FAST POLYMER SEPARATIONS ON MONOLITHS

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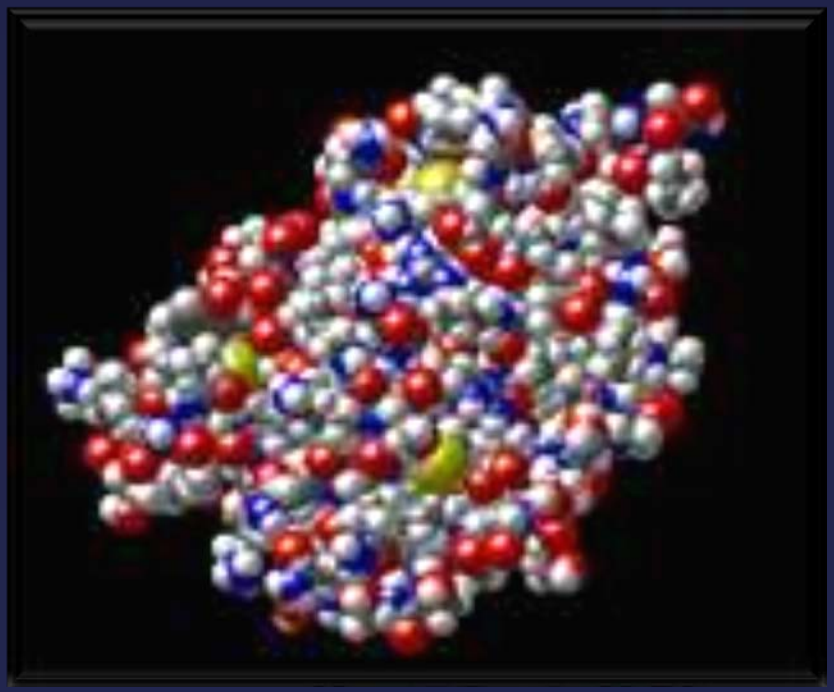
MAIN STREAM:

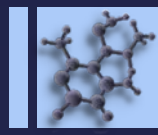
- FLEXIBLE CHAIN POLYMER *vs* GLOBULAR PROTEIN
- SEC (HDC) *vs* "ADSORPTION" SEPARATION MODE
- IMPORTANT SPECIALTIES
- PRACTICAL PERSPECTIVES

