INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

AN OVERVIEW OF INTRANASAL NANOSUSPENSION FOR BRAIN TARGETED DRUG DELIVERY

Kinjal Bhatt*, Ankita Patel, Ajay Talele, Dr. Anil G.Jadhav

ARTICLE INFO

Article history
Received 04/02/2015
Available online
28/02/2015

Keywords
Brain Targeting,
Nanosuspension,
Intranasal Delivery.

ABSTRACT

Problems regarding effective and convenient delivery of drugs to brain are larger as there are large number of barriers to brain and limited vascular access of brain. Many advances are been made in recent years to deliver drugs to brain. Intranasal drug targeting is one of these approaches. It is convenient and easily accessible. Also a desired drug candidate for brain targeting with poor aqueous solubility and high lipophillicity can be formulated into nanosuspension so the intranasal delivery can be convenient and simple. A novel approach to deliver drugs to brain can be in form of nanosuspension. The nanosuspension surpasses the BBB as size range is 1-1000nm. As compared to conventional formulations, nanosuspension serves a promising approach in drug targeting to required organ.

Corresponding author
Kinjal Bhatt
Department of Pharmaceutics,
Smt.B.N.B. Swaminarayan Pharmacy College,
Salvav, NH-8, Vapi - 396 191 Gujarat.
kinjalph@yahoo.com,
+91-9978582618.

*Please cite this article in press as **Kinjal Bhatt** et al. An Overview of Intranasal Nanosuspension For Brain Targeted Drug Delivery. Indo American Journal of Pharm Research.2015:5(02).*

INTRODUCTION

Intranasal delivery of a formulation is possible and easily accessible. The segments of nasal cavity include the olfactory epithelium which is a direct pathway for brain targeting. Nanosuspension has a size range from 1-1000nm. So the nanosuspension can be administered intranasal and it crosses the tight cell junctions of the Blood Brain Barrier easily. Thus the nanosuspension is targeted to brain and this therapy is painless and effortless. This has further its own uses and advantages.

Brain Drug Targeting:-

The brain is a delicate organ and evolution built very efficient way to protect it. Unfortunately the same mechanisms that protect it against intrusive chemicals also complicate therapeutic interventions. Many existing pharmaceuticals are rendered ineffective in the treatment of cerebral diseases due to inability to effectively deliver and sustain their delivery within brain [1]. Various strategies are been used for manipulating the blood-brain barrier for drug delivery to the brain, which include osmotic and chemical opening of the blood-brain barrier as well as the use of transport systems. Other approaches for drug delivery to the brain involve bypassing the BBB. Various pharmacological agents have been used to open the BBB and direct invasive methods can introduce therapeutic agents into the brain substance. It is important to determine not only the net delivery of the agent to the CNS but also the ability of the agent to access the relevant target site within the CNS [2, 3].

Strategies used for brain targeting:-

1. Nanomaterials are found to accelerate the safety and efficacy level of drug delivery devices in brain targeting. Nanoegineered devices are found to be directing the drugs at cellular levels through nano-fluidic channels [4].
2. Different drug delivery systems such as liposomes, microspheres, nanoparticles, nanogels and nanobiocapsules have been used to improve the bioavailability of the drug in the brain but microchips and biodegradable polymeric nanoparticulate careers have been found to be more effective therapeutically in treating brain tumour. The physiological approaches also utilized to improve the transcytosis capacity of specific receptors expressed across the BBB [5].
3. It is found that the low density lipo-proteins related proteing with engineered peptide compound (EpiC) formed the platform incorporating the Angiopep peptide as a new effective therapeutics.
4. The current challenges are to develop the drug delivery careers, which must be able to deliver the drug across the BBB at a non-toxic and effective manner. Nanoparticles are found to be effective careers in delivery of conventional drugs, recombinant proteins, vaccines as well as nucleotides [6].
5. Nanoparticlulate drug delivery systems are found to be improving in the pharmacokinetic strategies of the drug molecules such as biodistribution, bioavailability and drug release characteristics in a controlled and effective manner with site specific drug delivery targeting to tissue or cell with reduction in toxic manifestation. Therefore, the use of nanotechnology in the field of pharmaceutical biotechnology helps in improving the drug delivery strategy including the kinetics and therapeutic index to solve the delivery problems of some biotech drugs including the recombinant proteins and oligonucleotides [7].

INTANASAL DELIVERY:-

Nasal cavity: -

The nasal cavity is easily accessible part of nose. It functions to deliver air to the respiratory segment. It has high vascular plexus and larger surface area [8]. It is divided into two segments further olfactory and respiratory segments. The olfactory segment is a direct connection to brain which has been shown in figure 1as under.

