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Advances in the design of anion exchange stationary phases for Ion Chromatography with various bonding chemistries

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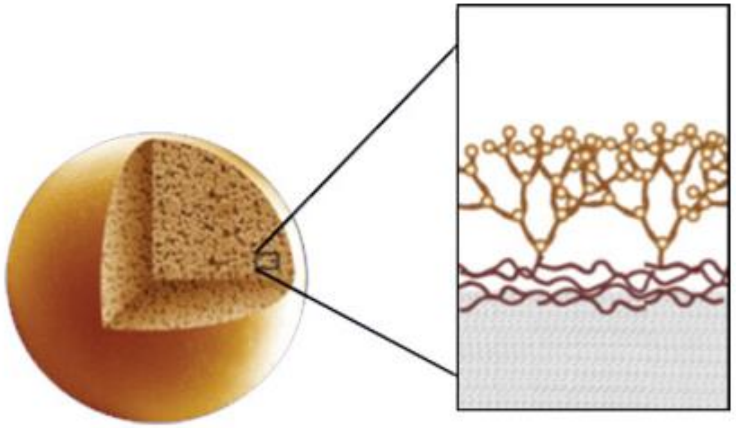
Hyperbranched anion exchangers

- Electrostatically bonded anion exchange materials
- Covered by original patent US 7,291,395
- Represent major portion of ICSP column revenues (at least 15 products)
- Simplified manufacturing process (automated in-column synthesis)
- High performance due to high surface hydrophilicity which minimizes hydrophobic interactions with analytes
- Many possibilities for selectivity variations



(12) **United States Patent**
Pohl et al.

(10) **Patent No.:** **US 7,291,395 B2**
(45) **Date of Patent:** **Nov. 6, 2007**

(54) **COATED ION EXCHANGED SUBSTRATE AND METHOD OF FORMING**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 536 days.

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(22) Filed: **Feb. 18, 2004**

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B05D 7/00 (2006.01)

(52) **U.S. Cl.** **428/407**; 427/221; 427/222

(58) **Field of Classification Search** 428/403, 428/407; 427/212, 221, 222
See application file for complete search history.

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Kopaciewicz, W., et al., "Stationary phase contributions to retention in high-performance anon-exchange protein chromatography: ligand density and mixed mode effects," *J. Chromatogr.* 318:157-172 (1985).

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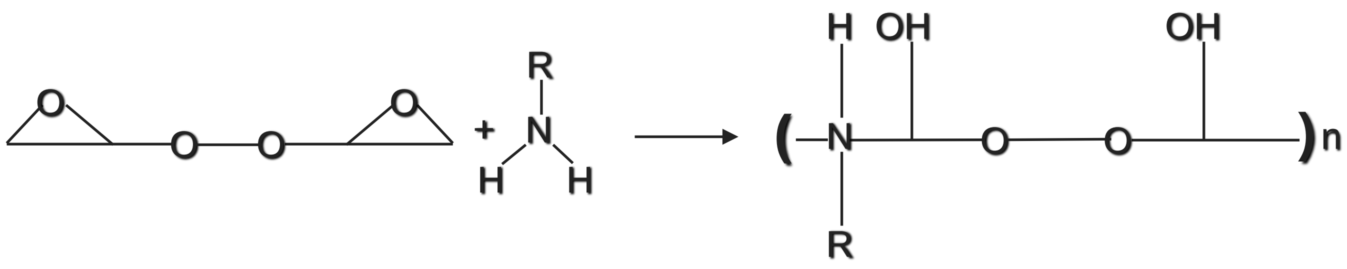
(57) **ABSTRACT**

A method for making an ion exchange coating (e.g., a chromatographic medium) on a substrate comprising (a) reacting at least a first amine compound comprising amino groups, with at least a first polyfunctional compound, in the presence of a substrate to form a first condensation polymer reaction product, with a first unreacted excess of either at least said first amino group or polyfunctional compound functional moieties, irreversibly attached to the substrate, and (b) reacting at least a second amine compound or at least

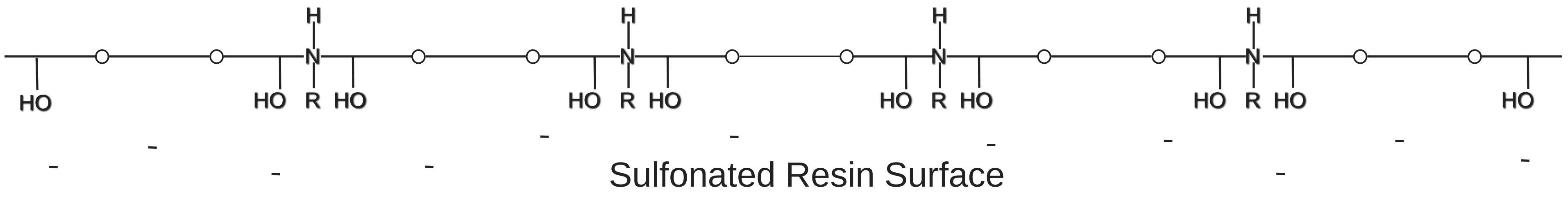
New selectivity → covalent attachment of hyperbranched layer → functionalized resin surface is required

Formation of electrostatically bonded basement coating

Hypothetical product of 1:1 ratio (diepoxide:amine)

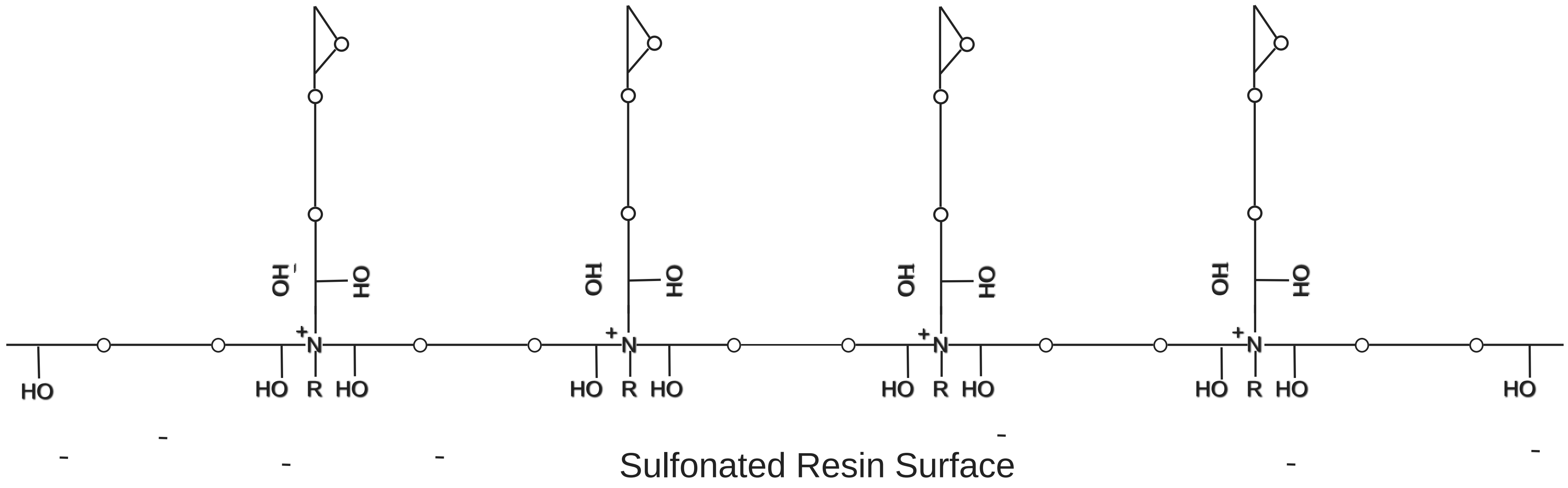


Basement coating [1:1 ratio (diepoxide:amine)]



