Demonstrating Equivalence of
Generic Complex Drug Substances and Formulations:
Advances in Characterization and In Vitro Testing
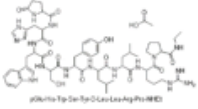
Characterizations of PLGA Polymers

Kinam Park

PLGA-based Long-Acting Depot Formulations (1 week ~ 6 months)

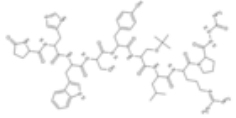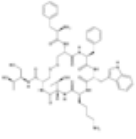## Poly(lactide-co-glycolide) (PLGA)

Lupron Depot®
leuprolide acetate for depot suspension
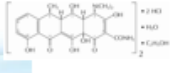1-4 months MP 1989
7.5 mg/month

Zoladex® 3-MONTH 10.8 mg DEPOT
GOSERELIN ACETATE IMPLANT
1, 3 months SI 1989
3.6 mg/month

Sandostatin LAR® Depot
(octreotide acetate for injectable suspension)
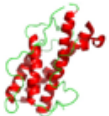1 month MP 1998
20 mg/month

ATRIDOX®
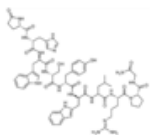(doxycycline hyclate) 10%
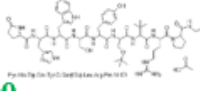Cost Effective
1 week, IS 1998
50 mg/week

Nutropin DEPOT®
[somatropin (rDNA origin) for injectable suspension]
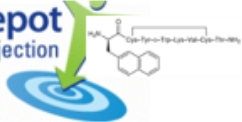1 month MP 1999
13.5 mg/month

TRELSTAR®
(triptorelin pamoate for injectable suspension)
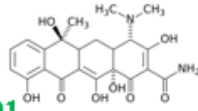1 month MP 2000
3.75 mg/month

Suprefact® Depot / Implantat/Implant/ti
Buserelin acetate
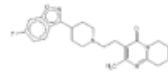2, 3 months SI 2000
6.3 mg/2 months

Somatuline® Depot
(lanreotide) Injection
1 month MP 2000
60 mg/month

Arestin®
minocycline HCl 1mg
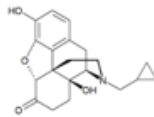MICROSPHERES
2 weeks MP 2001
1 mg/2 weeks

Risperdal CONSTA®
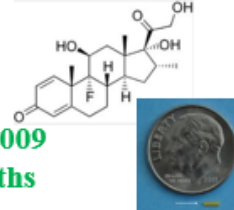risperidone Long-Acting Injection
2 weeks MP 2003
25 mg/2 weeks

Eligard®
(leuprolide acetate for injectable suspension)
1,3,4,6 months IS 2004
7.5 mg/month

Vivitrol®
(naltrexone for extended-release injectable suspension)
1 month MP 2006
380 mg/month

Ozurdex®
(dexamethasone intravitreal implant) 0.7 mg
3 months SI 2009
0.7 mg/3 months

TRELSTAR®
(triptorelin pamoate for injectable suspension)
6 months MP 2010
3.75 mg/month

Once-weekly
BYDUREON®
exenatide extended-release for injectable suspension
His–Gly–Glu–Gly–Thr–Phe–Thr–Ser–Asp–Leu–Ser–Lys–Gln–Met–Glu–Glu–Glu–Ala–Val–Arg–Leu–Phe–Ile–Glu–Trp–Leu–Lys–Asn–Gly–Gly–Pro–Ser–Ser–Gly–Ala–Pro–Pro–Pro–Ser–NH$_2$
1 week MP 2012
2 mg/week

Lupaneta Pack™
leuprolide acetate for depot suspension, 11.25 mg for intramuscular injection and norethindrone acetate tablets, 5 mg for oral administration
3 month, MP 2012
3.75 mg/month

Signifor® LAR
(pasireotide) for injectable suspension
1 month, MP 2014
20, 40, or 60 mg/month

Product Similarity

Q1: Same components

Q2: Same components in same concentration

Q3: Same components in same concentration with same arrangement of matter (microstructure)

Poly(lactide-co-glycolide) (PLGA)

Structure
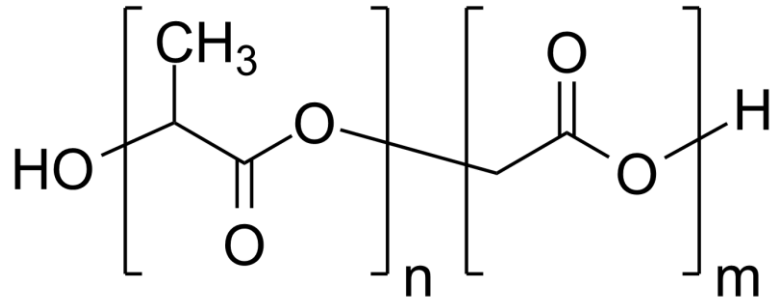
Lactide   **Glycolide**

L:G Ratio

| L | 100 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 | 0 | L |
| G | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | G |

Solvent-dependent solubility

Insoluble in most solvents

Solvent Solubility

Molecular Weight: GPC
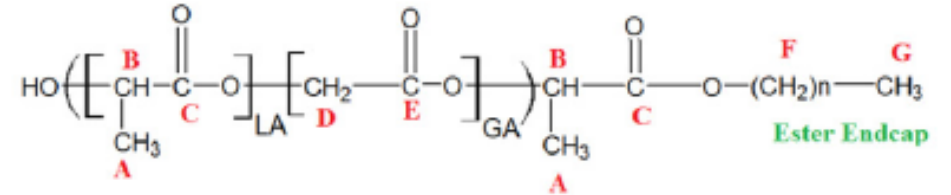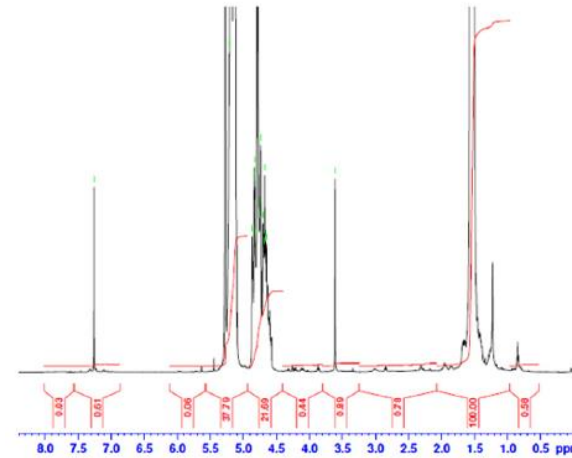
L:G Ratio: H-NMR   End Group: C-NMR
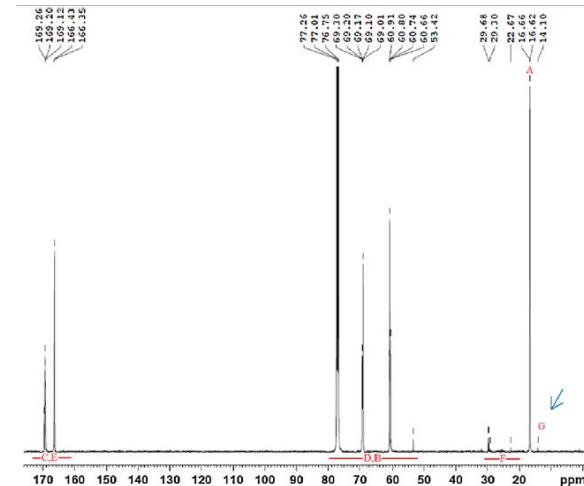
A protocol for assay of poly(lactide-co-glycolide) in clinical products.
J. Garner, S. Skidmore, H. Park, K. Park. S. Choi, & Y. Wang
International Journal of Pharmaceutics 495 (2015) 87–92

Characterization of Poly(lactide-co-glycolide) (PLGA)

Shape

Dynamic Light Scattering
V_h: Hydrodynamic volume

Intrinsic Viscosity
Mark-Houwink Equation
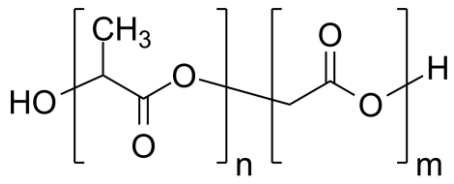
Multi-Angle Light Scattering
M: Molecular weight

