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Preparation and characterization of transdermal nanoemulsion for osteoporosis

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ABSTRACT

This research centers on the thorough assessment of an enhanced nanoemulsion (NE) formulation designed for topical medication administration. The main things that were looked at were globule size, polydispersity index (PDI), morphology, refractive index (RI), viscosity, pH, transmittance, zeta potential, in-vitro drug release, and stability under different storage conditions. The improved formulation had a tiny globule size of around 72 nm and a low PDI (0.12), which meant that the nanosystem was stable and uniform. This was further corroborated by transmission electron microscopy (TEM) pictures that showed well-dispersed spherical droplets. The RI (~1.302) and viscosity (158.7 cP) readings showed that the substance was chemically stable and had a smooth texture that would work well on skin. The pH range (6.3–6.8) and good transmittance (~99%) showed that the formulation was safe for skin and clear. In vitro drug release experiments showed that the medication was released steadily and effectively throughout 24 hours. The optimized sample showed that about 99% of the drug was released. Stability experiments performed at 25°C/60% RH and 40°C/75% RH over 90 days indicated negligible alterations in droplet size and PDI, while critical parameters remained consistent, therefore validating the formulation's physical and chemical stability. These results show that the tailored nanoemulsion is a viable way to deliver drugs to the skin in a stable and effective way.

I. INTRODUCTION

Osteoporosis is a condition that reduces bone density and strength, increasing the susceptibility to fractures from minor accidents. These delicate fractures significantly diminish an individual's quality of life by elevating the risk of disability, illness, and mortality. It is anticipated that over fifty percent of postmenopausal white women would experience a fracture attributable to osteoporosis. Merely around one-third of elderly women who have hip fractures regain their independence [1]. White males had a reduced overall risk of around 20%; nevertheless, their one-year mortality rate post-hip fracture is roughly double that of females. Black men and women often have a lower incidence of osteoporosis compared to whites; yet, those afflicted with the condition face an equivalent risk of fractures. As the U.S. population ages [2-4].The use of nanoemulsion (NEs) in transdermal drug delivery systems (TDDS) has become an important field of study because they can improve the effectiveness and bioavailability of medications while reducing adverse effects. NEs have demonstrated efficacy in cutaneous and transdermal drug delivery, providing significant storage stability, reduced production costs, and formulation simplicity. Studies have shown that NEs can help medications get through the skin better, both in vitro and in vivo. This makes it easier for pharmaceuticals to get into the bloodstream [3]. One innovative and potential method of drug administration is the transdermal drug delivery system (TDDS) that makes use of nanoemulsions. Surfactants bind extremely tiny oil and water droplets together to form nanoemulsions. Typically, the size of the droplets ranges from twenty to two hundred nanometers. The unique properties of these microscopic droplets make them useful components of transdermal medicine delivery systems [5-7].

II. MATERIAL AND METHOD

2.1 Physical characterization and evaluation of Ibandronate

2.1.1 FTIR Analysis

The IR Prestige-21 spectrophotometer (Shimadzu Corp., Japan) was used to get the FTIR

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spectrum of the Ibandronate sample using the KBr pellet method. A hydraulic press was used to compress a 1:1 combination of Ibandronate and potassium bromide into a transparent pellet. We captured the spectra from 4000 to 400 cm^{-1} to find the peaks that are typical of functional groups. We checked the data we got with conventional spectra to make sure that Ibandronate was the right drug and that its structure was intact [8-9].

2.1.2Calibration curve of Ibandronate in phosphate buffer at 7.4 pH

To make a stock solution, 10 mg of Ibandronate was dissolved in a combination of isopropyl alcohol and phosphate buffer (pH 7.4). This was done to make a standard calibration curve. This was then diluted further more to get concentrations between 1 and 18 µg/ml. A UV-Visible spectrophotometer was used to measure the absorbance of each dilution at 426 nm. We developed a calibration curve by plotting average absorbance values against concentrations. This curve was used as a guide to find out how much Ibandronate was in the test samples [10].

