RESEARCH ARTICLE

Characterization and *In-vitro* Study of Metformin and Repaglinide Nano Emulsion in the Treatment of Diabetes

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ABSTRACT

Repaglinide (RPG) and Metformin (MTF) are fast-acting glucose regulators that stimulate insulin release from pancreatic β-cells. Frequent dosing before meals is challenging. This study explores nanoemulsion as a promising carrier for RPG and MTF, aiming for sustained hypoglycemic effects. The drugs were incorporated into the oil phase, enhancing biopharmaceutical properties compared to traditional lipid-based systems. Formulations were chosen with a 5% w/w difference in oil content from the o/w nanoemulsion region. The optimized nanoemulsion to control nanodroplet size, ensure low viscosity, and incorporate distilled water. In vitro dissolution studies revealed that the nanoemulsion had higher drug release, finer droplet size, lower polydispersity, minimal viscosity, and excellent dilution capability compared to existing oral tablets. The optimized Repaglinide (RPG) and Metformin (MTF) nanoemulsion formulation demonstrated a superior hypoglycemic effect over the conventional tablet formulation.

Keywords: Diabetes mellitus, nano emulsion, repaglinide and metformin

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INTRODUCTION

Hyperglycemia, or elevated blood glucose, is a hallmark of diabetes mellitus, a long-term metabolic disease that is frequently accompanied by insulin resistance.[1,2] Uncontrolled hepatic glucose production and decreased skeletal muscle glucose absorption along with decreased glycogen synthesis led to hyperglycemia. Dehydration, thrust, and increased drinking (polydipsia) are the outcomes of glucose spilling into the urine (glycosuria) and causing an osmotic diuresis (polyurea). This occurs when the renal threshold for glucose re-absorption is exceeded.[3,4] Protein synthesis is decreased and breakdown is increased in cases of insulin insufficiency, leading to wasting. Diabetes mellitus is classified into two main categories.[5,6]

METHODOLOGY

Formulation development

Repaglinide

Screening of components

Every ingredient utilized to create the nano emulsion needs to be approved by pharmaceuticals for oral consumption. The following elements were chosen for the nano emulsion drug delivery system's development.

Phase solubility studies

To achieve equilibrium, 3 mL of specific polymers were placed in 5 mL vials. An excess amount of the drug was added to the oils, and the mixtures were agitated in a biological shaker for 72 hours at a constant temperature of 25±1.0°C. After removal from the shaker, the samples were centrifuged at 3000 rpm for 15 minutes. The supernatant was diluted and filtered using a 0.45 µm membrane filter. The concentration of Repaglinide (RPG) was then measured by high-performance thin-layer chromatography (HPTLC) at a maximum absorbance (λ_{max}) of 240 nm.

Pseudoternary phase diagram construction

Table 1 shows the surfactant and co-surfactant. A 100 mL stock was created for each group, and different volume ratios of the co-surfactant (Transcutol) and surfactant (Tween 80) mixture (S_{mix}) were further prepared. To ensure comprehensive coverage of the maximum ratios for the investigation, sixteen unique combinations of oil and S_{mix} were prepared in various volume ratios, ranging from 1:9 to 9:1, for each phase diagram. Table 2 show the nano emulsion region. The mixture of S_{mix} and the selected oils was titrated using distilled water. Distinct formulations were selected from each phase diagram shown for different S_{mix} ratios based on the following criteria. 1. The oil's content was such that a single dose of 2 mg of repaglinide promptly dissolved. 2. The oil concentration in each phase diagram was selected as a multiple of 5%, namely 5%, 10%, 15%, and 20%. The minimum surfactant concentration needed for the production of nano emulsions was determined for each selected oil percentage.

Thermodynamic stability

Table 1: Preparation of S_{mix} ratios

S. No.	Volume of Surfactant (mL)	Volume of Cosurfactant (mL)	Ratio of S_{mix}
1	50	50	1:1
2	33.3	66.7	1:2
3	25	75	1:3
4	66.7	33.3	2:1
5	75	25	3:1

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Table 2: Nano emulsion region.

Ratio Oil: Smix	Oil µl	Surfactant (Smix) µl	Water µl	Water added µl	Total µl	Oil %	Surfactant (S_{mix}) %	Water %
1:9	10	90	10	10	111	10.1	83.58	10.2
10	90	20	10	120	8.25	76.32	17.54	16.67
10	90	25	5	125	8.00	72.00	20.00	20.00
10	90	35	10	135	7.41	66.67	25.93	25.93
10	90	45	10	145	6.90	62.07	31.03	31.03
10	90	55	10	155	6.45	58.06	35.48	35.48
10	90	65	10	165	6.06	54.55	39.39	39.39
10	90	80	15	180	5.56	50.00	44.44	44.44
10	90	100	20	200	5.00	45.00	50.00	50.00
10	90	120	20	220	4.55	40.91	54.55	54.55
10	90	150	30	250	4.00	36.00	60.00	60.00
10	90	185	35	285	3.51	31.58	64.91	64.91
10	90	235	50	335	2.99	26.87	70.15	70.15
10	90	300	65	400	3	23	75	75
10	90	400	100	500	2.00	18.00	80.00	80.00
10	90	550	150	650	2	13	85	85
10	90	900	350	1000	1.00	9.00	90.00	90.00
10	90	2000	1100	2100	0.48	4.29	95.24	95.24