Step-growth polymer schematic

Layer 1 after diepoxide treatment



Step-growth polymer schematic

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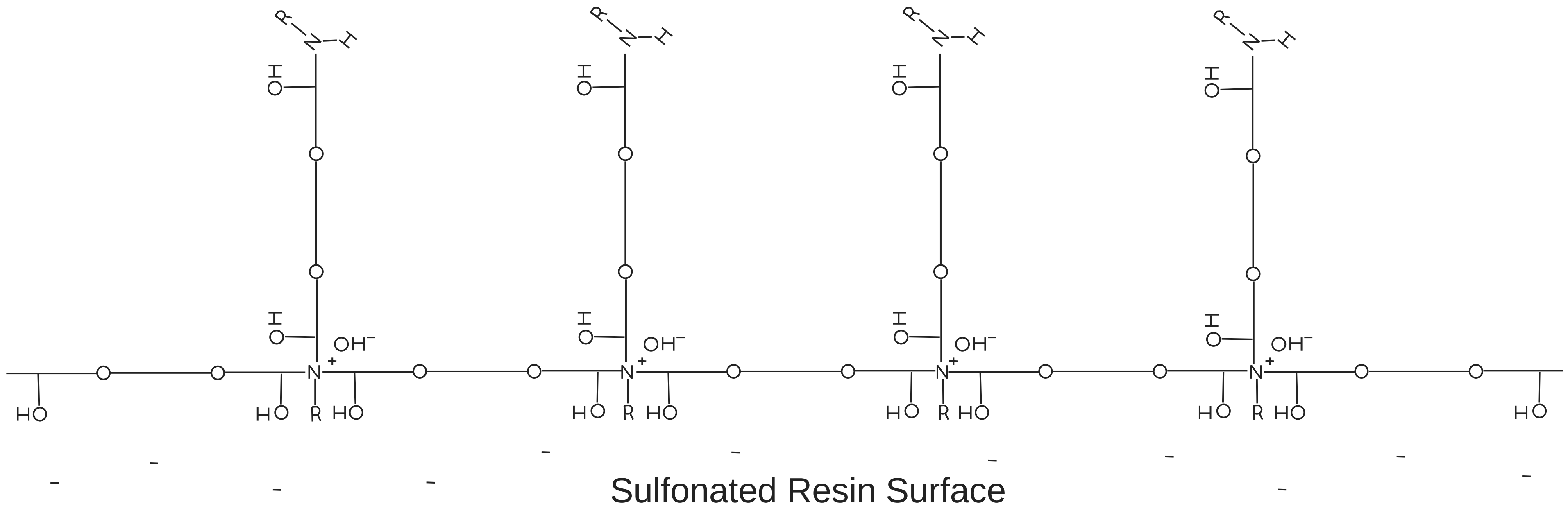
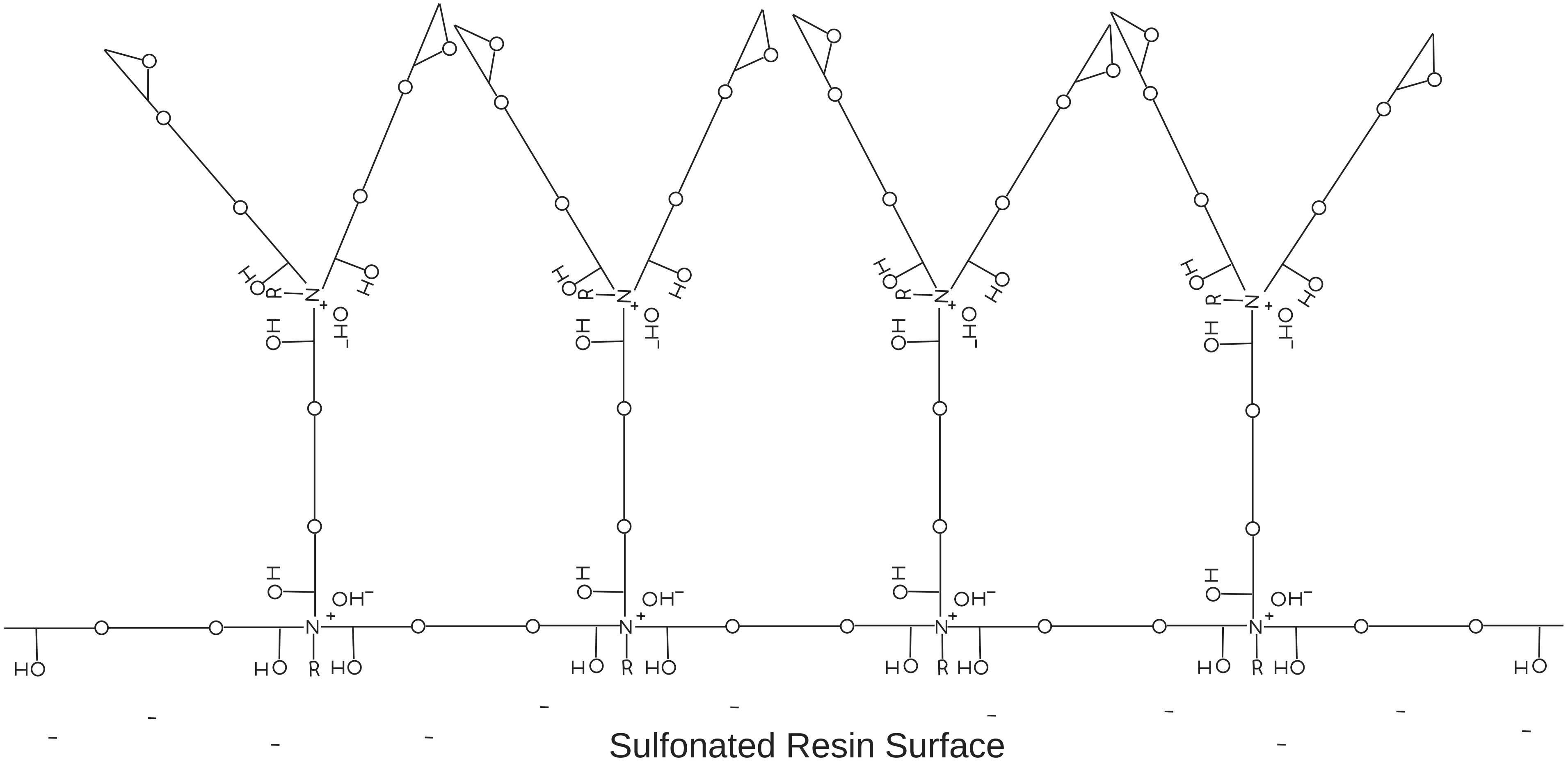
Layer 1 after diepoxide and amine treatment



