

DEVELOPMENT OF KETAMINE NANOEMULSION USING NITRILOTRIACETIC ACID AS AN ALTERNATIVE TO EDTA

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ABSTRACT

The present study was aimed at formulating and evaluating a ketamine-loaded nanoemulsion using suitable excipients and chelating agents such as nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA). Since ketamine has poor aqueous solubility, nanoemulsion was selected to improve its solubility and drug delivery.

A ketamine calibration curve was prepared by measuring the absorbance of different drug concentrations, and a linear relationship was observed. Soybean oil was used for the solubility study, and formulations were prepared using different concentrations of EDTA and NTA. The formulations were evaluated by centrifugation, heating–cooling cycles, and freeze–thaw cycles, and all formulations showed good physical stability.

FTIR studies confirmed the compatibility of the selected excipients. The prepared nanoemulsions showed oil-in-water (O/W) characteristics with a particle size of 191.8–206.1 nm and a PDI of 0.15–0.37. The zeta potential ranged from –29.9 to –57.5 mV, indicating good stability.

Overall, the study showed that ketamine can be successfully incorporated into a stable nanoemulsion system with satisfactory physicochemical properties. The developed formulation may improve ketamine solubility and drug delivery.

Keywords:

Ketamine; Nanoemulsion; Nitrilotriacetic acid (NTA); Ethylenediaminetetraacetic acid (EDTA); Solubility; Soybean oil; Drug delivery; Particle size; Zeta potential; Physical stability; FTIR.

1.Introduction

Ketamine is a class of drugs that is mostly utilized in human and veterinary applications as an anesthetic. It induces a dissociative state, a trance-like state that relieves pain, sedates, and temporarily erases memories.

Ketamine is sometimes referred to as a "unique drug" due to its hypnotic, analgesic, and amnesic properties; no other medication used in clinical practice combines these three crucial characteristics.

It was believed to be the ideal anesthetic agent due to these combined properties when it was first used in clinical settings in 1970.

Compared to propofol and etomidate, ketamine has the benefit of not requiring a lipid emulsion vehicle.

It is unknown how ketamine-induced analgesia and dissociative anesthesia work.

It engages with several CNS receptors.

Ketamine binds to N-methyl-D-aspartate (NMDA) receptors' phencyclidine recognition region in a noncompetitive manner.

Ketamine also affects muscarinic, nicotinic acetylcholine, and opioid receptors, among other sites.

Neurons' NMDA receptors are often activated by glutamate.

Ketamine binds into the receptor channel to inhibit NMDA receptors.

As a result, there is less excitatory neurotransmission via NMDA receptors.

The blockage indirectly boosts signaling via AMPA receptors.

Increased AMPA signaling facilitates synaptic plasticity and BDNF release, all of which are critical for the rapid antidepressant action of ketamine.

Higher doses cause analgesia, sedation, anesthesia, and dissociation due to the changed signaling.

High lipid solubility, quick onset of activity, and comparatively short duration of action.

Ketamine reaches peak plasma concentrations within one minute of IV treatment and within minutes of intramuscular injection. Ketamine is first delivered to highly perfused organs, like the brain, and its high hepatic clearance rate (1 L/min) is ensured by its great lipid solubility.

Peak plasma levels are reached almost immediately, with an immediate onset. Intramuscular (IM): Peak levels in 5 to 30 minutes, with a high bioavailability of about 93%. Intranasal: The bioavailability ranges from 45% to 50%. Oral: The liver's high first-pass metabolism results in a low bioavailability of 16% to 29%. It can swiftly pass across the blood-brain barrier since it is very fat-soluble. Distribution Volume: 3 to 5 L/kg is the steady-state volume. Protein Binding: The range of plasma protein binding is 10% to 50%. Redistribution: With a half-life of 10 to 15 minutes, the medication quickly shifts from the brain to peripheral tissues, causing awakening from anesthesia.

Mostly metabolized by cytochrome P450 enzymes (CYP3A4 and CYP2B6) in the liver. Active Metabolites: First broken down into hydroxynorketamine (HNK) and dehydronorketamine (DHNK), norketamine maintains mild-to-moderate activity (about one-third the potency of ketamine).

The parent medication has a terminal elimination half-life of two to three hours. Renal Route: Over the course of many days, approximately 91% of the medication and its metabolites are eliminated through the urine, with a little amount being eliminated through feces.

