Original Article

TIPS Trends in Pharmaceutical Sciences 2020: 6(4): 255-262.

Preparation and Characterization of Berberine loaded Micelle Formulations with Approach to Oral Drug Delivery

Roza Azadi[1], Seyyedeh Elaheh Mousavi[2,*], Negar Motakef Kazemi[1], Seyed Mahdi Rezayat[2,3,*], Mahmoud Reza Jaafari[4]

[1]Department of Medical Nanotechnology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.

[2]Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

[3]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

[4]Department of Pharmaceutical Nanotechnology, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Berberine (BBR) is a quaternary ammonium salt that possesses plentiful therapeutics properties. But notwithstanding the positive points, it has two negative points: poor aqueous solubility and permeability. These properties are important for achieving good bioavailability and therapeutic effect. Lately nano formulations developed to overcome these challenges through drug encapsulation. The aim of this study was preparation of nano formulations based on surfactant to achieve the best formulation with good characteristics. In this research, nano micellar formulations were prepared by thin film hydration method using poly sorbate 20 as surfactant and BBR as drug to get the good formulation based on high encapsulation efficiency (EE). Then nano micelles were characterized by particle size and polydispersity index (PDI) by DLS, drug encapsulation by UV-Vis spectrophotometer and drug release behavior in simulated gastro fluid (SGF) and simulated intestinal fluid (SIF). BBR successfully was encapsulated within micelles by thin film hydration method. DLS analysis showed average size of nano micelle samples between 9.247 and 18.46 nm, PDI was about 0.271, with maximum percentage of drug encapsulation of 78%. Also fluctuation of drug release was very low in elementary time points in SGF and SIF, and it was approximately sustained release profile. These results showed to achieve a good formulation and in order to have better drug delivery, physical attributes including the size distribution, PDI, and EE should be controlled. Our findings may be benefactress for different applications in variety research fields of pharmaceutical industry.

Keywords: Berberine, Nano Formulation, Drug Delivery, Encapsulation, Micelle

1. Introduction

Oral drug delivery is the most conventional method for drug administration due to patient friendly, convenient, cost effective, and noninvasiveness (1). Also the oral route provides a large surface area for absorb the drug molecules through epithelium of the human intestine due to the abundance of enterocytes in different parts of the intestine (2, 3). On the other hands, oral drugs for absorbing in the stomach and the small intestine need to be dissolved in gastrointestinal epithelium. Hence, formulations should be developed to have better delivery and therapeutic efficacy.

Preparation of drug formulations with approach of nanotechnology is one of strategies that

Corresponding Author: Seyyedeh Elaheh Mousavi & Seyed Mahdi Rezayat, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: semousavi@sina.tums.ac.ir & rezayat@sina.tums.ac.ir

Recieved: 01/10/2020; Accepted: 01/12/2020

shown promising results to improve the solubility of hydrophobic drugs. One of the hydrophobic herbal extracts which have many medicinal properties is BBR. It is an isoquinoline alkaloid found in plants includes barberry (Berberis vulgaris), coptis (Coptis chinensis), and tree turmeric (Berberis aristata). Also, compared with other chemical drugs, it has fewer side effects (4-6). Researches have suggested BBR can help to treat diabetes, obesity, and degenerative diseases; also it has shown promising activities in anti-inflammation, antioxidant and antimicrobial properties (7-12). But despite all the healing properties, it because of being hydrophobic nature, has poor aqueous solubility (log P=-1.3), poor absorption, and low bioavailability (<5%) (13-15). It is metabolized rapidly in the liver, and its clearance from blood is very fast, which lead to decreased therapeutic efficacy (16, 17). So to overcome these challenges using the new approaches such as the nanotechnology would be very promising.