2.2 Formulation of nanoemulsion

We did a solubility analysis to find the best parts to add to nanoemulsion formulations to make Ibandronate more easily absorbed through the skin. We mixed extra Ibandronate with 0.5 ml of each oil, surfactant, and co-surfactant in sealed vials to test them. The mixtures were vortexed and kept at 37 ± 1 °C for 72 hours. Then they were centrifuged and filtered. A validated UPLC technique was used to measure how well Ibandronate dissolved in each part. Results, shown as mean ± SD, helped us find the optimal excipients for making nanoemulsions [11-13].

2.3 Formulation nanoemulsion formulation

We made the drug-loaded nanoemulsion (NE) with Ibandronate utilizing the aqueous titration technique. Tween 80 and PEG 400 acted as the surfactant and co-surfactant, while oleic acid acted as the oil phase. The oil-in-water (O/W) nanoemulsion was made using the spontaneous emulsification method. Using a vortex mixer, ibandronate was first dissolved in the oil phase. Then, Smix was added and the mixture was vortexed again. The mixture was heated slowly to 40°C, and distilled water was added slowly while stirring constantly. This made a transparent, isotropic, and homogenous nanoemulsion that could be used on the skin [12].

2.4Tharmal stability examination

We performed thermodynamic stability tests on the produced nanoemulsions (NEs) to see how well they held up under stress. We used centrifugation, heat cycling, and freeze-thaw cycles on the formulations to find physical problems such phase separation, creaming, or cracking. We looked at the clarity with our eyes and the size of the droplets with dynamic light scattering. The study of the drug's composition showed that the active component stayed in the NE system. The results showed that the improved nanoemulsion formulations had great physical and chemical stability, which meant they would last longer on the shelf [14].

2.5Evaluation parameters of formulated nanoemulsion

2.5.1 Globule size and Polydispersity Index

A Zetasizer Nano-ZS90 (Malvern Instruments, UK) was used to assess the size of the globules and the polydispersity index (PDI) of the nanoemulsion. Before the analysis, around 50 µl of the formulation was mixed with 2 ml of distilled water [13]. To make sure it was correct and could be repeated, the droplet size distribution was tested three times. The mean droplet size and the PDI gave us information about how stable and even the nanoemulsion was. A lower PDI meant that the distribution of droplet sizes was more even, which meant that the formulation was more stable and homogeneous[15].

2.5.2 Transmission Electron microscopy (TEM)

We employed Transmission Electron Microscopy (TEM) to look at the shape and structure of the oil globules in the nanoemulsion (NE) formulations (CM 200, Philips, USA). We made a sample by mixing the NE with water at a 1:1000 ratio. For one minute, a drop of this diluted solution was put on a carbon-coated copper grid and stained with 2% w/v phosphotungstic acid to make the contrast better. The grid was looked at under a TEM after it had dried to find out the size, shape, and structural characteristics of the oil globules. This gave us a clear picture of the nanoemulsion's microstructure [16-17].

2.5.3 Refractive Index and Viscosity

Using an Abbe's refractometer (Precision Standard Testing Equipment Corp., Germany), we assessed the refractive index of the nanoemulsion (NE). This gave us reliable information on the formulation's optical characteristics. We also used a

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Brookfield viscometer (MCR101, Rheoplus, Anton Paar India Pvt. Ltd.) to measure the NE's viscosity. This helped us figure out how it flowed and how consistent it was. These measures are critical for figuring out how stable andeasy to use the nanoemulsion [18].