GPC
Mol. Wt. Standards
Polystyrene dissolved
in DCM or THF

Mixture
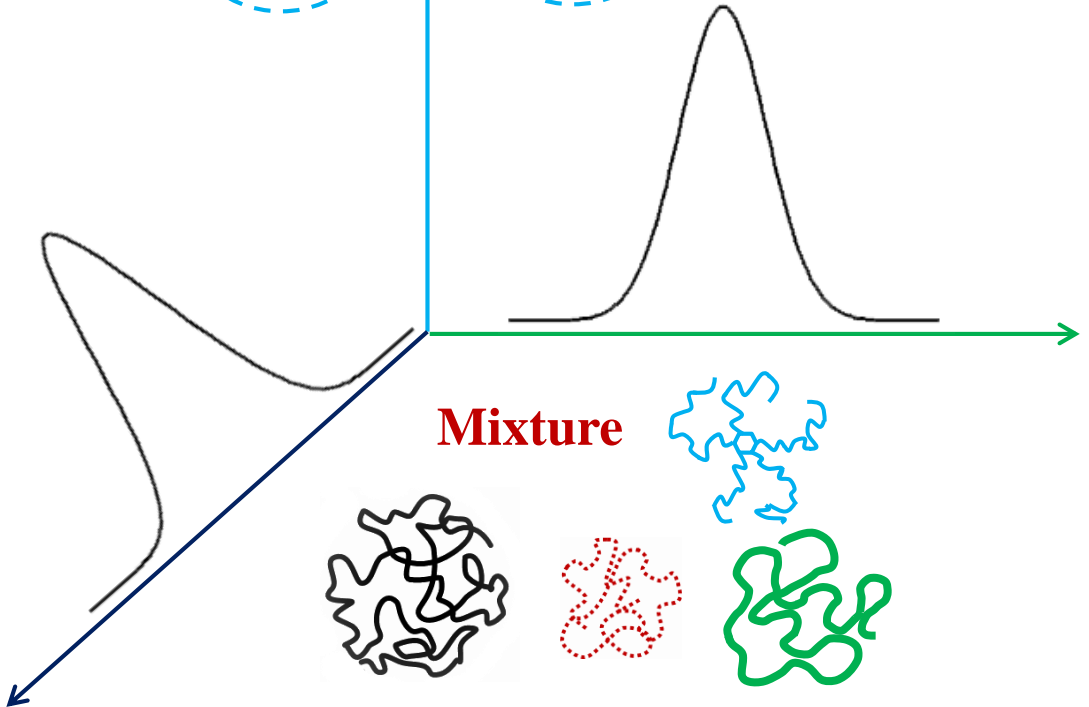
L:G (Lactodispersity)

Endcap

Hansen Solublity Parameters
Solvent solubility

Molecular Weight

Trelstar® (Triptorelin pamoate Injectable Suspension)

The TRELSTAR products are sterile, lyophilized biodegradable microgranule formulations supplied as single dose vials. Refer to Table 5 for the composition of each TRELSTAR product.

Table 5.   TRELSTAR Composition

	1 Month	3 Months	6 Months
Ingredients	TRELSTAR 3.75 mg	TRELSTAR 11.25 mg	TRELSTAR 22.5 mg
triptorelin pamoate (base units)	3.75 mg 2.7%	11.25 mg 8.7%	22.5 mg 11.0%
poly-*d,l*-lactide-co-glycolide	136 mg	118 mg	182 mg
mannitol, USP	69 mg	76 mg	68 mg
carboxymethylcellulose sodium, USP	24 mg	27 mg	24 mg
polysorbate 80, NF	1.6 mg	1.8 mg	1.6 mg

Molecular Weight: GPC. (Polystyrene standards)

L:G Ratio: H-NMR

End Group: C-NMR

PLGA Analysis of Trelstar® Formulations

Sample	M_n (Da)	M_w (Da)	L:G (mol:mol)	Endcap
Trelstar® 3.75 mg	25,192	85,207	52:48	Ester
Trelstar® 11.25 mg	47,214	72,286	74:26	Acid
Trelstar® 22.50 mg	46,368	74,042	77:23	N/A
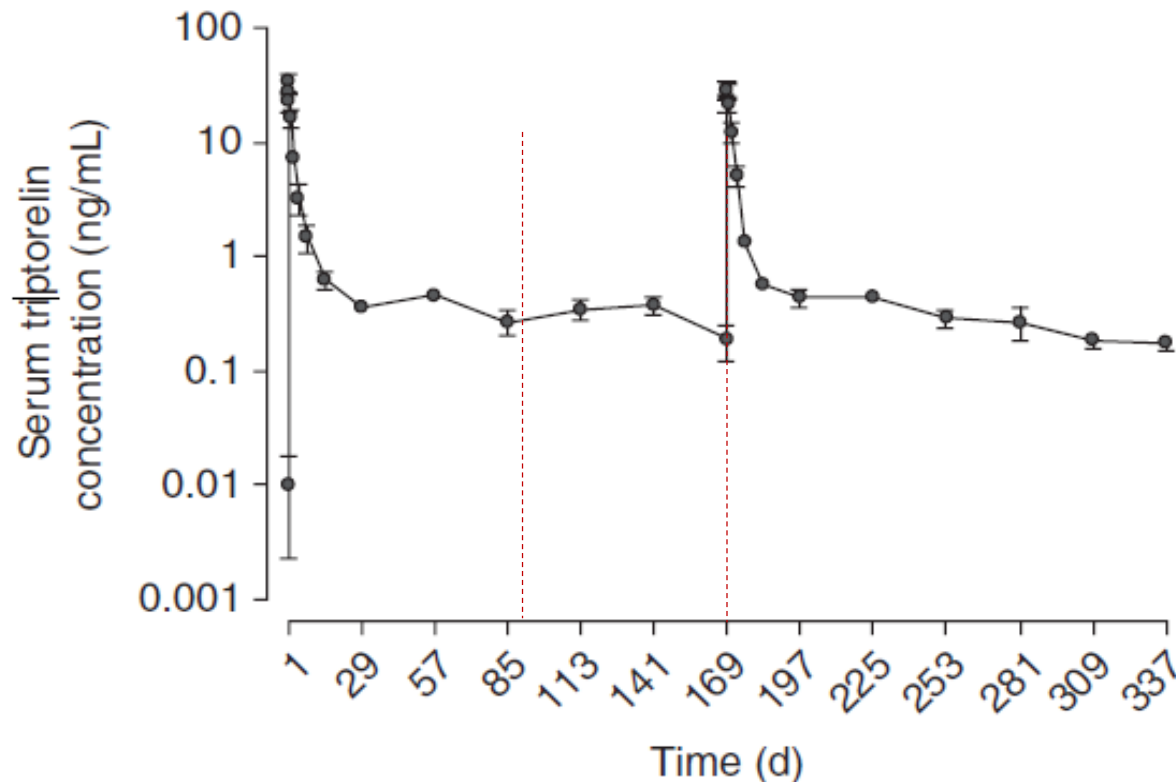
The same PLGA cannot be used for both 3-month & 6-month formulations.

PLGA degradation kinetics

Mixture of different PLGAs?

50:50, 65:35, 75:25, 85:15, 100:0

Analysis of PLGA Blends

PLGA Blend

Individual PLGAs with different L:G Ratios

Separation by GPEC

+

Analysis

Q1/Q2 Assay
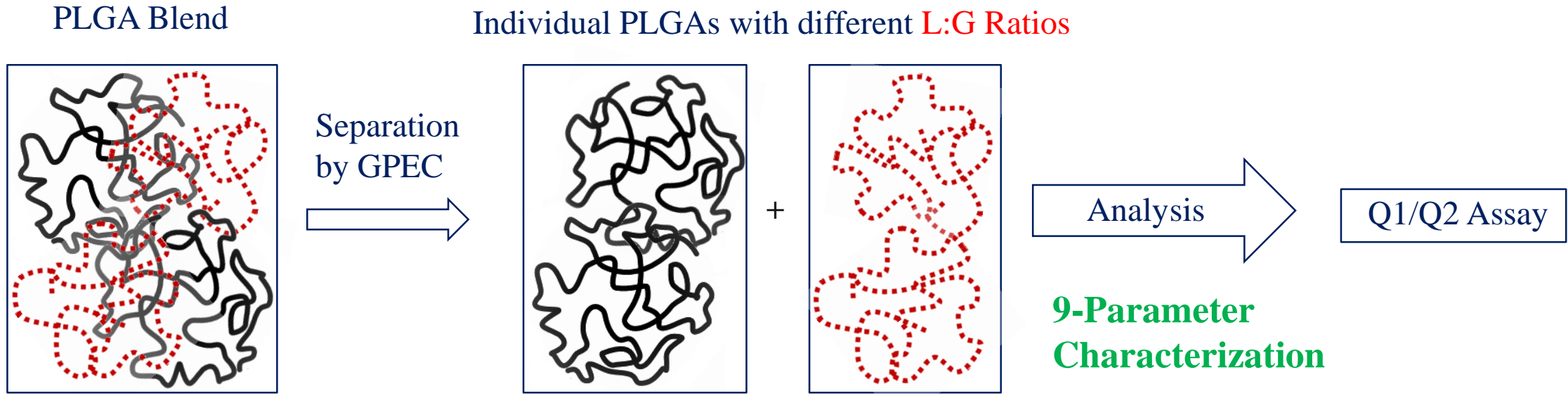
9-Parameter Characterization

M_w
M_n
PDI
L:G ratio
Endcap
Hansen solubility parameters
δ_d
δ_p
δ_h
δ_t

Parameter		Property
M$_w$		Weight average molecular weight
M$_n$		Number average molecular weight
PDI		Polydispersity index (PDI) of linear PLGAs
L:G ratio		Ratio of lactide:glycolide in the polymer
Endcap		Acid or ester at the end of a PLGA chain
Hansen	δ_d	The dispersive component
Solubility	δ_p	The polar component
Parameters	δ_h	The hydrogen bonding component
	δ_T	Hildebrand total solubility parameter

