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Ophthalmic Single and Mixed Polymeric Nanomicelles using Brimonidine as a model drug: Preparation, Characterization, and Physical Properties Evaluation

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Abstract

Nanomicelles are a nanocarrier drug delivery system applied in pharmaceutical research to deliver medicines to the targeted site. Their building units are amphiphilic molecules (surfactants or polymers) that are self-assembled into the hydrophobic core-hydrophilic shell-like structure when dispersed into an aqueous media. Polymeric nanomicelles had a lower critical micelle concentration by thousands of folds than surfactants of low molecular weight, resulting in a higher stability and circulation time after administration. Mixed nanomicelles have an extra advantage by improving the stability and encapsulation efficiency compared to single ones. This study aimed to prepare single soluplus and mixed soluplus-tocophersolan polymeric nanomicelles and compare their physical properties using brimonidine as a hydrophobic drug model. The saturated solubility study was used to determine different solubility parameters, and the thin-film hydration method was used to prepare three single and six mixed polymeric nanomicelles. Four variables, including brimonidine concentration, tocophersolan to soluplus ratio, the temperature of the hydration phase, and tocophersolan addition phase, were evaluated for their effects on the studied physical properties (appearance, particle size, polydispersity index, percentage of the entrapment efficiency, *in-vitro-release* profile, and the physical stability). The best-selected mixed polymeric nanomicelles formula was further characterized for its critical micelle concentration using the surface tension method, FTIR, and FESEM. The results showed that brimonidine solubility increased in direct proportion to soluplus concentration, with soluplus (59mg/ml) having the highest molar solubilization capacity (3.715) and fraction encapsulated (0.872) with a negative Gibbes standard-free (-3.453), indicating a spontaneous nanomicelles formation. The brimonidine concentration affected the physical appearance of the single soluplus nanomicelles and had a significant effect on the percentage of entrapment efficiency, while the particle size and the polydispersity index were not affected. The best single nanomicelles (F2) were transparent with particle size (75.75±1.13nm), polydispersity index (0.1243±0.02), and percentage of entrapment efficiency (53.49%±0.58), was selected to prepare the mixed one were all the studied variables had a highly significant effect on the particle size and the percentage of entrapment efficiency but, no effect on the physical appearance and polydispersity index. The best mixed polymeric nanomicelles (F7) were transparent, with a tocophersolan to soluplus ratio of (1:22.5) that added in the organic phase, and a particle size of (79.55±0.24nm), polydispersity index (0.1222±0.00) and percentage of entrapment efficiency (62.18%±0.23), with lower critical micelle concentration of (3.467*10-7M), and more stability than the F2 after 90 days storage at (4.0±2.0°C) with faster *in-vitro* release profile (78.4±0.28%) within 15 minutes. The FTIR spectra for the individual components and F7 indicated their compatibility, while the FESEM results showed a spherical morphology with particle sizes close to that detected by Malvern Zetasizer. In conclusion, soluplus and tocophersolan could be used successfully to prepare spherical and transparent mixed polymeric nanomicelles incorporating a hydrophobic drug in their core with the desired physical properties, higher *in-vitro* release, and more stability compared to single nanomicelles.

Keywords: Du Noüy ring, Mixed polymeric nanomicelles, Solubility parameters, Single polymeric nanomicelles, Tocophersolan.

Introduction

Nanotechnology has been used in many sectors, including engineering, agriculture, health, and others. In the health sector, it has been used for designing drug therapy and diagnostic tools as nanocarrier systems that put a prime fingerprint in pharmaceutics as they deliver their cargo to the targeted site efficiently [(1)].

Nanomicelles are a type of drug nanocarrier system that is built from amphiphilic units (surfactants or polymers) by self-assembling upon contact within an aqueous solution at a specific concentration and temperature (critical micelle concentration (CMC) and temperature) by arranging a core-shell like structure with hydrophobic tails aggregated in the core and hydrophilic head directed toward the surrounding media (the corona) [(2)]. This arrangement offers several advantages, including dissolving the hydrophobic drugs in their core, tunable physicochemical properties like (particle size (PS) in nanometer ranges, surface charges, targeting moieties, triggering to different factors like temperature and pH), stability, and others [(3)]. Polymers for nanomicelles could be natural as

hyaluronic acid and chitosan or synthetic biocompatible and biodegradable as a linear (diblock, triblock) or branched (grafted) copolymers mainly using polyethylene glycol as the hydrophilic chain [(4).] Polymeric nanomicelle has an extra advantage because of their low critical micelle concentrations compared to the surfactants, which render them more thermodynamically and kinetically stable and associated with fewer adverse effects [(5)], in addition to their biocompatibility and biodegradability [(6)]. The mixed nanomicelles are binary or ternary systems of various combinations prepared to obtain the desired hydrophilic and hydrophobic characteristics with the additional advantages of higher stability and encapsulation efficiency [(7)]. Soluplus® Figure. 1 is an amphiphilic grafted copolymer of polyvinyl caprolactam-polyvinyl acetate (hydrophobic chain) and polyethylene glycol (hydrophilic chain) that are self-assembled into nanomicelles upon hydration. Its nonionic characteristic and solubilizing effect, especially for hydrophobic drugs, make it a safe and better polymeric nanomicelle for loading hydrophobic drugs in its core [(8)].

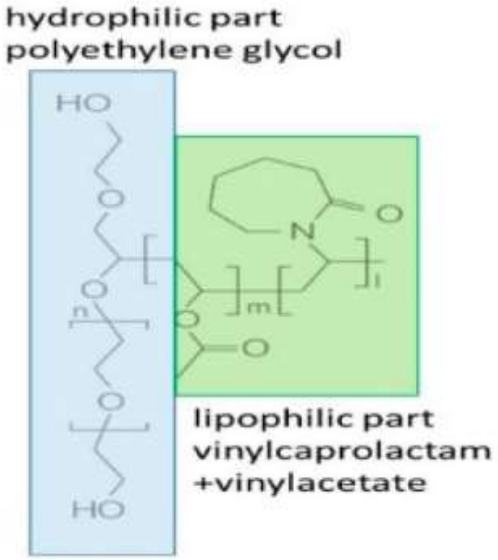

Figure 1. Soluplus® chemical structure [(9)]

Tocophersolan (TPGs) Figure. 2 is D-α-tocopheryl polyethylene glycol 1000 succinate, an FDA-approved excipient that has been used in drug formulation for its excellent solubilizing, stabilizing, and permeability-enhancing properties, in addition to safety, biocompatibility, and biodegradability. It has an amphiphilic surfactant characteristic with low CMC and is soluble in water and lipid media. Recently, it was used in nanocarrier drug preparations as a stability and entrapment efficiency enhancer. In ophthalmic drug carrier designing, it was used for its ability to overcome the corneal barrier with no irritation [(10-12)], in addition to its neuroprotective effect as an antioxidant and p-glycoprotein inhibitor [(13)].