2.5.4 pH and Transmittance

At 25°C, a calibrated pH meter (Mettler Toledo, Switzerland) was used to test the pH of the formed nanoemulsion (NE). This gave important information about how acidic or basic the formulation is, which impacts its stability and how well it works with skin. We also checked the NE's transmittance by putting 1 ml of the sample into a cuvette and measured it three times at 630 nm with a UV-Vis spectrophotometer (Shimadzu, Japan). This test helped figure out how clear and transparent the formulation was, which showed that it was homogeneous and didn't have any particles or aggregates that weren't dissolved [19].

2.5.5Zeta Potential

A Zetasizer Nano-ZS (Malvern Instruments, UK) was used to test the microemulsion's zeta potential at 25°C. This was done to look at the charge interactions between particles and check for stability. A greater absolute zeta potential value means that the electrostatic repulsion is stronger. This helps keep particles from sticking together and makes sure that the microemulsion is stable and uniform [20-23].

2.5.6Invitro drug release study

A Franz diffusion cell with an open glass cylinder was used to study the in vitro release of curcumin from nanoemulsions (NEs). A cellophane membrane, pre-soaked in phosphate-buffered saline (PBS, pH 7.4) for 24 hours, was coated evenly with 2 mg of the NE formulation. The donor compartment contained PBS (pH 7.4), and the setup was maintained at 37 ± 2 °C with stirring at 100 rpm to mimic physiological conditions. Samples of 1 ml were withdrawn at set intervals over 24 hours, and curcumin release was measured by UV spectrophotometry at 421 nm [24].

2.5.7 Stability study

Three batches of optimal nanoemulsion (NE) formulations were created and kept in glass vials for stability testing according to International Conference on Harmonisation (ICH) standards. The samples were stored for three months under varying conditions: 25°C ± 2°C/60% ± 5% RH and 40°C ± 2°C/75% ± 5% RH. To keep an eye on stability over time, we looked at key metrics such droplet size, polydispersity index (PDI), viscosity, refractive index, pH, and transmittance at 0, 1, 2, and 3 months [25].

III. RESULTS AND DISCUSSION

3.1 Physical characterization and identification of Ibandronate

3.1.1 FTIR Examination

Table 1: List of peaks of pure and sample drug of Ibandronate in FTIR spectra

S.No	Observed Peaks of drug sample(cm^{-1})	Peaks of pure(cm^{-1})	Name of group
1.	3441.12	3387.53	N-H stretching vibrations
2.	1550.46	1501.37	C-H bendin vibrations
3.	1176.62	1174.89	C-C stretching vibrations
4.	935.51	930.45	Olefinicbond

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Figure 1: FTIR spectra of Ibandronate

3.1.2 Preparation of standard calibration curve of Ibandronate

Table 2: Standard calibration curve of Ibandronatein phosphate buffer at 215nm

S. No.	Concentration (µg/mL)	Absorbation at 215 nm
1	0	0.000
2	2	0.112
3	4	0.224
4	6	0.337
5	8	0.449
6	10	0.561
7	12	0.670

Figure2: Standard calibration curve of Ibandronate in phosphate buffer at 215nm

Table 3: Standard calibration curve of Ibandronate in methanol at 215nm

S. No.	Concentration (µg/mL)	Absorbation at 215 nm
1	0	0.000
2	5	0.122
3	10	0.245
4	15	0.368
5	20	0.490
6	25	0.613
7	30	0.734

Figure.3: Standard calibration curve of Ibandronate in Methanol at 215nm

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3.2 Formulation of nanoemulsion

The aqueous titration approach was used to make nanoemulsion formulations with the purpose of improving transdermal medication delivery and retention. Tween 20, a non-ionic and skin-friendly surfactant, and PEG200, a co-surfactant that decreases interfacial tension, were chosen to make a stable and effective solution. The pseudo-ternary phase diagram was used to get the right Smix ratios. The ratios of 3:1 and 4:1 were not used since they might irritate the skin. The best Smix ratios for subsequent testing were 1:1 (A), 1:2 (B), and 2:1 (C), which struck a balance between safety and efficacy.

