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Research Article

Design and evaluation of polymeric micelles to enhance piperine dissolution

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ABSTRACT

Piperine, a bioactive compound derived from pepper fruit, exhibits anticancer potential but suffers from poor aqueous solubility, which limits its therapeutic application. This study aimed to develop polymeric micelles to enhance piperine release. Micelles were prepared using the thin-film hydration method, and seven formulations were initially evaluated based on particle size, polydispersity index (PDI), zeta potential, morphology, and entrapment efficiency. Quantification of piperine was performed using a validated reversed-phase high-performance liquid chromatography (RP-HPLC) method. Selected formulations underwent further testing, including release studies at pH 1.2 and 6.8, and stability assessments at 30°C and 40°C under 75% relative humidity (RH). The optimal formulation (F4, piperine: total polymer = 1:12.5 w/w; P123:F127 = 2:1 w/w) exhibited a particle size of 26 nm, a PDI of 0.0382, a zeta potential of -6.20 mV, a spherical morphology, and an entrapment efficiency of 6.20%. Release study revealed a 5.75-fold and a 4.55-fold increase in piperine release at pH 1.2 and pH 6.8, respectively, compared to pure piperine suspension. Stability study showed 40% and 47% reductions in piperine content after 3 months at 30°C and 40°C, respectively. These findings demonstrate that polymeric micelles are a promising nanocarrier system for improving the release profile of piperine.

Keywords:
Polymeric micelles; Piperine; Release enhancement; Drug delivery; Pluronic

1. INTRODUCTION

Piperine, a bioactive alkaloid found in black pepper (*Piper nigrum* L.), has attracted considerable attention due to its diverse pharmacological activities, including antioxidant, anti-inflammatory, and anticancer properties[1,2,3]. The anticancer mechanism of piperine involves inducing apoptosis[4,5] and inhibiting cancer cell proliferation[6], with the added advantage of being selectively toxic to cancer cells[7]. However, the clinical application of piperine is severely limited by its poor aqueous solubility and, consequently, poor release in biological fluids, leading to low oral bioavailability[8].

To overcome this limitation, various nanocarrier systems have been explored. Among them, polymeric micelles are particularly promising due to their unique core-shell architecture, which arises from the self-assembly of amphiphilic block copolymers in aqueous media. When the concentration of these copolymers exceeds the critical micelle concentration (CMC), their hydrophobic segments (e.g., poly(propylene oxide), PPO) associate to form an inner core, while the hydrophilic segments (e.g., poly(ethylene oxide), PEO) extend outward to form a stabilizing corona. This arrangement enables entrapment of poorly soluble hydrophobic drugs within the inner core, thereby increasing their apparent solubility in aqueous media. Simultaneously,

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the hydrophilic corona provides steric stabilization, preventing micelle aggregation and prolonging circulation time in the bloodstream[9,10]. The clinical potential of polymeric micelles has been demonstrated by several approved formulations, such as Genexol-PM (paclitaxel-loaded micelles) for the treatment of breast, lung, and ovarian cancers[10].

Among the various polymers, Pluronic block copolymers (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), PEO-PPO-PEO) have proven effective for micelle formation. A well-known example is SP1049C, which consists of doxorubicin entrapped in a binary mixture of Pluronic F127 and L61, and was the first anticancer polymeric micelle to enter clinical trials[11]. Combining two Pluronics and multi-polymer micelles, one more hydrophobic and one more hydrophilic, can optimise micelle stability and drug loading capacity[12,13,14,15]. In this study, a binary mixture of Pluronic P123 and Pluronic F127 in a 2:1 weight ratio was selected. This ratio was chosen based on the work of Fares et al.[16], who demonstrated that a 2:1 ratio of P123:F127 provided the optimal balance between drug affinity (contributed by the more hydrophobic P123) and micelle stability (conferred by the more hydrophilic F127). Furthermore, the same P123/F127 (2:1) system has been successfully utilised to entrap isoliquiritigenin, resulting in improved solubility, enhanced stability, and greater oral bioavailability[17]. Therefore, this study aims to develop piperine-loaded polymeric micelles using a Pluronic P123/F127 (2:1) binary system and to evaluate their particle size, polydispersity index, zeta potential, morphology, entrapment efficiency, *in vitro* release, release kinetics, and stability, in order to identify an optimal formulation for enhancing piperine release.

2. MATERIALS AND METHODS

2.1. Materials

Piperine, Pluronic F127, and Pluronic P123 were purchased from Sigma-Aldrich (St. Louis, MO, USA), and distilled water was purchased from Ikapharmindo (Jakarta, Indonesia). Absolute ethanol, methanol for chromatography, acetonitrile, hydrogen chloride, potassium chloride, sodium dihydrogen phosphate, sodium hydroxide, iodine, and potassium iodide were purchased from Merck (Darmstadt, Germany).

2.2. Critical micelle concentration (CMC) determination

An iodine-visible ultraviolet spectroscopy method, using iodine as a hydrophobic probe, was used to determine the CMC of polymers. KI/I_2 standard solution was prepared by dissolving 1 gram of potassium iodide and 0.5 gram of iodine in 50 mL of distilled water. Furthermore, polymer solutions with concentrations ranging from 0.000001% to 1% were prepared. A total of 25 µL of KI/I_2 standard solution was added to each of P123/F127 polymer solutions, which were then equilibrated in the dark at room temperature for 12 hours. The absorbance of the solution was measured at 366 nm using a UV-Vis spectrometer (Shimadzu, Japan). To determine the CMC, the absorbance was plotted against the logarithm of the pluronic concentration. A sharp increase in absorbance characterised CMC values[18].

2.3. Validation of analysis methods

Chromatography was performed using a Waters Alliance 2695 HPLC system (Waters, Milan, Italy), with a Zorbax Eclipse XDB C18 column (150 mm × 4.6 mm; 5 µm; Agilent, Santa Clara, CA, USA), and a mobile phase of methanol and water (75:25, v/v), which was maintained at 25°C and a flow rate of 1.0 mL/min. The HPLC method was validated in accordance with the AOAC International guidelines[19]. The following parameters were evaluated: linearity, accuracy, intra-day precision, limit of detection (LOD), limit of quantification (LOQ), system suitability, and selectivity. Linearity was assessed across concentrations from 0.05 to 10.00 µg/mL. Intra-day precision was evaluated by analyzing three replicates (n=3) at each concentration level on the same day. System suitability and selectivity were also confirmed. Full validation data are provided in Supplementary Materials (Tables S1–S2, Figure S1).

