Essentials of Centrifugal Partition Chromatography

Quick Reference Guide

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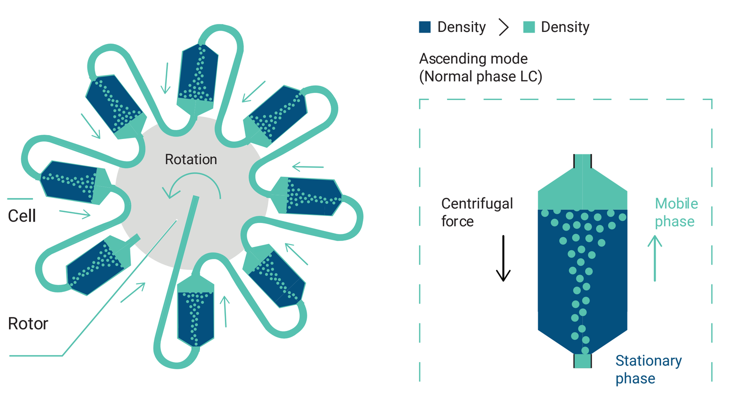
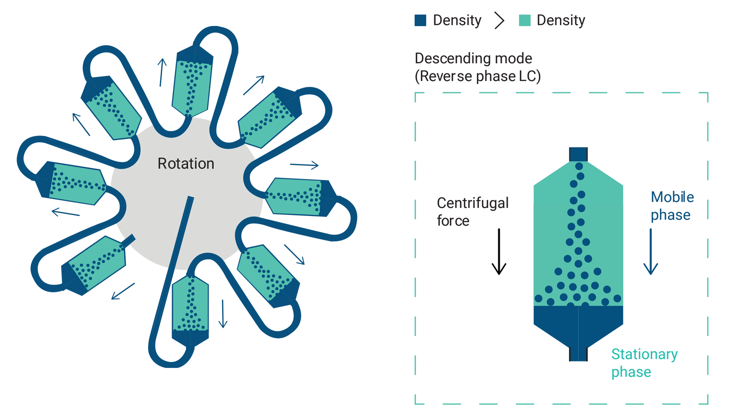
INSIDE A CELL - ASCENDING AND DESCENDING MODES



- Separation occurs between two immiscible liquid phases
- Stationary phase is immobilized by centrifugal force
- Mobile phase moves through the stationary phase in the form of tiny droplets (percolation)



AMONG CHROMATOGRAPHIC METHODS

Used phase: Stationary (Solid, Liquid); Mobile (Liquid, Gas, Supercritical fluid)