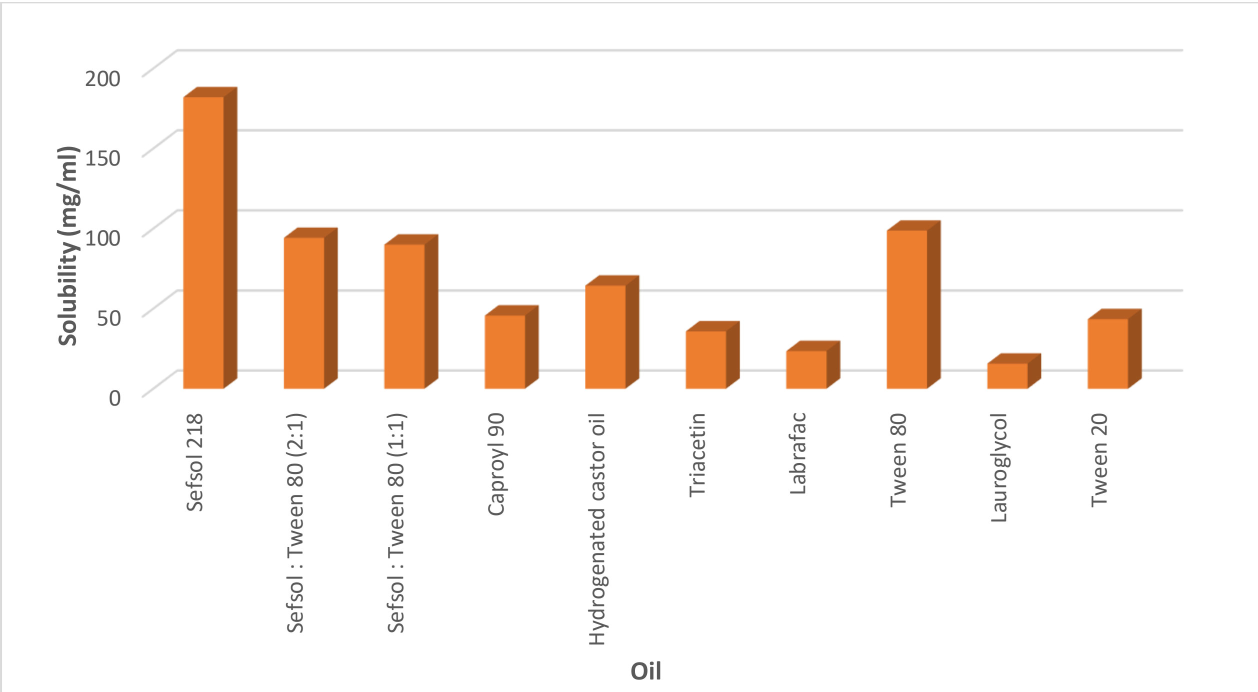


Figure 1: Bar diagram showing the highest solubility of repaglinide in different solvents.

Thermodynamic stability stress tests were applied to a subset of formulations: Centrifugation is followed by heating and cooling cycles between 45^{0}C and room temperature (25 ± 2^{0}C) with storage times of 24 h at each temperature. Six cycles of freeze-thaw were performed, with the samples being stored below -200C in a deep freezer and then thawed at ambient temperature (25 ± 2^{0}C) for twenty-four hours.

Dispersibility tests

With a typical USP XXII dissolving device, the effectiveness of the oral nano emulsion's self-emulsification process was evaluated. 500 mL of medium were combined with 1 mL of the nano emulsion and kept at 37 ± 0.50C. For gentle mixing, the dissolving paddle moved at a speed of 50 revolutions per minute.

Formulation of drug containing nano emulsion (repaglinide and metformin)

In a vortex mixer, 2 mg/kg body weight of medication was dissolved in oil and the corresponding Smix ratios to create drug-containing nano emulsion formulations. Nano emulsion was the resultant combination.

Characterization of repaglinide and metformin nano emulsion

Nano emulsions can be characterized by using different types of techniques.

Visual observation

Nano emulsion and macroemulsion were distinguished by visual inspection.

Surface morphology

The study of the nano emulsion surface morphology was conducted using TEM). A drop of nano emulsion was placed to a carbon-coated grid containing 2% phosphotungestic acid, diluted with distilled water, filtered, and left for 30 seconds. Placed on a slide, the dried-coated grid was covered with a cover slip. A light microscope running at 200 KV was used to examine the slide.

Droplet size analysis (particle size distribution)

The formulation's droplet size homogeneity increases with decreasing polydispersity value. The measurement of droplet size is a crucial metric for both optimizing the