Among the nano carriers (ranging from liposomes, polymer–drug conjugates and polymeric nano spheres), micelles possess very advantages as drug carriers such as small size (<50 nm), low toxicity, biodegradation, cost effective, easy preparation, good permeability, enhance solubility of hydrophobic drugs, and drug protection against enzyme degradation (18-27). In between, surfactant micelles are widely used in the pharmaceutical industry and food industry. Tween 20 is non-ionic surfactant that has low toxicity and do not usually interact with active ingredients, so has great significance in the pharmaceutical sciences (28). In this research, in order to achieve the optimal micellar formulation for better drug delivery and improve the solubility of hydrophobic drug, the synthesis of nano BBR-micelles was carried out using thin film hydration method (29). The other benefits of this method are the simplicity in micelle production and its ability to create small and uniform particles (30).

The aim of this study was use approach of nano technology to achieve the optimal BBR-micelle formulation and its characterization to get the high encapsulation percentage. Hence, it seems that encapsulating of BBR (as a hydrophobic drug) within micelles could be a novel formulation for oral administration of drug.

2. Materials and methods

2.1. Materials

Deionized water was used for the preparation of all formulations. All the chemicals used in the study were of the highest purity and were obtained from nano technology Research Centre, Pharmaceutical Technology Institute, Mashhad, Iran. The materials used for the preparation of the formulations were: BBR chloride, Polysorbate-20, methanol, chloroform, NaOH, KH_2PO_4, NaCL and HCL. Equipment used includes rotary (Buchi, Switzerland), centrifuge, microfuge, spectrophotometer UV-Vis (SPEKOL 1300; Analytik Jena, Germany), DLS (Malvern Zetasizer, Co.UK).

2.2 Preparation and characterization of nano micelle-BBR formulations

For the preparation of the first nano micellar formulation, amount of 0.4% (w/w) BBR was mixed with 49.6% (w/w) polysorbate 20 at room temperature, then was added at least amount of methanol to solution. Vortex was used to increase the solubility of the drug and the solution was place in the rotary apparatus for more than 2 hours to form the solid thin film. Also, methanol was removed by rotary evaporation under reduced pressure. Then, at the hydration phase, the mass of thin film was hydrated by 50% (w/w) of twice distilled deionized water at temperature 50-55 °C (provided by bain-marie) and stirring continued until was obtained a relatively clear solution. Then prepared solution kept under microfuge for 20 min with 14400 rpm. Supernatant moved to amicon filter (3000) and again kept under centrifuge for 20 min with 4000 rpm to remove the unentrapped or free drug. Finally, the supernatant was used to analysis in the spectrophotometer at λ=348 nm (29).

All formulations were prepared in exactly the same method and showed in (Table 1 and 2).

2.3. DLS

Average particle size (z-average), polydispersity index (PDI), and zeta potential of BBR-nano micelle samples were determined by laser dynamic light scattering (DLS) using Malvern Zetasizer (Nano ZS, Malvern Co.UK). The mea-

Table 1. Preparation of nano micellar formulations.

Formulation	Drug+ Surfactant+ water (W/W)
F1	0.4%+49.6%+50%
F2	0.5%+49.5%+50%
F3	1%+49%+50%
F4	2.5%+47.5%+50%
F5	5%+45%+50%

surements were repeated three times (Intensity, Number, and Volume).

2.4. Encapsulation Efficiency (EE)

Encapsulation efficacy was determined by an indirect method. Briefly, after the micellar solution was microfuge at 14,000 rpm for 20 minutes, the supernatant was filtrated through an amicon filter (3000) in centrifuge at 4000 rpm for 20 minutes. Then EE was calculated by equation 1 (31).

$$EE\% = \left(\frac{BBR\ Concentration\ in\ supernatant - filtrate}{initial\ concentration} \right) \times 100 \qquad \text{Eq. 1}$$

2.5. Drug release studies

In order to determine the stability of nano micelles, the release studies of BBR nano micelles were carried out in SGF (pH=2), and SIF (pH=6.5). SGF containing 246 µL HCl, 200 mg NaCl was added to 60 ml deionized water (DW), and pH was adjusted at 2, then the final volume was filled to 100 mL with DW.