Separation of PLGA polymers by L:G Ratio

Gradient Polymer Elution Chromatography
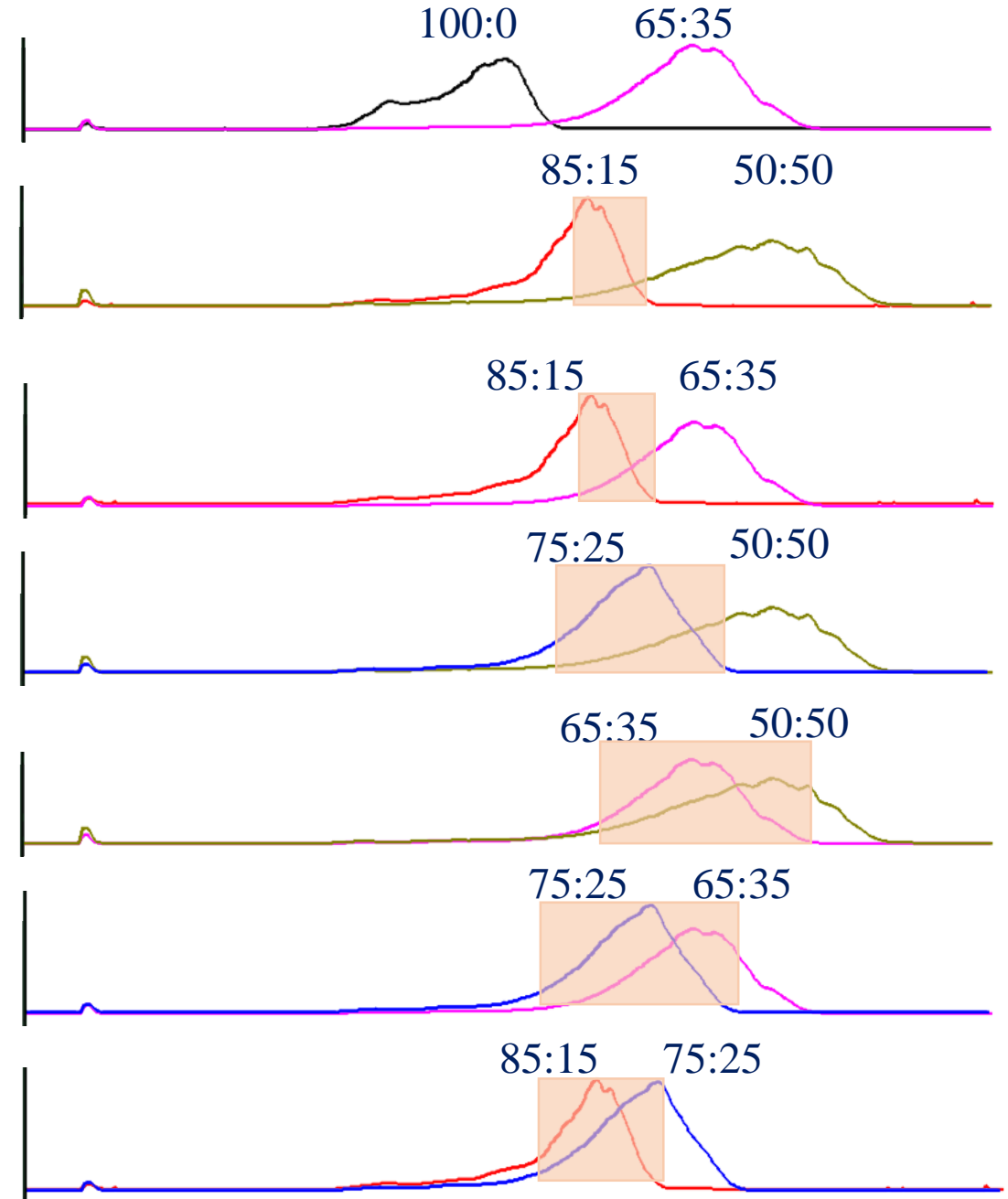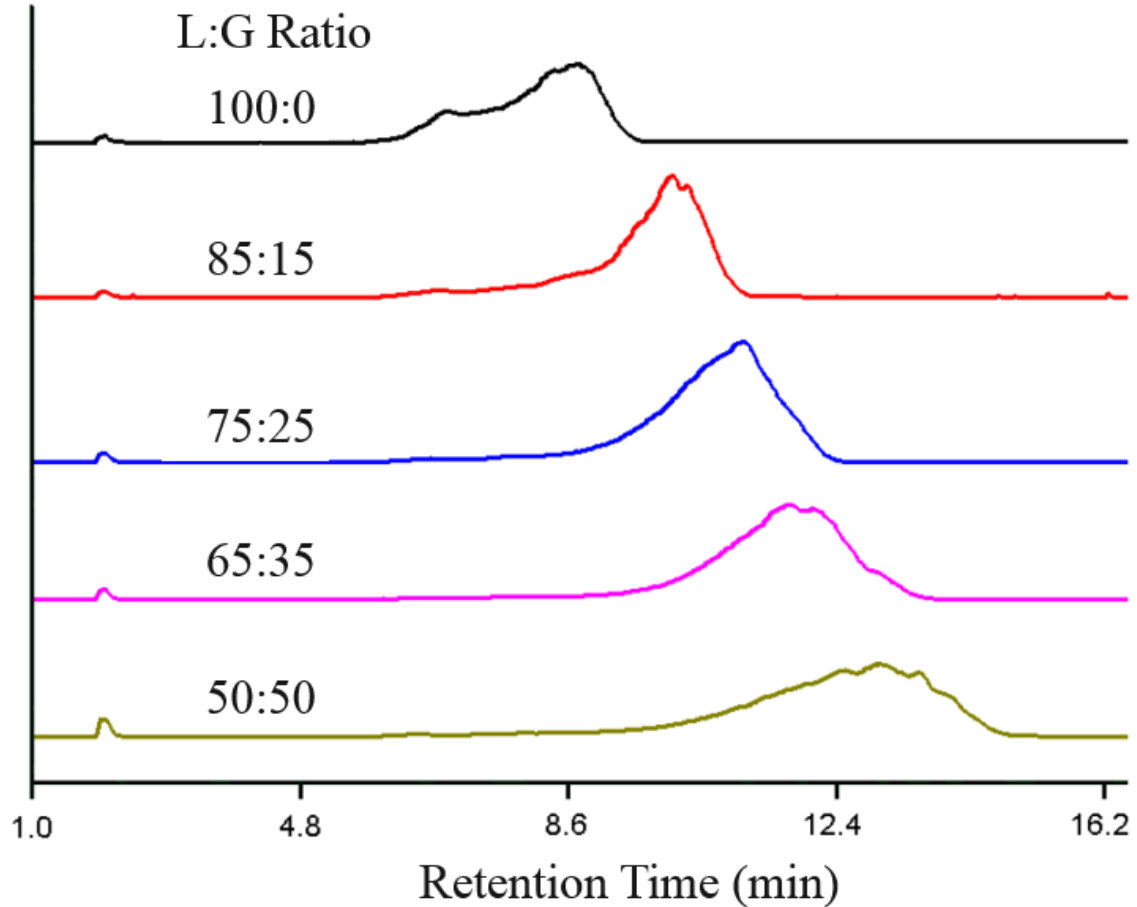
A 2-min hold in 99% methanol. Linear gradient of 1-99% THF in 10 min.

G.D. Saunders, J.M. Watkins, E. Meehan, L.L. Loberger. Characterisation of telechelic and copolymers by liquid chromatographic techniques. http://www.agilent.com/cs/library/

Solvent Solubility

At 20 °C

$\delta_p > 10$

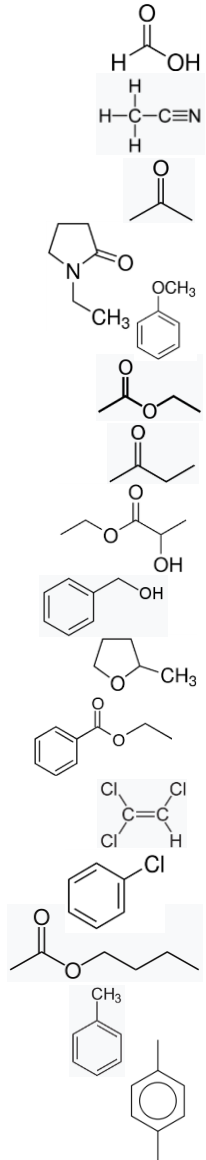| Solvent	L:G Ratio of PLGA			Hansen Solubility Parameters						
	88:12	75:25	50:50	δ_d	δ_p	δ_h	δ_t	f_d	f_p	f_h
Formic Acid				14.6	10.0	14.0	22.6	0.38	0.26	0.36
Acetonitrile				15.3	18.0	6.1	24.4	0.39	0.46	0.15
Acetone				15.5	10.4	7.0	19.6	0.47	0.32	0.21
1-Ethyl-2-Pyrrolidinone				18.0	12.0	7.0	22.7	0.49		
Anisole				17.8	4.4	6.9	19.6	0.61		
Ethyl Acetate				15.8	5.3	7.2	18.2	0.56	0.19	0.25
Methyl Ethyl Ketone				16.0	9.0	5.1	19.1	0.53	0.30	0.17
Ethyl-L-lactate				16.0	7.6	12.5	21.7	0.44		
Benzyl Alcohol				18.4	6.3	13.7	23.8	0.48	0.16	0.36
2-Methyl Tetrahydrofuran				16.9	5.0	4.3	18.1	0.65		
Ethyl Benzoate				17.9	6.2	6.0	19.9	0.59	0.21	0.20
Trichloroethylene				18.0	3.1	5.3	19.0	0.68	0.12	0.20
Chlorobenzene				19.0	4.3	2.0	21.0	0.75	0.17	0.08
n-Butyl Acetate				15.8	3.7	6.3	17.4	0.61	0.14	0.24
Toluene				18.0	1.4	2.0	18.2	0.84	0.07	0.09
p-Xylene				17.8	1.0	3.1	17.4	0.81	0.05	0.14

