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Chemical stability of reversed phase high performance liquid chromatography silica under sodium hydroxide regeneration conditions

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Abstract

Chromatographic sorbents used within the purification of peptide or protein based active pharmaceutical ingredients (APIs) are commonly subjected to caustic regeneration procedures, so-called CIP treatments. While polymeric materials remain unaffected by this treatment, silica-based sorbents are at an intrinsic risk of dissolution under high pH conditions, such as, e.g. 0.1 M NaOH. It is common misconception that silica-based materials simply cannot be subjected to alkaline conditions above pH 9. Moreover, most studies covering the chemical stability of HPLC sorbents above pH 9 have been limited to the chromatographic conditions used for the separations themselves. Such studies have used buffered mobile phases up to pH 11 or 12. Very little focus has been put on the stability of the stationary phases when subjected to shorter but harsher pH conditions required for regeneration purposes, such as 0.1 M NaOH (pH 13). Knowledge about the amount of so-called leachables, degradation products originating from the stationary phase, is of growing importance for the registration of pharmaceuticals for human use and is addressed in this work. This study compares the chemical stability of different commercially available reversed phase silica materials (C18) that are used in industrial scale preparative HPLC. The silica materials were subjected to NaOH regeneration conditions and it is shown that some materials are able to withstand 0.1 M NaOH conditions without significant harm. It is demonstrated that contaminants present in the effluent in the range of 10–50 μg/mL can lead to significant contamination of API product fraction.

Keywords: Reversed phase HPLC; Chemical stability; NaOH; Leachables

1. Introduction

Reversed phase HPLC is a commonly used purification technique within downstream processing of therapeutic peptides and smaller proteins [1]. Repetitive injections of large amounts of crude peptide often require periodic washing or regeneration procedures (CIP) of the column packing material [2]. While in most cases a washing step with a high content (>70%) of organic modifier is able to desorb and elute highly hydrophobic species from the column, it is also possible that an acidic or alkaline treatment is required in order to regenerate the packing material. NaOH is known to denature eventually aggregated peptide or protein species, which is a prerequisite for eluting them with a high content of organic modifier [3]. Caustic treatments (NaOH) are very common and uncontested for polymeric packing materials. In the case of silica-based materials, NaOH treatments that render pH >10 bring along an intrinsic risk of hydrolyzing siloxane bonds in the silica matrix, which are the backbone of the porous structure [4]. Continuous dissolution leads not only to deteriorating column performance, but also species from surface modification and/or parts of the silica matrix will elute from the column and are likely to contaminate product fractions in the case of preparative separations. Such leachables from the stationary phase are devastating for preparative chromatography and are gaining more and more attention from, e.g. FDA when approving drug manufacturing processes [5].

Claessens and van Straten list in their review article [6] all known factors that influence the observed stability of silica-based reversed phase materials. For the underlying silica matrix, the nature of manufacturing process is of decisive importance for the chemical stability of the final surface modified stationary phase. Properties such as metal content and surface topography are known to play an important role for the stability of the stationary phase. Compared to the traditional SiO_2 based materials, organic–inorganic hybrid materials, zirconia or polymer phases offer generally improved chemical stability, especially in the alkaline pH regime. Concerning

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Table 1
Column packing conditions

Packing material	V_{Slurry} [mL]	$c_{packing}$ [g/mL]	Slurry solvent	N [m^{-1}]	$As_{10\%}$ [–]
L1	80	0.04	D	70,500	1.06
L2	20	0.15	A	40,100	1.00
D1	20	0.15	A	48,300	0.87
D2	20	0.15	A	42,700	0.85
Y	20	0.15	E	25,500	1.13
F	40	0.08	C	55,000	1.04
K	40	0.12	B	63,800	0.90

All materials were packed into 4.6 mm × 250 mm columns (from Isolation Technologies, Hopedale, MA, USA). Slurry solvents: (A) 100% THF; (B) THF/IPA 95/5 (v/v); (C) THF/IPA 90/10 (v/v); (D) THF/IPA 85/15 (v/v); (E) THF/$CHCl_3$ 50/50 (v/v); (F) MeOH/acetone 95/5 (v/v); (G) MeOH/acetone 90/10 (v/v). Test conditions for column efficiency test: mobile phase: acetonitrile/water 70/30 (v/v), flow rate: 0.7 mL/min; detection: 247 nm; injection: 1 μL of acetophenone solution (1 μL/mL mobile phase).

