Chapter Three
PROOF OF CONCEPT - PRELIMINARY EVALUATION STUDIES
3.1. THE CONCEPT

As per the reference taken from Central Council for Research in Unani Medicine (CCRUM) Library (Razi et al., 2001) in fig. 20, stating clinical ethno practice of Nigella Sativa seeds used in epilepsy “Inhalation of roasted seed poultice is useful in fits”, indicating three important points:

1. Anticonvulsant activity of Nigella sativa
2. Role of route of administration e.g. Inhalation
3. Delivery system (size of the particle) having ability to cross BBB for quick onset of action e.g. vapours (nanometric in size)

Fig. 20: Text Image for the reference of the use of Nigella Sativa in epilepsy from CCRUM Library (Razi et al., 2001)

Based on the above mentioned facts our concept would be stated as:

Nanometric formulations can be used for the selected neurotherapeutic agent, amiloride for delivery through nasal route in order to get superior delivery system for seizure control with reduced dose in animal models of epilepsy.
3.2. EXPERIMENTAL WORK: Proof of Concept – Preliminary Evaluation

Proof of concept studies carried out to find out answers for the below mentioned questions:

A) Selection of Dose & route of administration
   by Pharmacodynamic methods (ICES and PTZ induced seizure models)

B) Selection of Prototype Novel formulation having nanometric size

3.2.1. Part A: Selection of Dose & route of administration

Animals:

Swiss albino mice of either sex weighing 20–35 g were used. Animals were housed in groups of six animals per cage and maintained in a natural light and dark cycle, with free access to food and water. The study was performed with prior approval of the institutional animal ethics committee (IAEC) of CPCSEA (Committee for the purpose of control and supervision of experiments on animals) with approval Reg. no. JH/CPCSEA/31-01-2000/Project no. 633.

Drug & Chemicals:

Amiloride Hydrochloride was procured from Ranbaxy laboratories Ltd, Gurgaon, India, Pentylenetetrazole (PTZ) was purchased from Sigma Aldrich, USA. Drug solutions were freshly prepared by dissolving in normal saline. In case of intranasal administration, the dose was reduced by keeping in view that it would work at lower dose by direct delivery to brain.

Pharmacodynamic methods:

i. Increasing Current Electroshock Seizure (ICES) Test

The ICES test is a model of grand mal-type seizure and is useful for evaluation of both the anti- and pro-convulsant activities of drugs. Here the ICES test, as proposed by Kitano et al. (1996) modified by Marwah et al. (1998), was used to evaluate the anticonvulsant effect of the drug. Starting with a current of 2 mA, electroshock was delivered to each mouse via ear electrodes as a single train of pulses (square wave, 20Hz for 0.2 sec) with a linearly increasing intensity of 2 mA/2 sec.
### Protocol for ICES test

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Seven groups of 6 mice each were be pre-treated with the Amiloride Hydrochloride Solution at five dose levels, i.e. 0.08, 0.12, 0.16, 0.35 and 0.65 mg/kg administered by intranasal (i.n.) route and 5 μl/per nostril distilled water as control and after 30 min. Electroshock was applied via ear electrodes (fig. 21) using an electric stimulator (Electro-convulsionometer)</td>
</tr>
<tr>
<td>2.</td>
<td>The electroshock consist of a single train of pulses (square wave, 20Hz for 0.2 sec) with a linearly increasing intensity of 2 mA/2 sec</td>
</tr>
<tr>
<td>3.</td>
<td>The current at which tonic hindlimb extension (HLTE) occurred was recorded as the seizure threshold current for each mouse. If no tonic hindlimb extension was observed by a current of 30 mA, electroshock was terminated, and this cut-off current will be used in the analysis.</td>
</tr>
<tr>
<td>4.</td>
<td>Observation were made immediately after the current applied in increasing manner</td>
</tr>
</tbody>
</table>
| 5.   | **Observations to be made:** (1) Threshold Current for tonic HLE  
(2) Post tonic HLE Recovery Time  
(3) Protection against Mortality |

**Fig. 21:** Experimental set-up for ICES-induced seizures model (right) and Hind Limb Extension (HLE) (left).
ii. Pentyleneetrazole (PTZ) induced seizures Test

Pentylenetetrazole (PTZ) is specifically used in seizure assays as a method of assessing the excitability of the central nervous system and GABA activity. Here seizures were induced chemically with PTZ as described by Vohora et al. (2000). Briefly, mice were given PTZ at a dose of 70 mg/kg i.p. The animals were observed immediately after PTZ injection for a period of 30 min. The latency to myoclonic jerks & clonic generalized seizures and percentage protection were recorded.

