CHAPTER 7

INVIVO STUDIES

- Invivo studies for Rifampicin Niosomes
- Invivo studies for Gentamicin Sulphate Niosomes
- Results
- Discussion

7.1 Experimental

7.1.1 Selection of animals

Albino rats (Wister strain) were selected for the study because of the ease in availability, handling and sampling. The rats selected for the study were weighing around 250gms. A group was designed to have three rats at each time intervals for each formulation The number of male and female rats in a group at the same time interval among all the formulations were kept constant to achieve homogenicity. The rats were obtained from Tetrex biological supply House, Madurai in Tamil Nadu

7.1.2 Calibration curves for the estimation of Rifampicin in Lung, Liver, Kidney and Serum

Albino rats fasted for an overnight, were sacrificed (hammering it on the head) and the lung, liver and kidney were separated, weighed and homogenized with 15ml Phosphate Buffer Saline pH 7.4. The organ homogenate was chilled and centrifuged in refrigerated centrifuge at 7375g for 20 mins. Supernatants separated, formed blank for the respective analysis of the drug. 1ml of blank supernatant from each of the organ was spiked with 25mcg of Rifampicin dissolved in 0.1ml of Phosphate buffer saline pH 7.4. Then 2ml of tungstic acid was added, vortered, for 30 sec. and centrifuged. The drug was extracted with 30% Methanol and (1000µl) filtered through membrane filter (Pore size 0.2µl).

Different volumes of filtrates equivalent to the amount of drug given in paranthesis Viz., 0.8µl (0.02µg), 1.6µl (0.04µg), 2.4µl (0.06µg), 3.2µl (0.08µg) and 4.0µl(0.1µg) were injected into HPLC column and the drug content was estimated as described in section 5.1.1.2, chapter 5 .

Calibration curve for estimation of Rifampicin in Serum

6 ml of blood sample was collected from retero-orbitary sinus puncture using capillary tube and kept for 10 mins. Serum (1 ml) was separated from the blood and spiked with 25mcg of Rifampicin contained in 0.1ml of Phosphate Buffer Saline pH 7 4. Then 2ml of tungstic acid was added, vortexed for 30 sec and centrifuged. The drug was extracted with 30% Methanol (1000µl) and filtered through membrane filter (pore size 0.2µl). Volumes of filtrate containing quantity of drug given in paranthesis viz., 0.8µl (0.02µg), 1.6µl (0.04µg), 2.4µl (0 06µg) and 4.0µl(0.1µg) were injected into the HPLC column, and the drug content was estimated as described in section 5.1.1.2, chapter 5

7.1.3 INVIVO ORGAN DISTRIBUTION STUDY OF RIFAMPICIN FOLLOWING INTRAVENIOUS ADMINSTRATION OF DRUG SOLUTION OR NIOSOMAL FORMULATION

Of niosomal suspension, 1ml equivalent to 5mg of Rifampicin was injected into the rat via the caudal vein into fifteen albino rats each weighing about 250g. . After administration 3 rats were killed at the periodic intervals of 1hr, 3rd hr, 6th hr, 12th hr and 24th hr. Blood sample was collected from retero-orbitory sinus puncture. Organs were isolated,

weighed and macerated with 30% Methanol. 1ml of homogenate was taken and 2ml of tungstic acid was added, vortexed for 30 sec, centrifuged. Drug was extracted using 30% Methanol from macerated tissues by multiple washings and centrifuged at 7357g for 20 mins. The drug contents were calculated from the area of chromatographic peak using the calibration curve given in the analytic method in section 5 1.1.2, chapter 5

7.1.4 PHARMACOKINETIC STUDY OF NIOSOMES CONTAINING RIFAMPICIN.

7.1.4.1 Estimation of Rifampicin in Serum.

Collected blood sample was kept for 10 mins Then 1ml of serum was separated from the blood and 2ml of tungstic acid was added to precipitate the protein, vortexed for 30 sec, centrifuged and the drug was extracted using 30% Methanol from serum by multiple washing. All washings were pooled and centrifuged at 7375g for 20 mins. The drug contents were estimated from the area of chromotagraphic peak using the calibration curve.

7.1.5 IN-VIVO STUDY FOLLOWING INTRATRACHEAL ADMINISTRATION OF NIOSOMES CONTAINING RIFAMPICIN.

Method:

Albino rats, 180-250gm were anaesthetized with 50mg kg^{-1} of sodium pentabarbitone intraperitoneally[161]. The trachea was cannulated with a 9cm length of PE-200 polyethylene tubing, with the tip of the

cannula positioned approximately at the tracheal bifurcation. This cannula served to guide the introduction of a 9.2 cm length of PE-50 polyethylene tubing, attached at one end to a 250µl glass Hamilton Syringe. The free drug or niosomes were administered to the animals by intratracheal instillation using the syringe. Each rat received 5mg of drug (1ml) niosomal suspension, followed by 50µl of 0.85% Nacl(Saline) to rinse the syringe and tubing. Cannula was removed and the tracheal incission was sutured, for those rats to be sacrificed after 1hr, 3rd hr, 6th hr, 12th hr and 24th hr.

The lung was isolated, macerated and added with 2ml of tungstic acid to precipitate protein. The drug was extracted by using 30% Methanol, from macerated tissues by multiple washing and centrifugation at 7375g for 20 mins. The supernatant from successive extracts of an organ from each rat was collected and the drug content was analysed by High performance liquid chromatography as described in the section 5 1.1.2. Drug content in blood serum was also estimated as described in section 5.1.1.2, chapter 5 .

7.1.6 INVIVO ORGAN DISTRIBUTION STUDY OF GENTAMICIN SULPHATE

7.1.6.1 Calibration Graphs Of Gentamicin Sulphate In Lung, Liver, Kidney And Blood

Albino rats fasted overnight, were sacrificed (hammering it on the head) and lung, liver and kidney were separated, weighed and homogenized with 15 ml Phosphate Buffer Saline. The organ

homogenate was chilled and centrifuged in refrigerated centrifuge at 7375g for 20mins. Supernatants separated formed blank for the respective analysis of the drug. 1ml of blank supernatant from each of the organ was spiked with 2000mcg of drug dissolved in 1ml of distilled water. Aliquots of 0.05ml (50mcg), 0.1ml(100mcg), 0.15ml (150mcg), 0 3ml(300mcg), 0.4ml(400mcg) and 0.5ml(500mcg) were taken and it was made into 10ml with distilled water. From this, 1 ml of each aliquote was taken and 1ml of ammonia was added and drug was extracted using 30 ml of chloroform. The chloroform layer was separated and reextracted and all portions were combined and the solvent was evaporated. The residue was dissolved in 5ml of water and 5ml of derivatization reagent was added and the drug was estimated as described in the section 5.1.1.3, chapter 5. The blank was prepared in the same manner, except that water was substituted for the sample.

Calibration curve for estimation of Gentamicin Sulphate in Serum

6ml of blood sample was collected from Retero-orbitory sinus puncture using capillary tube and kept for 10 mins. Serum (1 ml) was separated from the blood and spiked with 2000mcg of drug in 1 ml of distilled water. Then 1 ml of 10% Sodium tungstate and 1 ml of 2/3 N sulphuric acid were added to precipitate the serum proteins. Then aliquots of 0.05ml, 0.1ml, 0.15ml, 0.2ml, 0 3ml, 0.4ml and 0.5ml were taken and it was made into 10ml with distilled water. From this, 1 ml of each aliquote was taken and 1 ml Ammonia was added and drug was

extracted by using 30ml of chloroform. The chloroform layer was separated and reextracted and all the portions were pooled and the solvent was evaporated. After complete evaporation, the residue was dissolved in 5ml of distilled water, 5 ml of derivatization reagent was added and drug was estimated by the method described in the section 5 1.1 3, chapter 5.

7.1.7 Invivo Organ distribution Study of Gentamicin sulphate following intravenous administration of drug solution or niosomal formulation:

Of niosomal suspension, 2ml equivalent to 25mg of Gentamicin sulphate was injected through the caudal vein into fifteen albino rats each weighing about 250g. After administration, 3 rats were killed at the periodic intervals of 1hr, 3rd hr, 6th hr, 12th hr, 24th hr. Plain niosomal suspension was injected which was used as blank for study using niosomes containing drug. Distilled water was injected which was used as blank for study using drug solution. Blood sample was collected from Retero-orbitory Sinus puncture. Organs were isolated, weighed and macerated with 15ml of Phosphate Buffer Saline. 1ml of homogenate was taken and 2ml of tungstic acid was added, vortexed for 30 sec and centrifuged. The supernatant was separated and 1ml of ammonia was added and drug was extracted using chloroform. The

chloroform layer was separated and reextracted and all portions were pooled and the solvent was evaporated. After complete evaporation, the residue was dissolved in 5ml of distilled water and drug was estimated spectrophotometrically as described in the section 5.1.1.3, chapter 5.

7.1.8 Pharmacokinetic study of niosomes containing Gentamicin Sulphate

7.1.8.1 Estimation of Gentamicin Sulphate in serum

Collected bloodsample was kept for 10 mins. Then 1 ml serum was separated from the blood and 1ml of 10% sodium tunstate and 1ml of 2/3N sulphuric acid were added, vortexed for 30 sec, and centrifuged. The supernatant was separated and 1 ml of ammonia was added and drug was extracted by using chloroform. The extracted portions of chloroform was separated and all the reextracted portions were pooled and the solvent was evaporated. After complete evaporation, the residue was dissolved in 5 ml of water and drug content was estimated as described in the section 5.1.1.3, chapter 5

7.1.9 Invivo study following intratracheal administration of niosomes containing Gentamicin Sulphate.

Method:

Albino rats, each weighing about 250gm were anaesthetized with 50mg kg-1 of Sodium pentabarbitone intraperitoneally. The trachea was cannulated with a 9 cm length of PE-200 Polyethylene tubing, with the

tip of the cannula positoned approximately, at the tracheal bifurcation. This cannula served to guide the introduction of a 9.2 cm length of PE-50 polyethylene tubing, attached at one end to a 250ul glass Hamilton Syringe. The free drug or niosome entrapped drug was administered to the animals by intratracheal instillation using the syringe. Each rat received 25mg in 2ml niosomal suspension or free drug solution followed by 50µl of 0.85% Nacl (Saline) to rinse the syringe and tubing. Cannula was removed, and the tracheal incision was sutured, for those rats to be sacrificed after 1hr, 3rd hr, 6th hr, 12th hr and 24th hr.

The organ (lung) was removed, macerated in 15ml of Phosphate Buffer Saline 1ml of homogenate was taken and 2ml of tungstic acid was added, vortexed for 30 sec. and centrifuged. The supernatant was separated and 1ml of ammonia was added and drug was extracted using chloroform The chloroform layer was separated and all the reextracted portions were pooled and the solvent was evaporated. After complete evaporation, the residue was dissolved in 5ml of water, and the drug was estimated as described in section 5.1.1.3. The drug content in blood serum was estimated as described in section 5.1.1.3, chapter 5

7.1.10 RESULTS

TABLE No.25

CALIBRATION CURVE FOR RIFAMPICIN IN LIVER, LUNG, KIDNEY EXTRACT AND SERUM

Concentration	PEAK AREA			
	Liver Extract	Lung Extract	Kidney Extract	Serum
0.02	107	1079	90	3852
0.04	216	2121	189	7710
0.06	329	3235	279	12048
0.08	443	4298	368	15691
0.1	565	5400	452	20577
0 12	700	6393	544	23843

TABLE NO.26

INVIVO DISTRIBUTION OF RIFAMPICIN IN VARIOUS ORGANS AND SERUM OF RATS (INTRAVENOUS ADMINISTRATION)

Organs	Lung		Liver		Kidney		Serum	
Time (hr)	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM
1	311 72 (0.112)	1514.35 (0.215)	3.5693 (0.178)	3.859 (0.238)	32.65 (0.205)	8.3 (0.229)	0.9238 (0.125)	0.06342 (0.115)
3	174 44 (0.275)	3156.6 (0.302)	3.337 (0.318)	10.30 (0.214)	103.72 (0.234)	96.52 (0.315)	0.2886 (0.115)	0.0586 (0.205)
6	128.165 (0.148)	2421.83 (0.279)	2.9753 (0.198)	2.80 (0.251)	93.48 (0.188)	89.87 (0.195)	0.13606 (0.218)	0.0578 (0.126)
12	50.875 (0.224)	2228.70 (0.356)	2.1899 (0.150)	1.80 (0.254)	64.68 (0.341)	54.36 (0.304)	0.0893 (0.295)	0.0036 (0.280)
24	2.686 (0.326)	950.80 (0.280)	1.5779 (0.354)	1.073 (0.205)	58.81 (0.226)	13.32 (0.254)	0.00013 (0.258)	0.0012 (0.260)

n*=3

TABLE No.27

INVIVO DISTRIBUTION OF RIFAMPICIN IN VARIOUS ORGANS AND SERUM OF RATS (INTRATRACHEAL ADMINISTRATION).

