Chapter 10
Radiolabeling & Pharmacokinetics of Nitrendipine Formulations

10.1 MATERIALS AND METHODS

10.1.1 Materials

- Stannous chloride dihydrate ($SnCl_2.2H_2O$) were purchased from Sigma Chemical Company, St. Louis, MO.
- Sodium pertechnetate, separated from molybdenum-99 (99m) was provided by Regional Center for Radiopharmaceutical Division (Northern Region), Board of Radiation and Isotope Technology, New Delhi, India.
- Instant thin layer chromatography silica gel impregnated fiber sheets (ITLC - SG) were purchased from Gelman Sciences Inc., Ann Arbor, MI.
- MIAT® nasal monodose insufflator device was gift sample from MIAT S.p.A. Milan, Italy.
- Hard gelatin capsules No. 3 were received as the gift sample from Associated Capsules Pvt. Ltd. (A member of ACG Worldwide), Mumbai, India.
- All other chemicals and reagents used in the study were of analytical grade.

10.1.2 Equipments

- Shielded well-type gamma scintillation counter (Caprac-R, Capintec, USA).
- Dose calibrator (Capintec, CRC – 15R, Ramsey N. J., USA)
- Single Photon Emission Computerized Tomography gamma camera (SPECT, LC 75–005, Diacam, Siemens AG, Earlangan, Germany).
- Electronic weighing balance (Mettler AE 163)

10.2 Methods

10.2.1 Radiolabeling of Chitosan and Alginate Microspheres of Nitrendipine

The optimized formulations of NTD loaded chitosan (CHNT) and alginate microspheres (ALNT) were labeled with technetium-99m (^{99m}Tc) by direct labeling method as described in **Section 9.3.1.**

10.2.2 Radiolabeling of Lactose

Lactose powder was labeled as method described in **Section 9.3.2.**

10.2.3 Radiolabeling of Nitrendipine

NTD was labeled with technetium-99m (^{99m}Tc) by direct labeling method. Briefly, to 1 mL of drug solution in DMSO and water for injection (2:3) (1.25 mg/mL), solution containing 40 µL stannous chloride (5 mg/mL) and 0.5 mL technetium–99m pertechnetate eluate containing about 1 MCi of activity was added and pH was adjusted to 7.0 using 0.5 M sodium bicarbonate. Then the mixture was incubated for 30 min at room temperature.

10.2.4 Determination of Labeling Efficiency of Nitrendipine and Its Microspheres

The labeling efficiency was determined by ascending instant thin layer chromatography (ITLC) using silica gel (SG) coated fiber sheets (Gelman Sciences Inc, Ann Arbor, MI) using method described in **Section 9.3.4**.

The effects of stannous chloride concentration, incubation time, and pH on the labeling efficiency were studied by varying the factor in question and keeping the other factors constant.

10.2.5 Stability Study of ^{99m}Tc-NTD/Microsphere Complexes

The in vitro stability study of ^{99m}Tc-NTD/Microsphere Complexes was determined using 0.9% sodium chloride and rabbit serum by ascending thin layer chromatography. The complex (0.1 mL) was mixed with 1.9 mL of normal saline (0.9% sodium chloride) or rabbit serum and incubated at 37°C. ITLC was performed at different time intervals up to 24 hours to assess the stability of the complex.

10.2.6 In Vivo Studies

The in vivo studies were performed following the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The animal protocol was duly approved by the Institutional Animal Ethics Committee.

Twelve normal, healthy, New Zealand white rabbits weighing 2.5 – 3.5 kg were divided in three groups. The animals were fasted overnight prior to the experiment, with free access to water. To first two groups of rabbits, radiolabeled chitosan microspheres (CHNTD) and alginate microspheres (ALNT) (approximately 5-10 mg) of nitrendipine were administered intranasally using monodose insufflator (Miat, Milano, Italy). To third group, radiolabeled NTD was administered intravenously (0.5 mL) through the dorsal ear vein. At selected time intervals, blood samples were withdrawn from the marginal vein of other ear of the rabbits. The radioactivity in terms of counts per minute (CPM) was measured in a well-type gamma scintillation counter (Caprac-R, Capintec, USA). The animals were conscious during the whole experiment and between each blood sampling they were allowed to move freely within an enclosed area.