Protocol for PTZ-induced seizures (Vohora et al, 2000)

<table>
<thead>
<tr>
<th>STEP</th>
<th>PROCEDURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Seven groups of 6 mice each were be pre-treated with the Amiloride Hydrochloride Solution at five dose levels, i.e. 0.08, 0.12, 0.16, 0.35 and 0.65 mg/kg administered by intranasal (i.n.) route and 5 µl/per nostril distilled water as control</td>
</tr>
<tr>
<td>2.</td>
<td>PTZ (70mg/kg) administered to mice via i.p. 1 hour after administration of the drug Solution</td>
</tr>
<tr>
<td>3.</td>
<td>The onset of PTZ induced clonic spasms and the number of episode of clonic spasms persisting for at least 5 seconds were recorded during individual observation for 30 min.(Krall et al 1978). The absence of 5 sec clonic spasms was defined as protection. The latency period for any seizure as well as death was recorded for each animal. If none of these occurred during the time limit (1 hr.), the animals were considered protected (Krall et al 1978; Wang et al 2000). If no general clonus occurred during a 30-min period of observation, the animals were consider protected.</td>
</tr>
<tr>
<td>4.</td>
<td>Observations to be made: for the presence or absence of a threshold seizure (an episode of clonic seizure activity of at least 5s duration). Seizures were further characterise into four different patterns: (1) Latency to Myoclonic jerks (2) Latency to Generalized Seizures (3) Protection against Mortality</td>
</tr>
</tbody>
</table>

Statistical analysis

The results were presented as means ±SEM. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett’s t-test at the 95% confidence by using GraphPad Prism Version 5 Software.
Results & Discussion: Part A

I. Amiloride Hydrochloride (AMH) against ICES Seizure Model:

**Fig. 22**: Effect of AMH against ICES induced seizure threshold in mice.

**Fig. 23**: Effect of AMH against ICES induced Post HLTE recovery time in mice.
Chapter 3: Proof of Concept – Preliminary Evaluation Studies

II. AMH against PTZ induced seizure

Fig. 24: Percentage Protective Effect of AMH against ICES induced mortality in mice.

Fig. 25: Effect of AMH on latency to myoclonic jerks after PTZ induced seizures in mice.
Chapter 3: Proof of Concept – Preliminary Evaluation Studies

Fig. 26: Effect of AMH on the latency to generalized clonic seizure after PTZ induced seizures in mice.

Fig. 27: Percentage protection of AMH against mortality due to PTZ Induced seizures.
3.2.2. Conclusions: Part A
An improved anticonvulsant action was observed with intranasal route of administration (at lower dose) as compared to the reported route i.e. oral (at higher dose) in electrical induced seizure model (ICES model) and chemical induced seizure model (PTZ model). Following conclusions were made on the basis of proof of concept preliminary evaluation studies:

**Route of Administration**
Intranasal route showed improved brain uptake as compared to Oral route as the pharmacodynamic effects of AMH were statistically significant (**p<0.001**) when *i.n.* was compared to *p.o.*.

**Dose**
Experimental data showed that the intranasal dose of 0.16 mg/kg is equally effective as 0.65 mg/kg per oral, which was further evaluated for formulation development work.

3.2.3. Part B: Prototype Novel Nano-formulation Development
The strategy of applying drugs that are encapsulated into particulate vectors (such as synthetic nanoparticles) or nanosized colloidal dispersions (i.e. nanoemulsions) to the olfactory epithelium could potentially improve the direct CNS delivery of drugs—including biologics. If drugs could reach the CNS in sufficient quantity by this route, it could generate interest in previously abandoned drug compounds and enable an entirely novel approach to CNS drug delivery.

Various approaches have been tried for prototype development work for initial proof of concept studies with respect to various pharmaceutical factors for achieving maximum drug loading for achieving ultimate goal of better patient compliance and therapeutic effects.

Brain targeted nanocolloidal carrier systems were formulated using biodegradable polymers like chitosan, PLGA, PBCA. Nanoemulsion systems were prepared by using various surfactants ratios.

