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Formulation, Characterization and In-Vitro Drug Release Study of Simvastatin Nanosuspension for Bioavailability Improvement

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Abstract

Background: Simvastatin is a poorly water-soluble HMG-CoA reductase inhibitor for which dissolution can limit oral absorption. Nanosuspension technology reduces drug particle size and can increase surface area available for dissolution. Objective: The study aimed to formulate and characterize simvastatin nanosuspensions using poloxamer 188 and poloxamer 407 as stabilizers and to evaluate their in-vitro dissolution behaviour. Methods: Six formulations (F1–F6) containing 5% w/v simvastatin were prepared by high-pressure homogenization. Poloxamer 188 was used at 0.5%, 1.0% and 1.5% in F1–F3, while poloxamer 407 was used at the same concentrations in F4–F6. The nanosuspensions were lyophilized using 1% mannitol as cryoprotectant. Particle size, polydispersity index (PDI), zeta potential, saturation solubility, drug content, SEM, FTIR, DSC, PXRD, dissolution and stability were evaluated. Results: The reported particle sizes ranged from 92.6 to 281.6 nm. F3 showed the smallest reported particle size (92.6 nm), highest saturation solubility (177.50 µg/mL in 0.1 N HCl and 317.6 µg/mL in pH 7.0 phosphate buffer) and drug content of 99.63 ± 0.05%. In pH 7.0 phosphate buffer, the dissolution table reported 99.14 ± 0.19% release for F3 at 60 min, compared with 29.06 ± 0.48% for pure simvastatin. F3 drug content remained 98.72 ± 0.054% after 90 days at 4°C. Conclusion: The findings indicate that high-pressure homogenization with poloxamer 188, particularly F3, improved the in-vitro solubility and dissolution performance of simvastatin. The source thesis contains inconsistencies in selected reported values, and these require verification before journal submission.

Keywords: Simvastatin; nanosuspension; high-pressure homogenization; poloxamer 188; poloxamer 407; particle size; dissolution enhancement; lyophilization.

1. INTRODUCTION

The oral absorption of a drug is influenced by its aqueous solubility, dissolution rate, permeability and gastrointestinal conditions. Poor aqueous solubility can result in slow dissolution and variable drug exposure. Simvastatin is a lipid-lowering agent that inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. The source dissertation describes simvastatin as practically insoluble in water and reports oral bioavailability below 5%.

Particle-size reduction is a commonly investigated pharmaceutical approach for improving dissolution of poorly water-soluble compounds. According to the Noyes–Whitney relationship, increasing the effective surface area of a drug can increase its dissolution rate when other conditions remain comparable. Nanosuspensions are colloidal dispersions of drug particles stabilized by suitable surfactants or polymers. Their performance depends on particle size, size distribution, surface properties, stabilizer concentration and physical stability.

High-pressure homogenization is a top-down technique in which a coarse drug dispersion is forced through a narrow homogenization gap under high pressure. Cavitation, shear and particle collision contribute to particle-size reduction. Stabilizers such as poloxamers can absorb particle surfaces and reduce aggregation by steric stabilization. Poloxamer 188 and poloxamer 407 are non-ionic block copolymers with different molecular-weight ranges and have been used as pharmaceutical surfactants.

The present work compared simvastatin nanosuspensions prepared with increasing concentrations of poloxamer 188 or poloxamer 407. The study evaluated physicochemical characteristics, dissolution behaviour and short-term storage stability of lyophilized formulations.

2. AIM AND OBJECTIVES

The aim was to formulate, characterize and evaluate simvastatin nanosuspensions by high-pressure homogenization with a view to improving in-vitro dissolution.

- To prepare simvastatin nanosuspensions using poloxamer 188 and poloxamer 407 at three concentrations.
- To evaluate particle size, polydispersity index and zeta potential.
- To determine drug content and saturation solubility in selected media.
- To characterize morphology and solid-state properties using SEM, FTIR, DSC and PXRD.
- To compare in-vitro dissolution profiles of pure drug, physical mixtures and lyophilized nanosuspensions.
- To assess the storage stability of the selected formulation.

3. MATERIALS AND METHODS

3.1 Materials

Simvastatin was obtained from Micro Labs Limited, Hosur, India. Soya lecithin was obtained from HiMedia Laboratories, Mumbai. Poloxamer 407 and poloxamer 188 were sourced from Micro Lab, Bangalore. Sodium lauryl sulphate was obtained from S.D. Fine Chemical Ltd., Mumbai. Hydrochloric acid, potassium dihydrogen phosphate, sodium hydroxide, ethanol and mannitol were used as listed in the dissertation. Supplier details are reproduced from the source thesis.

3.2 Instruments

The instruments listed in the dissertation included an Ultra-Turrax T25 homogenizer (IKA-Werk, Germany), Micron-LAB 40 high-pressure homogenizer (APV System, Germany), magnetic stirrer, electronic digital balance, Virtis freeze dryer, UV–Visible spectrophotometer (Elico), FTIR spectrophotometer (Shimadzu), Microtrac Bluewave particle-size analyser, Malvern zeta analyser, scanning electron microscope, differential scanning calorimeter, stability chamber and dissolution test apparatus.

3.3 Formulation design

Six formulations were prepared with a constant simvastatin concentration of 5% w/v. Poloxamer 188 was used in F1–F3 and poloxamer 407 in F4–F6, each at 0.5%, 1.0% and 1.5%, respectively.

