Original Research Article

Open Access

Omniscient Pte.Ltd.

Green Fabrication of Berberine Nano Lipid Carriers by Hot Melt-ultrasonication Method

Van Hong Nguyen[1,*], Vy Thuy Nguyen, Hien Thi Dang[2]

[1] Life Sciences department, University of Science and Technology of Ha Noi, A21 building, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.
[2] Faculty of Pharmacy, Phenikaa University, Yen Nghia Ward, Ha Dong District, Hanoi, Vietnam.

***Correspondence to:** Van Hong Nguyen, Life Sciences department, University of Science and Technology of Ha Noi, A21 building, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. Email: chodomthanyeu@gmail.com

Abstract: The purpose of this research was to examine several factors such as lipids, surfactants and their own lipids were the blend of glyceryl monostearate with glyceryl dibehenate (C888), liquid lipid was Miglyol 812N (M812). Non-ionic hydrophilic surfactant-Poloxamer 407 was found to be suitable to provide nanostructure than its counterpart -Tween 80 (Tw 80). The *in vitro* drug dissolution was following oral administration model, through a dialysis bag, with the media of phosphate buffer pH 6.8, apparatus 2 at the speed of 50 rpm. As a result, NLCs were successfully fabricated from C888, M812 and P407. The value of hydrophilic-lipophilic balance should be from18 to 22 to ensure favourable preparation of BBR-NLCs. 15% liquid lipid over total used lipid (w/w) was shown best BBR encapsulation. The more liquid lipid quantity, the larger the particle size. In general, the best BBR-NLCs had a size of 102.30nm ± 5.49nm, uniform distribution, entrapment efficiency of 85% ± 0.96% and total release of nearly 70% after 24 hours, which was more sustained release than pure drug solution. The stability test proved BBR-NLCs could be considered as stable in nearly 30-day storage at room condition.

Keywords: Berberine; Nano lipid carrier; Ultrasonication; Hot - melt

1. Introduction

Like most of the herbal active agents, berberine (BBR) in this study was investigated to enhance its poor bioavailability. Berberine is an alkaloid family plant that has a long history of usage as folk medicine in Chinese, Native American and Indian medicine [1]. Physical appearance is yellow crystal powder and odourless. It is commonly in the form of salt with sulfate or chloride. Berberine is heat labile. Solubility of berberine is dependent on temperature, and buffer pH; in water, the solubility is 1-2 mg/ml at room temperature and permeability coefficient logP is -1.5 [2]. It has a numerous of therapeutic effects, majorly in treatment of gastrointestinal related diseases [3], and has been proved to prevent numerous intestinal tract infections including cholera and bacillary dysentery [4]. Moreover, BBR has been reported to have a variety of other promising beneficial roles on the applications in

treating infections [5], diabetes [6], anti-viral [7] and anti-cancer [8], etc.

Similar to almost natural compounds, BBR has a high molecular weight, low solubility and significantly poor permeability, which limits the absorption and therapeutic effectiveness [9]. Therefore, many modern drug carriers were designed to upgrade drug properties as well as promote drug absorption in the body. Lipid-based nanovehicles, including liposomes, niosomes, nano-emulsions, solid lipid nanoparticles (SLNs) and the most up-to-date nanostructured lipid carriers (NLCs), have beneficial impacts on encapsulated drug absorption for various administration routes over the last few decades. These upgraded types of lipid nanoparticles offered higher drug encapsulation proportion for both hydrophilic and hydrophobic drugs [10] and lower toxicity potential thanks to the utilization of biodegradable/ biocompatible lipid materials. In addition, they are feasible to manufacture mass production with a costly effective homogenization, compared to complicated polymerization processes [10], as well as green fabrication with no requirements of organic solvents. The appearance of liquid lipids enables NLCs better prevention of drug leakage during long term storage than SLNs [11]. For oral administration, BBR-NLCs were used for ulcer colitis treatment [12]. Briefly, BBR was incorporated with lipidic phase consisting of Compritol 888, olive oil, Cremophor EL d-α-tocopheryl polyethylene glycol 1000 succinate. These BBR-NLCs had good uptake in RAW 264.7 cells, Caco-2 cells as well as lessened inflammation symptoms in mice colitis. Another BBR-NLCs also showed sustained release with the hypoglycemia effect, which is promising for diabetes treatment [13]. In this study, BBR was coated outside with selenium to obtain synergetic treatment effects. The preparation NLCs was found in nano range size with entrapment efficiency reaching 90%. *In vitro* release, both BBR-NLCs and the coated one performed sustained release compared to pure drug solution in intestinal pH. *In vivo* administration, coated BBR-NLCs also showed better bioavailability, good intestinal absorption, and significantly decreased hypoglycemia in Wistar rats. BBR recently was incorporated in NLCs for Alzheimer's disease through oral administration [14]. The study revealed that oral bioavailability of the drug was improved more than 3 times of max concentration (C_{max}), along with the elevation of concentration in the brain as well. The similar results supported for brain targeting capability of BBR-NLCs was also performed elsewhere [15]. However, the screening of liquid types, and the effect of various liquid lipid ratios for NLC loading BBR were hardly found. Additionally, the surfactants used in BBR-NLCs are necessary to further study, as Tween 80 was the only one used in previous study [13, 14].