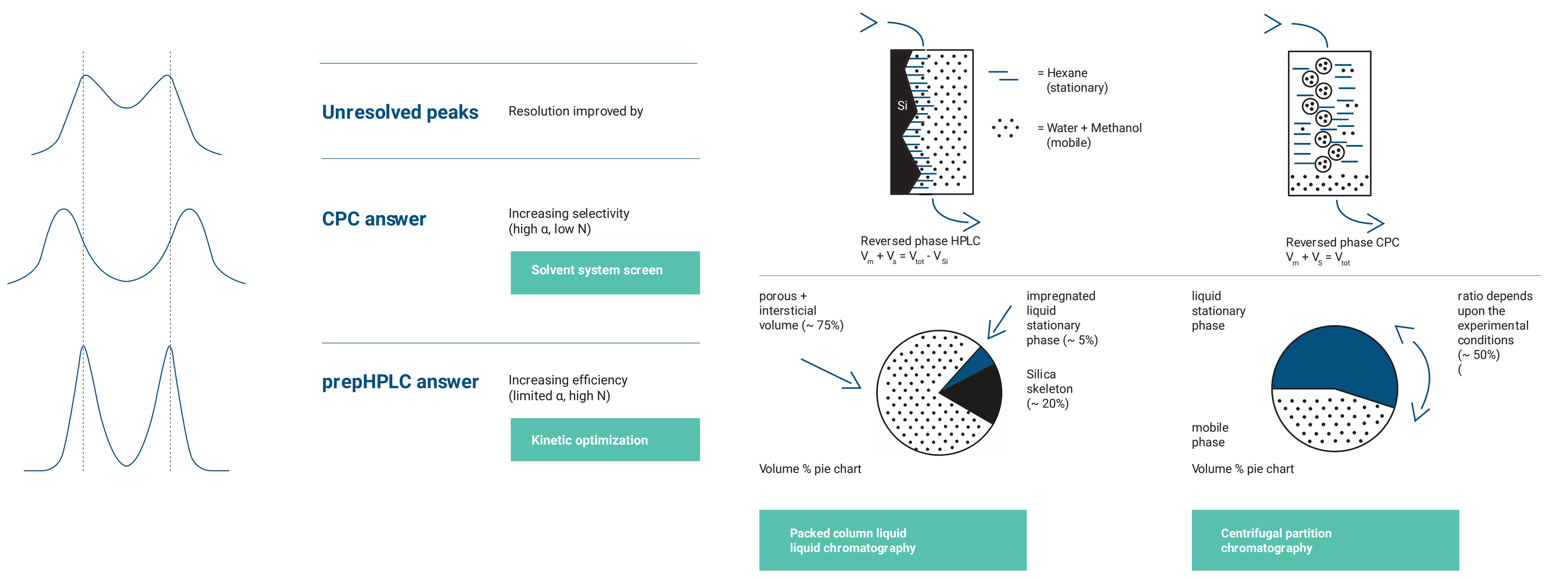
Scale and Goal: Preparative; Analytical

Driving force: Gravity; Density difference; Electric field; Capillary

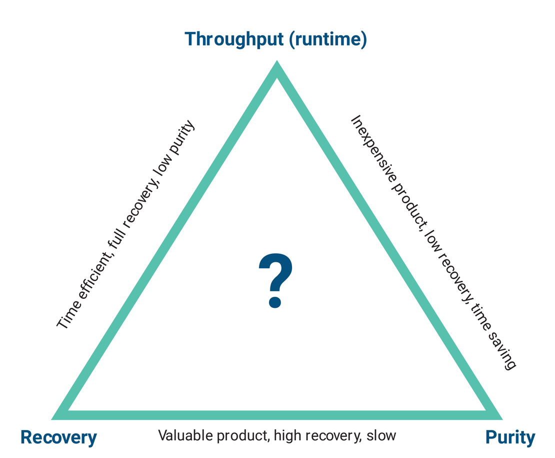
Geometry: Planar; Column; Rotor

Interaction: Physiochemical; Biospecific

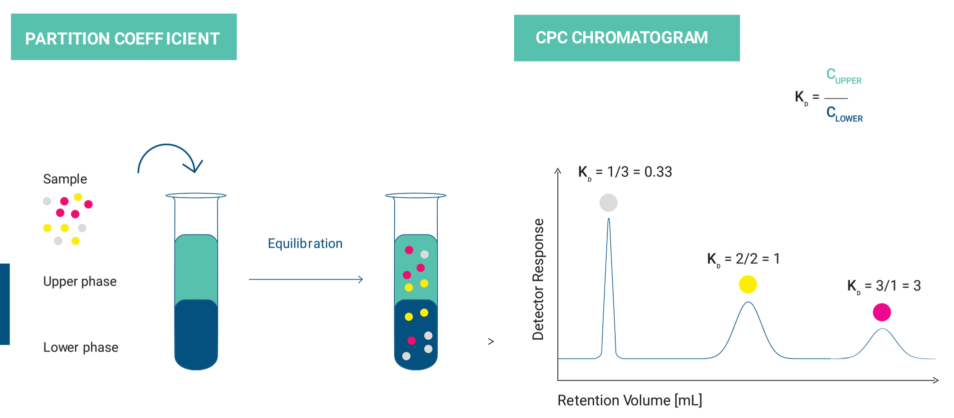
PREP HPLC VS CPC - DIFFERENCE IN WORKING PRINCIPLE