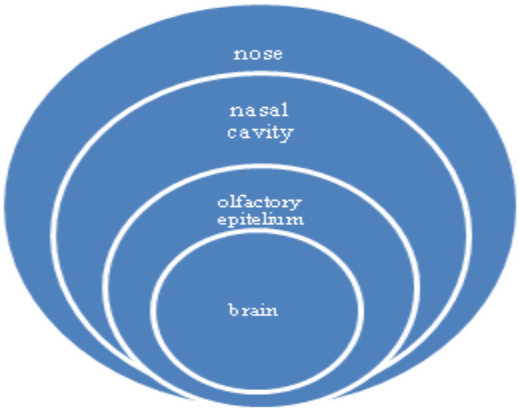

Figure 1 :- Schematic diagram showing connection of nose to brain.

Olfactory epithelium: -

Smell permits humans and animals with olfactory receptors to identify food, mates, predators, and provides both sensual pleasure as well as warnings of danger. The olfactory region of the two nasal passages in humans is an area of about 2.5 cm^2 containing in total of about 50 million primary sensory receptor cells. The olfactory region consists of cilia projecting down out of the olfactory epithelium into a layer of mucous which is about 60 microns thick. This mucous layer is a lipid-rich secretion that enriches

the surface of the receptors at the epithelium surface. The mucous layer is produced by the Bowman's glands which reside in the olfactory epithelium. The mucous lipids assist in transporting the odorant molecules as only volatile materials that are soluble in the mucous can interact with the olfactory receptors and produce the signals that our brain interprets as odour [10]. Olfactory cavity can be seen in figure 2 [11].

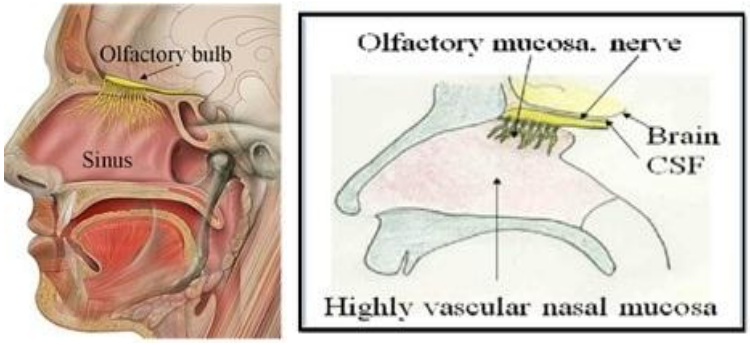

Figure 2:- Schematic diagram showing olfactory epithelium of nasal cavity.

Factors affecting nasal absorption:-

Effect of deposition on absorption: Deposition of the formulation in the anterior portion of the nose provides a longer nasal residence time. The anterior portion of the nose is an area of low permeability, while posterior portion of the nose is where the drug permeability is generally higher and provides shorter residence time [12].

Nasal mucosal membranes:- It is very rich in vasculature and plays a vital role in the thermal regulation and humidification of the inhaled air. The blood flow and therefore the drug absorption will depend upon the vasoconstriction and vasodilatation of the blood vessels [13].

Several enzymes:-Those that are present in the nasal mucosa might affect the stability of drugs. For example, proteins and peptides are subjected to degradation by proteases and amino peptidase at the mucosal membrane [14].

Effect of mucociliary clearance: - The absorption of drugs is influenced by the residence (contact) time between the drug and the epithelial tissue. The mucociliary clearance is inversely related to the residence time and therefore inversely proportional to the absorption of drugs administered.

Effect of pathological condition: - Intranasal pathologies may affect the nasal mucociliary transport process and/or capacity for nasal absorption [15].

Applications of nasal drug delivery:-

The applications are shown in table 1 [16].

Table 1:- Applications of nasal delivery.

Sr.No	Application	Example	Marketed product	Manufacturer
1.	Delivery of vaccines	Human Influenza vaccine	Flumist	MedImmune Inc.
2.	Delivery of peptide drugs for systemic effects	Buserelin	Profact Nasal	Aventis Pharma.
3.	Delivery of non-peptide drugs for systemic effects	Zolmitriptan	Asco Top Nasal	Astra Zeneca
4.	Delivery of Anti-migraine drugs	Ergotamine	Migranal nasal spray	Novartis
5.	Delivery for Diabetes therapy	Insulin	Novolin nasal spray	Novo nordisk
6.	Delivery of Pain relief drug	Morphine	Rylomine nasal spray	Nastech
7.	Delivery for control of urine output in patients with Diabetes Insipidus	Arginine-Vasopressin	Gel-vac nasal powder	Delsite Inc.
8.	Delivery for treatment of Erectile Dysfunction	Apomorphine	Otinose nasal spray	Britannia
9.	Delivery for treatment of menopause	Estradiol	Aerodil Nasal spray	Servier
10.	Delivery for treatment of Osteoporosis	Calcitonin	Miacalcin nasal spray	Novartis

Nanosuspension:-

One of the major problems related with poorly soluble drugs is very low bioavailability. The problem is even more complex for drugs like ropinorole, oxcarbazepine, and carbamazepine which are poorly soluble in both aqueous and nonaqueous media, belonging to BCS class II as classified by biopharmaceutical classification system[17]. Formulation of nanosuspension is an attractive

and promising alternative to solve these problems. Nanosuspension consists of the poorly water-soluble drug without any matrix material suspended in dispersion [18].