Organs	Lung		Serum	
Time (hr)	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/ml ±SEM	Niosomal drug µg/ml ±SEM
1	227.80 (0.156)	1207.82 (0.126)	0.21286 (0.128)	0.000639 (0.110)
3	139.24 (0.107)	2579.47 (0.109)	0.2856 (0.210)	0.0627 (0.107)
6	105.48 (0.175)	2537.81 (0.168)	0.01236 (0.146)	0.05583 (0.195)
12	6.1915 (0.270)	2337.83 (0.285)	0.05036 (0.193)	0.00127 (0.248)
24	3.0689 (0.289)	1772.09 (0.270)	0.000416 (0.160)	0.00041 (0.205)

n*=3

TABLE NO.28

PHARMACOKINETIC STUDY OF RIFAMPICIN NIOSOMES (INTRAVENEOUS ADMINISTRATION)

Organs	$[AUC]_0^{24}$ $\mu g\ hr^{-1}ml^{-1}$		$[AUMC]_0^{24}$ $\mu g\ hr^{-1}ml^{-1}$		$MRT_{(hr)}$	
	Free drug	Niosomal drug	Free drug	Niosomal drug	Free drug	Niosomal drug
Lung	1954.406	46824.255	11117.619	468948.53	5.6	10.015
Liver	56.2618	66.8335	574.46	508.1787	10.21	7.603
Kidney	1663.915	1227.3	18804.915	10951.84	11.30	8.92
Serum	3.5244 $\mu g\ hr^{-1}ml^{-1}$	0.42573	16.88532 $\mu g\ hr^{-1}ml^{-1}$	2.65683	4.7	6.24

TABLE NO.29

PHARMACOKINETIC STUDY OF RIFAMPICIN NIOSOMES (INTRATRACHEAL ADMINISTRATION)

Organs	$[AUC]_0^{24}$		$[AUMC]_0^{24}$		$MRT_{(hr)}$	
	Free drug	Niosomal drug	Free drug	Niosomal drug	Free drug	Niosomal drug
Lung	1238.61	51353.605 $\mu g\ hr^{-1}ml^{-1}$	5344.60	631792.77 $\mu g\ hr^{-1}ml^{-1}$	4.3	12.30
Serum	1.3732	0.422838 $\mu g\ hr^{-1}ml^{-1}$	5.2155	2.17445 $\mu g\ hr^{-1}ml^{-1}$	3.7	5.14

TABLE 30

CALIBRATION CURVE FOR GENTAMICIN IN LIVER, LUNG, KIDNEY EXTRACT AND SERUM

Concentration	ABSORBANCE			
	Liver Extract	Lung Extract	Kidney Extract	Serum
5	0.121	0.108	0.132	0.144
10	0.179	0.182	0.18	0.195
15	0.265	0.27	0.268	0.28
20	0.327	0.323	0.315	0.365
30	0.458	0.458	0.438	0.467
40	0.544	0.545	0.543	0.558
50	0.652	0.619	0.621	0.638

TABLE NO.31

INVIVO DISTRIBUTION OF GENTAMICIN IN VARIOUS ORGANS AND SERUM OF RATS (INTRAVENOUS ADMINISTRATION)

Organs	Lung		Liver		Kidney		Serum	
Time (hr)	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/g ±SEM	Niosomal drug µg/g ±SESM
1	273.13 (0.140)	715.81 (0.250)	72.01 (0.160)	33.63 (0.208)	270.76 (0.125)	135.38 (0.122)	10.3 (0.152)	2.5 (0.165)
3	221.33 (0.180)	1040.75 (0.283)	59.46 (0.305)	24.44 (0 297)	299.26 (0.314)	121.13 (0.150)	8.5 (0.205)	3.3 (0.157)
6	131.85 (0 164)	748.78 (0.315)	31.052 (0 178)	15.85 (0.276)	242 26 (0.278)	81.943 (0 315)	4.9 (0 204)	4.4 (0 128)
12	69.06 (0.322)	536.86 (0.302)	12.55 (0.182)	15.85 (0.284)	199.50 (0 326)	35.62 (0.324)	3 03 (0.276)	3.2 (0 108)
24	4.70 (0.308)	485.05 (0.265)	0.6606 (0.326)	5.94 (0.226)	163.88 (0.276)	10.68 (0.236)	0.08 (0.215)	1.7 (0.208)

n*=3

TABLE NO.32

INVIVO DISTRIBUTION OF GENTAMICIN IN VARIOUS ORGANS AND SERUM OF RATS (INTRATRACHEAL ADMINISTRATION)

Organs	Lung		Serum	
Time (hr)	Free drug µg/g ±SEM	Niosomal drug µg/g ±SEM	Free drug µg/ml ±SEM	Niosomal drug µg/ml ±SEM
1	518.02 (0.124)	626.33 (0.135)	9.1 (0.104)	0.53 (0.101)
3	414.41 (0.128)	1144.36 (0.130)	7.5 (0.115)	1.78 (0.122)
6	221.33 (0.162)	946.57 (0.158)	3.2 (0.126)	3.03 (0.205)
12	193.08 (0.218)	824.13 (0.225)	1.8 (0.193)	1.87 (0.184)
24	75.34 (0.207)	748.78 (0.296)	0.08 (0.312)	0.08 (0.256)

n* = 3

TABLE NO.33

PHARMACOKINETIC STUDY OF GENTAMICIN NIOSOMES (INTRAVENEOUS ADMINISTRATION)

Organs	$[AUC]_0^{24}$ $\mu g\ hr^{-1}ml^{-1}$		$[AUMC]_0^{24}$ $\mu g\ hr^{-1}ml^{-1}$		$MRT_{(hr)}$	
	Free drug	Niosomal drug	Free drug	Niosomal drug	Free drug	Niosomal drug
Lung	2206.19	14787.135	13764.9	156924.48	6.2	10.61
Liver	513.3126	361.155	2842.89	2372.955	5.5	6.57
Kidney	5023.24	1259.29	54336.33	8710.032	10.81	6.91
Serum $\mu g\ hr^{-1}ml^{-1}$	86.5	70.80	550.26 $\mu g\ hr^{-1}ml^{-1}$	737.7	6.36	10.419

TABLE NO.34

PHARMACOKINETIC STUDY OF GENTAMICIN NIOSOMES (INTRATRACHEAL ADMINISTRATION)

Organs	$[AUC]_0^{24}$ $\mu g\ hr^{-1}ml^{-1}$		$[AUMC]_0^{24}$ $\mu g\ hr^{-1}ml^{-1}$		$MRT_{(hr)}$	
	Free drug	Niosomal drug	Free drug	Niosomal drug	Free drug	Niosomal drug
Lung	4998 8	17504.49	40941	231909.95	8.19	13.24
Serum	274.08	36.19	495.18	309.435	7.8	8.5

Fig. No.9

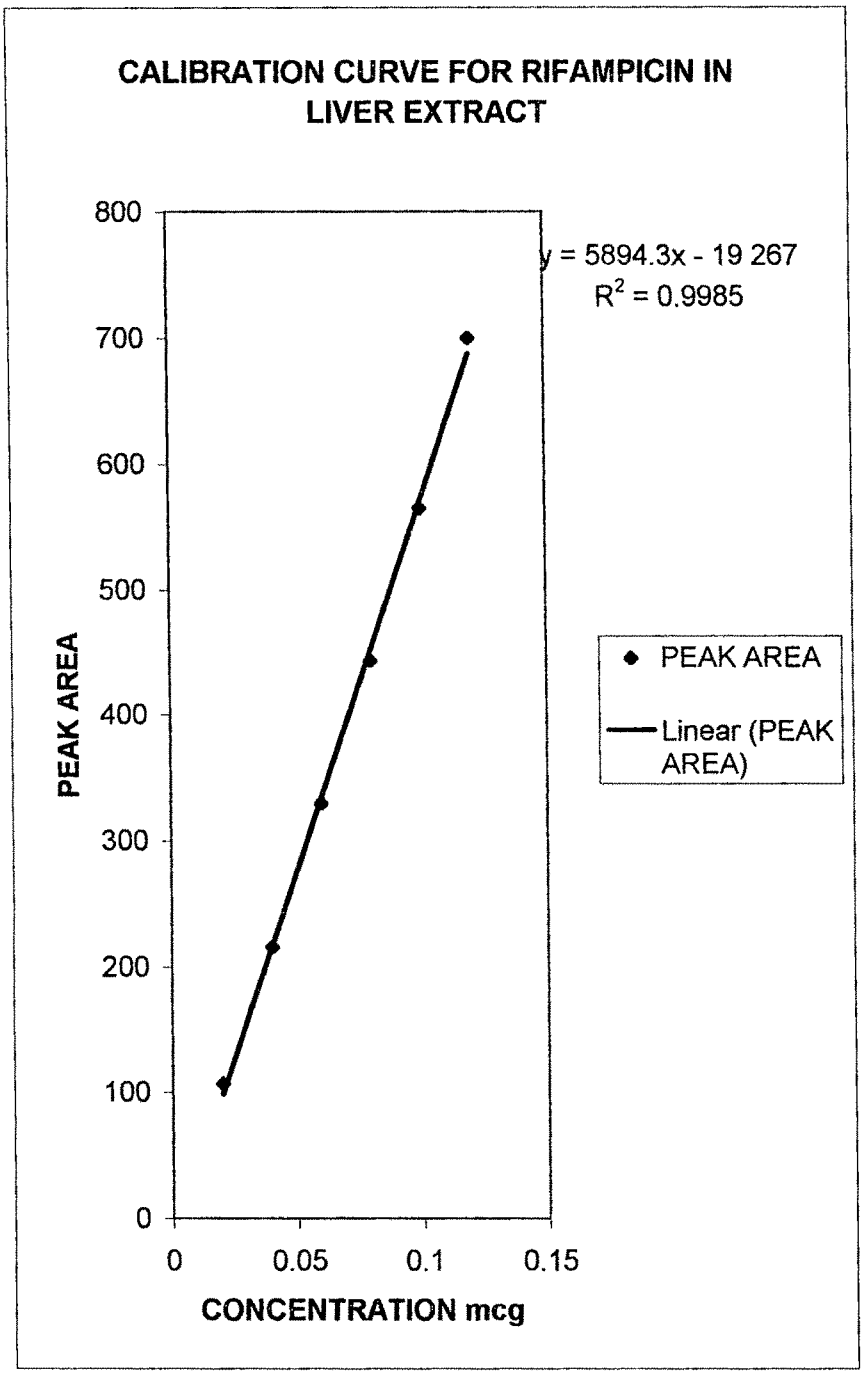
CALIBRATION CURVE FOR RIFAMPICIN IN LIVER EXTRACT
y = 5894.3x - 19 267
R2 = 0.9985
PEAK AREA
800
700
600
500
400
300
200
100
0
0
0.05
0.1
0.15
CONCENTRATION mcg
PEAK AREA
Linear (PEAK AREA)

Fig. No.10

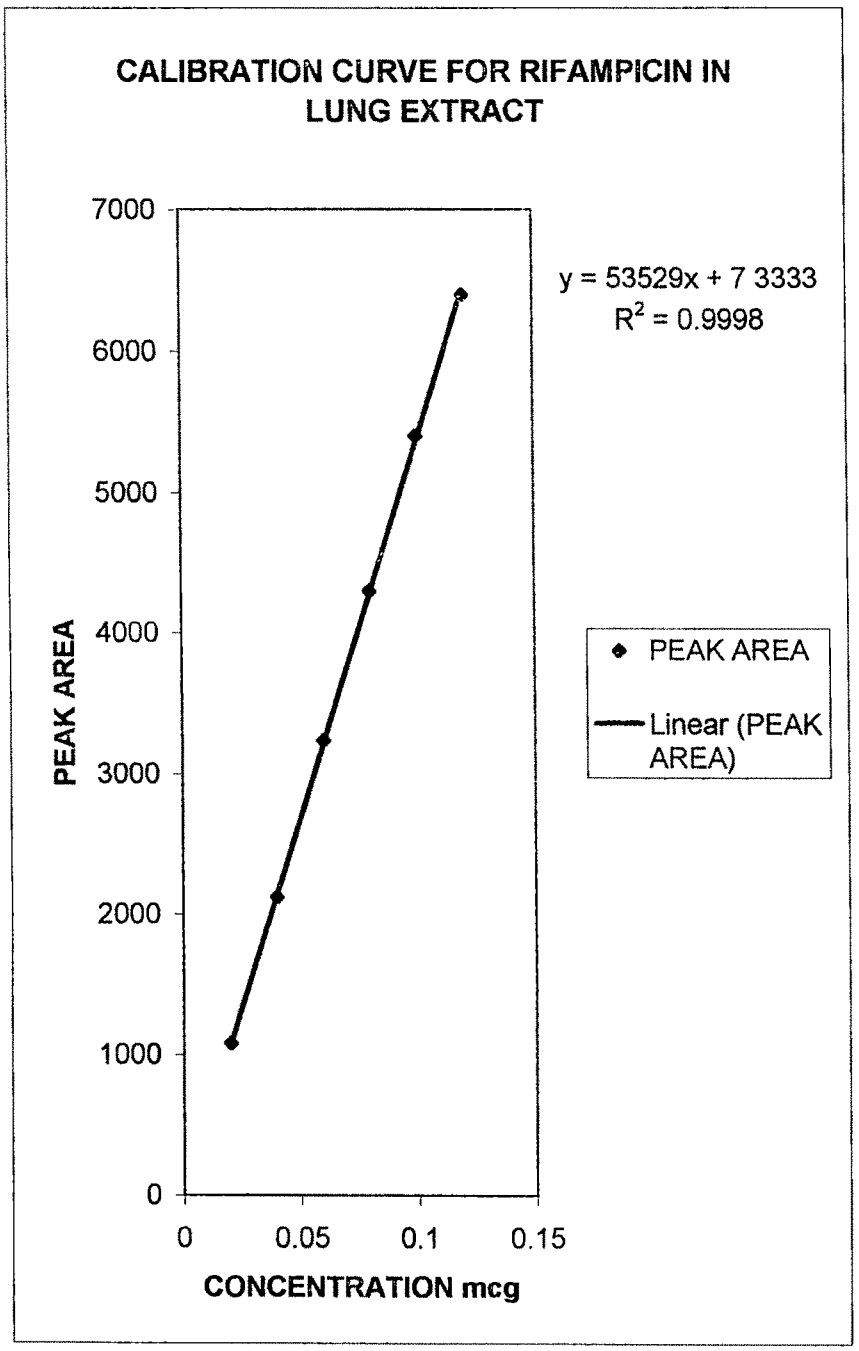
CALIBRATION CURVE FOR RIFAMPICIN IN LUNG EXTRACT
7000
6000
5000
4000
3000
2000
1000
0
PEAK AREA
0
0.05
0.1
0.15
CONCENTRATION mcg
y = 53529x + 7 3333
R2 = 0.9998
PEAK AREA
Linear (PEAK AREA)