2.4. Preparation of piperine polymeric micelles

The composition of the piperine polymeric micelle formulas used in this study is presented in Table 1. Polymeric micelles were prepared using the thin film hydration method by dissolving piperine in 20 mL of anhydrous ethanol, Pluronic P123 in 40 mL of anhydrous ethanol, and Pluronic F127 in 40 mL of anhydrous ethanol. The solution was transferred into a round-bottom flask, and the solvent was evaporated using a rotary evaporator (R-300, Buchi, Flawil, Switzerland) at 50°C and 96 rpm. The thin film was dried in a fume hood overnight and then hydrated with 100 mL of distilled water. It was then spun in a rotary evaporator at 50°C without vacuum and sonicated (Bronsonic, Branson, Danbury, CT, USA) for 5 minutes. Unincorporated piperine aggregates and crystals were removed by filtration through a 0.22 µm cellulose acetate filter membrane (Sartorius, Göttingen, Germany) to obtain a clear polymeric micelle[16,18]. The filtrated polymeric micelles were adjusted to a final volume of 100 mL with distilled water. The clear polymeric micelles were used for all further evaluations.

Table 1. Composition of piperine polymeric micelles.

No	Material piperine: polymer	F1 1:5	F2 1:7.5	F3 1:10	F4 1:12.5	F5 1:15	F6 1:17.5	F7 1:20
1	Piperine (mg)	400	400	400	400	400	400	400
2	Pluronic P 123 (mg)	1335	2000	2670	3333	4000	4667	5333
3	Pluronic F 127 (mg)	665	1000	1330	1667	2000	2333	2667
4	Aquades (mL)	100	100	100	100	100	100	100

*Polymers are a mixture of Pluronic P123 and Pluronic F127 at a weight ratio of 2:1.

2.5. Evaluation of piperine polymeric micelles

2.5.1. Particle size, polydispersity index (PDI), and zeta potential testing

Particle size distribution was determined by dynamic light scattering using a Particle Size Analyser (Microtrac, Montgomeryville, PA, USA) at 25°C. To preserve the native micelle structure, samples were analysed without dilution. An aliquot of 1 mL of piperine-loaded polymeric micelle solution was transferred directly into the measurement cell. The reported particle size corresponds to the intensity-weighted mean hydrodynamic diameter (Z-average), a standard parameter derived from dynamic light scattering for polydisperse colloidal systems. In addition to particle size, the polydispersity index and zeta potential were also obtained from the same measurement[20].

2.5.2. Morphological testing with Transmission Electron Microscopy (TEM)

The particle morphology of piperine polymeric micelles was evaluated using TEM (JEM-1400, JEOL, Japan). A drop of piperine polymeric micelles was placed on a carbon-coated copper grid, forming a thin film that was negatively stained with 2% uranyl acetate. The residual was allowed to dry in ambient air, and TEM was used to observe the morphology of piperine polymeric micelles.

2.5.3. Determination of piperine content in the polymeric micelles and entrapment efficiency

The filtered, clear polymeric micelle (obtained after filtration through a 0.22 µm membrane, as described in Section 2.4) was used to determine the piperine content and entrapment efficiency. A 0.5 mL of piperine polymeric micelles was mixed with methanol in a 5.0 mL volumetric flask and vortexed for 2 minutes to break the polymeric micelles. Afterwards, 0.1 mL solution was pipetted and mixed with the mobile phase in a 10.0 mL volumetric flask. Subsequently, the solution was filtered through a 0.45 µm syringe filter (Sartorius, Göttingen, Germany) into an autosampler vial and then injected into the HPLC. Piperine content was determined as the amount of piperine obtained from the polymeric micelles and reported as a weight/volume percentage (% w/v). In addition, the entrapment efficiency (EE) was also determined by calculating the percentage of piperine in the filtered polymeric micelles compared to the percentage of piperine initially weighed for the formulation.

$$\text{Piperine content after filtration (\%)} = \frac{\text{mass of piperine obtained from the polymeric micelles (g)}}{\text{total volume of polymeric micelles (100 mL)}} \times 100$$

$$\text{Entrapment Efficiency (\%)} = \frac{\text{total amount of piperine in filtered polymeric micelles}}{\text{total amount of piperine initially added}} \times 100$$

2.5.4. In vitro release study of piperine polymeric micelles

The release test was conducted on piperine polymeric micelles F3, F4, F5, and F6, and on a piperine suspension as a control. First, a 0.5% CMC-Na solution was prepared by hydrating 0.25 g of CMC-Na in 5 mL of warm water at 60°C for 30 min, grinding it, and adjusting the volume to 50 mL with distilled water. Then, a piperine suspension was prepared by dispersing 200 mg of piperine in 50 mL of CMC-Na solution, yielding a concentration of 4 mg/mL (0.4% w/v). Two media were employed: pH 1.2 (0.1 M HCl) and pH 6.8 (phosphate buffer). An aliquot (2 mL) of the filtered clear polymeric micelles (containing around 0.48 mg of piperine based on assay results) was placed in a dialysis bag (MWCO 4.5 kDa, Sigma Aldrich, St. Louis, MO, USA) and immersed in 100 mL of release medium at 37°C under orbital shaking (Yihder, New Taipei City, Taiwan) at 100 rpm. A sample withdrawal (2 mL) was performed at 5, 10, 15, 30, 45 min, 1, 3, 4, 5, 6, 7, 24, and 48 h, with the withdrawn volume replaced by an equal volume of fresh medium to maintain constant volume.

The 48-h sampling window was determined from preliminary experiments showing that piperine release from polymeric micelles continued for more than 24 h and then reached a stable plateau, consistent with piperine's extremely low aqueous solubility. Piperine concentrations in the withdrawn samples were quantified by HPLC[16,18]. Piperine has an aqueous solubility of 40 mg/L[21], which is equivalent at pH 1.2 and 6.8 due to its non-ionized nature (pKa ~11-12). Based on the assay, the actual piperine in the 2 mL sample was 0.48 mg, which would require a minimum of 12 mL to dissolve (0.48/0.04). According to USP, sink conditions require ≥ 3× the minimum volume (36 mL). As we used 100 mL (>36 mL), sink conditions were maintained.