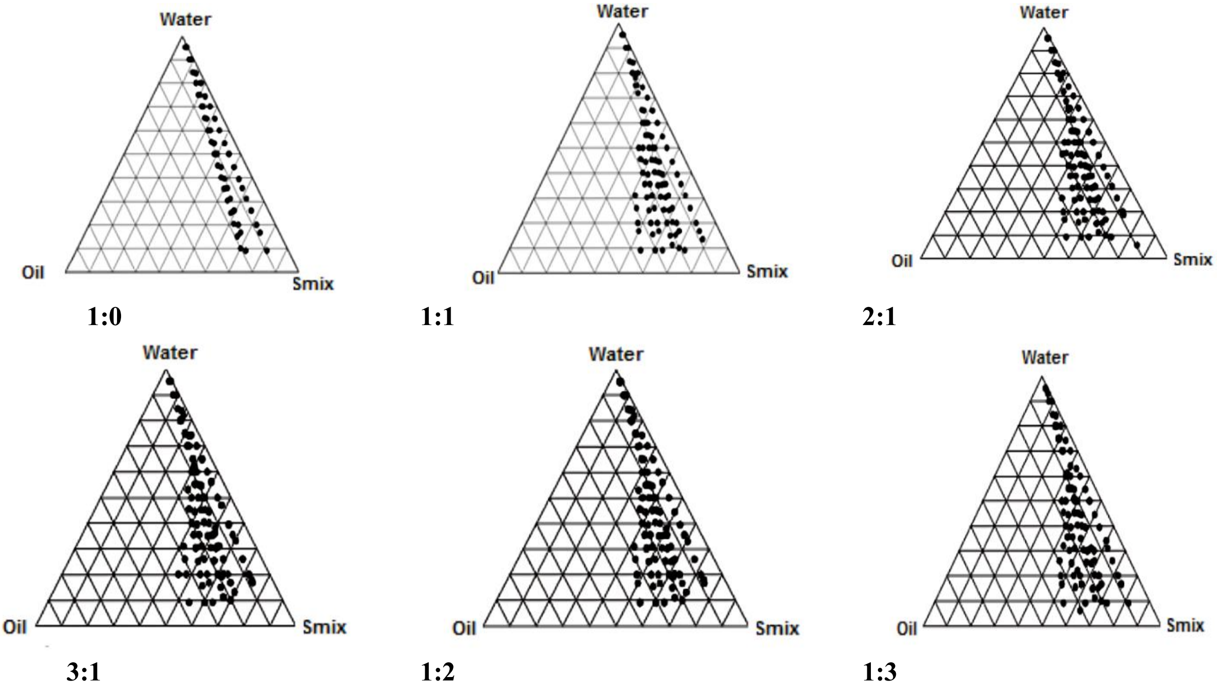


Figure 2: Phase diagram.

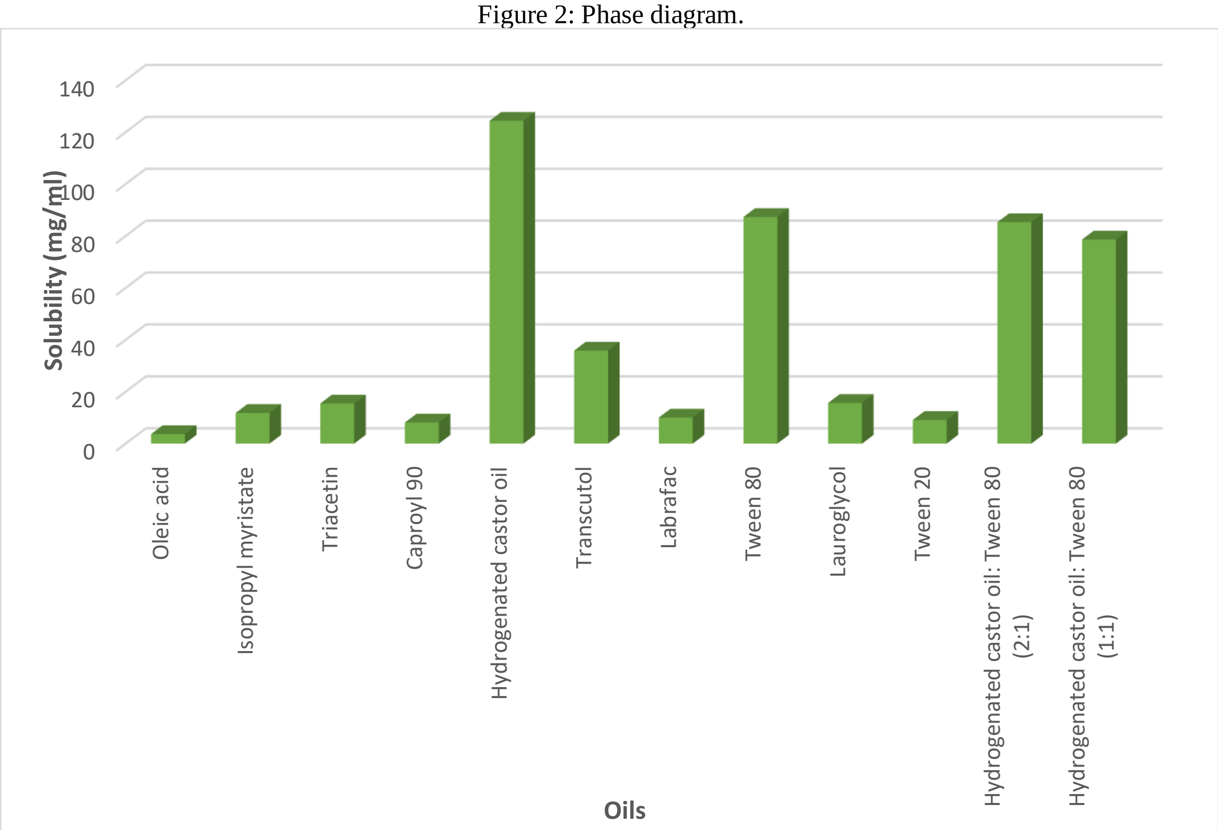


Figure 3: Bar diagram showing the highest solubility of metformin.

Table 3: Phase solubility determination.

S. No.	Oil	Average ± S.D. (mg mL^{-1})
1	Sefsol 218	182.06 + 0.04
2	Sefsol : Tween 80 (2:1)	94.23 + 0.08
3	Sefsol : Tween 80 (1:1)	89.98 + 1.06
4	Caproyl 90	45.7 + 0.75
5	Hydrogenated castor oil	64.37 + 9.35
6	Triacetin	35.85 + 2.20
7	Labrafac	23.54 + 1.22
8	Tween 80	98.73 + 8.39
9	Lauroglycol	15.68 + 3.59
10	Tween 20	43.48 + 7.12

formulation of the nano emulsion and differentiating it from the microemulsion.

