Preliminary Brain-targeting Studies on Intranasal Mucoadhesive Microemulsions of Sumatriptan

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Tushar K. Vyas,[1] A. K. Babbar,[2] R. K. Sharma,[2] Shashi Singh,[3] and Ambikanandan Misra[1]

[1]Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Kalabhavan, Post Box No. 51, Vadodara - 390 001, Gujarat, India
[2]Government of India, Ministry of Defense, Institute of Nuclear Medicine and Allied Sciences, Brig S. K. Mazumdar Marg, Timarpur, Delhi - 110054, India
[3]Center for Cellular and Molecular Biology (CCMB), Uppal Road, Hyderabad - 500 007 A. P., India

ABSTRACT

The aim of this investigation was to prepare microemulsions containing sumatriptan (ST) and sumatriptan succinate (SS) to accomplish rapid delivery of drug to the brain in acute attacks of migraine and perform comparative in vivo evaluation in rats. Sumatriptan microemulsions (SME)/sumatriptan succinate microemulsions (SSME) were prepared using titration method and characterized for drug content, globule size and size distribution, and zeta potential. Biodistribution of SME, SSME, sumatriptan solution (SSS), and marketed product (SMP) in the brain and blood of Swiss albino rats following intranasal and intravenous (IV) administrations were examined using optimized technetium-labeled (^{99m}Tc-labeled) ST formulations. The pharmacokinetic parameters, drug targeting efficiency (DTE), and direct drug transport (DTP) were derived. Gamma scintigraphy imaging of rat brain following IV and intranasal administrations were performed to ascertain the localization of drug. SME and SSME were transparent and stable with mean globule size 38 ± 20 nm and zeta potential between −35 to −55 mV. Brain/blood uptake ratios at 0.5 hour following IV administration of SME and intranasal administrations of SME, SMME, and SSS were found to be 0.20, 0.50, 0.60, and 0.26, respectively, suggesting effective transport of drug following intranasal administration of microemulsions. Higher DTE and DTP for mucoadhesive microemulsions indicated more effective targeting following intranasal administration and best brain targeting of ST from mucoadhesive microemulsions. Rat brain scintigraphy endorsed higher uptake of ST into the brain. Studies conclusively demonstrated rapid and larger extent of transport of microemulsion of ST compared with microemulsion of SS, SMP, and SSS into the rat brain. Hence, intranasal delivery of ST microemulsion developed in this investigation can play a promising role in the treatment of acute attacks of migraine.

Corresponding Author: Ambikanandan Misra, Professor and Head, Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Kalabhavan, Post Box No. 51, Kalabhavan, Vadodara - 390 001, Gujarat, India. Tel: +91-265-2434187; Fax: +91-265-2423898, 2418927; E-mail: misraan@hotmail.com, misraan@satyam.net.in

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INTRODUCTION

Migraine attack is a troublesome physiological condition associated with throbbing, intense headache in one-half of the head. During an attack, the blood vessels in the brain dilate and then draw together with stimulation of nerve endings near the affected blood vessels. These changes to the blood vessels and stimulation of nerves are probably what cause the pain, although migraine is still a poorly understood condition or phenomenon.[1] Migraine treatment has evolved into the scientific arena, but opinions differ on whether migraine is primarily a vascular or a neurological dysfunction.[2,3]

ST/SS, triptan derivatives are serotonin (5-hydroxytryptamine) agonists available in the market in oral tablets and subcutaneous injection dosage form for the treatment of migraine.[2,4] ST is also available in a rectal suppository dosage form for the treatment of migraine attacks. A substantial proportion of migraine patients not only suffer from gastric stasis but also have severe nausea and vomiting, which results in erratic absorption of ST from the gastrointestinal tract.[5] ST is rapidly but incompletely absorbed following oral administration and undergoes first-pass metabolism, resulting in a low absolute bioavailability of 14% in humans.[6] Moreover, the transport of ST across the blood-brain barrier (BBB) is very poor, although evidence of detection of some drug in cerebrospinal fluid (CSF) following high IV dose has been cited in the literature.[7] Therefore, an alternative route of drug delivery that can selectively target the drug directly into various regions of the brain, including vasculature,[8] is needed for the treatment of acute attacks of migraine.[9,10]

Previous studies have demonstrated that intranasal administration offers a practical, noninvasive, alternative route of administration for drug delivery to the brain.[7,9] Intranasal administration allows transport of drugs to the brain circumventing BBB, thus providing a unique feature and better option to target drugs to the brain.[11,12] However, reports in the literature reveal that the bioavailability for intranasal route of administration for ST has been found to be 17% compared with subcutaneous route.[13] Therefore, there is a need to design a delivery system that can provide rapid transport of drug across nasal mucosa and longer residence time in the nasal cavity.[14] Microemulsions have been explored widely as a delivery system by virtue of having considerable potential to enhance transport of a wide range of drug molecules.[15] The addition of a mucoadhesive agent such as polyelectrolyte polymer helps in retention of the formulation in the nasal cavity.[16,17]

The objective of this investigation was to prepare and characterize microemulsion/mucoadhesive microemulsion of ST and SS and to assess nose-to-brain delivery and biodistribution of the radiolabeled drug from the developed microemulsions, drug solutions, and a market formulation in rats. ST, being relatively more lipophilic compared with its salt, sumatriptan succinate (SS), was expected to absorb across nasal mucosa differently from microemulsion, hence both the drugs were included in this study. It was hypothesized that microemulsion-/mucoadhesive microemulsion-based alternative drug delivery systems would result in rapid nose-to-brain transport of ST, and therefore greater drug transport and distribution into and within the brain. This benefit would help to maximize the therapeutic index of the drug, reduce side effects, decrease the dose and frequency of dosing, and perhaps even the cost of the therapy.

Materials and Methods

Chemicals

ST was a gift from Sun Pharmaceuticals (Vadodara, India) and SS was gifted by Hetero Drugs Ltd (Hyderabad, India). Fatty acid ester of polyglycerol, caprylocaproyl macrogol glyceride, and purified diethylene glycol monoethyl ether were gifted by Gattefose (Saint-Priest, France). Polycarbophil (AA-1, pharmagrade, molecular weight (Mw) ~3.5 billion) was purchased from Noveon (Mumbai, India). Succinic acid was purchased from Merck Chemicals (Mumbai, India). Diethylene triamine penta acetic acid (DTPA) and stannous chloride dihydrate ($SnCl_2.2H_2O$) were purchased from Sigma Chemical Co (St Louis, MO). Sodium pertechnetate, separated from molybdenum-99 (99m) by solvent extraction method, was provided by Regional Center for Radiopharmaceutical Division (Northern Region), Board of Radiation and Isotope Technology (BRIT, Delhi, India). All other chemicals were of analytical reagent grade and used without further purification.