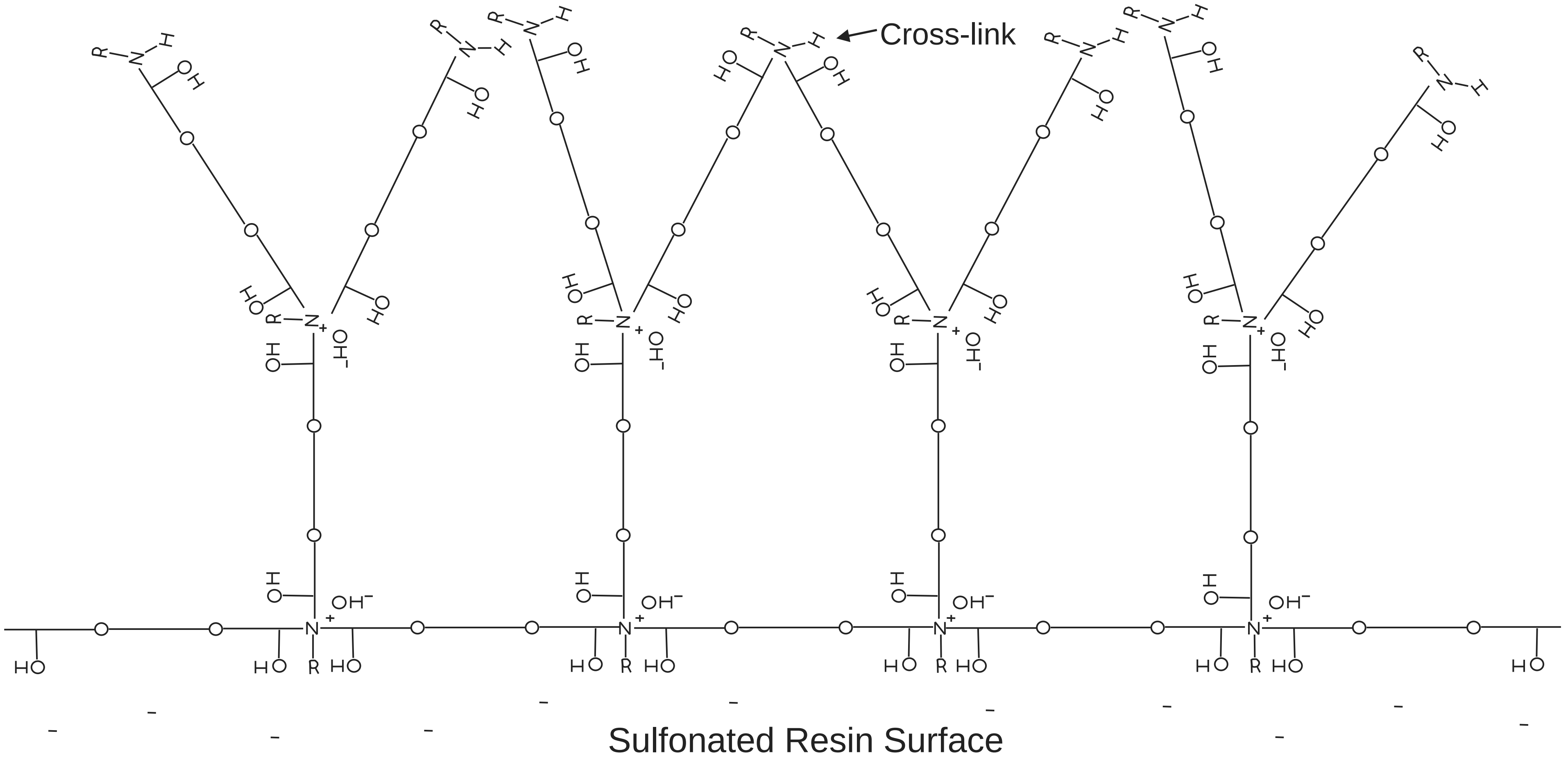
Step-growth polymer schematic

Layer 2 after diepoxide treatment

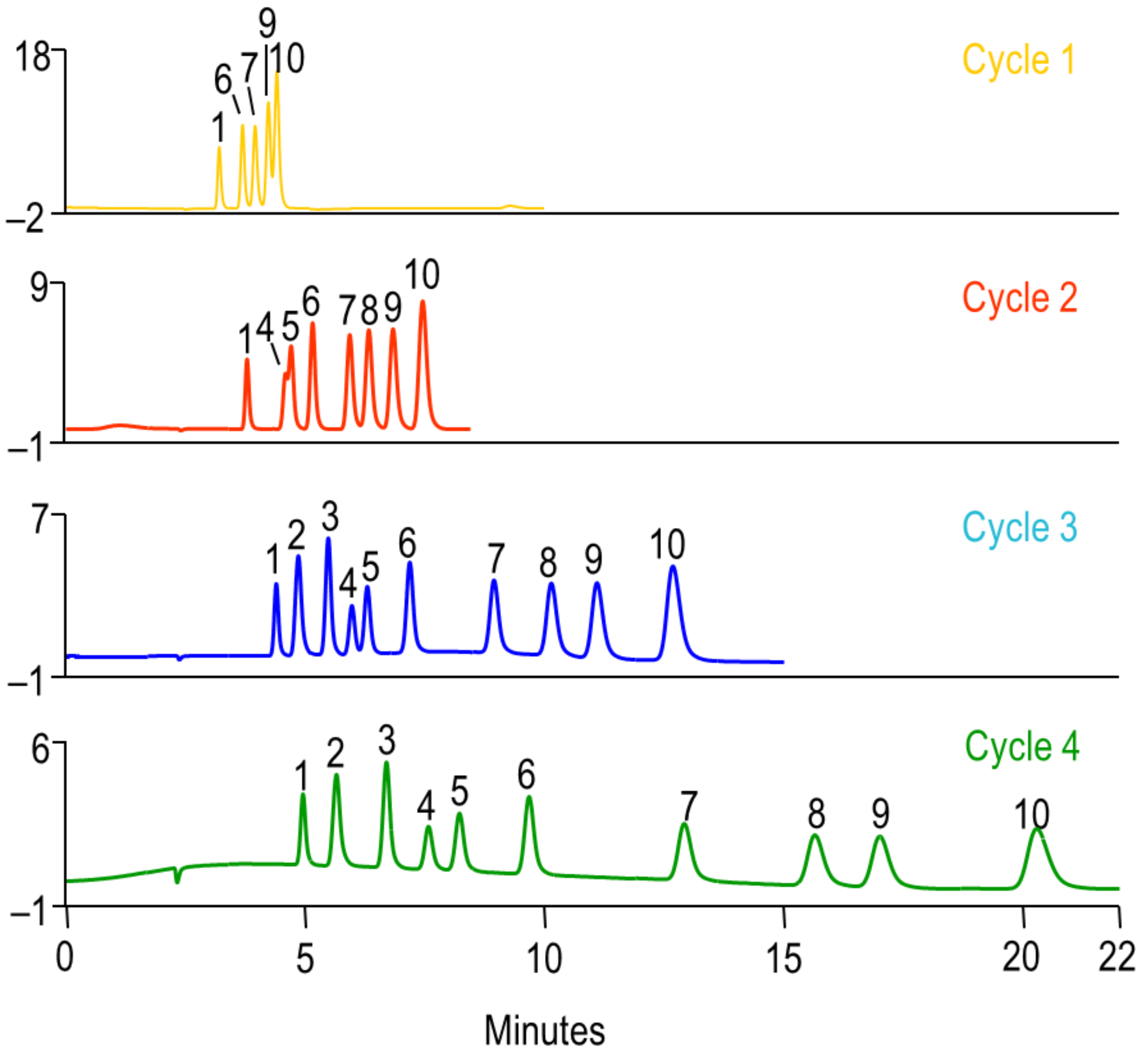


Step-growth polymer schematic

Layer 2 after diepoxide and amine treatment



In-column preparation of step-growth electrostatic graft



Column:	Prototype prepared using MA and 1.4-BDDGE
Eluent:	5 mM KOH
Flow Rate:	1 mL/min
Inj. Volume:	25 µL

Peaks:

1. Fluoride	1	ppm
2. Acetate	10	
3. Formate	5	
4. Chlorite	5	
5. Bromate	10	
6. Chloride	3	
7. Nitrite	5	
8. Chlorate	10	
9. Bromide	10	
10. Nitrate	10	

Covalently bonded anion exchangers

Major requirements:

- Functionalization should be limited to the resin surface
- Functionalization method should provide good surface hydrophilization

Solutions for covalent attachment proposed in literature:

- Chemical derivatization of substrate - difficult to accomplish surface modification → poor stationary phase performance