Viscosity determination

Viscosity determination is a key step in characterizing liquid formulations, such as nano emulsions, to understand their flow properties. Here's how viscosity is typically determined:

Sample Preparation

Prepare the nano emulsion or other liquid formulation, ensuring it is homogenous and at the desired concentration.

Temperature Control

Maintain the sample at a consistent temperature, typically 25 ± 1.0°C, as viscosity is temperature-dependent.

Viscometer Selection

Choose an appropriate viscometer, such as a rotational viscometer (e.g., Brookfield viscometer) or an oscillatory rheometer, depending on the sample's viscosity range.

Measurement

Place the sample in the viscometer's measurement chamber. For a rotational viscometer, the spindle is immersed in the sample and rotated at set speeds. The resistance to rotation, which correlates with viscosity, is measured. In an oscillatory rheometer, the sample is subjected to oscillatory stress or strain, and the resulting flow is measured.

Data Recording

Record the viscosity readings at different shear rates or rotational speeds to determine the flow behavior of the sample.

Analysis

Analyze the data to obtain the viscosity value, which can be used to assess the formulation's stability, ease of administration, and suitability for its intended application.

Refractive index

The refractive index is a measure of how much light bends, or refracts, as it passes through a substance. Determining the refractive index of a nanoemulsion or other liquid formulation can provide insights into the formulation's

Table 4: Observations for thermodynamic stability study of repaglinide nano emulsion formulations.

S_{mix} ratio	FC	% S_{mix}	% Oil	% Water	thermodynamic stability test		
	NR1	30	15	80	x	–	–
	NR2	35	20	70	x	–	–
	NR3	40	15	75	x	–	–
	NR4	30	30	60	x	–	–
1:0	NR5	35	20	70	✓	✓	✓
	NR6	40	15	70	✓	✓	✓
	NR7	40	20	60	✓	✓	x
	NR8	45	25	65	✓	✓	x
	NR9	35	15	70	✓	✓	x
	NR10	35	20	60	✓	x	10
	NR11	40	15	65	✓	x	–
1:1	NR12	40	15	60	✓	✓	✓
	NR13	45	15	70	✓	✓	✓
	NR14	30	15	75	✓	x	–
	NR15	35	20	60	✓	x	–
	NR16	40	15	65	✓	✓	✓
2:1	NR17	40	15	60	✓	✓	✓
	NR18	45	15	60	✓	✓	✓
	NR19	45	20	65	✓	✓	✓
	NR20	45	20	60	✓	✓	x
3:1	NR21	40	15	70	✓	✓	x
	NR22	40	20	70	✓	✓	x
	NR23	45	15	75	✓	✓	x
	NR24	50	15	65	✓	✓	x
	NR25	50	20	60	✓	✓	x
1:2	NR26	55	15	65	✓	✓	x
	NR27	40	30	65	x	–	–
	NR28	45	25	60	x	–	–
	NR29	45	16	60	✓	✓	x
1:3	NR30	50	15	65	✓	✓	x

Table 5: Phase solubility determination of MTF.

S. No.	Oil	Average ± S.D. (mg mL-1)
1	Oleic acid	3.67 ± 0.01
2	Isopropyl myristate	11.82 ± 0.04
3	Triacetin	15.45 ± 1.04
4	Caproyl 90	8.15 ± 0.64
5	Hydrogenated castor oil	124.37 ± 8.32
6	Transcutol	35.85 ± 1.30
7	Labrafac	10.04 ± 0.25
8	Tween 80	87.34 ± 9.36
9	Lauroglycol	15.68 ± 1.53
10	Tween 20	9.12 ± 8.14
11	Hydrogenated castor oil: Tween 80 (2:1)	85.34 ± 4.32
12	Hydrogenated castor oil: Tween 80 (1:1)	78.63 ± 3.43

composition and stability. Here's how it is typically measured:

Sample Preparation

Ensure that the nanoemulsion or liquid formulation is homogenous and free from bubbles. The sample should be clear, as turbidity can affect the accuracy of the measurement.

Temperature Control

Maintain the sample at a consistent temperature, usually around 25 ± 1.0°C, as the refractive index is temperature-dependent.

Refractometer Calibration

Calibrate the refractometer using a standard with a known refractive index, such as distilled water.

Measurement

Place a small amount of the sample onto the refractometer's prism. Close the prism cover and allow the sample to spread evenly across the surface.

Reading

Observe the light-dark boundary line (known as the shadow line) through the eyepiece or display of the refractometer. The refractive index is determined at the point where this boundary intersects the scale on the refractometer.

Recording

Record the refractive index value displayed by the refractometer.

Analysis

Compare the refractive index of the nanoemulsion to that of the pure components to ensure proper formulation and homogeneity. A consistent refractive index across different batches indicates uniformity and stability of the formulation.

Electrical conductivity

Electrical conductivity measurement is an important parameter for characterizing nanoemulsions, particularly to

Table 6: Observations for thermodynamic stability of metformin nano emulsion formulations.