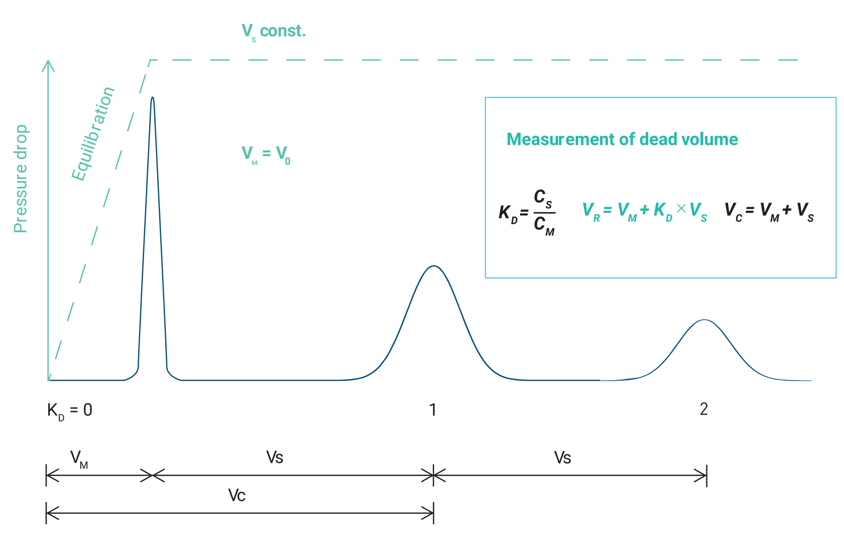
PURITY - RECOVERY - THROUGHPUT TRILEMMA



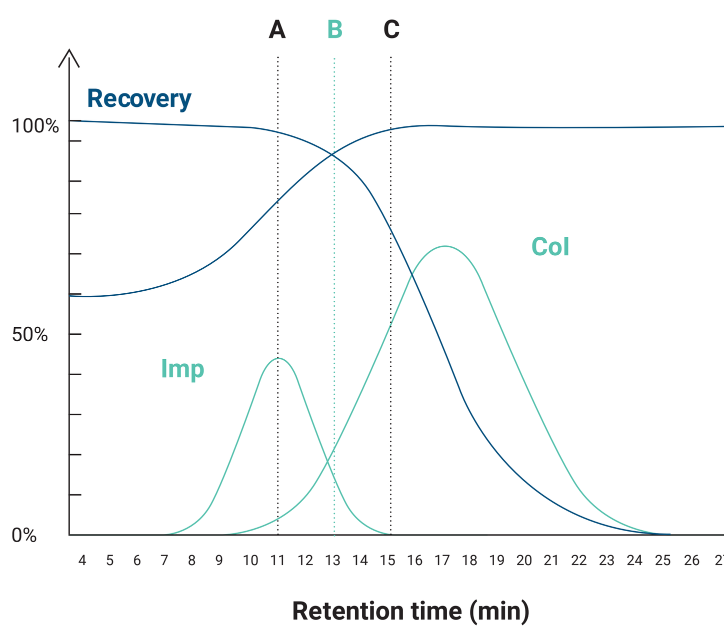
SIMPLE MECHANISM - DIFFERENCE IN PARTITION



DEAD VOLUME, PARTITION AND RETENTION



FRACTION COLLECTION: A TRADE-OFF BETWEEN RECOVERY AND PURITY



Col: Compound of Interest
Imp: Impurity

Crude: 57% Col and 43% Imp

Moderate overlapping

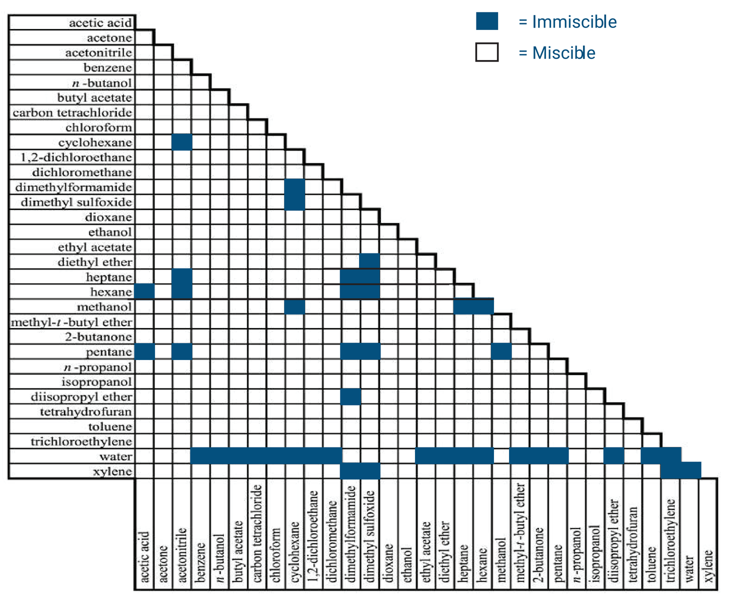
Fraction collection starts at:

