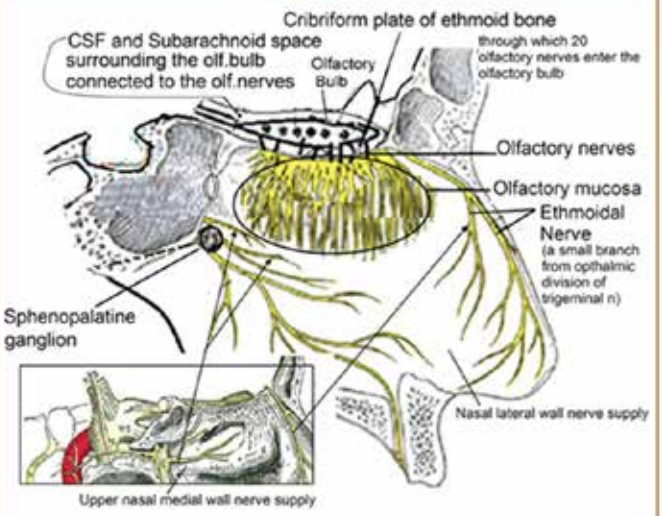
Transmucosal Drug Delivery to the Brain via the Nose

A recent paper has shown that macromolecules can pass rapidly from the nose into the brain along olfactory nerves and into the brain and brain stem along branches of the first and second trigeminal nerve structures, without any involvement of the cerebrospinal fluid (CSF), which was previously thought to be important[1]. In animals, transport along the olfactory and trigeminal nerves has been shown to occur within minutes through channels created by ensheathing cells, rather than simply by slow axonal transport or diffusion[1]. Recent data has shown that peptides administered intranasally were directly transported and distributed within the brain parenchyma fairly rapidly (within five minutes). As pointed out by the authors, simple diffusion within the brain parenchyma would be too slow to explain the broad distribution to all brain regions observed[2]. An interesting hypothetical mechanism for the observed speed with which a broad distribution within the brain is achieved, is bulk flow in the interstitial fluid, 'powered' by the 'perivascular pump' mechanism naturally produced by arterial pulsation and blood pressure[2,3].

The nose is divided into two nasal cavities by the septum, each with a volume of approximately 7.5ml and a surface area of 75cm^2 [4,5]. There are three distinct functional zones in the nose: the vestibular, respiratory and olfactory regions. The respiratory region is the largest and comprises approximately 65cm^2. It is highly vascularised and is the principal site of systemic drug absorption[4]. The respiratory epithelium consists of basal cells, mucus-containing goblet cells, ciliated columnar cells and non-ciliated columnar cells[4,5]. The cilia are surrounded by a film of mucus and move in a continuous wavelike fashion to transport mucus and entrapped particles to the pharynx for ingestion[4]. Mucus is a viscous colloid composed of mucins (glycoproteins) mixed with antibacterial proteins such as lysozyme and lactoferrin, immunoglobulins and inorganic salts[6]. The constantly regenerating mucus coating moves at 5–6 mm/min[7] and serves to protect epithelial cells from external viruses, bacteria and chemical irritants. By restricting drop size in nasally administered sprays to a diameter >10µm, deposition is typically targeted to the nasal cavity and lung exposure is minimised[8]. The pH of the nasal cavity is approximately 5.5–6.5 – hence nasal irritation is minimised when products are formulated within this pH range[9,10]. The total spray volume that can be reliably delivered to each nostril is 150µl and the upper limit of a drug dose has been suggested to be 25mg/dose[10].

The olfactory mucosa is situated within the recesses of the skull under the cribriform plate of the ethmoid bone that forms the roof of the nose, situated 7cm from the nostril, being positioned partly on the nasal septum and partly on the superior turbinate (Figure 1)[11].It is not easily accessible[12] hence, therapeutic agents need to be delivered to this narrow passage to treat CNS afflictions. The olfactory mucosa is made up of a mucus layer situated on the top of the receptor cells, supporting cells between the receptor cell, basal cells below the receptor and supporting cells, and goblet cells extending from the lamina propria opening on the olfactory mucosa supplying the mucosal coating to the olfactory mucosa. The lamina propria, which is below the receptor and basal cells, has 20 olfactory nerve bundles with BV (ethmoidal) and lymphatics (deep cervical) surrounded by connective tissue, which form the epineural and perineural connective tissue around the 20 olfactory nerve trunks that are connected to olfactory bulb leptomeninges and dura.