10.2.7 Pharmacokinetics Analysis

The noncompartmental pharmacokinetic analysis was performed by Kinetica 5.0 (Thermo Fisher Scientific, USA) using the procedure described in **Section 9.3.7**.

10.2.8 Gamma Scintigraphy

The deposition, distribution and subsequent clearance of microspheres and lactose were studied by gamma scintigraphy and imaging was performed as described in **Section 9.3.8**.

10.2.9 Statistical analysis

Data are presented as mean values ± SD of four determinations. The principal pharmacokinetic measures obtained for the intranasal dose groups were compared statistically using GraphPad™ Instat® Version 3.06 (GraphPad, San Diego, USA). A Student's t-test and one way ANOVA followed by Dunnett's multiple comparison test were performed.

10.3 Results and Discussion

10.3.1 Radiolabeling of Nitrendipine and Its Microspheres

The microspheres (CHNT and ALNT) and NTD were labeled with high efficiency by the direct labeling technique using reduced ^{99m}Tc. Table 10.1 depicts the effect of pH on labeling efficiency. As the pH increased from 6 to 6.5, the radiolabeling efficiency also increased from 91.68% to 97.34% for CHNT and 90.22% to 95.21% for ALNT. Further increase in the pH upto 8 led to reduction in the labeling efficiency of microspheres. As the pH increased from 6 to 7, the radiolabeling efficiency also increased from 92.74% to 97.16% for NTD. Further increase in the pH upto 8 led to reduction in the labeling efficiency of 91.58%.

Table 10.1 Effect of pH on the % radiolabeling efficiency of Microspheres and Nitrendipine

	% Radiolabeled		
pH	**CHNT**	**ALNT**	**NTD**
6	91.68±1.98	90.22±2.23	92.74±1.78
6.5	97.34±1.64	95.21±1.56	95.61±1.55
7	94.84±1.38	92.74±1.46	97.16±1.16
7.5	91.64±1.42	90.31±2.14	94.88±2.22
8	90.52±1.15	88.12±1.87	91.58±1.89

Table 10.2 depicts the influence of incubation time on labeling efficiency. The incubation time required for high labeling efficiency was found to be 10 min for CHNT and ALNT; and 30 min for NTD. Further increase in incubation time did not increase the labeling efficiency considerably.

Table 10.2 Effect of Incubation time on the % radiolabeling efficiency of Microspheres and Nitrendipine

Incubation time	% Radiolabeled		
(min)	CHNT	ALNT	NTD
0	90.32±1.45	92.79±1.55	94.11±1.56
10	97.12±1.84	95.61±1.96	95.22±2.37
20	96.88±1.75	94.72±1.34	96.86±2.11
30	95.23±2.14	93.43±1.92	97.94±1.94
40	95.12±1.88	93.28±1.95	95.32±1.17

In determining labeling efficiency, the amount of stannous chloride (reducing agent) used for labeling plays a very decisive role. A high amount of stannous chloride leads to the formation of radiocolloids (reduced/hydrolyzed $^{99m}TcO^{4-}$), which is undesirable. On the other hand, less amount of stannous chloride results in poor labeling (Arulsudar et al., 2003; Thakkar et al., 2004). Table 10.3 shows the effect of various concentrations of stannous chloride ($SnCl_2.2H_2O$) on labeling efficiency of microspheres and nitrendipine. By varying the amount of stannous chloride from 50 to 300 µg, but keeping the other factors like pH and incubation time constant, the effect on labeling efficiency was studied. With increase in stannous chloride amount from 50 to 250 µg, the labeling efficiency was increased from 86.98% to 97.11% for CHNT and 85.22% to 95.38% for ALNT. Further increase in the amount of stannous chloride led to a reduction in labeling efficiency. With increase in stannous chloride amount from 50 to 100 µg, the labeling efficiency was increased from 92.45% to 97.22% for NTD. Further increase in the amount of stannous chloride upto 300 µg led to a reduction in labeling efficiency to 85.58%.