Therefore, this study aimed to develop formulations of BBR-NLCs by determining the effect of the chosen material, and the excipient ratio by hot-melting and ultrasonication technique. The effect of different lipid compositions on BBR-NLC physical properties was evaluated. Specifically, the lipid types and different liquid lipid ratios were examined. Furthermore, numerous surfactant types and surfactant combinations were investigated in BBR-NLCs formation. Additionally, drug entrapment efficiency and *in vitro* release following oral uptake model were also quantified. Then, short-term stability of BBR-NLCs was tested.

2. Materials and Methods

2.1. Materials

Berberine hydrochloride (BBR) met pharmaceutical grade. Poloxamer 407 (P407) from BASF while Tween 80 (Tw80), and Span 80 (Sp80) from Xilong Scientific Co., Ltd were used. Glyceryl dibehenate (C888) was donated by Gattefossé. Miglyol 812N (M812) was from IOI Oleochemical. Glyceryl monostearate (GMS) was procured from INOLASTIC Company.

2.2. Methods

2.2.1. Preparation of BBR-NLCs

BBR-NLCs were prepared by the hot melt – ultrasonication method. Briefly, 2% w/v lipid phase was melted at 80 °C, followed by BBR addition to form a homogenous mixture in the water bath (Julabo TW12, Germany). Then, the water phase at the same temperature was added. Samples were probe-sonicated (Qsonica, USA), at the power of 210W in 15 minutes. Finally, these samples were immediately cooled at 4°C in 10 mins. The resulted samples were stored in tight glass bottle at room temperature for further investigations (**Figure 1**.).

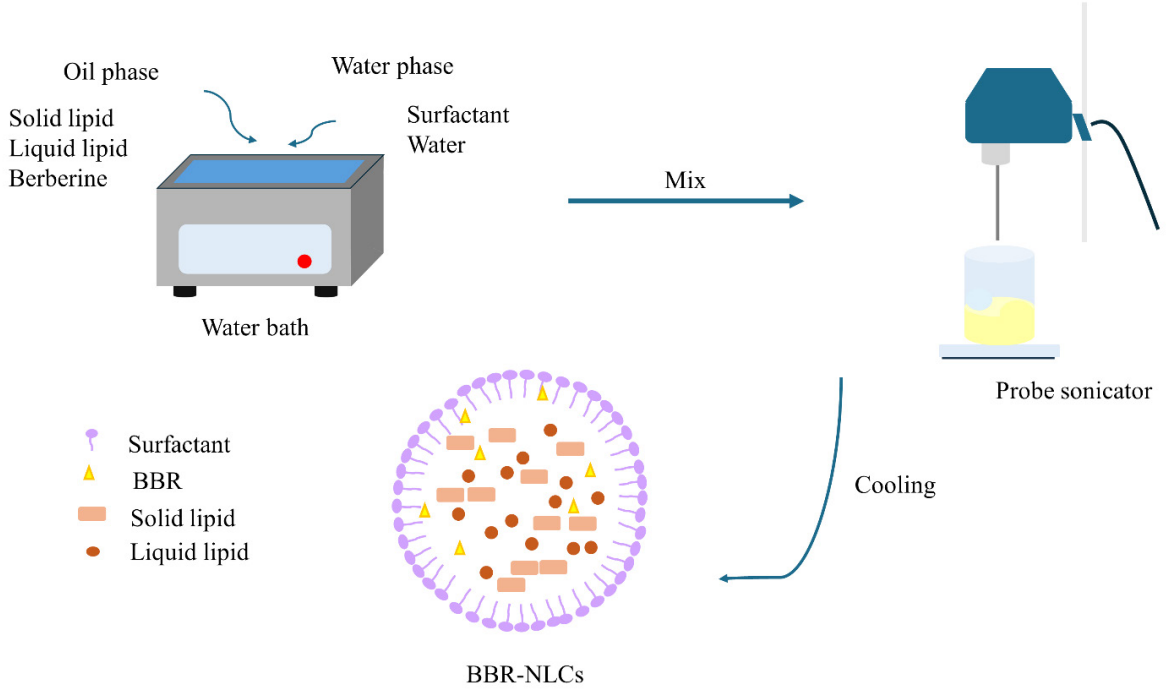

Figure 1. Preparation of BBR-NLCs by hot melt – ultrasonication methods

2.2.2. Size, polydispersity index and zeta potential

These above physical properties of BBR-NLC were checked by SZ-100 Zetasizer (Horiba, Japan). All samples were diluted with distilled water to ensure precise measurements. Samples were checked triplicate.