Hansen's Interaction Spheres (Solubility Parameter Plots)
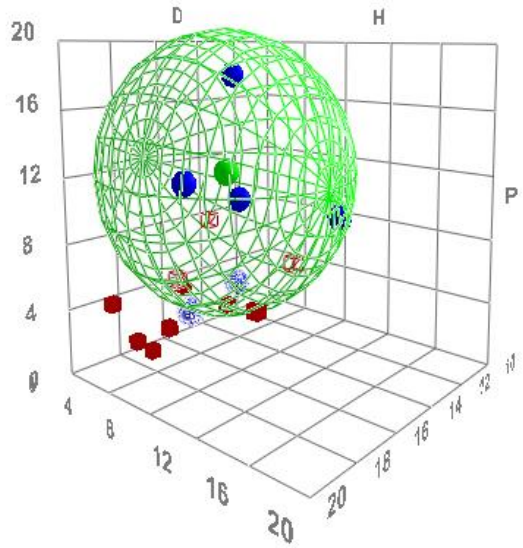
50:50
(n=17)
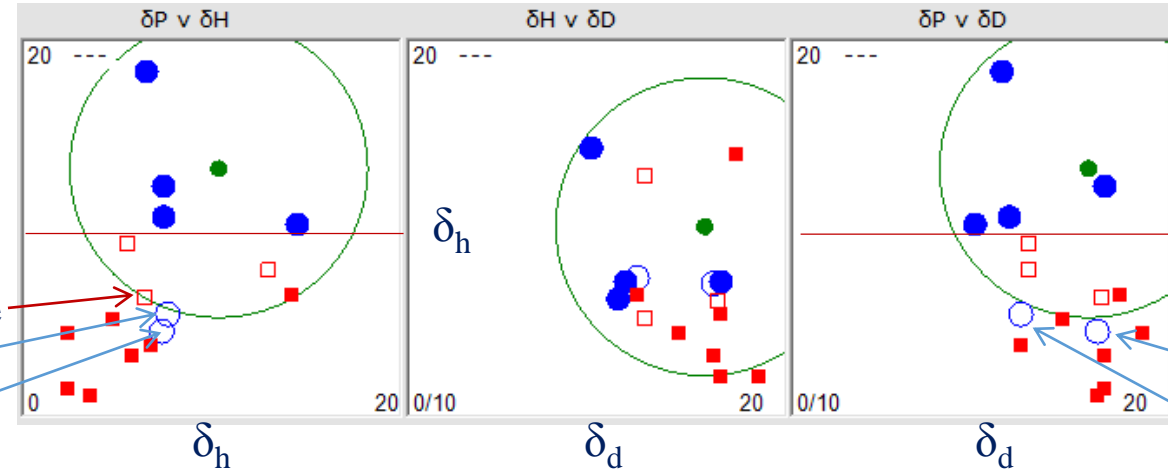
50:50
(n=44)
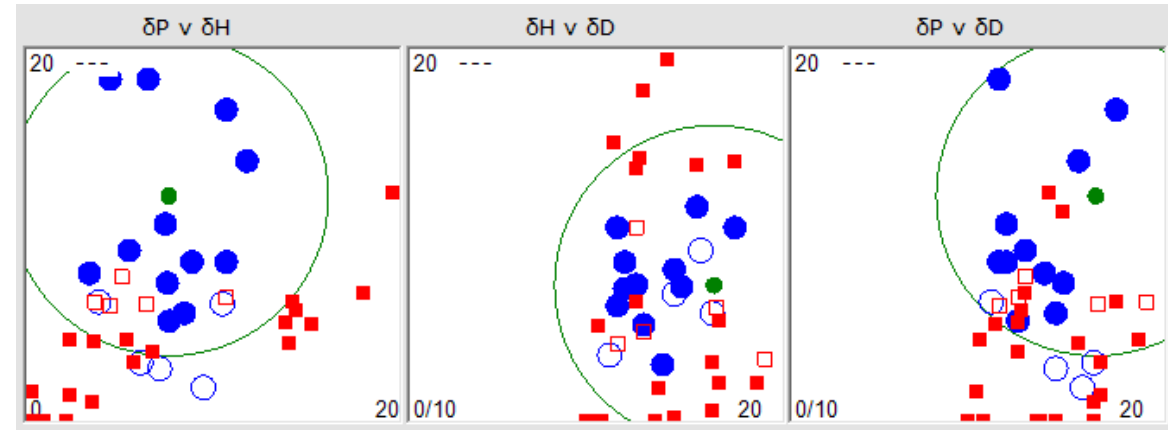
If we use a small number of solvents, we may see a certain trend in PLGA solubility. But as the number of solvents increases, many solvents are wrong in and/or wrong out of the Hansen's Interaction Spheres. Since the goal of this study is to separate PLGA of different L:G ratios, we can choose only the solvents relevant to differential dissolution of PLGAs. Thus, the solvents that dissolve, or does not dissolve, all types of PLGAs regardless of L:G ratios need to be excluded from the consideration. This leaves only the solvents that are able to dissolve PLGAs based on L:G ratios.

Hansen's Interaction Spheres (Solubility Parameter Plots)

50:50

In= 6 Out= 26 Total= 32
D= 19.03 P=14.23 H=9.16
Tot = 25.46.   R= 8.9. Fit= 1.000
Core= ±[0.30, 0.30, 1.15]
Wrong In= 0
Wrong Out= 0
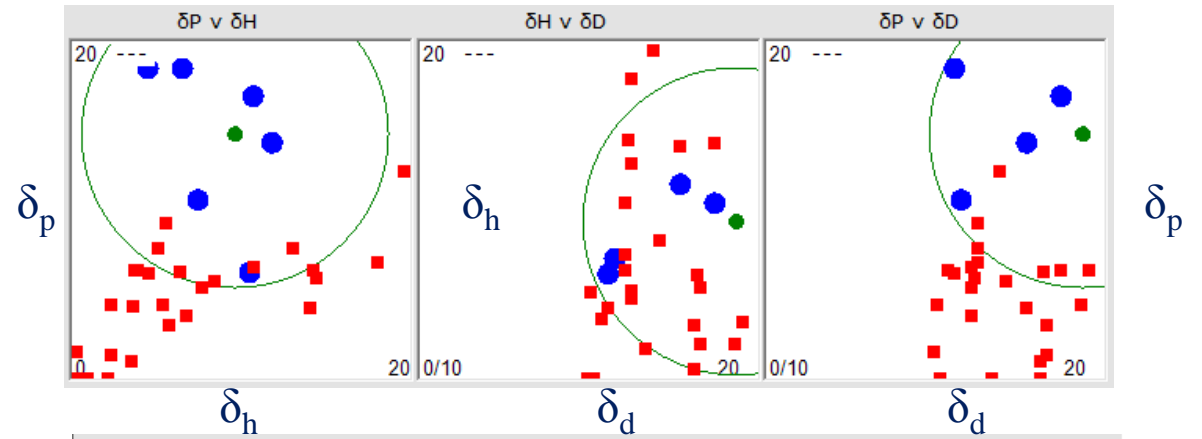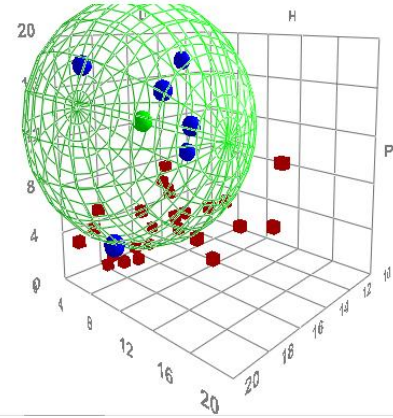
75:25

In= 17 Out= 14 Total= 31
D= 18.51 P=12.43 H=6.60
Tot = 23.25   R= 9.4 Fit= 0.945
Core= ±[0.15, 0.20, 0.40]
Wrong In= 2
   Butyl Lactate, Methyl Isobutyl Ketone (MIBK)
Wrong Out= 4
   Benzyl Alcohol, Chlorobenzene
   Diethyl Carbonate, Trichloroethylene
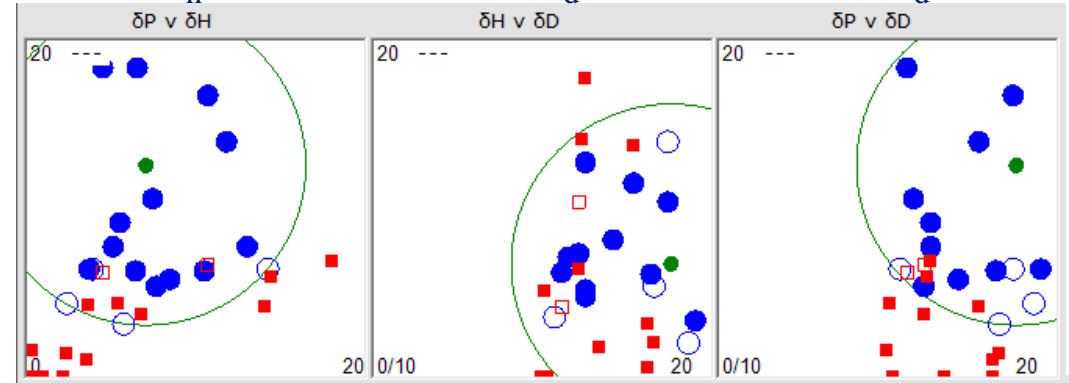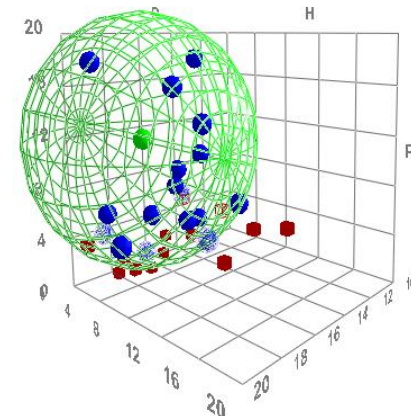
85:15