Preparation of nanosuspension:-

There are many techniques for preparing nanosuspension. These can be listed as under:-

1. Bottom up technology
2. Top bottom technology

a. Media milling

b. High pressure Homogenisation

3. Micro emulsion as template

Bottom up technology:-

In this method drug is dissolved in solvent and then solution is mixed with solvent to which drug is insoluble in the presence of surfactant. Rapid addition of solution to such solvent (generally water) leads to rapid super-saturation of drug in the solution and formation of ultrafine amorphous or crystalline drug. This method involves nuclei formation and crystal growth which are mainly dependent on temperature. High nucleation rate and low crystal growth rate are primary requirements for preparing a stable suspension with minimum particle size [19].

Advantages:-

- Simple process
- Low cost equipments
- Ease of scale up.

Disadvantages:-

- Drug has to be miscible with one solvent and this solvent should be a good solvent to the co-solvent.
- Surfactant addition is necessary to limit drug crystal growth.[20]

Example:-

Hydrocortisone nanosuspension was formulated using bottom-up technology. The technique included micro fluidic precipitation process that affect the size of generated drug particles. Altered parameters included flow rates of drug solution and antisolvent, microfluidic channel diameters, microreactors inlet angles and drug concentrations. The experimental results revealed that hydrocortisone nano-sized dispersions in the range of 80-450 nm were obtained and the mean particle size could be changed by modifying the experimental parameters and design of microreactors [21].

High pressure homogenisation:-

In this method the solvent used may be aqueous or non-aqueous. Following three steps are involved in this technique:-

First, drug powders are dispersed in a stabilizer solution to form pre-suspension; following this, pre-suspension is homogenized by homogenizer at 2800-21,300 psi sometimes for pre-milling; and finally homogenized at a high pressure for 10 to 25 cycles until the nanosuspensions are formed with desired size [22]. The process has been figured out in figure 3 [23].

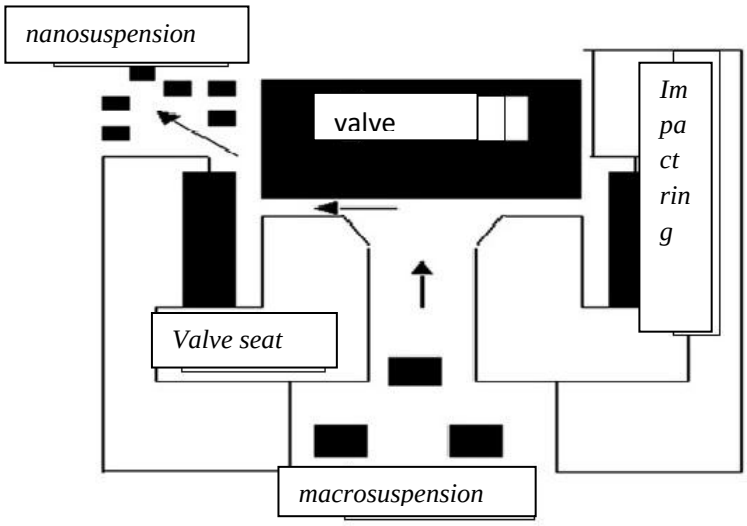

Figure 3 :- Figure representing high pressure homogenisation technique for production of nanosuspension.

Advantages:-

- Most applicable process.
- Useful for the formulation of very dilute as well as highly concentrated nanosuspension.
- Simple process.
- Aseptic production possible.
- Least risk of contamination.

Disadvantages:-

- Lengthy process
- Drug required of micronized size and suspension to be formulated before homogenisation.
- Possible risk of contamination of formulation due to metal ions present on the wall of homogeniser.

Example:-

Simvastatin nanosuspension is developed by high pressure homogenization method. Simvastatin powder (1% w/v) was dispersed in aqueous surfactant solution using magnetic stirrer. After drug dispersion first size reduction step is carried out using ultra turax T25 basic homogenizer at 950 rpm for 10 min.Then formulated mixture is homogenized using micron lab 40 homogenizer steps includes first two steps with 10 bar pressure and next two cycles with 50 bar pressure as initial step. Finally the suspension is homogenized for 15 cycles with 150 bar pressure to obtain nanosuspension [24].

