Characterization of Drug Nanosuspensions

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Workshop on Characterization of Nanomaterials for Medical and Health Applications
(jointly sponsored with ASTM, NCI, NIST, and FDA)
Reno, NV

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Agenda

- Technology Overview
- Formulation
- Characterization Tests
- Stability
- Dissolution Rate
- Pharmacokinetics
- Safety
- Efficacy
- Manufacturing

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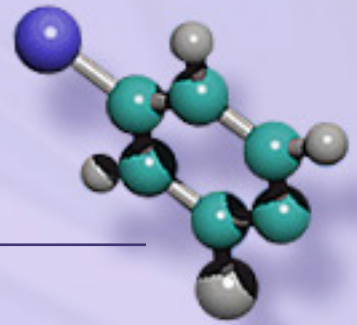
Technology Overview

Formulations



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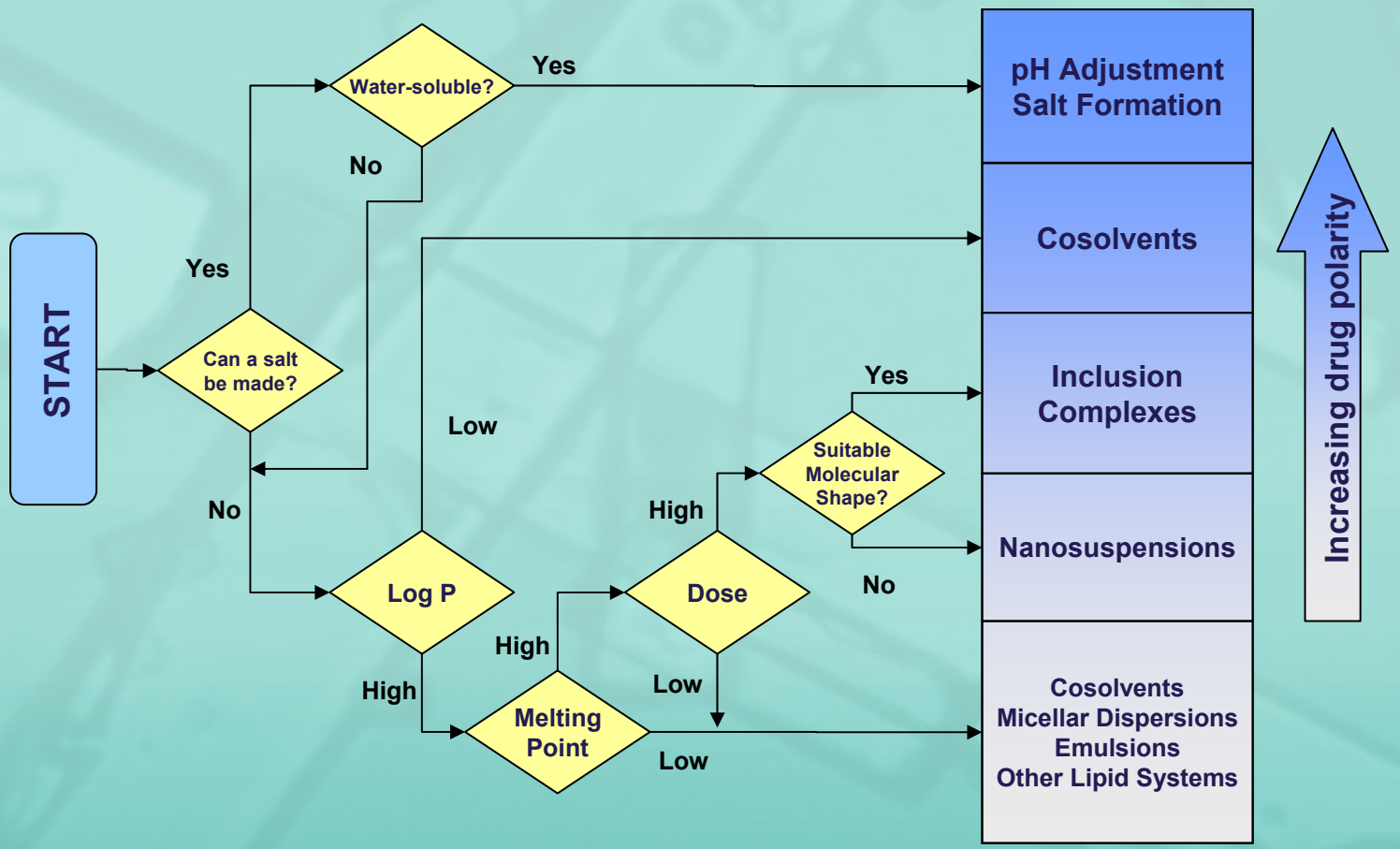
Decision Tree for Formulation of Water Insoluble Drugs



NANOEDGE Features

Broadly applicable:	Exploits non-specific property of insolubility
Small particle size:	300 nm to 10 microns (vol weighted mean)
High loading: **=Low volume doses**	10 – 200 mg/mL
Elimination of cosolvents:	increased safety may lead to increased dose
Long-term stability:	up to 2 yrs at RT or 5°C
Dosing:	injection: IV, IM, ID oral, respiratory and other routes
Tunable pharmacokinetics	rapid dissolution vs. targeting
Scalable processes:	benchtop (5 mL) to mfg (600 L)
Rapid screening tools:	computation and preformulation

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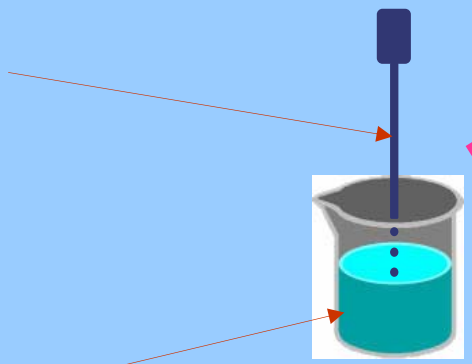
NANOEDGE Manufacturing Process

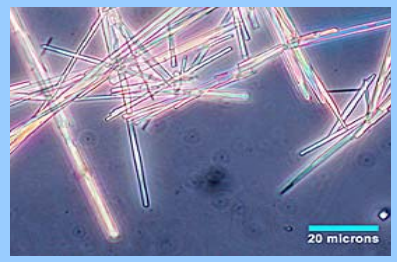
Drug Concentrate

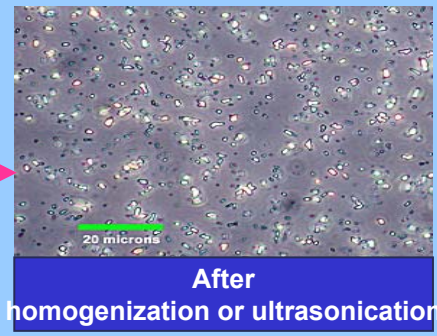
- Drug
- Surfactant(s)
- Water-miscible solvent (e.g. NMP)

Diluent

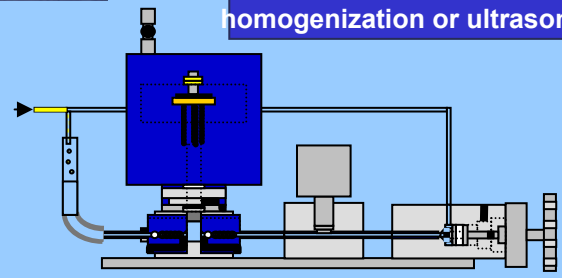
- Water
- Buffer
- Surfactant(s)



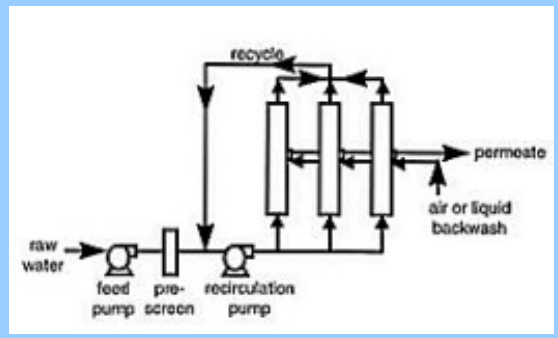




After homogenization or ultrasonication



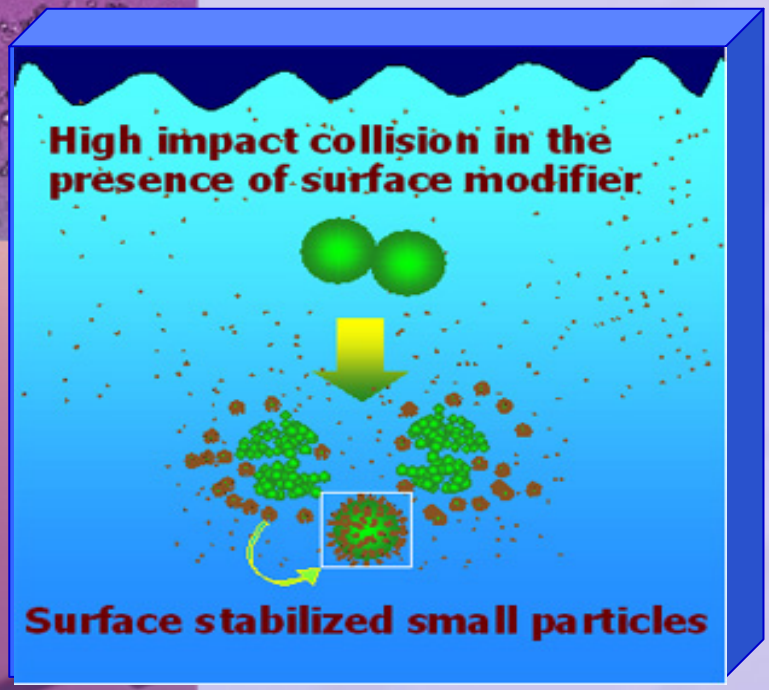
microprecipitation **homogenization**



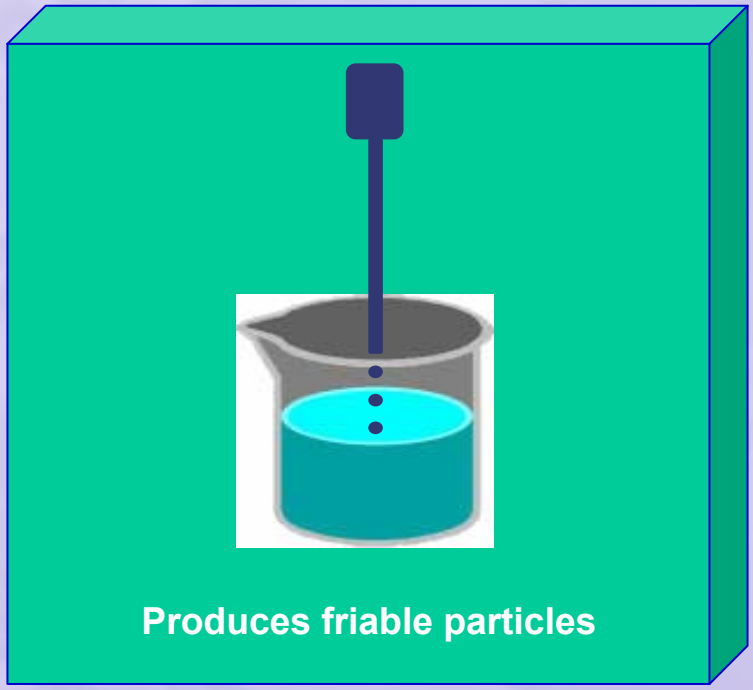
solvent removal

NANOEDGE Solid Particle Technologies

Direct Homogenization



Microprecipitation/ Homogenization



Features

- 300 to 600 nm particles
- High loading (10 – 200 mg/mL)
- Long-term stability (up to 2 yrs)

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Characterization Tests

Chemical
- **Active ingredient**
- **Excipients**
- **Degradants**
- **Moisture (for lyophilized)**
- **Preservatives**
- **pH**
- **Solvents/impurities**

Physical
- **Particle-size (PS) distribution**
- **Stress testing of size distribution**
- **High end of PS distrbn (USP<788>)**
- **Light microscope & SEM**
- **Drainability**
- **Syringeability, injectability**
- **Re-suspendability**
- **Dissolution rate**
- **Compatibility of admixture**
- **Zeta potential**
- **Polymorph (DSC/X-ray)**

Biological
- **Sterility**
- **Pyrogenicity**

- Preclinical
  - Acute toxicity
  - Pharmacokinetics
    - free
    - particulate
  - Efficacy
  - Subacute tox (2 sp)
  - ADME
  - genotoxicity