2.2.3. Drug entrapment

Samples were centrifuged for 20 minutes at 20,000 rpm. The supernatants were collected to quantify the free drug by Ultraviolet-visible (UV-vis) spectrometry (Jenway model 6750, UK) at 345 nm. Encapsulation efficiency (EE) and loading capacity (LC) [16] were calculated by the below equation:

$$\%EE = \frac{\text{total added BBR} - \text{free BBR}}{\text{total added BBR}} \times 100\%$$

$$\%LC = \frac{\text{total added BBR} - \text{free BBR}}{\text{total BBR NLCs}} \times 100\%$$

2.2.4. Drug dissolution

BBR release of BBR-NLCs were evaluated following United States Pharmacopoeia (USP) oral administration, with dialysis bad cut off at 14kDa. Specifically, BBR-NLCs and pure BBR at the same amount of 10 mg BBR were placed in the dialysis bags, and put in the baskets (apparatus 1) at 100 rpm, with 900mL of pH 6.8 phosphate buffer. 10 ml was collected from media, and an equal volume of fresh medium was added to maintain the required release volume, at the fixed time intervals. The taken samples were quantified by a UV-Vis spectrometer at $\lambda = 345$ nm.

2.2.5. Stability test

BBR-NLCs were stored at the room condition in 25 days. Samples were evaluated about size, PDI, EE and LC at each time point.

2.2.6. Statistical analysis

All data was analyzed by T-test and one-way ANOVA in Excel (Microsoft). A value of $p < 0.05$ indicated statistically significant. Each experiment was conducted triplicate.

3. Results & Discussions

3.1. Lipid Screening

Glyceryl dibehenate is a lipophilic mixture of various glycerol esters of behenate, with a melting point between 69 and 74°C. It appears in the form of white either semi-solid pellets, flake, or fine powder. In pharmaceutical applications, glyceryl dibehenate can be used as a lubricant for oral tablets, a solid matrix for sustained release formulation. Especially, it can be commonly used in lipid-based nanoparticles such as SLN and NLCs [17].

Miglyol 812N is a medium chain triglyceride

of caprylic acid and capric fatty acid and also the most used as a liquid lipid excipient in this colloidal system [18]. Its appearance is clear, colorless, and odourless. Miglyol 812 is miscible with natural oil at all ratios. In the preparation of NLCs, liquid lipid is not recrystallized during the cooling process, thereby forming crystals with an irregular lattice structure in lipid matrix, which will provide additional room for drug incorporation. Specifically, the melting point of Miglyol 812 is below 0°C, which is lower than the cooling temperature in BBR-NLC preparation method, while the melting points of GMS and C888 were in the range of 60-75°C. Moreover, the addition of triglycerides like Miglyol 812 enables the space in the lipid matrix for the drug solubilization and the prevention of drug aggregation during fabrication and storage [19].

The BBR-NLC formulations of separated solid lipid, as well as the combination with another lipid at the ratio of 1:1 (w/w) were investigated. As can be seen from **Figure 2**, the formulations consisting of C888, C888:M812 and GMS:M812 had low particle size as well as homogeneous (PDI < 0.3). By eye observation, all formulations with GMS formed a white thin layer on the surface of BBR-NLCs after cooling. Therefore, the mixture of C888 and M812 was chosen for further investigation.

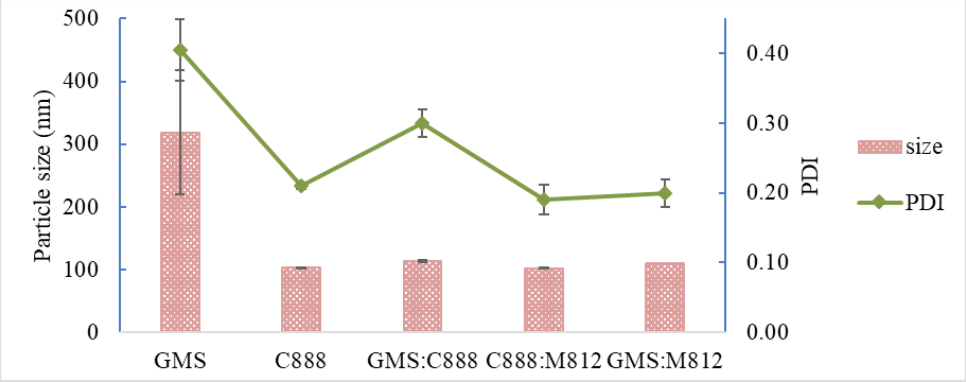

Figure 2. BBR-NLCs physical properties of different lipid ingredients

3.2. Surfactant screening

The hydrophilic-lipophilic balance (HLB) of a surfactant mixture determines emulsion types. Non-ionic surfactants are commonly used in pharmaceutical products for better solubilizing and as emulsifying agents. Thus, Tw80, Poloxamer 407 and Span 80 were non-ionic surfactants in this research. C888:M812 lipid at the ratio of 1:1, was prepared with an aqueous phase containing a combination of different types of surfactants including Sp80, Tw80 and P407. Span 80 (HLB 4.3) or sorbitan oleate is an ester of oleic acid and sorbitol, it is relatively lipophilic. While Tw80 (HLB 15) is a polysorbate with hydrophilic properties derived from esterification between ethoxylated sorbitan and oleic fatty acid. In contrast, P407 (HLB 22) is a hydrophilic polymer, contained numerous groups of poly(ethylene oxide) and poly(propylene oxide) [20]. The HLB system for investigation is 12 (**Table 1**).