2.5.5. Release kinetics analysis

The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models using linear regression in Microsoft Excel (Microsoft, Redmond, WA, USA). The best-fit model was selected based on the highest correlation coefficient (R). The Korsmeyer-Peppas exponent (n) was used to determine the release mechanism: $n \leq 0.43$ indicates Fickian diffusion (Case I transport); $0.43 < n < 0.85$ indicates anomalous (non-Fickian) transport; $n \geq 0.85$ indicates Case II transport (swelling-controlled).

2.5.6. Stability study of piperine polymeric micelles

Stability testing was conducted on piperine polymeric micelles F3, F4, F5, and F6 by storing the samples in a climatic chamber under two storage conditions: 30°C ± 2°C / 75% RH ± 5% RH (B-One, Taichung, Taiwan) and 40°C ± 2°C / 75% RH ± 5% RH (Climacell, MMM Group, Munich, Germany). Tests were conducted at weeks 4, 8, and 12, with testing parameters including piperine content, particle size, polydispersity index, and zeta potential. The initial piperine content of the formulations tested in the stability study (F3–F6) ranged from 2.49% to 3.88% w/v (equivalent to 24.9–38.8 mg/mL), with F4 showing 2.49% w/v (24.9 mg/mL). The results in Figure 4 are expressed as the percentage of piperine remaining relative to the initial content at week 0 (set as 100%).

2.5.7. Statistical analysis

All measurements were performed in triplicate (n=3) unless otherwise stated, and data are presented as mean ± standard deviation (SD). For release study data (Figure 3), unpaired t-tests were performed to compare F3 and F4 at each time point, and two-way ANOVA was used to evaluate differences between groups. A p-value < 0.05 was considered statistically significant. The similarity factor (f_2) was calculated to compare release profiles of F3 and F4 using the formula: $f_2 = 50 \log \{ [1 + (1/n) \sum (R_t - T_t)^2]^{-0.5} \times 100 \}$. An f_2 value between 50 and 100 indicates similar profiles[22] Statistical analyses were performed using Microsoft Excel (Microsoft, Redmond, WA, USA).

3. RESULTS AND DISCUSSION

3.1. CMC determination

The CMC of the Pluronic mixture was determined to confirm the polymer's ability to form micelles[16,18]. The P123/F127 mixture (2:1) was tested across a concentration range of 0.000001% to 1%. A sharp increase in absorbance was observed at a CMC of 0.0794% (w/v), determined by the intersection of the baseline and rising-phase regression lines (Figure 1).

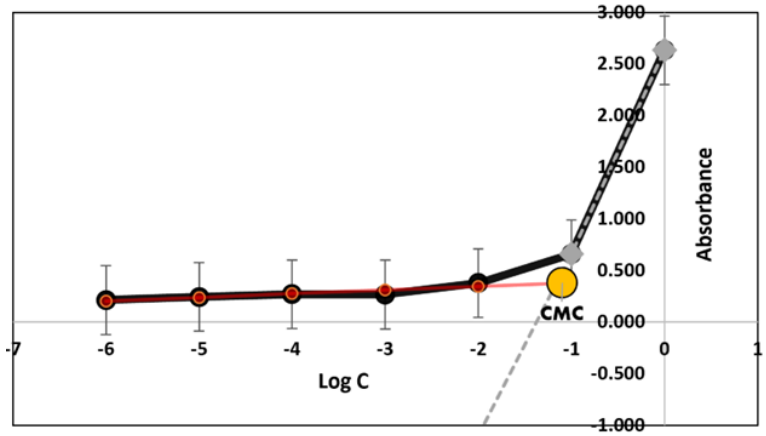


Figure 1. The plot of absorbance at 366 nm against the logarithm of the Pluronic P123/F127 (2:1) concentration. The CMC point (Log C = −1.1434, Y = 0.3743) has been clearly marked with a yellow circle and labeled as CMC = 0.0794%.

Table 2. Particle size, polydispersity index, and zeta potential (n=3).

Formula	Particle size (nm)	Poly dispersity index	Zeta potential (mV)
F1	54 ± 51	0.22 ± 0.30	12.93 ± 14.75
F2	26 ± 7	0.04 ± 0.01	-3.20 ± 5.20
F3	22 ± 1	0.04 ± 0.01	-0.20 ± 0.00
F4	26 ± 6	0.04 ± 0.01	-6.20 ± 0.00
F5	23 ± 1	0.04 ± 0.00	-0.07 ± 5.20
F6	88 ± 110	0.03 ± 0.03	-3.93 ± 5.20
F7	98 ± 126	0.03 ± 0.03	-0.07 ± 5.20

This low CMC reflects high micelle stability and resistance to dissociation upon dilution, a critical attribute for maintaining structural integrity under both *in vitro* and *in vivo* conditions[12,17]. The combination of Pluronic P123 and F127, which possess hydrophobic blocks of comparable length, yielded stable micelles with a narrow size distribution and small particle diameter[16]. Notably, the CMC value is inversely related to polymer hydrophobicity; hence, the greater the hydrophobicity, the lower the CMC[23].

3.2. Analytical method validation

Analytical method validation ensured that the analytical method was fit for purpose. The system suitability test (2.5 µg/mL piperine) showed a retention time of 3.901 min, an area of 338,342.7, a tailing factor of 1.13, theoretical plates of 4257.94, and an HETP of 0.0352, with RSD < 2%. Selectivity was confirmed by matching the retention times of the standard (3.846 min) and the test solution (3.852 min). The method was linear over the range of 0.05–10.00 µg/mL (R = 0.9996), with LOD = 0.35 µg/mL and LOQ = 1.16 µg/mL. Recovery values ranged from 81.40% to 91.63% (within 80–110%), with RSD < 2%, indicating good accuracy and precision. Full validation data are provided in Supplementary Materials (Tables S1–S2, Figure S1).