Table 10.3 Effect of Stannous Chloride Concentration on the % radiolabeling efficiency of Microspheres and Nitrendipine

Amount of stannous chloride (µg)	% Radiolabeled		
	CHNT	ALNT	NTD
50	86.98±1.62	85.22±1.98	92.45±2.12
100	90.45±1.55	88.33±1.36	92.64±1.66
150	91.34±2.21	91.36±2.41	95.44±1.88
200	94.68±1.68	93.42±1.67	97.22±1.85
250	97.11±1.84	95.38±2.47	89.22±1.59
300	93.92±1.56	90.88±1.64	85.58±2.24

10.3.2 Stability Study of ^{99m}Tc-NTD/Microsphere Complexes

The in vitro stability of the labeled formulations (^{99m}Tc-NTD/Microsphere Complexes) was evaluated in saline and in rabbit serum at 37 ^{0}C for 24 h. All formulations exhibited good in vitro stability as shown in Table 10.4 and 10.5. It is evident from the results that there is insignificant detachment of the radioisotope from the complex. There was no significant reduction in the radiolabeling efficiency upto 24 h which indicates its stability and suitability for in vivo use.

Table 10.4 In Vitro Stability of the ^{99m}Tc-NTD and ^{99m}Tc-Microspheres in Physiological Saline at 37 ^{0}C

Time (h)	% Radiolabeling efficiency in Saline		
	CHNT	ALNT	NTD
0.5	97.35±1.89	95.17±1.96	97.21±1.48
1	96.98±1.77	94.84±1.64	97.11±1.22
2	95.12±1.46	93.66±1.77	96.45±1.84
4	94.22±2.31	93.32±1.44	95.32±1.48
6	92.98±1.68	92.86±2.15	94.28±2.32
24	91.36±2.21	90.74±1.79	92.42±1.88

Table 10.5 In Vitro Stability of the ^{99m}Tc-NTD and ^{99m}Tc-Microspheres in Serum at 37 ^{0}C

Time (h)	% Radiolabeling efficiency in Serum		
	CHNT	ALNT	NTD
0.5	97.94±1.69	95.82±1.91	97.72±1.62
1	97.12±1.38	95.13±1.37	96.98±2.34
2	96.54±2.12	94.67±1.94	96.72±1.85
4	95.98±1.58	93.95±1.88	95.63±1.64
6	94.78±1.64	92.87±1.56	94.93±1.92
24	93.47±1.57	91.76±1.62	93.22±1.52

10.3.3 Pharmacokinetics Studies

The mean blood radioactivity (KCPM/gm) of Nitrendipine after intravenous (IV) and of microsphere formulations (CHNT and ALNT) after intranasal administration to rabbits is shown in Table 10.6 while blood kinetics data (blood radioactivity time profiles) of NTD solution intravenously and the microsphere formulations (CHNT and ALNT) intranasally to rabbits at various time intervals are shown in Figure 10.1.

Table 10.6 Mean blood radioactivity* (KCPM/gm) of Nitrendipine after IV and of CHNT and ALNT after Intranasal administration

Time(h)	NTD IV	CHNT Intranasal	ALNT Intranasal
0.25	31.24±3.68	11.35±2.55	8.58±1.72
0.5	26.68±1.92	39.22±3.48	21.62±2.61
1	16.47±2.21	86.49±5.27	45.76±3.72
2	10.34±1.87	69.44±4.62	76.85±4.95
3	9.62±1.14	52.38±3.21	53.72±4.38
4	7.84±2.26	41.65±4.08	41.14±2.86
6	5.21±1.38	27.54±2.26	31.38±2.35
8	2.12±1.49	18.56±1.74	17.54±1.62

* Each value represents the Mean ± SD (n = 4)