S_{mix} ratio	FC	% S_{mix}	% Oil	% Water	thermodynamic stability test		
					A	B	C
	NR1	30	15	80	x	–	–
	NR2	35	20	75	x	–	–
	NR3	35	15	80	x	–	–
	NR4	35	30	65	x	–	–
1:0	NR5	40	20	70	✓	✓	✓
	NR6	40	15	70	✓	✓	✓
	NR7	40	20	65	✓	✓	x
	NR8	40	20	75	✓	✓	x
	NR9	40	15	72	✓	✓	x
	NR10	40	20	62	✓	x	–
	NR11	50	15	62	✓	x	–
1:1	NR12	50	15	63	✓	✓	✓
	NR13	55	15	72	✓	✓	✓
	NR14	40	15	71	✓	x	–
	NR15	45	20	76	✓	x	–
	NR16	50	10	74	✓	✓	✓
2:1	NR17	50	10	62	✓	✓	✓
	NR18	50	10	63	✓	✓	✓
	NR19	50	20	61	✓	✓	✓
	NR20	50	20	64	✓	✓	x
3:1	NR21	40	10	71	✓	✓	x
	NR22	40	20	72	✓	✓	x
	NR23	45	15	73	✓	✓	x
	NR24	50	15	76	✓	✓	x
	NR25	50	20	62	✓	✓	x
1:2	NR26	55	10	67	✓	✓	x
	NR27	40	30	68	x	–	–
	NR28	45	20	63	x	–	–
	NR29	50	20	64	✓	✓	x
1:3	NR30	55	15	65	✓	✓	x

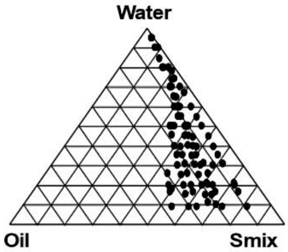
Table 7: Particle Size Distribution.

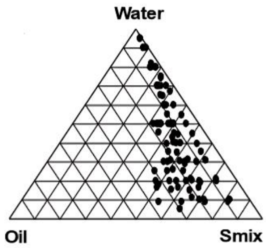
Formulation code	Droplet size ± S.D. (nm)a	Polydispersity indexa ± S.D.	Viscosity ± S.D. (cps)a	Refractive index ± S.Da	Conductivity (μS/cm) ± S.Da
RN 12	96.68 ± 9.68	0.317 ± 0.021	25.67 ± 0.417	1.578 ± 0.008	637.25 ± 3.19
RN 13	85.36 ± 5.64	0.219 ± 0.024	22.64 ± 0.317	1.764 ± 0.004	672.19 ± 4.81
RN 16	90.28 ± 7.59	0.247 ± 0.023	25.64 ± 0.564	1.785 ± 0.008	561.20 ± 5.67
RN 17	93.67 ± 7.58	0.329 ± 0.027	28.67 ± 0.567	1.732 ± 0.005	579.67 ± 2.92
RN 18	98.67 ± 7.59	0.537 ± 0.029	27.89 ± 0.375	1.864 ± 0.007	577.85 ± 2.71
RN 19	99.67 ± 8.67	0.267 ± 0.030	24.97 ± 0.674	1.917 ± 0.008	637.19 ± 6.74

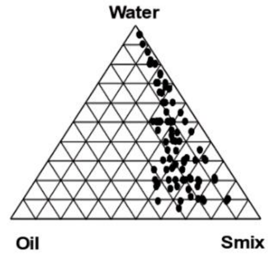
distinguish between oil-in-water (o/w) and water-in-oil (w/o) emulsions and to assess the emulsion's stability. Here's how it is typically determined:

Sample Preparation

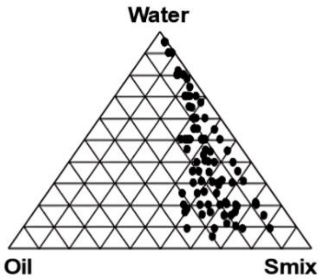
Ensure the nanoemulsion sample is well-mixed and

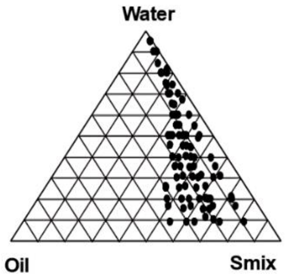


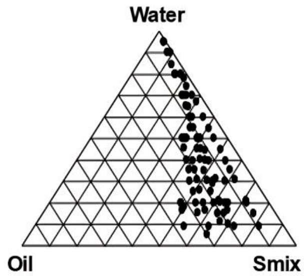




1:0 **1:1** **2:1**







3:1 1:2 1:3

Figure 4: phase diagram construction.

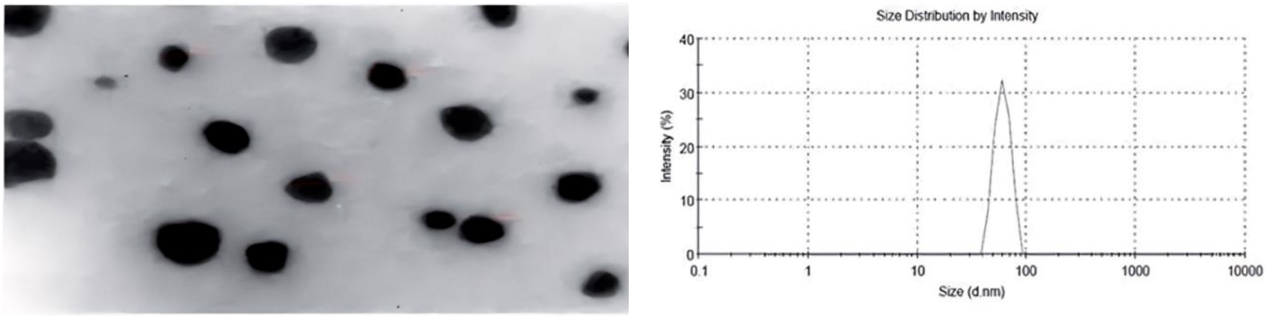


Figure 5: Transmission electron microscopic positive image of optimized repaglinide nano emulsion.