Table 1. Composition of simvastatin nanosuspension formulations as reported in the dissertation

Formulation	Simvastatin (% w/v)	Poloxamer 188 (%)	Poloxamer 407 (%)
F1	5	0.5	—

F2	5	1.0	—
F3	5	1.5	—
F4	5	—	0.5
F5	5	—	1.0
F6	5	—	1.5

3.4 Preparation of nanosuspension

Simvastatin powder (5% w/v) was dispersed in an aqueous solution containing the selected stabilizer using a magnetic stirrer. The dispersion was initially homogenized using an Ultra-Turrax T25 at 9500 rpm for 10 min. The pre-suspension was then processed using a Micron-LAB 40 high-pressure homogenizer. The homogenization sequence consisted of two cycles at 100 bar, two cycles at 500 bar and a further 15 cycles at 1500 bar.

3.5 Preparation of lyophilized nanoparticles

Mannitol (1%) was added to each nanosuspension as a cryoprotectant. The samples were frozen overnight at −70°C and subsequently lyophilized for two days at −50°C and 2 millitorr using a Virtis freeze dryer. The resulting dry products were used for solid-state and dissolution evaluations.

3.6 Preformulation and analytical method

The drug was examined for physical appearance and melting range. The reported melting range was 132–136°C. The UV absorption maximum was identified at 238 nm. Calibration curves were prepared separately in 0.1 N hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.0), using concentrations from 2 to 10 µg/mL. The reported linear regression equations were $y = 0.032x$ ($R^2 = 0.997$) in 0.1 N HCl and $y = 0.061x$ ($R^2 = 0.996$) in pH 7.0 buffer.

3.7 Particle size and PDI

Particle size and size distribution were determined using a Microtrac Bluewave particle-size analyser. Samples were diluted with deionized water to a suitable concentration before measurement. PDI values were recorded as an indicator of distribution breadth.

3.8 Zeta potential

Zeta potential was measured using a Malvern Zetasizer. Samples were diluted with deionized water and conductivity was adjusted using sodium chloride, as described in the dissertation.

3.9 Drug content

Nanosuspension powder equivalent to 10 mg simvastatin was dissolved in 10 mL ethanol, diluted with distilled water and analysed spectrophotometrically at 238 nm. Drug content was calculated using the absorbance-based equation described in the dissertation.

3.10 Saturation solubility

Pure simvastatin and lyophilized nanosuspension equivalent to 10 mg simvastatin were separately added to 10 mL of distilled water. Samples were agitated in a rotary shaker at 37°C for 24 h over two days. The samples were filtered and analysed at 238 nm. The dissertation reports triplicate determinations.

3.11 In-vitro dissolution study

Dissolution was performed using USP apparatus II (paddle) at 50 rpm. Lyophilized nanosuspension equivalent to 100 mg simvastatin was dispersed in 900 mL of dissolution medium maintained at 37.0 ± 0.5°C. Samples (5 mL) were withdrawn at predetermined intervals, filtered and suitably diluted before UV analysis at 238 nm. The withdrawn volume was replaced with fresh medium. The media included 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.0) containing 0.5% sodium lauryl sulphate. Each determination was reported in triplicate.

3.12 Morphology and solid-state characterization

For SEM, samples were mounted on double-sided adhesive tape attached to aluminium stubs and coated with platinum before imaging. FTIR spectra of pure simvastatin, stabilizers, physical mixtures and

lyophilized formulations were recorded over 4000–400 cm^{-1} using KBr discs. DSC measurements were performed using a Mettler Toledo DSC 821e instrument from 30°C to 300°C at 10°C/min under nitrogen. PXRD patterns were collected using Cu radiation at 40 kV/40 mA over 2θ = 5–70°.

3.13 Stability study

The selected F3 lyophilized formulation was packed in hard gelatin capsules and sealed in aluminium packaging coated internally with polyethylene. Samples were stored at 4°C, 27°C and 45 ± 2°C/75% RH. Drug content was evaluated at 0, 15, 30, 45, 60 and 90 days.

4. RESULTS AND DISCUSSION

4.1 Preformulation and UV analysis

The drug was described as a white crystalline powder. Its melting range was 132–136°C and the absorption maximum was 238 nm. Calibration curves were reported to be linear over 2–10 µg/mL in both media. The regression coefficients were 0.997 in 0.1 N HCl and 0.996 in pH 7.0 phosphate buffer, supporting the use of the selected wavelength for the reported assay and dissolution measurements.

Table 2. Calibration characteristics reported for simvastatin at 238 nm.

Medium	Concentration range (µg/mL)	Regression equation	R^2
0.1 N HCl	2–10	y = 0.032x	0.997
Phosphate buffer pH 7.0	2–10	y = 0.061x	0.996

4.2 Particle size and PDI

The reported mean particle sizes ranged from 92.6 to 281.6 nm. F3 exhibited the smallest reported mean particle size (92.6 nm), followed by F2 (116.6 nm) and F6 (131.1 nm). The PDI values ranged from 0.026 to 0.523, with F5 showing the lowest reported PDI (0.026) and F2 the highest (0.523). The broad PDI for F2 indicates a comparatively wider size distribution and should be considered when interpreting physical uniformity.

Table 3. Particle size and PDI values transcribed from Table 5 of the dissertation.

Formulation	Particle size (nm)	PDI
F1	281.6	0.167
F2	116.6	0.523
F3	92.6	0.167
F4	138.3	0.126
F5	190.2	0.026
F6	131.1	0.301

A unit discrepancy is present in the narrative preceding the table: the text refers to particle sizes in µm, whereas Table 5 labels the values in nm. The tabulated unit (nm) is retained here, but the original instrument report should be checked before submission.

4.3 Zeta potential

The dissertation reports zeta potentials of −38.5 mV for F3 and −37.1 mV for F6. These magnitudes indicate substantial electrostatic repulsion between similarly charged particles and are consistent with the authors' interpretation of favourable colloidal stability. Numerical values for F1, F2, F4 and F5 were not provided in the extracted results table and are therefore not inferred.

4.4 Saturation solubility

Table 4. Saturation solubility values reported in the dissertation. *The source prints F6 as "131..2"; formatted as 131.2 for readability and requires confirmation.

