5. BIOLOGICAL BUFFERS

Table 1 This table of frequently used buffers gives the pK_a value at 25°C and the useful pH range of each buffer. The buffers are listed in order of increasing pH

Acronym	Name	Mol. wt.	pK_a	Useful pH range
MES	2-(*N*-Morpholino)ethanesulphonic acid	195.2	6.1	5.5–6.7
BIS TRIS	*Bis*(2-hydroxyethyl)iminotris(hydroxymethyl)methane	209.2	6.5	5.8–7.2
ADA	*N*-(2-Acetamido)-2-iminodiacetic acid	190.2	6.6	6.0–7.2
ACES	2-[(2-Amino-2-oxoethyl)amino]ethanesulphonic acid	182.2	6.8	6.1–7.5
PIPES	Piperazine-*N*,*N*′-*bis*(2-ethanesulphonic acid)	302.4	6.8	6.1–7.5
MOPSO	3-(*N*-Morpholino)-2-hydroxypropanesulphonic acid	225.3	6.9	6.2–7.6
BIS TRIS PROPANE	1,3-*Bis*[*tris*(hydroxymethyl)methylamino]propane	282.3	6.8[a]	6.3–9.5
BES	*N*,*N*-*Bis*(2-hydroxyethyl)-2-aminoethanesulphonic acid	213.2	7.1	6.4–7.8
MOPS	3-(*N*-Morpholino)propanesulphonic acid	209.3	7.2	6.5–7.9
HEPES	*N*-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid)	238.3	7.5	6.8–8.2
TES	*N*-*Tris*(hydroxymethyl)methyl-2-aminoethanesulphonic acid	229.2	7.5	6.8–8.2
DIPSO	3-[*N*,*N*-*Bis*(2-hydroxyethyl)amino]-2-hydroxypropanesulphonic acid	243.3	7.6	7.0–8.2
TAPSO	3-[*N*-*Tris*(hydroxymethyl)methylamino)-2-hydroxypropanesulphonic acid	259.3	7.6	7.0–8.2
TRIZMA	Tris(hydroxymethyl)aminomethane			
HEPPSO	*N*-(2-hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulphonic acid)	121.1	8.1	7.0–9.1
POPSO	Piperazine-*N*,*N*′-*bis*(2-hydroxypropanesulphonic acid)	268.3	7.8	7.1–8.5
EPPS	N-(2-Hydroxyethyl)piperazine-*N*′-(3-propanesulphonic acid)	362.4	7.8	7.2–8.5
TEA	Triethanolamine	252.3	8.0	7.3–8.7
TRICINE	*N*-*Tris*(hydroxymethyl)methylglycine	149.2	7.8	7.3–8.3
BICINE	*N*,*N*-*Bis*(2-hydroxyethyl)glycine	179.2	8.1	7.4–8.8
TAPS	*N*-*Tris*(hydroxymethyl)methyl-3-aminopropanesulphonic acid	163.2	8.3	7.6–9.0
AMPSO	3-[(1,1-Dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulphonic acid	243.3	8.4	7.7–9.1
		227.3	9.0	8.3–9.7
CHES	2-(*N*-Cyclohexylamino)ethanesulphonic acid	207.3	9.3	8.6–10.0
CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulphonic acid	237.3	9.6	8.9–10.3
AMP	2-Amino-2-methyl-1-propanol	89.1	9.7	9.0–10.5
CAPS	3-(Cyclohexylamino)-1-propanesulphonic acid	221.3	10.4	9.7–11.1

[a] $pK_a = 9.0$ for the second dissociation stage.
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6A. CLASSIFICATION AND CHARACTERIZATION OF STATIONARY PHASES FOR LIQUID CHROMATOGRAPHY (IUPAC RECOMMENDATIONS 1997)

Descriptive Terminology

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Abstract

A wide range of stationary phases and column packing materials have been developed over the years for liquid chromatography and these need to be described accurately and unambiguously. The present paper, which is the first of a series planned for this area, recommends terms for the description of the stationary phase materials and their properties and expands the list of terms given in Nomenclature of Chromatography [*PAC*, 1993, **65**, 819–872.]. It concentrates on the chemical properties and chromatographic role of the materials. Many of the terms to describe their physical properties as particles have been discussed in a recent paper on the characterization of porous solids [*PAC*, 1994, **66**, 1739–1758].