Many factors, including as the patient's age, body weight, liver function, and concurrent medications, can affect the pharmacokinetics of ketamine. People with liver disease and the elderly may metabolize and remove ketamine more slowly, which could increase the risk of adverse effects. Obese individuals may require higher dosages of ketamine due to its large volume of distribution and high lipid solubility. Additionally, patients with low serum albumin levels may have higher free drug concentrations and perhaps harsher drug effects due to ketamine's high protein binding. Concurrent medications that affect liver enzymes or protein binding, such as cimetidine, erythromycin, and phenytoin, can also affect the pharmacokinetics of ketamine. Depending on these factors, ketamine dosage may occasionally need to be adjusted to achieve optimal outcomes and lower the risk of adverse effects.

Patients receiving ketamine need to be closely monitored in order to establish safe and effective sedation or anesthesia.

Ketamine is a commonly used intravenous anesthetic medication in clinical practice because of its rapid onset and offset of effect. Due to its high lipophilicity and water insolubility, ketamine presents a challenge for intravenous injection. Thus, the use of nanoemulsions as a ketamine drug delivery mechanism has become more popular in recent years.

Ketamine, a surfactant, and a co-surfactant are mixed with an aqueous phase at a high shear rate to produce a stable nanoemulsion. While the surfactant and co-surfactant are used to reduce the interfacial tension between the oil and water phases, high shear conditions are used to reduce the droplet size of the oil phase. The resultant nanoemulsion is stable and can be stored for long periods of time without phase separation.

NTA is an organic material that is widely used in many different applications as a chelating agent. In recent years, there has been an increasing interest in using NTA instead of EDTA when making

ketamine nanoemulsion. The benefits of NTA over EDTA and the rationale behind its use as a chelating agent in ketamine nanoemulsion will be discussed in this section.

Although both EDTA and NTA are polyamino carboxylic acids, NTA has one fewer carboxylic acid group than EDTA. NTA is a strong chelating agent and has a strong affinity for metal ions such as iron, copper, and zinc. NTA's molecular structure consists of three amine groups and three carboxylic acid groups, which enable it to bind metal ions.

In ketamine nanoemulsions, ethylenediaminetetraacetic acid (EDTA) is often replaced by the chelating chemical nitrilotriacetic acid (NTA). In pharmaceutical formulations, chelating agents—compounds that bind to metal ions—are used to prevent the formation of metal-ion complexes that could reduce the efficacy of the medication or cause it to degrade. EDTA has long been a commonly used chelating agent in medicines, especially in ketamine preparations. However, it has been connected to possible toxicity problems, which has increased interest in other chelating agents such as NTA. NTA can bind metal ions more well than EDTA since it is a smaller molecule. According to studies, it is less toxic than EDTA and less likely to result in acute toxicity and allergic reactions. Because NTA is biodegradable and does not linger in the environment, it is a more environmentally friendly option. Metal ions that may be present in ketamine nanoemulsions are chelated using NTA. These metal ions could come from a variety of sources, such as the water used in formulation or the equipment utilized in production. By chelating these metal ions, NTA helps maintain the stability of the ketamine nanoemulsion and stops drug breakdown. NTA in ketamine nanoemulsions provides a more efficient and potentially safer alternative to EDTA. It is an effective chelating agent that reduces the likelihood of toxicity issues caused by other chelating agents and helps to preserve formulation stability.

2. Materials and Methods

2.1 Ingredient List

Ketamine, soybean oil, glycerol, egg lecithin, ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), sodium hydroxide, and water for injection are the components needed to make the ketamine-loaded nanoemulsion. The active pharmacological component was 10 mg/mL of ketamine. Egg lecithin functioned as an emulsifier and soybean oil as the oil phase. An osmolality-regulating agent was glycerol. Sodium hydroxide was employed to change the pH, and EDTA and NTA were added as chelating agents. The vehicle used to create the final volume was water for injection. Suppliers such as Bachem, Croda, Merck KGaA, Lipoid, and Shiv Chem Industry provided the ingredients.

2.2 List of Stoppers, Seals, and Vials

Rubber stoppers, flip-off seals, and transparent molded USP Type I vials were among the packaging materials utilized for the formulation. Adit Pharma provided the flip-off seals, Datwyler provided the rubber stoppers, and SGD provided the vials. These elements are meant to give the formulation appropriate packaging and closing. It is necessary to confirm their appropriateness and compatibility for pharmaceutical usage.