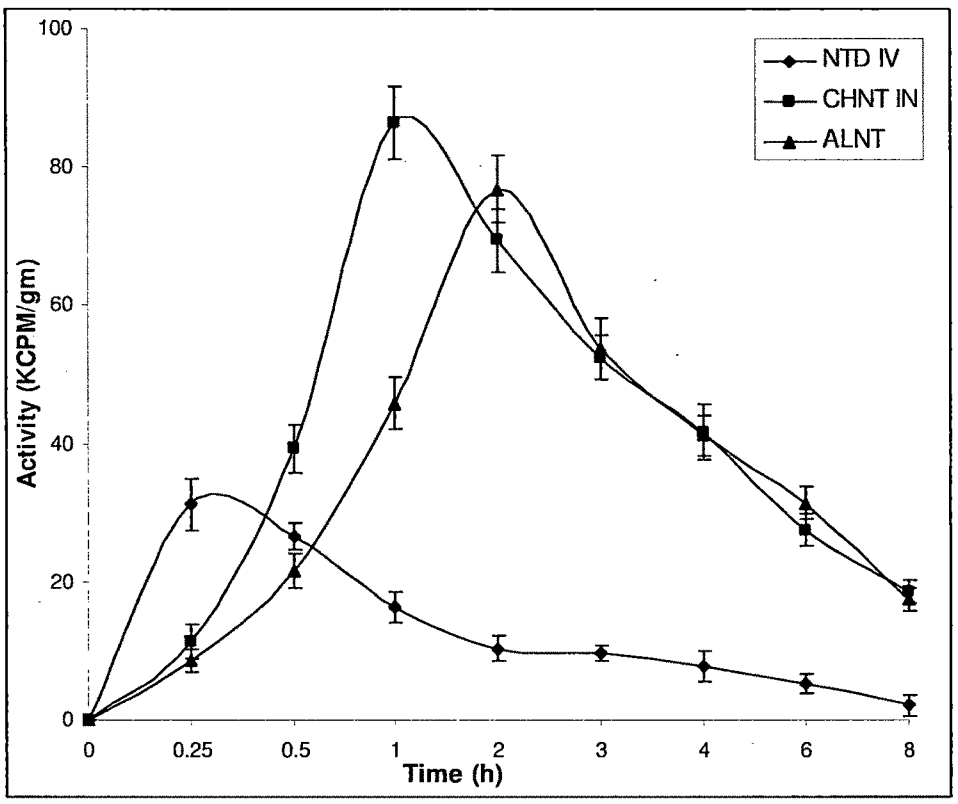

Fig. 10.1 Blood radioactivity time profiles of NTD after administration of microspheres intranasally (IN) (1.25 mg kg $^{-1}$) and NTD solution intravenously (IV) (0.2 mg kg $^{-1}$) in rabbits.

The relevant pharmacokinetic parameters including maximum concentration (C_{max}), time of maximum plasma concentration (T_{max}), the area under the curve (AUC), half life ($T_{1/2}$), mean residence time (MRT), elimination rate constant (K_{el}) and relative bioavailability (F) are shown in Table 10.7.

Table 10.7 Pharmacokinetic parameters of Nitrendipine after IV and of CHNT and ALNT after Intranasal administration in Rabbits

Parameters*	NTD IV	CHNT Intranasal	ALNT Intranasal
C_{max} (KCPM/gm)	-	86.49±5.27	76.85±4.95
T_{max} (h)	-	1.0	2.0
AUC_{0-8h} (KCPM.h/gm)	78.22±8.32	337.05±34.81	314.41±29.22
$AUC_{0-\infty h}$ (KCPM.h/gm)	86.2±9.54	426.52±41.12	390.76±32.48
$T_{1/2}$ (h)	2.60±0.54	3.34±1.18	3.01±0.86
MRT(h)	3.36±0.96	5.27±0.93	5.29±1.24
K_{el} (h^{-1})	0.265±0.073	0.207±0.085	0.229 ±0.097
F (%)	100	68.94	64.31

* Each value represents the Mean ± SD (n = 4)

The C_{max} values observed after intranasal administration of CHNT and ALNT were 86.49±5.27 KCPM/gm and 76.85±4.95 KCPM/gm respectively. The AUC after intranasal administration of CHNT and ALNT were about 337.05±34.81 KCPM.h/gm and 314.41±29.22 KCPM.h/gm respectively which was statistically not significant ($p>0.05$; Student's t test).

T_{max} values were 1.0 and 2.0 h for nasal administration of CHNT and ALNT. The average $T_{1/2}$ values were 3.34±1.18 and 3.01±0.86 h for CHNT and ALNT, respectively, as compared to 2.60±0.54 h following IV administration of NTD.

The MRT was considerably increased following nasal administration of the mucoadhesive formulations of nitrendipine (CHNT and ALNT) as compared to IV administration. The average MRT values after nasal administration of CHNT and

ALNT were 5.27±0.93 and 5.29±1.24 h, respectively, as compared to 3.36±0.96 h after IV administration of NTD and they were significantly different ($p<0.05$, ANOVA followed by Dunnett's multiple comparison test) from IV. Between the two mucoadhesive formulations i. e. CHNT and ALNT, the difference was non significant ($p>0.05$; Student's t test) indicating that there was no formulation variation.

The relative bioavailability (F) for CHNT and ALNT were 68.94% and 64.31% respectively which indicate that nasal administration results in improved absorption of nitrendipine from chitosan and alginate microspheres in rabbits.