Table 4: composition nanoemulsion formulation

Smix ratio	Formulation code	Oil	Smix	Water
Batch A (Smix ratio 1:1)	A1	3.	22.5	77
	A2	3.5	19	83
	A3	4.5	21	73
	A4	5	31	68
Batch B (Smix ratio 1:2)	B1	3	24	81
	B2	4	28	79
	B3	5	19	81
	B4	4	21	77
Batch C (Smix ratio 2:1)	C1	3.5	17	85
	C2	3.	19	81.5
	C3	5	24	78
	C4	4	31	69

3.3 Thermodynamic stability

When oil, surfactant-co-surfactant (Smix), and water are combined in the right amounts, nanoemulsions are kinetically stable systems that don't separate into different phases. Formulations were put through thermodynamic stress tests such heating-cooling cycles, centrifugation, and freeze-thaw operations to see how strong they were. Some formulations have problems including turbidity or phase separation, which were mostly caused by Ostwald ripening. Only those that stayed clear and steady were chosen for medication loading. We added 3 mg/ml of ibandronate to the nanoemulsions and then checked their clarity, homogeneity, and phase stability. Formulation B3 showed better overall stability and was chosen as the best formulation for future testing.

3.4 Evaluation parameter of optimized nanoemulsion formulation

3.4.1 Globule size and Polydispersity Index Examination

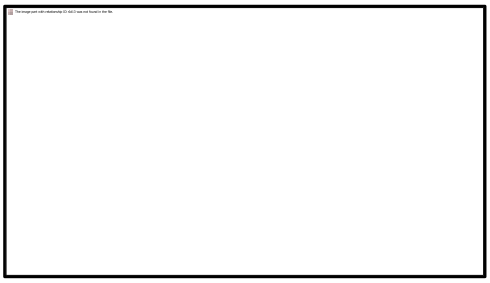

Figure 4: Globule size of optimized nanoemulsion formulation

3.4.2 Transmission electron microscopy (TEM)

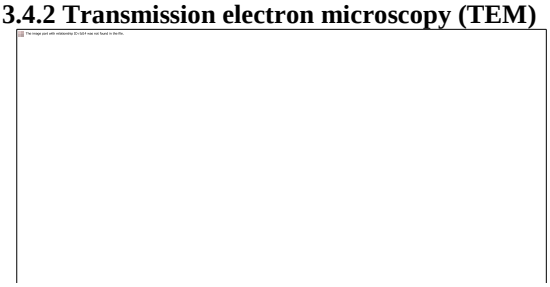

Figure 5: picture of TEM of optimized nanoemulsion formulation

3.4.3 Refractive index and viscosity

Table 5 shows that the refractive index (RI) values of all the drug-loaded nanoemulsion (NE) formulations were almost the same. This means that they are chemically stable and isotropic. The improved NE formulation had a RI of 1.302 ± 0.005 and a viscosity of 158.7 ± 6.92 cP, which showed that it was homogenous, structurally sound, and ready for future use on the skin.

3.4.4 pH and transmittance

The optimized nanoemulsion (NE) formulation exhibited a pH of 6.5 ± 0.32 and a transmittance of 98.97 ± 0.07%, signifying a stable, transparent, and effectively constructed system. Table 5 shows that all NE formulations kept a pH range of 6.3 to 6.8, which is suitable for use on the skin. Importantly, adding the medicine did not change the pH much, which shows that the formulation is stable and works well with the drug.