Also SIF containing 680 mg of KH2PO4 and 61.6 mg of NaOH dissolved in 60 mL DW, and the pH was adjusted 6.5, and then the final volume was filled to 100 mL with DW.

For prepare of SIF and SGF, samples were diluted in a ratio of 1:10, and incubated at 37 °C. Then sampling carried out at time points 30 min, 1, 2, 4, 8, 24 hr and were evaluated by spectrophotometer at λ=348 nm (32).

3. Results and discussion

3.1. Calibration curve

The calibration curve for BBR was created using spectrophotometer technique. The standard solutions were prepared at concentrations of 0.6, 1.25, 2.5, 5, 10 mg/mL, with a correlation coefficient R2=0.999 and Y=62.583, X+21.253 (with dilution coefficient), signifying that 99.9% of the absorbance values. Regression analysis revealed a significant relationship between concentration and light absorbance. Results of the assay verified linearity and accuracy of the method and showed in (Figure 1).

3.2. DLS

PParticle size and PDI of BBR-micelle nanoparticles were measured for all the prepared formulations by laser dynamic light scattering (Malvern Zeta sizer, UK). Results were shown in (Figure 2 A-E). In this research, results showed a single pick and the different sizes of nano micelles were between 9.247 and 18.46 nm, PDI was about less than 0.275 and zeta potential of F2 was achieved the negative and the other formulations were positive. The results are shown in the (Table 2).

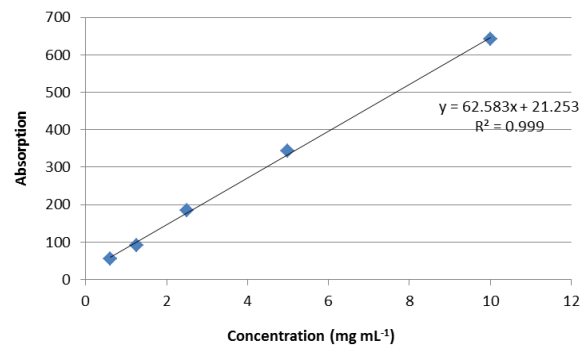

Figure 1. Standard curve (Considering the dilution coefficient).

3.3. Encapsulation Efficiency

According to spectrophotometer assay results, encapsulation efficacy was measured between 26.37 and 78%. Results have shown increasing in surfactant concentration lead to increase of drug encapsulation efficiency. This finding was in agreement with Deepak et al, 2014 (33). Furthermore, the hydrophobic nature of BBR enforces its maximum entrapment inside the core of nano micelles.