10.3.4 Gamma Scintigraphy

The nasal clearance characteristics of two microsphere drug delivery systems, CHNT and ALNT, were studied. Lactose powder was used as negative control. The percentage of the formulations cleared from the nasal cavity of rabbits in the time course of study (4 h) is shown in Table 10.8. The clearance data for each formulation from the nasal cavity (ROI) is shown in Fig. 10.2. This data shows that the control lactose powder was cleared rapidly (half-life of nasal clearance was less than 1.0 h), whereas the mucoadhesive delivery systems were retained within the nasal cavity for longer time (half-lives of nasal clearance were >2.5 h). It has been reported that the normal half-life of nasal clearance in man is about 20 min (Schipper et al., 1991). The nasal clearance half-lives of microspheres were higher than normal clearance half-life of human nose (at least four-fold higher), which is representative of high mucoadhesive strength of these particulate systems.

Table 10.8 The radioactivity remaining in the rabbit nasal cavity at each time point after administering chitosan microspheres (CHNT), alginate microspheres (ALNT) and lactose powder (control)

Time (h)	Radioactivity (%) in the nasal cavity		
	CHNT	ALNT	Lactose
0	100	100	100
1	67.98	62.48	46.00
2	54.46	52.34	28.32
3	49.28	45.43	21.48
4	43.92	35.24	12.64

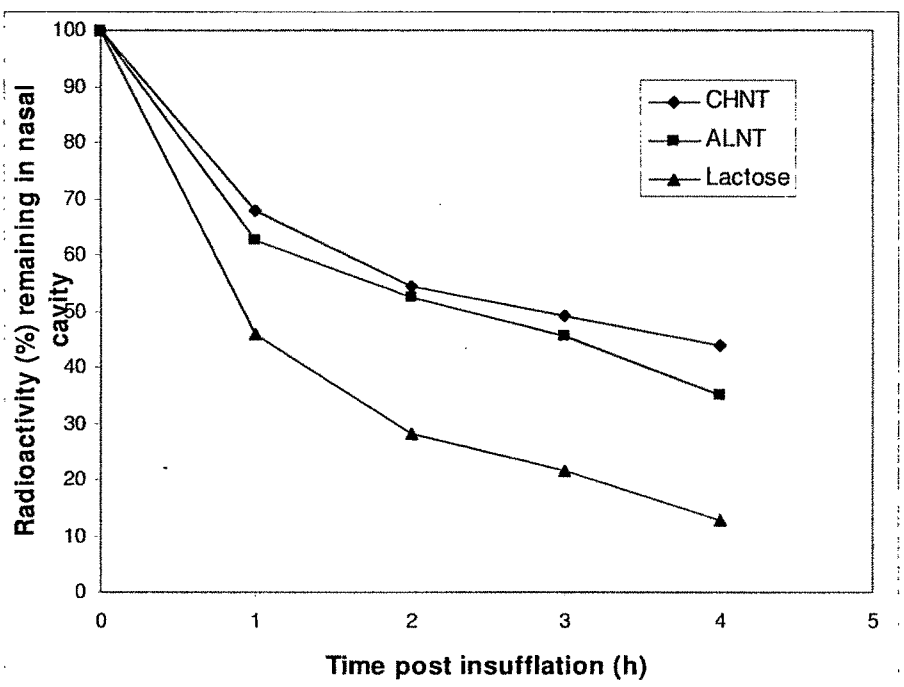

Fig. 10.2 The clearance characteristics of radiolabeled chitosan microspheres (CHNT) and alginate microspheres (ALNT) from the rabbit nasal cavity as compared to lactose powder a control.

Among microsphere formulations (CHNT and ALNT) studied, the lowest clearance rate and highest mucoadhesion was shown by chitosan microspheres (CHNT) followed by alginate microspheres (ALNT). After 4 h, 56.08% of chitosan and 64.76% of alginate microspheres have been cleared from nasal cavity while in the same time 87.36% lactose powder cleared, respectively.

Studies have shown that polymers with charge density can serve as good mucoadhesive agents (Park and Robinson, 1984; Chickering and Mathiowitz, 1995). Both the microspheres studied in the present work (chitosan and alginate) were ionic and showed good mucoadhesive potential. Compared to lactose powder as control, in nasal cavity, chitosan and alginate microspheres showed a significantly higher mucoadhesion.