Fig. No.11

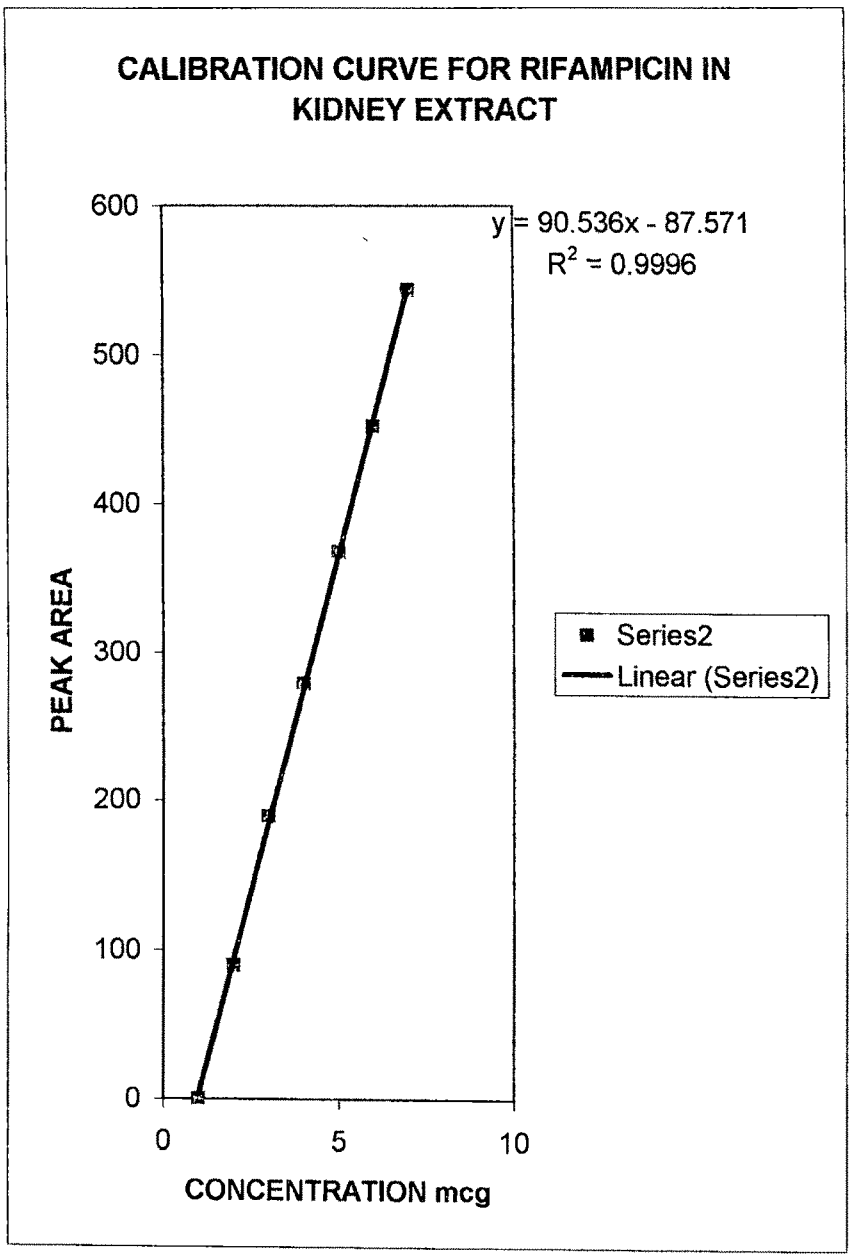
CALIBRATION CURVE FOR RIFAMPICIN IN KIDNEY EXTRACT
y = 90.536x - 87.571
R2 = 0.9996
600
500
400
300
200
100
0
PEAK AREA
0
5
10
CONCENTRATION mcg
Series2
Linear (Series2)

Fig. No.12

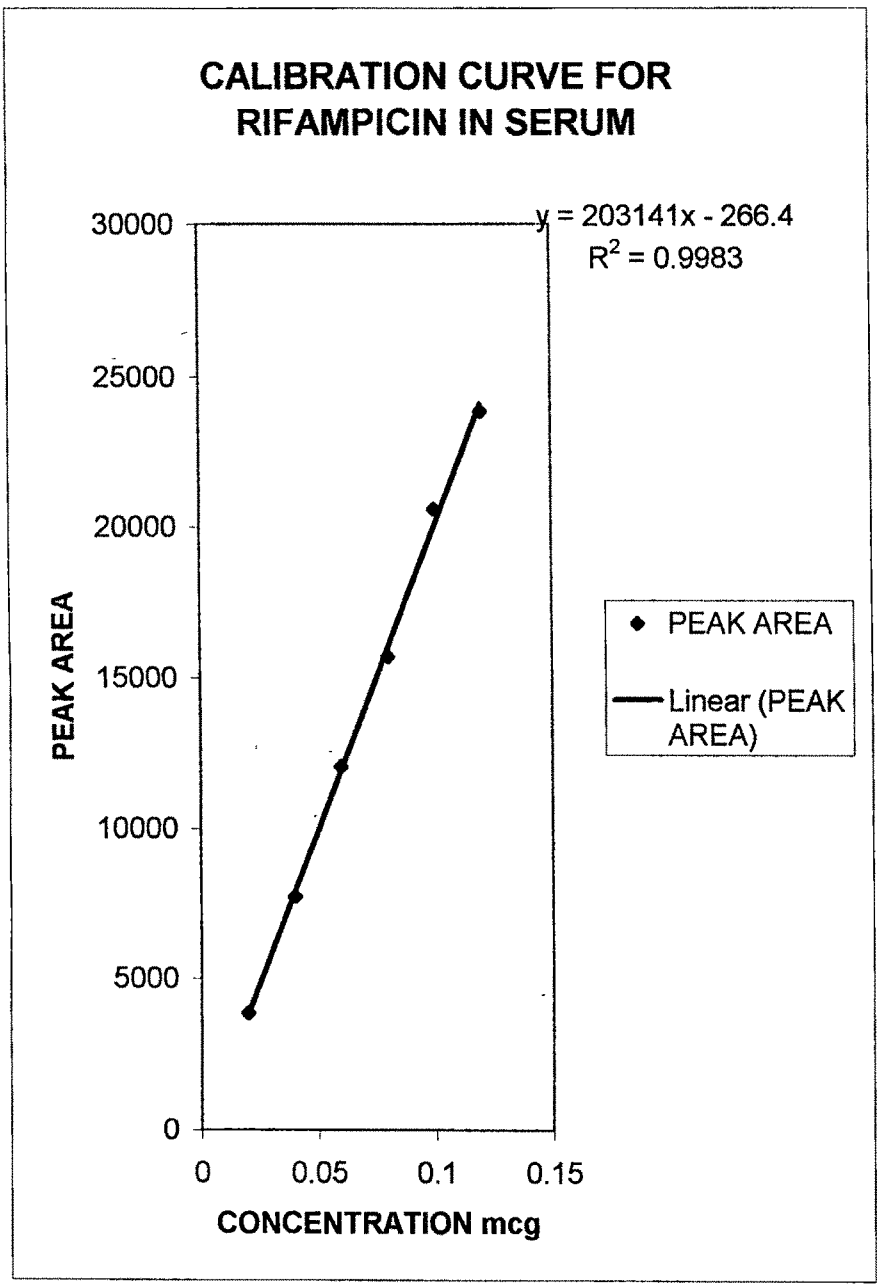
CALIBRATION CURVE FOR RIFAMPICIN IN SERUM
y = 203141x - 266.4
R2 = 0.9983
30000
25000
20000
15000
10000
5000
0
PEAK AREA
0
0.05
0.1
0.15
CONCENTRATION mcg
PEAK AREA
Linear (PEAK AREA)

Fig. No.13(a)

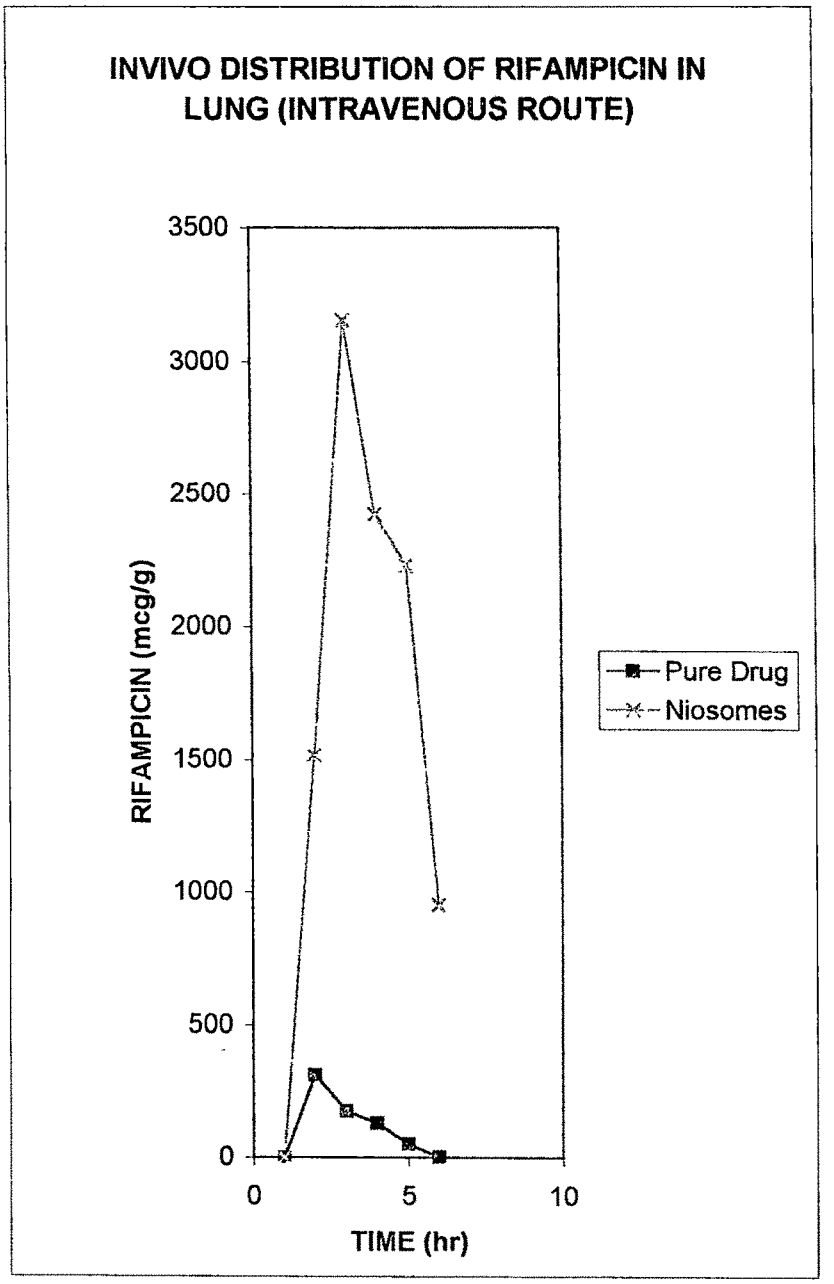
INVIVO DISTRIBUTION OF RIFAMPICIN IN LUNG (INTRAVENOUS ROUTE)
3500
3000
2500
2000
1500
1000
500
0
RIFAMPICIN (mcg/g)
0
5
10
TIME (hr)
Pure Drug
Niosomes

Fig. No.13(b)