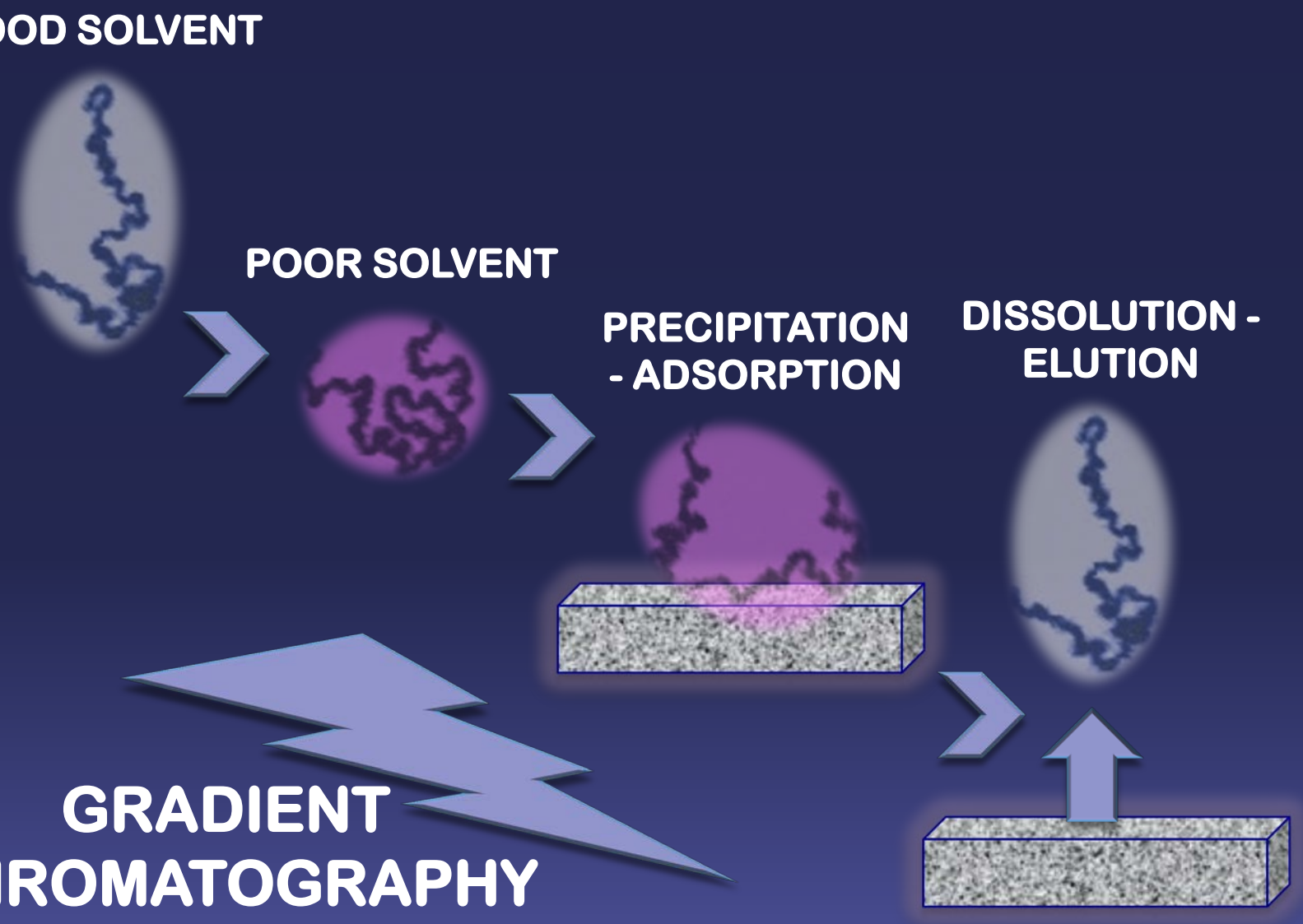


COMPACT PROTEIN GLOBULE

RANDOM COILED MACROMOLECULE (MMD>1)

PRECIPITATION-DISSOLUTION:





SEPARATION MEDIA FORMATS:

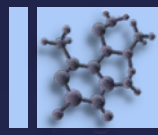
STAINLESS COLUMNS
4.6x50 mm

DISKS:

12x3 mm

5x5 mm

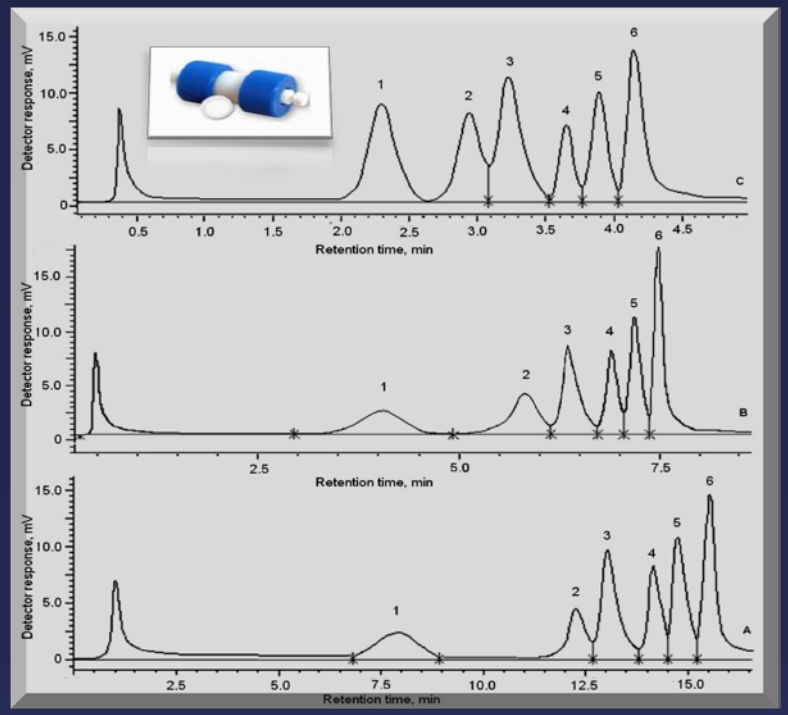