A: 11 min 99% recovery 70% purity

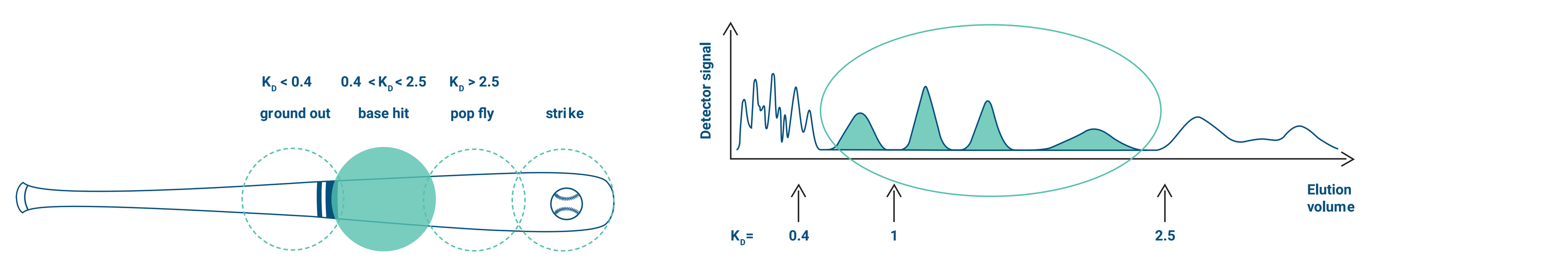
B: 13 min 93% recovery 93% purity

C: 15 min 70% recovery 99% purity

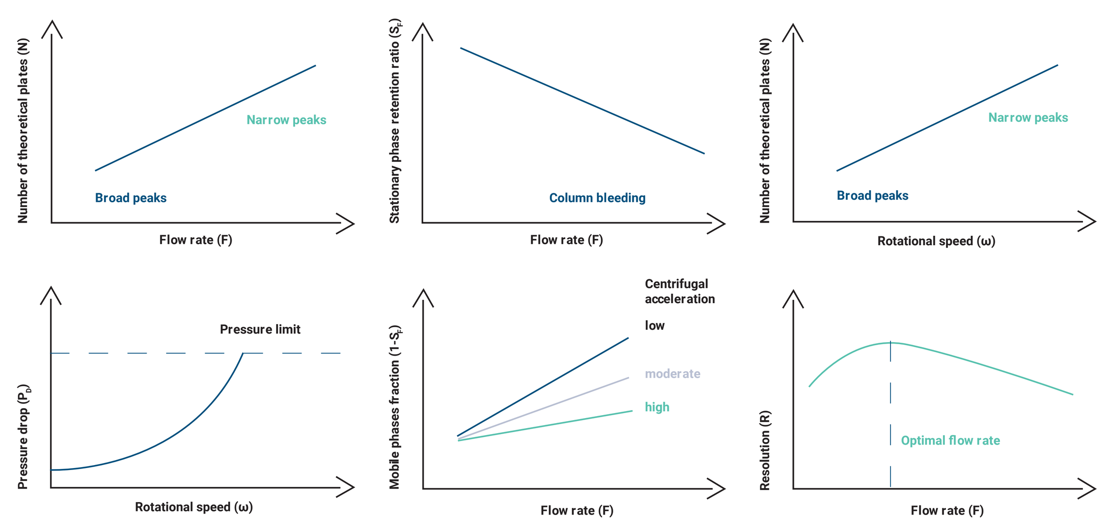
SOLVENT MISCIBILITY TABLE



SWEET SPOT OF PARTITION COEFFICIENT



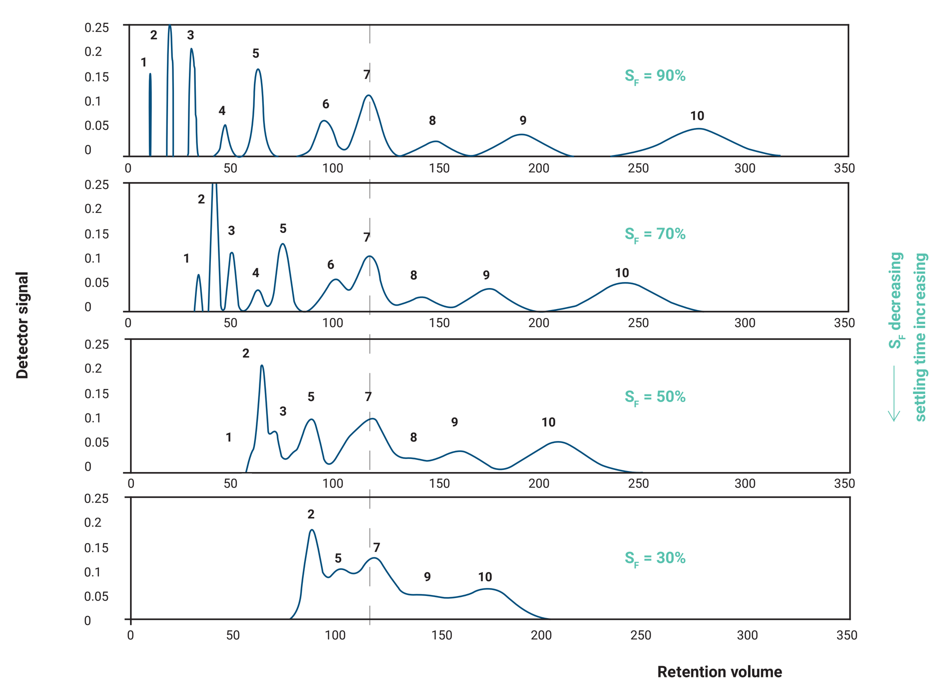
CORRELATION OF OPERATIONAL PARAMETERS



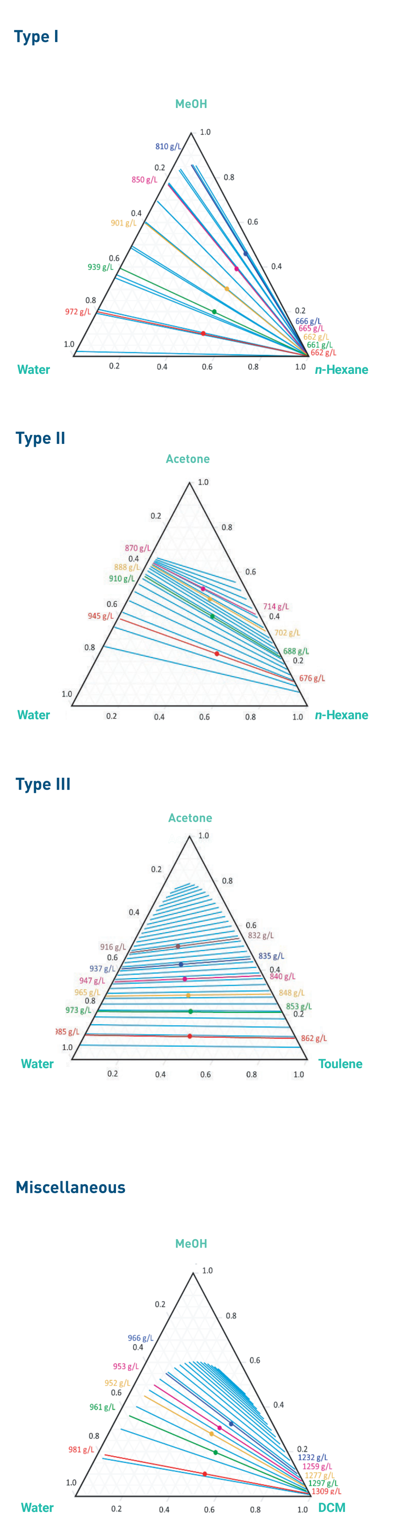
SOLVENT SYSTEM SELECTION ALGORYTHM

Samples		Two - Phase Partition Solvents
Water - Soluble MW > 2000	Bio high polymers (Proteins) – Denaturized/ sluggish in Organic solvents	Dextran / Polyethylene glycol (PEG)
	Bio high polymers (Nucleic acid, Sugars); No denaturalization/ sluggishess in high temperature solvents	PEG/ Aqueous salt solution EtOH/ Aqueous salt solution Acetonitrile/ Aqueous salt solution