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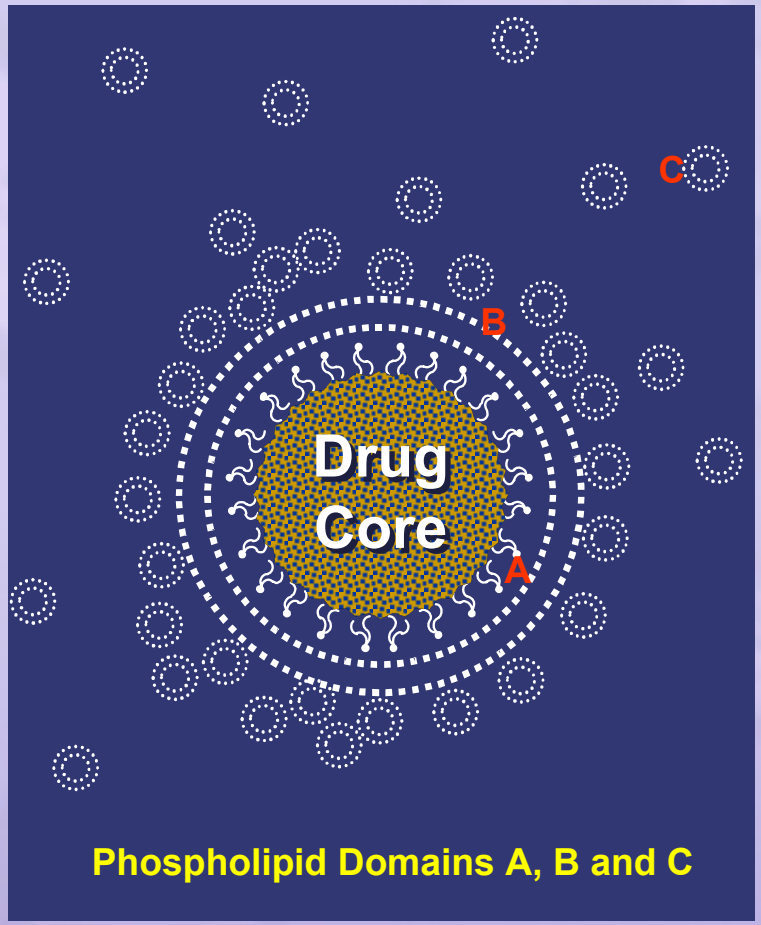
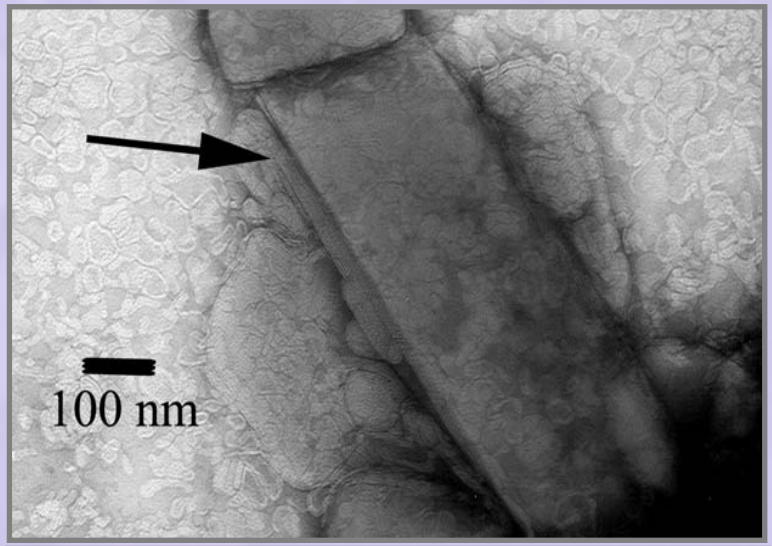
TEM Analysis of Particle Coating

Microstructure





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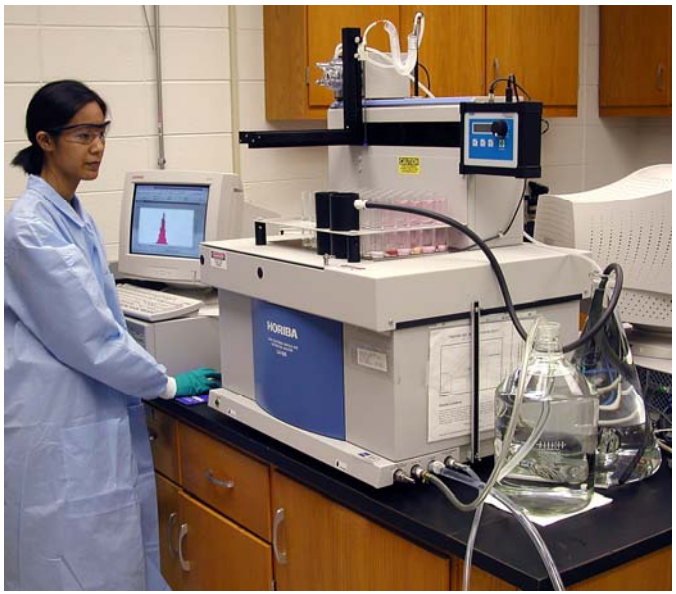
Particle Size Determination-Laser Diffraction



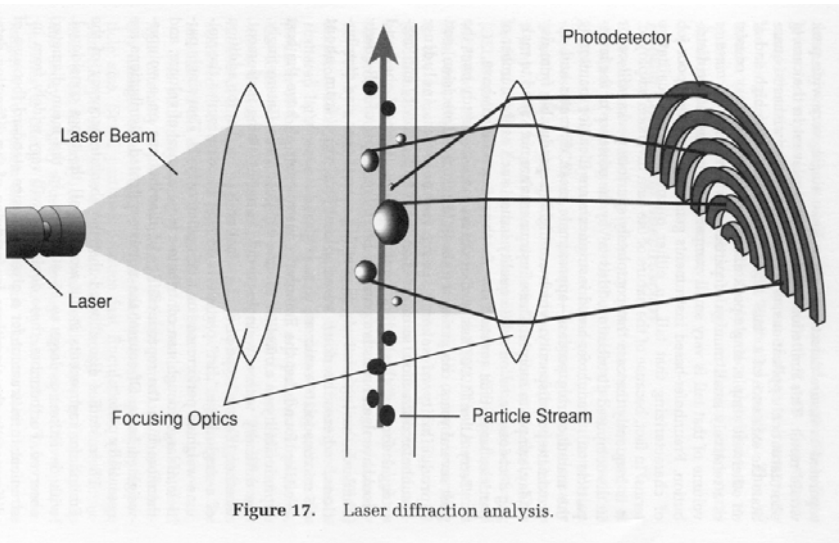


Figure 17. Laser diffraction analysis.

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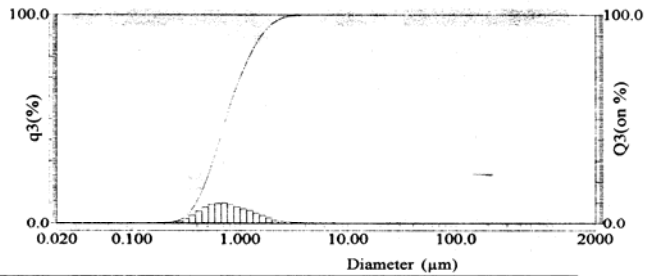
HORIBA LA-920 for Windows(TM) [WET(LA-920)] Ver 2.01
LA-920 system for Windows

Worksheet No. 95 Datasheet No. 9 01/25/2000 3:27 PM

Filename	:5542715250100
Circulation Speed	:2
Ultra sonic	:OFF
Laser T%	: 91.1(%)
Lamp T%	: 74.3(%)
Form of Distribution	:Standard
Distribution Base	:Volume
R.R.Index	:112A000I
Sample Name	:1% Ofloxacin Homogenized
Material	:Ofloxacin
Source	:20541-190
Lot Number	:Homogenized
Test or Assay Number	:2 of 2
Notebook	:22160-25
Study Number	:10087
Project Number	:IDN2122A
PAR	:16486

Median	: 0.739(μm)
Mean	: 0.880374(μm)
Mode	: 0.713298(μm)
Span	: 1.590412



No.	Diameter	q3(%)	Upper %	No.	Diameter	q3(%)	Upper %	No.	Diameter	q3(%)	Upper %	No.	Diameter	q3(%)	Upper %
1	0.022	0.000	0.000	25	0.584	9.275	32.901	49	15.172	0.000	100.000	73	394.244	0.000	100.000
2	0.026	0.000	0.000	26	0.669	9.796	42.697	50	17.377	0.000	100.000	74	451.556	0.000	100.000
3	0.029	0.000	0.000	27	0.766	9.956	52.653	51	19.904	0.000	100.000	75	517.200	0.000	100.000
4	0.034	0.000	0.000	28	0.877	9.303	61.957	52	22.797	0.000	100.000	76	592.387	0.000	100.000
5	0.039	0.000	0.000	29	1.005	8.196	70.152	53	26.111	0.000	100.000	77	678.504	0.000	100.000
6	0.044	0.000	0.000	30	1.151	7.257	77.409	54	29.907	0.000	100.000	78	777.141	0.000	100.000
7	0.051	0.000	0.000	31	1.318	6.359	83.768	55	34.255	0.000	100.000	79	890.116	0.000	100.000
8	0.058	0.000	0.000	32	1.510	5.121	88.889	56	39.234	0.000	100.000	80	1019.515	0.000	100.000
9	0.067	0.000	0.000	33	1.729	4.010	92.899	57	44.938	0.000	100.000	81	1167.725	0.000	100.000
10	0.076	0.000	0.000	34	1.981	2.909	95.808	58	51.477	0.000	100.000	82	1337.481	0.000	100.000
11	0.087	0.000	0.000	35	2.269	1.887	97.694	59	58.953	0.000	100.000	83	1531.914	0.000	100.000
12	0.100	0.000	0.000	36	2.599	1.153	98.847	60	67.523	0.000	100.000	84	1754.613	0.000	100.000
13	0.115	0.000	0.000	37	2.976	0.637	99.484	61	77.339	0.000	100.000	85	2000.000	0.000	100.000
14	0.131	0.000	0.000	38	3.409	0.342	99.826	62	88.583	0.000	100.000				
15	0.150	0.000	0.000	39	3.905	0.174	100.000	63	101.460	0.000	100.000				
16	0.172	0.000	0.000	40	4.472	0.000	100.000	64	116.210	0.000	100.000				
17	0.197	0.172	0.172	41	5.122	0.000	100.000	65	133.103	0.000	100.000				
18	0.226	0.356	0.529	42	5.867	0.000	100.000	66	152.453	0.000	100.000				
19	0.259	0.761	1.289	43	6.720	0.000	100.000	67	174.618	0.000	100.000				
20	0.296	1.397	2.686	44	7.697	0.000	100.000	68	200.000	0.000	100.000				
21	0.339	2.559	5.245	45	8.816	0.000	100.000	69	229.075	0.000	100.000				
22	0.389	4.262	9.507	46	10.097	0.000	100.000	70	262.376	0.000	100.000				
23	0.445	6.128	15.635	47	11.565	0.000	100.000	71	300.518	0.000	100.000				
24	0.510	7.990	23.625	48	13.246	0.000	100.000	72	344.206	0.000	100.000				

ANALYST: DATE: 1/28/2000 REVIEWER: DATE: 2/12/00

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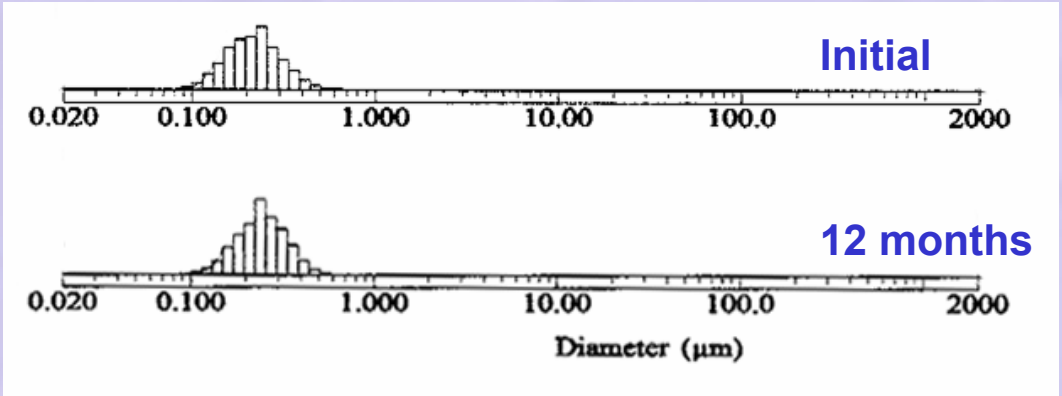
Particle Size Stability

- **1% itraconazole nanosuspension**
  - Particle size distribution (by laser diffraction) versus storage time at 25°C (12 months)



  - Effects of steam sterilization (121°C)

Exposure Duration (min)	Particle Diameter (nm)* Mean	Std Dev	99%
Initial	439	137	760
12	574	235	1125
20	554	237	1109

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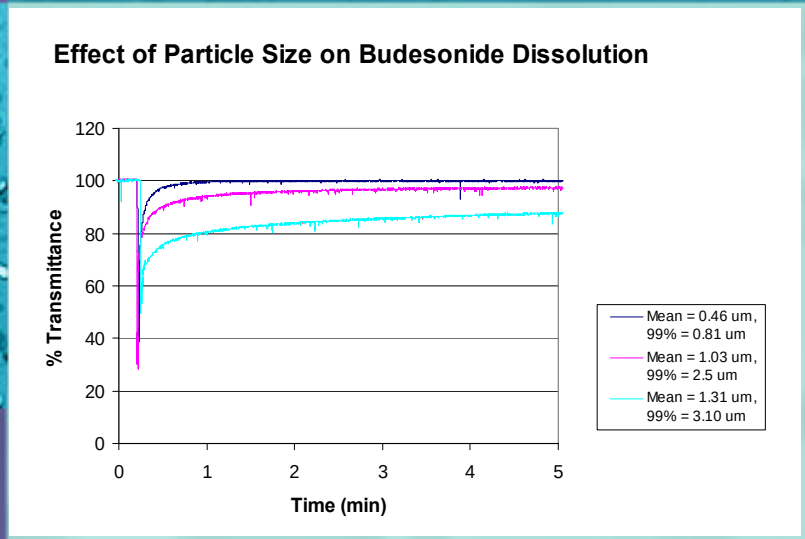
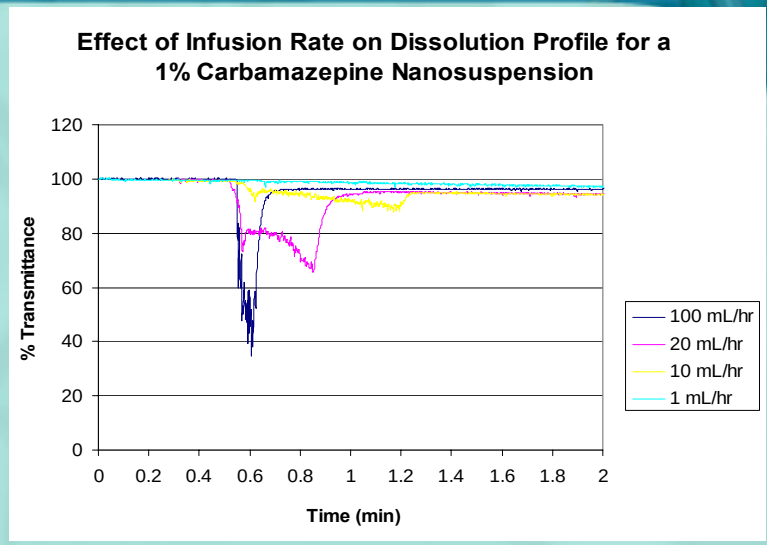
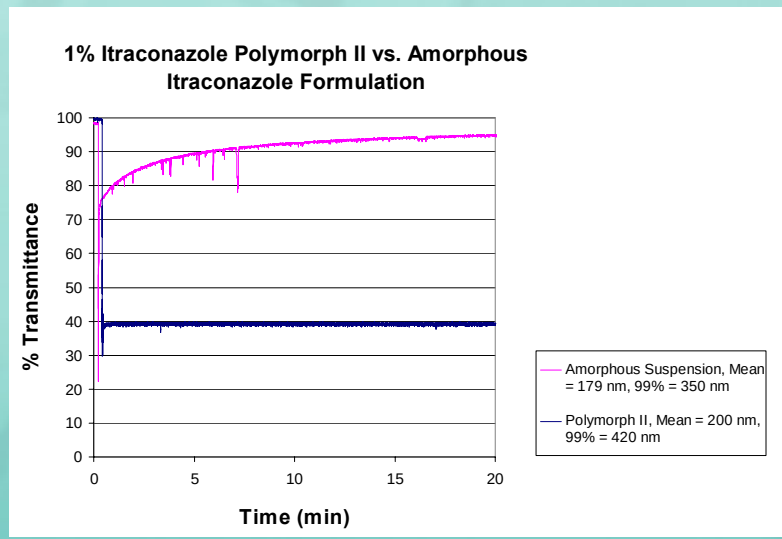
Controlling Dissolution Rate