The gamma scintigraphy images (Fig. 10.3, Fig. 10.4 and Fig. 10.5) showed that the microsphere powder was spread over a wide area within the nasal cavity of rabbits. The results indicated that the microspheres cleared slowly and were retained for extended periods in the nasal cavity, thereby providing sustained and enhanced drug absorption from the nasal mucosa, as confirmed from pharmacokinetic studies.

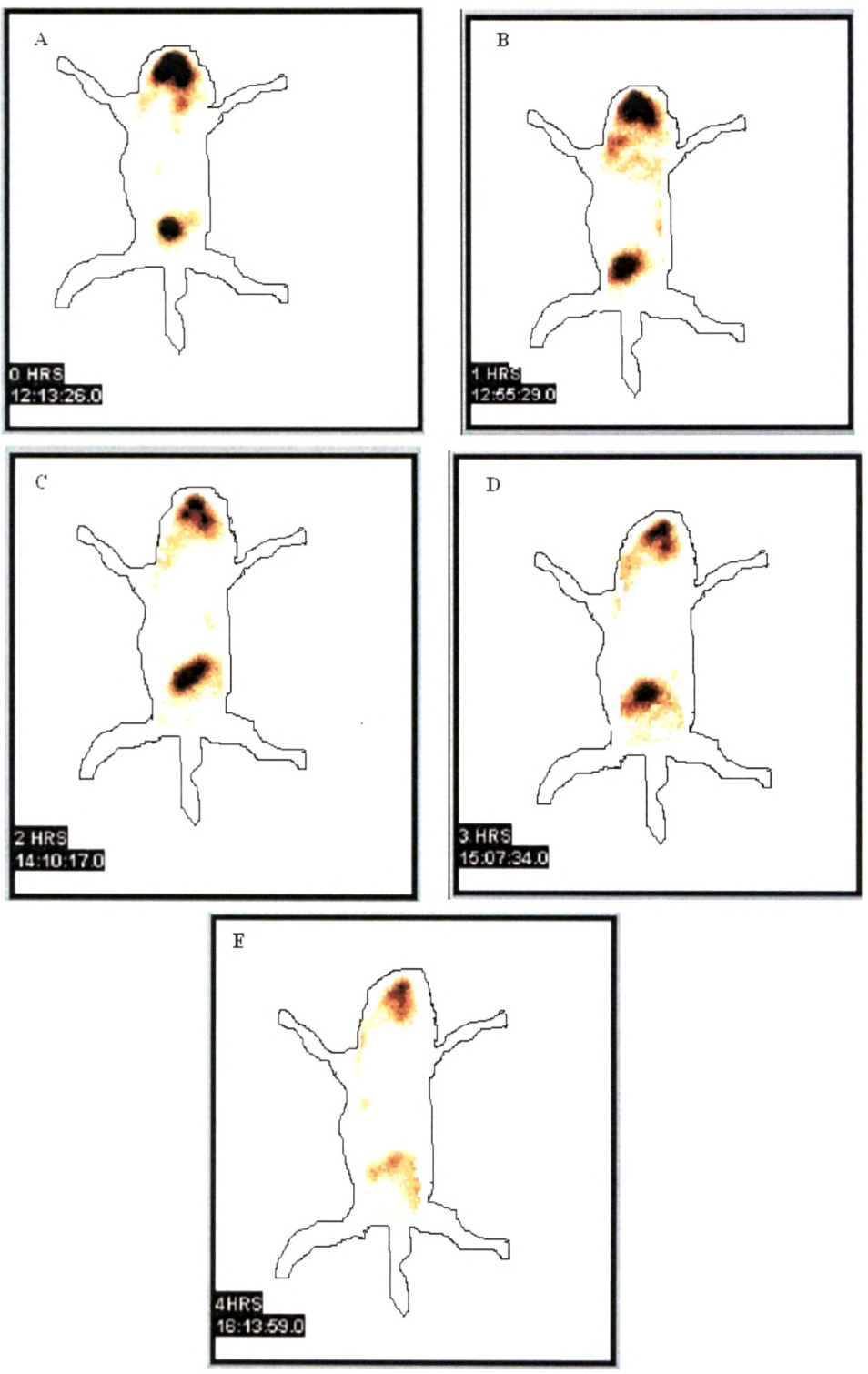

Fig. 10.3 Scintigraphic rabbit whole body images showing radioactivity in the nasal cavity after administration of ^{99m}Tc labeled chitosan microspheres of nitrendipine (CHNT) at different times of 0 h (A), 1 h (B), 2 h (C), 3 h (D) and 4 h (E) post insufflation.