- Incorporation of a reactive monomer as a comonomer in resin synthesis - uneven distribution of functional groups inside the particle → poor efficiency of the stationary phase.

Alternative solutions:

- To use reagents (monomers) that are not soluble in the resin
- To use the solvent that doesn’t cause resin swelling (highly polar solvents for PS-DVB functionalization)

Formation of covalently bonded basement coating

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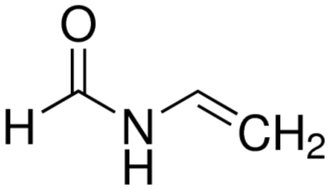
Highly Polar Monomer



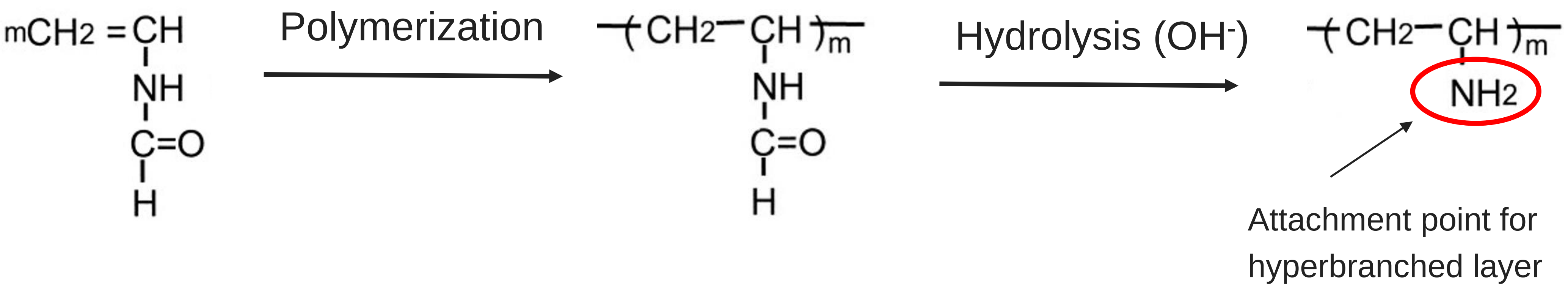
Polar Solvent

CH_3OH

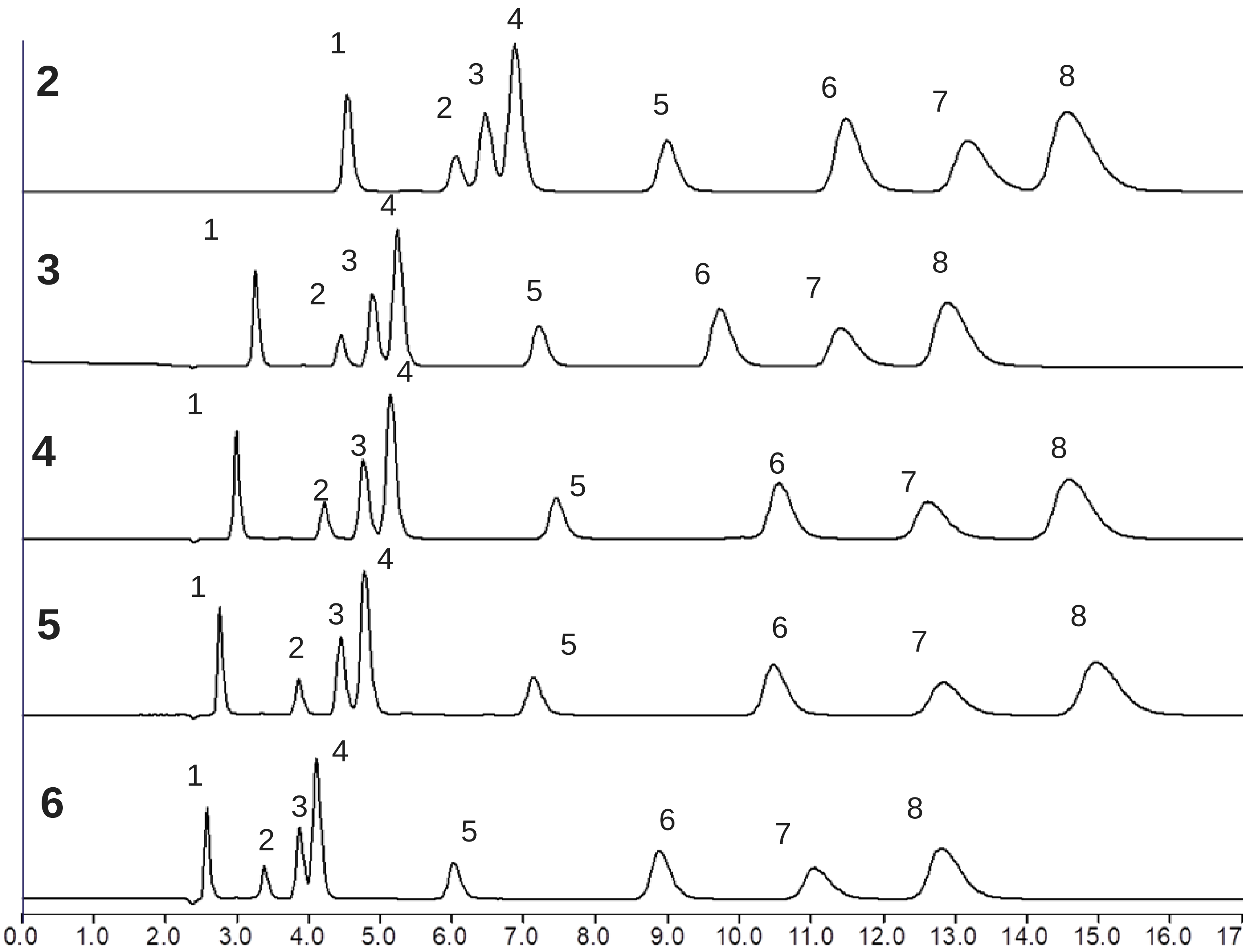
Initiator

AIBN or ACVA

N-Vinylformamide polymerization and hydrolysis



Effect of reaction cycles number on separation



Parameter	Analyte
N of cycles	2 – 6
Diepoxide	1,4-BDDGE
Amine	MA
Columns	250 mm x 4 mm i.d.
Flow	1.0 mL/min