Light transmittance through dissolution media, following bolus injection of drug suspension, indicates dissolution rate

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In Silico Dissolution Analysis

- Finite-difference equation:

$$r_{i+1} = r_i - \frac{DM}{\rho l}\left(C_0 - C_{bulk}\right)$$

$$r_{i+1} = r_i - \frac{kTM\Delta t}{6\pi\rho\eta r_m l}\left\{S_0 e^{\frac{2\gamma M}{RT\rho r_i}} - \left[C_{b,0} + \frac{m}{MV_L}\left(1 - \left(\frac{r_i}{r_0}\right)^3\right)\right]\right\}$$

k = Boltzmann's constant (ergs K^{-1})
T = Temperature (K)
R = Ideal gas constant (ergs K^{-1} $mole^{-1}$)
ρ = Particle density (g cm^{-3})
η = Viscosity (Poise, g cm^{-1} sec^{-1})
γ = Interfacial tension (ergs cm^{-2})
M = Molecular weight of drug (g $mole^{-1}$)
Δt = Time interval (sec)
r_m = Molecular radius (cm)

l = Hydrodynamic boundary thickness (cm)
D = Diffusion coefficient (cm^2 sec^{-1})
S_0 = Intrinsic solubility (moles cm^{-3})
$C_{b,0}$ = Concentration in bulk fluid at time zero
m = Mass of drug to be delivered (grams)
V_L = Volume of fluid compartment
r_i = Particle radius at interval i
r_{i+1} = Particle radius at interval i+1

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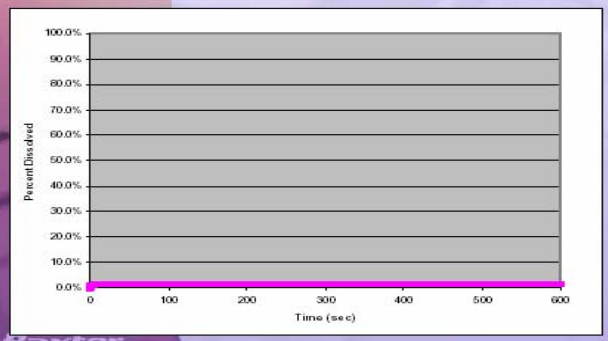
In Vitro Dissolution Comparison

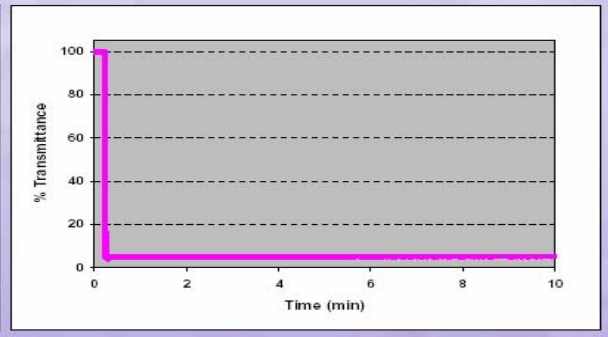
- Comparison of ab initio simulation with turbidity experiment (0.1 mL added to 10 mL PBS buffer). T = 37°C

Itraconazole

Ab initio (0.025 g/L)

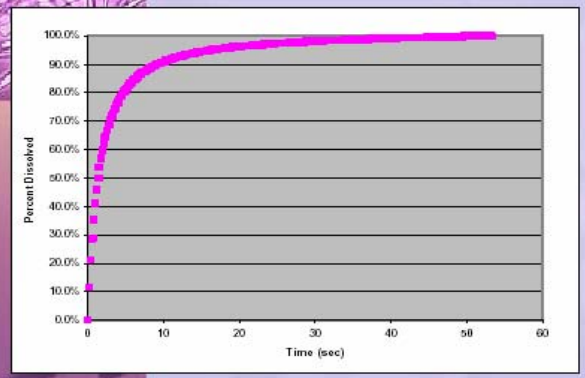


Actual

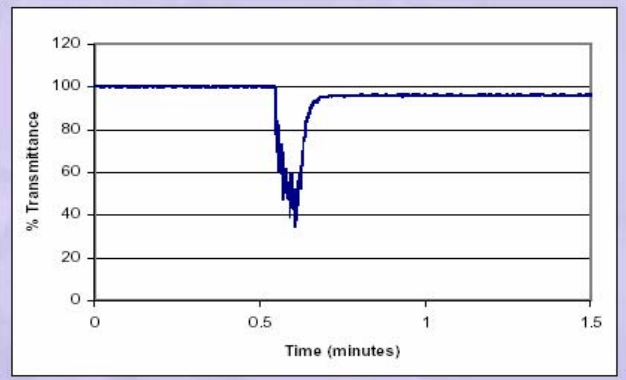


Carbamazepine

Ab initio (0.025 g/L)



Actual



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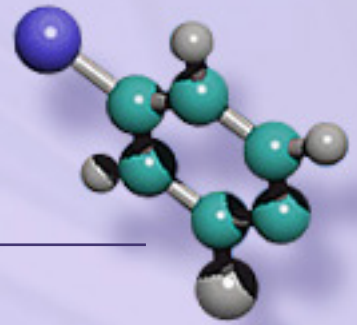
Pharmacokinetics



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Pharmacokinetics

Parenteral Delivery Modalities

- **Rapid Delivery (IV)**
  - Rapid dissolution in the blood
  - Moderately insoluble drugs (50-100 ppm)
  - Examples: Piroxicam, flurbiprofen, dantrolene
- **Sustained Release (SC, IM, ID)**
  - Sustained delivery by depot mechanism
  - Example: Tetracaine
- **Extended Release (IV)**
  - Particles captured by fixed macrophages before the drug can dissolve completely in the bloodstream
  - Highly insoluble drugs (<10 ppm) – “brickdust”

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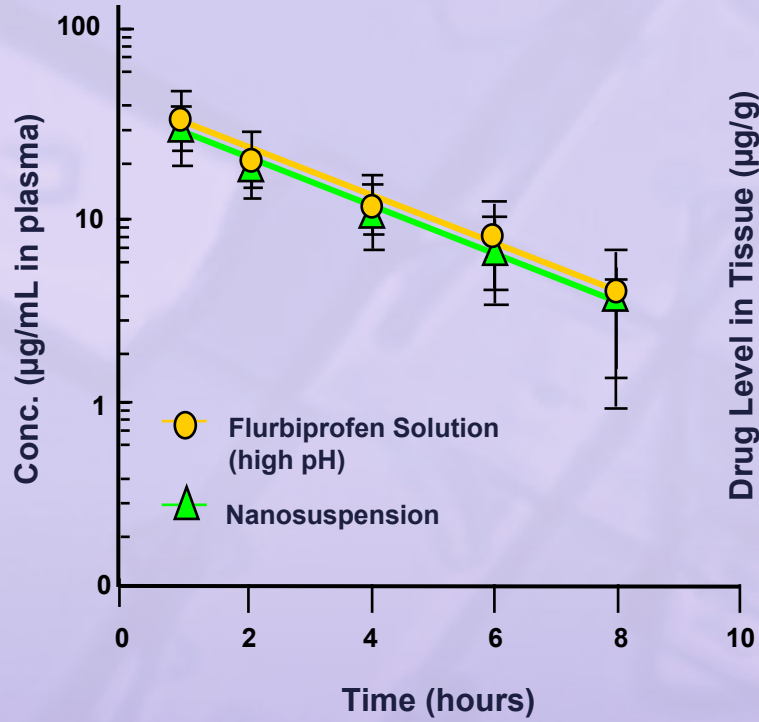
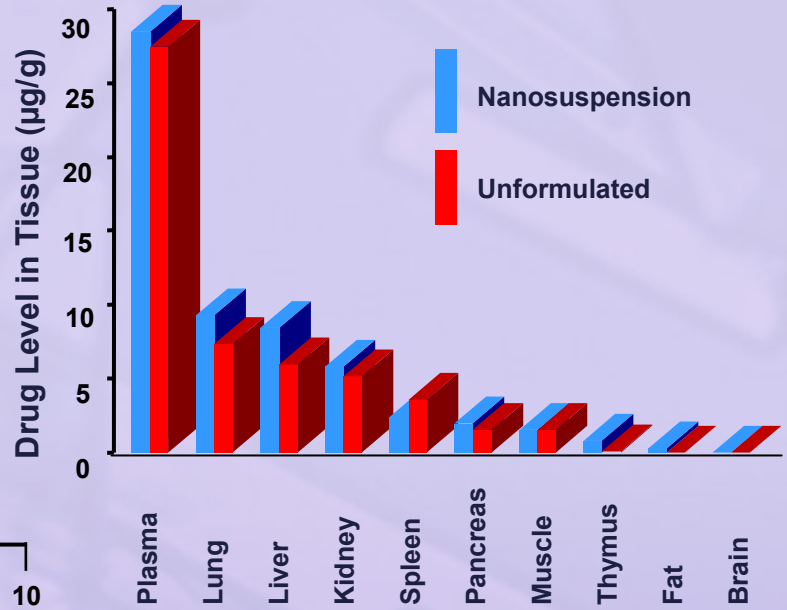
Rapid Plasma Dissolution Provides Tissue Distribution Equivalent to That for Solution Formulation

Flurbiprofen





MA Clement *et al.*; "Tissue Distribution and Plasma Clearance of a Novel Microcrystal Encapsulated Flurbiprofen Formulation"; *The Pharmacologist* 34(3), 204 (1992).

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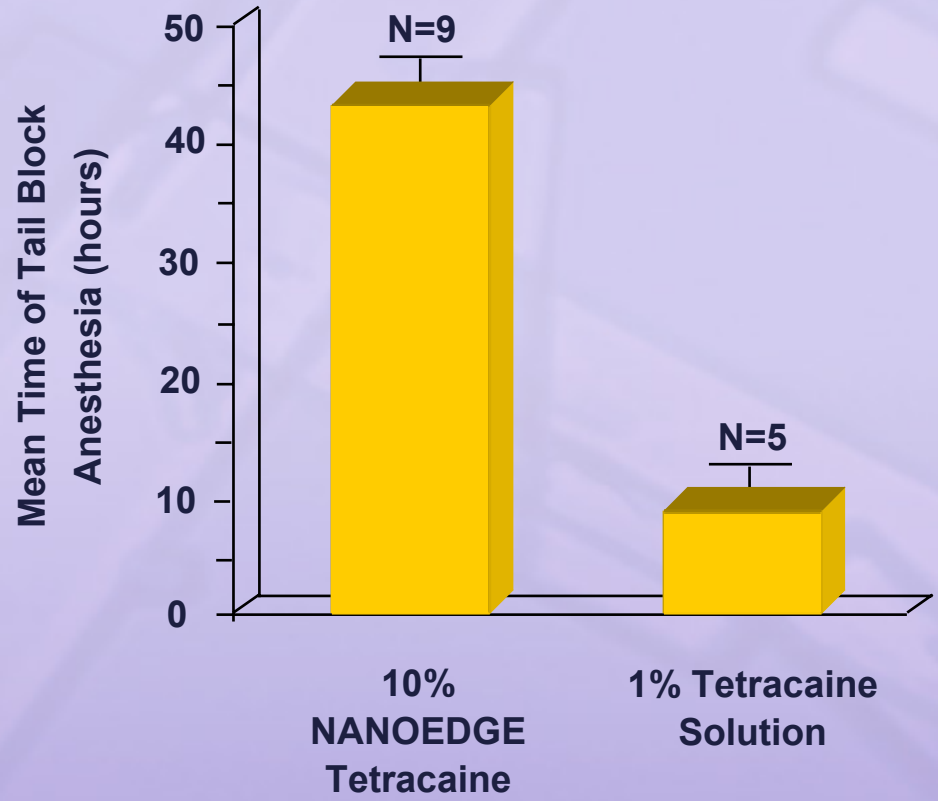
Sustained Release Depot

Sustained Release

NANOEDGE Tetracaine - Delivered intradermally
High dose/low volume depot possible



Boedeker *et al.*; *J. Clinical Pharmacol.* 34, 699 (1994)