Table 1. Physical properties of NLCs with different surfactants ($p < 0.05$)

	Blank NLCs		*Ber-NLCs*	
Blended surfactant	Tw80:Sp80	P407:Sp80	Tw80:Sp80	P407:Sp80
Size	102.00 ± 0.78	182.90 ± 1.83	1945.33 ± 636.85	248.57 ± 6.28
PDI	0.19 ± 0.02	0.26 ± 0.01	0.79 ± 0.19	0.23 ± 0.03
Zeta	-10.22 ± 0.41	-9.13 ± 0.45	0.63 ± 0.13	0.31 ± 0.32
Observation	Suspension	Suspension	Creamy	Suspension

Blank NLCs in two cases resulted in an acceptable range that was formulations with Tw80:Sp80 and

P407:Sp80 had a size and zeta potential of 102.00 ± 0.78 nm, -10.22 ± 0.41 and 182.90 ± 1.83 nm, -9.13 ± 0.45, respectively. However, when it comes to drug loading, BBR-NLCs containing Tw80 became heterogeneous (PDI = 0.79±0.19) and significantly enlarged in its size while that of P407 still remained unimodal dispersion. The HLB of P407 is higher than Tw80, which made the emulsion system more balanced, the nature of the molecular structure also contributes to the differences. Tw80 is a single-chain surfactant, which means they adsorb individual molecules at the interface of oil/water. Thus Tw80 stands singly at the oil/water interface. While P407 contains component blocks with different affinities [19], then strengthening the tiny lipidic droplets through a stronger steric impact [20]. Therefore, surfactant blends of P407 and Sp80 were used for HLB value investigation.

3.3. Effects of Total HLB Value of Surfactants

Surfactants have two crucial functions in the development of emulsions: formation and stabilization through prevention of particle aggregation and recrystallization [21]. Surfactant functionality in emulsion systems is influenced by a number of characteristics. There are examples of these factors such as surfactant ratio, HLB, volume, and chemical structure of surfactants [22]. Different combinations of surfactants Sp80:P407 (w/v) were prepared as an aqueous phase to obtain the hydrophilic – hydrophobic balance (HLB) values from 12 to 22, by varying the weight ratios of the two surfactants with different HLB [23].

All formulations without BBR at all HLB values presented at the preferable size range (**Figure 3**), with the size below 200 nm. The higher the HLB value increased, the smaller the BBR-NLCs size were. BBR-NLC size reduced lower than 100nm from HLB values larger than 20. When it comes to drug incorporation into these same formulations, the particle size had the increased trend in diameter. Additionally, the increase of HLB was inversely proportional to the particle size.

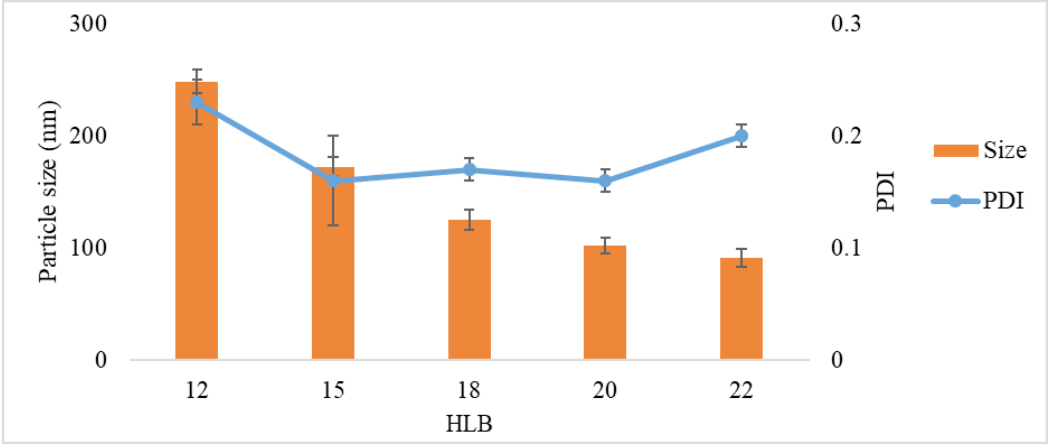

Figure 3. BBR-NLCs physical properties at various surfactant HLB

BBR-NLCs at different surfactant HLB from 12 to 22 showed a homogeneous distribution (PDI < 0.3). At HLB 20, the PDI of ber-NLC almost did not change compared to blank (0.16±0.01 compared to 0.16±0.02) ($p > 0.05$).

Although the usage of non-ionic surfactants Sp80 and P407, the blank NLCs formulations had a negative charge on the surface of particles. This can be due to the charge of lipids C888 and M812. When it comes to incorporating BBR inside, the positive charge of the cation group iminium in the BBR molecules had neutralized the negative charge, leading to the zeta potential of the particles close to 0mV.

Additionally, the EEs of BBR-NLCs were in the gap between 69% and 85% (**Figure 4**). The maximum efficiency was shown at HLB larger or equal value of 18, hence, it can be draw first conclusion that this o/w emulsion requires HLB from 18-22 to keep stable and able to encapsulate BBR greater than 80%.