Sample	0.1 N HCl (µg/mL)	Phosphate buffer pH 7.0 (µg/mL)
Pure drug	20.49	46.7
F1	141.50	181.84

F2	156.62	289.84
F3	177.50	317.6
F4	90.12	143.4
F5	124.50	179.0
F6	131.2*	218.0

All nanosuspension formulations showed higher reported solubility than pure simvastatin in both media. F3, containing 1.5% poloxamer 188, showed the highest values: 177.50 µg/mL in 0.1 N HCl and 317.6 µg/mL in pH 7.0 phosphate buffer. The improvement may reflect particle-size reduction, improved wetting and stabilizer-associated solubilization. The contribution of micellar solubilization cannot be separated from the particle-size effect using the reported data alone.

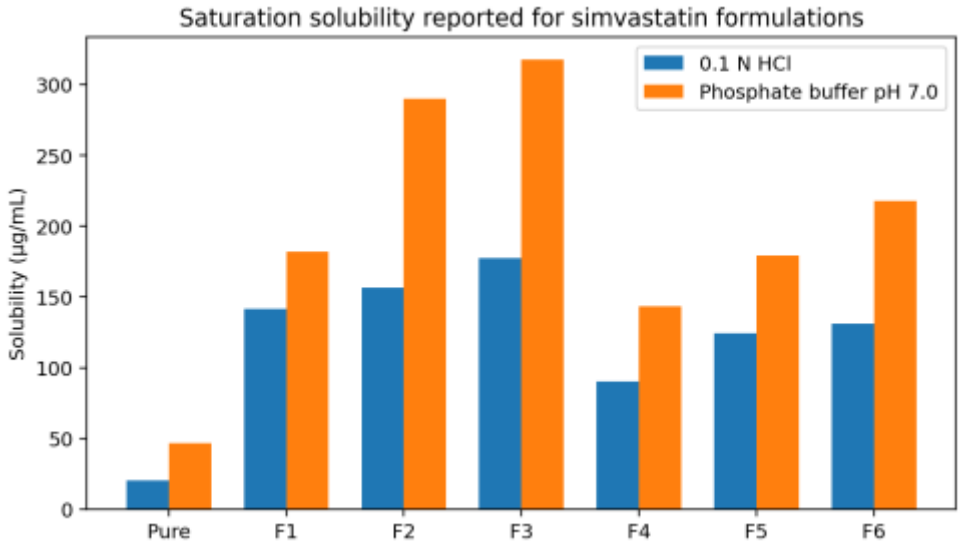


Fig 1: Comparative saturation solubility of pure simvastatin and F1–F6 in the two reported media.

4.5 Drug content

Table 5. Drug content of lyophilized nanosuspensions (reported mean ± SD).

Formulation	Drug content (%)
F1	98.52 ± 0.04
F2	98.97 ± 0.05
F3	99.63 ± 0.05
F4	98.13 ± 0.04
F5	98.79 ± 0.02
F6	99.16 ± 0.01

Drug content ranged from 98.13 ± 0.04% to 99.63 ± 0.05%. F3 showed the highest drug content, while all formulations were reported within the expected assay range in the dissertation. The low reported standard deviations suggest consistent assay results across the three observations.

4.6 SEM morphology

The dissertation describes pure simvastatin as large, irregular crystalline particles. Lyophilized nanosuspension samples were described as having smoother surfaces and smaller, more uniform or globular features. The authors attributed the altered surface appearance to adsorption of stabilizer onto the drug-crystal surface and formation of a stabilizer-rich layer during lyophilization. SEM images are included in the original thesis (pages 62–71); the original high-resolution micrographs should be supplied as figures in the journal submission rather than redrawn.

Representative scanning electron micrographs reproduced from the original dissertation are shown below. Original figure identifiers are retained.

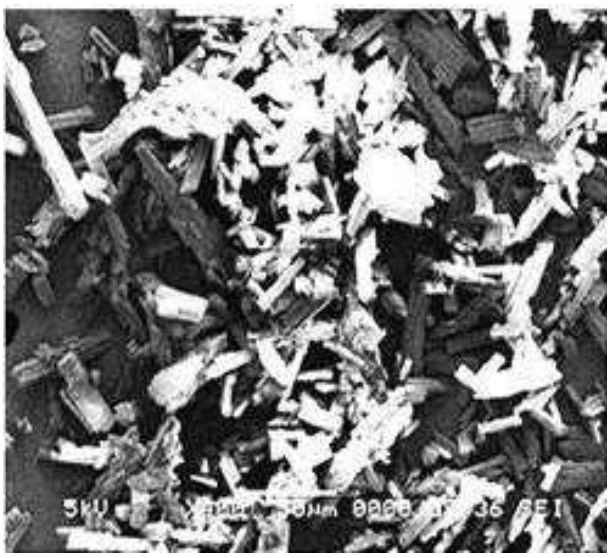


Fig 16: SEM microphotograph of pure simvastatin.

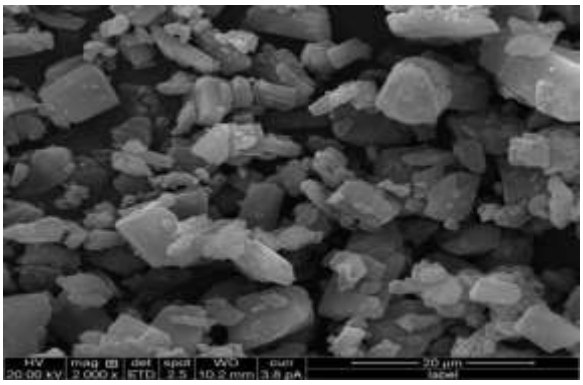


Fig 17A: Lyophilized nanosuspension F1 (20 µm).