2.3 List of Used Instruments

The ketamine-loaded nanoemulsion was prepared and assessed using a variety of tools. These featured a water bath with a magnetic stirrer for temperature-controlled processing, a magnetic stirrer with a hot plate and an overhead stirrer for mixing, and a top-loading and semi-micro balance for weighing components. A Julabo chiller assisted with temperature control, and a peristaltic pump was utilized for

liquid transport. The formulation was homogenized using an inline homogenizer and a high-pressure homogenizer, and its pH was measured using a pH meter. The temperature was tracked using an infrared (IR) pistol. For accurate identification and laboratory documentation, each instrument was recognized by its unique instrument ID.

3. PREPARATION PROCESS OF KETAMINE NANOEMULSION:

3.1 1N SODIUM HYDROXIDE SOLUTION PREPARATION:

Mix thoroughly after dissolving 40 grams of sodium hydroxide in 800 milliliters of injection-grade water (20–30 degrees Celsius). To change the pH, use this solution. Throw away any extra solution that wasn't used. The quantity of 1N NaOH solution needed for each batch:

1.25 ml × batch size, or 50 milligrams of NaOH per liter

3.2 AQUEOUS PHASE PREPARATION:

Gather 0.2 micron (PES OR PVDF) filtered water for injection, 110 percent of the batch size in a vessel with an SS316/SS316L jacket, and purge nitrogen gas until the dissolved oxygen is less than 2 ppm. Transfer water for injection in another 316/316L vessel for usage at subsequent stages of process and reagent production. This water is roughly 25% of the batch size (50l equivalent to 49.85kg based on the density of 0.997g/ml). Lower or raise the temperature of the leftover water for injection to 55–65°C. Stir for five minutes, or until a clear solution is seen at 250 rpm, after adding the prescribed amount of glycerol. Stir for ten minutes, or until a clear solution is seen at 250 rpm, after adding the amount of EDTA that was delivered. Keep the solution's temperature between 55 and 65 degrees Celsius. Set the rpm to 1100, add the amount of egg yolk lecithin that was dispensed, and homogenize the mixture for 30 minutes, or until all of the lecithin has been added. Stir for five minutes after adding 50% of the batch quantity of 1N NaOH, or 125 milliliters of solution. Throughout the procedure, keep the aqueous phase solution's temperature between 50 and 65°C.

3.3 NONAQUEOUS PHASE/OIL PHASE PREPARATION:

Pour the amount of soybean oil that has been dispensed into a suitable, jacketed SS316/316L vessel. After adding the amount of ketamine that was dispensed, heat the mixture to approximately 60°C (between 55 and 65°C) while stirring at a speed of 100 to 300 rpm. Throughout the procedure, keep the temperature constant.

3.4 MIXTURE OR PRE-EMULSIFICATION (high shear mixture or inline homogenizer mixing)

Keep the aqueous and non-aqueous phases' temperatures between 55 and 65°C, with an oil phase to aqueous phase ratio of roughly 1:7:14. Fill a beaker with about 10 liters of water to be injected, then set the peristaltic pump to deliver the water at a rate of around 2.5 liters per minute (the flow rate will be measured with a cylinder). To keep the aqueous phase flow rate at 2.5 liters per minute while mixing, remove the old tubing and replace it with new tubing. Fill a beaker with about 1 kg of extra soybean oil that has been dispensed, and set the peristaltic pump to present the oil phase at a flow rate of about 350 ml/min. To keep the nonaqueous phase flow rate at about 350 milliliters per minute while mixing, remove the old tubing and replace it with a new one. Charge the aqueous phase at a rate of 2.5 liters per minute and the oil phase at a rate of about 350 milliliters per minute (i.e., the oil phase to aqueous phase ratio is approximately 1:7:14) through an in-line homogenizer simultaneously at 8000 rpm. Gather the mixture in a vessel with an SS316/316L jacket and keep the temperature between 55 and 65 degrees Celsius. Repeat one or two passes at 8000 rpm with the aforementioned mixture or coarse

emulsion in the inline homogenizer. Maintain the mixture's temperature between 55 and 65°C. Stir for five minutes at 250 rpm while adding the remaining 50% of NaOH and 125 milliliters of 1N NaOH solution. For specific gravity, weight/ml, and pH, send the sample to QC. Fill the "Makeup vessel" with water for injection to reach 100% batch size, then stir at 250 rpm for less than ten minutes.