Micro emulsion as template:-

Nanosuspensions are also obtained by just diluting the emulsion, formed by using a partially water-miscible solvent as the dispersed phase. The emulsion technique is useful for drugs which are either partially water miscible or soluble in volatile organic solvents. Also, micro emulsion templates can also produce nanosuspensions [25].

Advantages:-

- High drug solubilisation.
- Long shelf life.
- Ease of scale up.

Disadvantages:-

- Use of hazardous solvent, which can produce toxicity.
- Use of high amounts of solvents and surfactant [26].

Example:-

Albendazole, a BCS Class II drug can be formulated into nanosuspension using micro emulsion template method. The stabilizer used includes PVP K30 and emulsifier includes Tween 80 [27].

Media Milling:-

In this method drug particles are subjected to media milling for nanoparticle production. Impaction is the main principle of this method. Drug, stabilizer, suitable buffer or water and milling media are subjected to high speed homogenisation on a Milling chamber. The media used can be either glass or zirconium oxide beads [28]. The disintegration of micronized drug occurs following impaction between milling media and drug, leading to nanosized drug particles or zirconium oxide beads. The disintegration of micronized drug occurs following impaction between milling media and drug, leading to nanosized drug particles. The process has been represented diagrammatically in figure 4 [29].

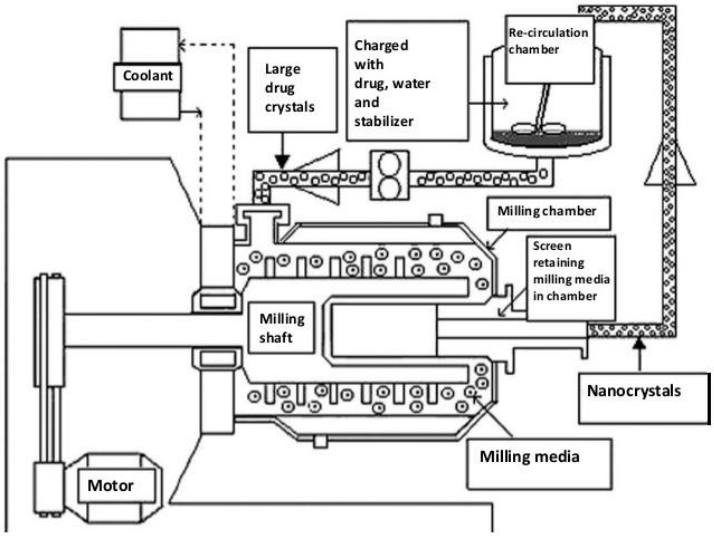

Figure 4 :- diagrammatic representation of media milling process for production of nanosuspension.

Advantages:-

- Little batch to batch variation
- Ease of scale-up
- High flexibility in handling large quantity of drugs.

Disadvantages:-

- Sometimes residues of milling media can be generated [30].

Example:-

Phenytoin and nifedipine are formulated into nanosuspension by wet media milling method. The method includes placing the drug in a conical test tube with milling media and stabilizers, which is been held by a holder. The holders are placed in a shaker and oscillated at 2700 rpm for 12hrs. The holder was then cooled at 0^0C and the formulation was collected later on [31].

Formulation consideration:-

Stabilizer:-

Stabilizer plays an important role in the formulation of nanosuspension. In the absence of appropriate stabilizer, the high surface energy of nanosized product can induce agglomeration or aggregation of the drug crystals. The main function of a stabilizer is to wet the drug particles thoroughly, and to prevent Ostwald's ripening and agglomeration of nanosuspension in order to yield a physically stable formulation by providing steric or ionic barriers [32].

The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behaviour of nanosuspensions. In some cases a mixture of stabilizers is required to obtain a stable nanosuspension.

Stabilizers that have been seen so far includes celluloses, poloxamers, polysorbates, lecithins and poviodones. Lecithin is of choice as stabilizer if the nanosuspension is autoclavable and parentrally acceptable [33].

Co-surfactant:-

The choice of co-surfactant is critical when using micro emulsions to formulate nanosuspensions. Since co-surfactants can greatly influence phase behaviour, the effect of co-surfactant on uptake of the internal phase for selected microemulsion composition and on drug loading should be investigated [34].

Eg: - transcutol, glycofurol, ethanol and isopropanol [35].

Advantages of nanosuspension:-

Increase in the dissolution velocity and saturation solubility of the drug:-

Actual cause of nanosuspensions being a promising drug delivery strategy behind the increase in the dissolution velocity and saturation solubility of the nanosuspensions can be given as according to the Nernst–Brunner and Levich modification of the Noyes Whitney dissolution model equation, the dissolution velocity of the nanosuspension increases due to an intense increase in the surface area of the drug particles from microns to particles of nanometre size i.e. µm to nm [36].