FIGURE 1



Shows the olfactory mucosa and other nerve structures on the walls of the nasal cavity. Note the olfactory mucosa (circled) is the solitary structure that is directly exposed to therapeutic agents, microorganisms, and amoeba that are transported rapidly to the CNS by 20 olfactory nerves from the olfactory mucosa to the olfactory bulb from where they are transported to the CNS, bypassing the BBB (Modified from Gray's Anatomy).

Absorption Enhancing Excipients

There are two primary mechanisms for absorption through the mucosa: Paracellular transport via opening of tight junctions between cells; and transcellular transport (or transcytosis) through cells via vesicle carriers that may be receptor mediated[13]. Potential barriers include that the compound is metabolised before reaching the systemic circulation and mechanical washout due to limited residence time in the nasal cavity. Some absorption enhancers may function by altering either or both the paracellular and transcellular routes, while others serve to extend time in the nasal cavity or alter or resist metabolic pathways of degradation (e.g. unwanted peptide hydrolysis).

Several excipients have been tested as absorption enhancers over the past three decades, but almost all of them have been found to elicit significant toxic responses and irritation. The mechanisms through which the tested excipients open cellular junctions often result in damage that cannot easily be reversed.

One of the primary factors for the lack of a broad acceptance for nasal delivery has been damage to the nasal mucosa, coupled with poor transmucosal absorption due to a shortened residence time and active mechanical washout due to irritation and resultant mucus flow. Therefore, the primary functions of a suitable excipient for nasal delivery would be I) lack of inflammation and irritation and II) increased residence time in the nasal cavity.

Selected excipients that have been used in clinical trials for nasal delivery are shown in Table 1.

Excipient Used in Clinical Trials (Reference)	Additional Irritation (Yes/No)
Benzalkonium chloride (14)	No
Chitosan (15)	No
Cyclodextrins (16)	No
Deoxycholic acid (17)	Yes
Alkylsachharides (18)	No
Glycocholic acid, sodium salt (19)	Yes
Laureth-9 (20)	Yes
Taurocholic acid, sodium salt (21)	Yes
Taurodihydrofusidic acid, sodium salt (22)	Yes

Some were not deemed suitable for further research. Bile salts, such as glycocholic acid, taurocholic acid and taurodihydrofusidic acid, can enhance absorption by increasing the fluidity of apical and basolateral membranes. However, they induce irritation, albeit to a lesser extent than compromising gap junctions. Likewise, while Benzalkonium chloride is a non-irritant, it has a direct inhibitory effect on cilia beat frequency and is typically used as a preservative rather than an enhancer.

The recommended excipients for further evaluation are therefore: chitosan, cyclodextrins and alkylsaccharides.

In the absence of an absorption enhancer, the apparent molecular weight cut-off for nasal absorption is approximately 1000 Da, with molecules less than 1000 Da showing better absorption[23]. Co-administration of drugs with absorption enhancers assists with the delivery of drugs ranging from 1000 Da to 31,000 Da. To summarise, the major mechanisms for increased absorption are loosening of the tight junctions between cells, vesicular transcytotic transport (which may be receptor-mediated), alteration of the rheological properties of mucus, alteration of surface (apical) membrane fluidity of epithelial cells, and/or alteration of ciliary beating or removal of cilia from the epithelial cells lining the nasal cavity[24, 25].

Toxicity Assessment

Extensive *in vitro* studies to examine the safety of approximately 50 absorption enhancers were conducted by Chen *et al.*[16], using a measurement of transepithelial electrical resistance across confluent cell layers composed of cultured human bronchial/tracheal epithelial cells in a microtiter well format. A reduction in transepithelial electrical resistance (TEER) across the cell layer was interpreted as an opening of tight junctions between cells. Attempts to use this cell-based model for toxicity assessment *in vivo* were not successful. It is not predictive due to major differences between the *in vitro* and *in situ* cell environments.

Within the nasal cavity, the epithelial cells are bathed in a constantly regenerating mucus coating that, in combination with the beat frequency of cilia, acts as a mucociliatory escalator, resulting in a clearance time of 15 minutes. As a result, both contact time of exposure to the nasal epithelium and the concentration of the drug and associated excipient are progressively reduced by three mechanisms – dilution into the mucus, absorption into the systemic circulation and mucociliary clearance.