3.3. Characterisation of piperine polymeric micelles

Piperine polymeric micelles were successfully prepared using the thin-film hydration method. The particle size, polydispersity index and zeta potential are summarised in Table 2. In addition, the piperine content and the entrapment efficiency are showed in Table 3. All seven formulations had particle sizes below 100 nm, which is suitable for polymeric micelles. PDI values were < 0.3 (except for F1), indicating a homogeneous size distribution[24]. Zeta potential values were predominantly low, ranging from −6.20 to −3.20 mV, which is typical for nonionic Pluronic micelles[25]. Entrapment efficiency was below 10% for F1–F6, while F7 showed 12.55%, likely due to poor interactions between piperine and the hydrophobic PPO core[12,26,27]. Notably, F1 was the only formulation that exhibited a positive zeta potential (+12.93 mV). Although positive surface charge in Pluronic-based systems at near-neutral pH has been previously attributed to partial protonation of ether oxygen groups on the PEO chain[28] or association of cationic species with the PEO corona[29], the exceptionally high standard deviation (±14.75 mV) accompanying this measurement suggests substantial variability. Given that F1 contained the lowest polymer concentration (2% w/v), the observed positive charge is more likely attributable to a combination of PEO chain orientation at the micelle-water interface under dilute conditions and variability stemming from incomplete micelle formation and colloidal instability, rather than a genuine electrostatic surface charge effect. At elevated polymer concentrations (formulations F6 and F7), the marked increase in viscosity promotes the formation of larger colloidal aggregates, which consequently accounts for the observed larger particle sizes and the high standard deviations.

Table 3. Piperine content and entrapment efficiency (n=3).

Formula	Piperine content (%)	Entrapment efficiency (%)
F1	1.17 ± 0.33	2.93 ± 0.01
F2	1.42 ± 0.24	3.54 ± 0.01
F3	2.51 ± 0.70	6.26 ± 0.02
F4	2.49 ± 0.44	6.20 ± 0.01
F5	3.24 ± 0.71	8.09 ± 0.02
F6	3.88 ± 0.02	9.68 ± 0.02
F7	5.03 ± 0.08	12.55 ± 0.00

The particle-size distribution histograms for the selected formulations are presented in the Supplementary Materials (Figure S2). The particle-size distribution histograms (Figure S2) show unimodal distributions, with most particles within a narrow size range, confirming the low polydispersity index values (PDI < 0.3) reported in Table 2. The size distributions for F3, F4, F5, and F6 are unimodal, indicating good homogeneity and the absence of large aggregates or multiple populations.

TEM images (Figure 2) revealed predominantly spherical to slightly cylindrical morphologies. The less spherical shape observed in specific formulations, such as F1 (lowest polymer ratio, 1:5) and F6/F7 (highest polymer ratios, 1:17.5 and 1:20), can be attributed to the hydrophobicity of the Pluronic P123 block, which favours cylindrical aggregates, as previously reported[16]. At low polymer concentration (F1), the polymer shell (PEO corona) is insufficient to stabilise

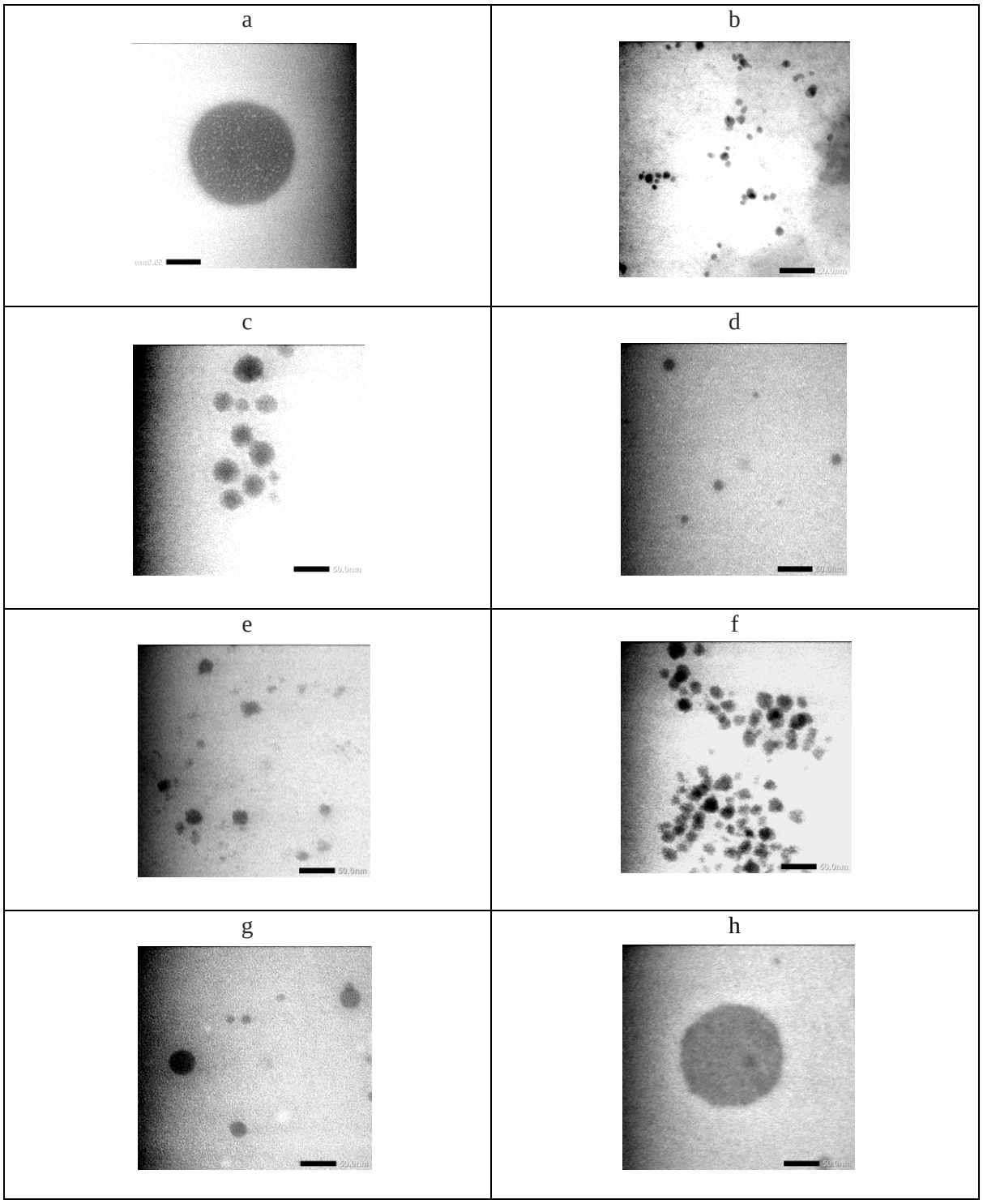


Figure 2. TEM photographs of piperine polymeric micelles at 80,000 magnification (a) F1, (b) F2, (c) F3, (d) F4, (e) F5, (f) F6, (g) F7, and (h) blank polymeric micelles.