Preparation of ST/SS Formulations

Sumatriptan succinate solution (SSS, 20 mg/mL ST) was prepared by addition of sumatriptan succinate (SS) (equivalent to 200 mg ST) to 8 mL distilled water with stirring. The pH was adjusted to 3.5 ± 0.25 using succinic acid (~ 0.12 mg/mL). The dispersion was stirred for 10 minutes and final volume was made up to 10 mL with distilled water. Sumatriptan succinate mucoadhesive solution (SSMS, 20 mg/mL ST) was prepared by addition of 0.5% wt/wt polycarbophil to SSS with continuous stirring.

Sumatriptan microemulsion (SME, 20 mg/mL ST) and sumatriptan succinate microemulsion (SSME, 20 mg/mL ST) were prepared using medium chain triglyceride (MCT) as an oil (20% wt/wt), caprylocaproyl macrogol glyceride as surfactant (S, 27.50% wt/wt). Mixture (1:1 wt/wt) of purified diethylene glycol monoethyl ether and fatty acid ester of polyglycerol was used as cosurfactant (CoS, 12.50% wt/wt) and distilled water (40% wt/wt) as aqueous phase. Formulations were prepared by dissolving ST/SS at 60°C ± 5°C in S, CoS, and oil mixture. The resultant solution was cooled to 30°C ± 5°C. Distilled water was added gradually with continuous stirring, which resulted in transparent and homogenous SME (transmittance at 630 nm > 99%). Sumatriptan- and sumatriptan-succinate mucoadhesive microemulsion (SMME/SSMME, 20 mg/mL ST) were prepared by addition of polycarbophil (0.5% wt/wt) to SME/SSME with continuous stirring.

Characterization

Sumatriptan (ST) content was analyzed using high-performance liquid chromatography (HPLC) method, wherein UV detector equipped at λ_{max} 228 nm was used for determination. C_{18} column at 25°C was used for separation, and a mixture of ammonium phosphate monobasic (0.05M):acetonitrile (84:16, vol/vol) was used as mobile phase.[18] Degassed mobile phase was isocratically run at a flow rate of 1 mL/min and the injection volume was 50 µL. Globule size was determined[19] using photon correlation spectroscopy (PCS) with built-in Zetasizer (model Nano ZS, Malvern Instruments, Worcestershire, UK) at 633 nm. Helium-neon gas laser having intensity of 4 mW was the light source. The equipment was programmed to provide 18-mm laser width. Measured electrophoretic mobility (µm/s) using small volume disposable zeta cell is converted to zeta potential[19] by built-in software based on Helmholtz-Smoluchowski equation. Compositions, globule size, zeta potential, and radiolabeling efficiency of the formulations are recorded in Table 1.

Table 1. Composition and Characterization of Sumatriptan and Sumatriptan Succinate Formulations*

Abbreviation	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Drug Content (%)	Globule Size (nm)	Zeta Potential (mV)	Radiolabeled Complex (%)
SMME	Sumatriptan mucoadhesive microemulsion	20.0	27.5	12.5	40.0	99.19 ± 0.12	30.15 ± 13.24	-48.66 ± 1.44	96.54 ± 0.20
SME	Sumatriptan microemulsion	20.0	27.5	12.5	40.0	98.94 ± 0.10	29.98 ± 15.42	-38.90 ± 2.05	97.69 ± 0.10
SSMME	Sumatriptan succinate mucoadhesive microemulsion	20.0	27.5	12.5	40.0	100.2 ± 0.08	34.51 ± 17.80	-51.20 ± 1.92	98.74 ± 0.09
SSME	Sumatriptan succinate microemulsion	20.0	27.5	12.5	40.0	97.99 ± 0.17	38.45 ± 20.38	-39.10 ± 2.02	98.22 ± 0.14
SSS	Sumatriptan succinate solution	-	-	-	100.0	98.39 ± 0.11	-	-	95.96 ± 0.10

*The results are mean values ± SEM derived from 6 different experimental batches. O indicates oil phase (medium chain triglyceride); S, surfactant (mixture (1:1) of caprylocaproyl macrogol glyceride and purified diethylene glycol); CoS, cosurfactant (fatty acid ester of polyglycerol); and AQ, aqueous phase (purified water). The formulations (SMME, SME, SSMME, SSME, and SSS) contain sumatriptan 20 mg/mL.

Radiolabeling of Sumatriptan Solution and Sumatriptan Microemulsions

SSS, SSMS, SME, SMME, SSME, SSMME, and Sumaneg NS (marketed product, SS aqueous solution, SMP) were radiolabeled using technetium-99m (^{99m}Tc) by direct-labeling method.[20-24] One milliliter of formulation was taken and 100 µg of stannous chloride dihydrate in 100 µL of 0.10N HCl was added, and pH was adjusted to 6.80 ± 0.20 using 50mM sodium bicarbonate solution. To the resultant mixture (filtered through 0.22-µm nylon 66 membrane), 1 mL of sterile ^{99m}Tc-pertechnetate (75 to 400 MBq) was added over a period of 60 seconds with continuous mixing and incubated at 30°C ± 5°C for 30 minutes with continuous nitrogen purging. The final volume was made up to 2.50 mL using 0.90% (wt/vol) sterile sodium chloride solution.

The radiochemical purity[22,23] of ^{99m}Tc-SSS (^{99m}Tc-labeled SSS), ^{99m}Tc-SSMS (^{99m}Tc-labeled SSMS), ^{99m}Tc-SME (^{99m}Tc-labeled SME), ^{99m}Tc-SMME (^{99m}Tc-labeled SMME), ^{99m}Tc-SSME (^{99m}Tc-labeled SSME), ^{99m}Tc-SSMME (^{99m}Tc-labeled SSMME), and ^{99m}Tc-SMP (^{99m}Tc-labeled SMP) were determined using ascending instant thin layer chromatography (TLC). Silica gel-coated fiberglass sheets (Gelman Sciences Inc, Ann Arbor, MI) and dual solvent systems consisting of acetone and pyridine:acetic acid:water (3:5:1.50 vol/vol) were used as mobile phases. The effect of incubation time, pH, and stannous chloride concentration on labeling were studied to achieve optimum reaction conditions. The radiolabeled formulations were challenged for bonding strength using diethylene triamine penta acetic acid[23] and in vitro stability in 0.90% (wt/vol) sodium chloride (normal saline) and in rat plasma were evaluated.[22] Optimized stable radiolabeled formulations were used to study biodistribution.