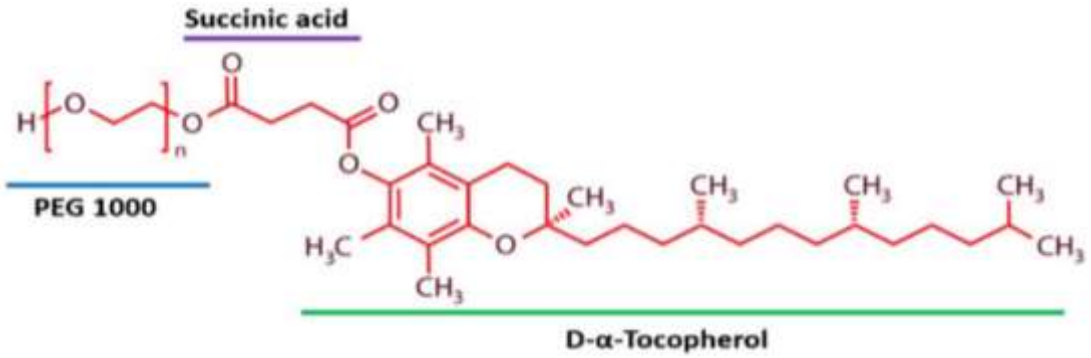

Figure 2. Tocophersolan® chemical structure [14]

Brimonidine, a hydrophobic, selective alpha-2 adrenergic agonist receptor, was approved in 1966 by the FDA as a topical eye drop (Alphagan® as brimonidine salt) to lower the increased intraocular pressure in open-angle glaucoma, moreover, it has a neuroprotective effect, that could reduce the chance for complications developments that needs an invasive treatment, such as surgical or laser procedures [15]. It is a hydrophobic molecule with molecular weight of 292.13 g/mol, pKa 7.78; which is metabolized primarily by the liver and eliminated mainly with the urine, with an approximately half life of 2 hours after ophthalmic solution application [16]. Ophthalmic medicines were administered using different dosage forms; the eye drop was the major due to its advantages represented by self-administration, noncomplicated application method, and cheap, although it has a main drawback of poor ophthalmic bioavailability resulting from ocular barriers and nonadherence of the patient to the treatment regimen [17, 18].

Other dosage forms like systemic and ocular injections have a higher bioavailability, but their administration involves an invasive method associated with many risks like adverse- effects and infection, besides the higher cost (administered by professionals mainly in clinics) [19]. Designing a drug nanocarrier system as a topical ocular dosage form capable of delivering the medication to the targeted site of the ophthalmic tissue could be an alternative for the injections.This research aimed to prepare a single soluplus® and mixed soluplus®-tocophersolan polymeric nanomicelles colloidal dispersion system loaded with the hydrophobic brimonidine particles within their cores and compare them regarding the best accepted physical properties as a topical eye drop.

Materials and Methods

Materials

Brimonidine (BR) was purchased from Anshi Pharmaceutical Co., Ltd. Soluplus® (SO) from BASF SE, Germany. Tocophersolan (TPGs) was purchased from Henan Guange Biotechnology Co., Ltd. Methanol HPLC was purchased from Chem-Lab NV, Belgium. Amicon® Ultracentrifuge tube, 10kDa MWCO, was purchased from Sigma-Aldrich, USA. Dialysis Bag MD34-5M, Wide flat: 34 MM, Mw: 8000 - 14000 D, was purchased from MYM Biomedical Technology Company Limited, USA. All other chemicals and reagents obtained are of analytical grade.

Solubility study

The shake-flask method was used to determine brimonidine solubility in deionized-distilled water (DDW), a series of soluplus® molar concentrations as a nanomicelle dispersion (0.5, 1.0, 1.5, 2.0, and 2.5 mM) at 25ºC, and in phosphate buffer saline (PBS) pH 7.4 at 37.5ºC. An excess of brimonidine was added to a specified volume (2 ml) of each studied media in a closed vial and placed in a water-bath shaker (Jeio Tech BS-11, 25Liter, Korea) for 48 hours at 50 rpm. The supernatant from each vial was taken (Centrifuge 5810 R, Eppendorf Company, Germany) and analyzed for the dissolved amount of brimonidine using the UV-spectrophotometer method (UV-VIS spectrophotometer UV-1900i, Shimadzu, Japan). Solubility parameters were determined, including the solubility factor, the micellar /water partition coefficient (P), molar partition coefficient (MP), and molar fraction of the drug encapsulated inside the micelles (f), Gibbs standard-free energy (ΔGS), and solubility enhancement factor [20,21].

Preparation of polymeric brimonidine-loaded nanomicelles

Different formulas were prepared by the thin film hydration method Table 1. Polymer (with TPGs) and brimonidine were dissolved in methanol with heat and stirring (Premium Hotplate Stirrer, Witeg Labortechnik.GmbH) and placed in a rotary-vacuum evaporator (Rotavapor® R-300a, Buchi, GmbH) to remove all the organic solvent, then left overnight for complete dryness and thin-film forming. Brimonidine-loaded nanomicelles were formed upon hydration of the thin film using DDW with stirring and heating [21]. The best formula (the one with accepted physical properties, including the physical appearance (PA), PS, polydispersity index (PDI), and the percentage of entrapment efficiency (%EE), was selected and subjected to further study. First, single polymeric nanomicelles were prepared using soluplus® alone (in a concentration equal to the one associated with the highest molar fraction of encapsulated drug obtained from the solubility study) and evaluate the effect of different brimonidine-to-soluplus weight ratios (1:45, 1:30, and 1:22.5) on the physical properties of the nanomicelles taking the marketed brimonidine eye drop (Alphagan®) as a reference for the starting amount of brimonidine. The best-selected single soluplus® nanomicelles were then used to prepare mixed polymeric (soluplus®-tocophersolan) nanomicelles (MPNs) by the same method. Different factors were studied for their effect on the MPN's physical properties, including tocophersolan-to-soluplus® weight ratios (1:30, 1:22.5, and 1:15), the temperature of the hydration phase (37.5±0.5, 55.5±0.5, and 80.0±0.5°C), and the tocophersolan phase addition (in the organic phase/ added to methanol with soluplus, then brimonidine was added to the dissolved mixtures, or in the aqueous phase/ added to the DDW and were used in the hydration phase).