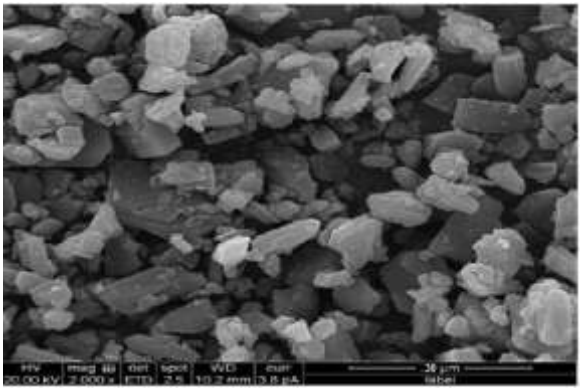


Fig 17B: Lyophilized nanosuspension F1 (30 µm).

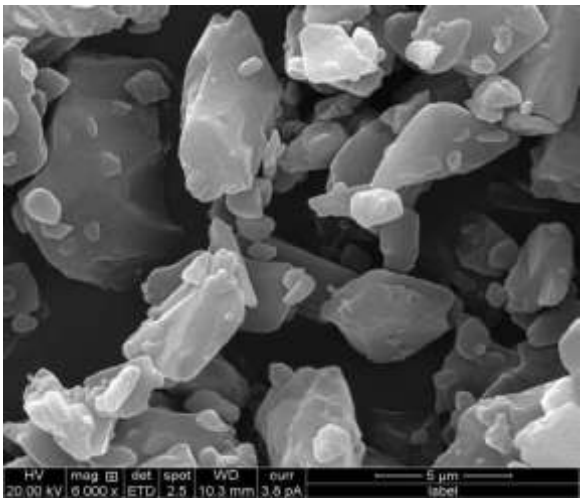


Fig 17C: Lyophilized nanosuspension F1 (5 µm).

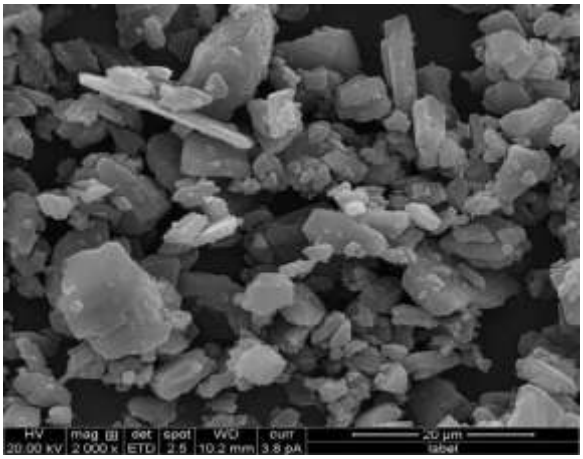


Fig 18A: Lyophilized nanosuspension F2 (20 µm).

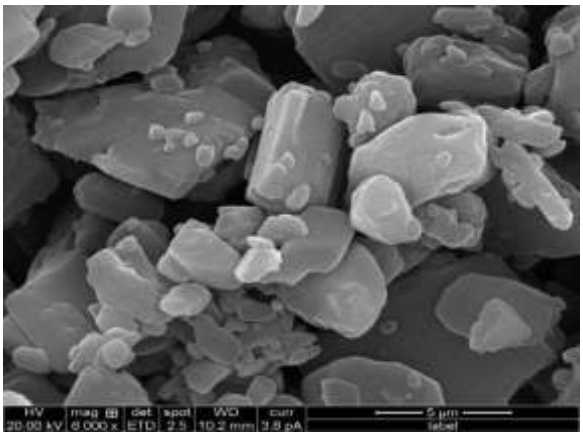


Fig 18B: Lyophilized nanosuspension F2 (5 µm).

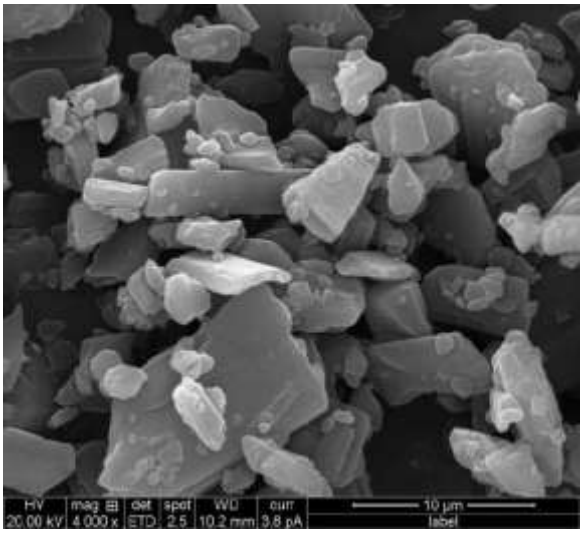


Fig 18C: Lyophilized nanosuspension F2 (10 µm).

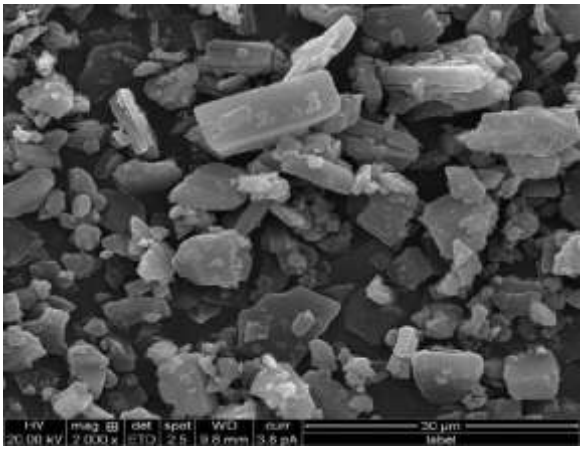


Fig 19A: Lyophilized nanosuspension F3 (30 µm).