Peak	Analyte
1	F^-
2	ClO_2^-
3	BrO_3^-
4	Cl^-
5	NO_2^-
6	Br^-
7	ClO_3^-
8	NO_3^-

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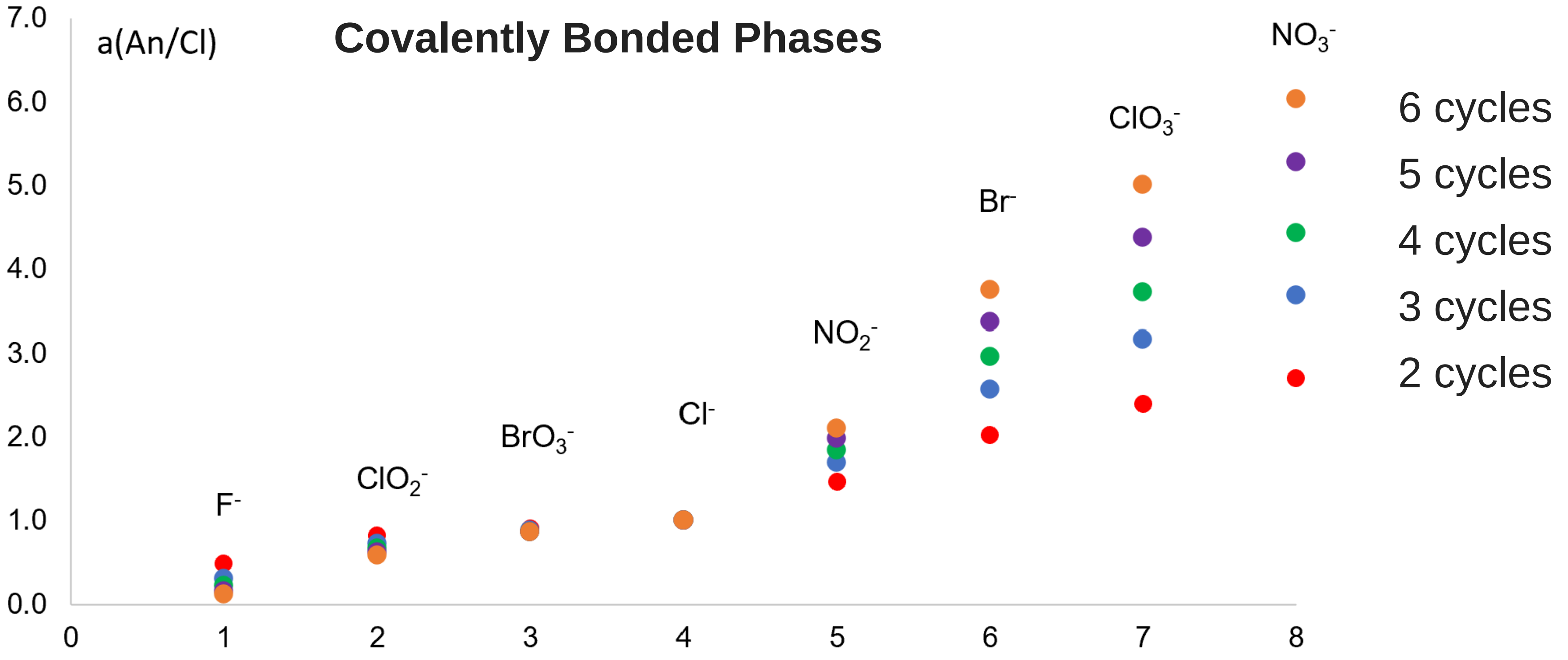
Effect of reaction cycles number on selectivity

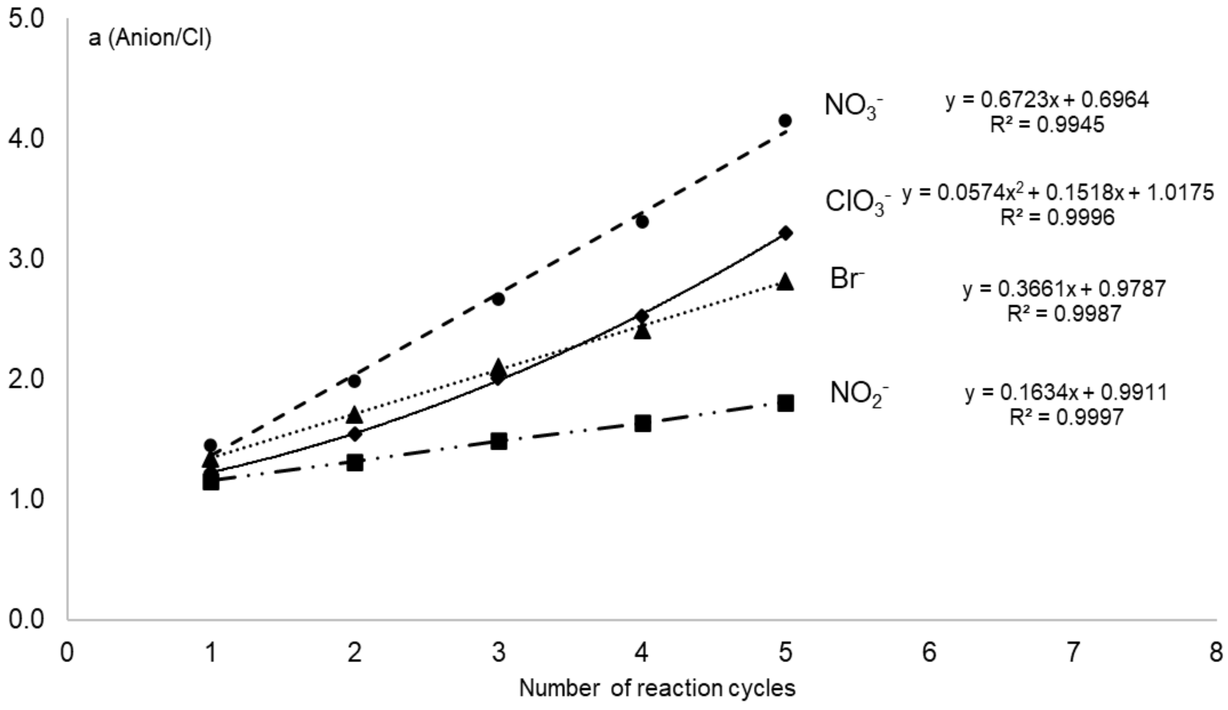


N of cycles	$a(ClO_3/Br)$	$a(NO_3/ClO_3)$	$a(NO_3/Br)$	$t_r(NO_3)-t_r(ClO_3)/ t_r(ClO_3)-t_r(Br)$
2	1.19	1.13	1.34	0.82
3	1.23	1.17	1.43	0.89
4	1.26	1.19	1.50	0.93
5	1.30	1.21	1.57	0.89
6	1.33	1.20	1.61	0.81