Table 1. Brimonidine-soluplus nanomicelles composition

F No.	BR mg/ml	BR-to-SO ratio	TPGs-to-SO ratio	Temp. of the aqueous hydration phase °C	TPGs addition phase
F 1	1.32	1:45	0	37.5±0.5	/
F 2	1.98	1:30	0	37.5±0.5	/
F 3	2.64	1:22.5	0	37.5±0.5	/
F 4	1.98	1:30	1:30	37.5±0.5	organic
F 5	1.98	1:30	1:22.5	37.5±0.5	organic
F 6	1.98	1:30	1:15	37.5±0.5	organic
F 7	1.98	1:30	1:22.5	55.5±0.5	organic
F 8	1.98	1:30	1:22.5	80.0±0.5	organic
F 9	1.98	1:30	1:22.5	55.5±0.5	aqueous

CMC determination study: The cmc of the mixed polymeric dispersion was determined by the du Noüy ring method using (Sigma 703D Attention-Force Tensiometers, Biolin Scientific, Gothenburg, Sweden) at 25±0.5°C to measure the surface tension. The cmc (molar concentration) for the mixed polymeric system represents the breaking point in the plot of the two extrapolated straight lines of the normal logarithm of molar concentration (x-axis) of the mixed polymeric system for serial concentrations including below and above the soluplus cmc versus the surface tension [22].

***In-vitro release*:** The dialysis membrane method (dialysis bag MD34-5M, Wide flat: 34 MM, Mw: 8000 - 14000 D) was used for the *in vitro* release using 50 ml PBS pH 7.4 as the receptor media. A specified amount of brimonidine from each of the aqueous drug suspension, single, and mixed best-selected polymeric nanomicelles were placed in a pre-soaked dialysis bag (24 hours in advance with the receptor media) in which both bag ends were closed using dialysis tubing closer-clips and dropped in a beaker containing the receptor media that was placed in the water bath shaker at 37.5±0.5 ºC and with 50 rpm. At 0,5,10,15 minutes, three milliliter samples were withdrawn from the receptor media and replaced immediately with an equivalent volume of PBS pH 7.4 at the same temperature to keep the experimental sink condition [23, 24]. The amount of *in vitro* brimonidine released was determined spectrophotometrically and plotted as a percentage accumulative amount against time.

Characterization of the prepared nanomicelles:

***Particle size determination*:** PS and PDI were determined by Malvern Zetasizer, UK, using the dynamic light scattering technique [25].

***Entrapment efficiency percentage (%EE) determination*:** Amicon® tube was used to separate the free unentrapped brimonidine by centrifuging at 6000 rpm for 15 minutes at room temperature, where the %EE was calculated by the indirect method. The %EE was calculated using Equation 1 [26]:

$$\%EE = \frac{BR\ total\ content\left(\frac{mg}{ml}\right) - BR\ content\ in\ the\ filter\left(\frac{mg}{ml}\right)}{BR\ total\ content\left(\frac{mg}{ml}\right)} * 100$$

Eq.1

***Physical stability study*:** A short-term physical stability study was performed by storing samples from the best-selected formulas of both single and MPNs loaded with brimonidine in a tightly closed glass container at room temperature (25.0±2.0°C)

and refrigerator (4.0±2.0°C) for three months, and then analyzed regarding their physical properties including PS and PDI comparing with that at the time of their preparation [20].

***Fourier Transform Infrared Spectroscopic (FTIR) Analysis*:** The FTIR analysis using (FTIR-1800 Shimadzu, Japan) was performed to determine any possible interaction between the drug and the polymers. The scanned samples include brimonidine and soluplus® crystalline powders, tocophersolan wax, and the liquid selected-best MPN formula. The range for FTIR spectroscopic analysis was 4000 – 400 cm^{-1} [27].

***Field emission scanning electron microscopy (FE-SEM) Analysis*:** The FE-SEM was used to analyze the morphology of MPNs for the selected best formula using (Inspect F50, FEI company, The Netherlands) [28].

***Statistical Analysis*:** Triplicate measurements for all the laboratory experiments were done, and the results were expressed as mean values ± standard errors (SE). The statistical significance of the variables was determined depending on the p-value using one-way analysis of variance (ANOVA) or student t-test with Tukey multiple comparison test, where p-value $\leq$ 0.001 is statistically highly significant, and p-value $\geq$ 0.05 is statistically non-significant.

Results and Discussion

***Solubility study*:** The aqueous saturated solubility of brimonidine increased by using soluplus® nanomicelle colloidal dispersion, as shown in Table 2, which was similar to that published by Hadi BM [29]. Soluplus® molar solubilization capacity (X) decreases with concentrations above 59mg/ml, indicating that more copolymer molecules will reduce the micellar concentration in the bulk system as it should be in equilibrium with monomers [30]. These findings were confirmed by the brimonidine solubility parameters that were determined, including the micellar /water partition coefficient (P), molar partition coefficient (MP), and molar fraction of the drug encapsulated inside the micelles (f). The (P) value was low at a soluplus® concentration of 59mg/ml (≈ 4.027), while it remained somewhat plateau at 118 and 177 mg/ml (≈ 6). When the concentration increased to 236 mg/ml, the (P) value increased remarkably (≈ 9), while the (MP) decreased. In addition, the (f)value was (87.2%) at 59mg/ml soluplus®, while this value decreased to (74.3%) for 236mg/ml, similar to Pignatello, R.'s findings [20]. The (ΔGS) value was negative, indicating the spontaneous formation of the self-assembled nanomicelles [31].

Table 2. Effect of different soluplus® concentrations on the aqueous saturated solubility of brimonidine and nanomicelles solubility parameters, (X) molar solubilization capacity, (P) micelle/water partition coefficient, (MP) molar partition coefficient, (F), molar fraction of drug encapsulated inside the micelles, (ΔGS) Gibbs standard-free energy Kj/mol, (FI) factor increment of solubility.