In= 21 Out= 10 Total= 31
D= 17.64 P=10.71 H=6.18
Tot = 21.54.   R= 8.8. Fit= 0.906
Core= ±[0.15, 0.20, 0.40]
Wrong In= 1
   Methyl-t-Butyl Ether
Wrong Out= 4
   Benzyl Alcohol, 2,4-Dichloroaniline
   Propylene Carbonate, Toluene
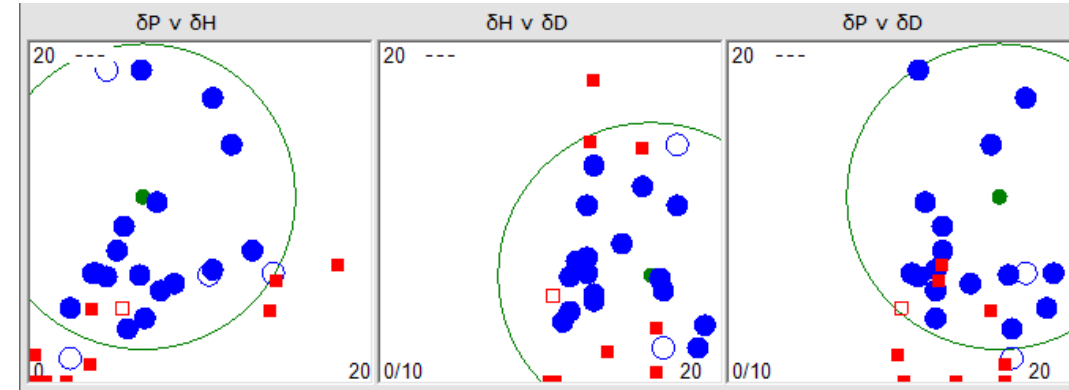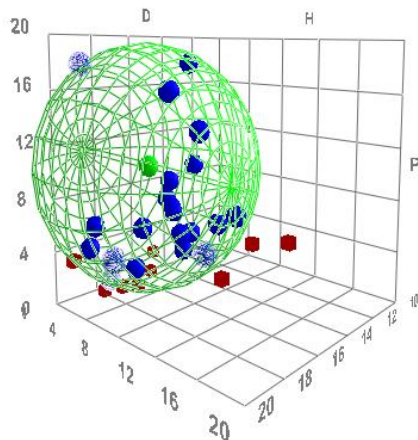
Benchtop Separation Techniques

- **Lactodispersity:** the dispersion of lactide content in a given PLGA batch
- **Lactoselectivity**: the 'cut-off' minimum lactide value for a given PLGA semi-solvent.
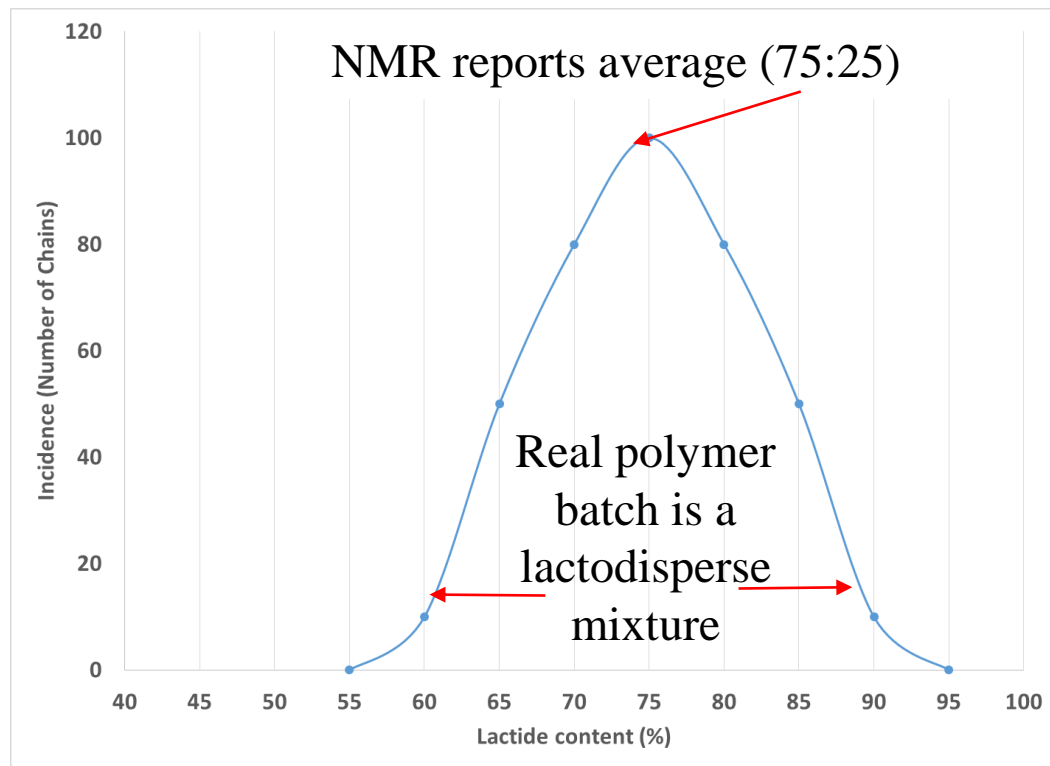
A theoretical example of lactodispersion
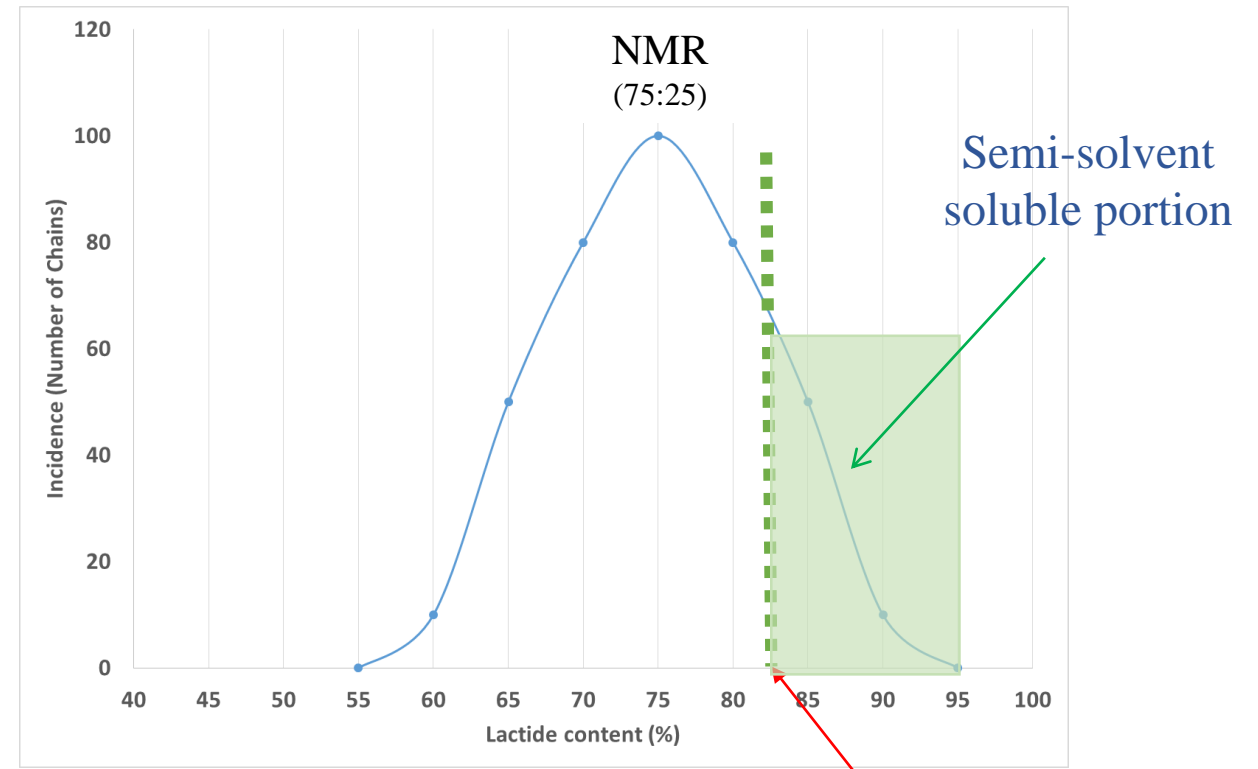
Exact 'cut-off' value depends on solvent

Benchtop Separation Techniques

- **Blockiness** the distribution of L:G in a given PLGA batch

- Glycolide-rich regions tend to self-crystalize making difficult to dissolve domains.