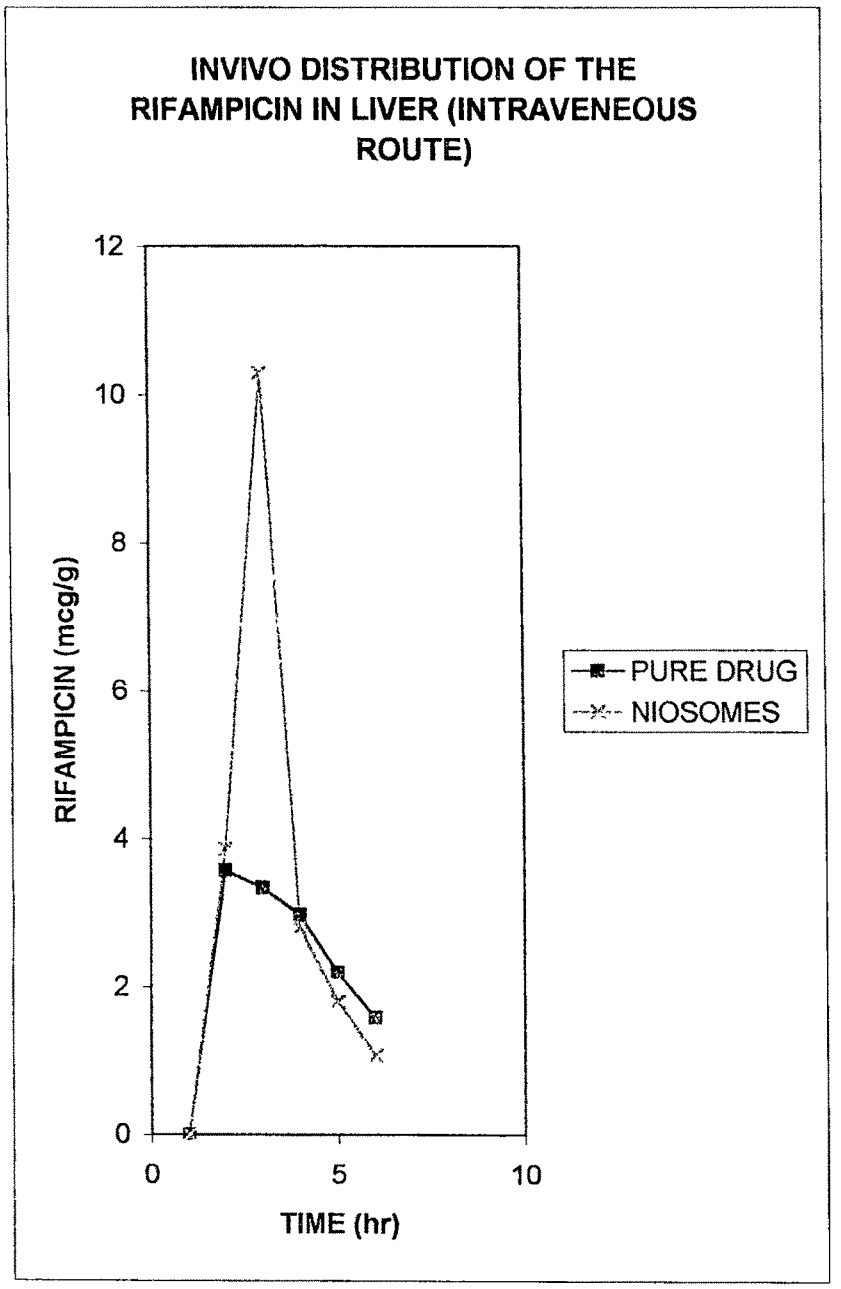
INVIVO DISTRIBUTION OF THE RIFAMPICIN IN LIVER (INTRAVENEOUS ROUTE)
RIFAMPICIN (mcg/g)
12
10
8
6
4
2
0
0
5
10
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.13 ©

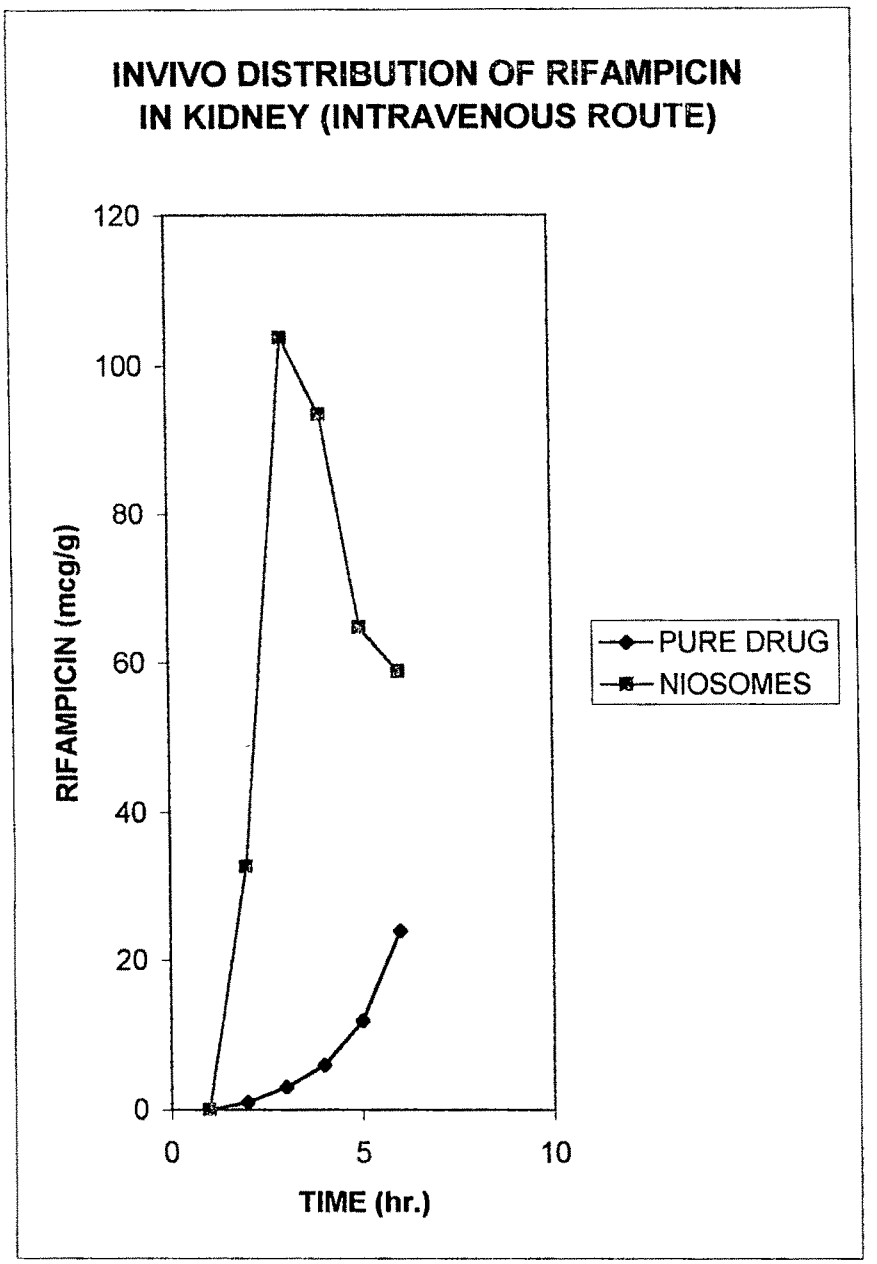
INVIVO DISTRIBUTION OF RIFAMPICIN IN KIDNEY (INTRAVENOUS ROUTE)
120
100
80
60
40
20
0
RIFAMPICIN (mcg/g)
0
5
10
TIME (hr.)
PURE DRUG
NIOSOMES

Fig. No.13(d)

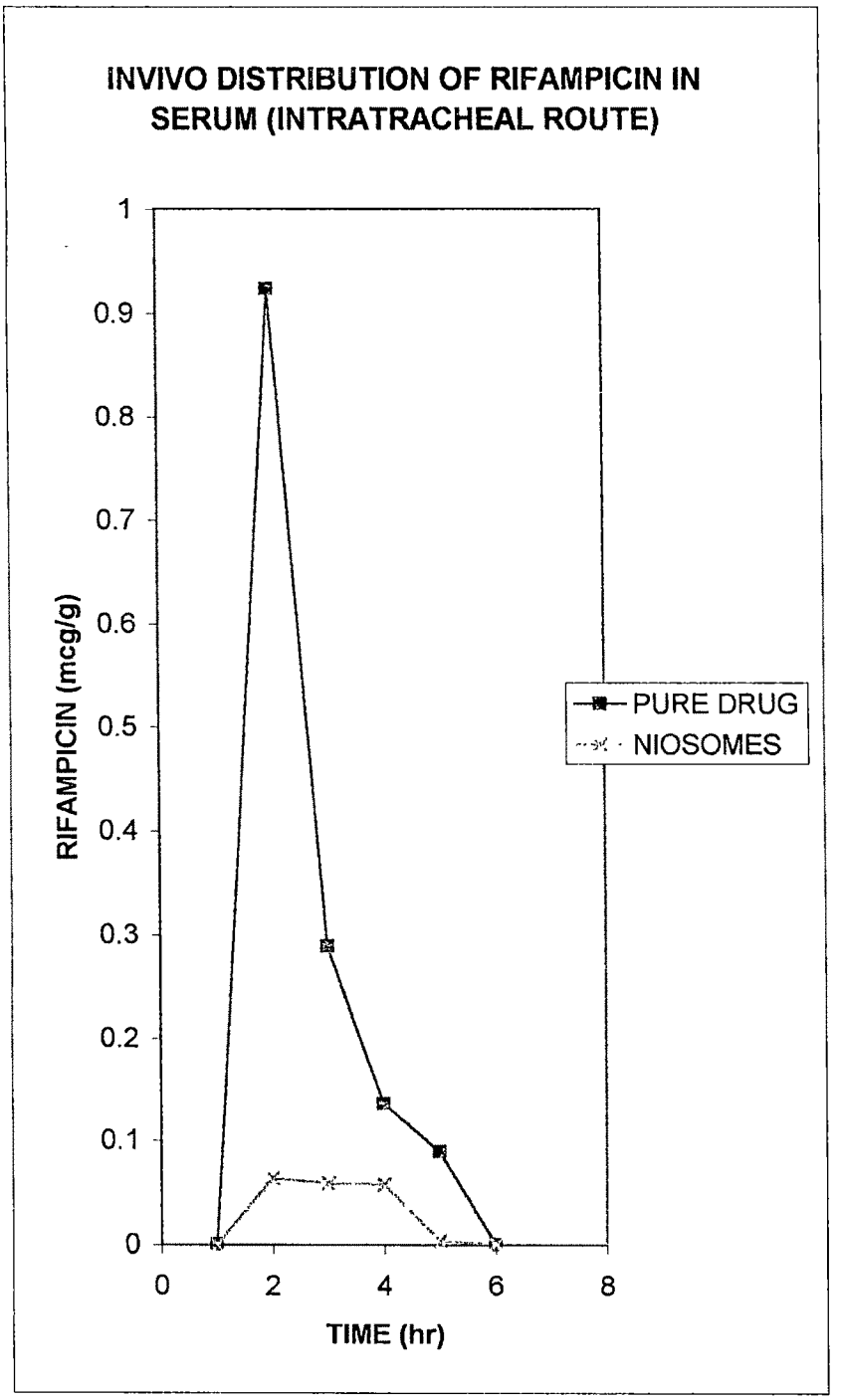
INVIVO DISTRIBUTION OF RIFAMPICIN IN SERUM (INTRATRACHEAL ROUTE)
RIFAMPICIN (mcg/g)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.14(a)