SEPARATION MEDIA:

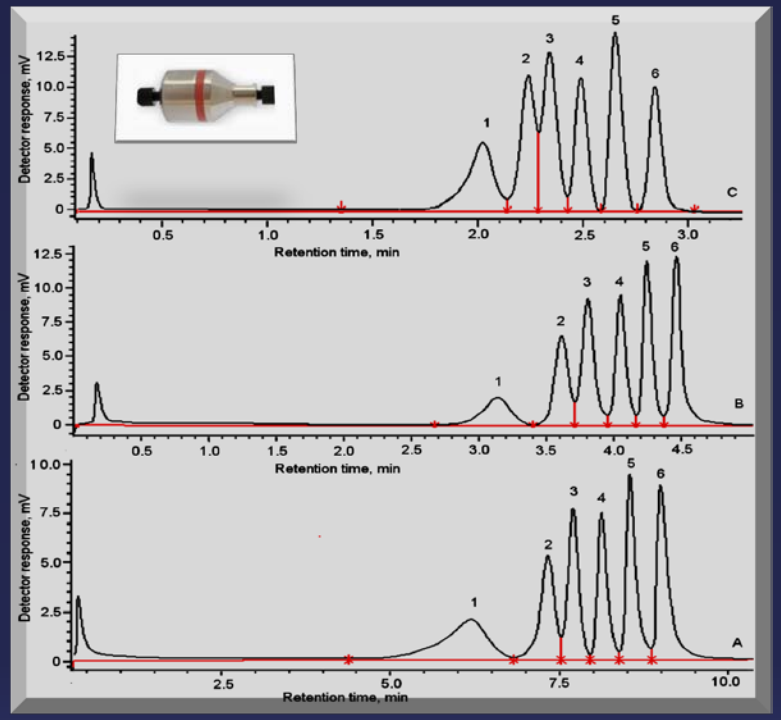


- STYRENE – DIVINYL BENZENE (ST-DVB)
- LAURYL METHACRYLATE – ETHYLENE DIMETHACRYLATE (LMA-EDMA)
- BUTYL METHACRYLATE – ETHYLENE DIMETHACRYLATE (BMA-EDMA)
- GLYCIDYL METHACRYLATE – ETHYLENE DIMETHACRYLATE (GMA-EDMA)

