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Review

Pharmaceutical and biomedical applications of affinity chromatography: Recent trends and developments

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ABSTRACT

Affinity chromatography is a separation technique that has become increasingly important in work with biological samples and pharmaceutical agents. This method is based on the use of a biologically related agent as a stationary phase to selectively retain analytes or to study biological interactions. This review discusses the basic principles behind affinity chromatography and examines recent developments that have occurred in the use of this method for biomedical and pharmaceutical analysis. Techniques based on traditional affinity supports are discussed, but an emphasis is placed on methods in which affinity columns are used as part of HPLC systems or in combination with other analytical methods. General formats for affinity chromatography that are considered include step elution schemes, weak affinity chromatography, affinity extraction and affinity depletion. Specific separation techniques that are examined include lectin affinity chromatography, boronate affinity chromatography, immunoaffinity chromatography, and immobilized metal ion affinity chromatography. Approaches for the study of biological interactions by affinity chromatography are also presented, such as the measurement of equilibrium constants, rate constants, or competition and displacement effects. In addition, related developments in the use of immobilized enzyme reactors, molecularly imprinted polymers, dye ligands and aptamers are briefly considered.

Contents

1. Introduction

Liquid chromatography and high performance liquid chromatography have been extensively used for decades in clinical and pharmaceutical laboratories. Examples of liquid chromatographic methods that are commonly used in these fields include reversed-phase, ion-exchange, size-exclusion and normal-phase chromatography [1,2]. However, another liquid chromatographic technique that has become increasingly important in work with biological samples and pharmaceutical agents is affinity chromatography [3]. This review will discuss the basic principles behind affinity chromatography and will examine recent developments that have occurred in the use of this method for biomedical and pharmaceutical analysis.

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2. Overview of affinity chromatography

Affinity chromatography can be defined as a type of liquid chromatography that uses a biologically related agent, or "affinity ligand", as a stationary phase to selectively retain analytes or to study biological interactions [4–6]. The affinity ligand can consist of a wide variety of binding agents, ranging from a protein or enzyme to an antibody, an antigen, a sequence of DNA or RNA, a biomimetic dye, an enzyme substrate or inhibitor, or a low mass compound (e.g., a drug or hormone). The affinity ligand is immobilized within a column and used to selectively bind a given target or group of targets within a sample. Because of the highly selective nature of many affinity ligands, the result is a column that can be used to isolate, measure, or study specific targets even when they are present in complex biological samples [4–8].

The immobilized ligand is an important factor that determines the success of an affinity chromatographic method. Most affinity ligands that are used in this technique are obtained from a biological source, such as antibodies, enzymes, transport proteins, and carbohydrate-binding proteins [3–8]. However, affinity chromatography can also use synthetic ligands such as metal ion chelates, boronates and biomimetic dyes [3–5,7]. The type of ligand that is employed in this method can be used to divide affinity chromatography into several categories. This review will discuss developments in many of these categories, including lectin affinity chromatography, boronate affinity chromatography, immunoaffinity chromatography, and immobilized metal ion affinity chromatography [3,5,7].

Another critical factor in the design and use of an affinity column is the type of support to which the ligand is immobilized. Many methods that use affinity chromatography for only sample pretreatment or target isolation employ a carbohydrate support such as agarose or cellulose. This type of material can be easily modified for ligand attachment, can be used with a wide range of elution conditions, and has low non-specific binding for many biological compounds. However, the limited mechanical stability and relatively low efficiency of many carbohydrate-based materials means that they tend to work best for off-line methods and for columns that will be operated at only low pressures and flow rates [5,9,10].

An alternative to traditional carbohydrate-based supports is to place the affinity ligand onto supports that consist of HPLC media like silica particles or, more recently, monolithic materials [5,9–13]. This alternative approach is known as high performance affinity chromatography (HPAC), or high performance liquid affinity chromatography (HPLAC). The use of affinity ligands with monolithic supports is also referred to as affinity monolith chromatography (see example in Fig. 1) [12–17]. The more rigid and efficient particles or synthetic polymers that are employed in HPAC are often capable of withstanding much higher flow rates and pressures than traditional carbohydrate-based supports and possess better mass transfer properties than these other materials. The result is that HPAC columns can be used on-line with other HPLC columns or analytical methods while providing an increase in speed, precision, and ease of automation versus traditional affinity supports [5,9–13]. This review will discuss recent examples in which both traditional and high performance affinity columns have been used in biomedical and pharmaceutical analysis. However, the emphasis will be placed on methods in which affinity columns are used as part of HPLC systems or in combination with other analytical methods (e.g., mass spectrometry).