the modified materials, it is known, that the way of ligand attachment (mono- versus polyfunctional and mono- versus bidentate RP-phases), as well as the length of the alkyl chain and the type of end-capping procedure all influence the life time of RP-HPLC columns at intermediate and high pH conditions [7–9]. Claessens concludes that few comparisons between different studies can be made, since some investigators recycled their eluents during the stability tests, while others used fresh ones. Consequently, the saturation capacity of the eluents was different which influences the dissolution rate [6].

Within this study, we compare several commercially available C18 modified silica-based packing materials upon their chemical stability under NaOH regeneration conditions, thus their ability to withstand alkaline CIP treatments without leading to impaired chromatographic performance or contamination of purified active pharmaceutical ingredients (APIs). The choice of the studied materials was based on the availability in large quantities, thus their potential suitability for industrial applications. To our knowledge, no comparative study has previously shown to which extent silica-based reversed phase materials are able to withstand harsh regeneration conditions (pH 13). Within this work, we have not studied the possible impact of regeneration procedure on the separation performance of any APIs.

2. Experimental

2.1. Chemicals

Methanol, acetonitrile, isopropanol, acetone and toluene (HPLC grade) were purchased from Labscan Ltd. (Dublin, Ireland). Water was purified using a Milli-Q Quantum^{TM} EX Ultrapure Organex Cartridge from Millipore (Billerica, MA, USA). Potassium dihydrogen phosphate (>99.5%) was purchased from Fluka (Buchs, Switzerland) and sodium nitrite, imipramine, nortriptyline and amitriptyline from Sigma–Aldrich (St. Louis, MO, USA).

Acetic acid glacial (analytical grade) and sodium hydroxide (reagent grade) were purchased from Scharlau Chemie S.A. (Barcelona, Spain) and Ethanol (99.5%) from Kemetyl AB (Haninge, Sweden).

The reversed phase packing materials Kromasil 100 Å–10 μm-C18 (K) were purchased from Eka Chemicals AB (Bohus, Sweden), Daiso SP-120-10-ODS-AP (D1) and Daiso SP-100-10-ODS-P (D2) from Daiso Co. Ltd. (Osaka, Japan), Luna 100 Å–10 μm-C18 (L1) and Luna 100 Å–10 μm-C18(2) (L2) from Phenomenex (Torrance, CA, USA), YMC 120 Å–10 μm-ODS-A (Y) from YMC (Kyoto, Japan) and Fuji 100 Å–10 μm-C18 (F) from Fuji Silysia Chemical Ltd. (Aichi, Japan).

2.2. Test solution

A test solution for the chromatographic evaluation was prepared by dissolving 1.0 mg of sodium nitrite in 1.6 mL of 25 mM KH_2PO_4 buffer pH 7.0. Amounts of 1.7 mg nortriptyline, 4.0 mg toluene, 3.9 mg imipramine and 3.0 mg amitriptyline were dissolved in 6.4 mL methanol. Thereafter the two solutions were mixed, forming the antidepressant test solution.

2.3. Column packing

All columns were slurry packed using a Haskel pump 51331-DS-122 from Haskel International Inc. (Burbank, CA, USA). Detailed packing conditions are found in Table 1. The packing pressure was 500 bar, and 200 mL of methanol was used as pushing solvent [10].

Prior to subjecting the columns to the test procedure, plate counts N [m^{-1}] and peak symmetry $As_{10\%}$ were measured with acetophenone in order to assure good initial column performance (Table 1). The acetophenone concentration in the test solution was 1 μL/mL mobile phase. The injection volume was 1 μL.

2.4. Test procedure

The columns (4.6 mm × 250 mm) were subjected to alkaline elution conditions using an 1100 Series liquid chromatograph from Agilent (Palo Alto, CA, USA). The entire test was conducted at 25 °C. Potential hydrolysis of the surface modification and/or the silica backbone was monitored by an appropriate chromatographic test, using basic analytes. Furthermore, the eluents were analyzed upon their silicon content by inductively coupled plasma-optical emission spectrometer (ICP-OES).