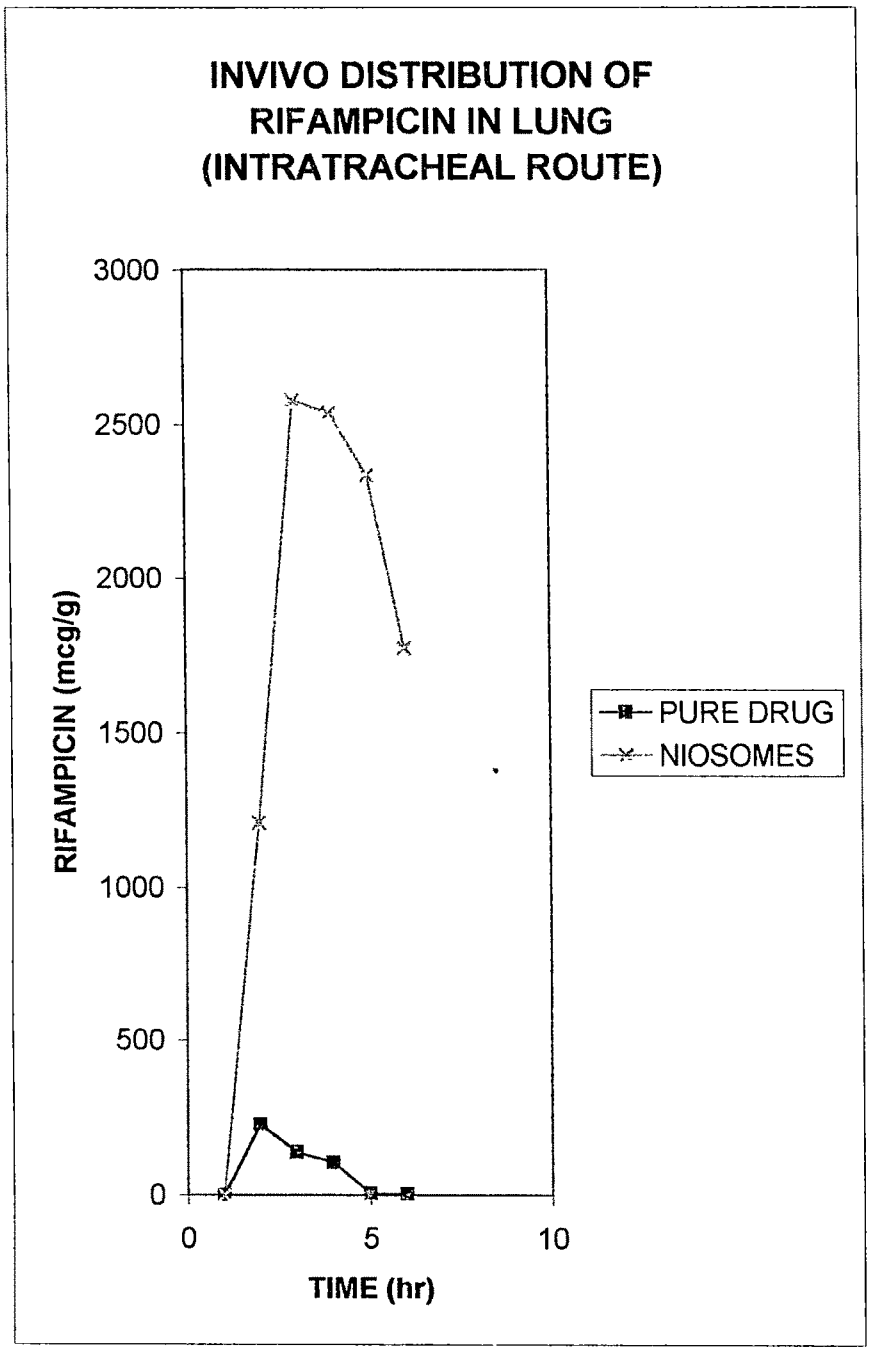
INVIVO DISTRIBUTION OF RIFAMPICIN IN LUNG (INTRATRACHEAL ROUTE)
RIFAMPICIN (mcg/g)
3000
2500
2000
1500
1000
500
0
0
5
10
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.14(d)

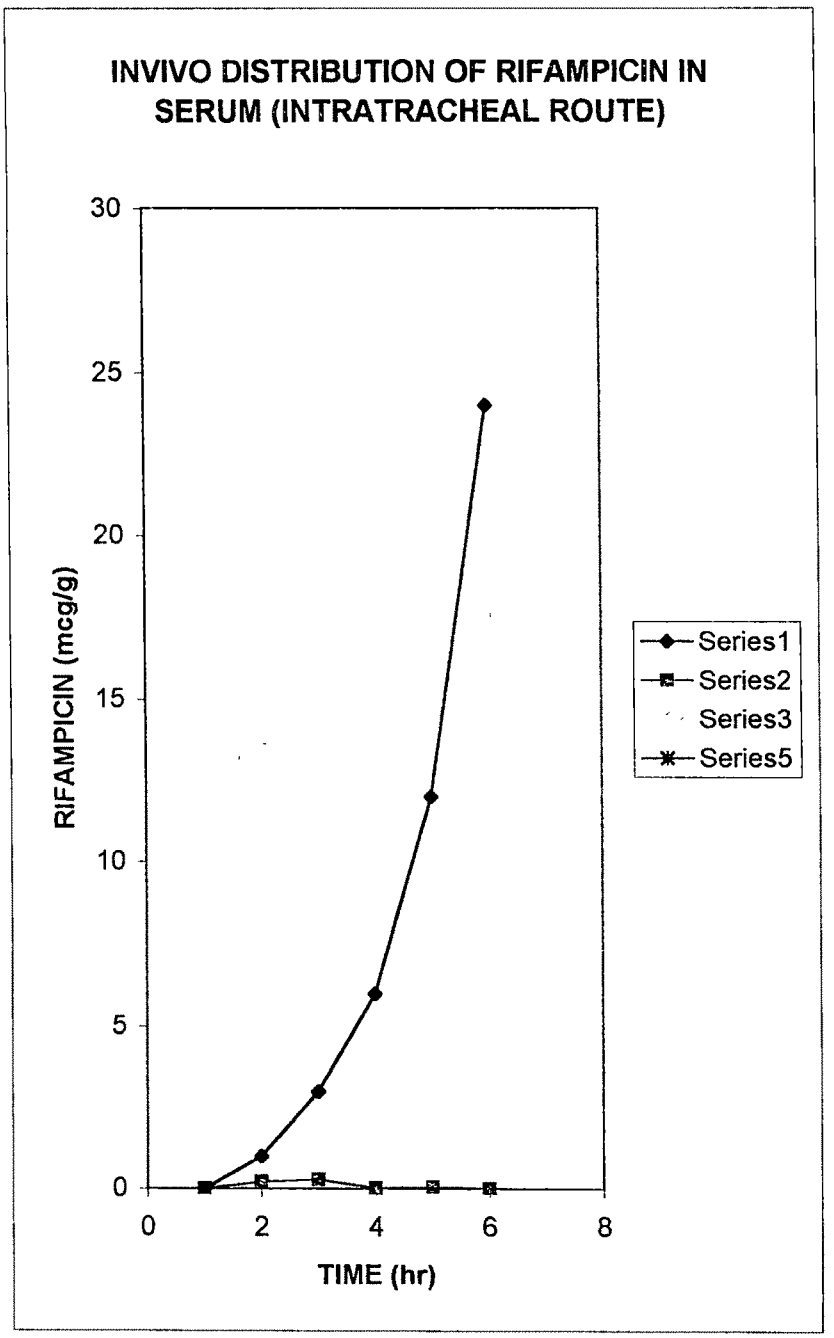
INVIVO DISTRIBUTION OF RIFAMPICIN IN SERUM (INTRATRACHEAL ROUTE)
RIFAMPICIN (mcg/g)
30
25
20
15
10
5
0
0
2
4
6
8
TIME (hr)
Series1
Series2
Series3
Series5

Fig. No.15

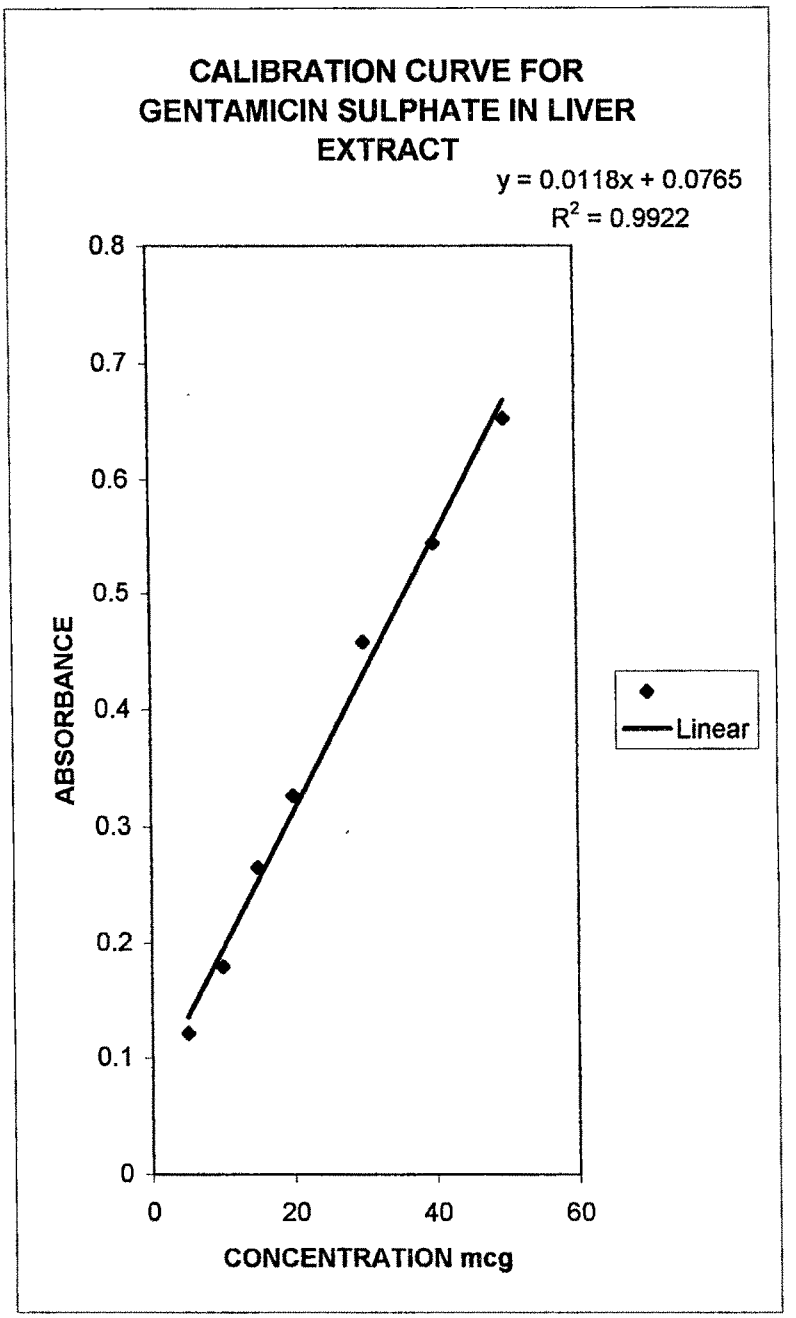
CALIBRATION CURVE FOR GENTAMICIN SULPHATE IN LIVER EXTRACT
y = 0.0118x + 0.0765
R2 = 0.9922
ABSORBANCE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
CONCENTRATION mcg
Linear

Fig. No.16

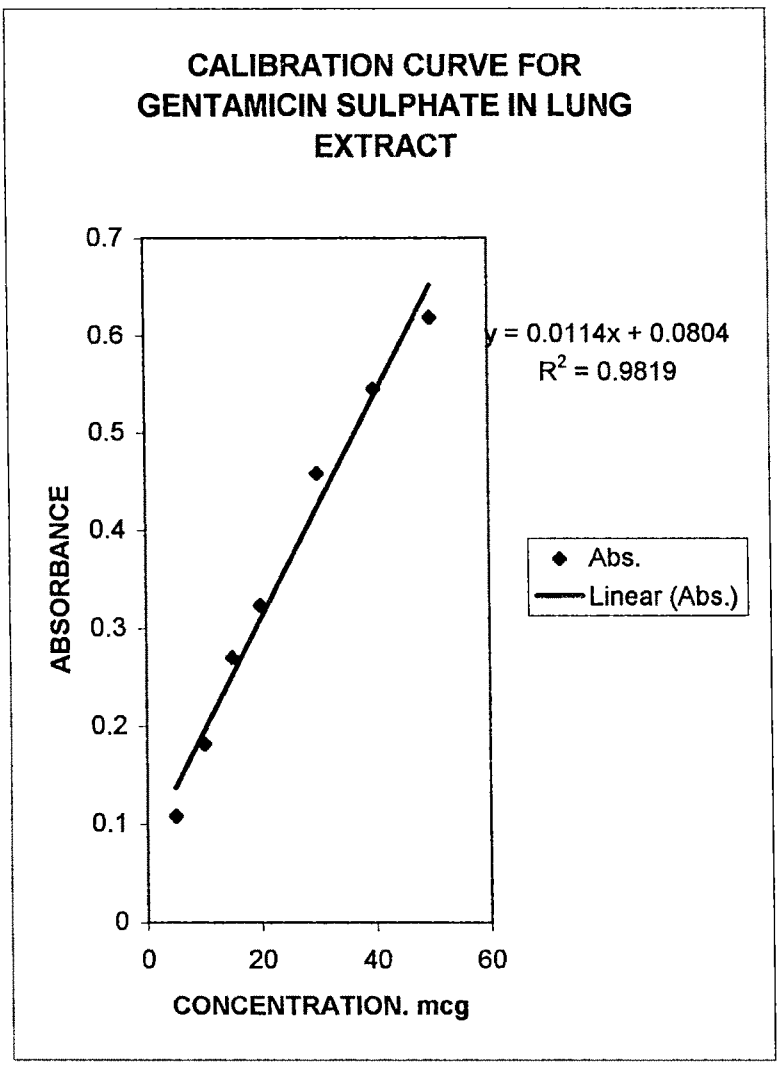
CALIBRATION CURVE FOR GENTAMICIN SULPHATE IN LUNG EXTRACT
ABSORBANCE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
CONCENTRATION. mcg
y = 0.0114x + 0.0804
R2 = 0.9819
Abs.
Linear (Abs.)