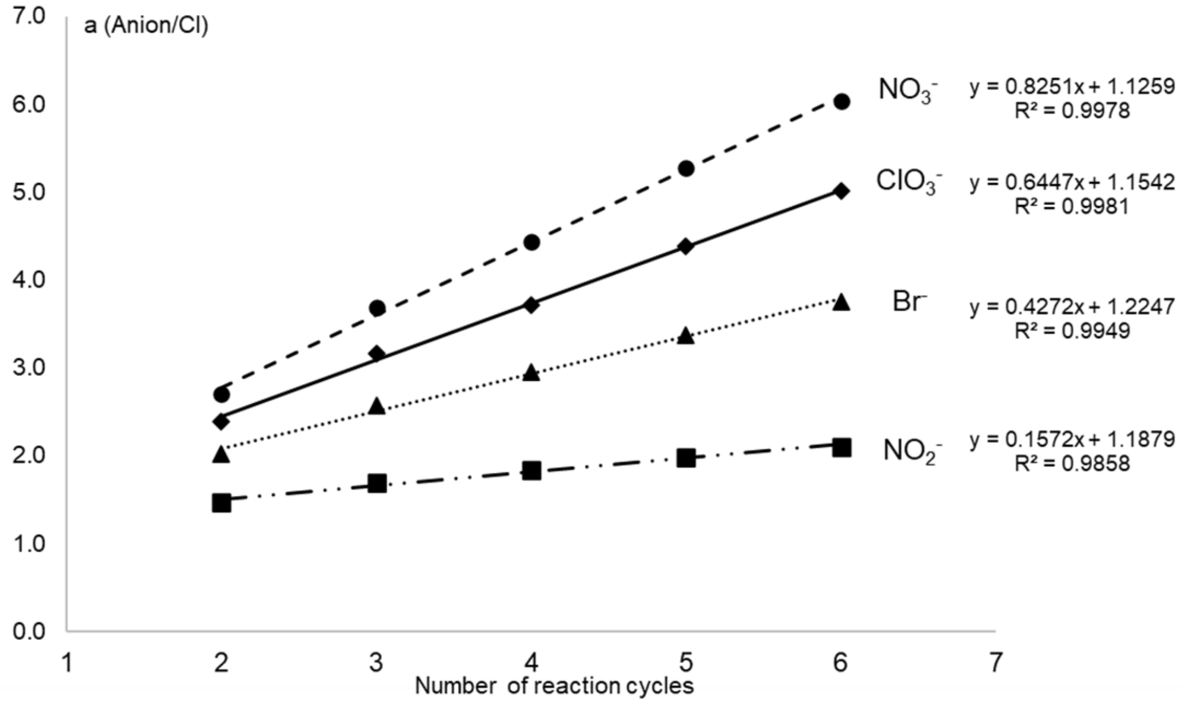
Effect of bonding chemistry on selectivity

Reaction cycles: 1,4-BDDGE + methylamine

Electrostatically Bonded

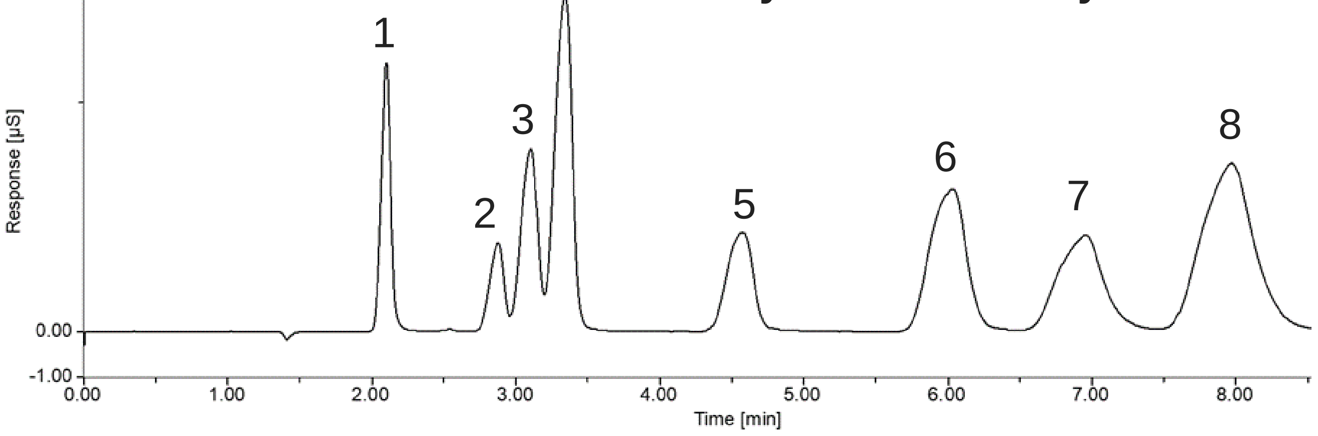


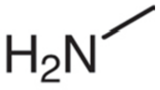
Covalently Bonded



Effect of amine in reaction cycle

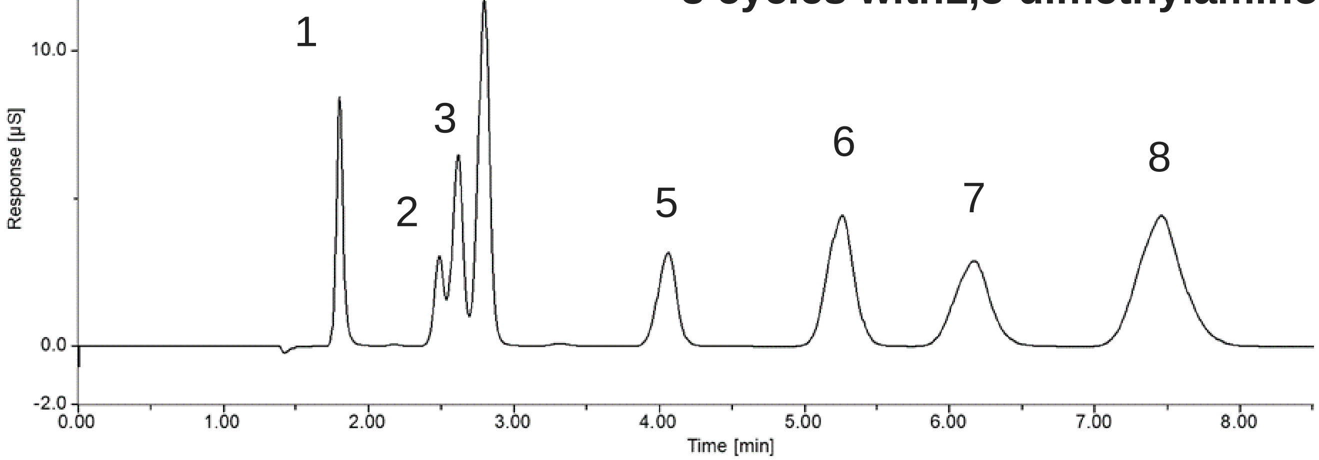
3 cycles with methylamine





Parameter	Value
N of cycles	3
Diepoxide	1,4-BDDGE
Amine	MA
Columns	250 mm x 4 mm i.d.
Eluent	5 mM KOH
Flow	1.0 mL/min