Therefore, the optimized formulation including C888, M812, P407, Sp80 having HLB value 20 with greatest EE at 85.50% ± 0.96%. The results were significant to that of HLB 12 and 15 ($p < 0.05$). Hence, this formulation would be further investigation.

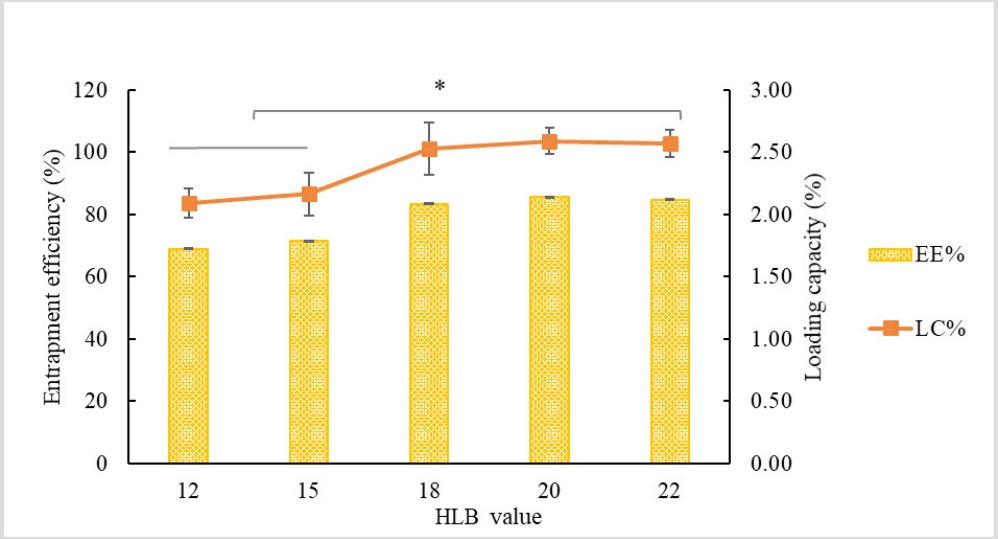

Figure 4. BBR-NLCs EE and LC at various HLB (* $p < 0.05$)

3.4. Effects of Liquid Lipid Proportion on BBR-NLCs Properties

With the PDI smaller than 0.3 and zeta potential close to 0mV, the particle diameter experienced a marginally increased trend when the proportion of liquid lipid increased (**Table 2**). That can be seen from **Figure 5**. of EE that the loading capacity of BBR-NLCs was not linear following the proportion of liquid lipid. The screening process is necessary for finding the most suitable lipid mixture.

Table 2. BBR-NLCs properties with different liquid lipid ratio in total lipid (w/w)

% liquid lipid	Size (nm)	PDI	Zeta (mV)	%EE	%LC
0%	91.78 ± 5.40	0.17 ± 0.02	-0.42 ± 0.22	73.94 ± 0.93	2.24 ± 0.16
15%	102.30 ± 5.49	0.16 ± 0.02	0.16 ± 0.16	85.50 ± 0.96	2.59 ± 0.20
25%	121.07 ± 5.50	0.20 ± 0.02	0.25 ± 0.21	69.02 ± 0.93	2.09 ± 0.21
37.5%	125.37 ± 6.73	0.19 ± 0.02	0.29 ± 0.39	67.42 ± 0.78	2.04 ± 0.39
62.5%	117.33 ± 4.74	0.16 ± 0.03	0.53 ± 0.28	73.72 ± 0.95	2.23 ± 0.28

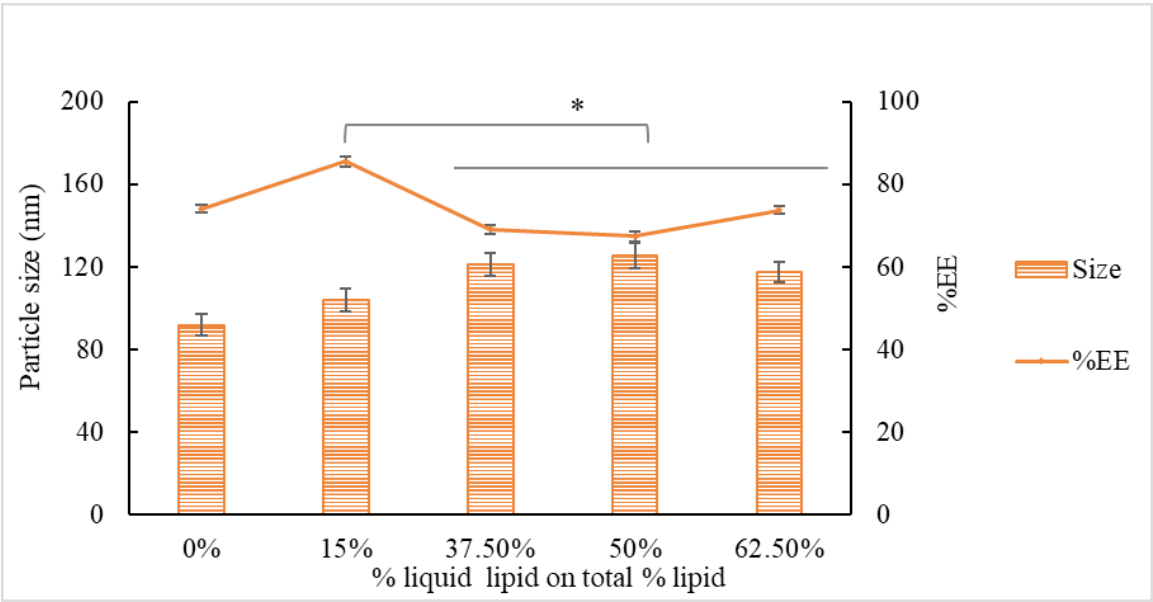

Figure 5. Size and EE of BBR-NLCs at different liquid lipid proportion (* $p < 0.05$)