Soluplus conc. mg/ml	Br solubility mg/ml	X	P	MP	F	ΔGS	FI
0	0.461±0.003	/	/	/	/	/	0
59	1.004±0.014	3.715	4.027	8.054	0.872	-3.453	2.17
118	1.287±0.026	2.826	6.126	6.126	0.814	-4.493	2.79
177	1.353±0.032	2.036	6.62	4.413	0.755	-4.685	2.93
236	1.692±0.027	2.106	9.13	4.566	0.743	-5.483	3.67

***Effect of different brimonidine-to-soluplus® ratios on the physical properties of single-loaded nanomicelles*:** From the solubility study, the soluplus® concentration of 59 mg/ml had the highest (f) value was used to study the effect of different brimonidine-to-soluplus® ratios. As shown in Table 3, brimonidine concentration had highly significant effects on the PA and %EE with a p-value≤0.001, while none on the PS and PDI with a p-value≥0.05. This effect could be due to the saturation of all the hydrophobic core of that soluplus® concentration nanomicelles with brimonidine, and all the excess of the drug settled down [32], further supporting the results obtained from the solubility study.

Table 3. Effect of different brimonidine concentrations on the different physical properties of nanomicelles

F No.	BR-to-SO ratio	BR conc. mg/ml	SO conc. mg/ml	Physical appearance after 24 hours	Mean PS(nm)±SE (P≥0.05)	Mean PDI±SE (P≥0.05)	Mean %EE± SE ***(P≤0.001)
F1	1:45	1.32	59.4	transparent	77.87±1.11	0.1292±0.01	47.45±0.47
F2	1:30	1.98	59.4	transparent	75.75±1.13	0.1243±0.02	53.49±0.58
F3	1:22.5	2.64	59.4	turbid	76.35±1.42	0.1464±0.02	60.58±0.16

Effect of tocophersolan-to soluplus ratios on the physical properties of MPNs

F2, with the highest %EE and accepted physical properties, was selected as the best single brimonidine-soluplus nanomicelles formula to prepare the mixed soluplus®-tocophersolan nanomicelles and to study the effect of different tocophersolan-to-soluplus® ratios on the previously analyzed physical properties. The tocophersolan ratios affected the PS and the %EE significantly (p-value≤0.001), while none on the PA and PDI, Table 4. The PS increment could have occurred due to the increment in the hydrophobic and hydrophilic segments of the formed MPNs compared to the single one [33]. From Table 4, the %EE for the MPNs was lower than the single one, which could be related to the strong interaction between the two polymers' hydrophilic segments, so it was difficult for the drug molecules to enter the core of the micelles. Furthermore, the %EE increased proportionally with tocophersolan ratios till 1:22.5 due to the increment in the polymer volume fraction. With more increment in the tocophersolan ratio, the %EE decreased again, which could be a consequence of the increment in the head-to-head interaction with higher strength compared to the head-to-solute interaction; thus, more drug molecules would be located on the surface of the micelles rather than in the core [34]. The transparent appearance and the low value of the PDI indicated the complete interaction between the two polymers to form a monodispersed system [34].

Table 4. Effect of tocophersolan ratios on the physical properties of brimonidine-loaded nanomicelles

F No.	TPGs conc.	TPGs-to-SO ratio	Physical appearance after 24 hours	Mean PS (nm)± SE *** (P≤0.001)	Mean PDI±SE (P≥0.05)	Mean % EE±SE *** (P≤0.001)
F2	0	0	transparent	75.75±0.92	0.1243±0.02	53.49±0.48
F4	1.98	1:30	transparent	90.72±0.13	0.1499±0.02	50.16±0.08
F5	2.64	1:22.5	transparent	91.43±0.65	0.2112±0.03	52.81±0.23
F6	3.92	1:15	transparent	92.58±1.39	0.1731±0.02	49.03±0.23

Effect of the temperature of the hydration phase on the physical properties of MPNs

F5, the MPN with the highest %EE and suitable PS and PDI, was selected as the best one to study the effect of different temperatures used for the hydration of the formed thin-layer on the physical properties of the nanomicelles as illustrated in Table 5. The temperatures had a high statistically significant effect with a p-value ≤0.001 on the PS and %EE, while not for the PA and PDI (p-value ≥0.05). Increasing the temperature from 37.5±0.5°C to 55.5±0.5°C decreases the PS and increases the %EE. On the other hand, further increases in the temperature to 80.0±0.5°C had the opposite effect. This effect of the temperature on the physical properties of the nanomicelles could be explained by the thermal behavior of the nanomicelles and the thermo-responsive properties of soluplus®. The micellization process for many surfactants is endothermic at low temperatures and exothermic at higher ones [36]. Soluplus® is a thermo-responsive polymer with a lower critical solution temperature of 40°C [37], which is also its cloud point temperature, as was reported by Maximiliano Cagel [34]. The values of these two temperatures will depend on the length of the hydrophilic and the hydrophobic chains of the MPNs. Below the low critical solution temperature, the thermodynamic process of the system was endothermic, and the micellization occurred spontaneously, a finding confirmed by the negative value of ΔGS found in the solubility study. Upon increasing the temperature to 55°C, the polymer hydrophilic chains-water hydrogen bonding became weakened, allowing easy entrapping of the hydrophobic drug into the core, and thus the %EE increased [34], and the system remained endothermic and monodispersed; above that temperature, the system became thermodynamically exothermic, and the hydrophobic polymers chains interaction became more predominant, thus releasing the drug from the hydrophobic core leading to a reduction in the %EE [38, 39].

Table 5. Effect of the temperature of the hydration phase on the physical properties of brimonidine-loaded nanomicelles

F No.	Temp. of the hydration phase °C	Physical appearance after 24 hours	Mean PS (nm) ± SE *** (P≤0.001)	Mean PDI ± SE (P≥0.05)	Mean % EE ± SE *** (P≤0.001)
F5	37.5±0.5	transparent	91.43±0.65	0.2112±0.03	52.81±0.23
F7	55.5±0.5	transparent	79.55±0.24	0.1222±0.00	62.18±0.23
F8	80.0±0.5	transparent	84.3±0.23	0.1528±0.00	31.38±0.36

Effect of the tocophersolan addition phase on the physical properties of MPNs

F7, with the highest %EE, was selected to study the impact of the addition of tocophersolan on the DDW used to hydrate the thin film on the physical properties of the nanomicelles [40]. Regarding the results of this study, Table 6, indicated an increase in the PS and a reduction in the %EE with the addition of tocophersolan in the aqueous phase F57 compared to the organic phase F7 with a statistically highly significant effect (a p-value≤0.001); on the other hand, no statistically significant effect (p-value≥0.05**) observed** on the PA and the PDI. These results could be explained by the tocophersolan dissolving in the aqueous phase before the hydration process, thus reducing the extension of the hydrophobic chains of the MPNs and increasing the interaction between the hydrophilic chains of the polymers.