	Oligomers (Peptides, Sugars)	Butanol/ Acetic Acid/ H_2O Butanol/ Methanol/ H_2O Butanol/ Ethanol/ H_2O
Water - Soluble MW < 2000	Ionic substances	Butanol/ Acetic Acid/ H_2O Butanol/ Methanol/ Buffer Butanol/ Ethanol/ Buffer Ethyl Acetate/ Buffer
	Non- ionics	Butanol/ Acetic Acid/ H_2O Butanol/ Methanol/ H_2O Butanol/ Ethanol/ H_2O Butanol/ Ethyl acetate/ H_2O
Water - Insoluble	High Polars (Soluble in Methanol, Acetone)	Butanol/ Acetic Acid/ H_2O Butanol/ Methanol/ H_2O Butanol/ Ethanol/ H_2O Butanol/ Ethyl acetate/ H_2O
	Normal Polars (Soluble in Chloroform)	Chloroform/ Methanol/ H_2O Methyl-tert, butyl ether/ Acetonitrile/ H_2O Methyl-isobutyl ketone/ Acetone/ H_2O Heptane/ Ethyl acetate/ Methanol/H_2O
	Low Polars (Soluble in Hexane)	Hexane/ Acetonitrile Hexane/ Methanol/ H_2O Hexane/ Ethanol/ H_2O Heptane/ Ethyl acetate/ Methanol/ H_2O