All the researched formulations resulted in the encapsulation efficiency from 67% to 85% (**Figure 5**). The formulations with 15% of liquid lipid over total lipid found to be in the nanosized range (102.30 ± 5.49, PDI = 0.16 ± 0.02) and had the best EE and LC at 85.50% ± 0.96% and 2.59% ± 0.20% compared to three others formulation with more liquid lipid proportion ($p < 0.05$), respectively. At the higher ratios of liquid lipid, from 15% to 50%, the BBR-NLCs sizes increased when the ratios of Miglyol 812 gained, which also occurred in previous studies [24, 25]. This could be explained by more formed space in irregular lipid structure, as well as NLC lipid core development at the higher liquid lipid ratios. Hence, Miglyol 812 at 15% of total lipid based would be expected as optimized formulation in this research.

3.5. BBR in vitro release

The drug dissolutions of pure BBR and BBR-NLCs were carried out at phosphate buffer pH 6.8 through dialysis bag (**Figure 6**). The solubility of BBR is around 1-2mg/ml, therefore after 1 hours, the release of pure BBR was 1.5 fold compared to BBR-NLCs. After 2 hours, BBR reached the plateau at greater than 90% of total BBR. In the case of BBR-NLCs, burst release occurred in the first 4 hours (61.29% ± 2.75%). This suggested that the drug molecules BBR were accumulating mostly on the particle, leading to fast release out of the lipid matrix. The burst release could be caused by the fast release of the BBR trapped on the surface of particles [26] by the steric hindrance effect of non-ionic surfactants (Sp80 and P407). The remaining of the loaded BBR continuously dissolved until 24 hours with the drug percentage of 67.66% ± 2.36% through the sustained - release control of the lipid matrix and surfactant layer, corresponding to continuous reduction of steric effect of surfactant [26] and drug diffusion of the lipidic matrix. This phenomenon may be due to the attraction of the lipid matrix to BBR molecules that hampered their diffusion out of the carriers, which tremendously influences on the drug release from NLCs [27]. Moreover, the drug released from nanoparticles further needs to diffuse across the dialysis membrane to the receptor chamber. The biphasic release pattern was observed in the case of NLCs as reported by [28-30]. Hence, BBR-NLCs performed more sustained release than that of BBR solution. The incomplete BBR dissolution maybe partly caused by high melting point of solid lipid in BBR-NLCs.

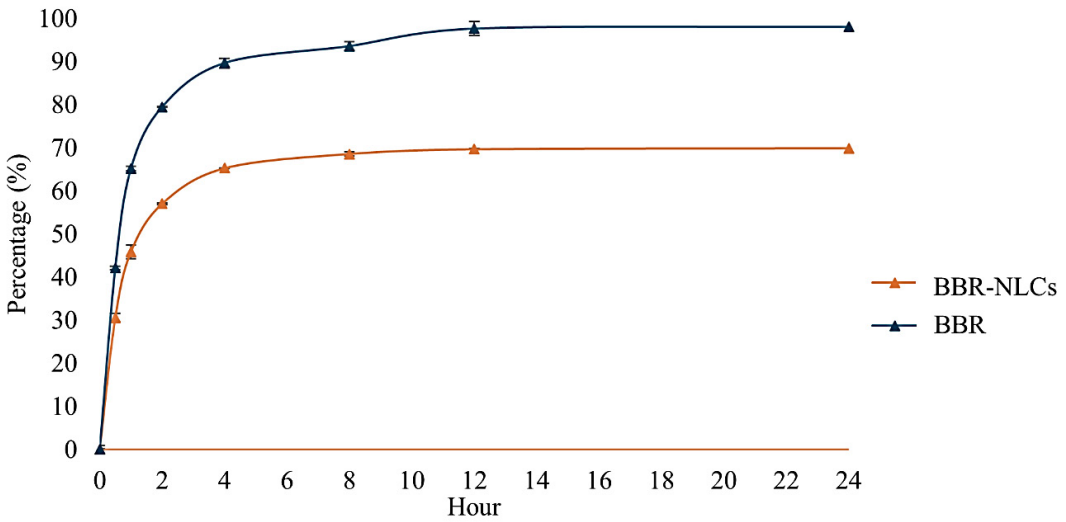

Figure 6. *In-vitro* dissolution profiles from BBR-NLCs

3.6. Stability Test

Comparing to **Table 2**, BBR-NLCs expressed minor changes in size, PDI and zeta after 25 days of storage at room temperature (**Table 3**). However, BBR tended to leak out of the carrier, leading the decrease of EE ($p < 0.05$) (**Figure 7**). Despite of that, the best formulation (in bold) remained high in drug entrapment and its LC (82.72% ± 2.03% and 2.51% ± 0.27%, respectively). Therefore, to obtain better stability, BBR-NLCs liquid system should be turned into a more stable solid

form such as powder by techniques spray-drying or lyophilization.