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Introduction

One of the major problems throughout analytical liquid (and supercritical fluid) chromatography has been in reproducibly transferring methods between columns and systems. One of the main factors is that there are large differences in the chemical and physical properties of stationary phase materials, even between those which are nominally the same, such as octadecylsilyl (ODS)-bonded silicas. In addition, the methods used to describe the physical and chemical properties of these stationary phase materials are not standardized and even the terms used have not been agreed. In the laboratory, the behaviour of the stationary phases is also dependent on the nature of the interaction of specific mobile phases and analytes with the stationary phase.

The present discussions will concentrate on chromatographic applications of stationary phases. The standardization of analytical stationary phase materials falls into two areas.

a) Terms needed to describe the physical and chemical properties of the stationary phase materials.
b) Methods and tests needed to describe the operational properties of these materials. This is an important area but one where research is still active and as yet no consensus of approaches and techniques has been reached. This topic still requires much experimental work and was considered inappropriate for the Commission to consider at this time. It is also the subject of work by ASTM committees and others who are in a better position to carry out experimental work and to organise comparative studies.

The present paper considers the first of these areas and recommends a number of terms for the description of stationary phase materials and their chemical and physical properties. Some descriptive terms for the stationary phase have already been defined in the Nomenclature of Chromatography (NC) [1] and in the recently published Nomenclature for Analytical Chiral Separation methods (CS) [2]. In addition many of the Recommendations for the Characterization of Porous Solids published by the Physical Chemistry Division [3] are relevant to the physical description of stationary phase materials.

Amended and expanded definitions are now recommended for a number of the terms. The original versions are included in the Appendix.

General Descriptive Terms for the Stationary Phase

A number of these terms are generally applicable throughout chromatography and were defined previously in the Nomenclature for Chromatography (NC) [1]. However, in a number of cases, an inappropriate capitalization was used in NC, particularly of the second word in terms and these have been corrected in the definitions reproduced here.

Stationary Phase [Replaces NC 1.1.05]

One of the two phases forming a chromatographic system. It is the part of a chromatographic system responsible for the retention of the analytes, which are being carried through the system by the *mobile phase*. It may be a solid, a gel or a liquid. If a liquid, it may be distributed on a *solid support*. This *solid support* may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (*bonded phase*) or immobilized onto it (*immobilized phase*).

The expression *chromatographic bed* or *sorbent* may be used as a general term to denote any of the different forms in which the stationary phase is used.

Note: Particularly in gas chromatography where the stationary phase is most often a liquid, the term *liquid phase* is used for it as compared to the *gas phase*, i.e. the mobile phase. However, particularly in the early development of liquid chromatography, the term 'liquid phase' had also been used to characterize the mobile phase as compared to the 'solid phase' i.e. the stationary phase. Due to this ambiguity the use of the term 'liquid phase' is discouraged. If the physical state of the stationary phase is to be expressed, the use of the adjective forms, such as *liquid stationary phase* and *solid stationary phase*, *bonded stationary phase* or *immobilized stationary phase*, are recommended.

Packing Material, Stationary Phase Material [Replaces NC 3.1.07]

The *packing* is the active solid, stationary phase plus solid support or swollen gel which is contained in the chromatographic column. In liquid chromatography the usage of the terms *packing material and stationary phase material* are often synonymous. The term *packing material* is preferred as a general term for a loose, usually particulate, material intended for chromatographic use before it is packed into the column. Once it is packed and in contact with the mobile phase, it becomes the *stationary phase* as one of the two chromatographic phases. The *stationary phase* usually consists of a specific *stationary phase material*, which has been packed into a column. Both are typically given the same description.

Solid Support (NC 3.1.03)

A solid that holds the stationary phase but, ideally, does not contribute to the separation process.

Continuous Bed Packing

A column packing, which is a single entity, rather than being composed of individual particles.

Carbon Loading (of the Packing Material)

Mass fraction of the packing material which is carbon. Usually taken as a guide to the extent of alkyl substitution on the surface. Usually reported as percentage carbon determined using elemental analysis.