Biodistribution Studies

The Social Justice and Empowerment Committee, Ministry of Government of India, approved all animal experiments conducted for the purpose of control and supervision on animals and experiments. Swiss albino rats (male, aged 4 to 5 months), weighing between 200 and 250 g were selected for the study. Four rats for each formulation per time point were used in the study. Radiolabeled complex of ^{99m}Tc-ST formulations (100 µCi/50 µL) containing 0.40 mg to 0.50 mg ST (equivalent 0.33 mg/kg body weight [BW]) were administered (10 µL) in each nostril. The rats were anaesthetized using ketamine intramuscular injection (50 mg/kg). Formulations were instilled into nostrils with the help of micropipette (100 µL) attached with low-density polyethylene (LDPE) tubing having 0.1-mm internal diameter at the delivery site. Similarly, radiolabeled complex of ^{99m}Tc-SME (100 µCi /20 µL) containing 0.40 mg to 0.50 mg ST (equivalent 0.33 mg/kg BW) injected through tail vein of Swiss albino rats.[20] The rats were killed humanely at different time intervals and the blood was collected using cardiac puncture. Subsequently, brain and spinal cord were dissected, washed twice using normal saline, made free from adhering tissue/fluid, and weighed. Radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter.

Table 2. Compartmental Distribution of ^{99m}Tc-SME (IV), ^{99m}Tc-SMME, SME, and SMP (intranasal) at Predetermined Time Intervals in Normal Swiss Albino Rats*

Formulation and Route of Administration	Distribution of ST in Blood and Brain Compartments at Predetermined Time Intervals					
		0.5 Hour	1 Hour	2 Hours	4 Hours	8 Hours
SME (IV)	Blood	4.256 ± 0.42	2.880 ± 0.28	2.050 ± 0.42	0.540 ± 0.14	0.120 ± 0.05
	Brain	0.860 ± 0.12	0.610 ± 0.15	0.430 ± 0.05	0.110 ± 0.06	0.080 ± 0.06
SMME (intranasal)	Blood	2.041 ± 0.18	2.672 ± 0.24	1.883 ± 0.16	0.924 ± 0.11	0.647 ± 0.05
	Brain†	1.214 ± 0.09	0.983 ± 0.11	0.779 ± 0.14	0.436 ± 0.15	0.211 ± 0.10
SME (intranasal)	Blood	1.953 ± 0.21	2.418 ± 0.22	2.594 ± 0.33	0.736 ± 0.16	0.229 ± 0.05
	Brain†	0.975 ± 0.14	0.851 ± 0.12	0.659 ± 0.13	0.430 ± 0.11	0.088 ± 0.03
SMP (intranasal)	Blood	1.093 ± 0.09	0.610 ± 0.15	0.380 ± 0.09	0.090 ± 0.02	0.028 ± 0.04
	Brain†	0.293 ± 0.09	0.234 ± 0.11	0.211 ± 0.12	0.132 ± 0.04	0.061 ± 0.03
SME (IV)	Brain/Blood	0.202 ± 0.09	0.212 ± 0.03	0.210 ± 0.04	0.204 ± 0.05	0.667 ± 0.14
SMME (intranasal)	Brain/Blood	0.595 ± 0.11	0.368 ± 0.08	0.414 ± 0.07	0.472 ± 0.06	0.326 ± 0.08
SME (intranasal)	Brain/Blood	0.499 ± 0.15	0.352 ± 0.05	0.254 ± 0.11	0.584 ± 0.11	0.384 ± 0.02
SMP (intranasal)	Brain/Blood	0.268 ± 0.05	0.384 ± 0.05	0.555 ± 0.09	1.467 ± 0.13	2.179 ± 0.16

*SMP indicates marketed product; all other abbreviations are explained in Table 1. The rats were administered with 100 μCi ^{99m}Tc-sumariptan formulations and the radioactivity was measured in percentage per gram of tissue of the administered dose. Each value is the mean ± SEM of 4 estimations.

†Difference was found significant ($P < .05$) when SME intranasal (brain) and SMME intranasal (brain) were compared with SMP intranasal (brain).

Radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose.[23] The results are recorded in Tables 2 and 3 (ST and SS, respectively) and the brain concentrations versus time (hours) for different formulations containing ST and SS are shown in Figures 1 and 2, respectively. Pharmacokinetic parameters for ST and SS formulations were calculated using Kinetica (Version 4.10, Innaphase, Philadelphia, PA) and recorded in Tables 4 and 5, respectively. Brain targeting efficiency was calculated using 2 equations mentioned below.[25,26]

Drug targeting efficiency (DTE)[12]: DTE represents time average partitioning ratio.

$$DTE(\%) = \left[\frac{\left\{\left(\frac{AUC_{brain}}{AUC_{blood}}\right)\right\}_{in}}{\left\{\left(\frac{AUC_{brain}}{AUC_{blood}}\right)\right\}_{IV}}\right] \times 100,$$

where AUC indicates area under the curve and is denoted for intranasal administration.

Table 3. Compartmental Distribution of ^{99m}Tc-SSS, SSME, SSMME, and SMP (intranasal) at Predetermined Time Intervals in Normal Swiss Albino Rats*