Fig. No.17

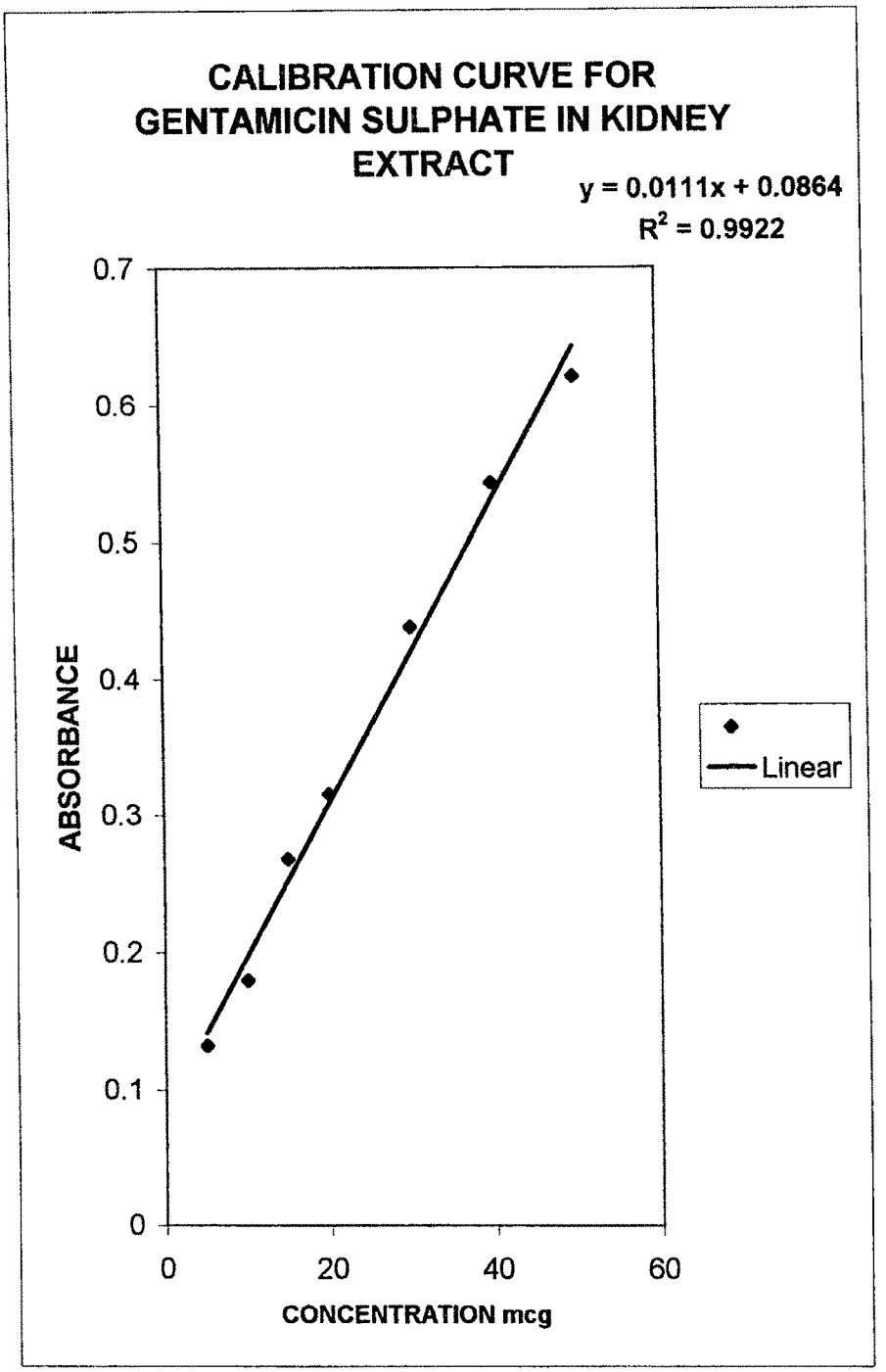
CALIBRATION CURVE FOR GENTAMICIN SULPHATE IN KIDNEY EXTRACT
y = 0.0111x + 0.0864
R2 = 0.9922
ABSORBANCE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
CONCENTRATION mcg
Linear

Fig. No. 18

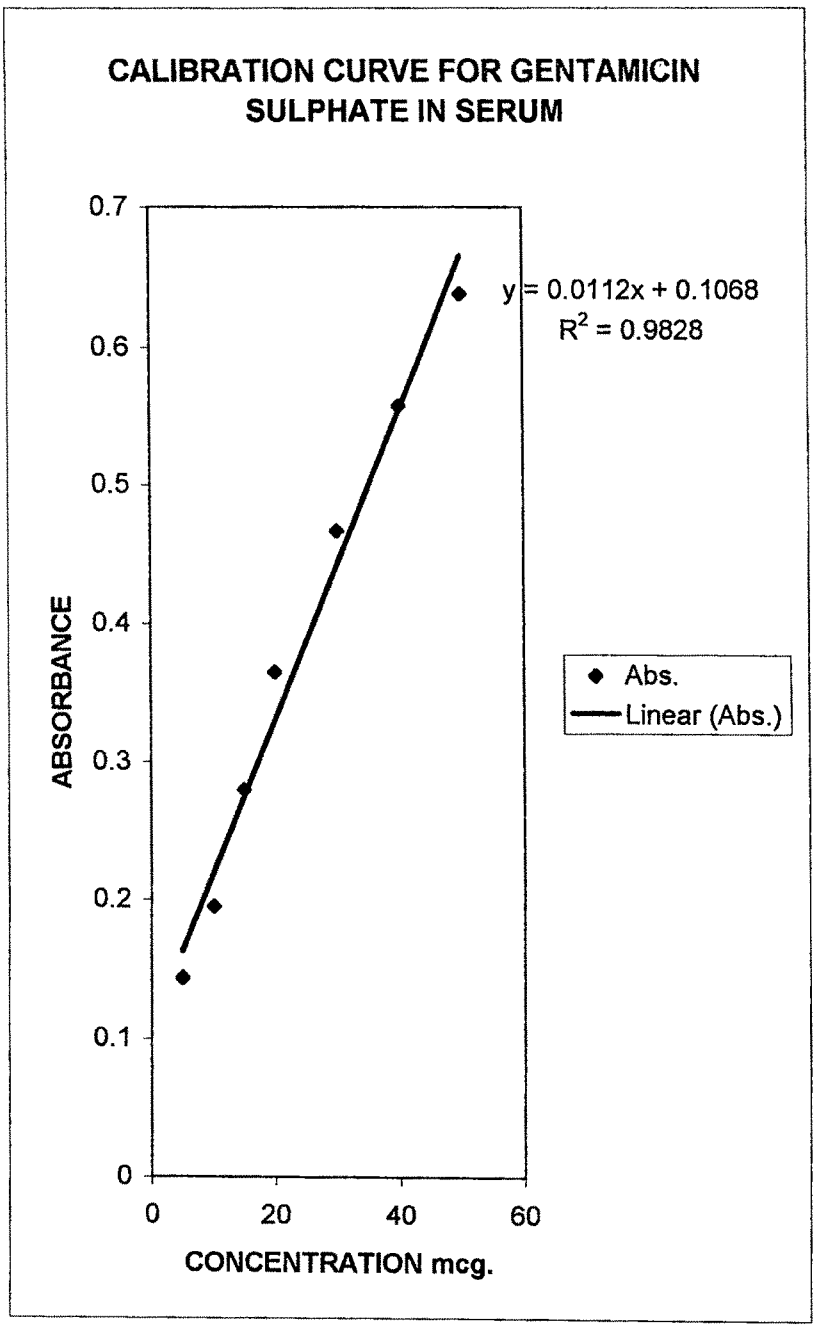
CALIBRATION CURVE FOR GENTAMICIN SULPHATE IN SERUM
y = 0.0112x + 0.1068
R2 = 0.9828
ABSORBANCE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
CONCENTRATION mcg.
Abs.
Linear (Abs.)

Fig. No.19(a)

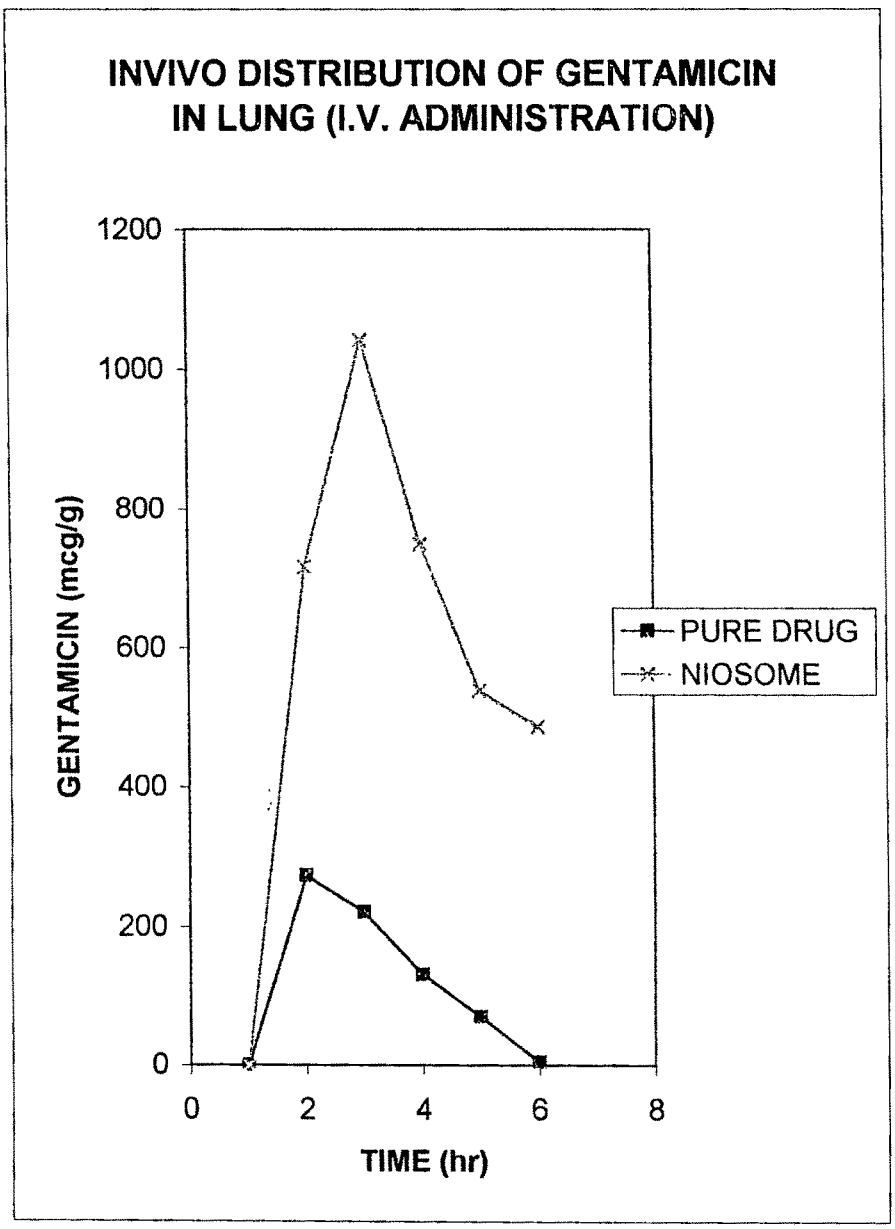
INVIVO DISTRIBUTION OF GENTAMICIN IN LUNG (I.V. ADMINISTRATION)
GENTAMICIN (mcg/g)
1200
1000
800
600
400
200
0
0
2
4
6
8
TIME (hr)
PURE DRUG
NIOSOME

Fig. No.19(b)