Terms for the Nature of the Stationary Phase Material

Immobilized Stationary Phase (Material) [Replaces, NC 1.1.05.2]

A stationary phase which has been immobilized on the support particles, or on the inner wall of the column tubing, e.g. by either a physical attraction (*coated stationary phase*), by chemical bonding (*bonded stationary phase*), or by *in situ* polymerisation (*cross-linked stationary phase*) after coating.

Coated stationary phase (material) A material in which a stationary phase is immobilized by a physical attraction to the surface of the solid support.

Filled stationary phase (material) An immobilized stationary phase (*material*) in which a liquid fills the pores of the solid phase.

Bonded stationary phase (material) [Replaces NC 1.1.05.1] A stationary phase which is covalently bonded to solid support particles or to the inside wall of the column tubing. Sometimes referred to as a *bonded phase* (*material*). The bonded stationary phase (material) may be *monomeric*, *polymeric* or *polymer-grafted phase* (*material*) and the *stationary phase* (*material*) can also receive additional treatment to give a *capped* (*end-capped*) *stationary phase* (*material*).

Bonded phase See *Bonded stationary phase* [Replaces NC 1.1.05.1]

Monomeric-bonded stationary phase (material) Bonded stationary phase (material) prepared using a reagent, usually monofunctional, which reacts with single sites on the surface of the solid support.

Polymeric-bonded stationary phase (material) Bonded stationary phase (material) prepared using a polyfunctional reagent which can react both with the surface of the solid support and/or with additional reagent molecules.

Polymer-grafted stationary phase (material) Bonded stationary phase (material) in which a pre-formed polymer has been bound to the surface by a chemical bond.

Capped stationary phase (material) (also known as end-capped stationary phase (material)) Bonded stationary phase (material) which has been treated with a second (usually less bulky) reagent, which is intended to react with remaining functional (e.g. silanol) groups which have not been substituted by the original reagent because of steric hindrance.

Alkyl-bonded stationary phase (material) Bonded stationary phase (material) in which the group bound to the surface contains an alkyl chain (usually between C_1 and C_{18}).

Phenyl-bonded stationary phase (material) Bonded stationary phase (material) in which the group bound to the surface contains a phenyl group.

Cyano-bonded stationary phase (material) Bonded stationary phase in which the group bound to the surface contains a cyanoalkyl–(–$[CH_2]_n$–CN) group.

Diol-bonded stationary phase (material) Bonded stationary phase in which the group bound to the surface contains a vicinal dihydroxyalkyl (–$[CH_2]_n$–CHOH–CH_2OH) group.

Amino-bonded stationary phase (material) Bonded stationary phase in which the group bound to the surface contains an aminoalkyl- (usually a –$[CH_2]_n$–NH_2) group.

Internal surface reversed-phase (ISRP) materials) Bonded stationary phase in which the external surface of the solid support carries different bonded groups from the internal pores (usually an external hydrophilic layer with a more hydrophobic internal layer). Examples include *restricted-access stationary phase* (*material*) in which polar macromolecules are excluded from the internal pores.

Cross-linked Stationary Phase (Material) A stationary phase (material) in which the liquid phase coating on a solid support has been polymerized or cross-linked after coating to make it insoluble in the mobile phase.

Polymeric Stationary Phase (Material)

Stationary phase (material) based on particles of a cross-linked organic polymeric material. Typical materials are *polystyrene divinylbenzene copolymers* (PS-DVB) and modified *PS-DVB* materials.

Liquid-coated Stationary Phase (Material)

A material in which a liquid stationary phase is coated on the surface of the solid support.

Modes of Application of Stationary Phase Materials

Stationary phases are often defined in terms of the mode of chromatography being employed in the separation.

Size Exclusion Chromatographic Phases

These phases are described in *Compendium of macromelecular nomenclature* [4] (term 3.4.6). or in the Nomenclature for Chromatography section 6 [1].

Ion-exchange Stationary Phases

The principle terms have already been defined in NC (section 5) and are included here for comparison.

Cation exchanger (NC 5.302) Ion-exchanger with cations as counter-ions. The term *cation-exchange resin* may be used in the case of solid organic polymers.