Formulation and Route of Administration	Distribution of SS in Blood and Brain Compartments at Predetermined Time Intervals					
		0.5 Hour	1 Hour	2 Hours	4 Hours	8 Hours
SSMME (intranasal)	Blood	2.284 ± 0.22	0.932 ± 0.16	0.418 ± 0.09	0.211 ± 0.08	0.023 ± 0.03
	Brain	0.677 ± 0.18	0.562 ± 0.13	0.420 ± 0.04	0.214 ± 0.06	0.076 ± 0.02
SSME (intranasal)	Blood	2.194 ± 0.24	0.977 ± 0.13	0.258 ± 0.03	0.174 ± 0.06	0.096 ± 0.04
	Brain†	0.582 ± 0.06	0.568 ± 0.07	0.443 ± 0.07	0.318 ± 0.11	0.110 ± 0.05
SSS (intranasal)	Blood	1.502 ± 0.11	0.545 ± 0.02	0.482 ± 0.12	0.310 ± 0.10	0.038 ± 0.03
	Brain	0.390 ± 0.05	0.288 ± 0.07	0.220 ± 0.08	0.149 ± 0.10	0.092 ± 0.05
SMP (intranasal)	Blood	1.093 ± 0.09	0.610 ± 0.15	0.380 ± 0.09	0.090 ± 0.02	0.028 ± 0.04
	Brain†	0.293 ± 0.09	0.234 ± 0.11	0.211 ± 0.12	0.132 ± 0.04	0.061 ± 0.03
SSMME (intranasal)	Brain/Blood	0.296 ± 0.07	0.603 ± 0.15	1.005 ± 0.17	1.014 ± 0.16	3.304 ± 0.21
SSME (intranasal)	Brain/Blood	0.265 ± 0.06	0.581 ± 0.13	1.717 ± 0.19	1.828 ± 0.09	1.146 ± 0.09
SSS (intranasal)	Brain/Blood	0.260 ± 0.12	0.528 ± 0.13	0.456 ± 0.21	0.481 ± 0.14	2.421 ± 0.19
SMP (intranasal)	Brain/Blood	0.268 ± 0.04	0.384 ± 0.08	0.555 ± 0.18	1.467 ± 0.24	2.179 ± 0.27

*SMP indicates marketed product; all other abbreviations are explained in Table 1. The rats were administered with 100 μCi ^{99m}Tc-sumariptan formulations and the radioactivity was measured in percentage per gram of tissue of the administered dose. Each value is the mean ± SEM of 4 estimations.

†Difference was found significant ($P < .05$) when SSME intranasal (brain) was compared with SMP intranasal (brain).

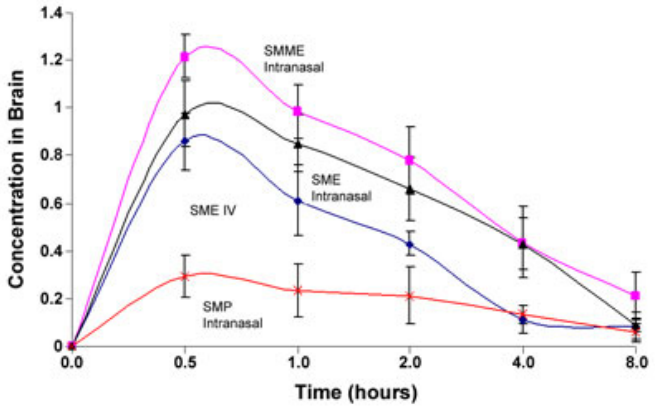

Figure 1. Brain concentrations versus time (hours) plot following administrations of sumatriptan ^{99m}Tc-formulations containing sumatriptan (ST).

Brain drug-direct-transport percentage (DTP [%]) has been calculated using Equations (2) and (3).

$$DTP\% = \left\{\frac{(B_{in} - Bx)}{B_{in}}\right\} \times 100, \quad (2)$$

$$where,\ Bx = \left(\frac{B_{IV}}{P_{IV}}\right) \times (P_{in}) \quad (3)$$

Bx indicates brain AUC fraction intranasal contributed by systemic circulation through the BBB; B_{IV}, $AUC_{0\rightarrow480}$ (brain) following IV administration; P_{IV}, $AUC_{0\rightarrow480}$ (blood) following IV administration; B_{in}, $AUC_{0\rightarrow480}$ (brain) following intranasal administration; and P_{in}, $AUC_{0\rightarrow480}$ (blood) following intranasal administration.

A study by Illum reveals that the drug uptake into the brain from the nasal mucosa occurs via 2 different pathways. One is a systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB. The other is the olfactory pathway by which part quantity of drug can travel from the olfactory region in the nasal cavity directly into CSF and/or brain tissue.[12] We can deduce that the amount of drug in the brain tissue after nasal application is attributed to these 2 parts. ST displays linear pharmacokinetics; the drug amount is proportional to AUC. Thus, we assume that the brain AUC fraction, contributed by systemic circulation through BBB (represented by Bx) and divided by plasma AUC from nasal route, is equal to that of IV route (see Equation 1). DTP represents the percentage of drug directly transported to the brain via olfactory pathway. DTP (%) and DTE (%) are calculated from tissue/organ distribution data following intranasal and IV administration and recorded in Table 6.

Gamma Scintigraphy Imaging

Swiss albino rats (200-250 g, male) were selected for the study. Radiolabeled formulation of ^{99m}Tc-SME (100 µCi/50 µL) containing 0.4 to 0.5 mg ST (equivalent to 0.33 mg/kg BW) was intravenously injected through the tail vein of the rat. Similarly, radiolabeled formulations ^{99m}Tc-SSS/SSMS/ SME/ SMME/ SSME/ SSMME/ SMP (100 µCi/50 µL) containing 0.4 to 0.50 mg ST (equivalent to 0.33 mg/kg BW) were administered (10 µL in each nostril). The rats were anaesthetized using 0.25 mL ketamine hydrochloride intramuscular injection (50 mg/mL) prior to administration of formulations. The rats were placed on board and images were captured using single positron emission computerized tomography (SPECT, LC 75-005, Diacam, Siemens AG, Erlanger, Germany) gamma camera.[24,27,28] The scintigraphy images following IV and intranasal administration of SMME are shown in Figure 3.

Transmission Electron Microscopy

Human nasal mucosa was collected after proper informed consent of donor and washed twice using phosphate buffered saline. The nasal mucosa was stored at 2°C to 4°C in a cotton gauze impregnated with normal saline solution till further use. Human nasal mucosa was kept within SSS, SME, and SMME for 12 hours to study the formulation uptake across nasal mucosa, mechanism of drug uptake and toxicity of the formulations on the nasal mucosa cells. Subsequently, formulation-treated nasal mucosa was exposed (3 hours) to 100mM phosphate buffer solution (pH 6.5) for removal of formulation, and toxicity of formulation on nasal mucosa cells was studied. Nasal mucosae, with/without formulation treatment and after washing, were fixed using 2.50% (vol/vol) gluteraldehyde solution in water for 3 hours at 25°C ± 2°C. The fixed nasal mucosae were washed thrice using 100mM phosphate buffer (pH 6.5). Washed nasal mucosae were postfixed in 1% wt/vol osmium

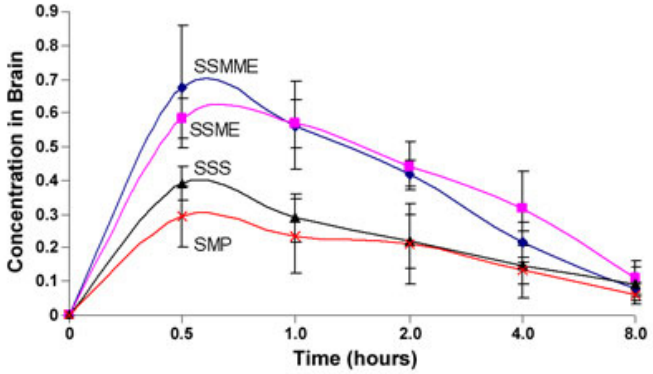

Figure 2. Brain concentrations versus time (hours) plot following administrations of sumatriptan succinate ^{99m}Tc-formulations containing sumatriptan succinate (SS).