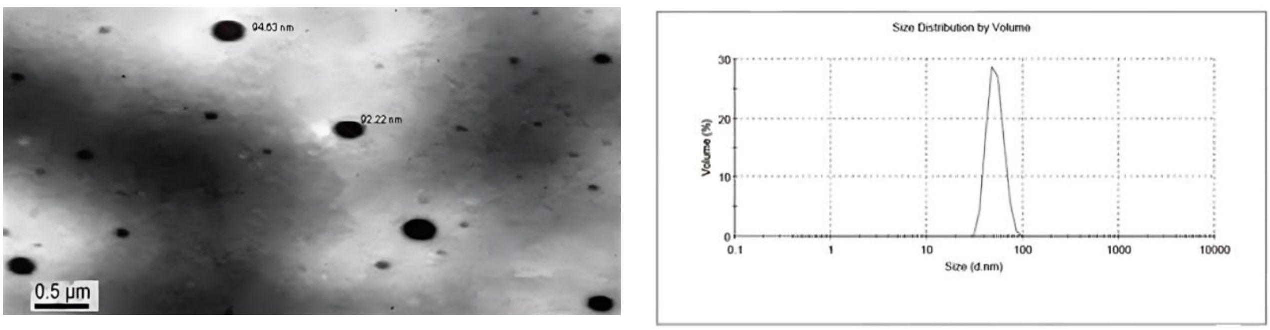


Figure 6: Transmission electron microscopic positive image of optimized repaglinide nano emulsion.

homogenous. The sample should be free of air bubbles, which can affect conductivity readings.

Temperature Control

Maintain the sample at a consistent temperature, typically around 25 ± 1.0°C, as temperature variations can influence conductivity.

Calibration

Calibrate the conductivity meter using a standard solution with a known conductivity value. This step ensures accurate measurements.

Measurement

Immerse the conductivity electrode into the nanoemulsion sample. Ensure the electrode is fully submerged and free from air bubbles, which could interfere with the measurement.

Reading

The conductivity meter measures the ability of the nanoemulsion to conduct electricity, which is typically dependent on the presence of free ions in the aqueous phase.

Recording

Record the conductivity value displayed by the meter.

Analysis

High Conductivity

Indicates an oil-in-water (o/w) emulsion, where the continuous aqueous phase allows for the movement of ions.

Low Conductivity

Suggests a water-in-oil (w/o) emulsion, where the continuous oil phase restricts ion movement, resulting in lower conductivity.

***In vitro* drug release**

Dissolution apparatus #2 was used to replicate stomach fluid in 500 mL of distilled water and 50 rpm of 37 ± 0.5^{0}C

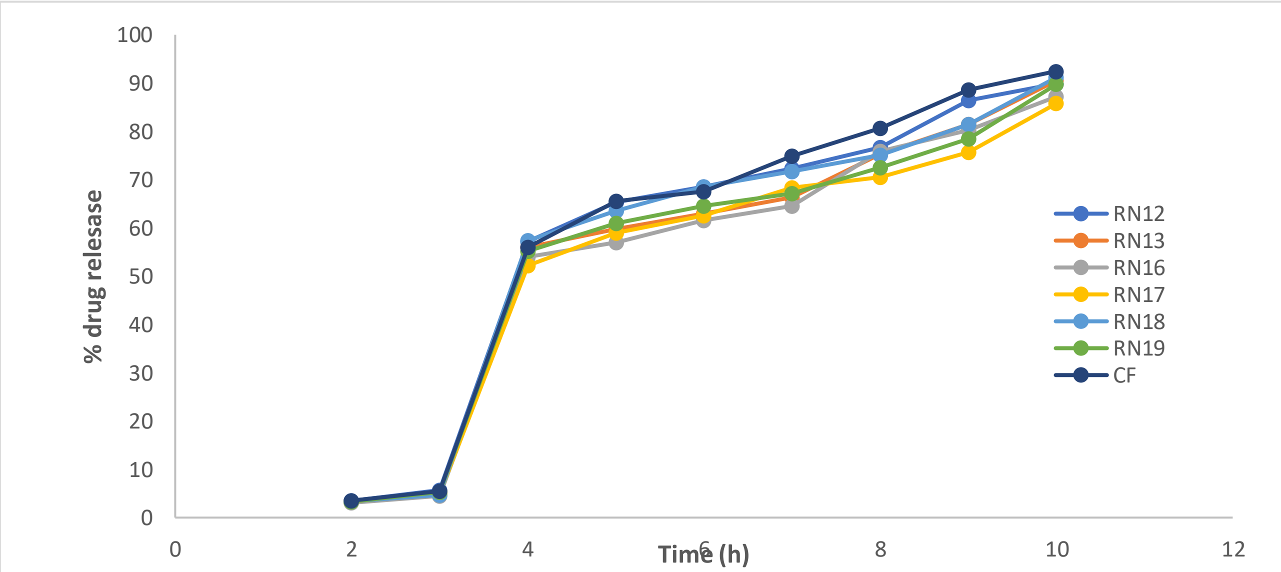


Figure 7: Comparative in vitro release profile of different formulations of repaglinide.