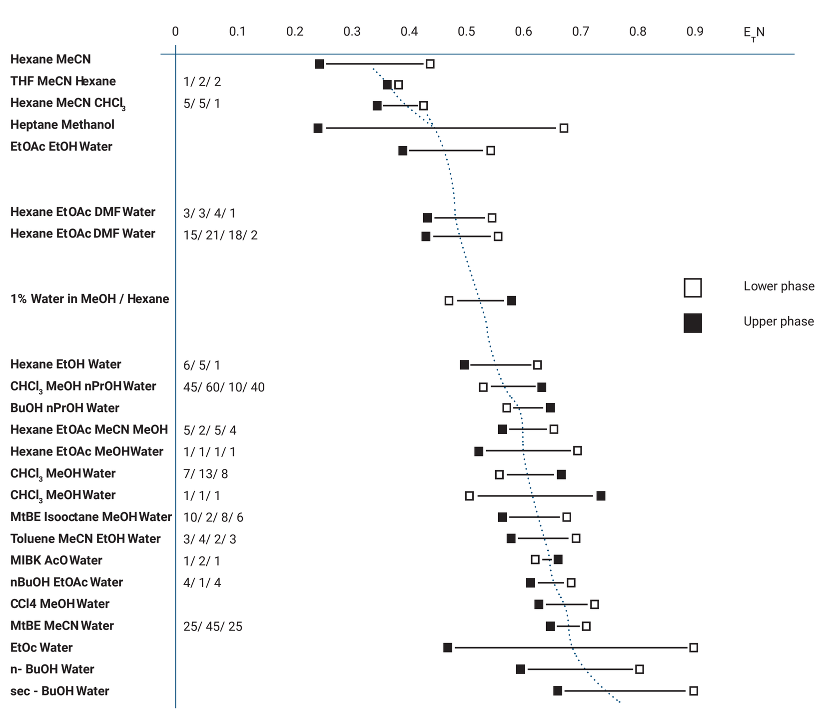
STATIONARY PHASE RETENTION RATIO A CRUCIAL PARAMETER

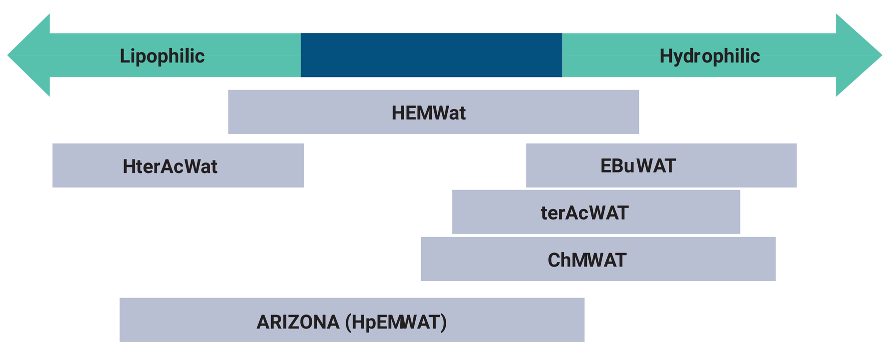


TERNARY DIAGRAMS



POLARITY MAPS





HEMWat: hexane/ethyl acetate/methanol/water
HTerAcWat: hexane/tert-butyl met hyl ether/acetonitrile/water
EBuWat: ethyl acetate/buthanol/water
TerAcWat: tert-butyl met hyl ether/acetonitrile/water
ChMWat: chloroform/methanol/water
Arizona or HpEMWat: heptane/ethyl acetate/methanol/water