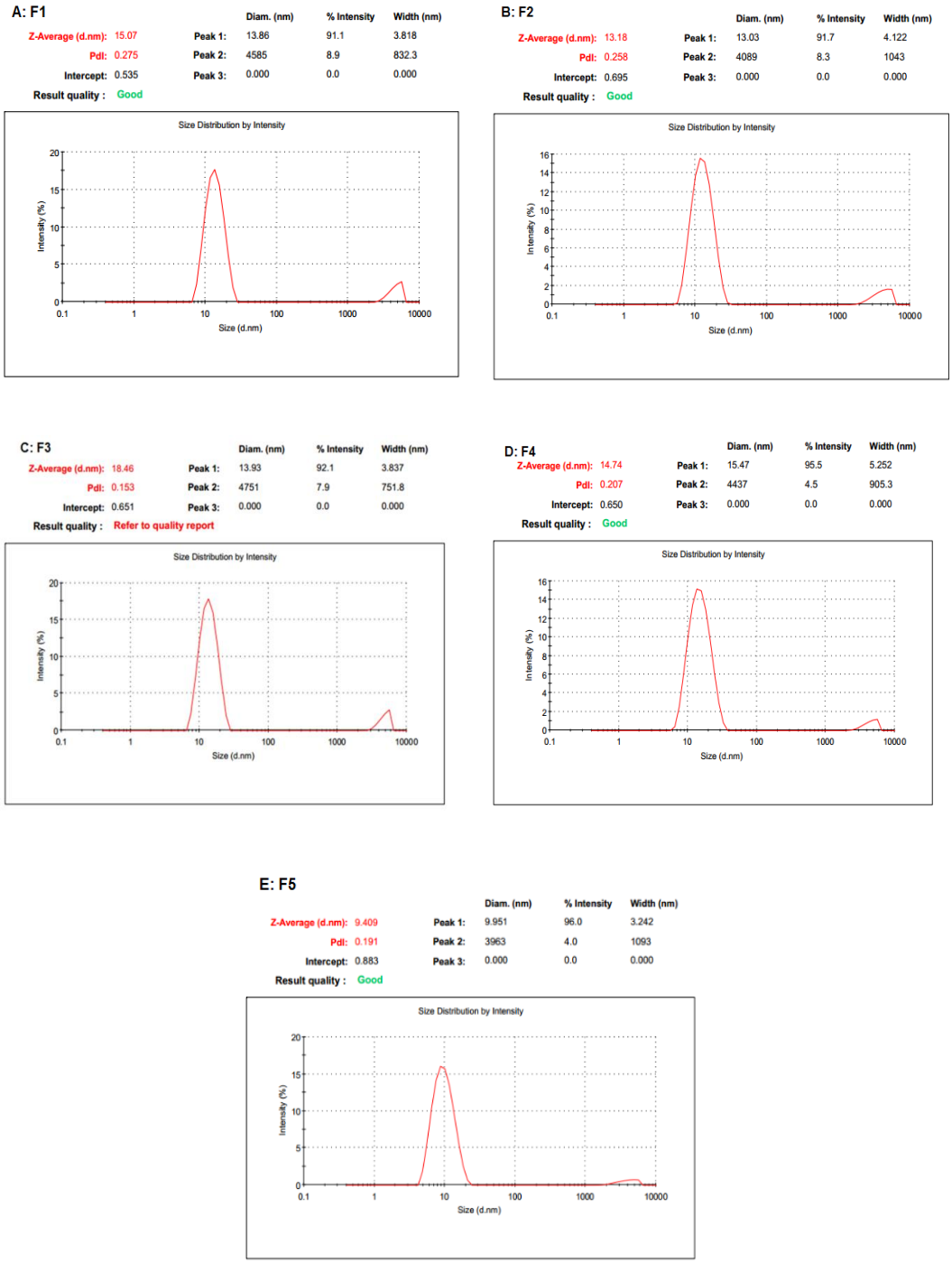

Figure 2. Size distribution graph of drug (BBR) loaded nanomicelle A: F1, B: F2, C: F3, D: F4, E: F5).

Table 2. Size distribution of nano micelle berberine formulations.

Formulation	Drug (W/W)	Size (nm)	PDI	Zeta potential	EE (%)	Result by malvern
1	0.4	15.07	0.275	1.5	72.86%	good
		14.64	0.172			
		15.10	0.202			
2	0.5	13.18	0.258	-1.33	78%	good
		12.38	0.259			
		12.92	0.222			
3	1	18.46	0.153	2.39	38.3%	---
		12.47	0.240			
		9.51	0.203			
4	2.5	14.7	0.207	2.07	26.37%	good
		14.73	0.263			good
		16.43	0.240			
5	5	9.409	0.191	2.08	25%	good
		9.247	0.175			good
		9.695	0.204			good

3.4. Drug release study

In vitro release studies were conducted in SGF and SIF. The change rate of drug release was very low in primary time points include 30 min, 1, and 2 hr in SGF and SIF (Figure 3 A, B). However, the concentration of BBR was reduced after 4 hr in SGF and SIF. According to the results, drug release in the SGF was less than in the SIF. So du-