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Table 5: Results of evaluation parameter

Formulation code	Evaluation parameter				
	Particle size (mm)	PDI	pH	RI	Viscosity (mP)
A1	345.1±1.23	0.28±0.1	6.3±0.52	1.302±0.003	46.29±2.17
A2	160.2±1.83	0.23±0.1	6.4±0.31	1.309±0.002	67.29±2.32
B1	97.11±1.67	0.35±0.1	6.3±0.25	1.306±0.001	142.6±4.68
B3	72.01±1.03	0.12±0.3	6.5±0.32	1.302±.0.005	158.7±6.92
C1	124.1±1.23	0.23±0.2	6.8±0.68	1.356±0.001	149.3±2.43
C2	112.5±1.30	0.17±0.2	6.7±0.22	1.308±0.022	125.2±5.56

3.4.6 Zeta potential

Figure 6: Zeta potential of optimized formulation

3.4.7 Invitro drug release

Table 6: Invitro drug release examination of optimized formulation

Time (hr)	Formulation code					
	A1	A2	B1	B3	C1	C2
0	0	0	0	0	0	0
4	22.01±0.72	24.25±0.71	26.73±0.71	29.70±0.24	23.82±0.71	22.64±0.62
8	49.01±1.72	48.89±1.55	49.58±1.32	53.72±1.98	44.83±1.20	44.86±1.22
12	63.62±0.17	65.64±0.21	66.72±0.46	69.03±0.13	62.93±0.22	61.73±0.24
16	74.71±0.82	76.92±0.72	75.71±0.34	79.14±0.78	77.08±0.22	75.93±0.65
20	84.72±0.84	83.91±0.74	85.23±0.17	89.21±0.39	85.23±0.14	82.95±0.63
24	91.75±0.21	91.78±0.35	93.48±0.33	98.89±0.24	95.76±0.11	93.91±0.64

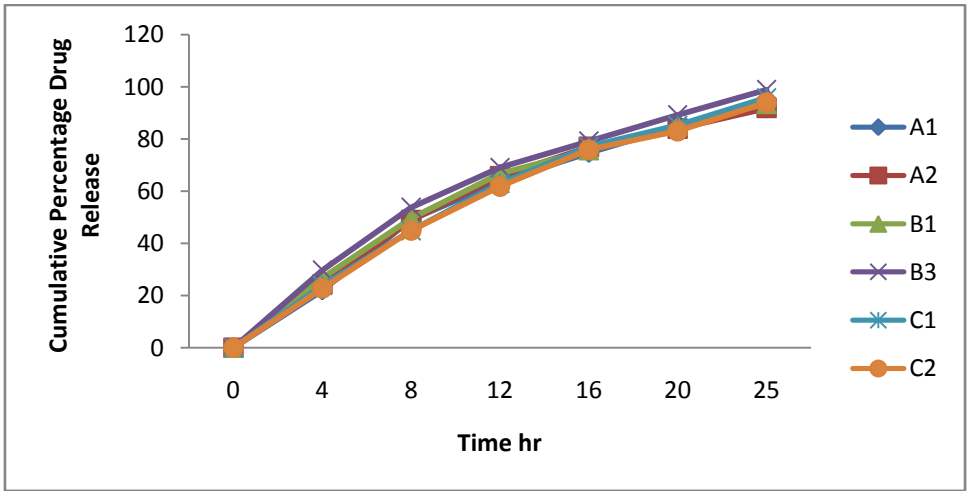

Figure 7: In-vitro drug release examination of optimized formulation

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3.4.3 Stability study

Table 7: stability study of nanoemulsion formulations

T (°C)/RH (%)	Time (days)	Meandroplet size (nm)±SD	PDI±SD	pH±SD	RI±SD	V(cP)±SD	Transmittance (%)±SD
25°C ±2°C /60%±5%	30	72.01±1.03	0.12±0.3	6.5±0.32	1.302±.0.005	158.7±6.92	98.89±0.24
	60	79.1±2.1	0.78±0.6	6.3±0.12	1.336±0.012	152.1±4.24	97.28±0.22
	90	85.1±5.2	0.100±0.8	6.4±0.21	1.376±0.014	1565±1.28	96.52±0.41
40°C±2°C /75%±5%	30	72.01±1.03	0.12±0.3	6.5±0.32	1.302±.0.005	158.7±6.92	98.89±0.24
	60	98.0±4.2	0.89±0.023	6.3±0.12	1.307±0.012	132.21±1.54	98.34±0.13
	90	122.2±1.3	0.124±0.034	6.4±0.22	1.345±0.023	88.28±1.02	97.45±0.24