Alkylsaccharides

Alkylglycosides and sucrose esters of fatty acids are nonionic alkylsaccharide surfactants consisting of an aliphatic hydrocarbon chain linked to a sugar moiety by a glycosidic or an ester bond, respectively. They provide controlled transient permeation of the nasal mucosal barrier with no irritation. Following absorption or ingestion, they metabolise to CO_2 and H_2O through the corresponding sugar and fatty acid[26,27].

Alkylglycosides are widely used in the food industry. They are sprayed on fruits and vegetables to prevent the growth of bacteria and fungi or to clean food processing equipment due to their antibacterial potency and lack of toxicity. The EPA has determined that there is no need to establish an upper limit of exposure for adults, children or infants[28]. Similarly, sucrose esters are widely used as food grade emulsifiers and in cosmetics. The No Observed Adverse Effect Level (NOAEL) in some of these molecules can be as high as 2000mg/kg body weight and are designated as Generally Recognised as Safe (GRAS) substances for food applications[24]. The World Health Organization (WHO) allowable daily intake (ADI) (oral) is 10,000 times the amount that would be used in a typical nasal spray-i.e. 2g/day ADI vs 200µg/spray[29].

The mechanism of action of enhancement of uptake by alkylglycosides is not clear. Transmission electron micrographs (TEM) of the nasal septa of rats show unstained regions consistent with cellular internalisation and areas of thinned cilia associated with vesicle formation[30]. The transcellular pathway is supported by fluorescence light micrographs showing the internalisation of fluorescein-labelled insulin administered intranasally with an alkyl maltoside[30].

Studies have shown that alkylglycosides and sucrose esters are effective nasal absorption enhancers for a variety of peptide, protein and non-peptide macromolecular drugs in rats, mice, cats, dogs and monkeys[31,32,33]. Dose-escalation studies conducted in rats showed that shorter chain alkylsaccharides coupled to glucose such as hexyl, heptyl, octyl or nonyl glucoses were ineffective or minimally effective at promoting insulin absorption from the nose[32]. Intermediate-length alkylsaccharides such as decanoyl sucrose were more effective in promoting nasal insulin absorption, and longer chain alkylsaccharides such as sucrose dodecanoate were very potent absorption enhancers even at concentrations as low as 0.03-0.06%. However, increasing the alkyl chain length beyond 14 carbons with excipients such as pentadecylmaltoside or hexadecyl maltoside decreased insulin absorption[32,33].

Cyclodextrins

Cyclodextrins are cyclic oligosaccharides composed of six or more monosaccharide units with a central cavity. They can form inclusion complexes with hydrophobic molecules and have been used to increase drug solubility and dissolution and to enhance low molecular weight drug absorption[34, 35]. Among the cyclodextrin (CD) derivatives evaluated as potential nasal insulin absorption enhancers, dimethyl-beta-cyclodextrin was found to be the most effective, while alpha-CD was less effective and beta- and gamma-CD had negligible effects on insulin absorption[36].

Since not all cyclodextrins are equally effective as absorption enhancers for peptides, it appears that the structure of the central cavity could play a possible role in its mechanism of action. Dimethyl beta-cyclodextrin has been shown to be effective at enhancing the delivery of insulin[37], calcitonin[22] and low molecular weight heparin[38].

Chitosan

Chitosan is a linear cationic polysaccharide produced from deacetylation of chitin, composed of randomly distributed β-(1, 4)-linked D-glucosamine and N-acetyl-D-glucosamine joined by glycosidic bonds. Chitosan has been shown to increase the bioavailability of insulin and other small peptides and polar macromolecules in animal models[39, 40]. Additionally, chitosan exhibits bioadhesive properties and interacts strongly with the nasal mucus layer, enhancing contact times for drug with the membrane. The addition of 0.2–0.5% chitosan to nasal formulations of insulin resulted in significant increases in plasma insulin and reductions in blood glucose in both sheep and rat models.

Studies in human volunteers demonstrated that nasal administration of chitosan formulations results in significantly longer nasal clearance times[36], suggesting that chitosan prolongs the residence time of peptides within the nasal cavity[42].