Table 6. Effect of the tocophersolan addition phase on the physical properties of brimonidine-loaded nanomicelles

F No.	Addition phase	Physical appearance after 24 hours	Mean PS (nm)±SE *** (P≤0.001)	Mean PDI±SE (P≥0.05)	Mean %EE±SE *** (P≤0.001)
F7	organic	transparent	79.55±0.24	0.1222±0.00	62.18±0.23
F9	aqueous	transparent	91.96±0.65	0.231±0.00	49.63±0.41

Critical micelle concentration (CMC) determination study

The cmc for the MPNs dispersion of the best-selected formula was found to be $3.467*10^{-7}$ M, Figure 3, which was higher than that of soluplus® ($6.44*10^{-8}$ M) [41] and lower than that of tocophersolan ($1.322*10^{-4}$) [34], which could be a result of the high cmc value for tocophersolan that leads to a negative effect on the self-assembling of soluplus® [42]. This low CMC indicates a higher physical stability upon dilution with the tear fluid.

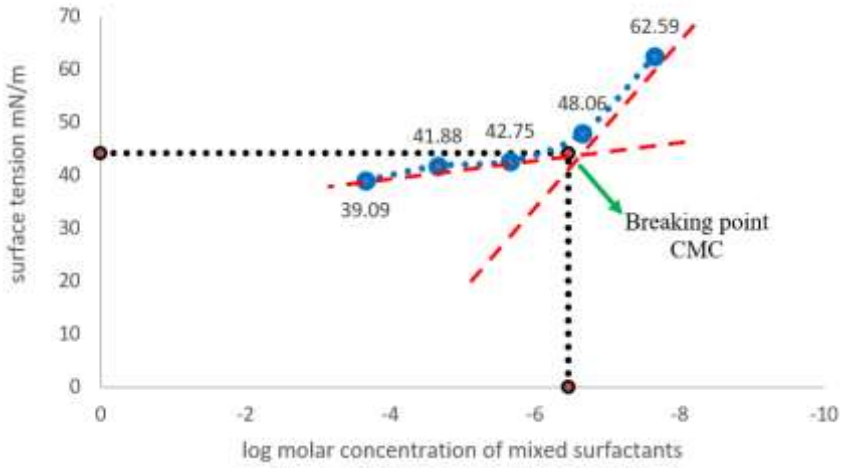

Figure 3. Surface tension versus a series of log molar concentrations of the mixed tocophersolan-soluplus (1:22.5) dispersion plot representing the CMC for the mixture.

In-vitro release

The best single F2, MPNs F7 formulas, and the aqueous brimonidine suspension were compared for their *in-vitro* release behavior. Figure .4 showed that both single (65.52±0.37%) and mixed (78.4±0.28) formulas had a fast *in-vitro* release profile compared to the pure aqueous suspension (23.88±0.05%) within 15 minutes (highly significant effect with p-value≤0.001). For the MPNs, in the first part of the *in-vitro* release profile (5 minutes), the percentage cumulative released was lower than the single nanomicelles, while in the second part (10-15 minutes), the pattern was inversed. Expectingly, the in-vitro release for the polymeric nanomicelles (single and mixed) will be faster and higher than the aqueous suspension due to the large specific surface area and the solubilizing effect of the polymer; on the other hand, the dual behavior of the MPNs compared to the single one could be related to the premium hydrophobic interaction (regarding numbers and affinity) in their core with the drug; consequently, they would be more stable [42], for the first five minutes, and the synergistic solubilizing effect (being tocophersolan more hydrophilic in nature) with the higher %EE for the second ten-fifteen minutes. [35].

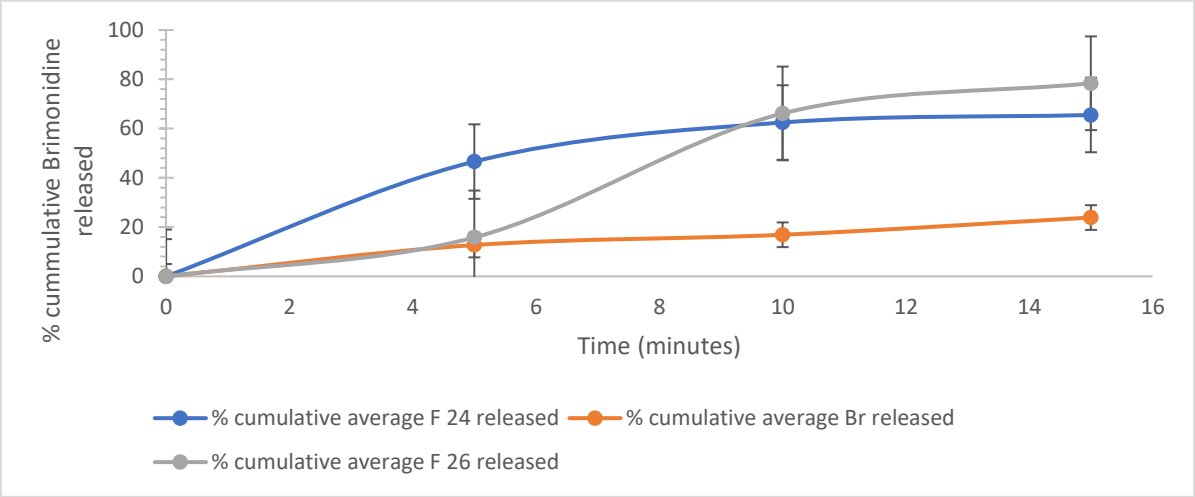

Figure 4. *In-vitro* release profile (mean ± SD, n=3) of F 2, F 7, and brimonidine aqueous suspension in PBS pH 7.4 at 37.5±0.5°C

Physical stability stud

The MPNs were more stable physically during storage time at the refrigerator (4.0°±2.0° Celsius) than the single one, Table 7. There was a statistically high significant effect of the temperature on the PS and PDI of the single polymeric nanomicelles; however, for the MPNs, the temperatures affected significantly only the PS with no effect on the PDI. The change in the PS for the MPNs was low and remained within the acceptable range (below 100 nm), while the PDI, in addition to the transparent physical appearance, indicated intact nanomicelles with a monodisperse system. These results suggested the ability to keep the MPNs stable in the refrigerator for 90 days, which was similar to that reported by Xue Feng [43].