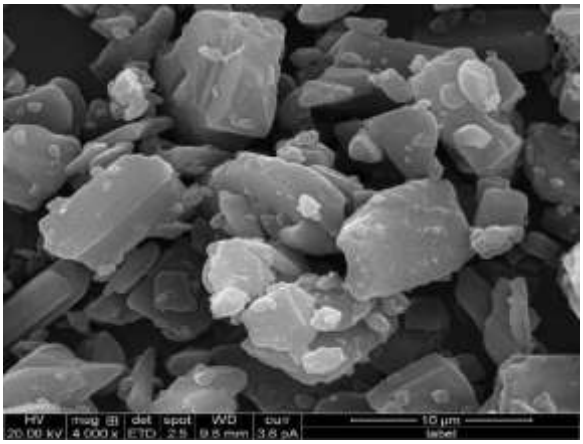


Fig 19B: Lyophilized nanosuspension F3 (10 µm).

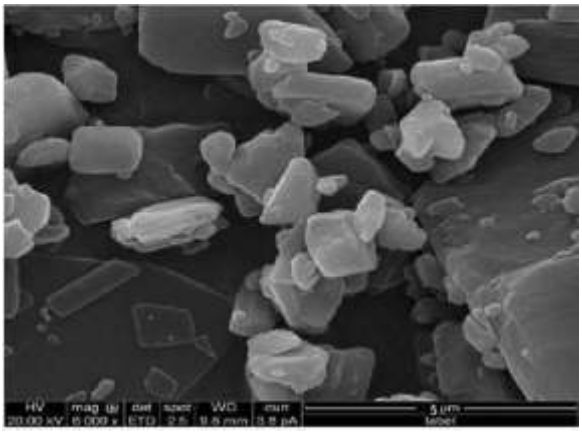


Fig 19C: Lyophilized nanosuspension F3 (5 µm).

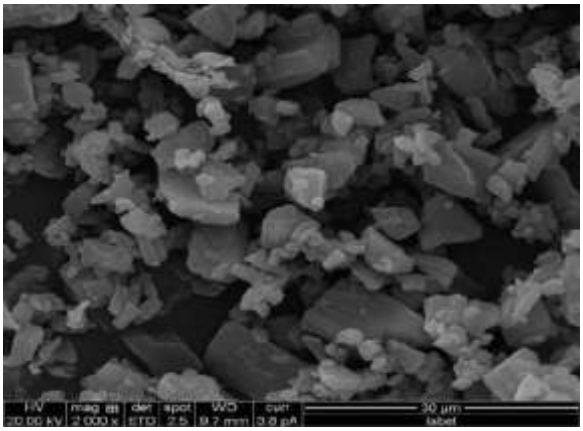


Fig 20A: Lyophilized nanosuspension F4 (30 µm).

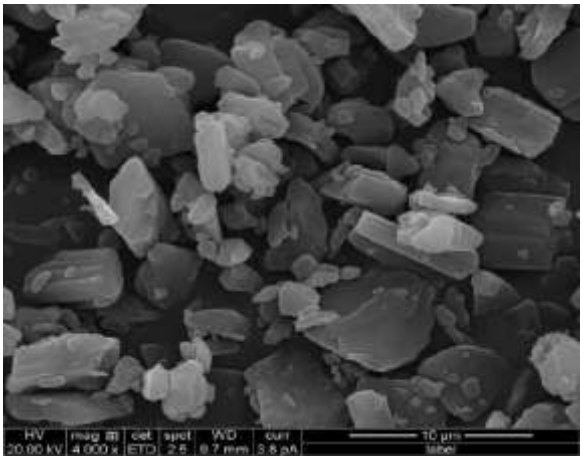


Fig 20B: Lyophilized nanosuspension F4 (10 µm).

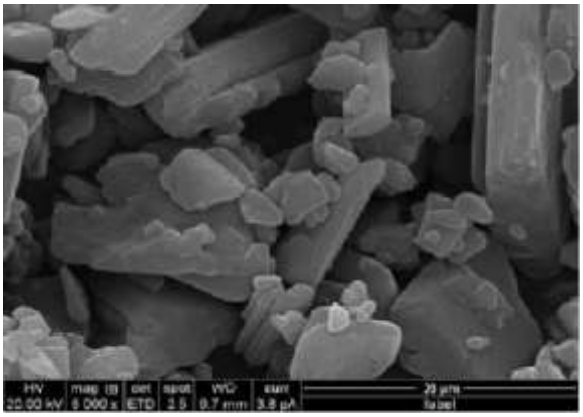


Fig 20C: Lyophilized nanosuspension F4 (20 µm).

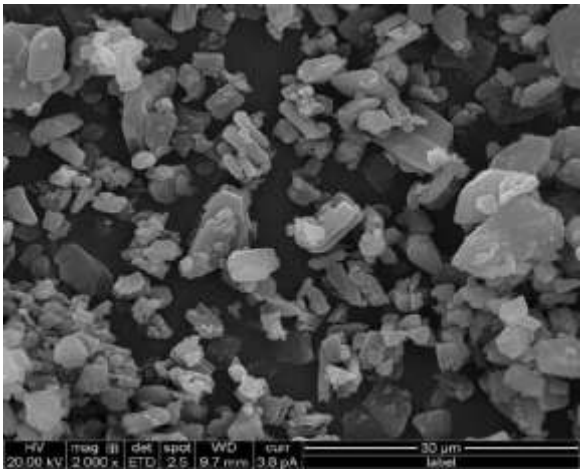


Fig 21A: Lyophilized nanosuspension F5 (10 µm).

