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Principles of
ion exchange

This chapter
provides a
general

introduction to
the theoretical
principles that

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underlie every
ion exchange
separation. An
understanding of
these principles
will enable the
separation power
of ion exchange
chromatography
(IEX) to be
fully
appreciated.
Practical
aspects of

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performing a
separation are
covered in
Chapter 2.

Ion Exchange Chromatography & Chromatofocusing

General

description This
Handbook
contains the
latest
information on

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the theoretical
and practical
aspects of ion
exchange and
chromatofocusing
techniques, the
prepacked
columns and
media available,
and how to
select them.

Ion Exchange Chromatography

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matography
And
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Ion exchange chromatography (IEX) separates proteins with differences in surface charge to give high-resolution separation with high sample loading

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capacity. The separation is based on the reversible interaction between a charged protein and an oppositely charged chromatography resin.

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What is ion
exchange (IEX)
chromatography?
IEX is a liquid
chromatography
technique to
separate
proteins that

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matography slight
differences in
their net
surface charge.

Even very
closely related
proteins will
have some
difference in
charge and

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Interaction and

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Chromatography

This handbook,
ÄKTA Laboratory-
scale

Chromatography
Systems, is
focused on
liquid
chromatography
systems used for
protein
purification at
research
laboratory

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scale. Beginners can use the handbook to obtain an overview of how purification systems work and to learn about important considerations for achieving successful results.

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Size Size
exclusion
chromatography
(SEC), also
called gel
filtration (GF)
Hydrophobicity
Hydrophobic
interaction
chromatography

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(HIC) Reversed
phase
chromatography
(RPC) Charge Ion
exchange
chromatography
(IEX)
Biorecognition
(ligand
specificity)
Affinity
chromatography
(AC) Isoelectric
point (pI)

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matofocusing
(CF) Fig I.1.

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matography
step for
partially
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purified
Healthcare
samples. The
fewer components
in the sample,
the better the
chance for a
well-resolved
separation of
individual
proteins.

Chromatofocusing

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: Principles and Methods | Sigma- Aldrich

For in-depth information about HIC, download our HIC handbook. How does hydrophobic interaction chromatography work? Proteins with different degrees of

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surface
hydrophobicity
can be separated
using

hydrophobic
interaction
chromatography.

The proteins are
bound to the
hydrophobic
ligand on the
HIC resin in a
binding buffer
with a high salt

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matography.
When the ionic
strength of the
buffer is ...

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chromatography.
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Hydrophobic
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mode
(multimodal)
chromatography.
In MM
chromatography,
ligands

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immobilized to
the resin
interact with
the target
protein molecule
through multiple
types of
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Protein

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1: Antibodies**

SOURCE 15S is a
synthetic high
performance,
preparative,
chromatography
resin, based on

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a 15 μm
monosized, rigid
polystyrene/divi
nyl benzene
polymer matrix.
It is modified
with sulphonate
(S) strong
cation exchange
groups. SOURCE
resins have
excellent
physical and
chemical

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matography,
allowing high
flow rates and
consistent
performance.

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In ion exchange
chromatography,
for example, the
pH optimum will

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change when
conductivity is
changed. Thus,
with the one-fac
tor-at-a-time
experimental
setup, there is
a great risk
that the true
optimum for the
studied process
is not
identified.

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Design of Experiments in Protein Production and Purification

Ion exchange
chromatography
(IEC)

Hydrophobic
interaction
chromatography
(HIC) Gel

filtration (SEC)
... My approach

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is based on
classical
combination of
ion-exchange,
hydrophobic and
size-exclusion
chromatography
for natively (no
tags) over-
expressed
proteins.
Nowadays the
high throughput
approach

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matography
dictates
increasing use
of tags in
protein
Healthcare
purification and
sometimes
classical
methods are ...

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