LA	GA	LA	GA	LA	GA

Uniform

LA	GA	GA	LA	LA	GA

Partial Block

GA	GA	GA	LA	LA	LA

Block polymer

Glycolide-rich regions
form crystalline domains

Solvent Solubility of PLGAs with Different L:G Ratios

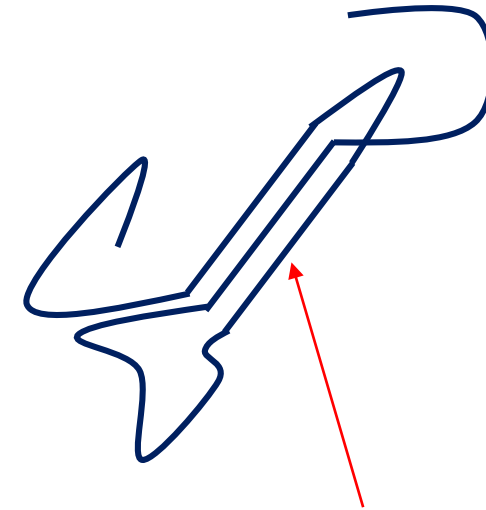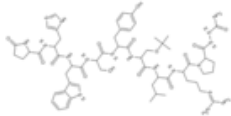| Solvent	PLGA (Mol. Wt. ~800k)										
	L:G Ratio										
	50:50	55:45	60:40	65:35	70:30	75:25	80:20	85:15	90:10	95:5	100:0
Dichloromethane	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Dimethyl sulfoxide	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Dimetyl formamide		■	▒	▒	▒	▒	▒	▒	▒	▒	▒
Ethyl acetate		■	▒	▒	▒	▒	▒	▒	▒	▒	▒
Methyl ethyl ketone			■	▒	▒	▒	▒	▒	▒	▒	▒
Tetrahydrofuran			■	▒	▒	▒	▒	▒	▒	▒	▒
Ethyl benzoate				■	▒	▒	▒	▒	▒	▒	▒
Chlorobenzene				■	▒	▒	▒	▒	▒	▒	▒
Benzyl alcohol					■	▒	▒	▒	▒	▒	▒
Methyl n-propyl ketone					■	▒	▒	▒	▒	▒	▒
n-Butyl acetate						■	▒	▒	▒	▒	▒
Trichloroethylene						■	▒	▒	▒	▒	▒
Ethyl-L-lactate							■	▒	▒	▒	▒
2-Methyl tetrahydrofuran							■	▒	▒	▒	▒
1,2-Dichlorobenzene								■	▒	▒	▒
Toluene								■	▒	▒	▒
Methyl isobutyl ketone									■	▒	▒
Butyl lactate									■	▒	▒
p-Xylene										■	▒
Methyl-t-butyl ether										■	▒
1,2,4-Trichlorobenzene											■
?											■

PLGA-based Long-Acting Depot Formulations (1 week ~ 6 months)

Poly(lactide-co-glycolide) (PLGA)

Lupron Depot®
leuprolide acetate for depot suspension
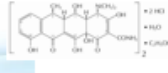1-4 months MP 1989
7.5 mg/month

Zoladex® 3-MONTH 10.8 mg DEPOT
GOSERELIN ACETATE IMPLANT
1, 3 months SI 1989
3.6 mg/month

Sandostatin LAR® Depot
(octreotide acetate for injectable suspension)
1 month MP 1998
20 mg/month

ATRIDOX®
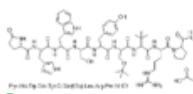(doxycycline hyclate) 10%
Cost Effective
1 week, IS 1998
50 mg/week

Nutropin DEPOT®
[somatropin (rDNA origin) for injectable suspension]
1 month MP 1999
13.5 mg/month

TRELSTAR®
(triptorelin pamoate for injectable suspension)
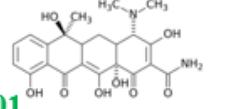1 month MP 2000
3.75 mg/month

Suprefact® Depot Implantat/Implant/itti
Buserelin acetate
2, 3 months SI 2000
6.3 mg/2 months

Somatuline® Depot
(lanreotide) Injection
1 month MP 2000
60 mg/month

Arestin®
minocycline HCl 1mg
MICROSPHERES
2 weeks MP 2001
1 mg/2 weeks

Risperdal CONSTA®
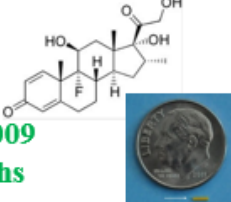risperidone Long-Acting Injection
2 weeks MP 2003
25 mg/2 weeks

Eligard®
(leuprolide acetate for injectable suspension)
1,3,4,6 months IS 2004
7.5 mg/month

Vivitrol®
(naltrexone for extended-release injectable suspension)
1 month MP 2006
380 mg/month

Ozurdex®
(dexamethasone intravitreal implant) 0.7 mg
3 months SI 2009
0.7 mg/3 months

TRELSTAR®
(triptorelin pamoate for injectable suspension)
6 months MP 2010
3.75 mg/month

Once-weekly BYDUREON®
exenatide extended-release for injectable suspension
His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH2
1 week MP 2012
2 mg/week

Lupaneta Pack™
leuprolide acetate for depot suspension, 11.25 mg for intramuscular injection and norethindrone acetate tablets, 5 mg for oral administration
3 month, MP 2012
3.75 mg/month

Signifor® LAR
(pasireotide) for injectable suspension
1 month, MP 2014
20, 40, or 60 mg/month

Product Similarity

Q1: Same components

Q2: Same components in same concentration

Q3: Same components in same concentration with the same arrangement of matter (microstructure)

11   DESCRIPTION

Octreotide is the acetate salt of a cyclic octapeptide. It is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Octreotide is known chemically as L-Cysteinamide, D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxy-methyl) propyl]-, cyclic (2→7)-disulfide; [R-(R*,R*)].

The molecular weight of octreotide is 1019.3 (free peptide, $C_{49}H_{66}N_{10}O_{10}S_2$) and its amino acid sequence is:

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol●xCH₃COOH

where x = 1.4 to 2.5
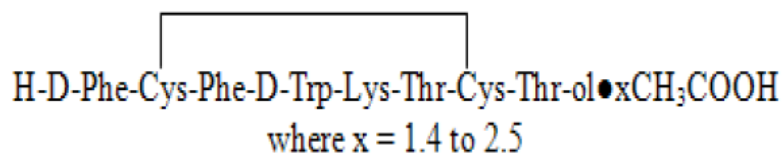
Sandostatin LAR Depot is available in a vial containing the sterile drug product, which when mixed with diluent, becomes a suspension that is given as a monthly intragluteal injection. The octreotide is uniformly distributed within the microspheres which are made of a biodegradable glucose star polymer, D,L-lactic and glycolic acids copolymer. Sterile mannitol is added to the microspheres to improve suspendability.

Sandostatin LAR Depot is available as: sterile 6-mL vials in 3 strengths delivering 10 mg, 20 mg, or 30 mg octreotide-free peptide. Each vial of Sandostatin LAR Depot delivers:

| Name of Ingredient	10 mg	20 mg	30 mg
octreotide acetate	11.2 mg*	22.4 mg*	33.6 mg*
D,L-lactic and glycolic acids copolymer	188.8 mg	377.6 mg	566.4 mg
mannitol	41.0 mg	81.9 mg	122.9 mg

*Equivalent to 10/20/30 mg octreotide base.
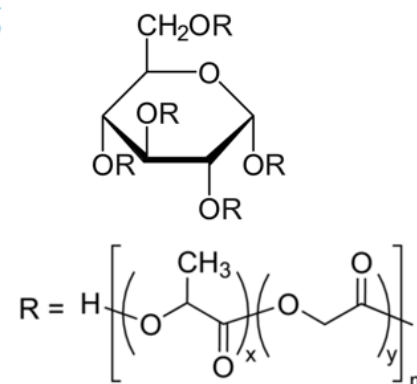
PLGA Analysis of Sandostatin® Formulation

Glu-PLGAs used to make octreotide formulations in the Bodmer patent.

	Molecular Weight	L:G Ratio	PI
Bodmer-Example 2	45,000 Da	55:45	ca. 1.7
Bodmer-Example 3	45,000 Da	50:50	N/A
Bodmer-Example 4	46,000 Da	50:50	ca. 1.7
Bodmer-Example 6	46,000 Da	50:50	ca. 1.7

GPC chromatograms of linear PLGA (A) and Glu-PLGA from Novartis (B). (From Reference [4]).
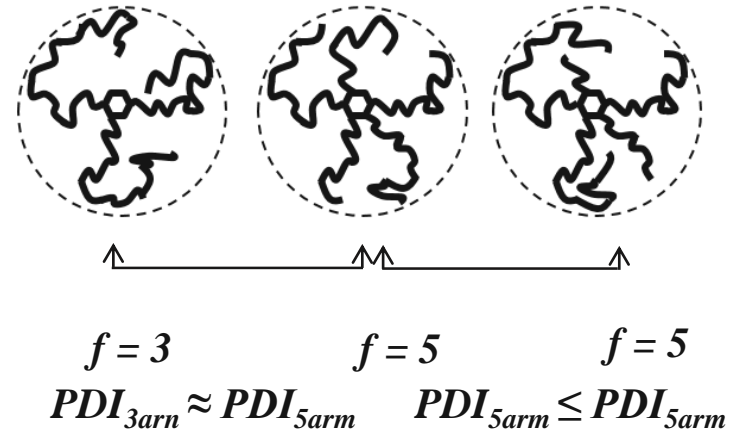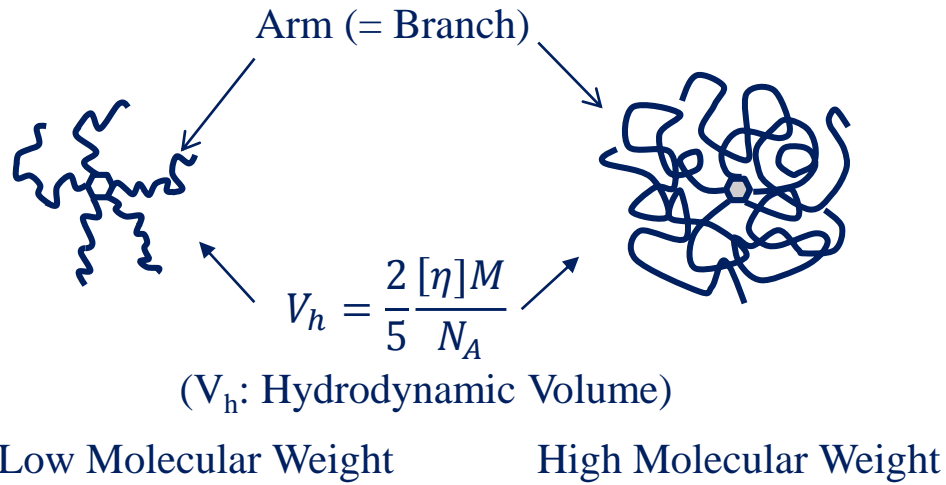