MEAN PORE SIZE ≈ 1.5-2.0 μM; SURFACE AREA ≈ 40-50 M^2/G; PERMEABILITY (*B*) ≈ 10^{-14}-10^{-15} M^2; POROSITY ≈ 0.5-0.6

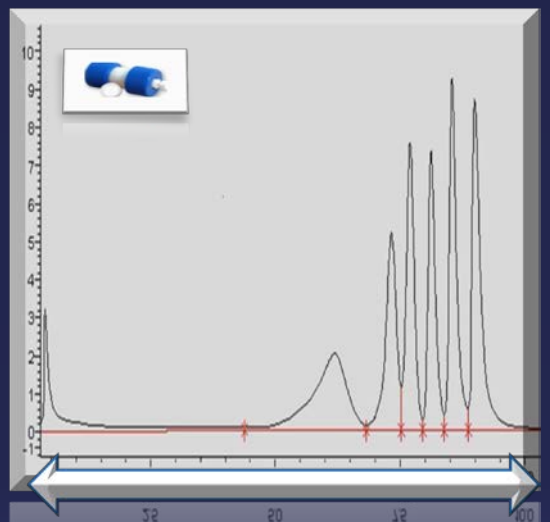
PRECIPITATION-ELUTION MODE

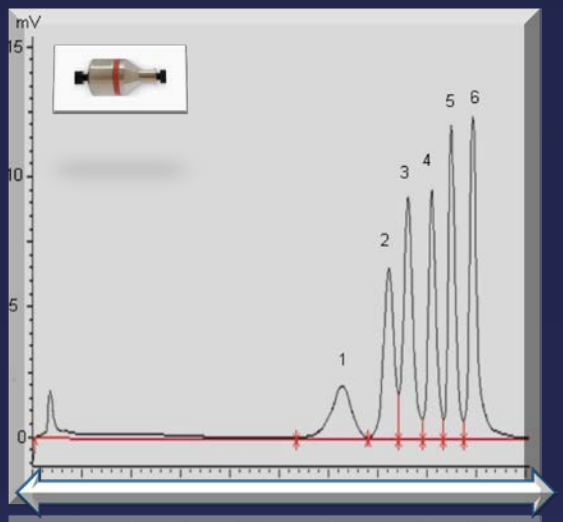


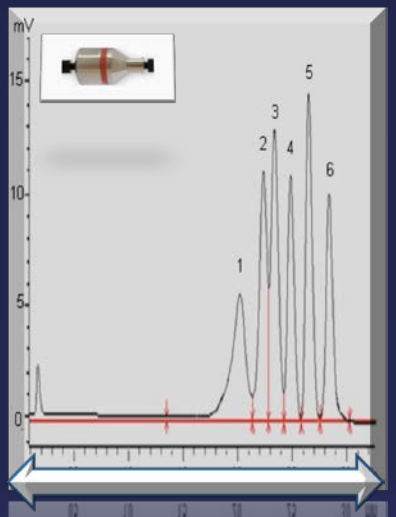


PS STANDARDS (M_w): *1* – 3 600, *2* – 30 000, *3* – 44 000, *4* – 110 000, *5* – 200 000, *6* – 2 000 000; LINEAR (RUN B,C) AND STEPWISE (RUN A) GRADIENTS ACN-THF; ST-DVB

DIFFERENT MONOLITH SIZE – CONSTANT GRADIENT VOLUME







PS STANDARDS; LINEAR (RUNS 1, 2) AND STEPWISE (RUN 3) GRADIENTS ACN-THF;