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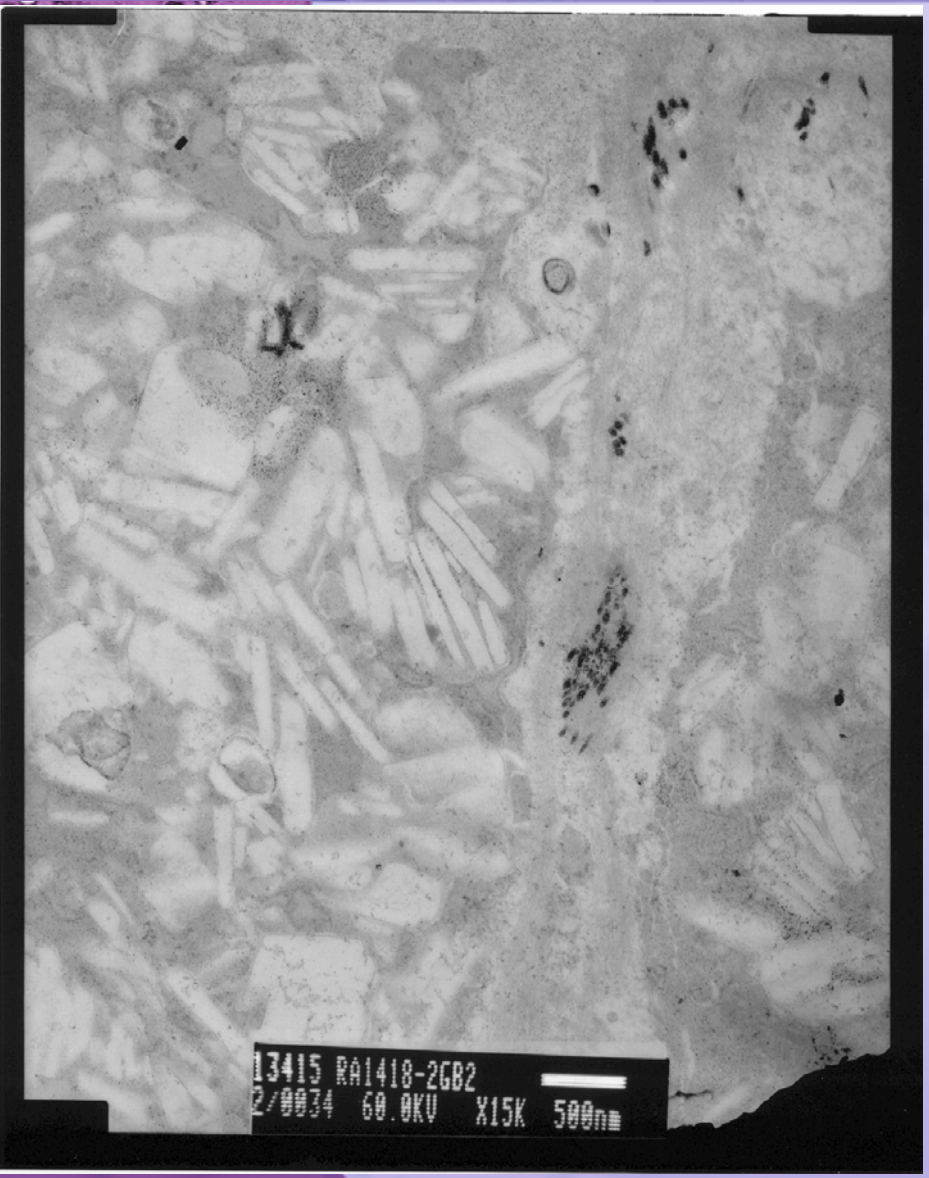
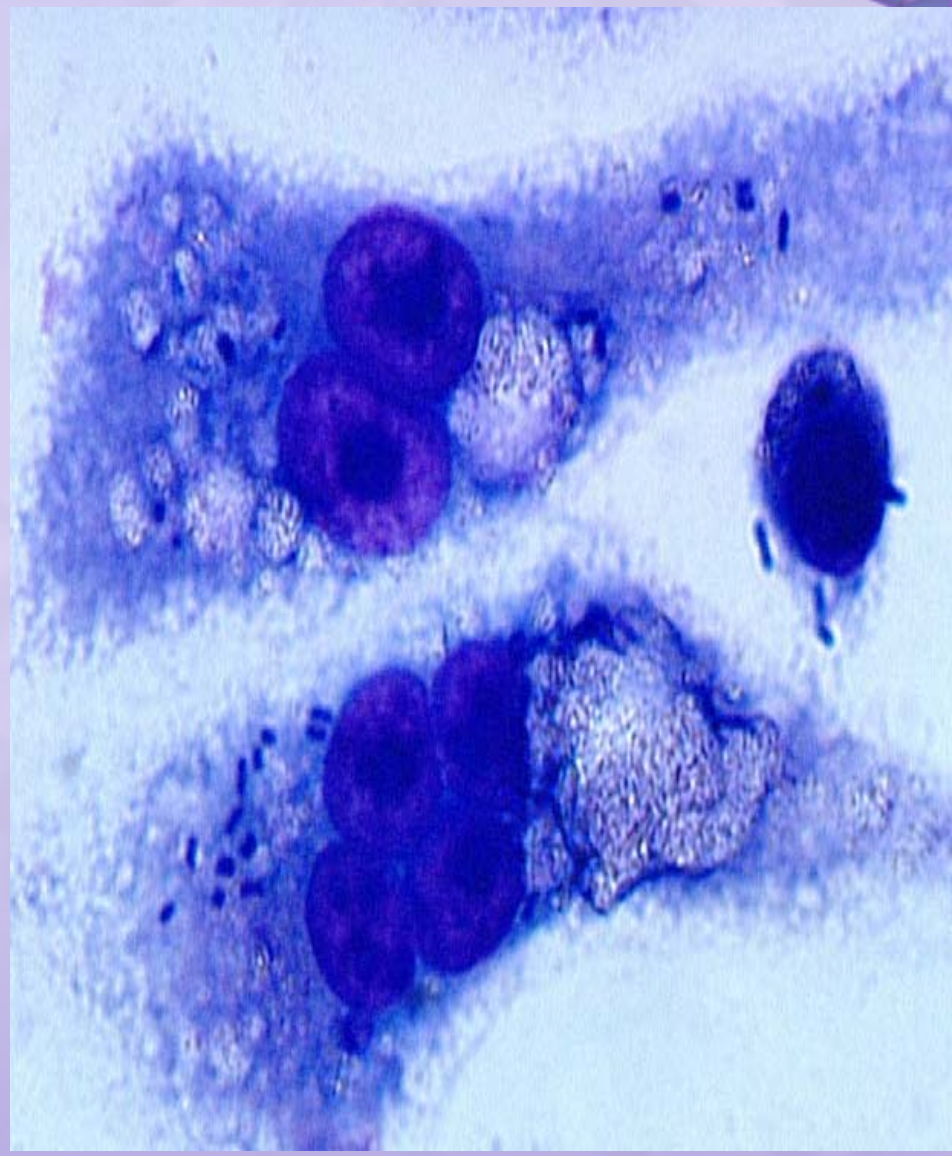
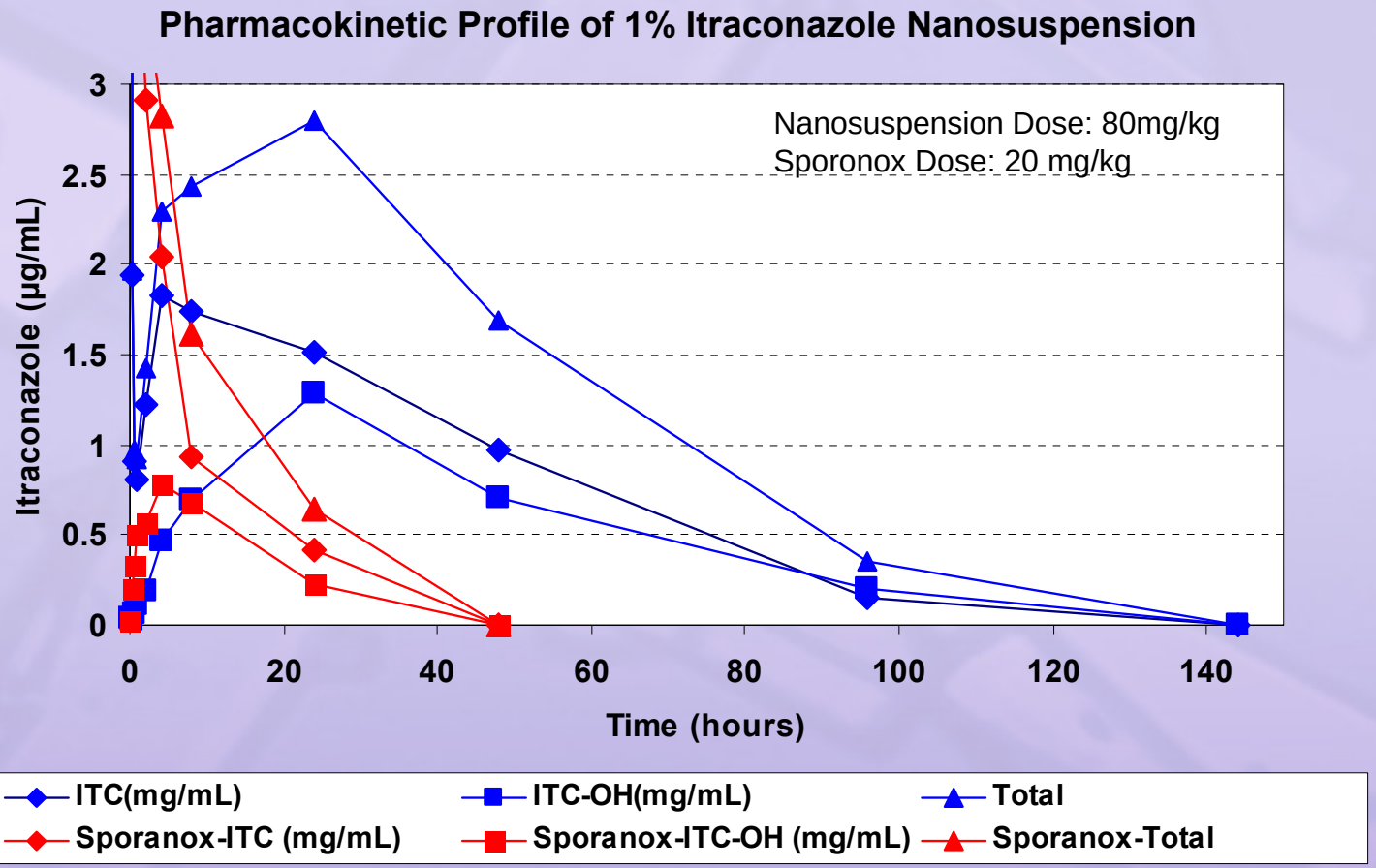
PK- IV Depot- Macrophage Sequestration





IV Depot Pharmacokinetics

Extended Release
Microprecipitated IV Itraconazole (1%)



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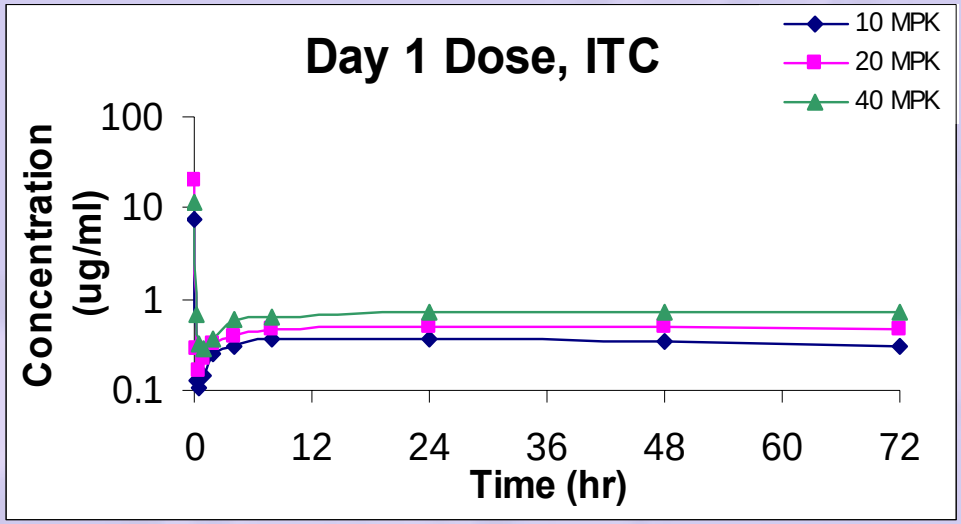
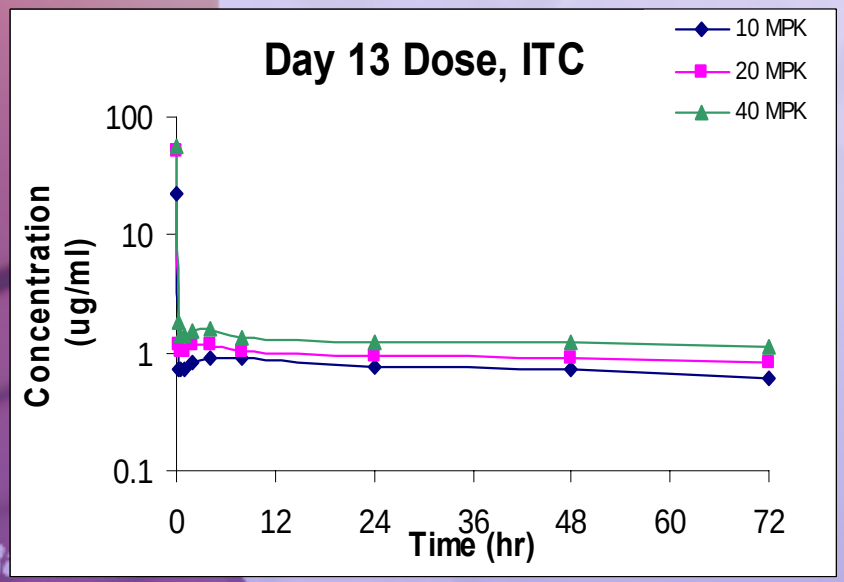
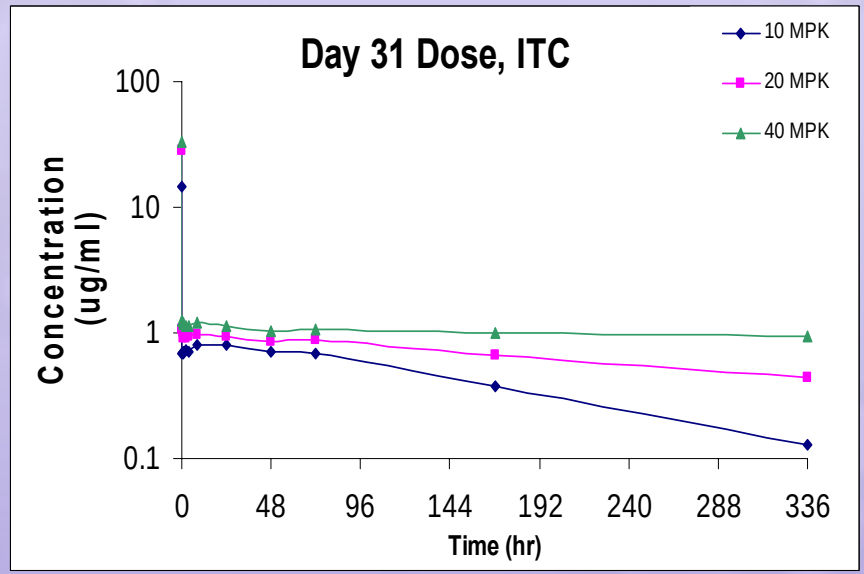
Itraconazole Nanosuspension PK Study in Dogs: 4-week Repeat Exposure IV Q3d

