TABLE 13

Properties of the Microspheres

Property	GJ040302 (MS prepared from stored "Leup DP")	GJ040902 (MS prepared from stored "Leup DP with LA"	Property of a typical MS batch
Drug Load, %	5.6 ± 0.35%	4.33 ± %	14-16
Drug encapsulation efficiency, %	31%	24%	78-89
% Leuprolide Related Substances*	6.04 ± 0.30%	0	2-4%
% Drug in to CP soon after MS formation	14.7%	17.7%	≈7%
% Drug found in CP-final	50.9%	58.2%	≈10%

*By extracting the drug in acetate buffer

The incorporation of lactic acid eliminated the related substance in the microspheres. The leuprolide DP which did not contain lactic acid showed 0.53% related substance initially, and increased to 6.04%. The related substance formation was gradual for leuprolide microspheres prepared from a fresh DP that did not contain lactic acid (data not shown). Lactic acid in the DP protected leuprolide from related substance formation during the entire microsphere process.

D. Effect of Solvent Used to Prepare DP on Molecular Weight Reduction of Polymer

DP formulations, as shown in Table-14, were incubated for 24 hours at 25° C. and at 40° C. to see the effect of DMSO and DMAc on molecular weight reduction compared to methanol. A DP, simply containing DCM and PLGA was also included in the experiment for comparison.

TABLE 14

DP Composition with Variety of Solvents

DP Code	RG503H	DCM	Other Solvent
RG503H + DCM	0.85 g	3.87 g	0
RG503H + DCM + MeOH	0.85 g	3.87 g	MeOH: 1.12 g
RG503H + DCM + DMSO	0.85 g	3.87 g	DMSO: 1.12 g
RG503H + DCM + DMAc	0.85 g	3.87 g	DMAc: 1.12 g

The DP samples were incubated at 40° C. for 24 hours in sealed tubes. The molecular weight of the PLGA after incubation was as shown in Table-15.

TABLE 15

Mw and the Reduction Mw of PLGA Upon Incubation - Effect of Solvent

	Mw
RG503H + DCM	52257
RG503H + DCM + MeOH	25907
RG503H + DCM + DMSO	52150
RG503H + DCM + DMAc	52876

As shown in Table 15, the Mw of RG503H in the DP system free from methanol remains unaffected. Leuprolide containing DP with DMSO instead of methanol is the appropriate system to compare the effect of various acids on preventing the leuprolide induced nucleophilic attack of PLGA ester bonds.

DP samples listed in Table 16 were prepared and incubated at 25 and 40° C. for 24 hours. Both molecular weight and the leuprolide impurity were followed.

TABLE 16

DP formulations prepared with DMSO solvent

DP components	RG503H	DCM	Leup. Act.	DMSO	Acid additive	% acid based on total DP
RG503H + Leup. Ac. + DMSO	0.85 g	3.87 g	0	1.52 g	0	N.A
RG503H + Leup. Ac. + DMSO + G. AA-1	0.85 g	3.87 g	0.21 g	1.52 g	GAA: 0.022 g	0.35
RG503H + Leup. Ac.. + DMSO + G. AA-2	0.85 g	3.87 g	0.21 g	1.52 g	GAA: 0.044 g	0.70
RG503H + Leup. Ac.. + DMSO + LA-1	0.85 g	3.87 g	0.21 g	1.52 g	LA: 0.026 g	0.35
RG503H + Leup. Ac. + DMSO + LA-2	0.85 g	3.87 g	0.21 g	1.52 g	LA: 0.052 g	0.70
RG503H + Leup. Ac. + DMSO + GA-1	0.85 g	3.87 g	0.21 g	1.52 g	GA: 0.022 g	0.35
RG503H + Leup. Ac. + DMSO + GA-2	0.85 g	3.87 g	0.21 g	1.52 g	GA: 0.044 g	0.70
RG503H + Leup. Ac. + DMSO + Oligomer-1	0.85 g	3.87 g	0.21 g	1.52 g	Oligomer: 0.022 g	0.35
RG503H + Leup. Ac. + DMSO + Oligomer-2	0.85 g	3.87 g	0.21 g	1.52 g	Oligomer: 0.044 g	0.70

Table-17 shows the Mw values before and after incubating the DP and compares the percentage loss in Mw.

TABLE 17

Mw Change Upon Storing DMSO Containing Leuprolide DP

		25° C.-24 Hours		40° C.-24 Hours	
DP components	Initial Mw	Mw	% Reduction in Mw	Mw	% Reduction in Mw
RG503H + Leup. Ac. + DMSO	44564	39155	12.1	23470	47.3
RG503H + Leup. Ac. + DMSO + G. AA-1	45925	40724	11.3	28063	38.9
RG503H + Leup. Ac.. + DMSO + G. AA-2	47019	42045	10.6	27897	40.7