the spherical curvature, leading to cylindrical or irregular shapes. At high polymer concentrations (F6 and F7), the increased aggregation numbers and chain crowding force the micelles to adopt elongated structures. In contrast, intermediate polymer ratios (F3–F5, 1:10–1:15) produced well-defined spherical particles. This variation in micellar shape, transitioning from spherical to more cylindrical or elongated structures, is consistent with findings reported for Pluronic P123/F127 systems, where micelle morphology is influenced by polymer composition and concentration[14]. Piperine appeared as dark cores[30].

The particle sizes observed by TEM differed from those measured by DLS. This discrepancy is not unexpected, as the two techniques measure fundamentally different physical properties of the particles[31,32]. DLS measures the hydrodynamic diamete r in aqueous dispersion, which includes the solvation layer and any surface-bound polymer chains, whereas TEM visualises the dehydrated particle core under vacuum. Additionally, DLS is intensity-weighted and inherently biased towards larger species or aggregates, while TEM provides a number-weighted distribution based on individual particle counting. These fundamental differences, well documented in the literature, explain why DLS typically yields larger values than TEM.

Based on these results, F3–F6 were selected for further release and stability studies. F1 and F2 were excluded due to low entrapment efficiency; F7 was excluded due to large particle size and high variability. Additionally, F1, F6, and F7 showed high standard deviations (SD as large as the mean), indicating instability or incomplete micelle formation, particularly at extreme polymer concentrations (F1: very low polymer; F6 and F7: very high polymer). Therefore, F1 and F7 were not investigated further.

3.4. *In vitro* release study and kinetics

Release tests of piperine from piperine polymer micelles and a piperine suspension were conducted to evaluate the effect of the polymers on piperine release (Figure 3). At pH 1.2, the amount of dissolved piperine (43.13–66.73%) was higher than that of the piperine suspension (10.63%). Among the four polymer micelle formulations, the highest amount of released piperine was observed in F3 (66.73%; 6.28-fold) and F4 (61.15%; 5.75-fold). At pH 6.8, the amount of dissolved piperine was also higher in piperine polymer micelles (44.41-73.21%) than in piperine suspensions (15.82%). Among the four polymeric micelles formulations, the highest dissolved piperine levels were observed in F3 (73.21%; 4.63-fold higher) and F4 (71.92%; 4.55-fold higher). The enhanced release is attributed to the small particle size and the formation of hydrophilic channels by Pluronic F127[33,34,35].

The amount of piperine released from polymeric micelles was greater at pH 6.8 than at pH 1.2. At pH 6.8, optimal hydration of the polyethylene oxide (PEO) corona maintains micelle stability and facilitates drug diffusion through a more open micelle structure. Conversely, at pH 1.2, the high ionic strength of the hydrochloric acid buffer induces dehydration of the PEO and PPO chains, making the micelles more compact and hindering drug release[36]. The low CMC of Pluronic P123 and F127 also correlated well with the sustained release profile of piperine from the optimized formulation, as the stable micelle structure ensured controlled drug diffusion over 48 h. Additionally, the acidic environment may promote partial protonation of piperine, potentially enhancing hydrogen bonding with the PEO groups and further increasing drug retention within the micelle corona.

3.5. Release kinetics analysis

Release kinetics were analysed using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The results are summarised in Table 4. At pH 1.2, F3 best followed the Higuchi model ($r = 0.9762$), while F4 followed Korsmeyer-Peppas ($n = 0.2613$, $r = 0.9926$). At pH 6.8, F3 followed Korsmeyer-Peppas ($n = 0.3069$, $r = 0.9737$), and F4 followed Higuchi ($r = 0.9916$). All n values were < 0.43, confirming Fickian diffusion (Case I transport) – piperine release occurs via passive diffusion from the micelle core without significant polymer swelling or erosion.

Statistical comparison of the release profiles of F3 and F4 using the similarity factor (f_2) yielded $f_2 = 63.75$ at pH 1.2 and 51.80 at pH 6.8. Both values are > 50, indicating that the overall release profiles are similar[22]. However, unpaired t-tests at individual time points revealed significant differences ($p < 0.05$) at almost all time points, confirming that the two formulations differ significantly at each observation time. The higher release at pH 6.8 compared to pH 1.2 is attributed to better hydration of the PEO corona at neutral pH, resulting in a more open micelle structure[37,38]. At pH 1.2, dehydration of PEO/PPO chains due to the salt effect of concentrated HCl buffer and disruption of hydrogen bonding likely cause the micelles to become more compact, hindering drug release[39,40].

3.6. Stability study

A stability test of piperine polymeric micelles was conducted for 12 weeks in a climatic chamber. The test conditions were in accordance with ASEAN Guideline[41], specifically at 30°C with 75% relative humidity (RH) and at 40 °C with 75% RH. The results