Table 7. Physical stability study for the single (F 2) and mixed (F7) polymeric nanomicelles at room and refrigerator temperatures for 90 days, * (p-value≤0.001).**

F No.	Mean PS (nm)±SE *** (P≤0.001)			Mean PDI±SE (P≥0.05)		
	At (0) days	After (90) days at room temp.	After (90) days at refrigerator temp.	At (0) days	After (90) days at room temp.	After (90) days at refrigerator temp.
F2	75.75±0.92	122.9±0.92	95.03±0.42***	0.1243±0.02	0.2601±0.014	0.1648±0.003***
F7	79.55±0.24	97.09±0.18	84.89±0.37***	0.1222±0.00	0.1799±0.001	0.1411±0.004

Fourier Transform Infrared Spectroscopy (FTIR) analysis

Figure. 5 previews the FTIR spectra for the pure drug, excipients, and the best-selected MPNs formula (F7). For brimonidine crude powder, the FTIR spectra Figure. 6a showed the representative peaks, including the C-C of the benzene ring at 1481.13 cm^{-1}, the N-H bending vibration at 1589.34 cm^{-1}, the N=C and C=C at 1645.28 cm^{-1}, and the N-H stretching vibration at 3163.26 cm^{-1}, similar to what was reported [44]. All these peaks are available in the spectrum of F 7 with shifting, indicating the compatibility of the drug with the excipients, and there was no chemical interaction between them.