D. Bodmer, J.W. Fong, T. Kissel, H.V. Maulding, O. Nagele, J.E. Pearson, Sustained release formulations of water soluble peptides, (1996) US Patent 5,538,739.
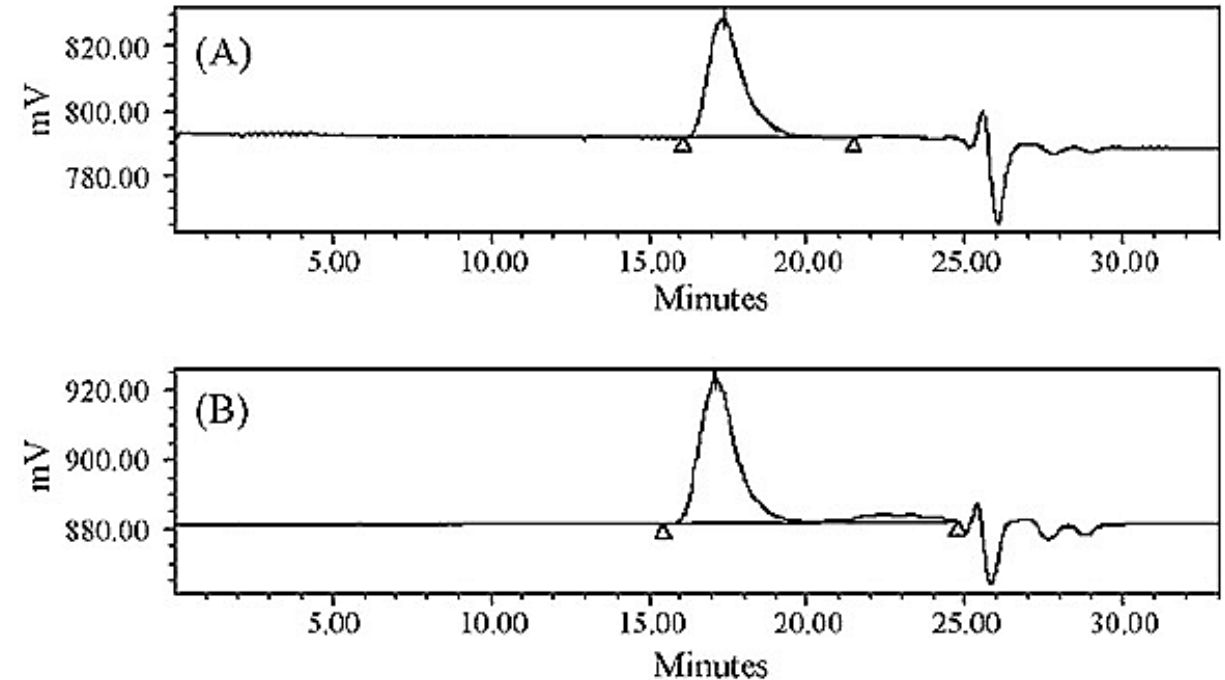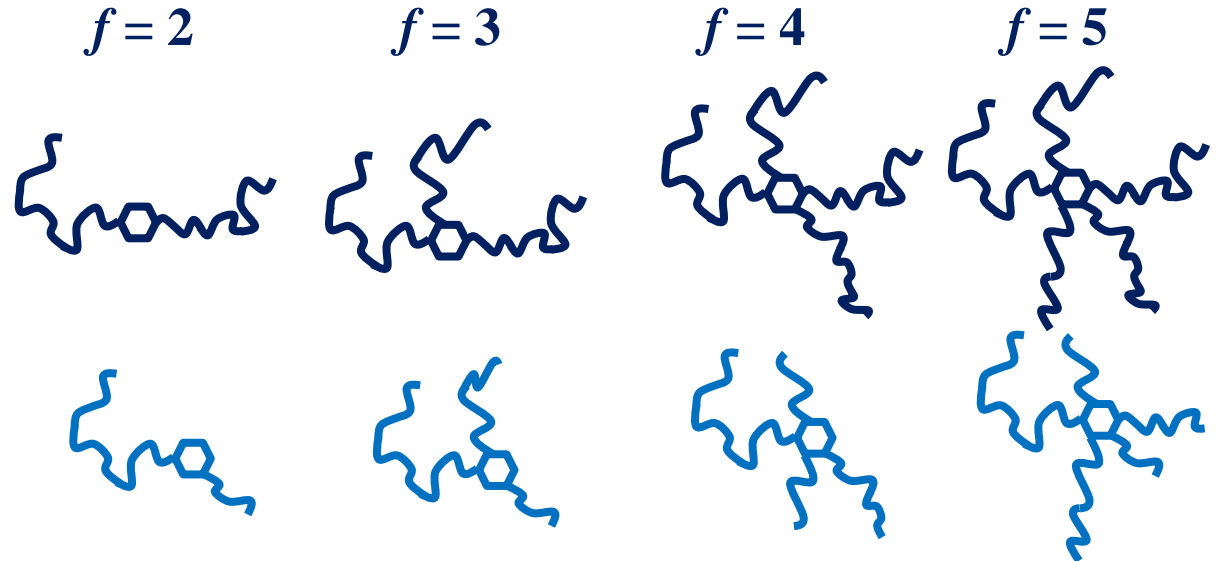
J. Kang, O. Lambert, M. Ausborn, S.P. Schwendeman. Stability of proteins encapsulated in injectable and biodegradable poly(lactide-co-glycolide)-glucose millicylinders. Int. J. Pharm., 357 (2008) 235-243.

Star-Shaped PLGA (Glucose-PLGA)

Arm (= Branch)

$$V_h = \frac{2}{5}\frac{[\eta]M}{N_A}$$

(V_h: Hydrodynamic Volume)

Low Molecular Weight   High Molecular Weight

$f = 3$
$PDI_{3arn} \approx PDI_{5arm}$

$f = 5$
$PDI_{5arm} \leq PDI_{5arm}$

$f = 5$
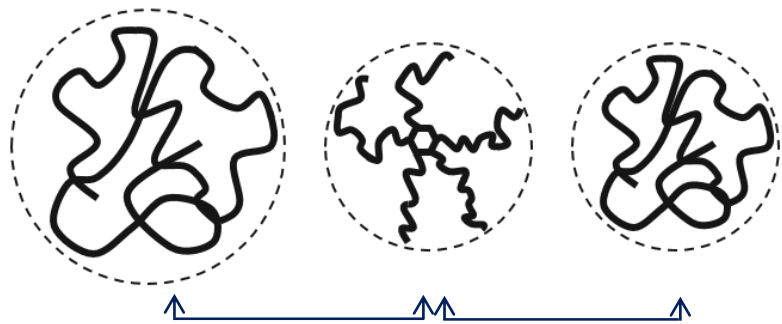
$M_{lin} = M_{star}$ $M_{star} > M_{lin}$

$V_{h,lin} > V_{h,star}$ $V_{h,star} = V_{h,lin}$

$f = 2$ $f = 3$ $f = 4$ $f = 5$

GPC with Quadruple Detectors

|	
1. Refractive index	This establishes the exact concentration of the polymer.
2. Multiangle static light scattering (MASLS)	The component measures the absolute weight average molecular weight (M_w) without any calibration using standard molecules, as well as the radius of gyration (R_g). The R_g obtained from MASLS is not dependent on the shape. R_g of molecules with radii less than 10 nm, however, cannot be measured by SLS, because they scatter light equally at all angles (Rayleigh scattering). For such small molecules small angle x-ray scattering (SAXS) or small angle neutron scattering (SANS) is used.
3. Dynamic light scattering	This yields hydrodynamic volume (V_h), and thus hydrodynamic radius (R_h). R_h describes the apparent size (i.e., radius) of the solvated, tumbling molecule. R_h values are calculated assuming the molecule is spherical.
4. Viscometer $$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$	The viscometer provides intrinsic viscosity ($[\eta]$) values which provide Mark-Houwink coefficients and distributive properties of long chain branching and hydrodynamic volume V_h of a polymer.

$$[\eta] = \frac{5}{2} N_A \frac{V_h}{M} = \frac{5}{2} N_A \frac{4\pi}{3} \frac{<R_\eta^2>^{\frac{3}{2}}}{M} (\approx \frac{5}{2} N_A \frac{4\pi}{3} \frac{<R_h^2>^{\frac{3}{2}}}{M}) = \phi' \frac{<R_g^2>^{\frac{3}{2}}}{M} = K M^\alpha$$
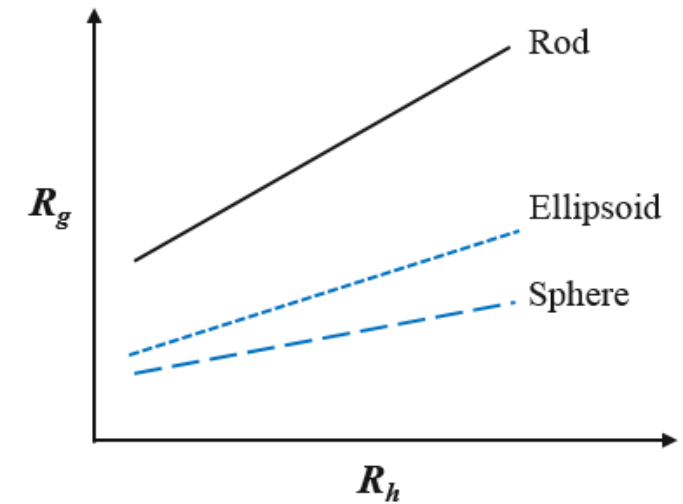
5. Osmometer	This measures the absolute number average molecular weight (M_w).