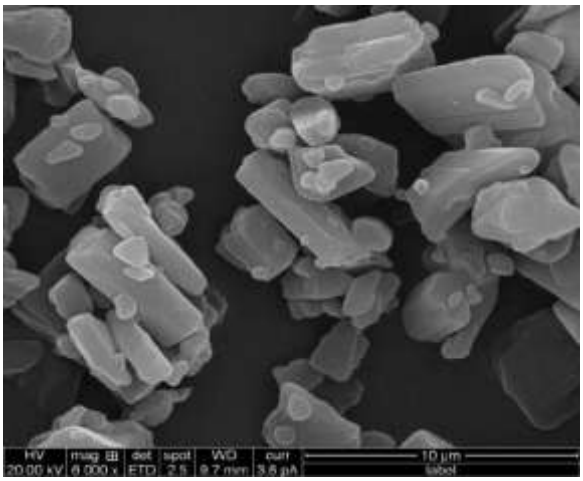


Fig 21B: Lyophilized nanosuspension F5 (30 µm).

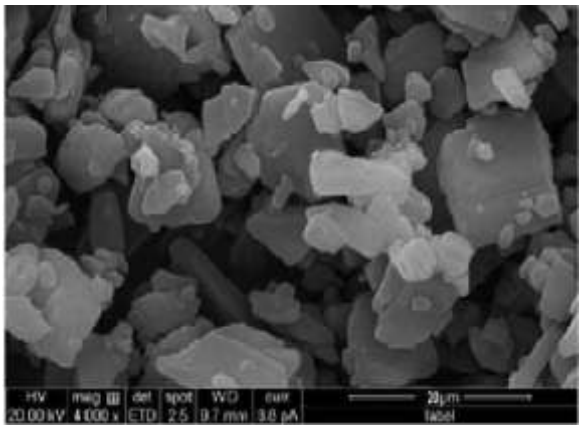


Fig 21C: Lyophilized nanosuspension F5 (20 µm).

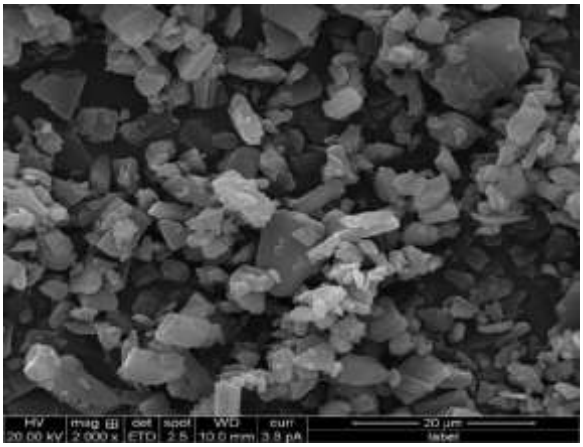


Fig 22A: Lyophilized nanosuspension F6 (20 µm).

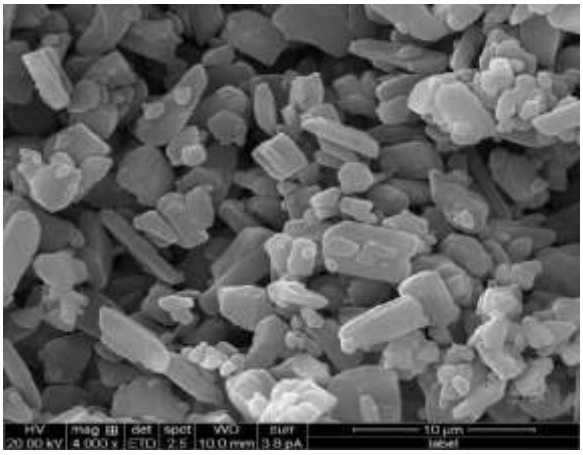


Fig 22B: Lyophilized nanosuspension F6 (10 µm).

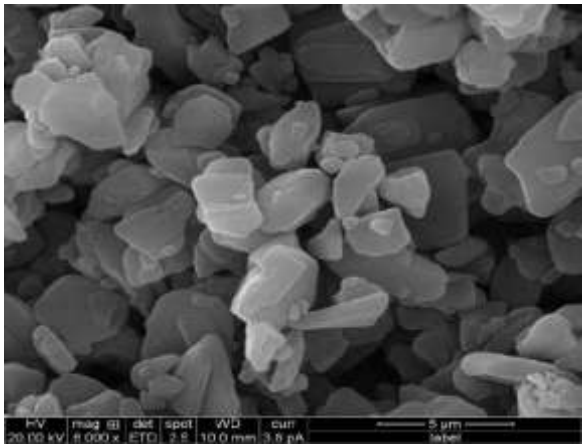


Fig 22C: Lyophilized nanosuspension F6 (5 µm).

Note: The original thesis labels the image scales as 5, 10, 20 and 30 µm. These annotations are reproduced as supplied; confirm the scale-bar interpretation from the original SEM acquisition files before submission.

4.7 FTIR compatibility

The major FTIR bands reported for pure simvastatin included 3549.14 cm^{-1} (free O–H stretching), 3010.98, 2968.55 and 2955.04 cm^{-1} (C–H stretching), 1697.41 cm^{-1} (lactone carbonyl-related band), and bands at 1467.88, 1460.16, 1390.72, 1369.50 and 1267.27 cm^{-1}. The lyophilized formulations retained characteristic drug-associated bands without marked shifts or disappearance, which the dissertation interpreted as absence of detectable drug–stabilizer interaction under the test conditions.

4.8 DSC and PXRD

The dissertation reports a sharp endothermic event for pure simvastatin at 135.66°C. The characteristic melting peak was not observed in the lyophilized nanosuspension thermograms, which was interpreted as reduced crystallinity or an amorphous/molecularly dispersed drug state. PXRD of pure simvastatin showed sharp diffraction peaks at 2θ values of 5.31°, 10.66°, 16.04° and 21.47°. The lyophilized nanosuspension patterns for F3 and F6 showed reduced or absent sharp peaks, suggesting a decrease in detectable crystallinity. These interpretations should be supported by the original DSC/PXRD traces and appropriate quantitative analysis.