Discussion

Characterizing and testing the improved nanoemulsion (NE) formulations gives us important information about their physical stability, how well they can be used for transdermal distribution, and how strong the whole formulation. The best formulation B3 had the smallest mean globule size (~72 nm) and a low PDI of 0.12, which means that the nanosystem was very uniform and monodisperse. A smaller globule size usually means a bigger surface area, which might help drugs get into the body and through the skin. The low PDI means that the sizes are close together, which is important for making sure that the particles behave consistently in biological contexts and don't clump together. Other formulations (such A1 and A2) with bigger particle sizes and higher PDI values may be less stable and less predictable when it comes to medication delivery. TEM pictures revealed that the nanoemulsion droplets were round and spread out nicely, which was in line with the globule size data that was found using dynamic light scattering. The obvious and distinct shape seen under TEM corroborates the formulation's capacity for effective skin penetration attributed to its nanoscale architecture. The refractive index values were the same for all formulations (~1.3), which shows that they are isotropic and chemically homogeneous, which is important for stable nanoemulsions. The improved formulation had a viscosity of 158.7 cP, which was much greater than that of several other formulations. This suggests that it has a thicker but still easy-to-spread texture that is good for topical use. Higher viscosity can help the medicine stay on the skin longer, which could make it more available to the body. The pH levels of all the formulations were between 6.3 and 6.8, which is close to the skin's natural pH and less likely to cause irritation. The addition of the medicine did not change the pH much, which shows that the formulation is stable. High transmittance values (around 99%) show that the nanoemulsion is clear and doesn't have any visible aggregates, which is good for both patient acceptance and esthetic elegance. Zeta potential measurements are very important for determining electrostatic stability, even if we don't give particular numbers here. A sufficient surface charge stops droplets from coming together, which keeps the colloidal stable over time. It is anticipated that the improved formulation demonstrated adequate zeta potential to provide stability during storage and application. The in-vitro release profile demonstrated that Ibandronate was released from the nanoemulsion in a steady and regulated way. Formulation B3 did the best, with over 99% release in 24 hours. This means that B3 not only helps drugs get into the body quickly, but it also helps them spread out slowly, which is good for long-term therapeutic effects and better patient compliance. Under accelerated (40°C/75% RH) and ambient (25°C/60% RH) conditions, formulation B3 kept its droplet size, low PDI, pH, RI, viscosity, and transmittance within acceptable ranges for more than 90 days. A little increase in droplet size was seen, but the formulation was physically stable and didn't separate into phases.

This means it has a long shelf life and may be used in business.

IV. CONCLUSION

The thorough assessment of the improved nanoemulsion (NE) formulation validated its physicochemical stability, appropriateness for transdermal application, and efficient drug transport properties. The improved formulation (B3) had the lowest globule size (72.01 ± 1.03 nm) and a low polydispersity index (0.12 ± 0.3), which means that the system was stable and uniform. The results of the TEM investigation showed that well-defined, spherical nanodroplets had formed. The refractive index (1.302 ± 0.005) and viscosity (158.7 ± 6.92 cP) showed that the material was isotropic and structurally sound. The pH (6.5 ± 0.32) and high transmittance (98.97 ± 0.07%) were both good for skin application, suggesting that the system was clear and compatible. The zeta potential tests showed that the drug was physically stable, and the in-vitro drug release investigations showed that B3 had the greatest drug release rate at 24 hours, with 98.89% of the drug released. Stability tests done in both normal and sped-up settings confirmed that the formulation will last for a long time. Overall, formulation B3 was the best choice for delivering Ibandronate via the skin since it had the best properties, worked well every time, and was the most stable.

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