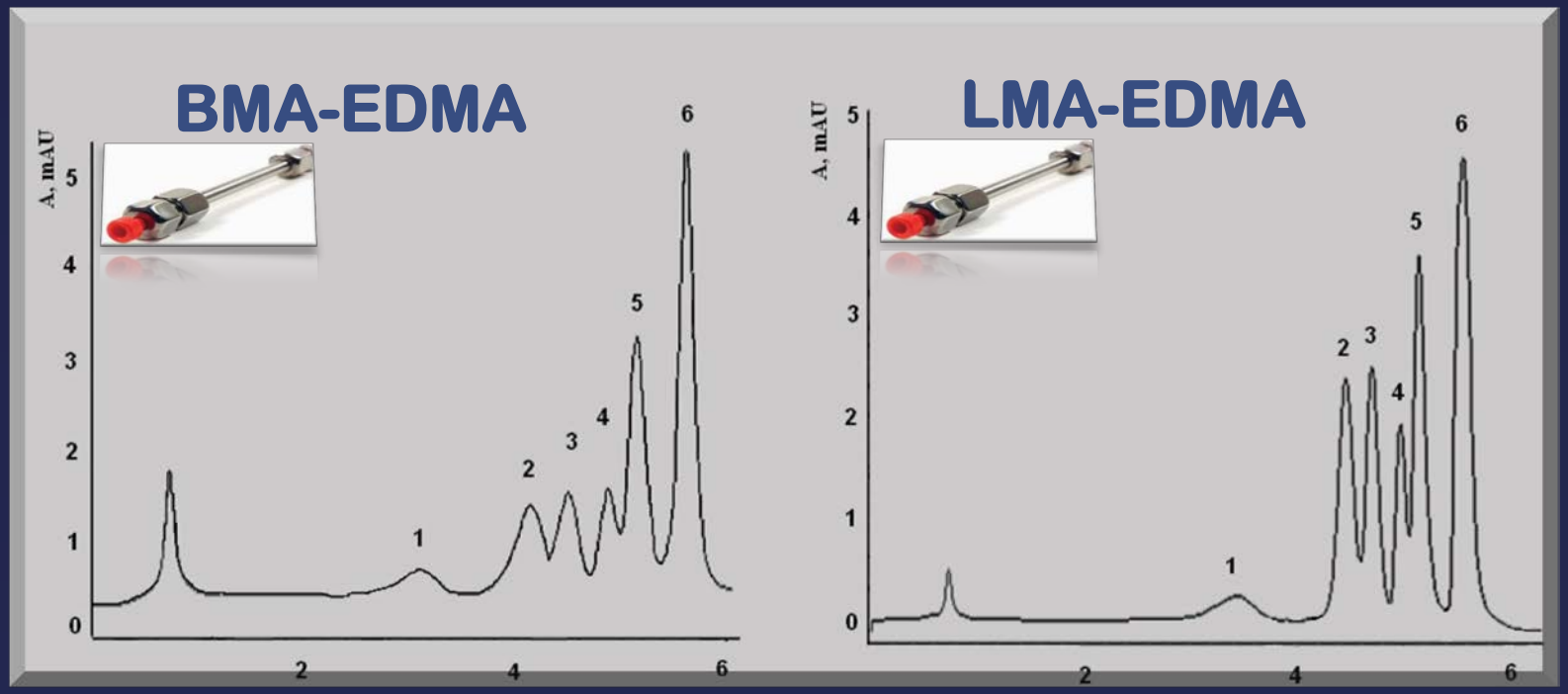
$V_G = Ft_G = CONST$

COLUMN LENGTH & RESOLUTION

Column Length (mm)	t_R (mL)		Peak Width (mL)		Rs
	44 000	200 000	44 000	200 000	
3	6.30	7.02	0.32	0.26	2.48
6	6.54	7.21	0.29	0.25	2.43
9	6.83	7.48	0.32	0.27	2.33

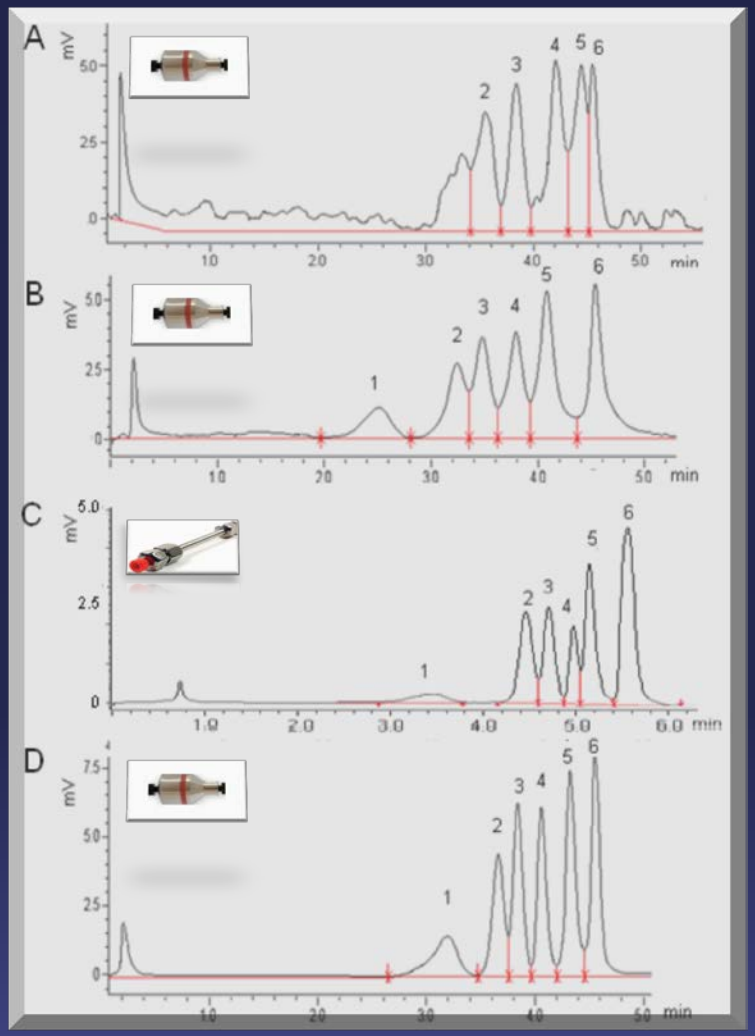
Column	t_R (mL)		Peak Width (mL)		Rs
	40 000	200 000	40 000	200 000	
C4 (Butyl) (L = 5 mm)	3.38	3.95	0.33	0.23	2.02
BMA-EDMA (L= 50 mm)	4.49	5.13	0.39	0.27	1.96