Conclusions

The three types of absorption enhancers that have been studied most extensively – alkylsaccharides, cyclodextrins and chitosan – have proven useful for nasal delivery of small molecule drugs as well as for peptide drugs. Each offers potential advantages in specific applications, e.g. chitosan may be used as an absorption enhancer in both aqueous and dry powder formats. Cyclodextrins can increase the solubility of hydrophobic drugs and enhance transmucosal absorption. Alkylsaccharides which provide the greatest degree of absorption enhancement are soluble in aqueous and oil based/organic liquid formulations and can be used in both formats.

Sinswat *et al.*[43] compared the absolute bioavailability of calcitonin administered nasally to rats using cyclodextrins and chitosan as absorption enhancers to intravenous calcitonin as the control. Ahsan *et al.* carried out a similar study in rats using the alkylsaccharide tetradecylmaltoside as the absorption enhancer, while using intravenous administration as the control[44]. In these side-by-side comparisons, the formulation containing the alkylsaccharide excipient tetradecyl maltoside was found to be significantly more effective than either of the formulations containing chitosan or dimethyl beta cyclodextrin.

Alkylsaccharides, cyclodextrins and chitosan have emerged as the leading candidates for broad clinical applications. Specific molecules of each type are able to exert the desired effects without causing transmucosal damage. All also have shown clinical utility in human studies together with demonstrated safety and lack of intranasal toxicity. A major mechanistic benefit shared by these excipients is the fact that they function independently of the drug and do not require a modification of the drug substance[45].

Due to the primary emphasis on safety, it is recommended that excipients which cause even a transient opening of gap junctions not be considered due to significant toxic effects following both acute and chronic (repeat) dosing.

Compound	Class	CNS Indication
Apomorphine	Dopamine agonist	Parkinson's disease (on-off symptoms)
Butorphanol	Opiate	Migraine
Diazepam	Benzodiazapene	Sedation/anxiolysis
Fentanyl	Opiate	Analgesia/postoperative pain
Ketamine	NMDA antagonist	Analgesia
L-Dopa	Nonproteinogenic amino acid	Parkinson's disease
Metoclopramide	D_2 receptor antagonist	Antiemesis
Midazolam	Benzodiazapene	Sedation/anxiolysis
Morphine	Opiate	Analgesia
Nicotine	Dopamine agonist (addictive)	Smoking cessation
Sildenafil	PDE inhibitor	Erectile dysfunction
Sumatriptan	Triptan	Migraine
Zolmitriptan	Triptan	Migraine

Table 2: Selected small molecule APIs in registered products administered via intranasal delivery (NMDA = N-methyl-D-aspartate; PDE = phosphodiesterase)

Lipophilic small molecules are in general absorbed efficiently across the nasal mucosa, but the rate of absorption decreases with increasing molecular weight until about 1kDa. The extent of absorption for lipophilic molecules > 1kDa is significantly lower.

Absorption of hydrophilic drugs is relatively low and highly dependent on molecular weight. Absorption through membranes is not only affected by lipophilicity/hydrophobicity and molecular weight, but also by the amount of drug existing as an uncharged species. This depends on the drug pKa and pH at and in the absorption site – the non-ionised fraction of the drug being more permeable than the ionised one. The pH of the nasal epithelium is 5.5–6.5. A pH lower than 5.5 or higher than 6.5 combined with a buffer capacity higher than that of the nasal epithelium may cause local adverse effects and may therefore affect drug permeation. As only the molecular species of the drug at the absorption site can cross the nasal epithelium, sufficient drug solubility is a prerequisite for any absorption[46]. Drug candidates that fit the Class I classification profile (high permeability, high solubility) therefore have the best potential for being good candidates for nasal delivery.

REFERENCES

1. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Adv. Drug Deliv Rev 2012; 64:614-628.
2. Liu Q, Shen Y, Chen J, Gao X, Feng C, Wang L et al. Nose-to-brain transport pathways of wheat germ agglutinin conjugated PEG-PLA nanoparticles. Pharm Res 2012; 29: 546-558.
3. Hadaczek P, Yamashita Y, Mirek H, Tamas L, Bohn MC, Noble C et al. The "perivascular pump" driven by arterial pulsation is a powerful mechanism for the distribution of therapeutic molecules within the