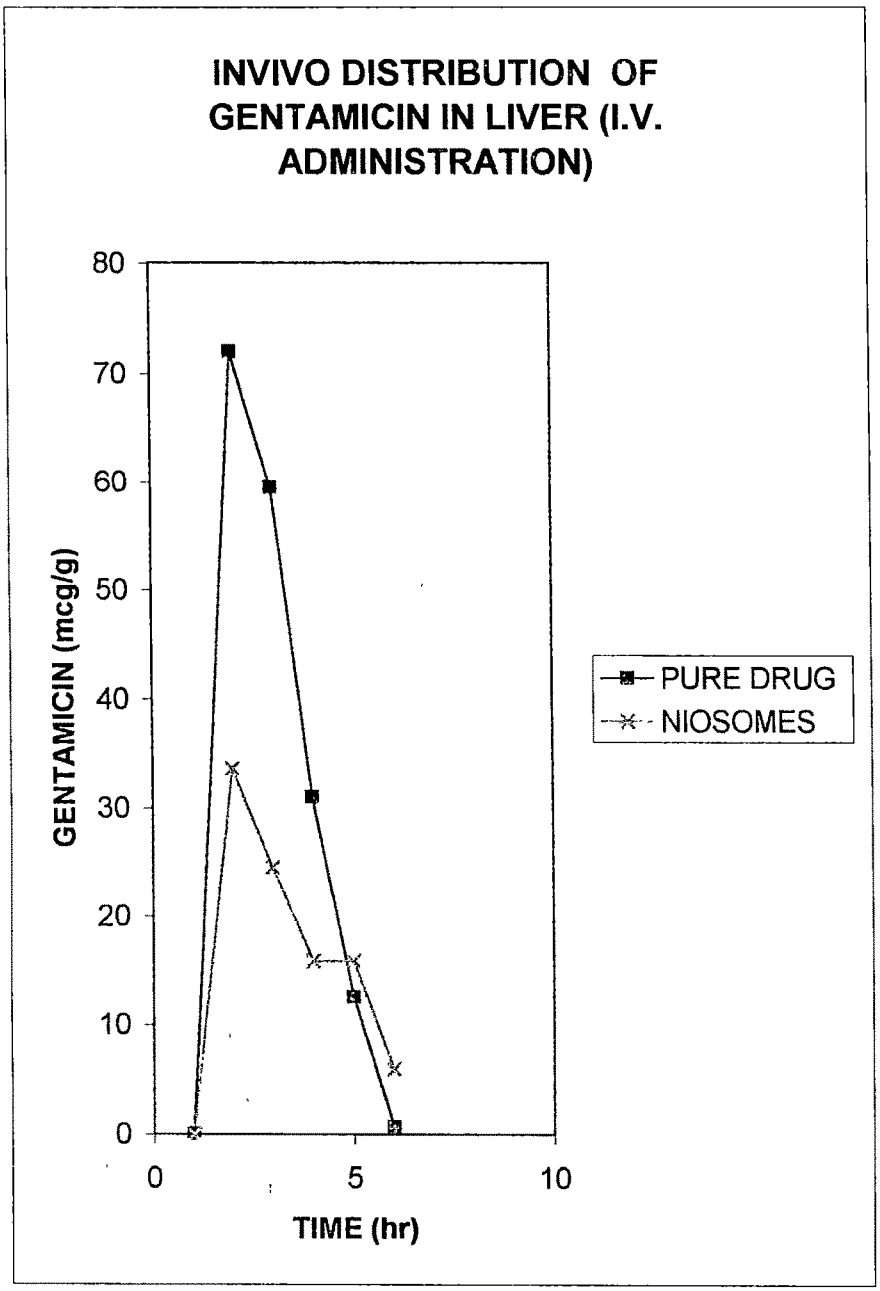
INVIVO DISTRIBUTION OF GENTAMICIN IN LIVER (I.V. ADMINISTRATION)
GENTAMICIN (mcg/g)
80
70
60
50
40
30
20
10
0
0
5
10
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.19©

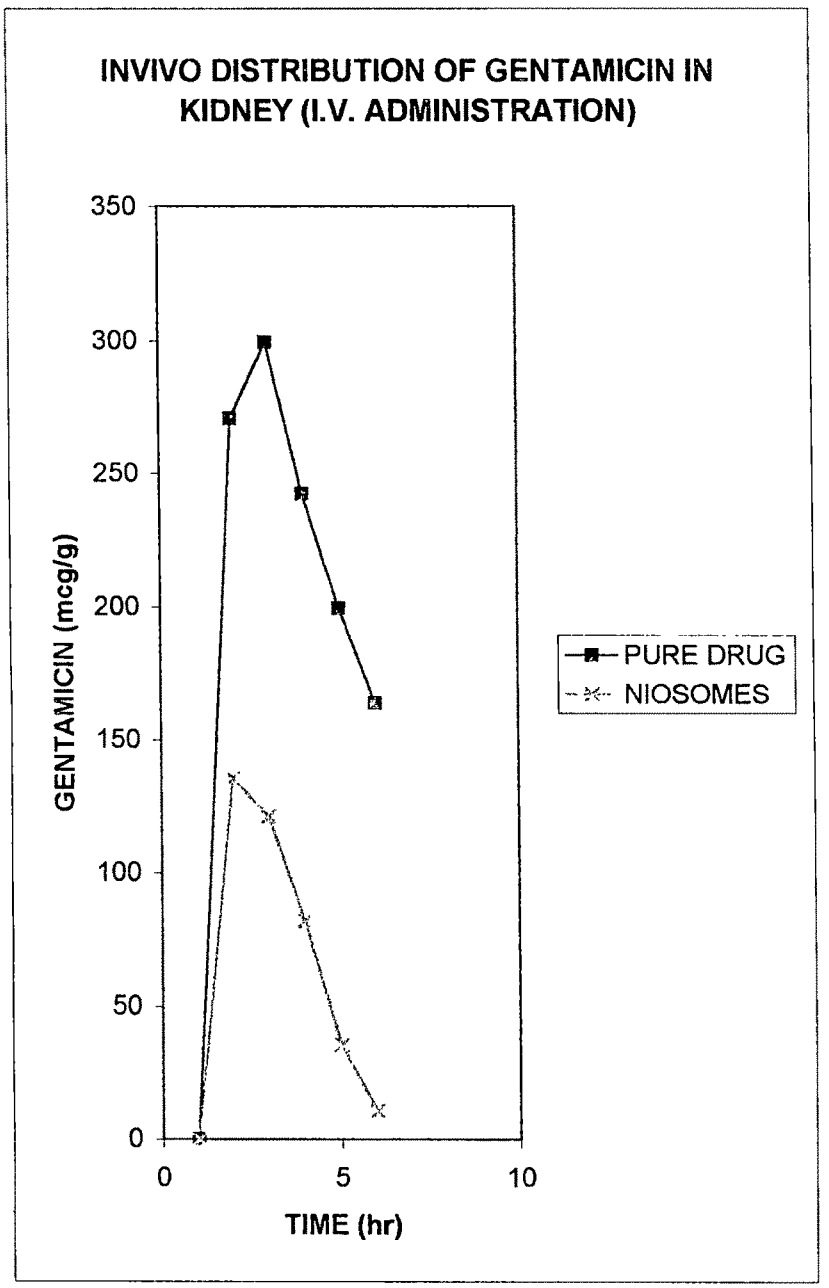
INVIVO DISTRIBUTION OF GENTAMICIN IN KIDNEY (I.V. ADMINISTRATION)
350
300
250
200
150
100
50
0
GENTAMICIN (mcg/g)
0
5
10
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.19(d)

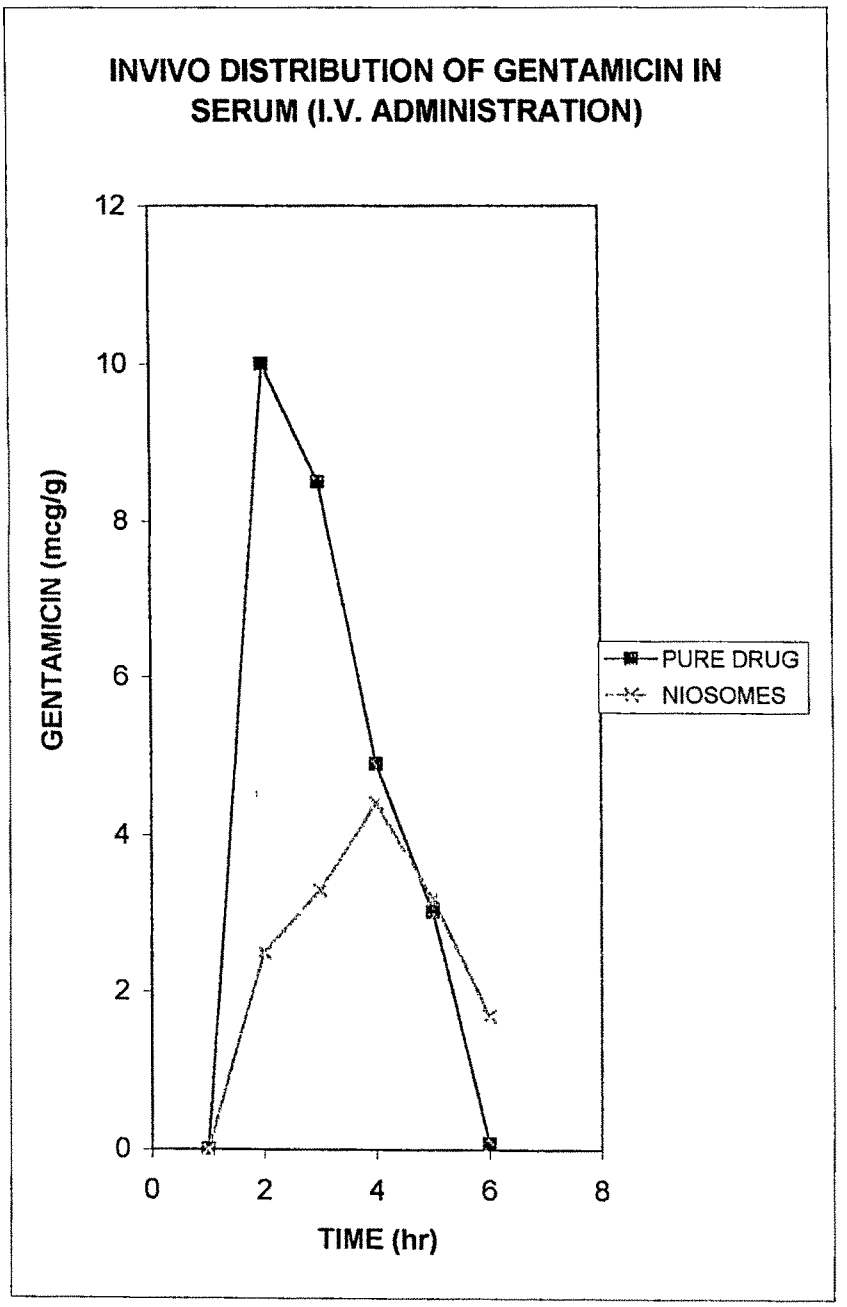
INVIVO DISTRIBUTION OF GENTAMICIN IN SERUM (I.V. ADMINISTRATION)
GENTAMICIN (mcg/g)
12
10
8
6
4
2
0
0
2
4
6
8
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.20(a)

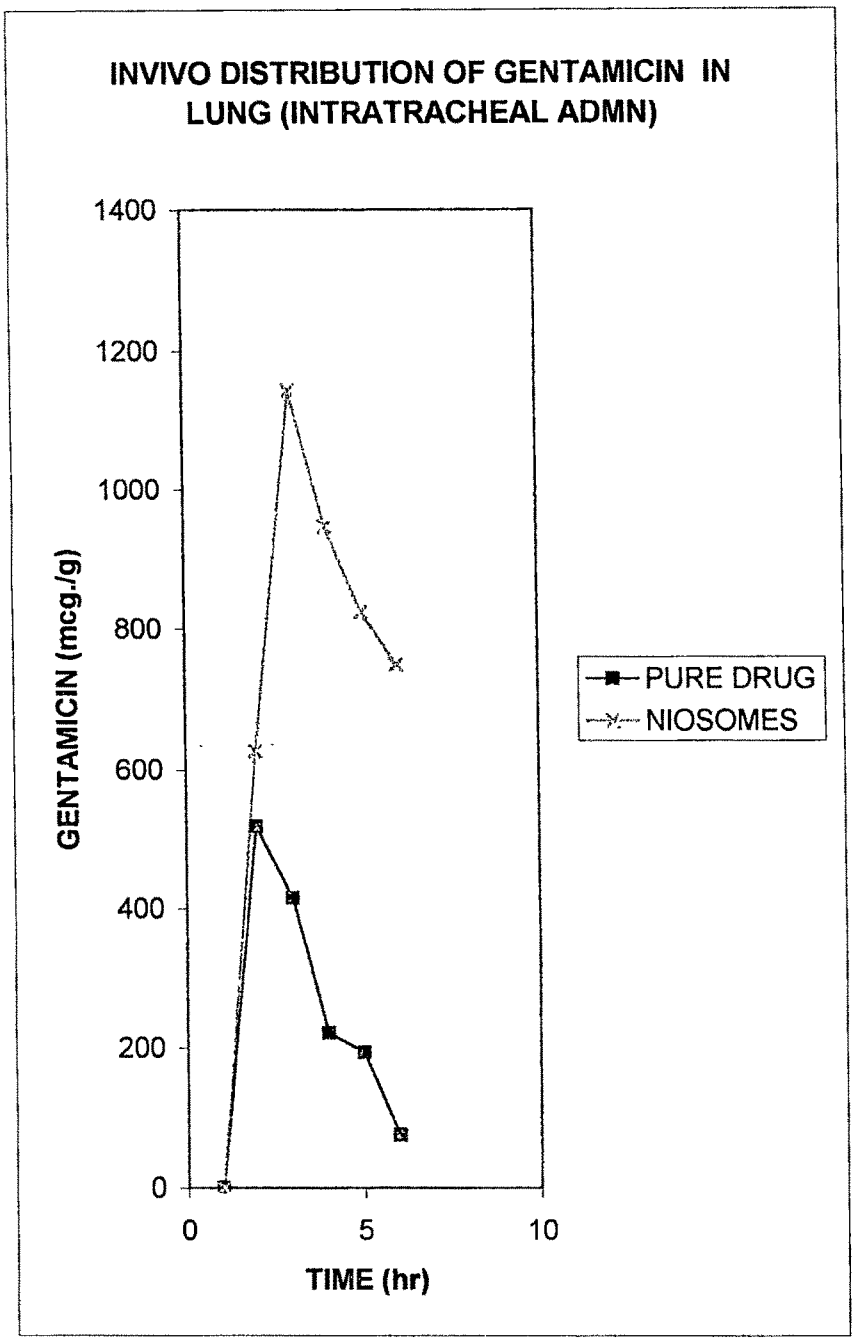
INVIVO DISTRIBUTION OF GENTAMICIN IN LUNG (INTRATRACHEAL ADMN)
1400
1200
1000
800
600
400
200
0
GENTAMICIN (mcg./g)
0
5
10
TIME (hr)
PURE DRUG
NIOSOMES