3. General formats for affinity chromatography

The most common scheme for performing a separation in traditional affinity chromatography and HPAC is shown in Fig. 2 [5]. First, a sample is injected onto the affinity column under conditions that allow strong binding by the target or analyte of interest with the immobilized ligand. These application conditions typically involve the use of an aqueous buffer that has a pH and ionic strength that mimic the native environment of the ligand and its target. Compounds in the sample that have little or no interaction with the ligand will elute from the column during this step, giving a non-retained peak. The next step utilizes an elution buffer to dissociate the target from the affinity ligand. This elution step often requires changing the mobile phase composition to promote target elution, as can be accomplished by altering the pH or by adding a competing agent to displace the target from the column. During this elution step, the released target can be collected for later analysis or use. If an HPLC support is used in the affinity column, it may also be possible during this step to monitor the eluting target directly by an on-line method. Both the on-line and off-line approaches can be combined with detection methods such as absorbance, fluorescence or mass spectrometry [18]. After the target has been eluted, the column can then be regenerated prior to the next sample application by passing through the original application buffer.

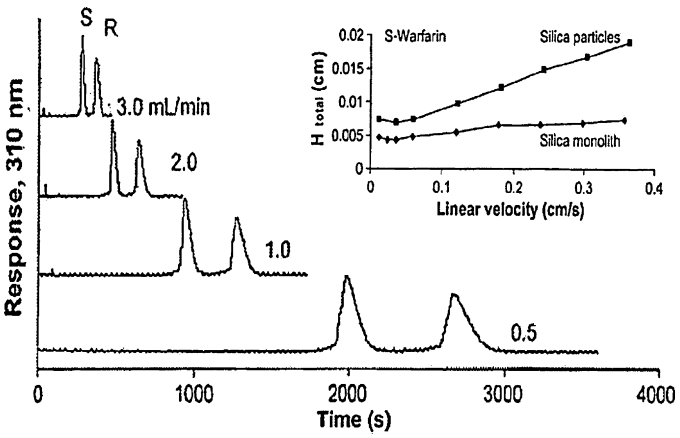

Fig. 1. Separation of *R*- and *S*-warfarin on a 10 cm × 4.6 mm I.D. silica monolith column containing immobilized α_1-acid glycoprotein. These separations were obtained at room temperature using pH 7.0, 0.067 M phosphate buffer as the mobile phase. The inset compares the total plate height (H_{total}) that was measured for *S*-warfarin on the AGP silica monolith column to the total plate height that was determined for the same target and affinity ligand when using 7 μm silica particles as the support. Adapted with permission from Ref. [17].

The format that is shown in Fig. 2 for target capture and elution is sometimes known as the "on/off" or step elution mode of affinity chromatography [5,19]. This approach has been widely employed for compound isolation and sample pretreatment in biomedical and pharmaceutical analysis because of its simplicity, flexibility, selectivity, and ease of use [5–8,18]. This format can also be utilized with many types of affinity ligands, such as lectins, boronates, and antibodies [3]. In addition, it is relatively easy to automate this format when using affinity columns that are appropriate for work in HPAC or as part of HPLC systems; it is even possible to use this mode in some cases for the direct detection of analytes [18]. Direct detection in the step elution mode is commonly performed using on-line absorbance or fluorescence detectors, but detection based on mass spectrometry or a post column reactor is also possible [18–22].

Methods that employ isocratic elution can be developed in affinity chromatography if the retained target has weak or moderate affinity for the immobilized ligand. This situation allows a single mobile phase to be used as both the sample application and elution buffer. The result is an approach known as weak affinity chromatography (WAC) or dynamic affinity chromatography [19,23–27]. WAC has been used in several pharmaceutical and biomedical applications. One common example is the use of isocratic elution for chiral