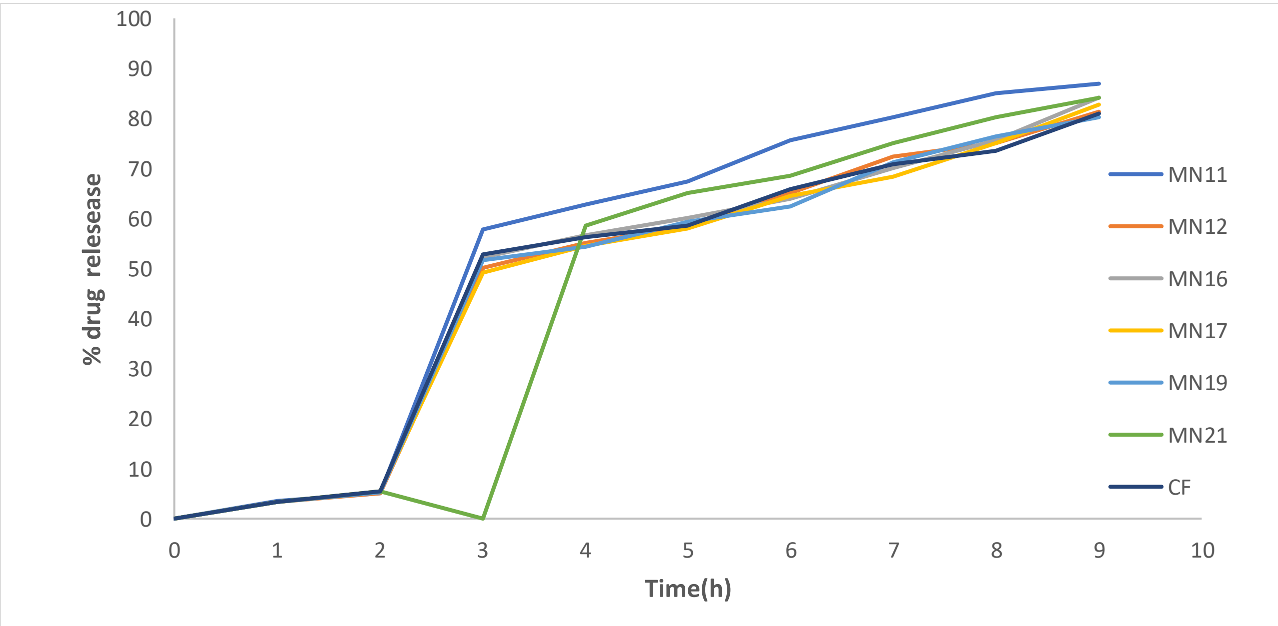


Figure 8: *In vitro* release profile of different metformin nano emulsions.

for an in vitro release test of specific repaglinide and metformin nano emulsions. In the treated dialysis bag, one milliliter of the nano emulsion formulation was added. At regular intervals of 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 24 hours, a milliliter sample was taken out and replaced with an aliquot of distilled water or simulated stomach fluid. The samples were subjected to a validated HPTLC technique at 240 nm to determine the drug content. For a few chosen metformin nano emulsions, an in vitro release test was also carried out utilizing dissolution apparatus #2 in 500 mL of distilled water and simulated stomach juice at 50 rpm at 37± 0.5^{0}C. In the treated dialysis bag, one milliliter of the nano emulsion formulation was added. At regular intervals of 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 24 hours, a milliliter sample was taken out and replaced with an aliquot of distilled water or simulated stomach fluid. Using the HPLC technique at 234 nm, the samples were examined to determine the drug content.

RESULTS

Formulation development and optimization of repaglinide nano-emulsion

Screening of components

Oils

Since these excipients created effective emulsification systems with several surfactants approved for oral administration and shown improved drug solubility qualities, modified or hydrolyzed vegetable oils have found widespread application. They have benefits for formulating and physiology, and the breakdown products they produce are similar to the byproducts of intestine digestion.

Surfactants

The HLB value of >10 was a crucial requirement for choosing the surfactants in the o/w nano 0emulsion formation process. In order to facilitate the dispersion process during the manufacture of the nano emulsion, the selected surfactant must be able to reduce the interfacial tension to a very small value.

Cosurfactants

It is unusual to obtain transient negative interfacial tension with a single surfactant; instead, a cosurfactant must usually be added. Another way to create a fluid interfacial film is to include a cosurfactant.

Phase solubility studies

Table 3 and Figure 1 shows Study on phase solubility were conducted to identify the best oil to use in the creation of a repaglinide nano emulsion. Figure 2 show the phase diagram of repaglinide nano-emulsion. Table 4 shows the Observations for thermodynamic stability study of repaglinide nano emulsion formulations

Formulation development and optimization of metformin

Screening of components

Oils

Vegetable oils that have been modified or hydrolyzed are commonly utilized because they are effective emulsifiers, possess a high number of surfactants that are permitted for oral administration, and have superior drug solubility qualities. They have benefits for formulating and physiology, and the breakdown products they produce are similar to the byproducts of intestine digestion.

Surfactants

The HLB value of >10 is a crucial requirement for choosing the surfactants that will produce the o/w nano emulsion.

Cosurfactants

It is unusual to obtain transient negative interfacial tension with a single surfactant; instead, a cosurfactant must usually be added. Another way to create a fluid interfacial film is to include a cosurfactant.