As a first step towards the development of polymeric nanoformulations, placebo nanoformulations were prepared with different polymers and different excipients at different concentrations to screen out the concentration ranges of the important
excipients. Formulation feasibility studies were carried out with different selected polymers by varying the individual formulation parameters viz. monomer/polymer/drug concentration, solution spraying rate, stirring speed, pH of the medium etc. and by visual observation of the formulation.

### 3.2.3.1. Formulation considerations

Following factors were considered for designing the formulation to administer through intranasal route (Vyas et al. 2005):

1. **Drug Molecule**

   a) Molecular weight and size: <1000 Da

   b) Solubility: Higher to get solubilized in the nasal fluid and thereby to get permeated (important for particulate drug delivery).

   c) Compound lipophilicity: Should be high for better absorption (through transcellular route), although hydrophilic small molecular weight compounds absorb through aqueous channels.

   d) Partition coefficient and pKa: Unionized molecules easily permeate, although ionized species also permeate through different pathways.

   e) Therapeutic dose: < 25 mg per dose

2. **Formulation Characteristics**

   a) Drug concentration: Higher the concentration, higher the permeation (up to certain extent)

   b) Dose volume: 0.01 - 0.15 mL/dose

   c) Formulation pH: 4.5 – 6.5 to avoid nasal irritation.

   d) Osmolarity: Isotonic formulation (less irritant), higher salt concentration increases permeability but is irritant to nasal mucosa.

   e) Viscosity: Higher the viscosity, longer the residence time of formulation. But it also hinders normal physiological functions like ciliary beating and mucociliary clearance, thus affecting permeability.
### 3.2.3.2. Materials Used

<table>
<thead>
<tr>
<th>Material Name</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride Hydrochloride</td>
<td>Ranbaxy laboratories Ltd., Gurgaon, India.</td>
</tr>
<tr>
<td>Pluronic F68 (Poloxamer 188) &amp; Pluronic F127 (Poloxamer 407)</td>
<td>BASF India</td>
</tr>
<tr>
<td>Low molecular weight chitosan (LM-chitosan, number average molecular weight (Mn)=41,000 Da) and Medium molecular weight chitosan (MM-chitosan, Mn=72,000 Da)</td>
<td>Primex ehf Oskarsgata 7580 Siglufjordur Iceland,</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>CDH Labs, Mumbai, India</td>
</tr>
<tr>
<td>Isobutyl cyanoacrylate monomer</td>
<td>Henkel Loctite, USA.</td>
</tr>
<tr>
<td>Poly (dl-lactide-co-glycolide) (PLGA), with a copolymer ratio of dl-lactide/glycolide of 60/40 and an inherent viscosity of 0.5 dl/g</td>
<td>PURAC Biochem, Gorichem, The Netherlands</td>
</tr>
<tr>
<td>Polyvinyl alcohol (PVA) with 86- 89% hydrolysis degree and molecular mass range of 15,000–100,000 g/mol</td>
<td>Fluka, Buchs, Switzerland</td>
</tr>
<tr>
<td>The dialysis membrane (molecular weight cut-off between 12 and 14 kDa)</td>
<td>HiMedia, India.</td>
</tr>
<tr>
<td>Polysorbate 80 (Tween 80), polysorbate 20 (Tween 20), oleic acid, isopropyl myristate (IPM), olive oil, glycerol triacetate (Triacetin), castor oil, Octylphenoxy polyethoxy ethanol (triton x 100), poly ethylene glycol 200 (PEG 200), poly ethylene glycol 400 (PEG 400), propylene glycol, sorbitan monolaurate (Span 20), diethylene glycol monoethyl ether (Carbitol), soyabean oil and coconut oil</td>
<td>CDH Labs, Delhi, India</td>
</tr>
<tr>
<td>Oleyl macrogolglyceride (Labrafil M), polyethylene glycol dicaprylocaprate (Labrafac), caprylocaproyl macrogol-8-glyceride (Labrasol) and propyleneglycol mono laurate (Lauroglycol 90)</td>
<td>Gattefosse, Saint-Priest, France</td>
</tr>
<tr>
<td>Polyethoxylated castor oil (Cremophore EL) was gifted by</td>
<td>Signet Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Propylene glycol mono caprylic ester (Safsol 218)</td>
<td>Nikko chemicals, Tokyo, Japan</td>
</tr>
</tbody>
</table>