brain. Mol Ther 2006; 14:69-78.
4. Mygind N, Anggard A. Anatomy and physiology of the nose-pathophysiologic alterations in allergic rhinitis. Clin Rev Allergy. 2:173-188, 1984.
5. Illum L. Transport of drugs from the nasal cavity to the central nervous system. Eur J Pharm Sci. 11(1):1-18, 2000.
6. Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. Nature, 417(6888):552-5, 2002.
7. Chien YW, Su KSE, Chang, S. Nasal Systemic Drug Delivery. New York: Marcel Dekker Inc., 1989:1-38.
8. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. Toxicol. Lett., 205(2): 97-104, 2011.
9. England RJ, Homer JJ, Knight LC, Ell SR. Nasal pH measurement: a reliable and repeatable parameter. Clin. Otolaryngol. Allied Sci., 24(1):67-8, 1999.
10. Behl CR, Pimplaskar HK, Sileno AP, deMeireles J, Romeo VD. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv. Drug Delivery Rev., 29:89-116, 1998.
11. Gopinath PG, Gopinath G, Kumar TCA. Target site of intranasally sprayed substances and their transport across the nasal mucosa: a new insight into the intranasal route of drug delivery. Curr Ther Res. 1978;23,596-607.
12. Shantha TR. Alzheimer's disease treatment with multiple therapeutic agents delivered to the olfactory region through a special delivery catheter and iontophoresis. US20120323214, US20140012182, US20150080785, WO/2015/013252A1, and WO/2009/149317A3.
13. Martin E, Verhoef JC, Cullander C, Romeijn SG, Nagelkerke JF, Merkus FWHM. Confocal laser scanning microscopic visualization of the transport of dextrans after nasal administration to rats: effects of absorption enhancers. Pharm Res., 14:631-637, 1997.
14. Green K. 1993. The effects of preservatives on corneal permeability of drugs. In Biopharmaceuticals of Ocular Drug Delivery, ed Edman P. 43 Boca Raton, FL: CRC Press.
15. Davis SS, Illum I. Absorption enhancers for nasal drug delivery. Clin. Pharmacokinet. 42 (13):1107-1128, 2003.
16. Chen-Quay SC, Eiting KT, Li A, Lanharzin, Quay SC. Identification of Tight Junction Modulating Lipids. J Pharm Sci., 98(2):606-19, 2009.
17. Chavanpatil MD, Vavia PR. Nasal drug delivery of sumatriptan succinate. Pharmazie. 60(5):347-9, 2005.
18. Maggio ET, Meezan E, Ghambeer DKS, Pillion DJ. High bioavailability formulation of salmon calcitonin-potential opportunities for expanded use in analgesia. Drug Deliv. Technol., 10:58-63, 2010.
19. Donovan, MD, Flynn GL, Amidon GL. The Molecular Weight Dependence of Nasal Absorption: The Effect of Absorption Enhancers. Pharmaceutical Research 7(8):808-815, 1990.
20. Aungst BJ, Rogers NJ. Site dependence of absorption-promoting actions of laureth-9, Na salicylate, Na2EDTA, and aprotinin on rectal, nasal and buccal insulin delivery. Pharm. Res. 5:305-8, 1998.
21. Yetkin G, Celebi N, Agabeyoglu I, Gokcora N. The effect of dimethyl-beta-cyclodextrin and sodium taurocholate on the nasal bioavailability of salmon calcitonin in rabbits. STP. Pharma. Sci. 9:249-53, 1999.
22. Baldwin PA, Klingbeil CK, Grimm CJ, Longenecker JP. The effect of sodium tauro-24,25-dihydrofusidate on the nasal absorption of human growth hormone in three animal models. Pharm. Res., 7(5):547-552, 1990.
23. McMartin C, Hutchinson LEF, Hyde R, Peters GE. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. J Pharm Sci., 76:535-540, 1987.
24. Illum L. Nasal drug delivery: new developments and strategies. Drug Discov. Today, 7:1184-89, 2002.
25. Aungst BJ, Rogers NJ, Shefter E. Comparison of nasal, rectal, buccal, sublingual, and intramuscular insulin in efficacy and the effects of a bile salt absorption enhancer. J. Pharm. Exp. Ther., 244:23-27, 1988.
26. Material Safety Data Sheet TEGOSOFT LSE 65 K SOFT Evonik Goldschmidt GmbH Version: 1.11 Date Issued: 01/21/2010 Gold-schmidtstr. 100 Essen, 05 45127.