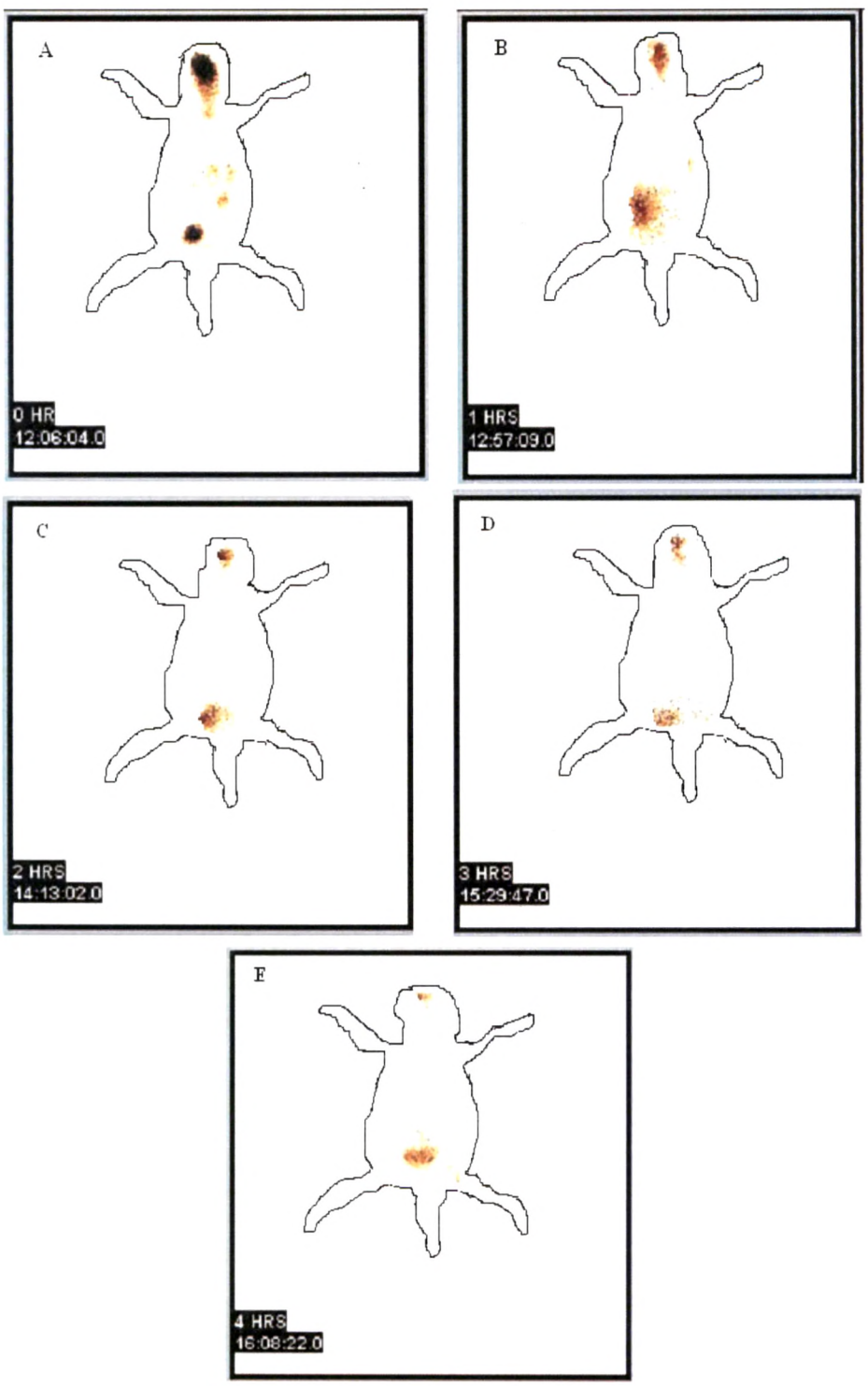

Fig. 10.4 Scintigraphic rabbit whole body images showing radioactivity in the nasal cavity after administration of ^{99m}Tc labeled alginate microspheres of nitrendipine (ALNT) at different times of 0 h (A), 1 h (B), 2 h (C), 3 h (D) and 4 h (E) post insufflation.