SURFACE CHEMISTRY



LINEAR GRADIENT FROM 100% ACN TO 100% THF. GRADIENT TIME 7.1 MIN, FLOW RATE 0.85 ML/MIN. PS STANDARDS (M_W): *1* – 3 600, *2* – 30 000, *3* – 44 000, *4* – 110 000, *5* – 200 000, *6* – 2 000 000

SURFACE CHEMISTRY



GMA-EDMA

BMA-EDMA

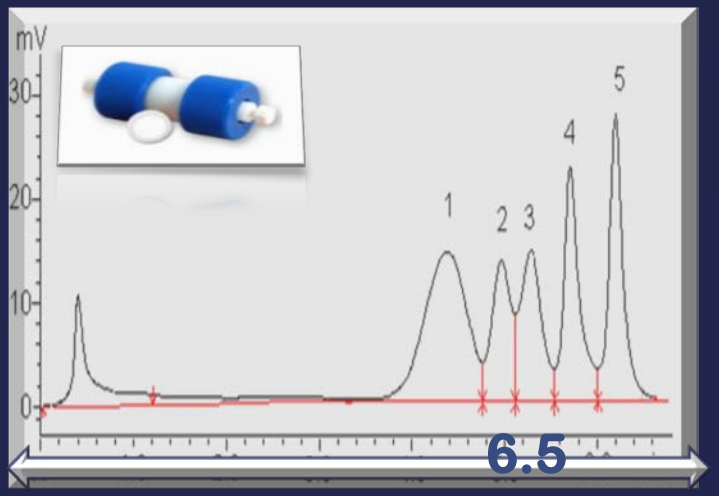
LMA-EDMA

ST-DVB

V_G = CONST

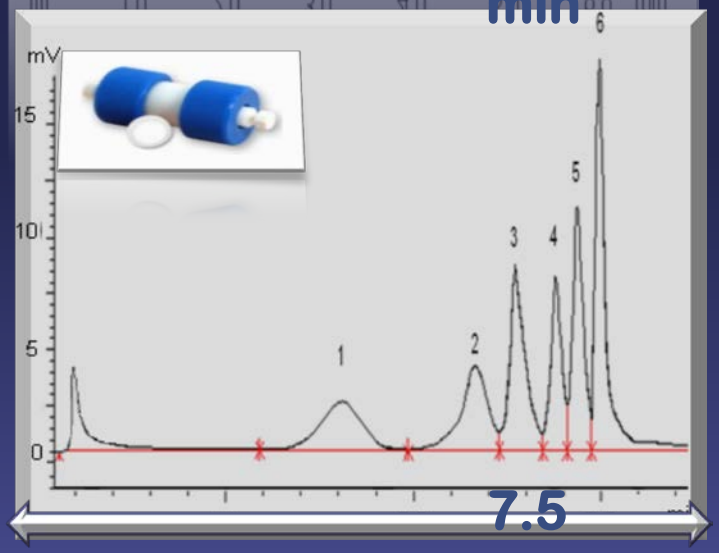
GMA-EDMA < BMA-EDMA < LMA-EDMA < ST-DVB

SAMPLE CHEMISTRY



PMMA STANDARDS: *1* – 27 000, *2* – 60 000, *3* – 107 000, *4* – 400 000, *5* – 1 500 000

GRADIENT: 100% MeOH – 100% THF

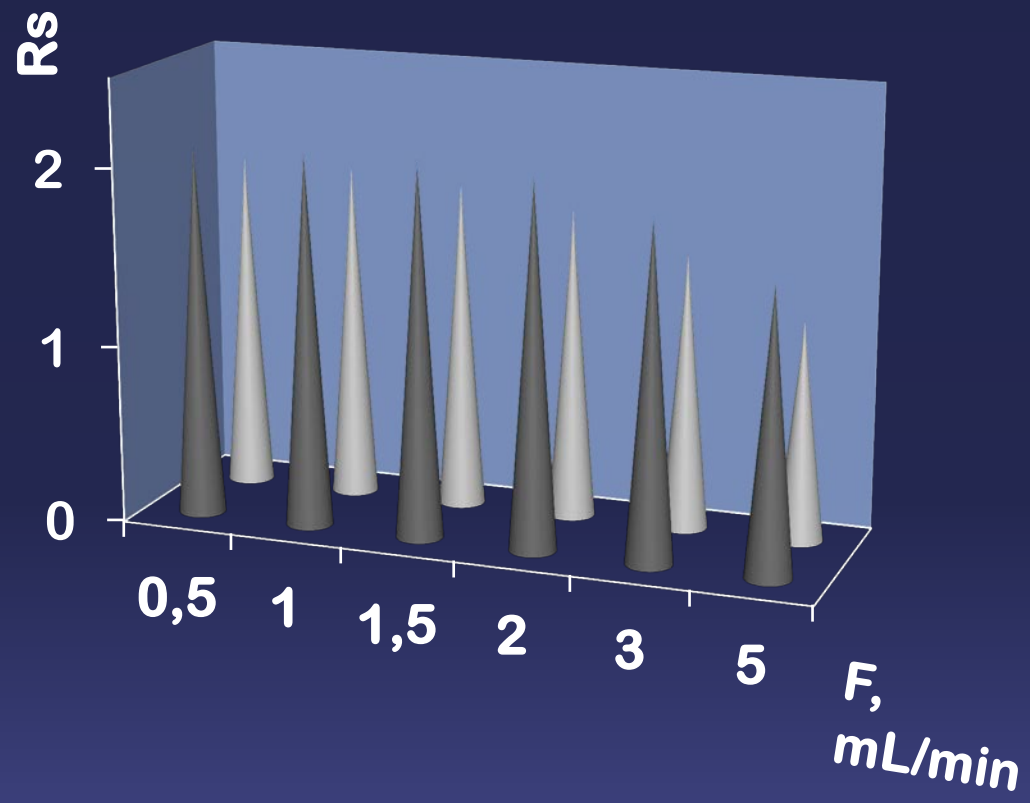