Note: Remaining chemicals were procured from Merck Ltd., Mumbai, India.
3.2.3.3. Method of Nanoformulation Preparations

1. Chitosan-sodium alginate nanoparticles

Chitosan-sodium alginate nanoparticles (Nps) were prepared by the modified complex coacervation method as reported by Calvo et al. (1997). Briefly, an aqueous solution of polysaccharide chitosan (CS, polycation) was mixed with an aqueous solution of sodium alginate (ALG, polyanion) under controlled magnetic stirring resulting in the formation of nanoparticles under mild conditions because of ionic interactions.

ALG was dissolved in distilled water at various concentrations. CS was dissolved in aqueous acetic acid solution (2% w/v) at various concentrations under stirring. About 50 mL of ALG solution was sprayed into 50 mL of CS solution containing the drug and 0.50% surfactant under continuous magnetic stirring (Remi 5MLH, REMI Corp., India) at 1500 ± 25 rpm for 60 min. The formation of the nanoparticles could be observed as the appearance of turbidity and opalescent suspension formation. The rate of the ALG solution spraying to CS solution was controlled (approximately 0.5 g min-1) so as to avoid the formation of microparticles or precipitate. An aliquot of the nanoparticles suspension was centrifuged (REMI high speed, cooling centrifuge, REMI Corp., India) at 18,000 rpm for 30 min. at 4°C and the drug content in supernatant was measured by validated UV spectrophotometric method.

2. Poly (butylcyanoacrylate) nanoparticles

Poly (butylcyanoacrylate) (PBCA) nanoparticles were prepared by anionic polymerisation of Isobutylcyanoacrylate (IBCA, n-butyl-2-cyanoacrylate) monomer in acidic media as described previously by Douglas et al. (1984) and Reddy et al. (2004).

About 50 mL solution of IBCA monomer in acetone was sprayed into 50 mL acidic solution of drug in 0.1N HCl containing 1% surfactant under continuous magnetic stirring (Remi 5MLH, REMI Corp., India) at 1500 ± 25 rpm. After 3 h, the reaction was finalized by neutralization with 0.1N NaOH. The formation of the nanoparticles could be observed as the appearance of bluish tinge or light turbidity and opalescent suspension formation. The rate of the monomer solution spraying to acidic solution of drug was controlled (approximately 1.0 g min-1) so as to avoid the formation of microparticles or precipitate. An aliquot of the nanoparticles suspension was
centrifuged (REMI high speed, cooling centrifuge, REMI Corp., India) at 18,000 rpm for 30 min. at 4°C and the drug content in supernatant was measured by validated UV spectrophotometric method.

3. Poly (lactide-co-glycolide) nanoparticles

Poly (lactide-co-glycolide) (PLGA) nanoparticles were prepared by nano-precipitation or emulsion-solvent evaporation method as described by Némati et al. (1996). The nature of the organic solvent, the nature of the aqueous phase (water or buffer) and the concentration of the PVA was varied. The solvent, which gave smallest particle size of PLGA nanoparticles was acetone.

PLGA was first dissolved in 10 mL of acetone and sprayed at controlled rate (approximately 1.0 g min⁻¹) into 20 mL of an aqueous phase containing drug and 2% PVA under continuous magnetic stirring (Remi 5MLH, REMI Corp., India) at 1500 ± 25 rpm. PLGA nanoparticles formed instantaneously as observed by the appearance of slight turbidity and formation of opalescent suspension. The organic solvent was finally removed by evaporation under vacuum at 40°C, until a final volume of 20 ml was reached. An aliquot of the nanoparticles suspension was centrifuged (REMI high speed, cooling centrifuge, REMI Corp., India) at 18,000 rpm for 30 min. at 4°C and the drug content in supernatant was measured by validated UV spectrophotometric method.