Table 4. Pharmacokinetics of ^{99m}Tc-SME (IV), ^{99m}Tc-SMME, SME, and SMP (intranasal) at Predetermined Time Intervals in Normal Swiss Albino Rats*

Formulation and Route of Administration	Organ/Tissue	C_{max} (%/g)	T_{max} (hours)	$AUC_{0\rightarrow480}$ (hours* %/g)	$AUC_{0\rightarrow\infty}$ (hours* %/g)	K_{el} (L/h)	$T_{1/2}$ (hours)
SME (IV)	Blood	4.26 ± 0.42	0.50 ± 0.05	9.85 ± 0.37	10.65 ± 0.69	0.48 ± 0.05	1.46 ± 0.21
	Brain	0.86 ± 0.12	0.50 ± 0.10	2.23 ± 0.28	2.50 ± 0.22	0.32 ± 0.08	2.15 ± 0.28
SMME (intranasal)	Blood	2.67 ± 0.24	1.00 ± 0.14	9.75 ± 0.44	12.79 ± 0.82	0.20 ± 0.01	3.51 ± 0.35
	Brain	1.21 ± 0.09	0.50 ± 0.18	4.15 ± 0.29	5.16 ± 0.52	0.22 ± 0.03	3.15 ± 0.23
SME (intranasal)	Blood	2.59 ± 0.33	2.00 ± 0.21	8.77 ± 0.51	9.88 ± 0.39	0.39 ± 0.04	1.78 ± 0.17
	Brain	0.98 ± 0.14	0.50 ± 0.11	3.39 ± 0.18	3.89 ± 0.37	0.32 ± 0.03	2.18 ± 0.31
SMP (intranasal)	Blood	1.09 ± 0.09	0.50 ± 0.19	1.79 ± 0.17	1.95 ± 0.24	0.48 ± 0.05	1.45 ± 0.08
	Brain	0.29 ± 0.09	0.50 ± 0.09	1.13 ± 0.19	1.45 ± 0.16	0.21 ± 0.11	3.38 ± 0.37

*AUC indicates area under the curve; SMP, marketed product; all other abbreviations are explained in Table 1.The rats were administered with 100 μCi ^{99m}Tc-sumatriptan formulations and the radioactivity was measured in percentage per gram of tissue of the administered dose. The pharmacokinetic parameters are derived using mean ± SEM of 4 estimations.

tetroxide solution for 3 hours; fresh osmium tetroxide was replaced every 30 minutes. The nasal mucosae samples were washed, dehydrated through acetone grades, and in-filtered in araldite:dodeceny succinic anhydride mixture (1:1.32) for 24 hours. The resin mixture was removed and nasal mucosa samples were embedded in pure resin; samples were cured by subjecting at 60°C ± 2°C for 72 hours. Ultra-thin sections (20-30 μm) were taken using microtome and placed on 200-mesh formwar-coated copper grids and were stained using uranyl acetate:lead citrate (Reynolds, Kettering, OH). To study morphological changes of epithelial cells and tight junctions, nasal mucosa samples were scanned using JEOL 100 CX transmission electron microscope (Jeol, Tokyo, Japan) equipped with 20-μm aperture at 80 kV. The electron micrographs are shown in Figure 4 (A to F).

Statistical Analysis

All data are reported as mean ± SEM and the difference between the groups were tested using Student *t* test at the level of $P < .05$. More than 2 groups were compared using analysis of variance (ANOVA) and differences greater than $P < .05$ were considered significant.

Results and Discussion

ST content was found to be 98.94%, 99.19%, 97.99%, and 100.02% for SME, SMME, SSME, and SSMME, respectively. The mean globule size and zeta potential of SME were found to be 29.98 nm ± 15.42 nm and −38.90 ± 2.05 mV and for SSME were found to be 38.45 nm ± 20.38 nm and −39.10 mV ± 2.02 mV, respectively (Table 1). SME and SSME showed net negative charge and the addition of mucoadhesive agent further contributed negatively to the system. With increase in surfactant level, surface tension and surface energy of the formed micelles decreases, therefore net negative charge (anionic) of the microemulsion increases.[29] Prepared microemulsions were expected to have good physical stability with respect to phase separation and/or flocculation since zeta potential was less than −30 mV.[30,31]

Radiochemical purity of SSS, SME, SMME, SSME, and SSMME were found to be 95.96%, 97.69%, 98.22%, 96.54%, and 98.74%, respectively. Optimum $SnCl_2.2H_2O$ concentration was found to be 100 μg/mL at pH 6.80 ± 0.20 and incubation time of 30 minutes. ^{99m}Tc labeled formulations were found to be stable in 0.90% (wt/vol) sodium chloride solution (saline) and in rat serum up to

Table 5. Pharmacokinetics of ^{99m}Tc-SME (IV), ^{99m}Tc-SSME, SSS, and SMP (intranasal) at Predetermined Time Intervals in Normal Swiss Albino Rats*