The equation can be given as under:-

$$Dx/dt = A.D/h. (Cs - X_d/ V)$$

Where, Dx/dt = dissolution velocity,

A = surface area of particle,
D = diffusion coefficient,
h = thickness of the diffusion layer,
X_d = concentration in surrounding liquid,
Cs = saturation solubility of drug.

Improved biological performance:-

An increase in the dissolution velocity and saturation solubility of a drug leads to an improvement in the in-vivo performance of the drug irrespective of the route used, whether parental or nasal or any other [37].

Ease of manufacture and scale-up:-

Nanosuspensions are easy to manufacture as compared to nanoparticles. The production processes are easily scaled up for commercial production. Also, the methods are possible to perform on lab scale and require less effort with lower cost and time [38].

Long-term physical stability:-

One of the special feature of nanosuspension is the absence of Ostwald ripening, the phenomenon in which smallest particles in solution dissolve and deposit on large particles in order to reach a more thermodynamically stable state in which surface to area ratio is minimized. Ostwald ripening is responsible for crystal growth and subsequently formation of microparticles [39].

The absence of particles with large differences in their size in nanosuspensions prevents the existence of the different saturation solubility's and concentration gradients in the vicinity of differently sized particles, which in turn prevents the Ostwald ripening effect.

Versatility:-

The flexibility offered in the modification of surface properties and particle size, and ease of characterization of formulated nanosuspensions enables them to be incorporated in various dosage forms, such as tablets, pellets, suppositories and hydrogels, for various routes of administration, thus proving their versatility [40].

Evaluation of nanosuspension:-

The evaluation parameters of nanosuspension are as follows:-

Particle size and particle size distribution:-

The particle size was measured using Malvern Zetasizer ZS200 at 25 ± 0.5^0C. Each sample was measured at least 3 times and the average values were employed for calculation of response surfaces [41].

Scanning electron microscopy:-

This technique is used to investigate the shape and surface morphology of the drug nanocrystals and to measure the particle size. Aqueous suspensions of nanoparticles are placed on a sample holder for analysis. Nanocrystals are further observed with a scanning electron microscope [42].

X-ray Diffraction:-

X-ray diffraction studies are used in order to characterise the crystalline nature of the formulated nanosuspension. The molecules with random arrangement in noncrystalline substances makes them poor and coherent scatters of x-ray, which gives broad, diffuse maxima in diffraction patterns. The crystalline patterns thus, become easy to distinguish from non-crystalline particles as they show sharply defined diffraction peaks. The X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing [43].

Surface charge (zeta potential):-

Surface charge properties of the nanosuspensions are studied through zeta potential. The value of zeta potential indicates the stability of nanosuspensions at the macroscopic level. A maximum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions and a minimum of ±20 mV for steric stabilization. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential. Electroacoustic technique is also used for the determination of the zeta potential in the areas of material sciences [44].

In-vitro drug release study of nanosuspension:-

Dialysis bag diffusion technique was used to study in-vitro release of drug from the prepared nanosuspension. The formulation (2ml) has to be placed in the dialysis bag, hermetically sealed and immersed into a 100 ml beaker containing 50 ml of the release media maintained at 37±0.5°C and stirred at 50 rpm. Aliquots of 5 ml are to be withdrawn at pre-determined time intervals and immediately restored with the same volume of fresh media maintained at the same temperature. The study has to be carried out in phosphate buffer 6.5, 1% v/v Tween 80 can be used to maintain the sink condition.

Dissolution:-

The dissolution of a nanosuspension can be performed using Dissolution Apparatus USP Type II Paddle at 37±0.5°C, 50 rpm and specific buffer solution. Aliquots of 5 ml are to be withdrawn at pre-determined time intervals and immediately restored with the same volume of fresh media maintained at the same temperature. The study has to be carried out in phosphate buffer 6.5, 1% v/v Tween 80 can be used to maintain the sink condition [46].

In-vitro permeability study of nanosuspension:-

In-vitro permeability studies for nanosupensions are performed using semi permeable artificial membranes such as cellophane membrane or biological membrane. Diffusion cell is used for performing study and phosphate buffer 6.5.

In-vivo study:-

The animals are to be divided into four groups of five rats each. Group I, Normal control and receives standard pellet diet, water and orally administered with 5% CMC. Group II, Negative control and receives a single dose of triton administered at a dose of 400mg/kg, p.o. After 72 hours of triton injection, this group receives a daily dose of 5% CMC (p.o) for 7 days. The Group III receives a daily dose of nanosuspension nasally, for 7 days, after inducing specified disease. Group IV will receive standard drug (10mg/kg nasally).