SOLVENT PROPERTY CHART

Name of substance	Molecular formula	CAS	Boiling point, °C	Molar mass (g/mol)	Relative polarity	Density (g/mL)	Viscosity (10^{-3} Pa s)	Class*	Price**
Acetic acid	$C_2H_4O_2$	64-19-7	117.9	60.05	0.648	1.049	1.12	3	$
Acetone	C_3H_6O	67-64-1	56.05	58.08	0.355	0.786	0.30	3	$
Acetonitrile	C_2H_3N	75-05-8	81.6	41.05	0.460	0.786	0.34	2	$$
Ammonia (25 w/w%)	NH_3	1336-21-6	37.3	17	-	0.903	-	-	$
Anisole (Methoxybenzene)	C_7H_8O	100-66-3	155.5	108.1	0.198	0.996	-	3	$$$
1-Butanol	$C_4H_{10}O$	71-36-3	119	74.12	0.586	0.810	2.59	3	$
2-Butanol	$C_4H_{10}O$	78-92-2	100	74.12	0.506	0.808	3.10	3	$$
Chlorobenzene	C_6H_5Cl	108-90-7	131	112.6	0.188	1.106	0.75	2	$$
Chloroform	$CHCl_3$	67-66-3	61	119.4	0.259	1.498	0.54	2	$$
Cyclohexane	C_6H_{12}	110-82-7	80.7	84.16	0.006	0.779	0.89	2	$
1,2-Dichloroethane	$C_2H_4Cl_2$	107-06-2	83.6	98.97	0.269	1.235	0.78	1	$$
Dichloromethane	CH_2Cl_2	75-09-2	40	84.93	-	1.325	0.44	2	$
Diethylene glycol	$C_4H_{10}O_3$	111-46-6	244	106.1	0.713	1.118	30.20	-	$$$
Diisopropyl ether	$C_6H_{14}O$	108-20-3	67	102.2	-	0.725	0.27	-	$$
N,N-Dimethylformamide (DMF)	C_3H_7NO	68-12-2	153	73.09	0.386	0.944	0.80	-	$
Dimethyl glycol (1,2-dimethoxyethane)	$C_4H_{10}O_2$	110-71-4	84	90.12	0.231	0.868	1.10	2	$$$$
Dimethyl sulfoxide	C_2H_6OS	67-68-5	189	78.13	0.444	1.092	2.00	3	$$$
Dioxane	$C_4H_8O_2$	123-91-1	101	88.11	0.164	1.033	1.18	2	$$$
Ethanol	C_2H_6O	64-17-5	78	46.07	0.654	0.789	1.08	3	$
Ethyl acetate	$C_4H_8O_2$	141-78-6	77.1	88.11	0.228	0.894	0.22	3	$
Ethylene glycol	$C_2H_6O_2$	107-21-1	197.3	62.07	0.790	1.115	16.10	2	$
Formic acid	CH_2O_2	64-18-6	101	46.03	-	1.220	1.61	3	$$
n-Heptane	C_7H_{16}	142-82-5	98.2	100.2	0.012	0.684	0.39	3	$
n-Hexane	C_6H_{14}	110-54-3	69	86.18	0.009	0.655	0.29	2	$
Hydrochloric acid (HCl) (37 w/w%)	HCl	7647-01-0	-	-	-	1.190	2.30	-	$
Isooctane (2,2,4-Trimethylpentane)	C_8H_{18}	540-84-1	99.2	114.2	-	0.692	0.50	-	$$
Isopentanol (Isoamyl Alcohol)	$C_5H_{12}O$	123-51-3	131	88.1	-	0.810	3.74	-	$$$
Limonene	$C_{10}H_{16}$	5989-27-5	176	136.2	-	0.841	0.93	-	$
Methanol	CH_4O	67-56-1	65	32.04	0.762	0.791	0.54	2	$
Methyl acetate	$C_3H_6O_2$	79-20-9	57.5	74.08	0.253	0.933	0.36	3	$
Methyl Ethyl Ketone (Butane-2-on, MEK)	C_4H_8O	78-93-3	79.6	72.11	-	0.806	0.40	3	$
Methyl isobutyl ketone	$C_6H_{12}O$	108-10-1	114	100.2	-	0.802	0.59	2	$$$
Methyl tert-butyl ether	$C_5H_{12}O$	1634-04-4	55.3	88.15	0.124	0.741	0.36	-	$
2-Methyl-tetrahydrofuran	$C_5H_{10}O$	96-47-9	78	86.14	-	0.854	0.46	-	$$$
Methyl-cyclohexane	C_7H_{14}	108-87-2	100.9	98.19	-	0.770	0.68	2	$$$
Nitromethane	CH_3NO_2	75-52-5	101.2	64	-	1.139	0.61	2	$$
N-Methyl-2-Pyrrolidone	C_5H_9NO	872-50-4	204.3	99.13	-	1.028	1.65	2	$$
n-Pentane	C_5H_{12}	109-66-0	36.1	72.15	0.009	0.626	0.23	3	$
PEG 200	$HO(C_2H_4O)_nH$	25322-68-3	>150	190-210	-	1.124	63.80	-	$
PEG 400	$HO(C_2H_4O)_nH$	25322-68-3	>250	375-450	-	1.126	115.10	-	$$
1-Propanol	C_3H_8O	71-23-8	97	60.1	0.617	0.803	1.95	3	$
2-Propanol (Isopropyl alcohol, IPA)	C_3H_8O	67-63-0	82	60.1	0.546	0.785	2.07	3	$
Propionitrile	C_3H_5N	107-12-0	96.8	55.08	-	0.792	0.44		$$$$
Pyridine	C_5H_5N	110-86-1	115.2	79.1	0.302	0.982	0.88	2	$$$
Tetrahydrofuran	C_4H_8O	109-99-9	65	72.11	0.207	0.886	0.46	2	$$
Toluene	C_7H_8	108-88-3	110.6	92.14	0.099	0.867	0.55	2	$
Triethylamine	$C_6H_{15}N$	121-44-8	88.8	101.19	-	0.670	0.35	3	$
Water	H_2O	7732-18-5	100	18.02	1.000	0.998	0.89	-	$
Xylenes	C_8H_{10}	1330-20-7	139.1	106.2	-	0.864		2	$

FORMULA COLLECTION

Partition coefficient

$$K_D = \frac{C_S}{C_M} = \frac{\text{solute concentration in the stationary phase}}{\text{solute concentration in the mobile phase}}$$

Phase ratio

$$\beta = \frac{V_S}{V_M} = \frac{\text{volume of the stationary phase}}{\text{volume of the mobile phase}}$$