Formulation and Route of Administration	Organ/Tissue	C_{max} (%/g)	T_{max} (hours)	$AUC_{0\rightarrow480}$ (h* %/g)	$AUC_{0\rightarrow\infty}$ (h* %/g)	K_{el} (L/h)	$T_{1/2}$ (hours)
SSMME (intranasal)	Blood	2.28 ± 0.22	0.50 ± 0.14	2.91 ± 0.22	3.19 ± 0.23	0.51 ± 0.09	1.36 ± 0.14
	Brain	0.68 ± 0.18	0.50 ± 0.08	2.11 ± 0.26	2.43 ± 0.18	0.29 ± 0.11	2.38 ± 0.18
SSME (intranasal)	Blood	2.19 ± 0.24	0.50 ± 0.11	2.79 ± 0.35	3.51 ± 0.24	0.16 ± 0.04	4.27 ± 0.32
	Brain	0.58 ± 0.06	0.50 ± 0.07	2.47 ± 0.15	3.05 ± 0.43	0.23 ± 0.07	2.98 ± 0.28
SSS (intranasal)	Blood	1.50 ± 0.11	0.50 ± 0.14	2.46 ± 0.24	2.77 ± 0.28	0.41 ± 0.07	1.70 ± 0.18
	Brain	0.39 ± 0.05	0.50 ± 0.13	1.36 ± 0.37	2.01 ± 0.22	0.14 ± 0.09	4.89 ± 0.41
SMP (intranasal)	Blood	1.09 ± 0.09	0.50 ± 0.06	1.79 ± 0.11	1.95 ± 0.29	0.48 ± 0.11	1.45 ± 0.16
	Brain	0.29 ± 0.09	0.50 ± 0.06	1.13 ± 0.14	1.45 ± 0.38	0.21 ± 0.05	3.38 ± 0.18

*AUC indicates area under the curve; SMP, marketed product; all other abbreviations are explained in Table 1. The rats were administered with 100 μCi ^{99m}Tc-sumatriptan succinate formulations and the radioactivity was measured in percentage per gram (%/g) of tissue of the administered dose. The pharmacokinetic parameters are derived using mean values ± SEM of 4 estimations.

Table 6. Drug Targeting Efficiency and Direct Nose-to-Brain Transport Following Intranasal Administration of ^{99m}Tc-SSS/ SME/ SMME/ SSME/ SSMME/ SMP Against SME (IV) Administration*

Formulation	Drug Targeting Efficiency (%DTE)*	Direct Nose-to-Brain Transport (%DTP)*
SMME (intranasal)	225 ± 3	47 ± 2
SME (intranasal)	186 ± 2	41 ± 2
SSMME (intranasal)	147 ± 2	69 ± 1
SSME (intranasal)	131 ± 3	74 ± 2
SSS (intranasal)	129 ± 2	59 ± 1
SMP (intranasal)	133 ± 1	64 ± 2

*Parameters are derived using mean ± SEM values of 4 different estimations.

24 hours (degradation < 4% wt/wt). Percentage transchelation of the labeled complex was 1.69% wt/wt at 25mM DTPA concentration, while at 100mM, it increased to 3.47% wt/wt. These results suggest high bonding strength and stability of ^{99m}Tc-labeled formulations and hence were found suitable to study the biodistribution of the drug in rats.

Drug concentrations in brain following intranasal administrations of SME and SMME were found to be significantly higher at all sampling time points compared with IV administration of SME. The brain/blood ratio at 0.5 hour for SME (intranasal) and SMME (intranasal) was found to be 2.5- to 3-fold higher as compared with SME (IV). This finding may be attributed to direct nose-to-brain transport. Reports in the literature revealed that following intranasal administration, preferential nose-to-brain transport bypassing the BBB occurred due to the unique connection between the nose and the CNS.[11,12,32]

The SME (intranasal) shows significantly higher brain/blood ratio at 0.5 hour compared with SSS and SMP (intranasal) and showed rapid nose-to-brain transport of ST from microemulsion. SME and SMME show 2-fold higher C_{max} and 8-fold higher AUC compared with SMP. Higher DTP (%) and DTE (%) values were observed for SMME compared with SME, demonstrating the role of mucoadhesive agent (Table 6). This may be attributed to longer residence time of mucoadhesive microemulsion in the nasal cavity. This observation corroborates the findings reported indicating that microemulsion enhances nose-to-brain transport of drug.[25,33]

SSMME and SSME showed comparable direct nose-to-brain transport (DTP [%]) to that of SSS and SMP. The difference in DTE (%) for SSMME and SSME was found to be nonsignificant compared with SSS and SMP. However, SSMME and SSME show approximately 2-fold higher C_{max} and 2-fold higher AUC compared with SSS and SMP. $T_{1/2}$ was also extended to 3 hours from 1.5 hour for SSMME compared with SSS and SMP. Higher C_{max} and $T_{1/2}$ for SSMME/SSME (intranasal) compared with SSS/SMP (intranasal) may be attributed to longer residence time of microemulsion due to more viscosity and better mucoadhesion.[16,17]

Drug concentrations in brain following intranasal administrations of SSME and SSMME were found to be significantly higher at all sampling time points compared with IV administration of SME.[34,35] SMME and SME showed significantly higher brain concentrations compared with SSMME and SSME (Tables 2 and 3). SMME showed 2-fold higher uptake (C_{max}) of ST in brain compared with SSMME, which is suggestive of higher ST nose-to-brain transport compared with SS. Substantially higher uptake of SMME compared with SSMME in the brain compartment at all sampling points suggests greater extent of selective transport of ST to the brain. It is likely that the higher partition coefficient (lipophilicity) of the ST compared with that of SS resulted in higher drug uptake. Significantly higher DTP of SMME compared with SSMME also proved more uptake of ST compared with SS. SME and SMME showed enhanced rate and extent of transport of drug compared with SMP. Extended $T_{1/2}$ for SMME (blood, 3.51 hours; brain, 3.15 hours) compared with SSMME (blood, 1.33 hours; brain, 2.38 hours) suggest role of microemulsion in delaying the mucociliary clearance of lipophilic molecule (ST) and lesser extent to hydrophilic molecule. Higher DTP for mucoadhesive microemulsion was observed and the better brain-targeting efficiency may be attributed to substantial direct nose-to-brain transport. These findings are in congruence with the observations reported by Qizhi Zhang et al[25] and Li et al.[33] Microemulsion containing lipophilic and hydrophilic drug will have different mucociliary clearance owing to their presence in lipophilic and hydrophilic phase in the microemulsion.

Gamma scintigraphy images of rat 0.5-hour postintravenous and -intranasal injection are shown in Figure 3A-3C. Significantly high radioactivity was noticed in the rat brain for SMME (intranasal) compared with SME (IV) and SME

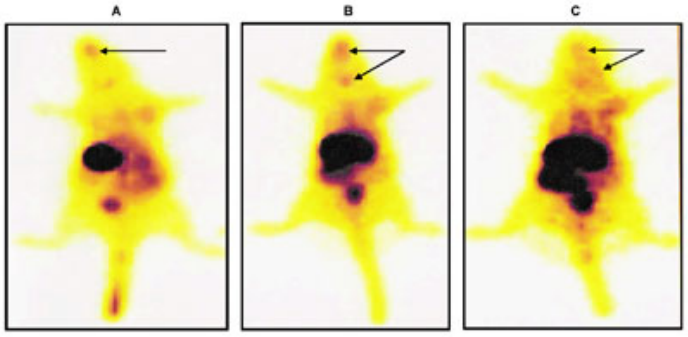

Figure 3. Gamma scintigraphy images of rat (A/P view) showing the presence of radioactivity into the brain (arrows). (A) IV and (B) intranasal administration of ^{99m}Tc- SME (100 μCi), and (C) intranasal administration of ^{99m}Tc-SMME (100 μCi).