Mark-Houwink Plots of a PLGA in Solvents

PLGA (L:G ratio, Mol. Wt.)
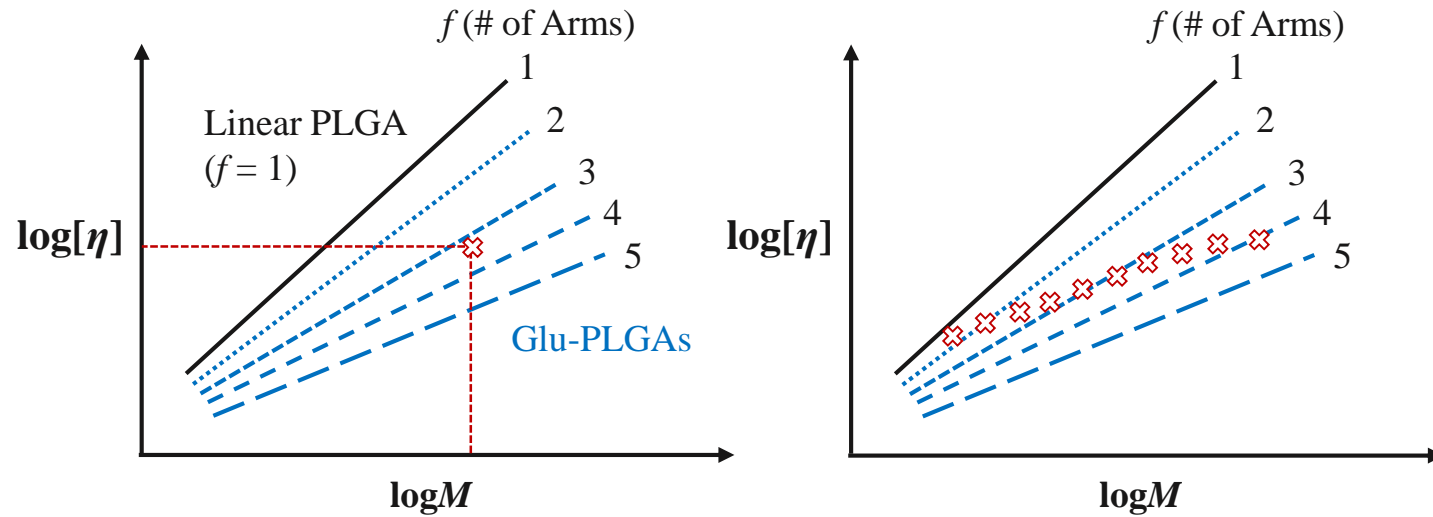
Shape Factor (ρ)

Mark-Houwink plots of a PLGA with a given L:G ratio in different solvents.

The molecular weight (M) remains the same but the V_h of the polymer changes in different solvents. The intrinsic viscosity [η] increases as the V_h increases in good solvents. Thus, the solvent quality for each PLGA can be characterized by using the K and α values.
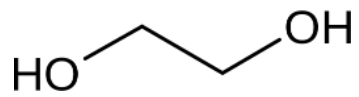
Dependence of the R_g/R_h ratio on the molecular shape.

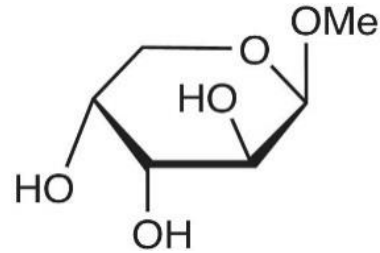# Core Structures of Star PLGA Polymers
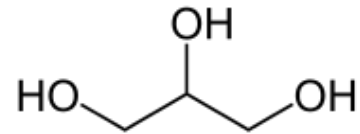

$f = 2$
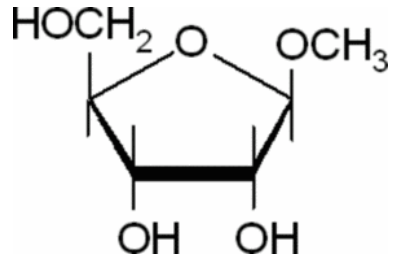
Methyl β-D-ribofuranoside
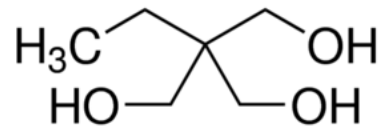
Ethylene glycol
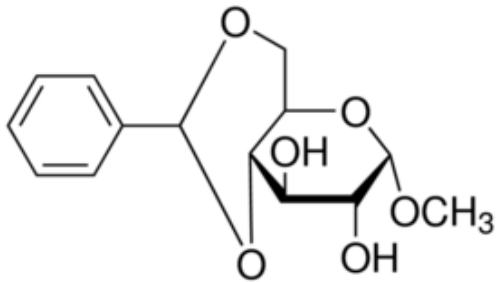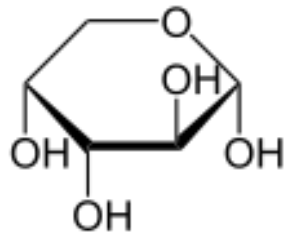
Methyl 4,6-O-benzylidene-α-D-glucopyranoside
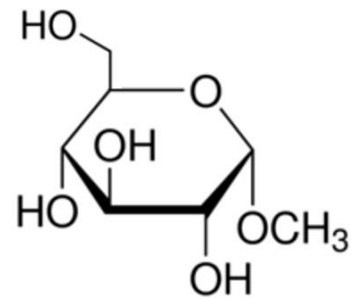
$f = 3$
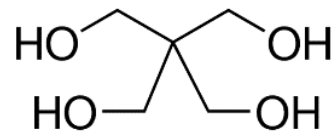
Methyl-β-D-arabinopyranoside

Glycerol

Trimethylolpropane

α-D-Arabinopyranose
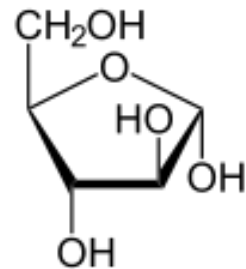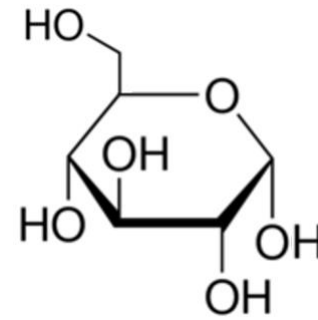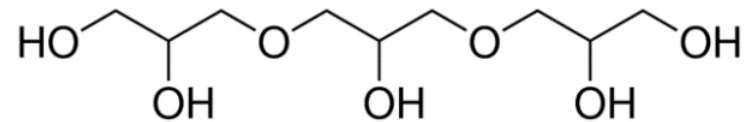
$f = 4$

Methyl α-D-glucopyranoside
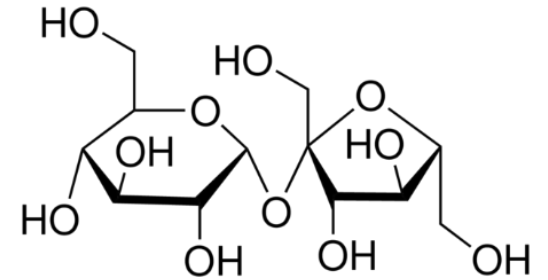
Pentaerythritol

α-D-Arabinofuranose

$f = 5$

α-D-glucose

$f = 8$

Sucrose

Triglycerol

Ten Quantitative Parameters Defining Glu-PLGA in Comparison with Linear PLGA.

Parameter	Method to Obtain the Value	Property
1. Absolute weight average molecular weight (M_w)	Static light scattering detector connected to GPC	Absolute M_w values of all types of polymers
2. Absolute number average molecular weight (M_n)	Osmometer	Absolute M_n values of all types of polymers
3. Polydispersity index of Glu-PLGA (PDI_{star})	$\dfrac{M_w}{M_n}$	Broadness of a molecular weight distribution
4. L:G ratio	^{1}H-NMR	Affecting the PLGA property
5. Intrinsic viscosity $[\eta]$	Online Viscometer for GPC	Mark-Houwink plots allow information on the f value
6. Radius of gyration	Multiangle static light scattering	Distance from the center of mass
7. Hydrodynamic radius R_h	Dynamic light scattering detector of GPC-TDS*	Size of polymer molecules moving in solution
8. Shape factor (ρ)	R_g/R_h	Distinguish polymers based on molecular shape
9. Number of arms (or branches) (f)	Mark-Houwink plot	Degree of branching from a glucose core
10. Polydispersity index of arms (PDI_{arm})	$f\left(\dfrac{M_{w,star}}{M_{n,star}} - 1\right)$	Heterogeneity of PLGA arms on a glucose core

Q1, Q2, and Q3 of PLGA-based Long-Acting Depot Formulations

Product Similarity	Example	Future
Q1: Same components	PLGA Glu-PLGA PLGA Mixture	New structures. Mixtures difficult to analyze.
Q2: Same components in same concentration	PLGA Mixture	Mixtures difficult to analyze (Similar molecular weights, Similar L:G ratios)
Q3: Same components in same concentration with same arrangement of matter (microstructure)	Current clinical products	Isolation of PLGA frames. Impacts on drug release kinetics.