3 cycles with1,3-dimethylaminopropane



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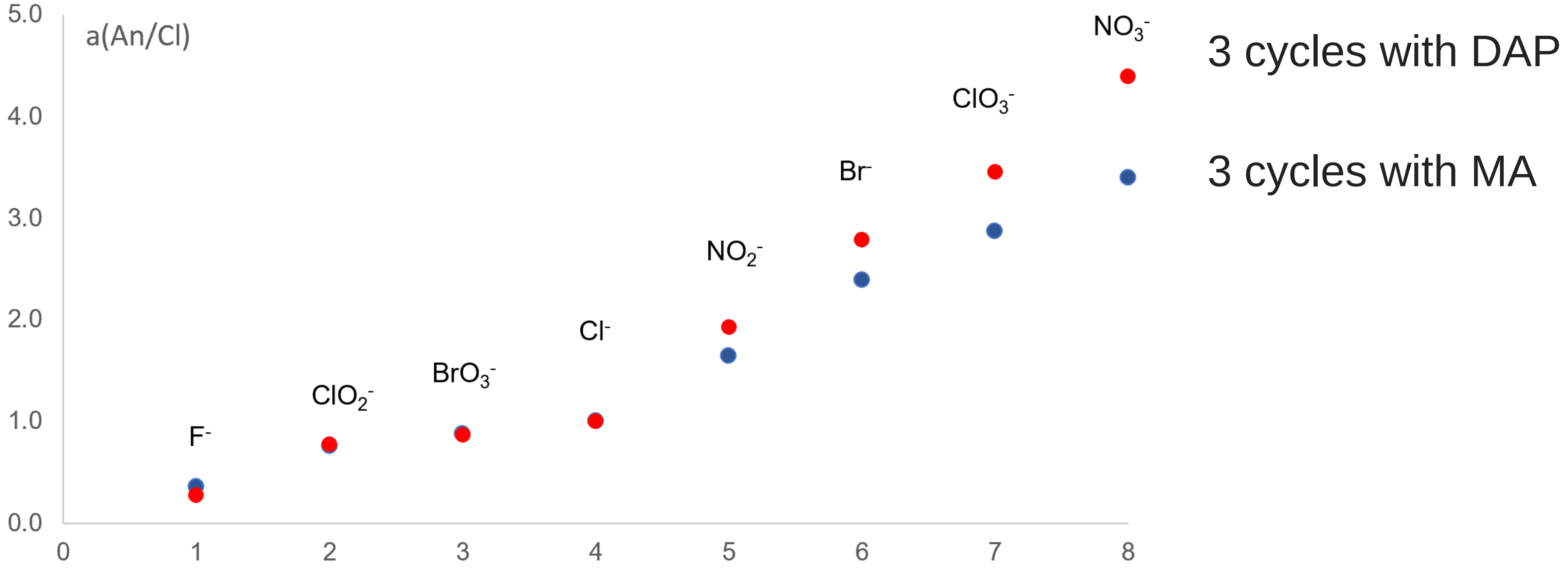
Effect of amine in reaction cycle on selectivity



	a(ClO_3/Br)		a(NO_3/ClO_3)		a(NO_3/Br)	
N cycles/Amine	MA	DAP	MA	DAP	MA	DAP
3 cycles	1.20	1.24	1.18	1.27	1.42	1.57
4 cycles	1.26	1.26	1.19	1.28	1.50	1.62
5 cycles	1.30	1.30	1.21	1.29	1.57	1.67

Effect of monomer used for grafting

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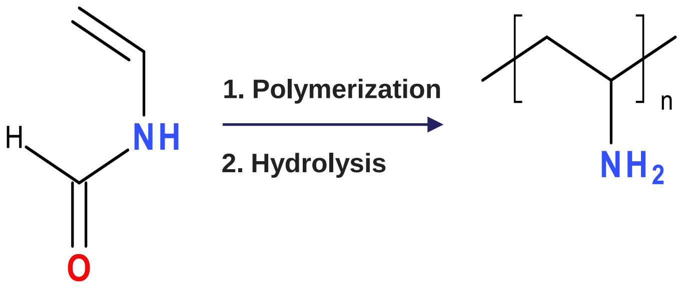
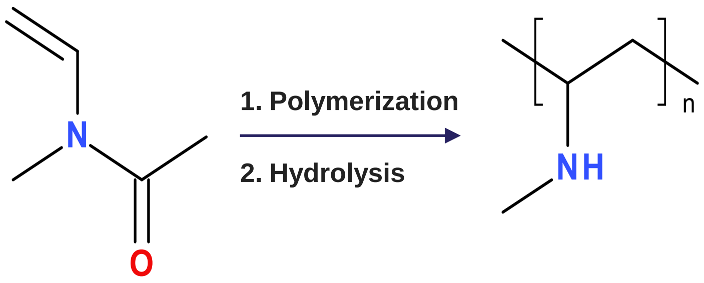
Monomers in the base layer