PS STANDARDS: *1* – 3 600, *2* – 30 000, *3* – 44 000, *4* – 110 000, *5* – 200 000, *6* – 2 000 000

GRADIENT: 100% ACN – 100% THF

ST-DVB **V_G = CONST**

EFFECT OF FLOW RATE ON R_S



PS

44 000 : 200 000

PMMA

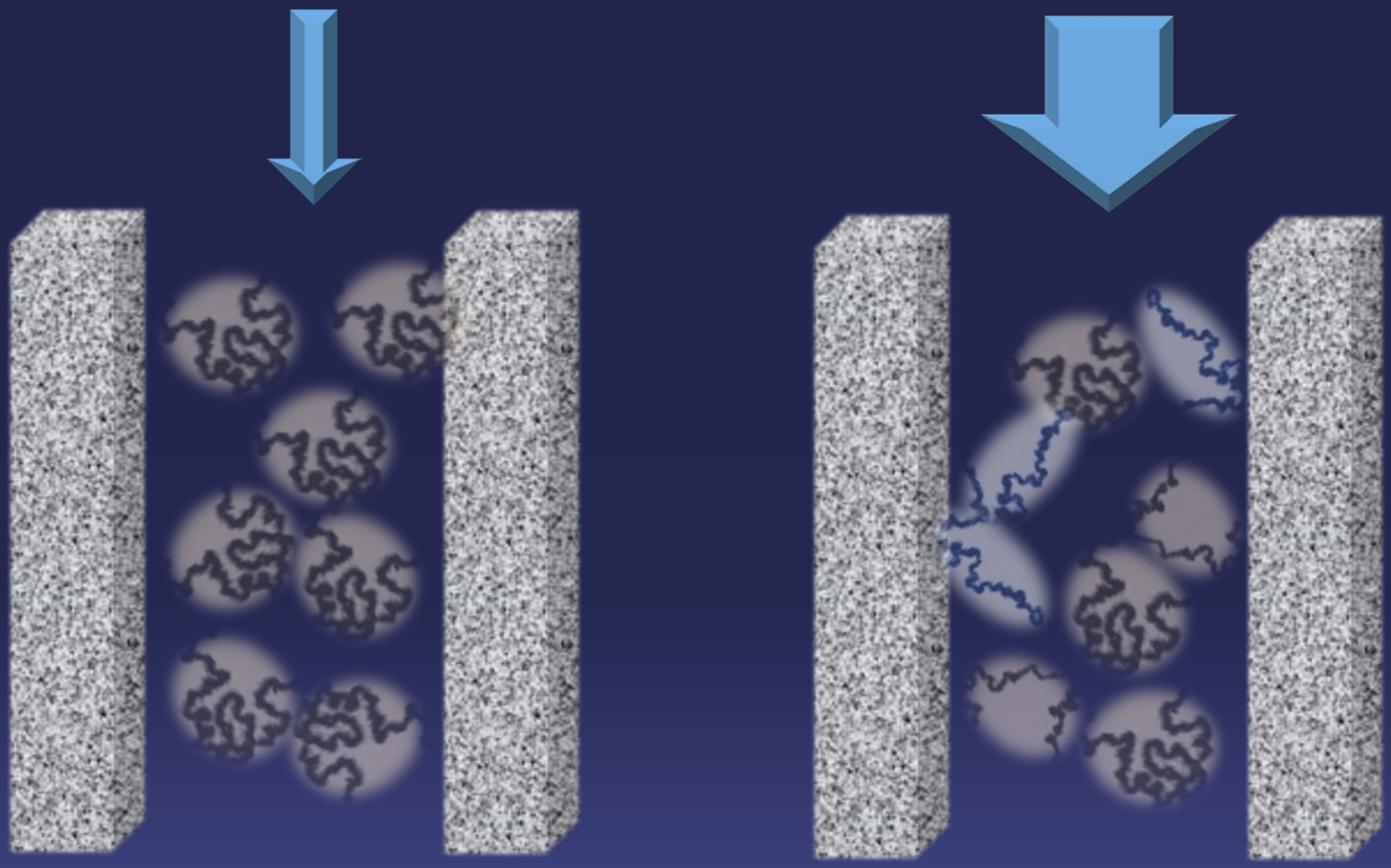
60 000 : 400 000

EFFECT OF FLOW RATE ON R_S

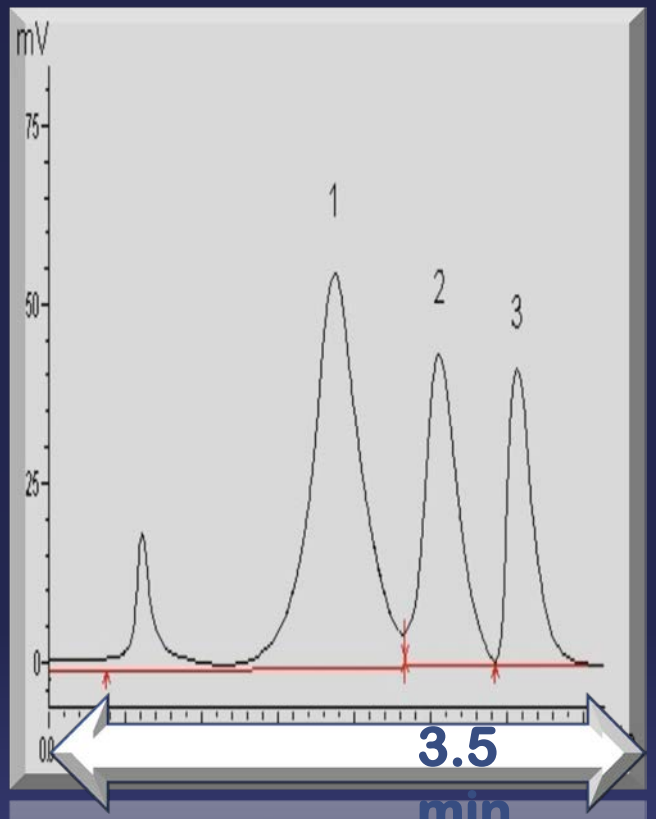
F, mL/min	t_G, min	t_R, min		$W_{1/2}$		R_s
		44 000	200 000	44 000	200 000	
0.5	24	12.5	14.1	0.37	0.27	2.48
1.0	12	6.4	7.2	0.19	0.13	2.52
1.5	8	4.4	5.0	0.20	0.16	2.84
2.0	6	3.4	3.8	0.21	0.17	2.49
3.0	4	2.4	2.7	0.29	0.22	1.90
5.0	2.4	1.6	1.7	0.40	0.33	1.20

$$R_s = \frac{t_R(2) - t_R(1)}{\sigma_{\frac{1}{2}}(1) + \sigma_{\frac{1}{2}}(2)}$$