4. Nanoemulsions

AMH loaded nanoemulsions (NE) were prepared by titration followed by pseudoternary phase diagramme construction. Maximum solubility of AMH was measured and one oil phase was selected. In order to optimize surfactant and cosurfactant, phase diagrams for each surfactant and cosurfactant combinations were constructed by using aqueous titration method. After selection of surfactant and cosurfactant, their optimum concentration ranges were determined by detailed study of phase diagrams using different ratios of Smix (1:0, 1:1, 2:1, 3:1, 4:1, 1:2, 1:3, 1:4). For each Smix ratio, oil: Smix ratio was varied. Different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 1:0.7, 1:0.43, 1:0.25, 1:0.1) were made so that maximum ratios was covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Slow titration with aqueous phase was
performed to each weight ratio of oil and Smix and visual observation were carried out for transparency, flowability and physical state of NE.

![Phase diagram](image)

**Fig. 28:** Pseudoternary Phase diagramme for Nanoemulsion systems; illustrating interpretation from various regions of the phase diagramme for individual colloidal system.

### 3.2.3.4. Preliminary Characterization of the Prototype Nanoformulations

Various factors are reported in the reported text for nanoformulations; however we have listed down and studied the following critical parameters for preliminary characterization of the developed prototypes:

**Particle/Droplet size and polydispersity index:** It controls the entry to and residence time of the nanoparticles/nanoemulsion at the site of action and is a vital parameter controlling endocytosis and drug release kinetics. The nanoparticles/droplet size was determined by dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) at 25°C at an angle of 173°, taking an average of three measurements. The polydispersity index (PI) indicating the width of the size distribution was also determined.
Zetapotential: It influences the physical stability and possible mucoadhesion of the nanoparticles/nanoemulsion to the biological surfaces. It was measured by Zetasizer Nano ZS, Malvern Instruments, Malvern, UK.

**Entrapment efficiency & Drug Loading:** It defines the efficiency of the manufacturing process and should be maximized. It was calculated as given formula below:

\[
\text{Drug loading (\%)} = \left( \frac{\text{drug encapsulated in nanoparticles}}{\text{nanoparticle weight}} \right) \times 100
\]

\[
\text{Drug encapsulation (\%)} = \left( \frac{\text{drug encapsulated in nanoparticles}}{\text{total drug added}} \right) \times 100
\]

**Drug release:** It should be optimised for the specific purpose of onset/sustained and/or targeted drug delivery. A 10 mg (nanoparticles) & 100 \(\mu\)L (for nanoemulsion) quantity of drug-loaded nanoformulation (individually) were enclosed within dialysis bags containing 2.0 mL of PBS, pH 6.5 (containing 30% ethanol), and immersed in 20.0 mL of the same medium in a beaker under mild agitation. At stipulated time points, samples were withdrawn from the incubation medium and analyzed using analytical method detailed in next section of analytical method development. The measurements were done in triplicate, and the average and standard deviations were calculated. An identical volume of fresh PBS was added back into the beaker after each removal. The percent drug released was determined using a standard curve.

### 3.2.3.5. Results & Discussion: Part B

**Table 10:** Preliminary Characterization results of amiloride hydrochloride (AMH) loaded Prototype Nanoformulations:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulations</th>
<th>% EE</th>
<th>% Drug loading</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
<th>Zeta-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CS-ALG Nps</td>
<td>24.42</td>
<td>2.02</td>
<td>273.28 ± 11</td>
<td>0.27 ± 0.08</td>
<td>31.22 ± 2.7</td>
</tr>
<tr>
<td>2</td>
<td>PBCA Nps</td>
<td>13.58</td>
<td>2.41</td>
<td>320.12 ± 12</td>
<td>0.20 ± 0.03</td>
<td>-36.73 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>PLGA Nps</td>
<td>16.94</td>
<td>2.85</td>
<td>304.44 ± 19</td>
<td>0.29 ± 0.07</td>
<td>-35.78 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>Nanoemulsion</td>
<td>34.70</td>
<td>4.8</td>
<td>39.44 ± 1.9</td>
<td>0.12 ± 0.07</td>
<td>-25.78 ± 1.3</td>
</tr>
</tbody>
</table>

*Note: The measurements were done in triplicate, and the average and standard deviations were calculated (n=3)*
Chapter 3: Proof of Concept – Preliminary Evaluation Studies

The entrapment efficiency (% EE) of drug amiloride hydrochloride (AMH) is very poor in all the formulations as observed in above table 10; this could be ascribed to the relatively hydrophilic nature of the drug. To improve its % EE in various formulations, an attempt was made to make this drug less hydrophilic and so its free base form i.e. amiloride free base (AMB) was prepared in laboratory (detailed in chapter – 4) and used further to prepare again these formulations. The results are given below in table 11.