Table 3. BBR-NLCs characteristics after 25 days

%liquid lipid	Size (nm)	PDI	Zeta (mV)	%EE	%LC
0%	93.22 ± 0.52	0.18 ± 0.00	0.52 ± 0.12	68.51 ± 2.53	2.08 ± 0.30
15%	108.63 ± 0.38	0.22 ± 0.01	0.73 ± 0.32	82.72 ± 2.03	2.51 ± 0.27
25%	119.40 ± 0.17	0.20 ± 0.00	0.67 ± 0.06	65.50 ± 3.76	1.98 ± 0.42
37.5%	127.37 ± 0.55	0.20 ± 0.01	0.66 ± 0.15	56.71 ± 2.45	1.72 ± 0.28
62.5%	121.57 ± 0.68	0.17 ± 0.02	0.53 ± 0.30	66.03 ± 2.59	2.00 ± 0.32

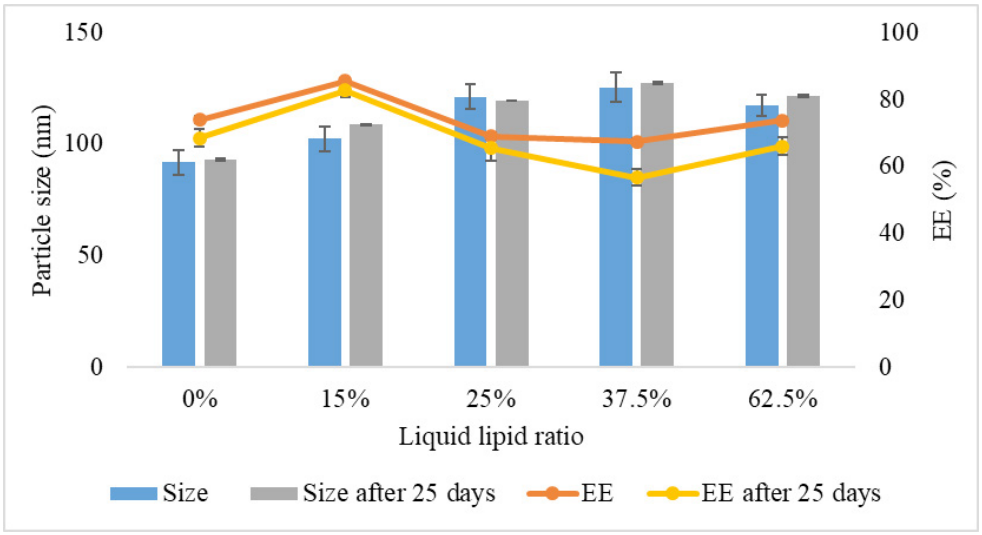

Figure 7. Size and EE of BBR-NLCs freshly prepared and after 25 days

Conclusions

BBR-NLCs were successfully prepared by the hot melt - ultrasonication method. Lipid types, lipid compositions, surfactants and their relative parameters strongly affted on the properties of BBR-NLCs. The BBR-NLC particles was found in nano-range size, above 100 nm and homogeneous (PDI of 0.16). The formulation fabricated from P407, Span80 at HLB of 20, and 15% (w/w) Miglyol 812 in the lipid matrix for oral *in vitro* release. The dissolution performances through dialysis bag in pH 6.8 phosphate buffer indicated that BBR-NLCs had more sustained release than BBR solution.

Conflicts of interest

None

References

[1] Chander, V., et al., *A review on Pharmacological potential of Berberine; an active component of Himalayan Berberis aristata.* J Phytopharmacol, 2017. 6(1): p. 53-58.

[2] Battu, S.K., et al., *Physicochemical characterization of berberine chloride: a perspective in the development of a solution dosage form for oral delivery.* AAPS PharmSciTech, 2010. 11(3): p. 1466-1475.

[3] Imenshahidi, M. and H. Hosseinzadeh, *Berberine and barberry (Berberis vulgaris): a clinical review.* Phytotherapy Research, 2019. 33(3): p. 504-523.

[4] Muluye, R.A., Y. Bian, and P.N. Alemu, *Anti-inflammatory and antimicrobial effects of heat-clearing Chinese herbs: a current review.* Journal of traditional and complementary medicine, 2014. 4(2): p. 93-98.

[5] Tan, J., et al., *Antimicrobial characteristics of Berberine against prosthetic joint infection-related Staphylococcus aureus of different multi-locus sequence types.* BMC Complementary and Alternative Medicine, 2019. 19(1): p. 218.

[6] Zhang, Y., et al., *Gut microbiome-related effects*

of berberine and probiotics on type 2 diabetes (the PREMOTE study). Nature communications, 2020. 11(1): p. 1-12.