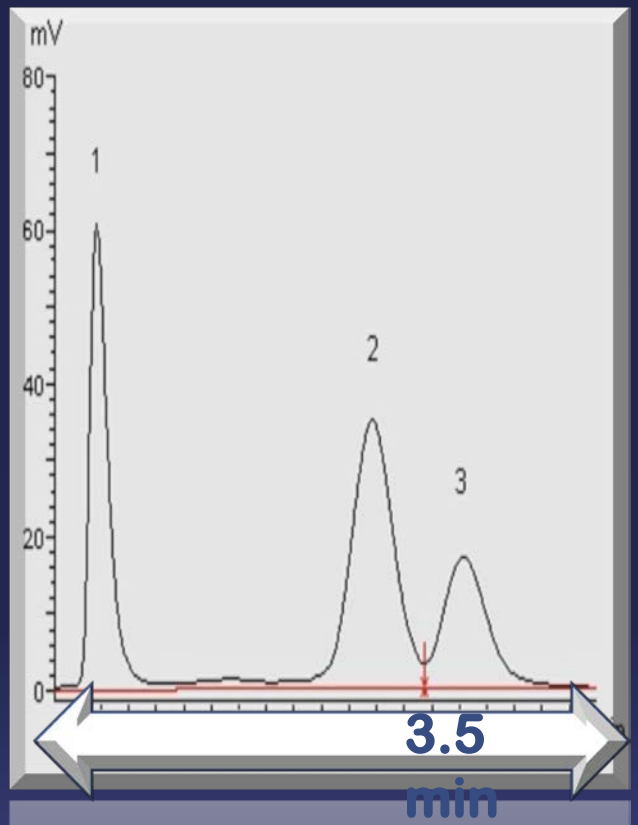
EFFECT OF FLOW RATE ON $W_{0.5}$



PRACTICAL EXAMPLES

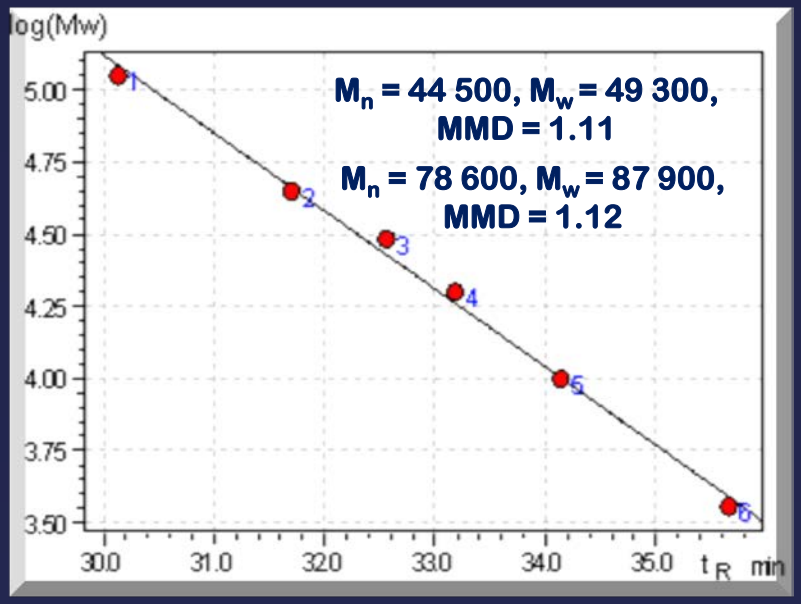


POLY-tret-BUTYL METHACRYLATES:
MM: *1* – 7 500, *2* - 14 400, *3* - 56 700

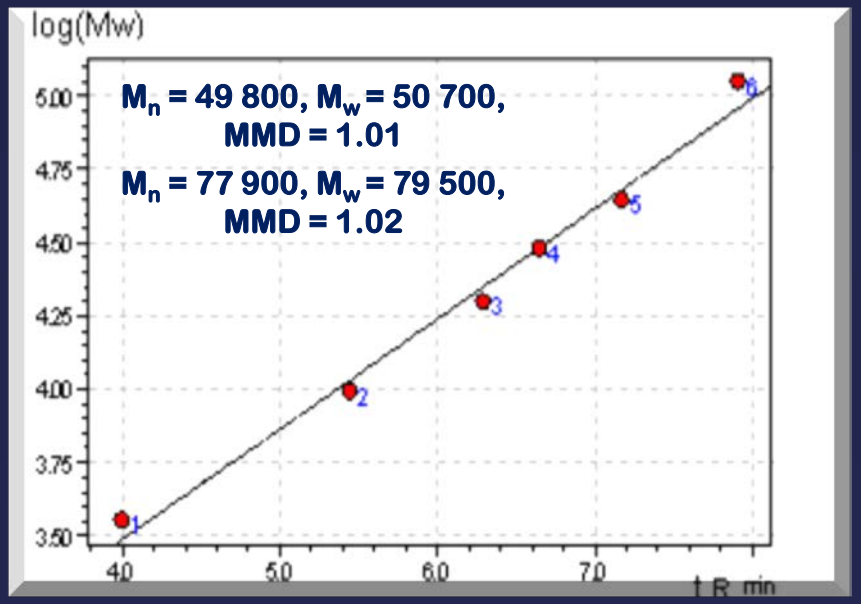


MIXTURE: *1* – POLY-2-VINYL PIRIDYNE, *2* – BLOCK COPOLYMER VP-ST, *3* - PS

SEC *vs* "ADSORPTION" MODE



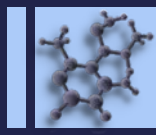
GPC:
St-DVB column Waters Styragel HMW 6E, 4.6x300 mm, THF, flow rate 0.3 mL/min



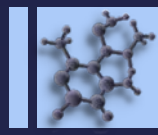
Monolith:
St-DVB Disk, 12x3 mm, gradent elution from 100% ACN to 100% THF in 12 min, flow rate 1 mL/min

PS standards of M_W 3 550, 9 800, 19 750, 30 000, 44 000, 111 000

CONCLUSIONS:



- PRECIPITATION-DISSOLUTION MECHANISM CAN BE SUCCESSFULLY REALIZED FOR FAST POLYMER SEPARATION ON MONOLITHS
- SURFACE CHEMISTRY PLAYS AN IMPORTANT ROLE FOR SEPARATION EFFICIENCY
- THE OPTIMUM OF ELUTION FLOW RATE NEEDS TO BE FOUND TO REACH MAXIMUM SEPARATION EFFICIENCY
- THE METHOD DISCUSSED CAN BE COUNTED AS FAST AND ROBUST TOOL FOR EXPRESS POLYMER CHARACTERIZATION



FINANCIAL SUPPORT:

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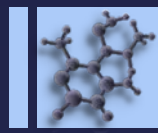
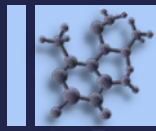
- RUSSIAN FEDERAL PROGRAM “RESEARCH AND SCIENTIFIC-PEDAGOGICAL PERSONNEL OF INNOVATIVE RUSSIA IN 2009-2013” (CONTRACT #14.740.11.0382)



ACNOWLEDGEMENTS:

PROFS. LYUDMILA VINOGRADOVA, GEORGE PAVLOV AND VALERY KRASIKOV (ALL FROM IMC RAS) FOR POLYMER SAMPLES, KIND INTEREST AND FRUITFUL DISCUSSIONS



THANK YOU VERY MUCH!