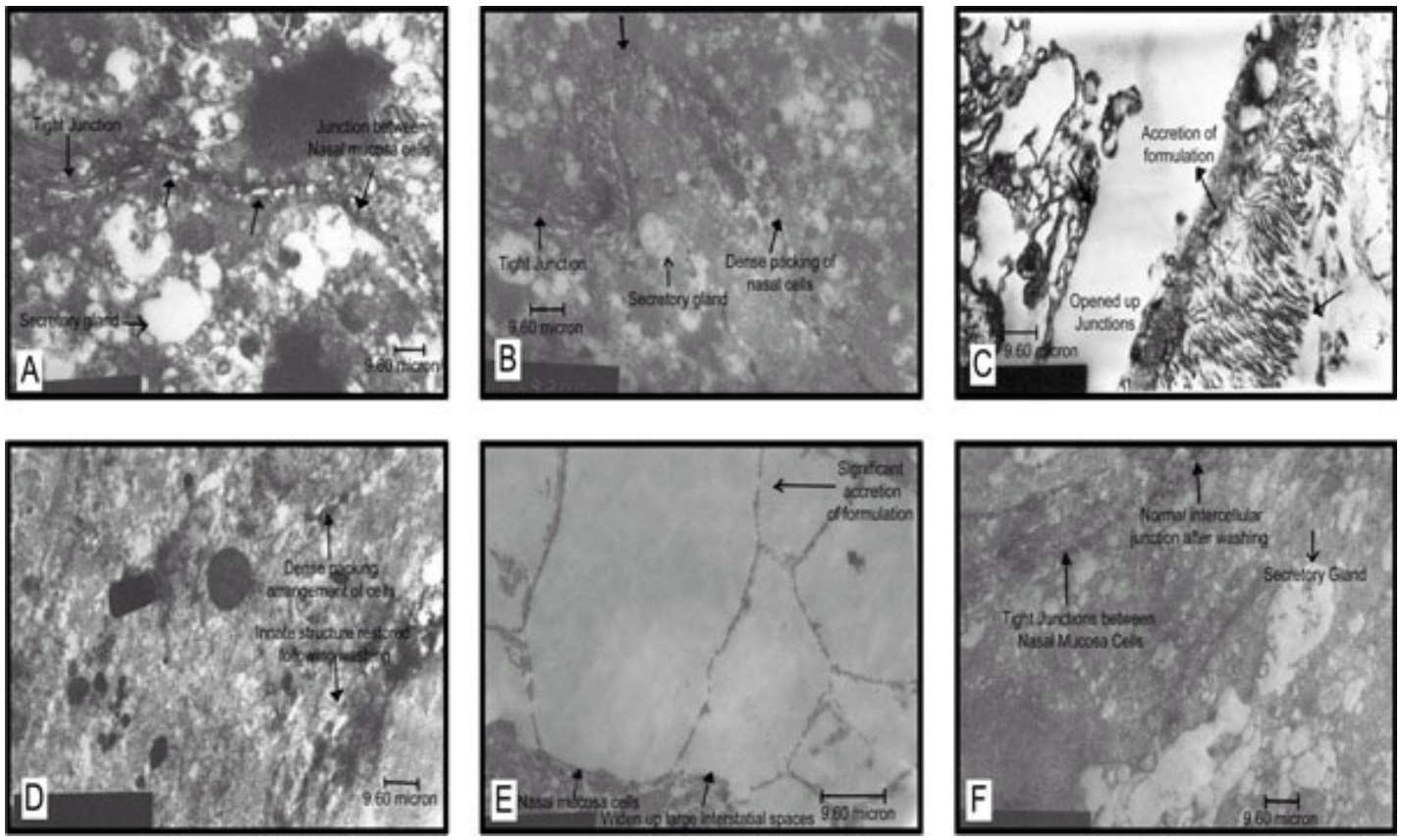

Figure 4. Electron micrograph of human nasal mucosa: (A) normal nasal mucosa (B) SSS-treated nasal mucosa, (C) SME-treated nasal mucosa, (D) nasal mucosa washed, followed by SME treatment, (E) SMME-treated nasal mucosa, and (F) nasal mucosa washed after SMME treatment. Significant accretion was noticed between the cells of nasal mucosa treated with SMME.

(intranasal). Scintigraphy images are consistent with the biodistribution data shown in Tables 2 and 3.

Electron micrographs of human nasal mucosa following formulation treatment and washing are shown in Figure 4. Electron micrographs of nasal mucosa treated with various formulations revealed that SSS-treated nasal mucosa (Figure 4B) showed presence of unaltered tight junctions identical to untreated nasal mucosa (Figure 4A). However, higher uptake of SME (Figure 4C) was found as compared with SSS. Significant accrual of SMME (Figure 4E) as compared with SME was noticed within the junctions of nasal mucosa cells. Nasal mucosa washed (Figure 4D and 4F) after formulation treatment was found to restore the innate cellular structure when compared with the normal nasal mucosa suggesting reversal of dilation of tight junctions. Presence of SMME within the interstitial spaces of tight junctions of nasal mucosa cells indicated paracellular mode of transport of SMME.

CONCLUSION

The studies demonstrated rapid and larger extent of selective ST nose-to-brain transport compared with SS and SMP in rats. Enhanced rate and extent of transport of ST following intranasal administration of SMME may help in decreasing the dose and frequency of dosing and possibly maximize the therapeutic index. However, clinical benefits to the risk ratio of the formulation developed in this investigation will decide its appropriateness in the clinical practice.

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REFERENCES

1. Humphrey PP, Feniuk W, Marriott AS, Tanner RJ, Jackson MR, Tucker ML. Preclinical studies on the anti-migraine drug, sumatriptan. *Eur Neurol.* 1991;31:282–290.