On the 8th day blood has to be collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples are to be centrifuged for 10 minutes and serum samples are used for various biochemical experiments. Then animals are to be sacrificed by using 5ml Diethyl ether and collected the liver, brain, heart and spleen. The liver, heart, brain and spleen were homogenized in cold 0.15M KCl and extracted with $CHCl_3$:CH_3OH (2%v/v). The extracts are to be assayed after dilution using HPLC. The AUC for each extract has to be calculated and compared. The broader the AUC, the higher the amount of drug in that organ. So here, the brain extract should have the broadest peak of all, as the nanosuspension is for brain targeting [47, 48].

Stability studies:-

The formulated nanosuspension was divided in to 3 samples and stored at different temperatures for a period of 3, 6 months.

- 4°C , refrigerated temperature
- 27°C ± 2°C at 65% RH, room temperature
- 40°C ± 2°C at 65% RH, accelerated temperature.

After 3, 6 months formulation is evaluated for remaining drug content in by specific methods [49].

Toxicity studies:-

A general range of compounds formulated as nanosuspension are of BCS Class II/IV with higher log P values. Formulation in nanosuspension increases solubility so it can be said that a dose reduction is required for drugs. A nanosuspension can result in highest exposure of drug, so a higher plasma concentration of drug is achieved. So a specific toxicity study has to be performed in different animal species such as Sprague-Dawley rats, Rhesus Monkeys, Beagle Dogs and Yukatan Minipigs in fed as well as fasted state. Parameter to be studied includes Tmax, Cmax and AUC. After assessing safety of nanosuspension in these animals, human volunteer studies have to be performed [50].

Comparative studies of intranasal delivery to delivery via other routes:-

Intranasal vs. Buccal route:-

Anderson et al. (Jan 2012) compared buccal lorazepam to nasal midazolam using a crossover design volunteer study they found that buccal drug does not absorb as rapidly as nasal drug and so is not as clinically effective for disease states that require rapid onset of action (seizure, breakthrough pain, opiate reversal for examples). Nasal lorazepam achieves therapeutic levels in the blood and CSF more rapidly and therefore its clinical onset of action is earlier. The figure 5 shows resulting serum levels in the first 10 minutes.

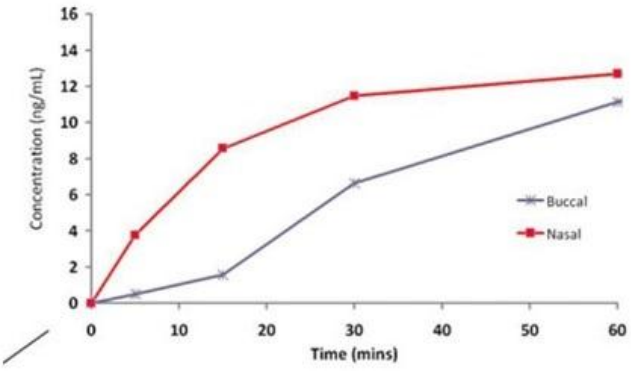

Figure 5 :- Comparison graph of nasal vs buccal formulation.

Intranasal vs. Oral route:-

In case of diabetes, Metoclopramide oral tablet is ineffective in symptoms of gastro paresis so it has to be given in form of nasal spray. As studied by Parkman HP et al. 88.9% of subjects who received oral 10 mg, 91.2% who received nasal 10 mg, and 97.1% who received nasal 20 Metoclopramide were classified as responders. The side-effect profile of the Metoclopramide nasal spray was favourable. More side effects, especially nausea, occurred with the oral tablets [51].

Equipments required for formulation and evaluation of nanosuspension:-

The equipments are listed in table 2 [51

Table 2:- Equipments required for formulation and evaluation of nanosuspension.

Sr no.	Equipments
1.	High speed magnetic stirrer
2.	Fourier Transform Infrared Spectrophotometer
3.	X-ray Diffraction
4.	Scanning Electron Microscopy
5.	Zeta analyser
6.	Franz Diffusion Apparatus
7.	UV-Vis Spectrometer
8.	Dissolution Apparatus

CONCLUSION

From the present review, the use of nanosuspensions for brain targeting has been highlighted. Nanosuspension serves the criteria for nasal administration and is very beneficial over other nasal formulations. A further research on formulation of any drug for CNS disorder into nanosuspension can be performed to serve the basic criteria for brain targeting and obtaining the required efficacy in treatment of the specific CNS disorder (Eg:- Epilepsy).

ACKNOWLEDGEMENT

The first author sincerely acknowledges the Managing trust of SMT BNB Swaminarayan Pharmacy College, Salvav and Principal Dr Anil Jadhav for providing basic requirements to conduct research. A sincere thanks to HOD, Pharmaceutics Mr Ajay Talele and Guide Miss Ankita Patel for providing an expertise in conducting the review. A sincere thanks to Mr. Harsh Upadhyay and Pranshu for all the help provided.