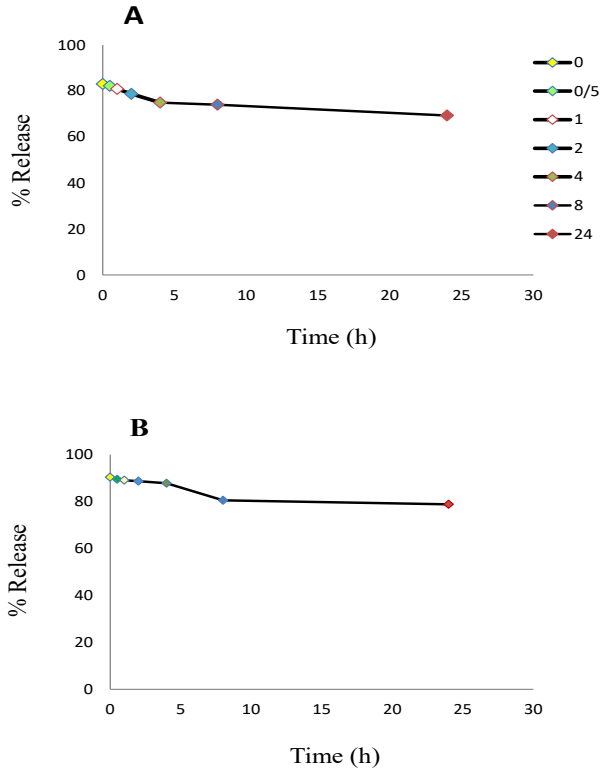

Figure 3. Release study of BBR from Nanomicelle at 37 °C.
A: Simulated Gastric Fluid (SGF), B: Simulated Intrinsic Fluid (SIF).

rability of nano micelles was higher in SGF than SIF.

In this research, micellar technique was utilized to prepare nano BBR-micelle formulations using thin film hydration method. BBR encapsulated due to its low solubility in water (0.000354 mg/mL) and hydrophobic nature (Log P=-1.3). In brief, BBR (at specified concentrations) was added to tween 20 as surfactant and then carried out hydration step, then to get the good formulation, were evaluated physicochemical characteristics of micellar formulations based on size, size distribution, homogeneity, encapsulation efficiency, drug release in SGF and SIF.

Previous studies have shown particle size is an important factor in drug encapsulation and release (34). Various particle sizes for routes of drug administration including: Lymphatic (10-50 nm), Intravenous (200-2000 nm), Ocular (100-3000 nm), Aerosol (1-10Mm), and Nasal (8-20 Mm) (35). So, we conclude that the size of prepared micelles is appropriate to the oral route. In the other hand, PDI explain the size distribution or the degree of non-uniformity of particles or nano carriers (36). In drug delivery applications, the samples with PDI values of 0.3 and less than indicate a homogenous sample of phospholipid vesicles (37,38), whatever PDI values bigger than 0.7 show size distribution of particles is widespread and not suitable to be analyzed by the dynamic light scattering (DLS) technique. In this research PDI was less than 0.25 that shown good size distribution.

According to previous studies, it was observed that drug concentration hadn't effect on size average and PDI, whereas increase or decrease of surfactant caused change in particle size (39).

4. Conclusion

Our results showed that this technique was successfully usage for the preparation of nano BBR-micelles with high EE% and good distribution. These results were shown in Tab. 1 and 2, which found increase in surfactant concentration lead to increase to EE%. The probable reason could be sufficient amount of surfactant for micelles formation. This study suggests that surfactant concentration has an important effect on the properties of the final particle.

Acknowledgments

The authors thank the Department of medicine, Faculty of Pharmacology, Tehran University of Medical sciences, Tehran, Iran. (Grant No: 98023042949)

Nanotechnology research center, Pharmaceutical technology institute, Mashhad University of medical sciences, Mashhad, iran.

Conflict of Interest

None declared.