4.9 In-vitro dissolution in phosphate buffer pH 7.0

In phosphate buffer pH 7.0 containing 0.5% sodium lauryl sulphate, pure simvastatin released 29.06 ± 0.48% at 60 min. The lyophilized nanosuspensions showed substantially higher release, ranging from 87.67 ± 0.59% (F4) to 99.14 ± 0.19% (F3) at 60 min. F3 showed the highest release at the 60-min endpoint in Table 11.

Table 6. In-vitro dissolution of lyophilized nanosuspensions in phosphate buffer pH 7.0 (mean ± SD; n = 3), transcribed from Table 11.

Time (min)	Pure	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	7.22 ± 0.03	34.08 ± 0.43	35.41 ± 0.15	36.15 ± 0.06	33.78 ± 0.15	34.08 ± 0.42	35.26 ± 0.46
20	10.18 ± 0.40	45.73 ± 0.43	50.21 ± 0.76	55.32 ± 0.32	44.11 ± 0.80	47.36 ± 0.42	51.63 ± 0.13
30	15.63 ± 0.42	61.81 ± 0.75	64.17 ± 0.41	68.01 ± 0.70	56.23 ± 0.26	61.84 ± 0.36	65.58 ± 0.60
40	20.65 ± 1.40	73.64 ± 0.83	75.54 ± 0.34	79.53 ± 0.13	69.34 ± 0.21	73.94 ± 0.93	77.45 ± 0.31
50	24.34 ± 0.55	84.57 ± 0.52	86.45 ± 0.33	90.14 ± 0.66	79.85 ± 0.20	83.83 ± 0.21	88.22 ± 0.78
60	29.06 ± 0.48	90.32 ± 0.28	93.71 ± 0.50	99.14 ± 0.19	87.67 ± 0.59	89.92 ± 0.22	91.54 ± 0.32

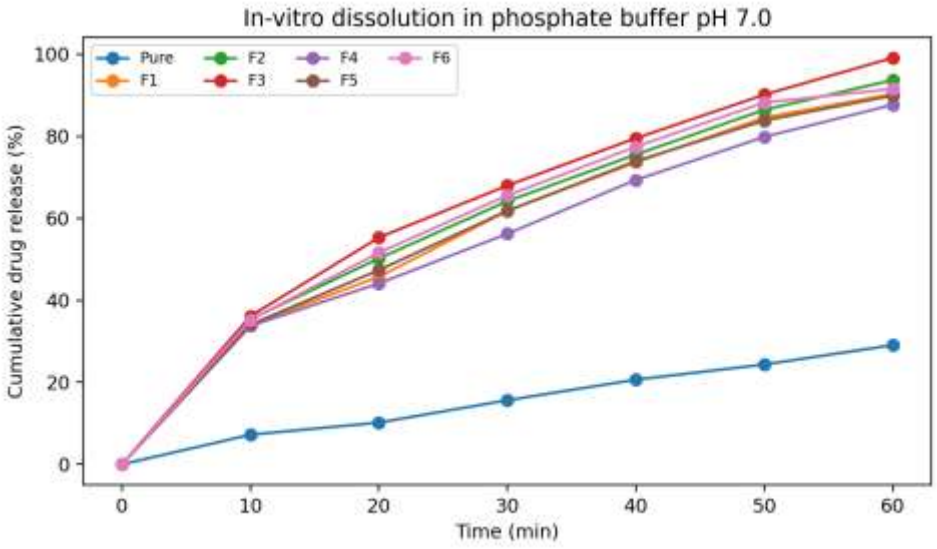


Fig 2: Comparative in-vitro dissolution profiles in phosphate buffer pH 7.0, plotted from the mean values in Table 11.

The increased dissolution may be associated with the greater effective surface area of the nanosized drug particles and improved wetting in the presence of stabilizers. However, the source thesis contains an internal discrepancy: the narrative on page 87 attributes 91.54% to F3 and 99.14% to F6, whereas Table 11 assigns 99.14% to F3 and 91.54% to F6. This manuscript follows the numerical table and flags the narrative discrepancy for author verification.

4.10 Dissolution in 0.1 N HCl

Table 7. In-vitro dissolution of lyophilized nanosuspensions in 0.1 N HCl (mean ± SD; n = 3), transcribed from Table 10.

Time (min)	Pure	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	3.40 ± 0.31	9.06 ± 0.13	8.92 ± 0.13	11.89 ± 0.33	8.49 ± 0.37	10.76 ± 0.28	11.60 ± 0.20
20	6.23 ± 0.45	13.87 ± 0.22	14.44 ± 0.54	17.27 ± 0.30	12.73 ± 0.28	14.15 ± 0.66	15.29 ± 0.21
30	8.78 ± 0.45	20.10 ± 0.50	23.21 ± 0.17	25.76 ± 0.34	18.96 ± 0.30	21.51 ± 0.08	25.19 ± 0.25
40	9.91 ± 0.15	29.16 ± 0.24	30.85 ± 0.28	31.99 ± 0.28	27.45 ± 0.80	30.57 ± 0.25	31.14 ± 0.24
50	11.89 ± 0.22	36.52 ± 0.52	37.08 ± 0.06	39.35 ± 0.25	33.67 ± 0.31	35.10 ± 0.08	36.52 ± 0.62
60	14.47 ± 0.07	40.20 ± 0.13	42.46 ± 0.38	46.71 ± 0.64	38.77 ± 0.25	41.61 ± 0.43	44.16 ± 0.80

At 60 min in 0.1 N HCl, F3 showed the highest reported release (46.71 ± 0.64%), followed by F6 (44.16 ± 0.80%) and F2 (42.46 ± 0.38%). All formulations released more drug than pure simvastatin (14.47 ± 0.07%) at the same time point.