[7] Warowicka, A., R. Nawrot, and A. Goździcka-Józefiak, *Antiviral activity of berberine.* Arch Virol, 2020. 165(9): p. 1935-1945.

[8] Zhang, C., et al., *Effects of Berberine and Its Derivatives on Cancer: A Systems Pharmacology Review.* Frontiers in Pharmacology, 2020. 10: p. 1461.

[9] Yasir, M., et al., *Biopharmaceutical classification system: An account.* International Journal of PharmTech Research, 2010. 2(3): p. 1681-1690.

[10] Fundarò, A., et al., *Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after iv administration to rats.* Pharmacological Research, 2000. 42(4): p. 337-343.

[11] Makoni, P.A., K. Wa Kasongo, and R.B. Walker, *Short Term Stability Testing of Efavirenz-Loaded Solid Lipid Nanoparticle (SLN) and Nanostructured Lipid Carrier (NLC) Dispersions.* Pharmaceutics, 2019. 11(8): p. 397.

[12] Deng, J., et al., *Berberine-Loaded Nanostructured Lipid Carriers Enhance the Treatment of Ulcerative Colitis.* International journal of nanomedicine, 2020. 15: p. 3937-3951.

[13] Yin, J., et al., *Selenium-coated nanostructured lipid carriers used for oral delivery of berberine to accomplish a synergic hypoglycemic effect.* International journal of nanomedicine, 2017. 12: p. 8671-8680.

[14] Lohan, S., et al., *Galactosylated nanoconstructs of Berberine with enhanced Biopharmaceutical and cognitive potential: A preclinical evidence in Alzheimer 's disease.* Journal of Drug Delivery Science and Technology, 2021. 66: p. 102695.

[15] Raju, M., et al., *Berberine loaded nanostructured lipid carrier for Alzheimer's disease: Design, statistical optimization and enhanced in vivo performance.* Life Sciences, 2021. 285: p. 119990.

[16] Nguyen, V.H., K.N. Manh Le, and M.C.N. Nguyen, *Spray-dried solid lipid nanoparticles for enhancing berberine bioavailability via oral administration.* Current Pharmaceutical Design, 2023. 29(38): p. 3050-3059.

[17] Aburahma, M.H. and S.M. Badr-Eldin, *Compritol 888 ATO: a multifunctional lipid excipient in drug delivery systems and nanopharmaceuticals.* Expert opinion on drug delivery, 2014. 11(12): p. 1865-1883.

[18] Jenning, V., M. Schäfer-Korting, and S. Gohla, *Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties.* Journal of Controlled Release, 2000. 66(2): p. 115-126.

[19] Grumezescu, A.M., *Design of nanostructures for versatile therapeutic applications*. 2018: William Andrew.

[20] Bodratti, A.M. and P. Alexandridis, *Formulation of Poloxamers for Drug Delivery.* Journal of functional biomaterials, 2018. 9(1): p. 11.

[21] Han, F., et al., *Effect of surfactants on the formation and characterization of a new type of colloidal drug delivery system: nanostructured lipid carriers.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2008. 315(1-3): p. 210-216.

[22] Ici Americas, i., *The HLB system : a time-saving guide to emulsifier selection*. 1984, Wilmington: ICI Americas, Inc.

[23] Lieberman, H., M. Rieger, and G.S. Banker, *Pharmaceutical dosage forms: Disperse systems.* 2020: CRC Press.

[24] Fitriani, E.W., et al., *Development of nanostructured lipid carrier containing tea tree oil: Physicochemical properties and stability.* Journal of Pharmacy & Pharmacognosy Research, 2023. 11(3): p. 391-400.

[25] Ortiz, A.C., et al., *Development of a nanostructured lipid carrier (NLC) by a low-energy method, comparison of release kinetics and molecular dynamics simulation.* Pharmaceutics, 2021. 13(4): p. 531.

[26] Khosa, A., S. Reddi, and R.N. Saha, *Nanostructured lipid carriers for site-specific drug delivery.* Biomedicine & Pharmacotherapy, 2018. 103: p. 598-613.

[27] Patel, S.G., et al., *Chapter 8 - Solid Lipid Nanoparticles for Targeted Brain Drug Delivery*, in *Nanotechnology-Based Targeted Drug Delivery Systems for Brain Tumors*, P. Kesharwani and U. Gupta, Editors. 2018, Academic Press. p. 191-244.

[28] Chaudhari, V.S., U.S. Murty, and S. Banerjee, *Nanostructured lipid carriers as a strategy for encapsulation of active plant constituents:*

Formulation and in vitro physicochemical characterizations. Chemistry and Physics of Lipids, 2021. 235: p. 105037.

[29] Shrivastava, S., B. Gidwani, and C.D. Kaur, *Development of mebendazole loaded nanostructured lipid carriers for lymphatic targeting: Optimization, characterization, in-vitro and in-vivo evaluation.* Particulate Science and Technology, 2021. 39(3): p. 380-390.

[30] Müller, R.H., M. Radtke, and S.A. Wissing, *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations.* Advanced Drug Delivery Reviews, 2002. 54: p. S131-S155.