the weight gained (W) by the chromatographic beads upon absorption of the liquid, where ρ is the density of the liquid (g/mL) and C is the capillarity (m) of the packed bed [33].

$$W^2 = \frac{C\rho^2\gamma\cos\theta}{\eta}t \quad (3)$$

There are two unknowns in the Washburn equation, the capillarity (C) and the contact angle (θ). These unknown terms can be determined by performing the capillary rise experiment with a liquid that wets the solid substrate completely, i.e., for which the contact angle equals to zero [34]. In the case of hydrated chromatographic adsorbents, water was chosen as completely wet-