Table 11: Preliminary Characterization results of amiloride free base (AMB) loaded Prototypes Nanoformulations:

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Formulations</th>
<th>% EE</th>
<th>% Drug loading</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
<th>Zeta-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CS-ALG Nps</td>
<td>22.42</td>
<td>1.02</td>
<td>213.28 ± 10</td>
<td>0.29 ± 0.02</td>
<td>32.22 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>PBCA Nps</td>
<td>43.54</td>
<td>3.41</td>
<td>340.12 ± 11</td>
<td>0.24 ± 0.02</td>
<td>-35.73 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>PLGA Nps</td>
<td>45.64</td>
<td>3.85</td>
<td>314.44 ± 15</td>
<td>0.25 ± 0.05</td>
<td>-36.78 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>Nanoemulsion</td>
<td>94.89</td>
<td>10.78</td>
<td>56.41 ± 1.7</td>
<td>0.12 ± 0.07</td>
<td>-24.71 ± 1.3</td>
</tr>
</tbody>
</table>

3.2.3.6. Correlation between formulation characteristics versus associated factors with disease condition: Selection of one Formulation from the developed prototypes

Since epileptic disorder requires onset of action with maximum exposure of drug at site of action e.g. brain, we have compared various developed prototype formulation characteristics (particle size, drug loading) to the patient compliance (e.g. route of administration) and disease requirement (onset or sustained action) summarized below (Table 12). For selecting one formulation & its extensive development studies were the next steps of this project.
Table 12: Comparative studies for selection of amiloride formulation from the developed prototypes for establishing the requirement/fulfilment of disease condition and patient compliance with respect to maximum dose loaded formulation and process feasibility as drug attributes

<table>
<thead>
<tr>
<th>Formulation Assessed</th>
<th>Dose Loading</th>
<th>Drug per mL</th>
<th>Process Scalability</th>
<th>Preliminary Stability</th>
<th>Pharmacodynamic requirement (% release within 30 min)*</th>
<th>Nasal Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-ALG Nps</td>
<td>43%</td>
<td>1.0 mg/mL</td>
<td>Not Feasible</td>
<td>√</td>
<td>28.4%</td>
<td>Yes</td>
</tr>
<tr>
<td>PBCA Nps</td>
<td>23%</td>
<td>0.4 mg/mL</td>
<td>Not Feasible</td>
<td>√</td>
<td>18.6%</td>
<td>No</td>
</tr>
<tr>
<td>PLGA Nps</td>
<td>28.5%</td>
<td>0.7 mg/mL</td>
<td>Not Feasible</td>
<td>√</td>
<td>20.3%</td>
<td>No</td>
</tr>
<tr>
<td>Nanoemulsion</td>
<td>100%</td>
<td>5.0 mg/mL</td>
<td>Feasible</td>
<td>√</td>
<td>81.4%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*On the Basis of the in-vitro release studies in phosphate buffer pH 6.5 (drug release from the formulation over the time)
3.2.4. Conclusions: Part B

Prototype Selection:

On the basis of the various attributes mentioned in the Table 12 for amiloride formulations, Nanoemulsion was selected as proficient novel prototype formulation with respect to following advantages:

1. Maximum drug loading
2. Most feasible method of preparation
3. Anticipated stability profile of the formulation
4. Quick drug release (Prime Pharmacodynamic requirement of epileptic condition)
5. Maximum Drug delivery per minimum dose of the formulation with respect to mg/mL
6. Maximum Suitability/acceptability for Intranasal route of administration

Nanoemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multi component fluids composed of oil, water and surfactant (usually in conjunction with a cosurfactant). The droplets in a Nanoemulsion are in the range of 1 nm-100 nm in diameter (Schott, 2000; Kumar & Mittal, 1999; Hoar & Schulman, 1943). The basic difference between emulsions and nanoemulsions is that emulsions exhibit excellent kinetic stability but they are thermodynamically unstable as compared to nanoemulsions. In recent years Nanoemulsions have attracted a great deal of attention because of their biocompatibility, biodegradability, ease of preparation and handling and most importantly solubilization capacity for both water and oil soluble drugs (Moulik & Mukherjee, 1996; Paul & Moulik, 2001).

Next step was to extensively optimize and characterize the nanoemulsion formulation.