3.5 HOMOGENIZATION

Move the mixture from the previous stage to the homogenizer's input feed, subject it to 14000 psi, and collect it in an appropriate jacketed SS 316/316L vessel. Throughout the HPH procedure, keep the temperature between 55 and 65°C. To get the necessary particle size, follow the homogenization procedure for the first and second passes at roughly 14000 PSI. Check the sample's particle size after the second pass. If necessary, homogenize it until the desired particle size (d99NMT 450nm) is reached.

In process check the final pH and clarity of the solution (Limit: 8.0 to 10.0)

TEST PARAMETERS	SPECIFICATION
CLARITY	White oil-water emulsion
Ph	8.0-10.0

Use a sterile 1.2/1.0 micron PP prefilter to filter the bulk solution mentioned above. The prefilter is followed by 0.8+0.45 micron PES employing 1 to 2 kg/sq.cm pressure of 0.2 micron filtered nitrogen, which is collected in an SS316/316L holding vessel while the bulk temperature is kept between 55 and 65°C. Until the bulk solution is removed for additional processing, keep it under nitrogen pressure. After determining the necessary fill volume, stoppering and sealing should be done. Filling vials are terminal sterilized at 121°C for 15 minutes using 2RPM.

4. PREPARATION OF KETAMINE NANOEMULSION WITH NITRILOTRIACETIC ACID:

In this there are three main formulations with NTA which is (0.02mg/ml, 0.05mg/ml and 0.09mg/ml).

4.1 1N SODIUM HYDROXIDE SOLUTION PREPARATION:

Mix thoroughly after dissolving 40 grams of sodium hydroxide in 800 milliliters of injection-grade water (20–30 degrees Celsius). To change the pH, use this solution. Throw away any extra solution that wasn't used. The quantity of 1N NaOH solution needed for each batch: 50 milligrams of NaOH per liter (1.25 ml × batch size)

4.2 AQUEOUS PHASE PREPARATION:

Gather 0.2 micron (PES OR PVDF) filtered water for injection, 110 percent of the batch size in a vessel with an SS316/SS316L jacket, and purge nitrogen gas until the dissolved oxygen is less than 2 ppm. Transfer water for injection, which is around 25% of the batch size (50L, or 49.85 kg based on the density of 0.997g/ml), to another 316/316L vessel for use in subsequent steps of the process and reagent production. Lower or raise the temperature of the leftover water for injection to 55–65°C. Stir for five minutes, or until a clear solution is seen at 250 rpm, after adding the prescribed amount of glycerol. For each batch, add the prescribed amount of nitrilotriacetic acid (0.02 mg/ml, 0.05 mg/ml, and 0.09 mg/ml). Stir for ten minutes, or until a clear solution is visible at 250 rpm. Keep the solution's temperature between 55 and 65°C. Set the speed to 1100, add the amount of egg yolk lecithin that was dispensed, and homogenize the mixture for 30 minutes, or until all of the lecithin has been incorporated.

Stir for five minutes after adding 50% of the batch quantity of 1N NaOH, or 125 milliliters of solution. Throughout the procedure, keep the aqueous phase solution's temperature between 50 and 65°C.

4.3 NONAQUEOUS PHASE/OIL PHASE PREPARATION:

Pour the amount of soybean oil that has been dispensed into a suitable, jacketed SS316/316L vessel. Add the amount of ketamine that was dispensed, then heat the mixture to around 60°C (between 55 and 65°C) while stirring at a speed of 100 to 300 rpm. Throughout the procedure, keep the temperature constant.