Retention volume

$$V_R = V_M + K_D V_S$$

Rotor volume

$$V_C = V_M + V_S$$

$$V_R = V_C + (K_D - 1)V_S = V_C[1 + (K_D - 1)S_F]$$

Stationary phase retention ratio

$$S_F = \frac{V_S}{V_C}$$

Retention factor

$$k = \frac{V_R - V_M}{V_M}$$

$$k = K_D \frac{V_S}{V_M} = K_D\left(\frac{1-S_F}{S_F}\right) = K_D\beta$$

Retention time (t_R), dead time (t_0) and flow rate (F)

$$V_R = t_R F \qquad t_0 = \frac{V_M}{F} \qquad t_R = t_0(1+k)$$

Partition coefficient measured on the rotor

$$K_D = \frac{V_R - V_M}{V_C - V_M} = \frac{(t_R - t_0)F}{V_C - Ft_0}$$

Selectivity

$$\alpha = \frac{k_2}{k_1} = \frac{t_{R,2} - t_0}{t_{R,1} - t_0} \geq 1.05$$

Theoretical plate number (Efficiency)

$$N = 4\left(\frac{V_R}{w_{0.6h}}\right)^2 = 16\left(\frac{V_R}{w_b}\right)^2$$

$w_{0.6h}$ peak width at 60% of peak height
w_b peak width at baseline

Resolution

$$R_s = 2\frac{V_{R,2} - V_{R,1}}{w_{b,2} + w_{b,1}} \geq 1,5$$

$$R_s = \frac{\sqrt{N}}{4}\,\frac{\alpha - 1}{\alpha}\,\frac{k}{1+k}$$

$$R_S = S_F\frac{\sqrt{N}}{4}\frac{(K_{D,2} - K_{D,1})}{1 - S_F(1 - \frac{K_{D,1} - K_{D,2}}{2})}$$

Pressure drop on the rotor

$$P_d = n\,(\Delta d\omega^2 Rh \ + \ \nu\gamma F)$$

Hydrostatic term Hydrodymanic term

n	is the number of cells
Δd	is the phase density difference
ω	is the rotor spin rate
R	is the rotor radius
h	is the stationary phase height in a cell
v	is the mobile phase viscosity
γ	is a constant for the geometry of the apparatus
F	is the mobile phase flow rate

Centrifugal force

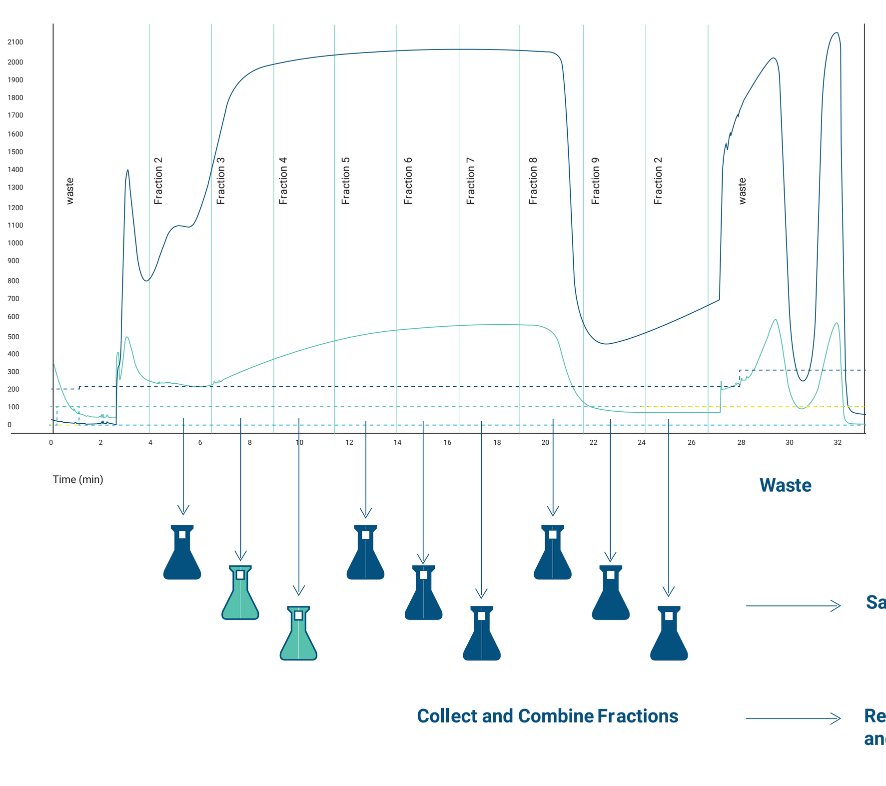
$$g = R\omega^2$$

WORKFLOW

rCPC hardware unit overview

- rCPC rotor unit (1)
- Input selector valve (2)
- Liquid pump (3)
- UV-VIS detector (4)
- Fraction collector (5)

rCPC chromatogram



Construct Fractogram