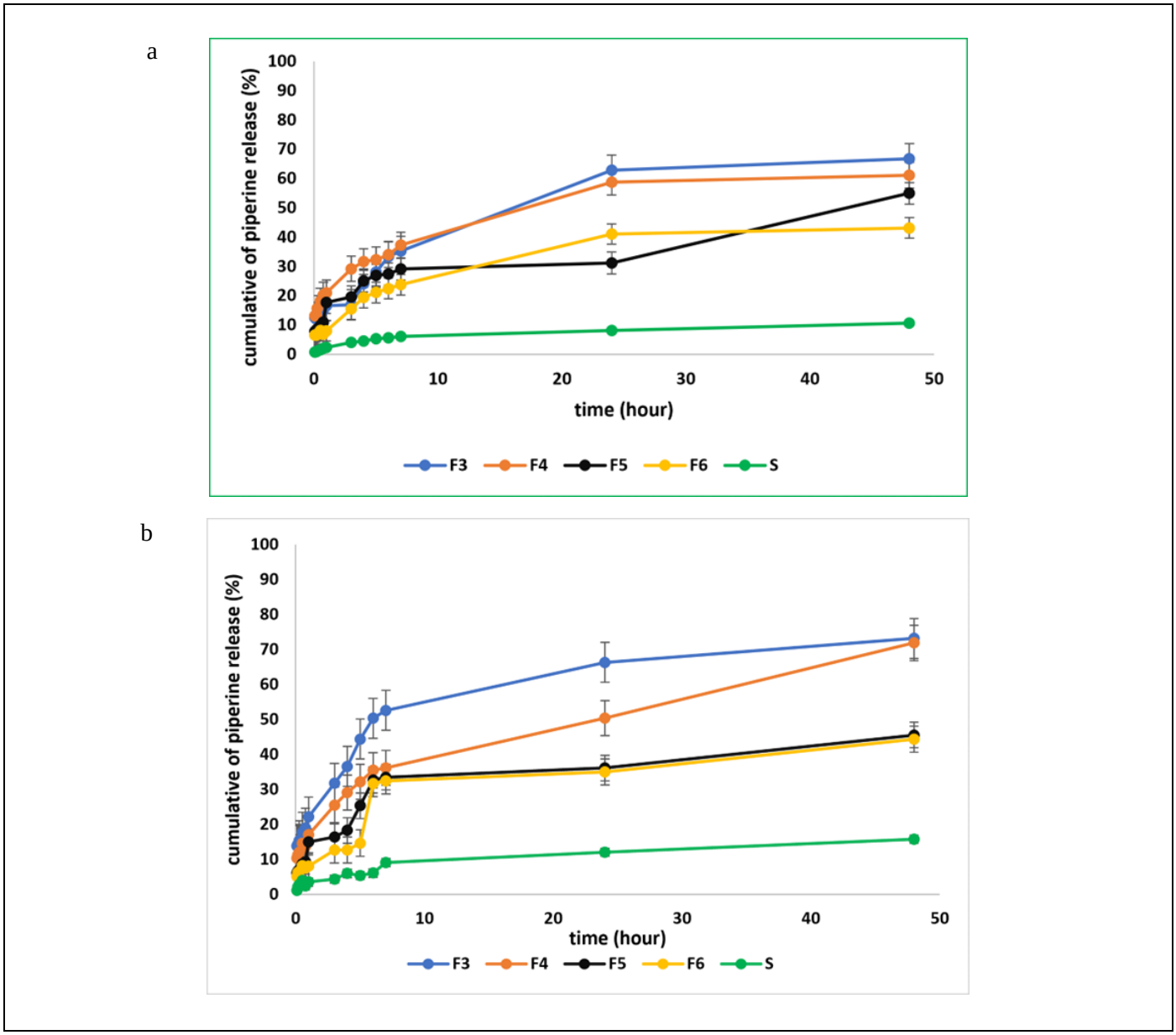


Figure 3. The *in vitro* release of piperine from polymeric micelles (F3–F6) and piperine suspension (S, control) under different conditions: (a) at pH 1.2 and (b) at pH 6.8. Data are presented as mean ± SD (n = 3).

were shown in Figure 4. The decrease in piperine content in polymer micelles at 30°C from highest to lowest is F5>F6>F4>F3, while the decrease in piperine content in polymer micelles at 40°C from highest to lowest is F3>F6>F5>F4. The initial piperine content of the formulations tested in the stability study (F3–F6) ranged from 2.49% to 3.88% w/v (equivalent to 24.9–38.8 mg/mL or 24,900–38,800 µg/mL), with F4 showing 2.49% w/v (24.9 mg/mL). The piperine percentage in Figure 4 was calculated relative to the initial content at week 0 (set to 100%). Piperine degradation can occur through several mechanisms, including acid hydrolysis,

Table 4. Release kinetics parameters for F3 and F4.

Condition	Formula	Zero-order (r)	First-order (r)	Higuchi (r)	Korsmeyer-Peppas (r)	n	Mechanism
pH 1.2	F3	0.9169	0.8326	0.9762	0.9455	0.2937	Fickian diffusion
pH 1.2	F4	0.8829	0.7784	0.9768	0.9926	0.2613	Fickian diffusion
pH 6.8	F3	0.8230	0.7154	0.9460	0.9737	0.3069	Fickian diffusion
pH 6.8	F4	0.9286	0.7904	0.9916	0.9868	0.3174	Fickian diffusion

basic hydrolysis, oxidation, and photolysis[42]. The degradation was more pronounced at higher temperature (40°C), consistent with accelerated degradation kinetics typically observed for bioactive compounds under elevated temperature and humidity[39,40]. Overall, the piperine content of the polymeric micelle formulations decreased by approximately 40% after 3 months of storage at 30°C/75% RH and by approximately 47% after 3 months at 40°C/75% RH.

The stability test also evaluated the changes in particle size, polydispersity index, and zeta potential (Figure 5). At both 30°C and 40°C, particle size increased in polymer micelles F3 and F6, but remained relatively stable in F4 and F5. Specifically, F5 exhibited an increase in particle size as early as week 4, whereas F4 showed a similar increase at week 8. This difference is attributed to the higher polymer concentration in F5 than in F4, which accelerates the intermicelle aggregation kinetics driven by F127 association at the particle surface. A closer examination of the particle size evolution revealed a distinct behaviour for F4: after week 8, its particle size decreased from approximately 35 nm to 28 nm, indicating redispersion of transient aggregates. This phenomenon occurs because the cohesive forces between particles in F4 are insufficient to maintain the aggregated structure, allowing thermal fluctuations and steric repulsion from the PEO corona to overcome interparticle attraction and promote disaggregation.