Phase solubility studies

Table 5 show the Phase solubility determination of metformin. Figure 3 show the Bar diagram showing the highest solubility of metformin. Table 6 show the observations for thermodynamic stability of metformin nano emulsion formulations. Figure 4 show phase diagram construction of metformin.

Formulation of drug containing nano emulsion

Six formulations, selected based on previous studies, were further evaluated after drug incorporation to assess their performance and suitability for advanced studies and potential therapeutic application.

Characterization of repaglinide nano emulsion

Visual observation

Whereas the macroemulsion seemed opaque and milky/cloudy white, the nano emulsion was a clear, transparent liquid that flowed readily.

Surface morphology

Figure 5 show the Transmission Electron Microscopy (TEM). It was employed to examine the morphology and structure of the nano emulsion droplets. The nano emulsion appeared dark against a light background, producing a "positive" image. TEM's high point-to-point resolution allowed for precise measurement of droplet sizes, providing detailed insights into the nano emulsion structural characteristics.

Droplet size analysis (particle size distribution)

The formulation RN13 demonstrated the smallest droplet size of 76.23 ± 4.14 nm, while formulations RN17 and RN18 displayed larger droplet sizes as a result of higher oil concentration. The formulation RN13 exhibited a distribution of droplets within the 76–89 nm range, with the majority (82%) falling below the 90 nm size threshold. Low polydispersity nanodroplets in the formulation demonstrated homogeneity in the nano emulsion formulation. For the various formulations, the polydispersity values were 0.214, 0.183, 0.195, 0.229, 0.407, and 0.198, respectively.

Formulation of drug containing nano emulsion

Six formulations were selected and were subjected to additional investigations following the introduction of MTF (125 mg added to each selected formulation).

Characterization of metformin nano emulsion

Visual observation

The macroemulsion appeared opaque and milky, while the nano emulsion was a clear, transparent liquid with easy flow characteristics.

Surface morphology

Figure 6 show the Transmission electron microscopy (TEM). It was used to determine the morphology and structure of the nano emulsion droplets. The surrounding lightened, giving the nano emulsion a black appearance. With TEM, a "positive" picture was observed. Because of its point-to-point resolution, TEM was used to measure droplet sizes.

Droplet size analysis

The measurement of droplet size is a crucial metric for both optimizing the formulation of the nano emulsion and differentiating it from the microemulsion. The homogeneity of droplet size within the formulation is shown by polydispersity, which is calculated as the ratio of standard deviation to mean droplet size. The formulation's droplet size homogeneity increases with decreasing polydispersity value. The MN21 formulation demonstrated the lowest droplet size of 92.25 ± 3.54 nm, while the MN19 formulation displayed an increase in droplet size as a result of a higher oil concentration. The distribution of droplets in formulation MN21 fell between 92 and 98 nm, with 80% of the maximum droplets being smaller than 96 nm. Low polydispersity nanodroplets in the formulation demonstrated homogeneity in the nano emulsion formulation. For the various formulations the polydispersity values were, in order, 0.198, 0.185, 0.187, 0.196, 0.204, and 0.172.

***In vitro* drug release for repaglinide**

Figure 7 show the Comparative in vitro release profile of different formulations of repaglinide. Figure 8 show the In vitro release profile of different metformin nano emulsions.

CONCLUSIONS

An optimized nano emulsion for Repaglinide and Metformin was successfully formulated using 5% v/v of Sefsol 218 as the oily phase, 30% v/v of Smix (surfactant and cosurfactant mixture), and 65% v/v of distilled water as the aqueous phase. The formulation mentioned above was refined based on factors such as the ideal globule size, the lowest polydispersity index, the drug release, the viscosity, the concentration of surfactant, and the drug's higher solubilization in the least amount of oil. The formulation that was optimized demonstrated strong antidiabetic effects. It might be because of its greater surface area and nanosize, which enable better absorption and a faster rate of drug release, leading to improved bioactivity at lower doses of the medication.

REFERENCES

1. Porter CJ, Pouton CW, Cuine JF, Charman WN. Enhancing intestinal drug solubilization using lipid-based delivery systems. Advanced drug delivery reviews. 2008 Mar 17;60(6): page no.673-91.
2. Porter CJ, Kaukonen AM, Taillardat-Bertschinger A, Boyd BJ, O'Connor JM, Edwards GA, Charman WN. Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine. Journal of pharmaceutical sciences. 2004 May 1;93(5): page no.1110-21.
3. Porter, W.N. Charman, *In vitro* assessment of oral lipid-based formulations, Adv. Drug Deliv. Rev.50 (1), 2001: page no.127–147.
4. Fatouros DG, Nielsen FS, Douroumis D, Hadjileontiadis LJ, Mullertz A. In vitro–in vivo correlations of self-emulsifying drug delivery systems combining the dynamic lipolysis model and neuro-fuzzy networks. European Journal of Pharmaceutics and Biopharmaceutics. 2008 Aug 1;69(3): page no.887-98.
5. Dixon JB, Greiner ST, Gashev AA, Cote GL, Moore Jr JE, Zawieja DC. Lymph flow, shear stress, and lymphocyte velocity in rat mesenteric prenodal lymphatics. Microcirculation. 2006 Jan 1;13(7): page no.597-610.
6. Dongaonkar RM, Laine GA, Stewart RH, Quick CM. Balance point characterization of interstitial fluid volume regulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2009 Jul;297(1): page no.6-16.
7. Fores B, Ehrhardt C. Human respiratory epithelial cell culture for drug delivery applications, Eur. J. Pharm. Biopharm., 60(2), 2005: page no.193-205.