4.4 MIXTURE OR PRE-EMULSIFICATION (high shear mixture or inline homogenizer mixing)

Keep the temperature of the aqueous and non-aqueous phases between 55 and 65°C. The oil phase to aqueous phase ratio should be approximately 1:7:14. Fill a beaker with about 10 liters of water to be injected, then set the peristaltic pump to deliver the water at a rate of around 2.5 liters per minute (the flow rate will be measured with a cylinder). To keep the aqueous phase flow rate at 2.5 liters per minute while mixing, remove the old tubing and replace it with new tubing. Fill a beaker with about 1 kg of extra soybean oil that has been dispensed, and set the peristaltic pump to present the oil phase at a flow rate of about 350 ml/min. To keep the nonaqueous phase flow rate at about 350 milliliters per minute while mixing, remove the old tubing and replace it with a new one. Charge the oil phase at a rate of about 350 milliliters per minute and the aqueous phase at a rate of 2.5 milliliters per minute (i.e., the ratio of oil phase to aqueous phase is about 1:7:14). Using an in-line homogenizer at 8000 rpm concurrently, collect the mixture in a jar with an SS316/316L jacket and keep the temperature between 55 and 65°C. Repeat one or two passes at 8000 rpm with the aforementioned mixture or coarse emulsion in the inline homogenizer. Maintain the mixture's temperature between 55 and 65°C. Stir for five minutes at 250 rpm while adding the remaining 50% of NaOH and 125 milliliters of 1N NaOH solution. For pH and specific gravity or weight/ml, send the sample to quality control.

Fill the "Makeup vessel" with water for injection to reach 100% batch size, then stir at 250 rpm for less than ten minutes.

4.5 HOMOGENIZATION

Move the mixture from the previous step to a homogenizer's input feed, subject it to 14000 psi, and collect it in an appropriate jacketed SS 316/316L vessel. Throughout the HPH procedure, keep the temperature between 55 and 65 degrees Celsius. To get the necessary particle size, follow the homogenization procedure for the first and second passes at roughly 14000 PSI.

After the second pass, measure the sample's particle size (if necessary, homogenize it until the desired particle size is reached).

Table 6- In process check the final pH and clarity of the solution (Limit: 8.0 to 10.0)

TEST PARAMETERS	SPECIFICATION
CLARITY	White oil-water emulsion
pH	8.0-10.0

The aforementioned bulk solution should be filtered through a sterilized 1.2/1.0 micron PP prefilter, then 0.8+0.45 micron PES utilizing 1 to 2 kg/sq.cm pressure of 0.2 micron filtered nitrogen. The collected material should be placed in an SS316/316L holding tank to keep the bulk temperature between 55 and 65 degrees Celsius. Until the bulk solution is removed for additional processing, keep it under nitrogen pressure.

After determining the necessary fill volume, stoppering and sealing should be done.

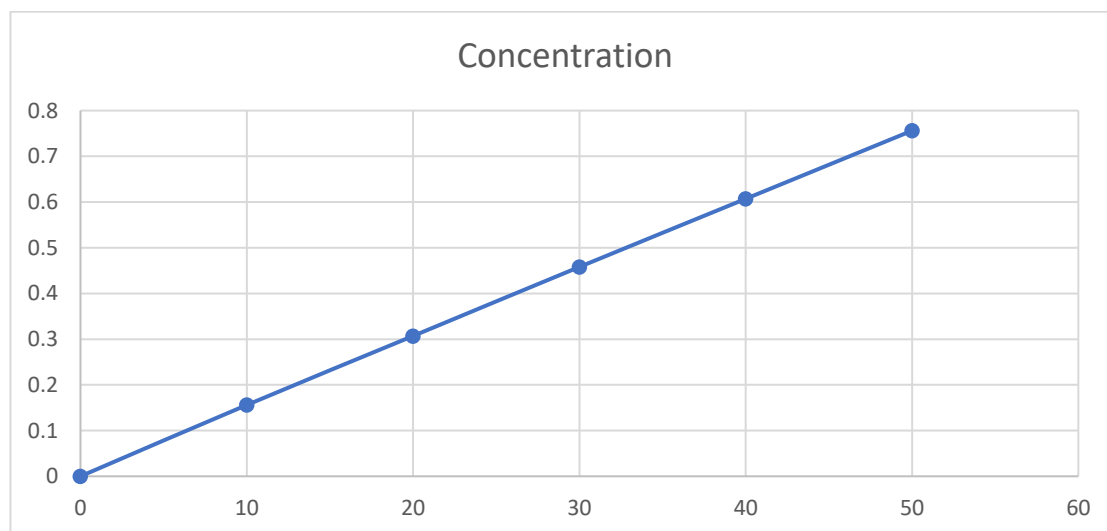
5.RESULT AND DISCUSSION:

5.1 CALIBRATION CURVE STANDARD:

To determine the linearity between drug concentrations in the solution, the drug's calibration curve was created. It was determined that when the concentration range was between 10µg/ml and 60µg/ml, a linear relationship between the concentration and absorbance was seen.