Conflict of Interests :-

The Author declares none conflict of interests.

ABBREVIATIONS AND SYMBOLS

BBB:- blood brain barrier
Nm :- nanometre
CNS :- central nervous system
BCS :- biopharmaceutical classification system
Psi :- pounds per square inch
PVP :- polyvinyl pyrollidone
Rpm :- rounds per minute
KCl :- potassium chloride
$CHCl_3$:- chloroform
CH_3OH :- methanol
HPLC :- high performance liquid chromatography
CSF :- cerebro-spinal fluid
SLN :- solid lipid nanoparticles
AUC :- area under curve
C_{max} :- maximum plasma concentration
T_{max} :- maximum time required
SEM :- scanning electron microscopy
FTIR :- Fourier transform infrared spectroscopy
CMC :- carboxy methyl cellulose
RH :- relative humidity
p.o :- orally

REFERENCES

1. William M Pardridge, Drug Delivery to the Brain, Journal of Cerebral Blood Flow & Metabolism ,1997, 17:713–731.
2. Vyas T K et al. Intranasal Drug Delivery for Brain Targeting, Current Drug Delivery, 2005, 2(2):165-75.
3. Leah R Hason et al. Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease, BMC Neuroscience, 2008, 9:3, S5.
4. Gabriel A Silva, Neuroscience Technology: progress, opportunities and challenges, Nature Review Neuroscience, 2006, 7: 65-74.
5. Anita Schnyder et al. Drug transport to Brain with Targeted Liposomes, NeuroRx, Jan 2005, 2(1):99-107.
6. William A Banks, Characteristics Of compounds that crosses the Blood Brain Barrier, BMC Neurology 2008, 9(1), 3-5.
7. Xie X et al. PLGA nanoparticles improves the oral bioavailability of curcumin in rats: characterisation and mechanisms, J Agric Food Chemicals, Epub 2011, 59(17): 9820-9.
8. G.J.Tortora, Principles of Anatomy and Physiology, Wiley International Edition, Biological Sciences Textbook Inc, 2006: 19:847-850.
9. Rupali Sharma et al. Nasal mucosa as a Novelistic platform for brain targeting, American Journal of Pharmaceutical Technology and Research, 2014, 4(1): 12.
10. Ming Ming Wen, Olfactory Targeting Through Intranasal Delivery of Biopharmaceutical Drugs to the Brain — Current Development, Drug Discovery-Pharmacology, 2011: 1-6.
11. Jogani V et al. Recent patents review on intranasal administration for CNS drug delivery, Recent Pat Drug Delivery Formulations. 2008; 2(1):25-40.
12. Khafagy E et al. Effect of cell-penetrating peptides on the nasal absorption of insulin, Journal Of Control Release. 2009, 133: 103-108.
13. Illum L et al. Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides. International Journal of Pharmaceutics. 2001, 222: 109-119.
14. Farraj N F et al. Nasal administration of insulin using bioadhesive microspheres as a delivery system. Journal of Control Release. 1990, 13: 253-261.
15. Illum L. Transport of drugs from the nasal cavity to the central nervous system. European Journal of Pharmaceutial Sciences. 2000, 11: 1-18.
16. Lakshmi P et al. Nanosuspension technology: A review. International Journal of Pharmaceutical Sciences. 2010;2:35–40.
17. Vermaa S et al. Quality by design approach to understand the process of nanosuspension preparation. International Journal of Pharmaceutics. 2009; 377:185–98.
18. Nagaraju P et al. Nanosuspensions: A promising drug delivery systems. International Journal of Pharmaceutial Science Nanotechnology, 2010; 2:679–84.
19. Werling et al**.** Nanosuspensions of anti-retroviral agents for increased central nervous system delivery, United States Patent Application, 60540718. [USPTO]
20. Kocbek P et al. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. International Journal of Pharmaceutics. 2006;312,179–186.
21. Ali H S et al. Preparation of Hydrocortisone Nanosuspension by precipitation technology using microfluidic reactors, International Journal of Pharmaceutics, 2009, 375:1-2.
22. Maincent P et al. Preparation and characterization of nanoparticles containing an antihypertensive agent. European Journal of Pharmaceutics and Biopharmaceutics. 1998, 46: 137-143.
23. V R Patel et al. Nanosuspension: An approach to enhance solubility of drugs, Journal of Advances in Pharmaceutical Technology and Research, 2011, 2(2):81-87.
24. Athul P V, Preparation and Characterisation of Simvastatin Nanosuspension By High Pressure Homogenisation Technology, Journal Of Pharmaceutical Technology, 59(1):193-197.
25. Elena Adina Rogozea et al, Highly homogeneous nanostructured templates based on environmental friendly microemulsion for nanomaterials processing, Materials Letters- Elsevier, 2014, 132(1), 346-348.
26. V. B. Patravale et al, Nanosuspensions: a promising drug delivery strategy, Journal of Pharmacy And Pharmacology, 2004, 56(1): 827-840.
27. Akshay Koli et al. Development of Albendazole nanosuspension by Various Techniques, Drug Delivery Letters, 4(2): 1-2.
28. Singh S K et al. Investigation of preparation parameters of nanosuspension by top-down media milling to improve the dissolution of poorly water-soluble glyburide, European Journal Of Pharmaceutics and Biopharmaceutics.2011;78(3):441-6.
29. E M Liversidge et al. Nanosizing for oral and parenteral drug delivery: A perspective on formulating poorly-water soluble compounds using wet media milling technology, Advances Drug Delivery Reviews, Elsevier, 63(6): 427-440.
30. Filippos Kesisoglou et al. Nanosizing Oral Formulation Development and biopharmaceutical evaluation, Advanced Drug Delivery Reviews, Elseveir, 2007, 59(7): 631-644.
31. J Hecq et al. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine, International Journal of Pharmaceutics, 2005, 299:167-77.
32. P Kockbek et al. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly water soluble drugs, International Journal Of Pharmaceutics, 2006, 312:179-86.
33. Kayser O et al. The impact of Nanobiotechnology on the development of new drug delivery systems. Current Pharmaceutical Biotechnology. 2005;6:3–5.