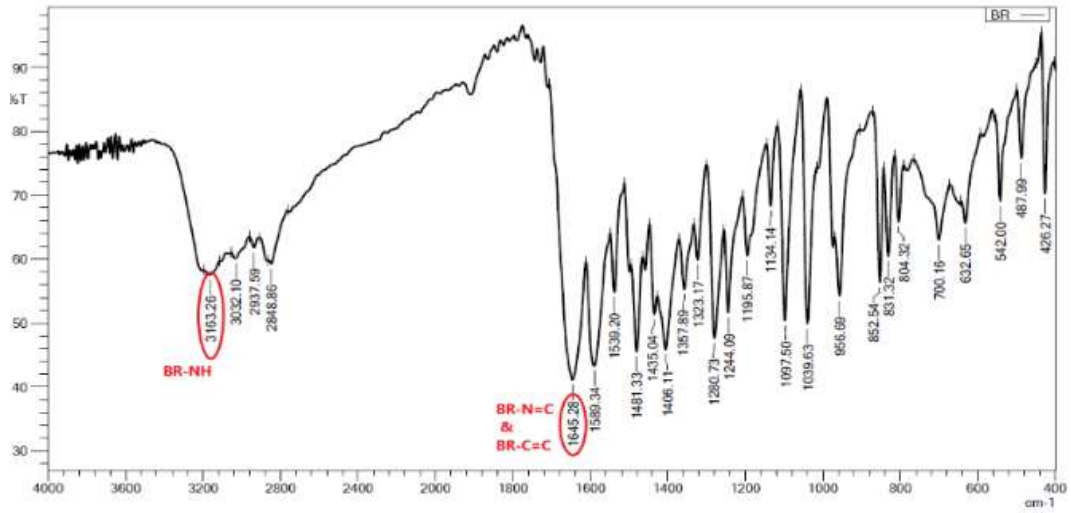

Figure 5a

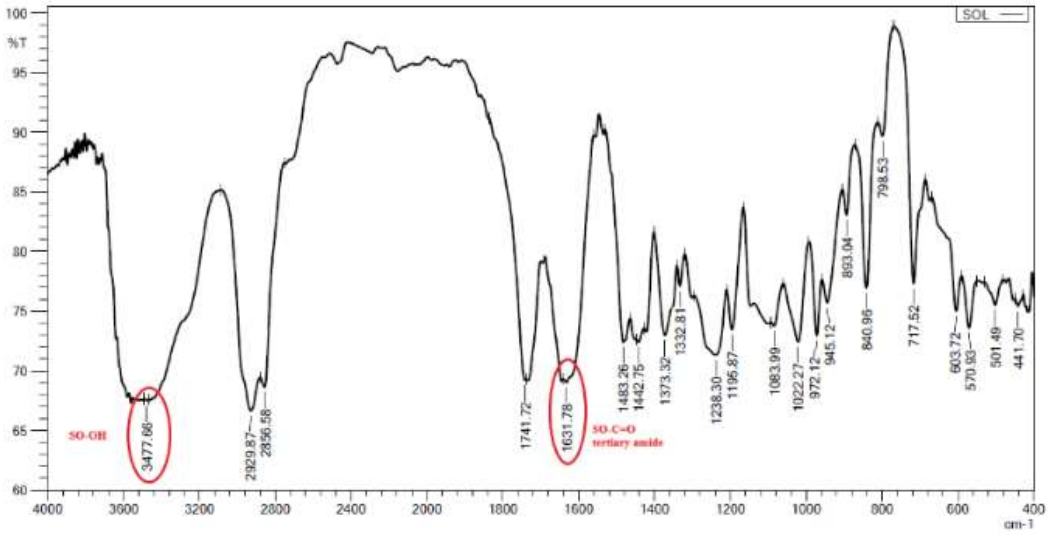

Figure 5b

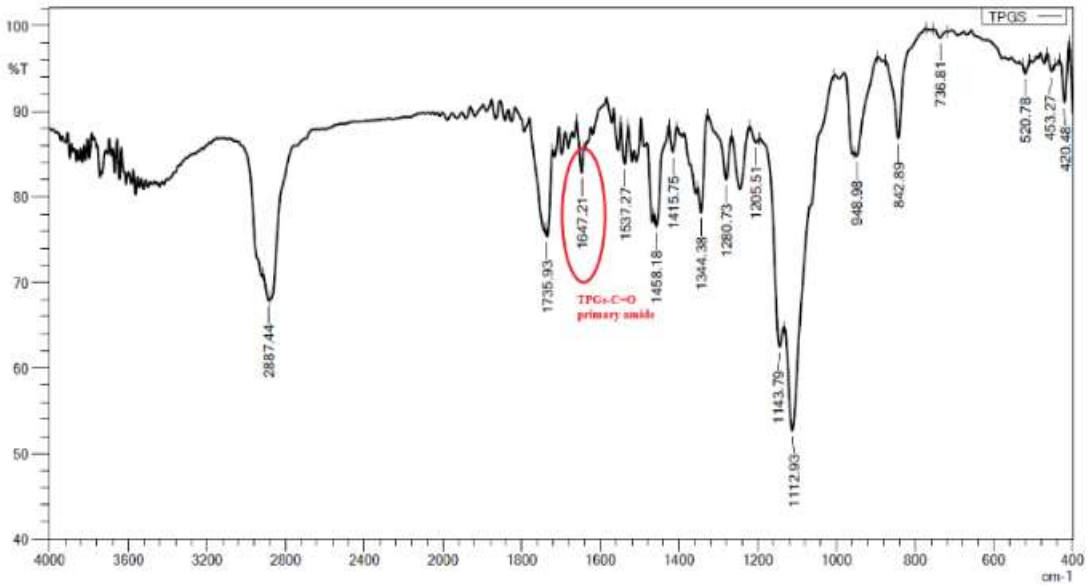

Figure 5c

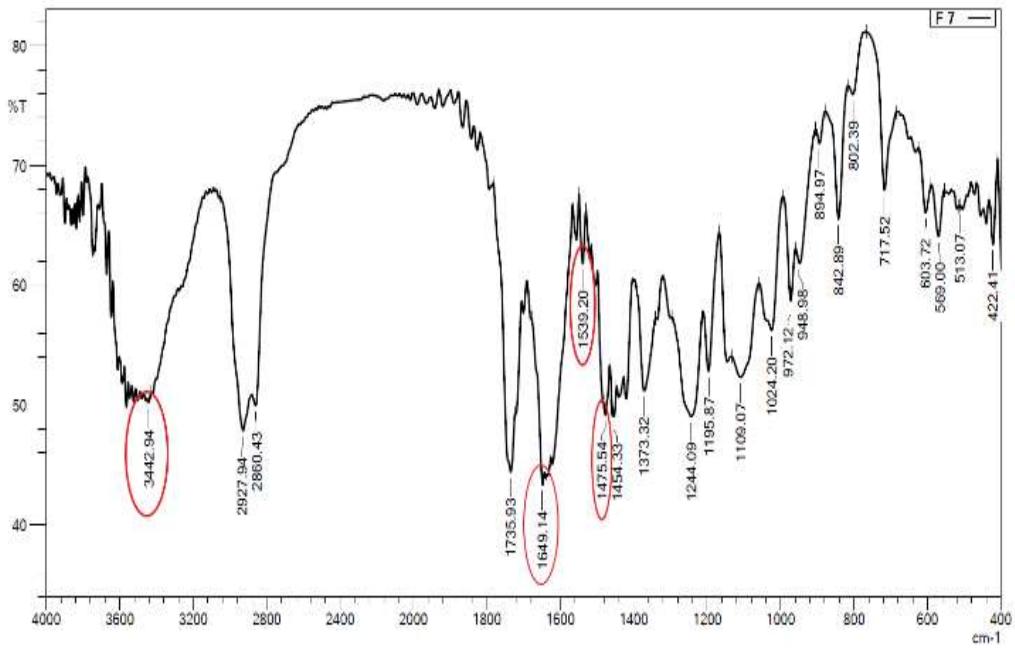

Figure 5d

Figure 5. FTIR spectrum of brimonidine powder(a), soluplus® powder(b), brimonidine/soluplus® physical mixture(c), and F 6 (d)

***Field emission scanning electron microscopy (FE-SEM)*:**

The taken image Figure. 6, for F7, showed a spherical particle morphology for the MPNs with sizes ranging between 80.1 – 84.82 nm, which was close to that measured by Malvern Zetasizer.

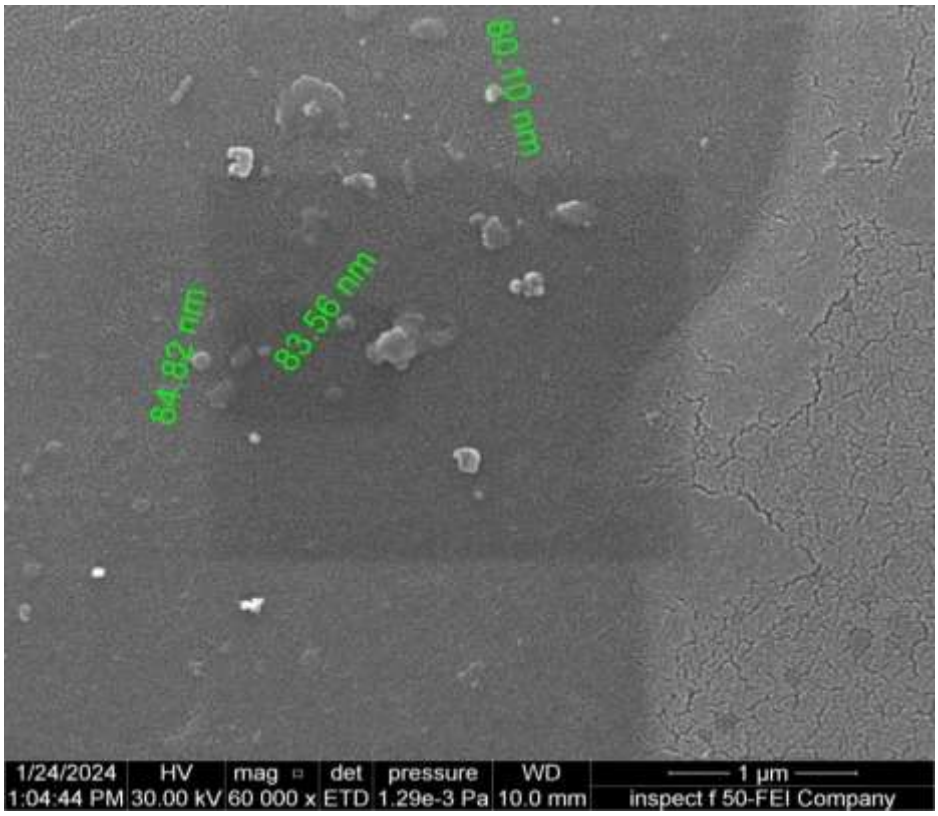

Figure 6. FESEM image of (F 7)

Conclusion

In conclusion, soluplus® and tocophersolan could be used successfully to prepare mixed polymeric nanomicelles incorporating a hydrophobic drug physically in their core that are spherical in shape, transparent with accepted physical properties, and had a faster and higher in vitro release profile, and could remain stable to about 90 days at 2.0±2.0°C.