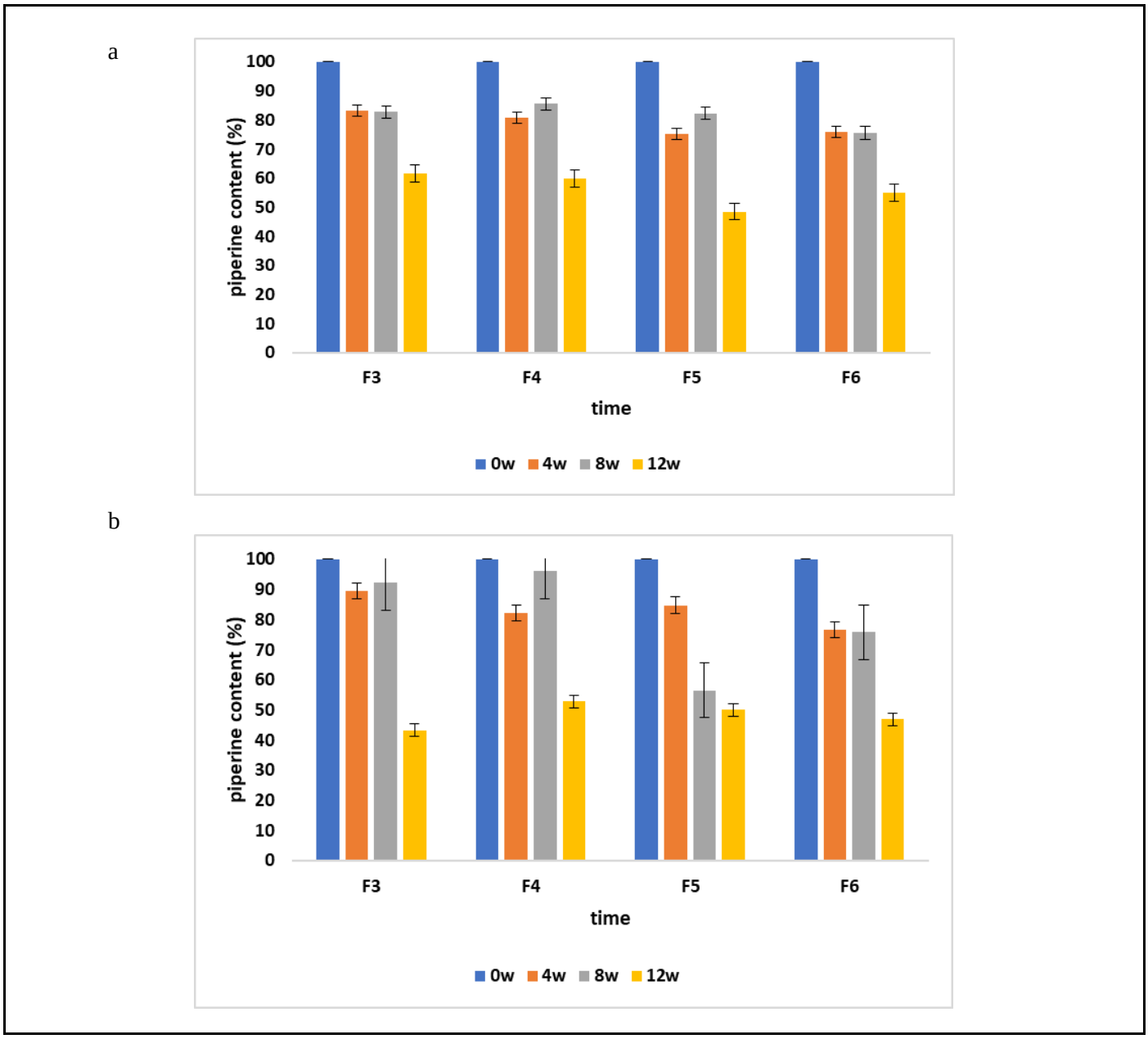


Figure 4. Stability test of piperine content in polymeric micelles considering different conditions: (a) temperature 30ºC and 75% RH, and (b) temperature 40ºC and 75% RH (n=3).