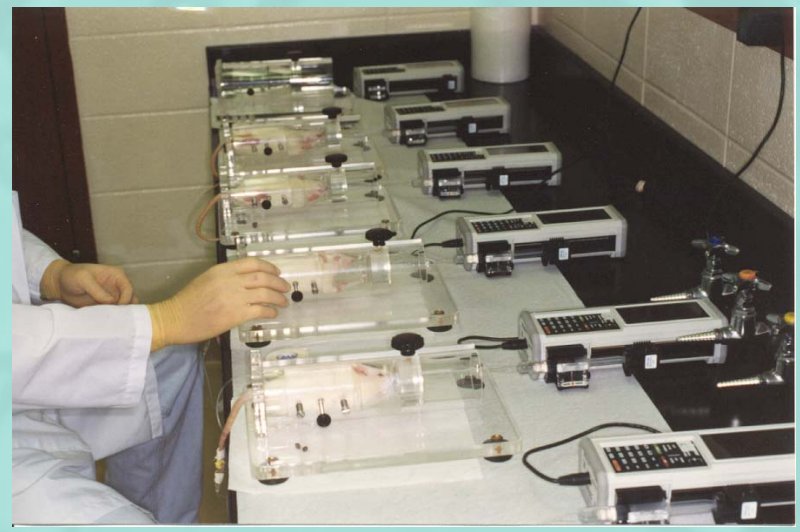


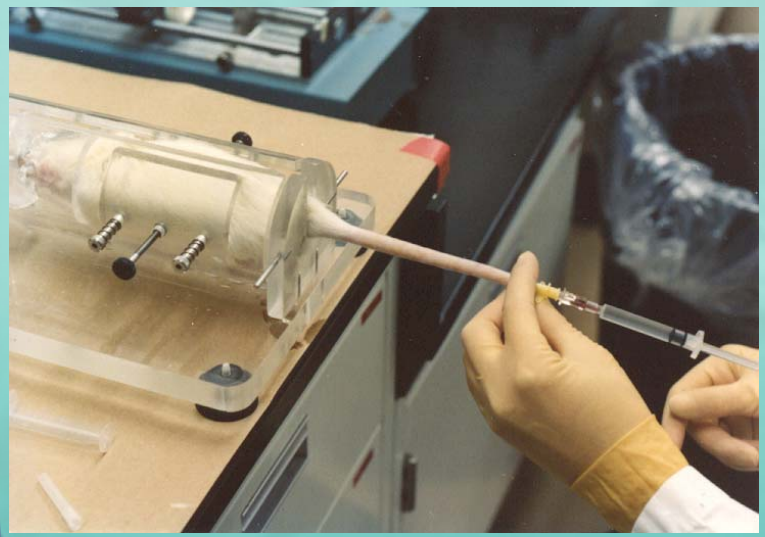


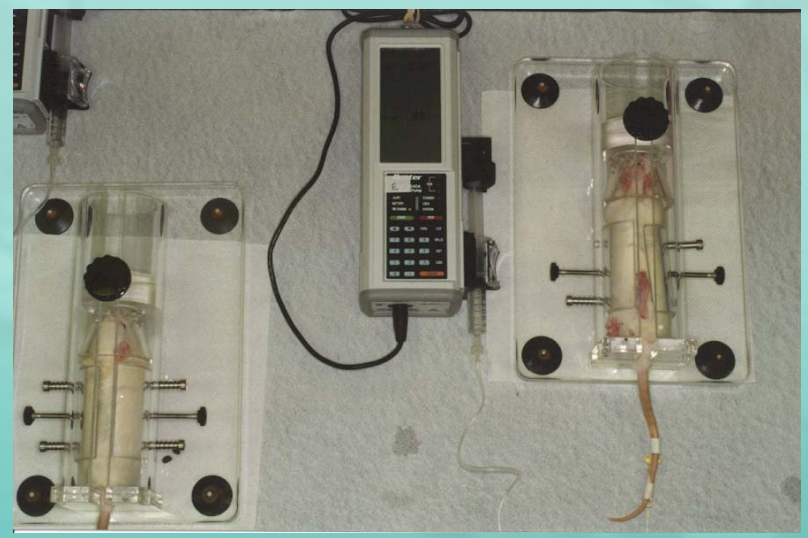


Acute Toxicity Animal Study

Single Dosing Regiment:
Monitor: Weight, Behavior, Appearance of Test Subject, Urine, and Feces
Necropsy: Gross Changes in Organs







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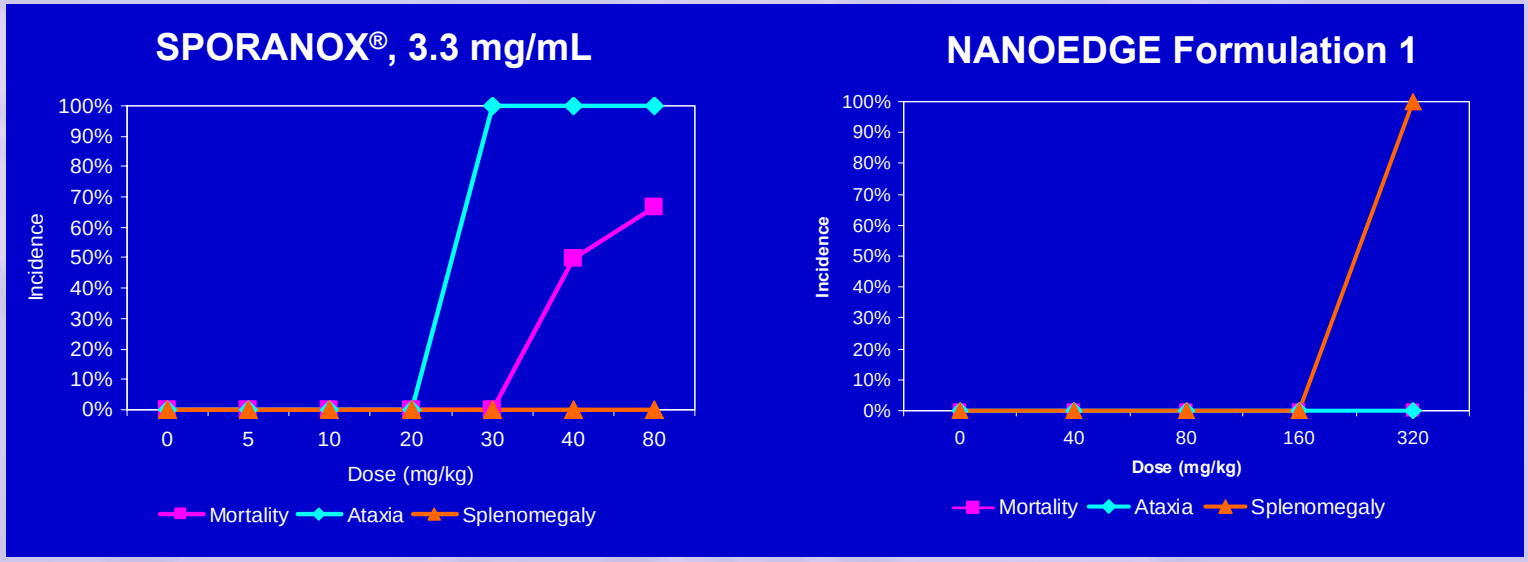
Acute Toxicity

1% Itraconazole Formulation



- **Commercial product**
  - Itraconazole : 2-hydroxypropyl-β-cyclodextrin complex (Sporanox® IV, Janssen Pharmaceuticals).
- **LD_{50} in rats:**
  - Sporanox: <40 mg/kg
  - NANOEDGE: >320 mg/kg (above the highest dose studied)

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Acute Toxicity

Itraconazole Formulations

Methods: Body weight, gross observations, day 7 gross necropsy

Formulation Composition	MTD (mg/kg)	NOEL (mg/kg)
Sporanox® IV 13% cyclodextrin	20	20
Poloxamer, glycerol, bile salt	320	80
Phospholipid, glycerol	320	80

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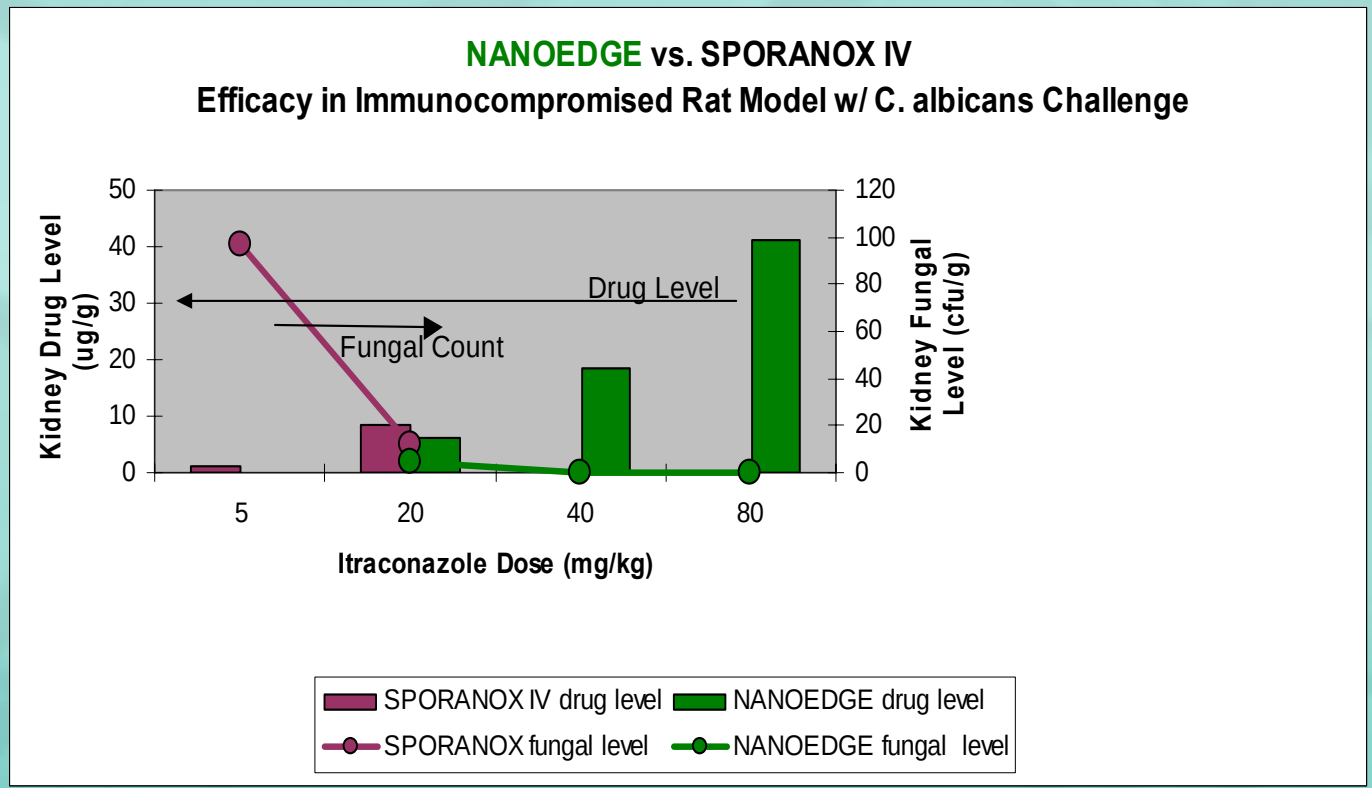
Slowly Dissolving Nanosuspension Enhances Efficacy



Elevated Target Organ Drug Levels via Slowly Dissolving Nanosuspension

PROCEDURE

Animal Dosing: Single 80 mg/kg IV NANOEDGE itraconazole bolus, sacrifice at 48h.

Tissue Sampling: 250 mg of each tissue was combined with 1.1 mL, 99:1 acetonitrile: H_3PO_4

Digestion: BOSTON BAROCYCLER Pressure Cycling Technology BAROCYCLER NEP2017 ,35 kpsi, 5× 1 min cycles at 4 °C

Recover crude lysate and transfer maximum amount of solution to 1.5 mL microcentrifuge tubes for HPLC analysis

Organ	Nanosus-pension (µg/g)	Solution (µg/g)	Enhance-ment Factor
Brain	0.43	0.022	19
Kidney	3.98	0.29	14
Lung	12.20	0.14	87

The brain/kidney ratio is 0.1. This is in generally good agreement with the ratio observed for SPORANOX. ["The Pharmacokinetics of Itraconazole in animals and man", Recent Trends in the Discovery, Development and Evolution of Anitfungal Agents, 1987]The absolute brain concentration is however 20x higher for NANOEDGE, than for the SPORANOX experiment. The SPORANOX experiment was run as organ analysis 24h after a single oral dose of 10mg/kg, while the NANOEDGE was run as 48h after a single IV injection of 80mg/kg.

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Efficacy -Resistant Strain

Hypothesis: Will greater drug loading, attendant with nanosuspension injection, permit treatment of what are currently considered itraconazole-resistant C. *albicans* infections?

Strain: C. *albicans* strain c43 (ATCC number 201794) MIC_{80}= 16 μg/ml for SPORANOX itraconazole; 8-16 for VFEND, and 0.1 for CANCIDAS

Host: Immunocompromised rat (prednisolone daily).