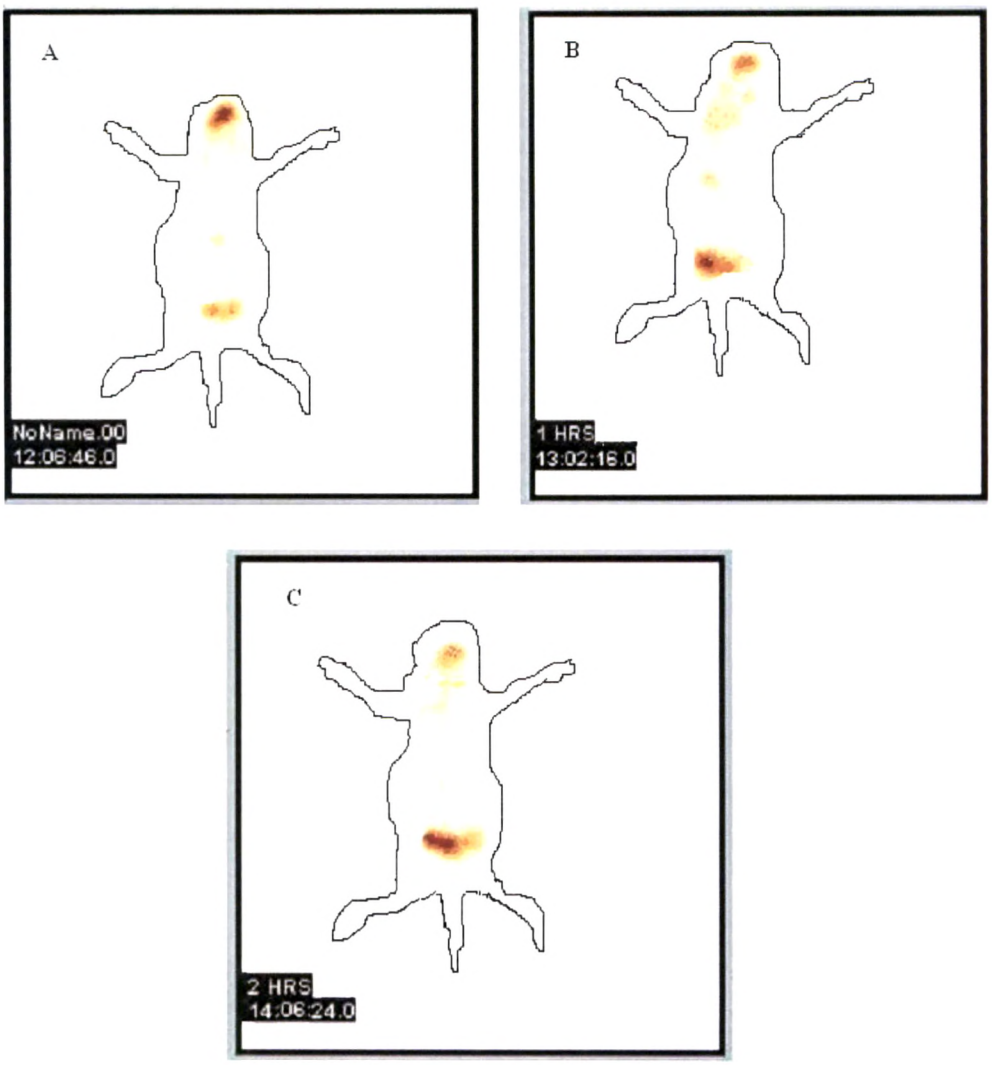

Fig. 10.5 Scintigraphic rabbit whole body images showing radioactivity in the nasal cavity after administration of ^{99m}Tc labeled lactose powder (control) at different times of 0 h (A), 1 h (B) and 2 h (C) post insufflation.

REFERENCES

Chickering, D.; Mathiowitz, F. Bioadhesive microspheres: **1**. A novel electrobalance-based method to study adhesive interactions between individual microspheres and intestinal mucosa. *J. Controlled Release* **1995**, 34, 251–261.

Park, K., Robinson, J. R. Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion. *Int. J. Pharm.* **1984**, 19, 107–127.

Peppas, N. A.; Buri, P. A. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. *J. Controlled Release* **1985**, 2, 257–275.