34. Liang Y C et al. Effect of triblock copolymer non-ionic surfactants on the rheology of 3 mol% yttria stabilised zirconia nanosuspensions. Ceram Int. 2008;34:293–7.
35. Scholer N et al. Atovaquone nanosuspensions show excellent therapeutic effect in a new murine model of reactivated toxoplamosis. Antimicrobial Agents Chemotherapy. 2001; 45:1771–9.
36. Yang J Z et al. Fluticasone and budesonide nanosuspensions for pulmonary delivery: Preparation, characterization, and pharmacokinetic studies. Journal of Pharmaceutical Sciences. 2008; 97:4869–78.
37. K Peters et al. Preparation of a clofazamine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. Journal of Antimicrobial Chemotherapeutics, 2000,45: 77-83.
38. P Kocbek et al. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drug. International Journal of Pharmaceutics, 2006, 312:179-186.
39. J Moschwitzer et al. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. European Journal of Pharmaceutics and Biopharmaceutics. 2004, 58: 615-619.
40. Z Zili et al. Preparation and characterization of poly-ε- carprolactone nanoparticles containing griseofulvin. International Journal of Pharmaceutics, 2005, 294:261-267.
41. Francso L et al. Diclofenac nanosuspensions, Influence of preparation procedure and crystal form on drug dissolution behaviour, International Journal Of Pharmaceutics, 2009, 373: 124-132.
42. Hanafy et al. Pharmacokinetic evaluation of oral fenofibrate nanosuspension and SLN in comparison to conventional suspension of micronized drug. Advanced Drug Delivery Reviews; 2007: 59(6) : 419-426.
43. Rosario P et al. Eudragit RS100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen, European Journal of Pharmaceutical Science, 2002, 16: 53-61.
44. Bindu et al. Novel strategies for poorly water soluble drugs, International Journal of Pharmaceutical Sciences Review and Research, 2010, 4(3): 76-84.
45. Ravichandran R, Physico-chemical evaluation of gymnemic acid nanocrystals, International Journal of Nanoparticles, 2010, 3(3):280-286.
46. Deng J et al. Understanding the structure and stability of Paclitaxel nanocrystals, International Journal Of Pharmaceutics, 2010, 399(1-2):242-249.
47. N Arunkumar et al. Nanosuspension Technology and its application in drug delivery, Asian Journal Of Pharmaceutics, 2009, 3(3): 168-173.
48. Bohm BH, Muller RH. Lab-scale production unit design for nanosuspensions of sparingly soluble cytotoxic drugs. PSTT 1999; 2:336-9.
49. Chen Ye. Oleanolic acid nanosuspensions: Preparation, in vitro characterization and enhanced hepato protective effect. Journal of Pharmaceutics and Pharmacology, 2005, 57:259-64.
50. Praveen Khirwadka et al. Magnetic Nanoparticles for Drug Delivery, Indo American Journal Of Pharmaceutical Research, 2014,4(12):5599-5610.
51. Langutth P et al. Nanosuspension formulation for low soluble drugs: Pharmacokinetics evaluation using spiranolactone as model compound. Drug Delivery Ind Pharm 2005, 31:319-29.
52. James C Boylan, Encyclopedia Of Pharmaceutical Technology,2nd edition, Marcel- Decker Inc, Vol I-III, NY, 2002.

5487847845**1150206**