27. Weber N, Benning H. Metabolism of orally administered alkyl beta-glycosides in the mouse. J. Nutr., 114(2):247-54, 1984.
28. Federal register ref. CFR Part 180. Alkyl (C10-C16) polyglycosides; exemption from the requirement of a tolerance. Federal register 70(177), 54281-54286, 2005.
29. Sucrose esters of fatty acids and sucroglycerides (WHO Food Additives Series 40), the forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additivers (JECFA) Geneva 1998. http://www.inchem.org/documents/jecfa/jecmono/v040je04.htm.
30. Arnold JJ, Ahsan F, Meezan E, Pillion DJ. Correlation of tetradecyl-maltoside induced increases in nasal peptide drug delivery with morphological changes in nasal epithelial cells. J. Pharm. Sci., 93(9):2205-13, 2004.
31. Pillion DJ, Hosmer S, Meezan E. Dodecylmaltoside-mediated nasal and ocular absorption of lyspro-Insulin: independence of surfactant action from peptide multimer dissociation. Pharm. Res., 15:1637-39, 1998.
32. Pillion DJ, Atchison JA, Gargiulo C, Wang RX, Wang P, Meezan E. Insulin delivery in nosedrops: new formulations containing alkylglycosides. Endocrinology, 135:2386-91, 1994.
33. Pillion DJ, Ahsan F, Arnold JJ, Balasubramaniam BM, Pirana O, Meezan E. Synthetic long-chain alkylglycosides as enhancers of nasal insulin absorption. J. Pharm. Sci., 91:1456-62, 2002.
34. Rajewski RA, Stella VJ. Pharmaceutical applications of cyclodextrins 2. In vivo drug delivery. J. Pharmaceut. Sci., 85:1142-69, 1996.
35. Merkus FW, Verhoef JC, Marttin E, Romeijin SG, van der Kuy PH, Hermens WA, Schipper NG. Cylcodextrins in nasal drug delivery, Adv. Drug Del. Rev., 36:41-57, 1999.
36. Merkus FW, Verhoef JC, Romeijin SF, Schipper NG. Absorption enhancing effect of cyclodextrins on intranasally administered insulin in rats. Pharm. Res., 8:588-92, 1991.
37. Shao Z, Krishnamoorthy R, Mitra K. Cyclodextrins as nasal absorption promoters of insulin: mechanistic evaluations. Pharm. Res., 9(9):1157-63, 1992.
38. Yang T, Hussain A, Paulson J, Abbruscato TJ, Ahsan F. Cyclodextrins in nasal delivery of low-molecular-weight heparins: in vivo and in vitro studies. Pharm. Res., 21:1127-36, 2004.
39. Illum L, Dodane V, Iqbal K. Chitosan technology to enhance the effectiveness of nasal drug delivery. Drug Deliv. Tech., 2:40, 2002.
40. Illum L, Farraj NF, Davis SS. Chitosan as a novel delivery system for peptide drugs. Pharm. Res., 11:1186-89, 1994.
41. Soane RJ, Frier M, Perkins AC, Jones NS, Davis SS, Illum L. Evaluation of the clearance characteristics of bioadhesive systems in humans. Int. J. Pharm., 178:55-65, 1999.
42. Aspden TJ, Illum L, Skaugrud O. Chitosan as a nasal delivery system: Evaluation of insulin absorption enhancement and effect on nasal membrane integrity using rat models. Eur. J. Pharmaceut. Sci., 4:23-31, 1996.
43. Sinswat P, Tengamnuay P. Enhancing the effect of chitosan on nasal absorption of salmon calcitonin in rats: comparison of hydroxyl propyl and dimethyl-beta-cyclodextrins. Int. J. Phar. 257:15-22, 2003.
44. Ashan F, Arnold J, Meezan E, Pillion DJ. Enhanced bioavailability of calcitonin formulated with alkylglycosides following nasal and ocular administration in rats. Pharm. Res., 18:1742-46, 2001.
45. Maggio ET. Absorption enhancing excipients in systemic nasal drug delivery. J. Excipients and Food Chem., 5(2):1-13, 2014.
46. Sastre J, Mosges R. Local and Systemic Safety of Intranasal Corticosteroids. J Investig Allergol Clin Immunol 22(1):1-12, 2012.

Aditya Das

Aditya Das is a Director of Business Development at Recipharm AB. Dr Das coordinates the interface between client needs and services/capabilities to facilitate optimal product development and global registration. Recipharm is a global CDMO and the facility at Research Triangle Park is focused on transmucosal drug delivery and development.

Email: aditya.das@recipharm.com