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Conflicts of Interest

Both authors declared that no conflict of interest related to this work.

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Ethics Statements

Both authors assured that no animals were used for this work

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design, data collection, analysis, and interpretation of results were done by the first author, Noor N. Abdulla. Draft manuscript preparation, reviewing the results, and approving the final version of the manuscript was done by both authors, Noor N. Abdulla and Fatima J. Al-Gawahari.

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المذيلات النانويات البوليمرية العينية المفردة والمختلطة باستخدام البريمونيدين كنموذج دوائي: تحضير, توصيف, و تقييم الخواص الفيزيائية

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الخلاصة

المذيلات النانوية هي حاملات دوائية نانوية ناقلة تستخدم في البحوث الصيدلانية لتوصيل الأدوية إلى الموقع المستهدف. تكون الوحدات البنائية لها عبارة عن جزيئات محبة للماء (مواد خافضة للتوتر السطحي أو بوليمرات) تتجمع ذاتيًا في بنية شبيهة بالصدفة بمركز كاره للماء و قشرة محبة للماء عندما تنتشر في وسط مائي. تمتلك المذيلات النانوية البوليمرية تركيز مذيل حرج أقل بآلاف المرات من المواد الخافضة للتوتر السطحي ذات الوزن الجزيئي المنخفض، مما يؤدي إلى ثباتية ووقت توفرأكثر بعد الإعطاء. تتمتع المذيلات النانوية المختلطة بميزة إضافية تتمثل بتحسين الثباتية وكفاءة حجز الدواء مقارنة بالمذيلات المفردة. هدفت هذه الدراسة إلى تحضير مذيلات نانوية بوليمرية أحادية من السولوبلس ومختلطة من السولوبلس- توكوفيرسولان ومقارنة خصائصهما الفيزيائية باستخدام البريمونيدين كنموذج دوائي كاره للماء. تم استخدام دراسة الذوبان المشبع لتحديد معاملات الذوبان المختلفة، و استخدام طريقة ترطيب الغشاء الرقيق لإعداد ثلاث مذيلات نانوية بوليمرية أحادية وستة مختلطة. تم تقييم أربعة متغيرات، تشمل تركيز البريمونيدين، نسبة التوكوفيرسولان إلى السولوبلس، درجة حرارة مرحلة الترطيب، ومرحلة إضافة التوكوفيرسولان، بالنسبة لتأثيراتها على الخصائص الفيزيائية المدروسة(المظهر، حجم الجسيمات، مؤشر تعدد التشتت، نسبة كفاءة حجز الدواء، تحرر الدواء خارج الجسم الحي، و الثباتية الفيزيائية). تم اختيارأفضل عينة من المذيلات النانوية البوليمرية المختلطة وتم دراسة التركيز المذيل الحرج لها باستخدام طريقة التوتر السطحي، بالاضافة الى دراسة FTIR, FESEM. أظهرت النتائج أن ذوبان البريمونيدين زاد بشكل طردي بنسبة متناسبة مع تركيز السولوبلس، حيث كان تركيز السولوبلس (٥٩ مجم / مل) له أعلى سعة إذابة مولارية (٣,٧١٥) و نسبة حجز دواء تساوي (٠,٨٧٢) مع قيمة سالبة لطاقة جبس القياسية الحرة (-٣,٤٥٣)، مما يشير إلى تكوين المذيلات النانوية بصورة تلقائية. أثر تركيز البريمونيدين على المظهر الخارجي للمذيلات النانوية المفردة وكان له تأثير كبير على نسبة كفاءة حجز الدواء، في حين لم يكن له تأثير على حجم الجسيمات ومؤشر تعدد التشتت. كانت أفضل المذيلات النانوية المفردة (F2) شفافة, بحجم جسيمات (٧٥,٧٥ ± ١,١٣ نانومتر) ومؤشر تعدد التشتت (٠,١٢٤٣ ± ٠,٠٢) ونسبة مئوية لكفاءة حجز الدواء (٥٣,٤٩٪ ± ٠,٥٨)، قد تم اختيارها لإعداد المذيلات النانوية المختلطة والتي وجد ان جميع المتغيرات المدروسة كان لها تأثير كبير للغاية على حجم الجسيمات ونسبة كفاءة حجز الدواء في حين لم يكن لها تأثير على المظهر الخارجي ومؤشر تعدد التشتت. كانت أفضل المذيلات النانوية البوليمرية المختلطة (F7) شفافة، بنسبة توكوفيرسولان إلى السولوبلس تساوي (١:٢٢,٥) و المضاف في المرحلة العضوية، وبحجم جسيمات (٧٩,٥٥±٠,٢٤ نانومتر)، ومؤشر تعدد التشتت (٠,١٢٢٢±٠,٠٠) ونسبة كفاءة حجز الدواء (٦٢,١٨٪ ± ٠,٢٣)، مع تركيز مذيل حرج قدره (3.467 * 10-7M)، واستقرار أكثر من F2 بعد التخزين لمدة ٩٠ يومًا في (٤,٠±٢,٠ درجة مئوية) مع تحرر دوائي سريع خارج الجسم الحي (٧٨,٤±٠,٢٨٪) في غضون ١٥ دقيقة. أشارت نتائج FTIR للمكونات و F7 إلى توافق المكونات مع بعضها، بينما أظهرت نتائج FESEM ان شكل المذيلات هو كروي و بأحجام جسيمات قريبة من تلك التي تم اكتشافها بواسطةMalvern Zetasizer . كمحصله للدراسة من النتائج, انه يمكن استخدام السولوبلس و التكوفيرسولان بنجاح لإعداد المذيلات النانوية البوليمرية المختلطة بأشكال كروية شفافة تحتوي على عقار كاره للماء في مركزها تحمل الخصائص الفيزيائية المرغوبة، بالاضافة الى تحرر دوائي سريع خارج الجسم الحي، وثباتية أكبر مقارنة بالمذيلات النانوية المفردة.

الكلمات المفتاحية: حلقة دو نوفيه, المذيلات النانوية المختلطة, معاملات الذوبانية, المذيلات النانوية المفردة, تكوفيرسولان.