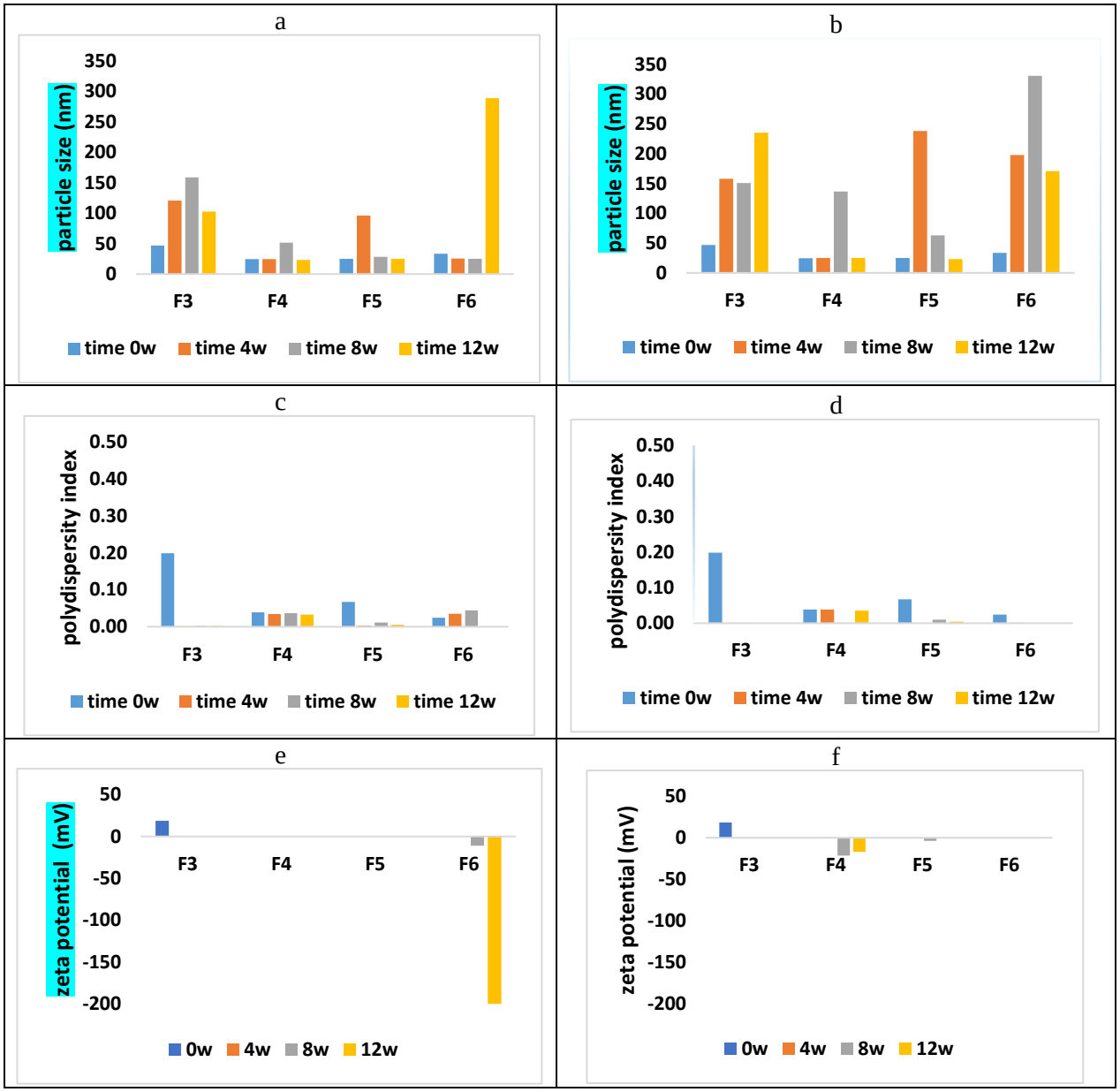


Figure 5. Stability tests of piperine polymer micelles considering different condition: (a) particle size at 30ºC and 75%RH, (b) particle size at 40ºC and 75% RH, (c) polydispersity index at 30ºC and 75% RH, (d) polydispersity index at 40ºC and 75% RH, (e) zeta potential at 30ºC and 75% RH, and (f) zeta potential 40ºC and 75% RH (n=1).

In contrast, F5 did not exhibit redispersion throughout the 12-week study period. The higher polymer concentration in F5 leads to more interparticle crosslinks and a higher cohesive energy, thereby rendering the aggregates thermodynamically stable. This observation is consistent with Tilley et al.[36], who reported that the F127 association becomes irreversible at higher polymer concentrations.

Furthermore, the agglomeration was closely related to the low zeta potential value of the polymeric micelles system, below 30 mV. This could be due to the formation of piperine polymeric micelles that agglomerated, resulting in an increase in the particle size of the polymeric micelles after 12 weeks. The polydispersity index increased at both 30°C and 40°C in F3, F5, and F6, but remained unchanged in F4. All formulas had a good polydispersity index, with values below 0.05. At 30°C, F3, F4, and F5 showed stable zeta potential, while F6 experienced an increase. Polymeric micelles with Pluronic are neutral and tend to have a low negative charge due to the negative charge on the Pluronic polymer surface, resulting from the partial negative charge of the oxygen atoms in the Pluronic structure[25]. Based on several studies, Pluronic polymeric micelles have been reported to exhibit negative zeta potentials of approximately -0.2 mV[43]. Although zeta potential values of piperine polymeric micelles were low, it was assumed that the presence of the nonionic polymer was sufficient to stabilise

piperine polymeric micelles, as the PEO group of Pluronic sterically stabilised polymeric micelles.

3.7. Selection of optimal formulation

Among the four formulations that passed the initial screening (F3, F4, F5, and F6), F4 emerged as the most superior and balanced candidate. The assessment was guided by standard parameters: particle size (<30 nm), polydispersity index (PDI < 0.3), zeta potential, spherical morphology, entrapment efficiency (~6%), release enhancement, and stability. F4 exhibited a particle size of 26.37 nm, a PDI of 0.0382, a zeta potential of −6.20 mV, an entrapment efficiency of 6.20%, and a spherical morphology. In release studies, F4 significantly enhanced piperine release ($p < 0.05$) by 5.75-fold at pH 1.2 and 4.55-fold at pH 6.8 compared to pure piperine suspension. The release profile of F4 was similar to that of F3, with f_2 values of 63.75 (pH 1.2) and 51.80 (pH 6.8), and was further supported by two-way ANOVA, which revealed no significant difference between groups ($p > 0.05$). Moreover, F4 demonstrated the greatest stability, with minimal changes in particle size and PDI over 12 weeks. In contrast, F3 exhibited greater variability in stability, while F5 and F6 showed higher PDI and poorer stability. With a release profile not significantly different from that of F3 and the best stability among the four formulations, F4 was objectively selected as the optimal formulation.

3.8. Limitations of the study

Due to limited sample volume, stability parameters (particle size, PDI, and zeta potential) were measured only once per time point, precluding statistical analysis. As all other experiments were performed in triplicate, these stability trends should be interpreted with caution. Future studies should ensure sufficient sample volume for triplicate measurements. In addition to the stability study limitation, this research has other limitations. The study is limited to *in vitro* evaluations; *in vivo* studies in animal models are needed to confirm the bioavailability enhancement of piperine from the polymeric micelles. Additionally, the stability study was conducted only at two temperatures (30°C and 40°C); other storage conditions, such as room temperature (25°C) or refrigerated conditions (4°C), have not yet been evaluated.

4. CONCLUSION

This study demonstrated that piperine-loaded polymeric micelles formulated via the thin-film hydration method significantly enhanced piperine release compared to pure piperine, with 5.75- and 4.55-fold increases at pH 1.2 and 6.8, respectively, after 48 hours. Regarding release kinetics, the optimal formulation (F4) followed the Korsmeyer-Peppas model at pH 1.2 and the Higuchi model at pH 6.8, with diffusion exponent (n) values below 0.43 in both media, confirming Fickian diffusion as the predominant release mechanism. F4 was identified as the optimal formulation, exhibiting a particle size of 26.37 nm, a low polydispersity index (0.0382), a zeta potential of −6.20 mV, spherical morphology, a piperine entrapment efficiency of 6.20%, and the most favorable stability profile among all formulations over 12 weeks at 30°C and 40°C. These physicochemical and stability attributes suggest that F4 is a promising nanocarrier for enhancing the release of lipophilic compounds, such as piperine.

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Author contribution

ASM: Conceptualisation, Methodology, Data Collection, Data curation, Formal Analysis, Investigation, Project administrator, Validation, Writing-original draft preparation, S: Supervision, Writing - review & editing, YH: Supervision, Writing - review & editing, SS: Conceptualisation, Methodology, Data evaluation, Formal analysis, Supervision, Validation, Funding acquisition, Writing – review & editing

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Conflict of interest

None to declare.

Ethics approval

None to declare.

Data availability
Supplement information is provided upon request.

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