List of concentration and absorbance value

CONCENTRATION	ABSORBANCE
0	0.00
10	0.156
20	0.307
30	0.458
40	0.607
50	0.756



5.2 RESEARCH ON SOLUBILITY:

Using the chelating agents EDTA and nitrilotriacetic acid, the saturation solubility of ketamine was measured in soybean oil. According to the findings, ketamine is a lipophilic medication that has a low water solubility. Therefore, at 55 degrees, the drug ketamine is soluble in soybean oil 65°C.

5.3 TEST OF THERMODYNAMICS STABILITY:

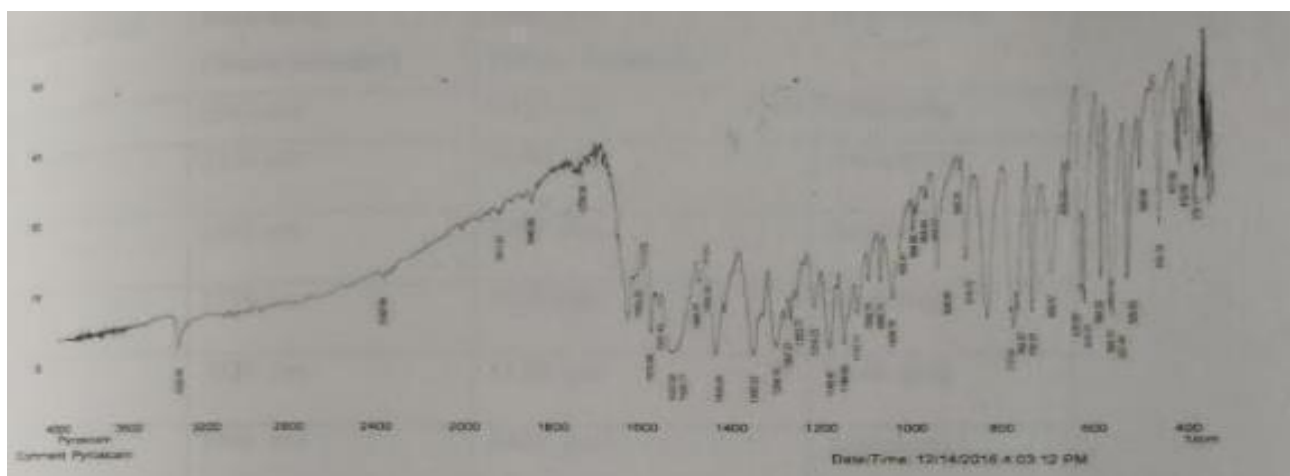
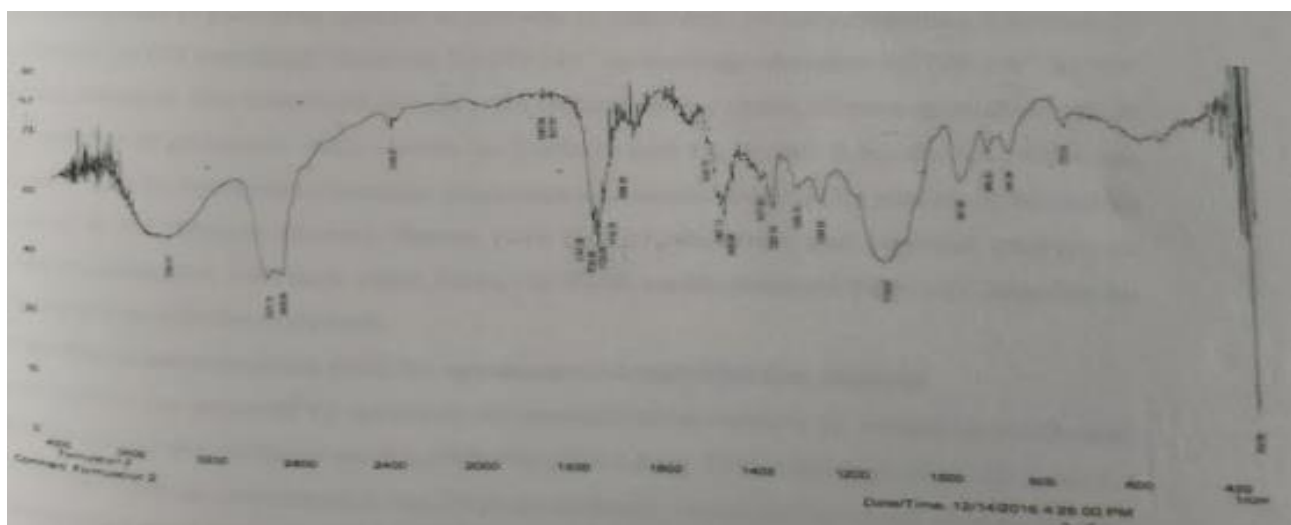
Different thermodynamic stability studies were conducted on the chosen formulation from various concentrations of nitrilotriacetic acid on the aqueous titration, and the formulation that passed the thermodynamic stability study was taken for a dispersibility test. Studies of thermodynamic stability distinguish NE formulations from kinetically stable ones that go through phase separation. This suggests that the formulation has the proper concentration needed for NE formulation.