2. Pakalnis A, Kring D, Paolicchi J. Parenteral satisfaction with sumatriptan nasal spray in childhood migraine. *J Child Neurol.* 2003; 18:772–775.

3. Villalon CM, Centurion D, Valdivia LF, de Vries P, Saxena PR. Migraine: pathophysiology, pharmacology, treatment and future trends. *Curr Vasc Pharmacol.* 2003;1:71–84.

4. Ahonen K, Hamalainen ML, Rantala H, Hoppu K. Nasal sumatriptan is effective in treatment of migraine attacks in children: a randomized trial. *Neurology.* 2004;62:883–887.

5. Fuseau E, Petricoul O, Moore KH, Barrow A, Ibbotson T. Clinical pharmacokinetics of intranasal sumatriptan. *Clin Pharmacokinet.* 2002;41:801–811.

6. Martindale PK. *The Complete Drug Reference*. London, UK: Pharmaceutical Press; 1999:450–452.

7. Gladstone JP, Gawel M. Newer formulations of the triptans: advances in migraine treatment. *Drugs.* 2003;63:2285–2305.

8. Chien YW. Nasal Drug Delivery and Delivery Systems. In: Chien YW, Su KSE, Chang S, eds. *Nasal Systemic Drug Delivery.* New York, NY: Marcel Dekker Inc; 1989:239.

9. Bigal ME, Bordini CA, Antoniazzi AL, Speciali JG. The triptan formulations: a critical evaluation. *Arq Neuropsiquiatr.* 2003;61:313–320.

10. Dahlof C. Clinical applications of new therapeutic deliveries in migraine. *Neurology.* 2003;61:S31–S34.

11. Illum L. Nasal drug delivery: problems, possibilities and solutions. *J Control Release.* 2003;87:187–198.

12. Illum L. Transport of drugs from the nasal cavity to central nervous system. *Eur J Pharm Sci.* 2000;11:1–18.

13. Logemann CD, Rankin LM. Newer Intranasal Migraine Medications. *Am Fam Physician [serial online].* 2000. Available at: http://www.aafp.org/afp/20000101/180.html. Accessed: June 15, 2004.

14. Behl CR, Pimplaskar HK, Sileno AP, De Meireles J, Romeo VD. Effects of physicochemical properties and other factors on systemic nasal delivery. *Adv Drug Deliv Rev.* 1998;29:89–116.

15. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev.* 2000;45:89–121.

16. Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *J Pharm Pharmacol.* 2001;53:3–21.

17. Sinswat P, Tengamnuay P. Enhancing effect of chitosan on nasal absorption of salmon calcitonin in rats: comparison with hydroxypropyl and dimethyl-beta-cyclodextrins. *Int J Pharm.* 2003;257:15–22.

18. United States Pharmacopoeial Convention.*United States Pharmacopeia.* Asian edition, *USP* 27. Rockville, MD: United States Pharmacopoeial Convention; 2003.

19. Roland I, Piel G, Delattre L, Evrard B. Systemic characterization of oil-in-water emulsions for formulation design. *Int J Pharm.* 2003; 263:85–94.

20. Jha SK, Dougall P, Behari M, Ahuja GK. Interictal brain 99mTc-HMPAO SPECT study in chronic epilepsy. *J Assoc Physicians India.* 1998;46:438–441.

21. Eckelman WC. Radiolabeling with technetium-99m to study high-capacity and low-capacity biochemical systems. *Eur J Nucl Med.* 1995;22:249–263.

22. Saha GB. *Physics and Radiobiology of Nuclear Medicine*. New York, NY: Springer-Verlag; 1993.

23. Babbar AK, Singh AK, Goel HC, Chauhan UPS, Sharma RK. Evaluation of 99mTc-labeled Photosan-3, a hematoporphyrin derivative, as a potential radiopharmaceutical for tumor scintigraphy. *Nucl Med Biol.* 2000;27:419–426.

24. Koziara JM, Lockman PR, Allen DD, Mumper RJ. In situ blood-brain barrier transport of nanoparticles. *Pharm Res.* 2003; 20:1772–1778.

25. Zhang Q, Jiang X, Jiang W, Lu W, Su L, Shi Z. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation of the targeting efficiency to brain. *Int J Pharm.* 2004;275:85–96.

26. Chow HS, Chen Z, Matsuura GT. Direct transport of cocaine from the nasal cavity to brain following intranasal cocaine administration in rats. *J Pharm Sci.* 1999;88:754–758.

27. Ueda T, Torihara Y, Tsuneyoshi N, Ikeda Y. Effect of sumatriptan on cerebral blood flow during migraine headache: measurement by sequential SPECT used 99mTc-ECD background substraction method. *No To Shinkei.* 2001;53:625–630.

28. Stein DJ, Van Heerden B, Wessels CJ, Van Kradenburg J, Warwick J, Wasserman HJ. Single photon emission computed tomography of the brain with Tc-99m HMPAO during sumatriptan challenge in obsessive-compulsive disorder: investigating the functional role of the serotonin auto-receptor. *Prog Neuropsychopharmacol Biol Psychiatry.* 1999;23:1079–1099.

29. Espinosa-Jimenez M, Padilla-Weigand R, Ontiverous-Ortega A, Ramos-Tejada M, Perea-Carpio R. Interpretation of colloidal dyeing of polyester fabrics pretreated with ethyl xanthogenate in terms of zeta potential and surface free energy balance. *J Colloid Interface Sci.* 2003;265:227–233.

30. Block LH. Pharmaceutical Emulsions and Microemulsions. In: Lieberman HA, Rieger MM, Banker GS, eds. *Pharmaceutical Dosage Forms.* 2nd ed. 2001;47–110.

31. Nash RA. Pharmaceutical Suspensions. In: Lieberman HA, Rieger MM, Banker GS, eds. *Pharmaceutical Dosage Forms.* 2nd ed. 2001;1–47.

32. Fuseau E, Petricoul O, Moore KH, Barrow A, Ibbotson T. Clinical pharmacokinetics of intranasal sumatriptan. *Clin Pharmacokinet.* 2002;41:801–811.

33. Li L, Nandi I, Kim KH. Development of an ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam. *Int J Pharm.* 2002;237:77–85.

34. Vyas TK, Shahiwala A, Marathe S, Misra A. Intranasal drug delivery for brain targeting. *Curr Drug Deliv.* 2005;2:165–175.

35. Sakane T, Yamashita S, Yata N, Sezaki H. Transnasal delivery of 5-fluorouracil to the brain in the rat. *J Drug Target.* 1999; 7:233–240.