Treatment: 24h post-innoculation, continued for 10 days. q2d with 20, 40, or 80mg/kg NANOEDGE itraconazole nanosuspension. No treatment arm, SPORANOX (10 mg/kg qd)

Measures: Body weight, survival and kidney cfu/g were assessed

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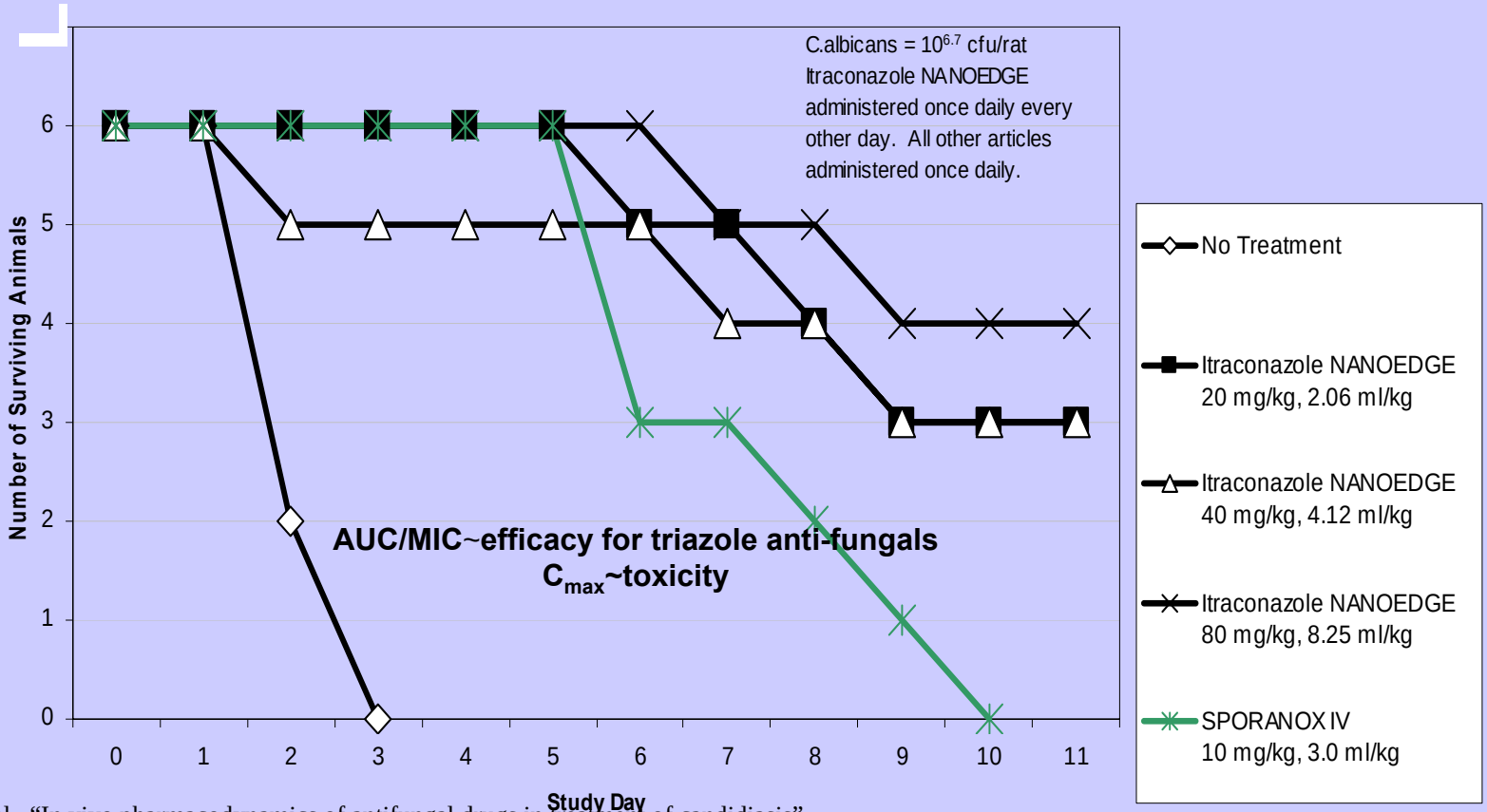
Survival Curves - Resistant Strain

Mortality/Moribundity Profile after Daily or Every Other Day Dosing With Antifungal Drugs for 10 Days in Rats Systemically Infected with Itraconazole Resistant C. *albicans*

- 80 mg/kg NANOEDGE formulation resulted in greater survival than did SPORANOX IV (p<.02)
- The combined groups of 20, 40, and 80 mg/kg NANOEDGE formulation resulted in greater survival than did SPORANOX IV (p<.01)



D. Andes, et al., "In vivo pharmacodynamics of antifungal drugs in treatment of candidiasis", Antimicrob Agents and Chemotherapy, Apr 2003, 1179-1186.

Optimized PK for Antifungal Therapy

- **Efficacy of azole antifungal agents is determined by AUC (ref 2) but toxicity is determined by peak levels**
- **Hence, optimum PK would maximize AUC and minimize peak values, yielding constant, sustained kinetics**
- **NANOEDGE itraconazole nanosuspension approximates this ideal PK, utilizing the MPS as an IV depot**
- **Lower toxicity permits higher dosing, enabling higher tissue drug levels, and lower colony counts. Brain levels are increased 20x**
- **This permits attaining zero colony counts for some susceptible organisms**
- **Efficacy toward itraconazole-resistant fungal strains has been demonstrated by reduced colony counts, increased survival, and decreased body weight loss**

•Ref. 1: J. Heykants, et al., “The Pharmacokinetics of Itraconazole in animals and man", Recent Trends in the Discovery, Development and Evolution of Anitfungal Agents, R. A. Fromtling (Ed) 1987.

•Ref. 2: D. Andes, et al., “In vivo pharmacodynamics of antifungal drugs in treatment of candidiasis”, Antimicrob Agents and Chemotherapy, Apr 2003, 1179-1186.

$T_{1/2}$ of Cell-cycle Sensitive Oncolytic Is Dramatically Increased by NANOEDGE Formulation in Cynomolgus Monkey

Dose [mg/kg]	$T_{1/2}$ [h]	AUC [(ng/ml)h]	C_{max} [ng]
0.2 sol.prop.	1.85	319	401
5.0 **NANOEDGE**	26.7	6374	796

IV administration

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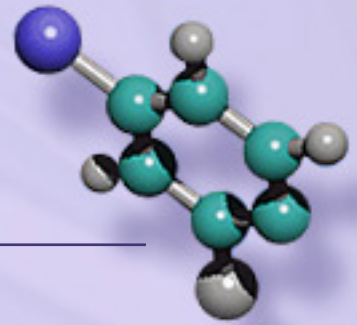
NANOEDGE

Toxicology



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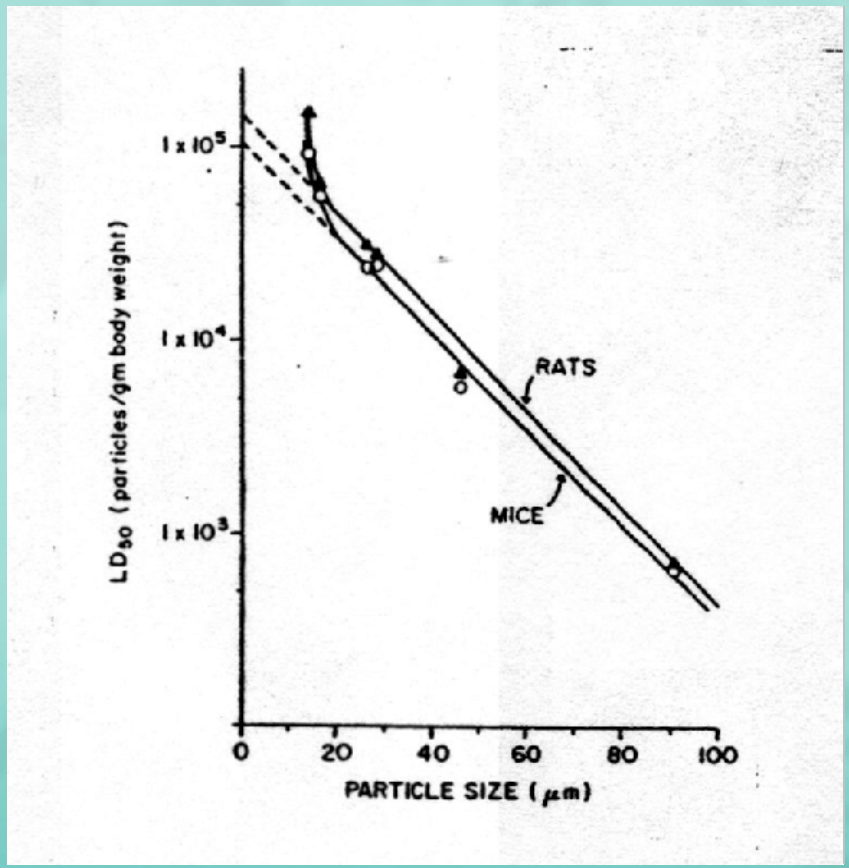
LD_{50} Vs. Particle Size

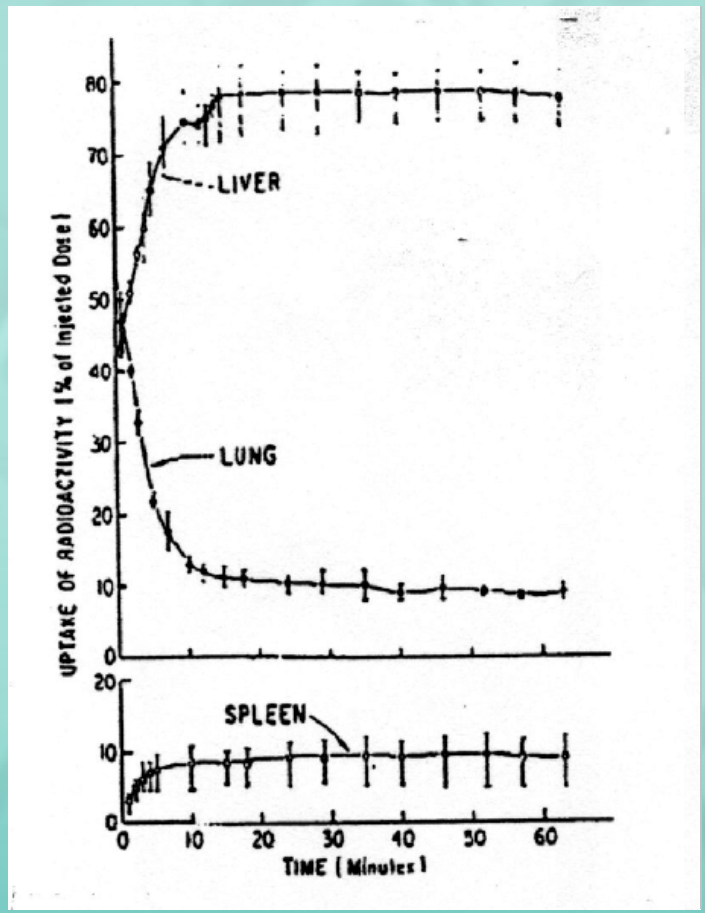
Relationship between particle size (µm) and LD_{50} (particles per gram body weight) in mice and rats for particle diameter 13.7µm to 91µm.



MA Davis and RA Taube.Pulmonary perfusion imaging: acute toxicity and safety factors as a function of particle size. J. Nucl. Med 19:1209-1213 (1978).

Distribution - Microparticles

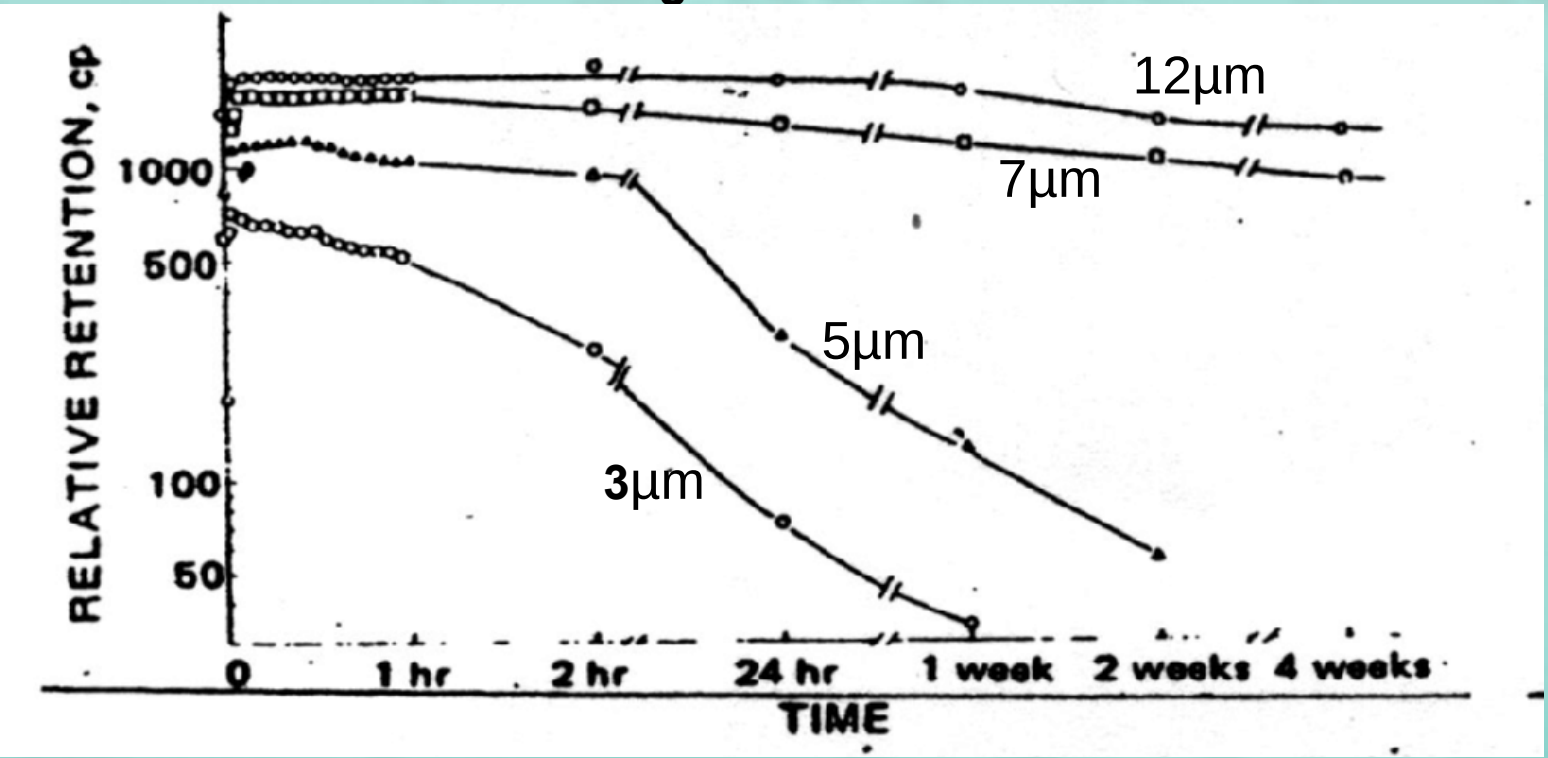
Distribution pattern of ^{141}Ce labelled 3.2µm spheres in the rat following IV injection, by rapid frame sequential scanning.