Anion exchanger (NC 5.3.03) Ion-exchanger with anions as counter-ions. The term *anion-exchange resin* may be used in the case of solid organic polymers.

Chiral Stationary Phase (CS 2.4 [2])

A stationary phase which incorporates a *chiral selector*. If not constituent of the stationary phase as a whole, the chiral selector can be chemically bonded to (*chiral bonded stationary phase*) or immobilized onto the surface of a solid support or column wall (*chiral coated stationary phase*), or simply dissolved in the liquid stationary phase.

Affinity Stationary Phase (Material)

Bonded stationary phase (material) containing attached (adsorbed or covalently bonded) ligand molecules with a specific biological interaction for a particular molecule or small group of related molecules.

Perfusion Stationary Phase (Material)

Stationary phase in which the mobile phase primarily travels through the pores of the stationary phase.

Physical Properties of the Stationary Phase Material

Recommendations for the characterization of porous solids have recently been published by the Commission on Colloid and Surface Chemistry [3] and many of these are relevant to the characterization of stationary phase materials. In particular the conclusions presented in that paper should be noted, **especially, that in many cases absolute values of the parameters such as pore diameter and surface area cannot be obtained**. The measured value frequently depends on the method of measurement (and this should always be stated) and the selection of a method of characterization starts from the intended use of the material. These comments would also apply to the determination of particle diameter. In addition, the calculation methods for average particle diameters, such as number average or weight average, must be reported.

Previously Defined General Terms

Particle diameter (d_p) (NC 3.1.08) The average diameter of the solid particles.

Pore radius (r_p) (NC 3.1.09) The average radius of the pores within the solid particles.

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Appendix 1.

Terms from the 'Nomenclature for Chromatography' [1] which have been Redefined.

1.1.05. Stationary phase

One of the two phases forming a chromatographic system. It may be a solid, a gel or a liquid. If a liquid it may be distributed on a solid. This solid may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (*bonded phase*) or immobilized onto it (*immobilized phase*).

The expression *chromatographic* bed or sorbent may be used as a general term to denote any of the different forms in which the stationary phase is used.

Note: Particularly in gas chromatography where the stationary phase is most often a liquid, the term *liquid phase* is used for it as compared to the *gas phase*, i.e., the mobile phase. However, particularly in the early development of liquid chromatography, the term 'liquid phase' had also been used to characterize the mobile phase as compared to the 'solid phase', i.e. the stationary phase. Due to this ambiguity the use of the term 'liquid phase' is discouraged. If the physical state of the stationary phase is to be expressed the use of the adjective forms, such as *liquid stationary phase* and *solid stationary phase*, *bonded phase* or *immobilized phase*, are recommended.

1.1.05.1. Bonded phase

A stationary phase which is covalently bonded to the support particles or to the inside wall of the column tubing.

1.1.05.2. Immobilized phase

A stationary phase which immobilized on the support particles, or on the inner wall of the column tubing, e.g. by *in situ* polymerization (cross-linking) after coating.

3.1.07. Packing

The active solid, stationary phase plus solid support or swollen gel contained in a tube.

6B. Characterization of Ion Exchange Chromatographic Stationary Phases

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Abstract

In order to characterize ion exchange chromatographic stationary phases the thermodynamic exchange constant and the free energy interaction parameters are recommended. These parameters are calculated from the experimentally available corrected selectivity coefficient *vs*. exchanger phase composition functions. The equations used for the calculations have been obtained by introducing the Friedman equation (developed for the calculation of the excess free energy change) into the thermodynamic derivation. The suggested parameters also make possible the estimation of the value of the selectivity coefficient at an arbitrary exchanger phase composition. The characteristic parameters of the ion exchange resins and the equations in a directly suitable form for the estimation of the selectivity coefficient are calculated and presented for several systems.

Introduction

Parameters for the physical and chemical characterization of chromatographic stationary phases including ion exchangers have already been defined [1]. The purpose of this paper is to introduce sensitive numerical parameters for the comparison of operation ion exchange chromatographic stationary phases based on their selectivity coefficient exhibited in a particular ion exchange equilibria. For two competing counter ions (*e.g.* A^+ and B^{z+}) the problem arises not only because various selectivity coefficients may be assigned to the various commercially available products, but also because the exact value of the selectivity coefficient