Results of different thermodynamic stability studies.

FORMULATION	CENTRIFUGATION	HEATING AND COOLING CYCLE	FREEZE THRAW CYCLE	RESULTS
0.05 EDTA	Pass	Pass	Pass	Stable
0.02 NTA	Pass	Pass	Pass	Stable
0.05 NTA	Pass	Pass	Pass	Stable
0.09 NTA	Pass	Pass	Pass	Stable



Picture of the formulations.

COMPATIBILITY STUDIES BETWEEN 0.05 EDTA AND 0.05 NTA.**FTIR result for 0.05mg/ml EDTA****FTIR result for 0.05mg/ml N****FTIR results for EDTA and NTA**

FUNCTIONAL GROUP	0.05mg/ml EDTA	0.05mg/ml NTA	INTERFERENCE
C-H	2362cm	2925cm	Stretching
O-H	3336cm	3390cm	stretching
N-H	1693cm	1698cm	bending
C=O	1729cm	1729cm	stretching
C=C	1466cm	1461cm	stretching

5.4 DRUG CONTENT PERCENTAGE IN NE:

It is assumed that the entire medication would be contained in the nano emulsion because the amount of drug integrated (10%) is significantly less than the saturation solubility.

Stability data of optimized formulation at room temperature.

S.NO	COURSE OF STUDY	PHYSICAL APPEARANCE	PARTICLE SIZE	PDI	ZETA POTENTIAL
1	0.05mg/ml EDTA	White, O/ W Emulsion	191.8	0.15	-29.9
2	0.02mg/ml NTA	White, O/ W Emulsion	204.3	0.24	-52.4
3	0.05mg/ml NTA	White, O/ W Emulsion	206.1	0.28	-57.5
4	0.09mg/ml NTA	White, O/ W Emulsion	199.8	0.37	-55.3

6. SUMMARY AND CONCLUSION:

The creation of ketamine nano emulsion involves several steps that require precise measurements, temperature control, and homogenization. After being manufactured separately, the aqueous phase and non-aqueous phase are combined in a specific ratio using an inline homogenizer. It is necessary to add 1N sodium hydroxide solution and homogenize under high pressure in order to get the proper particle size and pH level for the final product. After going through sterile pre-filters, the final solution is gathered in an appropriate container. This process produces a transparent, stable ketamine nano emulsion with a pH range of 8.0–10.0 and a particle size of d99NMT 450 nm. To ensure the production of a safe and efficient product, strict adherence to the protocol and quality control measures is necessary throughout the preparation process. It is possible to conclude that 0.05 mg/ml nitrilotriacetic acid is a more effective chelating agent than 0.05 mg/ml EDTA based on the data provided and assessed. Protein purification yield was greater when nitrilotriacetic acid was used instead of EDTA. This may be due to nitrilotriacetic acid's greater affinity for metal ions than EDTA, which enables it to attach to the target protein and elute it more successfully. Nitrilotriacetic acid is an excellent and economical chelating agent. It is a less costly substitute for EDTA, which occasionally yields disappointing outcomes.

In protein purification, 0.05 mg/ml nitrilotriacetic acid is a better chelating agent than 0.05 mg/ml EDTA. It is more economical and has a higher protein purification yield. Nitrilotriacetic acid is therefore recommended for use in upcoming protein purification studies.

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