RA Yokel, JP. Sabo, GH Simmons and PP. DeLuca. Acute toxicity of latex microspheres. Toxicol. Lett. 9:165-170 (1981).

Distribution - Microparticles

Relative retention and average time dependence of microspheres in beagle dog lungs following IV administration.



M Kanke, G Simmons, D Weiss, B Bivens and P DeLuca. Clearance of ^{141}Ce labelled microspheres from blood and distribution in specific organs following intravenous and intraarterial administration in beagle dogs. J. Pharm. Sci 69:755-762 (1980).

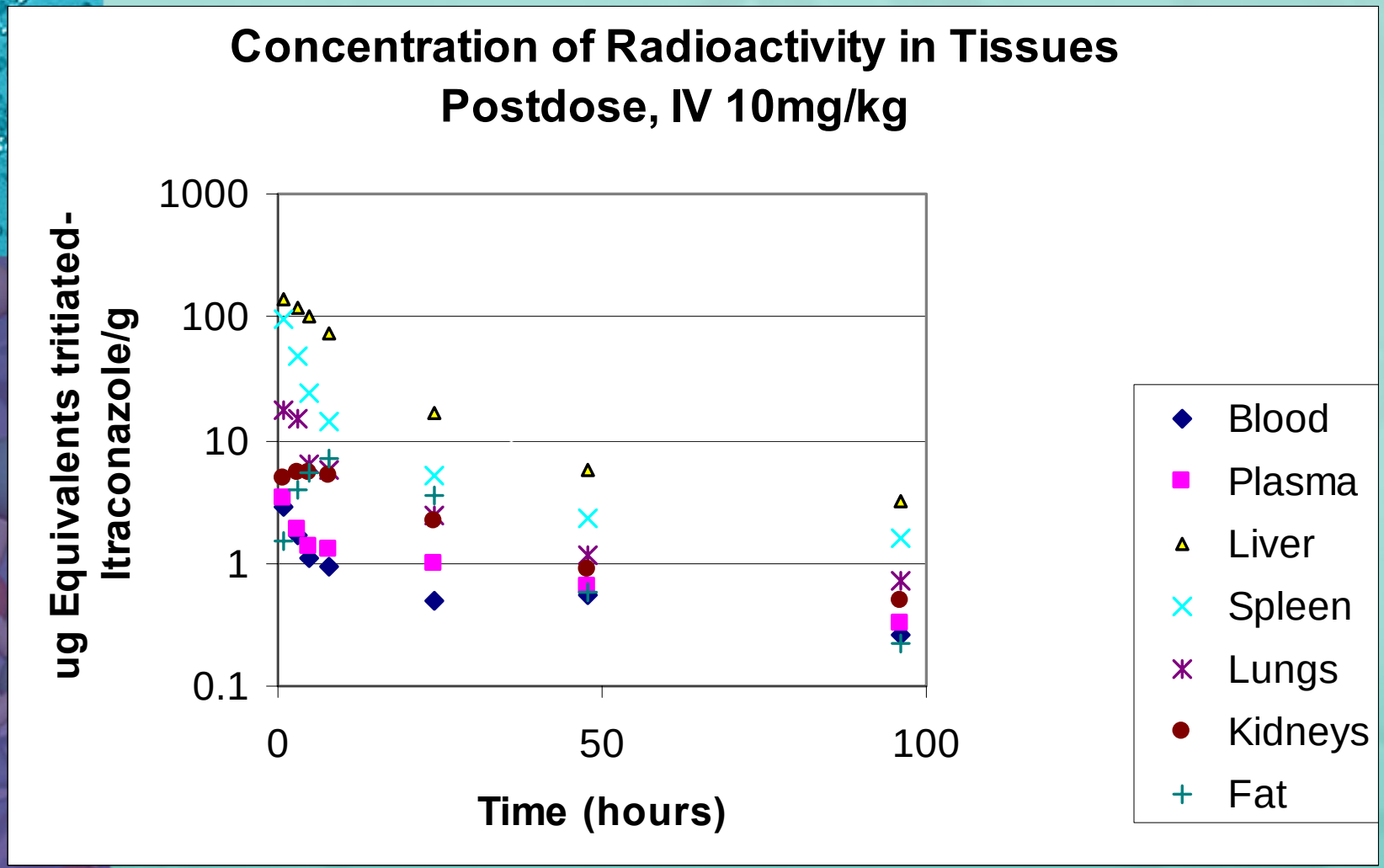
Distribution - Microparticles

Summary of Particle Distribution and Incidence in Tissues of Animals Surviving for the Time Indicated (number of animals per group of 5 in which particles were found in indicated organ)

Part. Size	Dose Part./kg	1 hour				1 day				7 days							28 days				
		Lung	Heart	Liver	Spleen	Lung	Heart	Liver	Spleen	Lung	Heart	Kidney	Spleen	Brain	Pancrs	Liver	Lung	Heart	Brain	Liver	Spleen
0.4	10^3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2×10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4×10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	0	1*
4	10^3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	2×10^4	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
	4×10^5	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0
	8×10^6	2	1	1	2	0	0	2	1	0	0	0	2	0	0	2	0	0	0	1	1
10	10^3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2×10^4	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	4×10^5	5	0	0	0	5	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0
	8×10^6	4	1	2	0	5	0	0	0	5	1	1	1	1	1	0	5	0	0	1	2
40	10^3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2×10^4	2	0	0	0	3	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
	4×10^5	5	2	1	0	5	1	0	0	5	0	0	0	0	0	0	5	2	0	0	0
	$8 \times 10^{6**}$	1	1	0	0	2	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0

R Gesler,et al. The biological effects of polystyrene latex particles administered intravenously to rats-A collaborative study., Bull. Parent. Drug. Assoc. 27(3):101-113, 1973.

Distribution - Nanoparticles



Distribution - Microparticles

- **Particles smaller than 7μm escape from their initial lung sequestration rather quickly[1] within minutes, and**

- **Undergo phagocytosis by the fixed macrophage cells of the liver and spleen[2]**

- **This is a normal behavior of these cells, when presented with microbes, and foreign material of size less than about 8 microns. In several rat studies, no evidence of an inflammatory reaction was found.**

- **Apparently safe levels of 8 x 10^6 particles/kg of size 0.4μm to 10μm or 4x 10^5 particles/kg of particles of size 40μm could be administered**

1] Singer, J.M., J. RES. Soc 6:561, 1969 found that an initial accumulation of 0.23um particles in lung tendeed to disappear between 10 to 60min, as accumulation in liver and spleen increased
[2] Gesler, R.M., et al, The biological effects of polystyrene latex particles administered intravenously to rats-A collaborative study., Bull. Parent. Drug. Assoc. 27(3):101-113, 1973.
Schroeder, H.G., Distribution of radiolabeled subvisible microspheres after intravenous administration to beagle dogs. J. Pharm. Sci 67(4):508-513, 1978.

Maximal Levels Of Injected Particles With Outcomes

Particle Size (μm)	Protocol, Dose/kg	Outcome, Reference
1.3	Bolus, 6 x 10^9	PK study[1]
0.5-1.17	Bolus, 1.6 x 10^{12}	PK study[2]
0.4, 4, 10	Rats, Bolus, 8 x 10^6	Well tolerated[3]
3.4	Dogs, Bolus, 1x 10^{10}	Well tolerated[4]
3.7	Dogs, Repet. Bolus, 2.4x 10^8	Well tolerated[5]
3.4	Dogs, 2min Bolus,8.9x10^7	Well tolerated[6]
2.0-4.5	Humans, Bolus, 9.9 x 10^7	Optison, approved product[7]
0.4μm	Rats, Bolus, 2.5 x 10^{12}	Well tolerated[8]
0.4 μm	Dogs, Infusion, 1.3 x 10^{12}	Well tolerated[9]

[5] Schroeder, H.G., id
6] Kanke, M., id.
7] Optison, id
[8] Baxter Healthcare, itraconazole nanosuspension.
[9] Baxter Healthcare, itraconazole nanosuspension

DRAXIMAGE™ MAA
Kit for the Preparation of
Technetium Tc 99mAlbumin
Aggregated Injection
DIAGNOSTIC
For Intravenous Use

Macroaggregated Albumin

DESCRIPTION
The kit consists of reaction vials which contain the sterile, non-pyrogenic, nonradioactive ingredients necessary to produce Technetium Tc 99m Albumin Aggregated Injection for diagnostic use by intravenous injection.
Each 10 mL reaction vial contains 2.5 mg of albumin aggregated, 5.0 mg of albumin human, 0.1 mg of stannous chloride dihydrate

The aggregated particles are formed by denaturation of human albumin in heating and aggregation process. Each vial contains 4-8 million particles. By light microscopy, more than 90% of the particles are between 10 and 70 micrometers, while the typical average size is 20 to 40 micrometers; none is greater than 150 micrometers.

ACTION
Immediately following intravenous injection, more than 80% of the aggregated albumin is trapped in the pulmonary alveolar capillary bed. The imaging procedure can thus be started as soon as the injection is complete. Assuming that a sufficient number of radioactive particles has been used, the distribution of radioactive aggregated particles in the normally perfused lung is uniform throughout the vascular bed, and will produce a uniform image. Areas of reduced perfusion will be revealed by corresponding decreased accumulation of the radioactive particles, and are imaged as areas of reduced photon density. Organ selectivity is a direct result of particle size. Below 1-10 micrometers, the material is taken up by the reticuloendothelial system. Above 10 micrometers, the aggregates become lodged in the lung by a purely mechanical process. Distribution of particles in the lungs is a function of regional pulmonary blood

Macroaggregated Albumin

The aggregated albumin is sufficiently fragile for capillary micro-occlusion to be temporary. Erosion and fragmentation reduce the particle size, allowing passage of the aggregates through the pulmonary alveolar capillary bed. The fragments are then accumulated by the reticuloendothelial system.

DOSAGE AND ADMINISTRATION

The recommended intravenous dose range for the average (70 kg) adult patient is 37 to 148 megabecquerels (MBq) (1-4 mCi) of Technetium Tc 99m Albumin Aggregated Injection after reconstitution with oxidant-free Sodium Pertechnetate Tc 99m Injection. **The suggested range of particle numbers for a single injection is 200,000-700,000 with the recommended number being approximately 350,000.** Depending on the activity added and volume of the final reconstituted product, the volume of the dose may vary from 0.2 to 1.4 mL.

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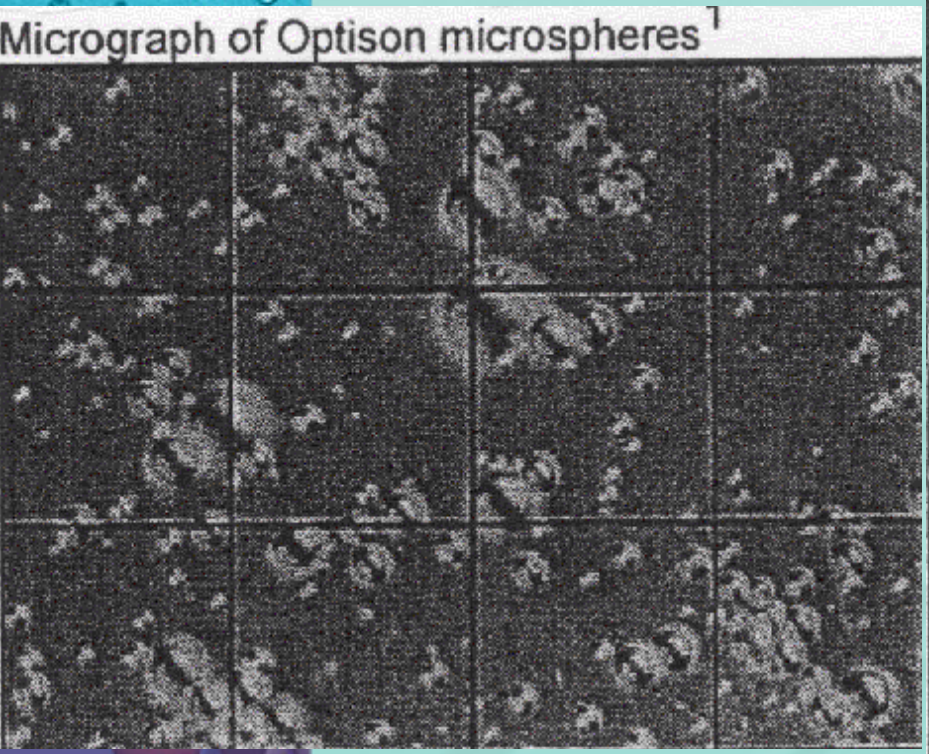
Optison

Micrograph of Optison microspheres[1]



Microsphere concentration	$5.0\text{-}8.0 \times 10^8$
Microsphere mean diameter	2.0-4.5 µm
Size distribution	93% less than 10 µm
Maximum diameter	32.0 µm
pH	6.4-7.4
Osmolarity	Iso-osmolar
Sterility	Sterile
Pyrogenicity	Non-pyrogenic

The maximal number of particles that can be injected for this approved product is therefore 7×10^9 , or 9.9×10^7/kg

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Particle Size Limits

By way of comparison, the USP <788> microscopic test for particulate matter in SVP intravenous solutions permits 300 particles >25μm. Therefore, conformance of IV nanosuspensions to the USP <788> standard will ensure safety factors of 1000 relative to current practice of pulmonary and cardiac perfusion of radio- and echo-graphic particulate injections.