4.11 Dissolution of physical mixtures

The dissertation also reports a separate table for physical mixtures in pH 7.0 medium. At 60 min, the reported values were 46.32 ± 0.24% (F1), 53.1 ± 0.21% (F2), 61.8 ± 0.82% (F3), 43.67 ± 0.18% (F4), 51.78 ± 0.19% (F5) and 60.49 ± 0.47% (F6), compared with 29.06 ± 0.48% for pure drug. These physical-mixture results are distinct from the lyophilized nanosuspension results and should not be combined.

4.12 Stability study

F3 was selected for stability evaluation. Drug content decreased from 100% at day 0 to 98.72 ± 0.054% at day 90 under 4°C, 99.15 ± 0.054% at 27°C and 98.62 ± 0.056% at 45 ± 2°C/75% RH. The source reports no significant change in drug content during the 90-day observation period.

Table 8. Stability study of F3 expressed as residual drug content (%), transcribed from Table 12.

Day	4°C	27°C	45 ± 2°C / 75% RH
0	100 ± 0.00	100 ± 0.00	100 ± 0.00
15	99.92 ± 0.032	99.97 ± 0.045	99.73 ± 0.019
30	99.77 ± 0.050	99.46 ± 0.033	99.16 ± 0.023
45	99.60 ± 0.011	99.27 ± 0.030	99.12 ± 0.063
60	99.21 ± 0.043	99.16 ± 0.041	99.25 ± 0.34
90	98.72 ± 0.054	99.15 ± 0.054	98.62 ± 0.056

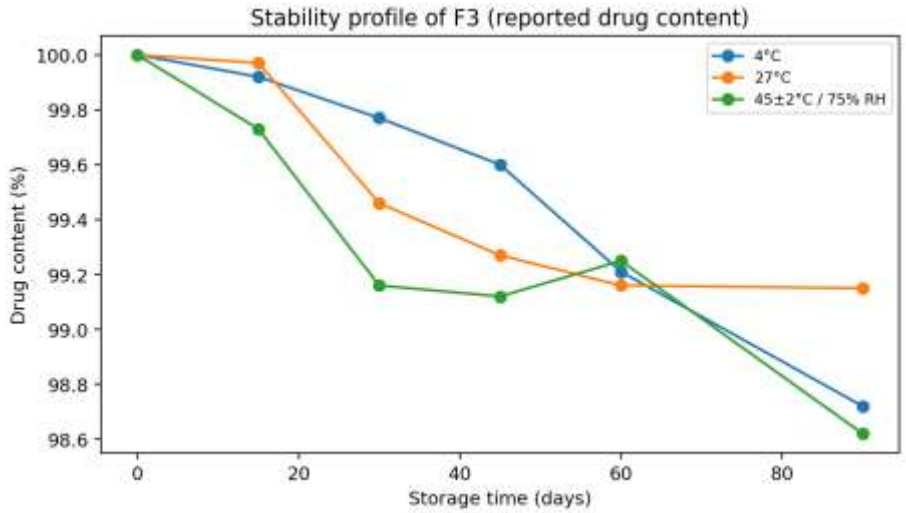


Fig 3: Drug-content profile of F3 during storage at the three reported conditions.

5. CONCLUSION

Simvastatin nanosuspensions were prepared using high-pressure homogenization with poloxamer 188 or poloxamer 407 as stabilizers. Among the tested batches, F3 (5% w/v simvastatin with 1.5% poloxamer 188) showed the smallest reported particle size (92.6 nm), highest saturation solubility in both tested media, highest drug content (99.63 ± 0.05%) and highest 60-min dissolution value in the pH 7.0 dissolution table (99.14 ± 0.19%). F3 also retained 98.72 ± 0.054% drug content after 90 days at 4°C. These results support the potential of poloxamer-stabilized nanosuspension as an in-vitro dissolution-enhancement approach for simvastatin. The study did not report in-vivo pharmacokinetic or bioavailability experiments; therefore, improved oral bioavailability remains a hypothesis requiring further investigation.

6. LIMITATIONS AND DATA VERIFICATION REQUIRED

- Particle-size narrative gives values in µm, while the corresponding table labels them as nm. Verify against the original analyser printouts.
- The PDI narrative states a range of 0.227–0.301, but Table 5 contains values from 0.026 to 0.523. Table values are reproduced pending confirmation.
- The dissolution narrative swaps the F3 and F6 60-min pH 7.0 release values; Table 11 is used in this manuscript.
- The dissolution method mentions pH 7.0 phosphate buffer with 0.5% sodium lauryl sulphate, while the table heading only says phosphate buffer pH 7.0. Confirm whether SLS was present for Table 11.
- The F6 solubility entry is printed as “131..2” in the source. It is displayed as 131.2 µg/mL but requires checking against laboratory records.

- The thesis contains inconsistencies in SEM instrument model identifiers and DSC figure numbering. Confirm figure labels and instrument details before final submission.
- The source reports 90-day stability for F3; this does not establish a commercial shelf life or full ICH stability profile.
- No animal pharmacokinetic study or human bioavailability study is reported. Do not claim demonstrated bioavailability improvement.

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CONFLICT OF INTEREST

The source dissertation did not provide a conflict-of-interest statement. The authors should confirm and insert the appropriate declaration.

AUTHOR CONTRIBUTIONS

To be completed and approved by the authors according to their actual contributions.

DATA AVAILABILITY

The data presented in this manuscript are derived from the M.Pharm dissertation supplied for manuscript preparation. Original instrument outputs and raw datasets should be retained by the authors and made available where required by the journal.

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