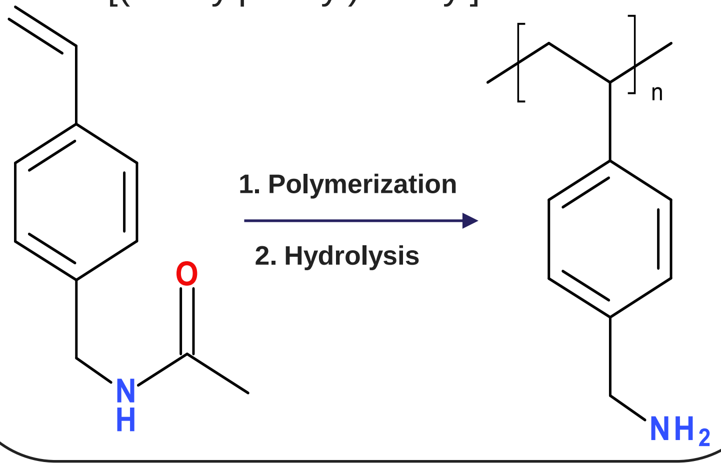
N-VFA
N-vinylformamide



N-MVAcA
N-methyl-N-vinylacetamide



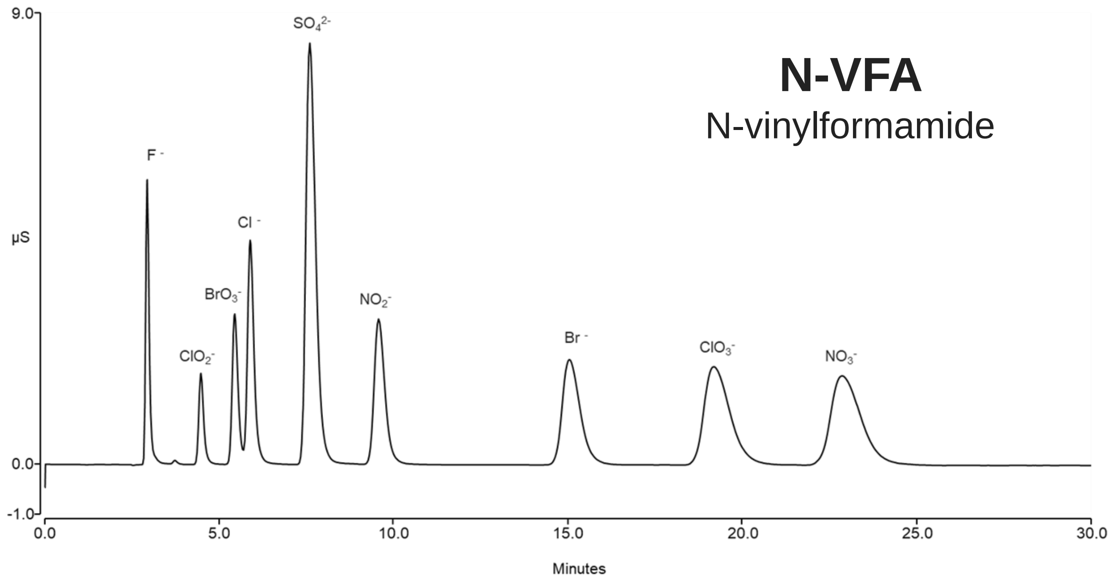
N-VPhMAc
N-[(4-vinylphenyl)methyl]acetamide

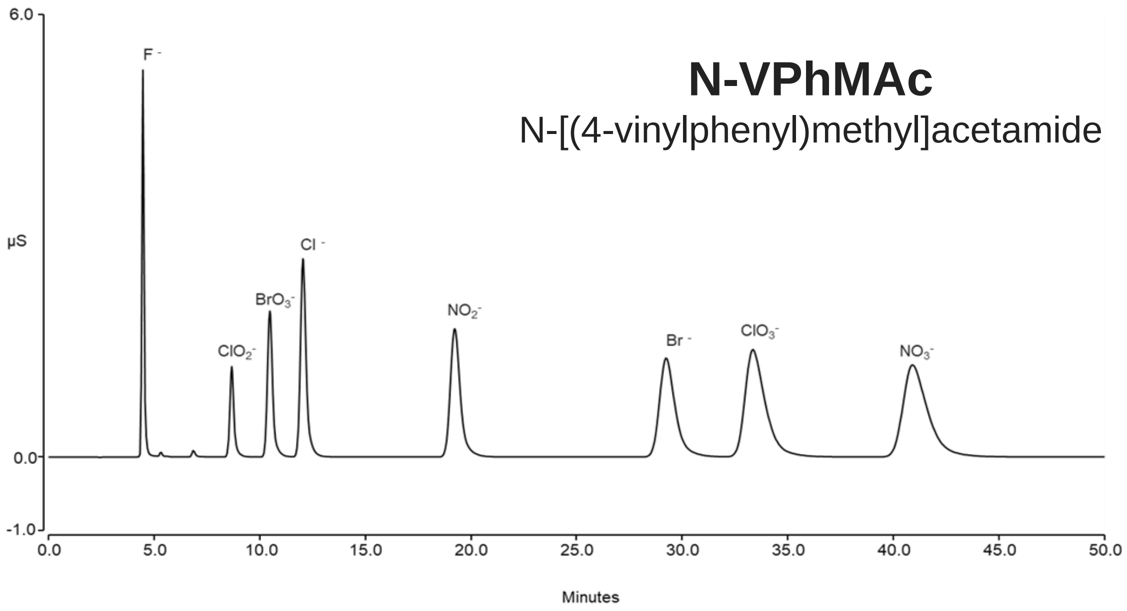


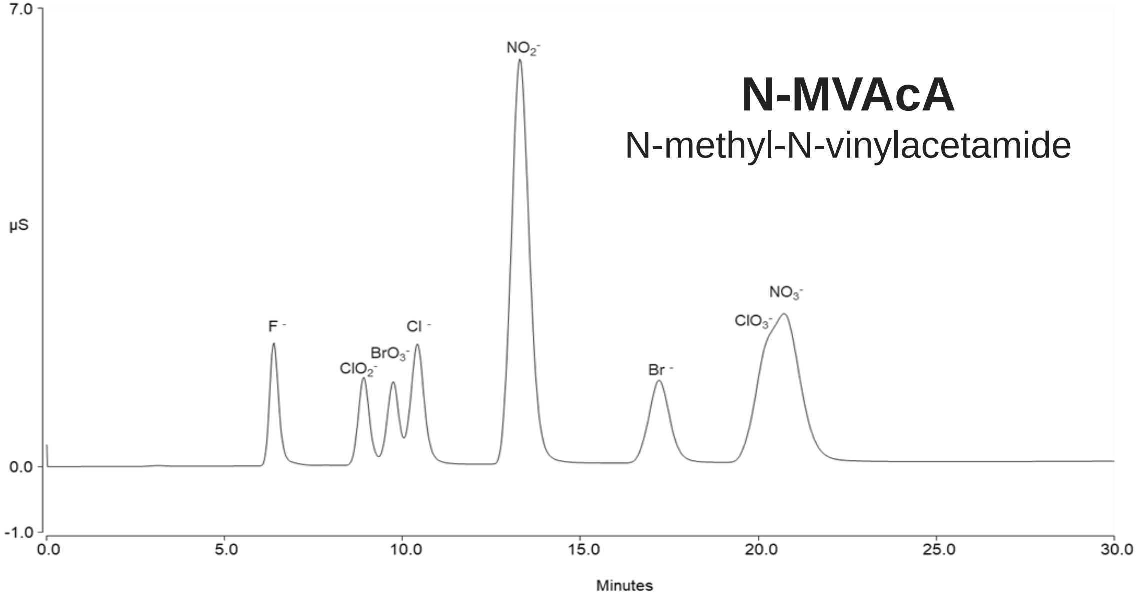
Monomer in the base layer	*Capacity after base layer (μEq)
N-vinylformamide	91.2
N-methyl-N-vinylacetamide	1.7
N-[(4-vinylphenyl)methyl]acetamide	22.9

*Per 250 mm × 4 mm i.d. column

Effect of monomer used for grafting







Monomer in the base layer	N of cycles/ capacity	Eluent
N-VFA	6 / 154 µeq	20 mM KOH
N-MVAcA	6 / 35 µeq	1 mM KOH
N-VPhMAc	5 / 127 µeq	5 mM KOH

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Conclusions

- The proposed grafting approach allows one to limit the modification of the resin to the surface thus preparing covalently bonded hyperbranched phases with high chromatographic performance
- Proposed method of functional layer attachment allows for the preparation of hyperbranched anion exchangers with new selectivities and controlled crosslinks throughout the layer
- Elution order for anions on the covalently bonded hyperbranched anion exchangers is not dependent on the number of the reaction cycles and amine structure used for hyperbranching
- The most effective way to influence selectivity of covalently bonded phases is by changing the structure of grafted monomer and grafting conditions

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