Effects upon MPS

If the MPS becomes overloaded by phagocytic activity, then reticuloendothelial blockage could occur[1]

- **but only if the phagocytic overload is continued and heavy [2,3]**
- **because these cells can digest all biodegradable substances[4]**

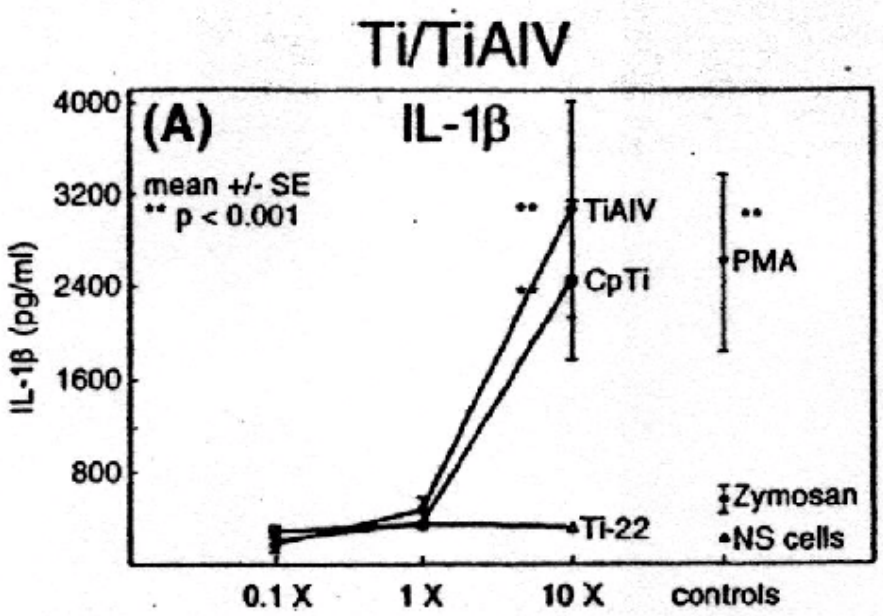
- **Compared with inert particles[5] such as metal, plastic and latex microspheres, a metabolizable drug nanoparticulate will be processed through the phagolysozomes of the macrophages much faster**

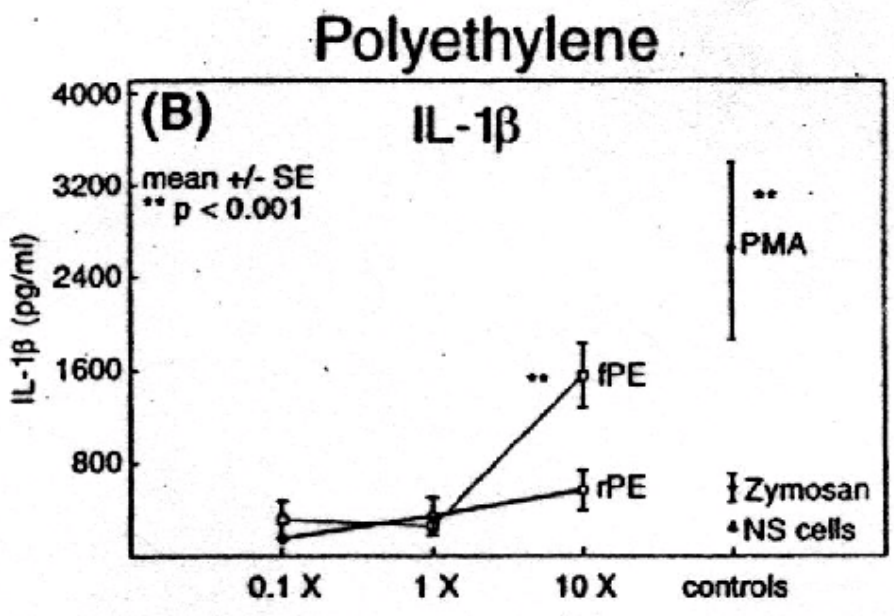
- **This poses much less of burden upon the macrophages and enables them to cycle faster**

1. Daemen, T. Liposomal doxorubicin-induced toxicity:depletion and impariment of phagocytic activity of liver macrophages. Int. J. Cancer. 61: 716-721 (1995)
2. VanEtten, E.W.M., Administration of liposomal agents and blood clearance capacity of the mononuclear phagocyte system. Antimicrobial Agents and Chemotherapy, 42: 1677-1681 (1998)
3. Bakker-woudenberg, I.A.J.M., Administration of liposomal agnets and the phagocytic function of the mononuclear phagocytic system. Int. J. Pharm. 162: 5-10 (1998).
4. Pesko, L.J., Physiological consequences of injected particulates in Liquid- and Surface-Forne Particle Measurement Handbook, eds. Knapp, J.Z., Barber, T.A., and Lieberman, A, Marcel Dekker, New York, 1996.
5. Kreuter, J., Nanoparticles in Colloidal Drug Delivery Systems, ed. Jorg Kreuter, Marcel Dekker, Inc., New York, 1994.

Effects upon MPS

Model: Human peripheral monocytes
Challenged: with TiAlV, Ti, PE particles of size<1µm, on basis of surface area ratio
Monitoring: inflammatory mediators: IL-1, IL-1β, IL-6, and PGE-$_2$
Results: A No Observable Effect Dose was identified.





AS Shanbhag, JJ Jacobs, J. Black, J. Galante, T. Glant, Human monocyte response to particulate biomaterials generated in vivo and in vitro. J. Orthopaedic Research 13:792-801 (1995).

Effects upon MPS

- **Clinically, administration of liposomal doxorubicin (a cytotoxic agent and macrophage targeter) did not result in more frequent opportunistic infections in patients with AIDS-related Kaposi's sarcoma compared to patients treated with combinations of doxorubicin, bleomycin and vincristine[1]**
- **This probably results from the compensatory increase in macrophage numbers and activity when subjected to high phagocytic loads[2]**

1. Bogner, J.R., Liposomal doxorrubicin in the treatment of advanced AIDS-related Kaposi sarcoma. J. Acquir. Immune. Defic. Syndr. 7:463-468 (1994)

2.Daemen, T, et al, Toxicity of docorubicin entrapped within long-circulating liposomes. J. Cont. Rel. 44:1-9 (1997).

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Repeat Dose Toxicology Studies

- **The safety of injected nanosuspensions has been confirmed in several subchronically dosed toxicology studies[1,2] For the most part, clinical chemistry, hematology, and body weight data did not differ from controls.**

Mononuclear-Phagocytic System Expansion:

Histologically, particles were found in a dose dependent manner in the MPS cells of the liver, spleen, and lymph nodes, consistent with their physiological role, as was found by previous studies.

- **–Reversibility was demonstrated**
- **–Sequelae at high doses can be clinically monitored (in rat)**

1.White, R.D., et al, Intravenous safety study in rats given paramagnetic, polystyrene beads with covalently bound sheep anti-mouse immunoglobulin G (IgG). J. Amer. Coll. Tox. 14: 251-265 (1995).

2. Baxter Healthcare, 28 day chronically dosed rat and dog studies involving itraconazole nanosuspensions

Approved Product: Abraxane

130nm nanoparticles of paclitaxel in albumin matrix

Phase III: 460 breast cancer patients

- **Taxol® protocol: 175mg/m^2 / 3 hour, + premedication with steroid and antihistamines**
- **Abraxane protocol: 260mg/m^2 / 30 min, without premedication or G-CSF support**

- **Abraxane ® toxicity was no worse: no hypersensitivity reactions; neutropenia decreased; while neuropathy increased somewhat.**
- **Abraxane ® efficacy: higher tumor response rate vs. paclitaxel (33% vs. 19%) and a longer time to tumor progression (21.9wk vs. 16.1wk)**

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NANOEDGE

Manufacturing

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Equipment Design Considerations

- Size reduction & reproducibility
- Reliability & wear
- Fluid path isolation
- Clean-in-place, steam-in-place
- Operating pressure
- Cooling capacity
- Scalability
- Aseptic barrier isolation
- Portability & fluid path chg components
- In-process seal change-out

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cGMP Pilot Line – 20L Batch Capacity

Equipment Scale

Primary Use	Batch size	# of machines
feasibility	1 mL	1
optimization	10 mL	10
pilot	100 mL	7
preclin supply	1 L	3
clinical	50 L	3
commercial	500 L	1

All processes are barrier isolator based

Cleaning methods established for all drugs

Client dedicated fluid contact parts for all cGMP work

Up to 15% drug concentration

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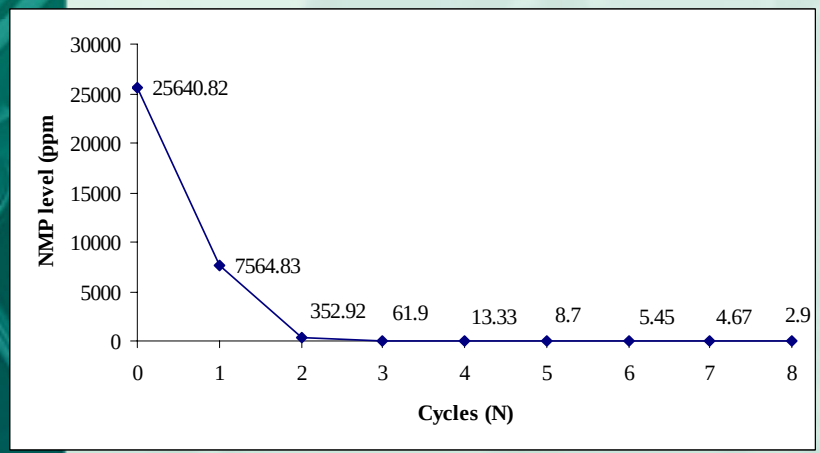
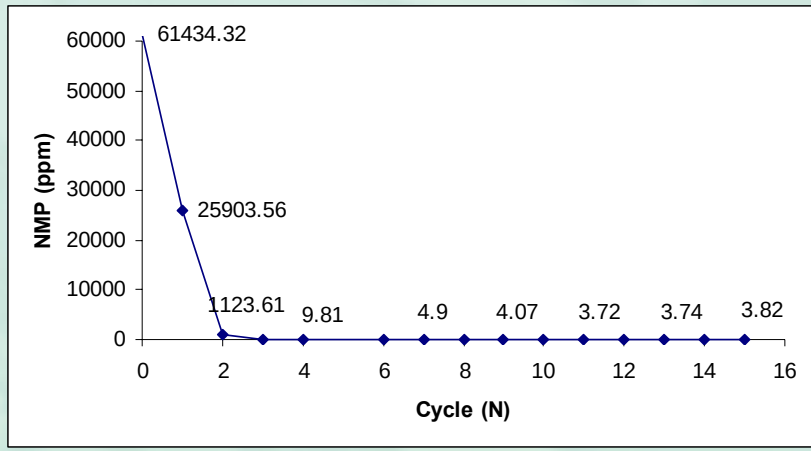
NMP Solvent Removal Scale-Up

NMP is reduced upon successive cycles, comparably at different scales

200 mL batch



10 L batch



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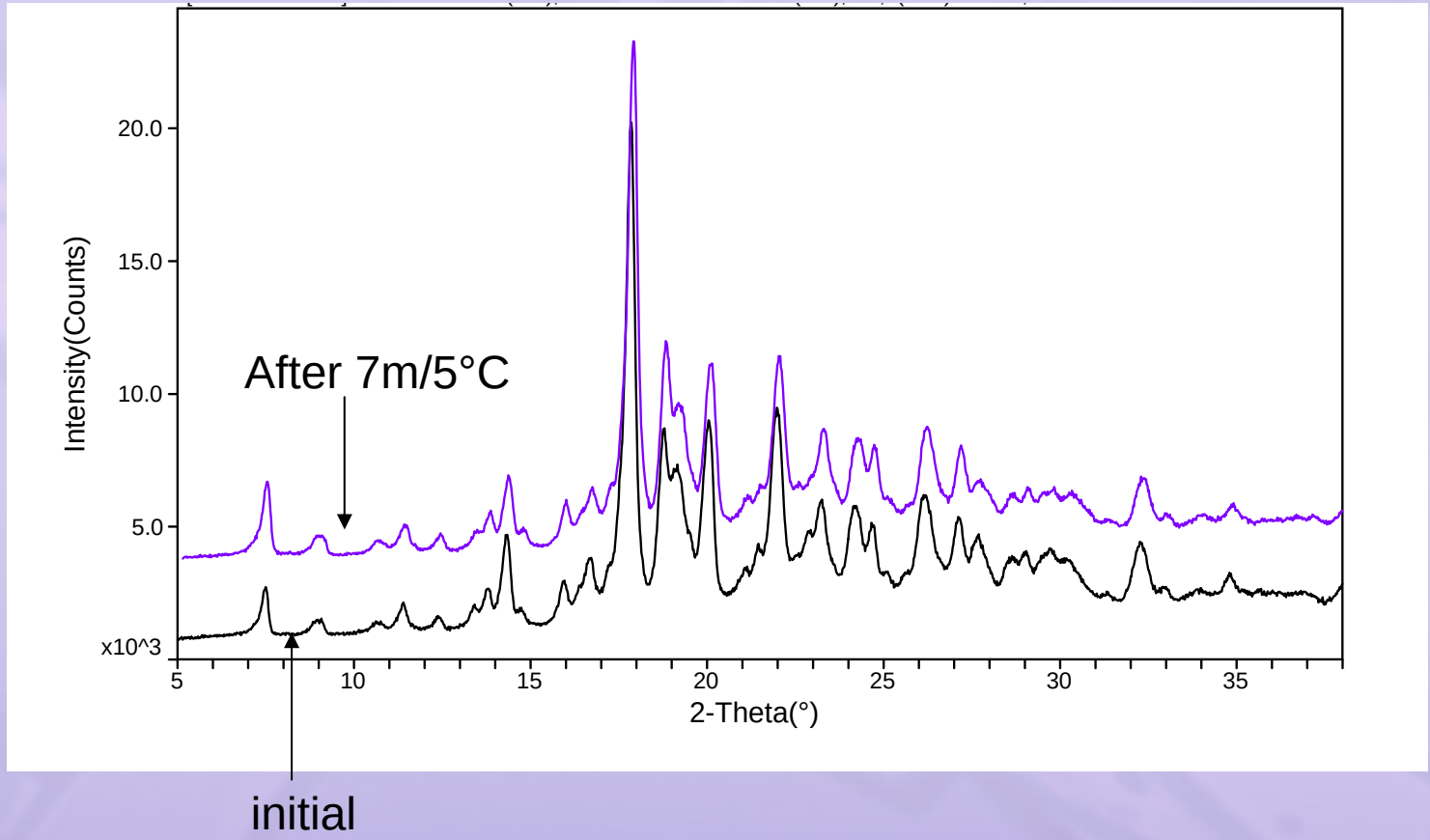
Stability of Less Stable Polymorph

Stability demonstrated over 7 months at 5°C



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Conclusions

- Nanosuspensions: generally applicable approach to the pre-clinical testing of water-insoluble drug candidates
- High drug loading without interfering excipients
- Can modulate the disposition of drug in the body and potentially improve the safety & efficacy profile of drug
- Current characterization methodology is applicable to nanosuspensions with appropriate adaptation

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