Fig. No.20(d)

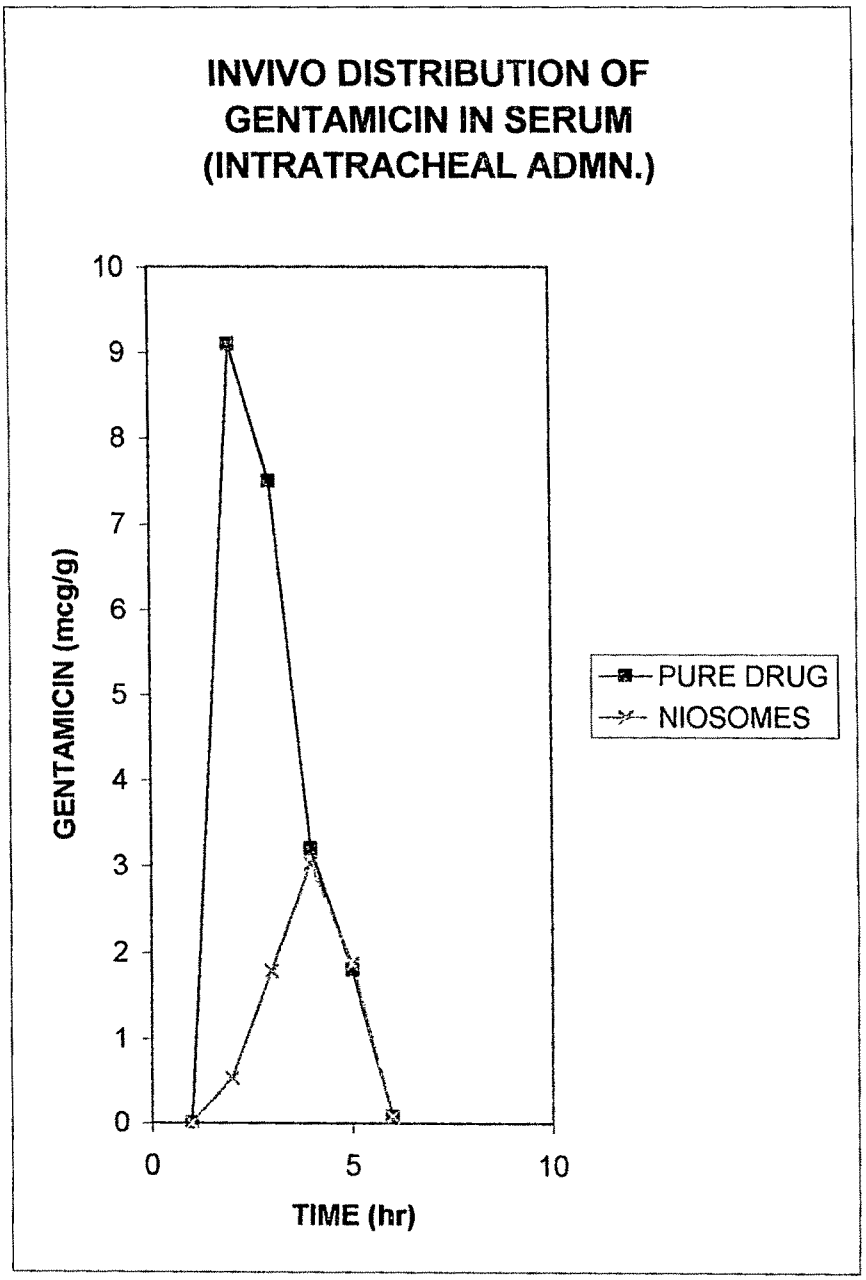
INVIVO DISTRIBUTION OF GENTAMICIN IN SERUM (INTRATRACHEAL ADMN.)
GENTAMICIN (mcg/g)
10
9
8
7
6
5
4
3
2
1
0
0
5
10
TIME (hr)
PURE DRUG
NIOSOMES

Plate No.6

Photograph of cannulated rats after intravenous injection in the caudual vein

Plate No.7

Photograph of cannulated rats after intratracheal instillation

7.1.11 DISCUSSION

7.1.11.1 *INVIVO ORGAN DISTRIBUTION STUDIES FOR NIOSOMES CONTAINING RIFAMPICIN:*

1. Calibration curves for estimation of Rifampicin in Lung, Liver, Kidney and Blood Serum.

The data for calibration curves for the estimation of Rifampicin in Liver, Lung, Kidney and Serum was given in Table No.25. The corresponding calibration curves were plotted after regression analysis of the data. The calibration curves for Rifampicin in liver, lung, kidney and serum were shown in Fig No 9,10,11 and 12 respectively.

2. Organ distribution pattern of Rifampicin from two different route of administration of niosomal drug delivery system.

The organ distribution pattern of Rifampicin from niosomes was studied in albino rats in comparison with free drug solution to determine the potential of this delivery system for site specific delivery of Rifampicin and also to find out the effective route of administration of this delivery system.

The amount of Rifampicin distributed in each organ was calculated as percentage of administered dose distributed in each organ at 1hr, 3rd hr, 6th hr, 12th hr and 24th hr after administration of formulation by both intravenous route and intratracheal route.

The amount of Rifampicin distributed in blood serum was calculated as concentration per ml of administered dose at 1hr, 3rd hr, 6th hr, 12th hr and 24th hr after the administration of formulation by both the intravenous and intratracheal route.

The data for organ distribution of Rifampicin from Rifampicin solution and niosomes were presented in Table No.26 & 27 and corresponding plots are given in Fig No.13 & 14.

Some important observations are enlisted below:

1. Appreciable drug levels are estimated in the lung after 3 hours of the administration by both the routes.
2. Following intravenous administration of niosome sample (equivalent to 5 mg of Rifampicin), 88.19% of drug administered in the form of niosomes was recovered from the lung at 3rd hour and the remainder was found to be distributed in liver (1.98%), Kidney (3.65%) and Serum (0.0082%).
3. Following intratracheal Administration of niosome sample (equivalent to 5mg of Rifampicin) 72.04% of drug administered in the form of niosomes was recovered from the lung at 3rd hour and the remainder was found to be distributed in Blood (0.00877%).

In case of Intravenous administration only 4.87% of drug was found in the lungs at 3hr when an equivalent amount of plain drug was administered and the remainder was found to be distributed in the liver (0.64%), kidney (3.93%) and serum (0.040%).

In case of intratracheal administration only 3.79% of drug was found in lungs at the 3rd hr when an equivalent amount of plain drug was administered and amount of drug in serum was found to be 0 039%.

The invivo organ distribution studies of Rifampicin niosomes showed a slow drug release behaviour with MRT (Mean Residence Time) for lung following intravenous administration which was found to be 10.015 hours and it was 12.30hrs in case of intratracheal administration.

The values of AUC, AUMC and MRT of Rifampicin from Rifampicin solution and niosomes were presented in Table No.28 & 29.

Compared to free drug solution, niosomes showed the concentration of 950.80µg/g in lung, 1.0738µg/g in liver, 13.32µg/g in kidney and 0.0012 µg/ml in serum at 24^{th} hrs, after intravenous administration. After intratracheal administration, the concentration was 1772.09µg/g in lung and 0.00041µg/ml in serum at 24^{th} hr. Significant difference in total drug concentration in lung, liver, kidney and blood serum was found between Rifampicin loaded niosomes and free Rifampicin Solution ($P<0.001$)

The tissue distribution of Rifampicin niosome was examined after intravenous route and intratracheal administration in rats and the results were compared with the plain drug solution. The niosomes encapsulated form showed significantly higher accumulation in the lung

as compared with the plain drug solution following both the routes of administration.

Ranked means from lowest to highest values listed out were **0.842 (serum), 5.17 (kidney), 7.318 (liver) 39.398 (lung)**. The underlined means which were not significantly different from each other statistically. These multiple comparisons in ANOVA were carried out by calculating at 5% allowance[162].

The preferential distribution of Rifampicin entrapped niosomes to lung indicate the potential use of niosomes for targeting of Rifampicin to lungs. This observation is in conformity with the observations of other researchers.

Kazukiro Morimoto et al[163] reported that the particle size is considered to be one of the main factor affecting the bioavailability of drugs after pulmonary administration. He also reported that small particles of microspheres like 10μm may well have reached the lower region of lung. The fact that smaller particles can reach the lower region of the lung may be true not only for powder formulation but also for suspension form.

It has been reported that absorption of certain plasma proteins (opsonins) will facilitate phagocytic uptake and removal of particles primarily to the liver, but also to the spleen. This gives an opportunity to target drugs to the cells of the Mononuclear phagocytic system (MPS) but prevent distribution to the rest of the body. If sites other than the MPS are to be reached, then the delivery system must circulate freely

without get opsonized. The small particles (less than 7μm) were taken up by the MPS, whilst large particles (7μm and above) were lodged in the first capillary bed that was reached. Hence vesicle particle size is the first parameter that could be used to avoid the uptake by the MPS.

The pulmonary route, in particular the deep lung, has recently attracted considerable interest as a route of administration for drugs because of the large surface area, thin epithelial membrane, relatively low metabolic activity, High vascularization of the alveolar epitheluim[3] and absence of a hepatic first pass effect. One or more of these factors may be responsible for the significant distribution in the lung.

The present study is an attempt to provide a maximum pulmonary concentration of the drug at the affected site (lung) to get safe and effective therapy.

7.1.11.2 INVIVO ORGAN DISTRIBUTION STUDIES FOR NIOSOMES CONTAINING GENTAMICIN

1) Calibration curves for estimation of Gentamicin in Lung, Liver, Kidney and Blood Serum.

The data for calibration curve for the estimation of Gentamicin in liver, lung, kidney and serum was given in Table 30. The corresponding calibration curves were plotted after regression analysis of the data. The calibration curves for Gentamicin in liver, lung, kidney and blood serum were shown in Fig No.15,16,17 & 18. respectively.

2) Organ distribution pattern of Gentamicin following two different route of administration of niosomal drug delivery system.

The organ distribution pattern of Gentamicin from niosomes was studied in albino rats in comparision with free drug solution to determine the potential of this delivery system for site specific delivery of Gentamicin and find out the effective route of administration of this drug delivery system.

The amount of Gentamicin distributed in each organ was calculated as percentage of administered dose distributed in each organ at 1hr, 3^{rd}hr, 6^{th}hr, 12^{th}hr and 24^{th}hr after administration of formulation by both intravenous route and intratracheal route.

The amount of Gentamicin distributed in blood serum was calculated as concentration per ml of administered dose at 1hr, 3^{rd}hr, 6^{th}hr, 12^{th}hr and 24^{th}hr after administration of formulation by both intravenous and intratracheal route.

The data for the organ distribution of Gentamicin from Gentamicin solution and niosomes was presented in Table No.31 & 32 and corresponding plots in Fig No. 19 & 20.

Some important observations are enlisted below.

1. Appreciable drug levels were detected in the lung at the 3^{rd} hr of the administration by both the routes.

2. Following intravenous administration of Niosome sample (equivalent to 25 mg of Gentamicin sulphate), 58 15% of drug

administered in the form of niosomes was recovered from the lung at 3rdhour and the remainder was found to be distributed in Liver (9.40%), Kidney (9.18%) and Serum (0.92%).

3. Following intratracheal administration of Niosome sample (equivalent 25mg of Gentamicin Sulphate) 63.94% of drug administered in the form of niosomes was recovered from the lung at 3rdhr and the remainder was found to be distributed in blood serum (0.4984%).

In case of intravenous administration, only 12.36% of drug was found in the lungs at 3rdhr, when an equivalent amount of plain drug was administered and the remainder was found to be distributed in the liver (22.88%) Kidney (22.70%) and Serum 2.38%.

In case of intratracheal administration, only 23.15% of drug was found in lungs at 3rdhr when an equivalent amount of plain drug was administered and amount of drug in serum was observed as 2.1%.

The invivo-organ distribution studies, Gentamicin niosomes showed a slow drug release behaviour with MRT value for lung following intravenous administration was 10.61 hours while it was 13.24 hours in the case of intratracheal administration.

The values of AUC, AUMC and MRT of Gentamicin from Gentamicin niosomes and free drug solution are presented in Table No. 33 & 34

Compared to free drug solution, niosomes showed the concentration of 485.05µg/g in lung, 5.94 µg/ml in Liver, 10.68µg/g in Kidney, 1.7µg/ml in Serum at 24th hrs after intravenous administration.

Following intratracheal administration, the concentration was 748.78µg/g in the lung and 0.08µg/ml in serum at 24^{th}hr. Significant difference in total drug concentration in the lung, liver, kidney and blood serum was found between niosomes and free Gentamicin solution ($P<0.001$). Out of the four organ, significant accumulation of gentamicin was found in the lung following niosome administration as compared to the drug solution.

Ranked means from lowest to highest values listed out were **0.762 (liver), 1.9859 (kidney), 6.386(serum), 57.398 (lung)**. The underlined means are not significantly different from each other statistically. These multiple comparisions in ANOVA were carried out by calculating at 5% allowance.