References

1. Homayun B, Lin X, Choi HJ. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. *Pharmaceutics.* 2019 Mar 19;11(3):129. doi: 10.3390/pharmaceutics11030129.
2. Ma S, Wang L, Huang X, Wang X, Chen S, Shi W, Qiao X, Jiang Y, Tang L, Xu Y, Li Y. Oral recombinant Lactobacillus vaccine targeting the intestinal microfold cells and dendritic cells for delivering the core neutralizing epitope of porcine epidemic diarrhea virus. *Microb Cell Fact*. 2018 Feb 9;17(1):20. doi: 10.1186/s12934-018-0861-7.
3. Kwon K, Daniell H. Oral delivery of protein drugs bioencapsulated in plant cells. *Mol Ther.* 2016; 24: 1342-50.
4. Pund S, Borade G, Rasve G. Improvement of anti-inflammatory and anti-angiogenic activity of berberine by novel rapid dissolving nanoemulsifying technique. *Phytomedicine.* 2014 Feb 15;21(3):307-14. doi: 10.1016/j.phymed.2013.09.013.
5. Singh N, Sharma B. Toxicological Effects of Berberine and Sanguinarine. *Front Mol Biosci.* 2018 Mar 19;5:21. doi: 10.3389/fmolb.2018.00021.
6. Fan D, Liu L, Wu Z, Cao M. Combating Neurodegenerative Diseases with the Plant Alkaloid Berberine: Molecular Mechanisms and Therapeutic Potential. *Curr Neuropharmacol*. 2019;17(6):563-579. doi:10.2174/1570159X16666180419141613
7. Li Z, Geng YN, Jiang JD, Kong WJ. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *Evid Based Complement Alternat Med*. 2014;2014:289264.

doi: 10.1155/2014/289264.
8. Xu JH, Liu XZ, Pan W, Zou DJ. Berberine protects against diet-induced obesity through regulating metabolic endotoxemia and gut hormone levels. *Mol Med Rep.* 2017;15(5):2765-2787. doi:10.3892/mmr.2017.6321
9. Zou K, Li Z, Zhang Y, et al. Advances in the study of berberine and its derivatives: a focus on anti-inflammatory and anti-tumor effects in the digestive system. *Acta Pharmacol Sin.* 2017;38(2):157-167. doi:10.1038/aps.2016.125
10. Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, Buzoianu AD. Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. *Front Pharmacol*. 2018 Aug 21;9:557. doi: 10.3389/fphar.2018.00557.
11. Yuan NN, Cai CZ, Wu MY, Su HX, Li M, Lu JH. Neuroprotective effects of berberine in animal models of Alzheimer's disease: a systematic review of pre-clinical studies. *BMC Complement Altern Med.* 2019 May 23;19(1):109. doi: 10.1186/s12906-019-2510-z.
12. Hasanein P, Ghafari-Vahed M, Khodadadi I. Effects of isoquinoline alkaloid berberine on lipid peroxidation, antioxidant defense system, and liver damage induced by lead acetate in rats. *Redox Rep.* 2017 Jan;22(1):42-50. doi: 10.1080/13510002.2016.1140406.
13. Sahibzada MUK, Sadiq A, Faidah HS, Khurram M, Amin MU, Haseeb A, Kakar M. Berberine nanoparticles with enhanced in vitro bioavailability: characterization and antimicrobial activity. *Drug Des Devel Ther*. 2018 Feb 14;12:303-312. doi: 10.2147/DDDT.S156123.
14. Cui HX, Hu YN, Li JW, Yuan K, Guo Y. Preparation and Evaluation of Antidiabetic Agents of Berberine Organic Acid Salts for Enhancing the Bioavailability. *Molecules.* 2018 Dec 28;24(1):103. doi: 10.3390/molecules24010103.
15. Liu CS, Zheng YR, Zhang YF, Long XY. Research progress on berberine with a special focus on its oral bioavailability. *Fitoterapia.* 2016 Mar;109:274-82. doi: 10.1016/j.fitote.2016.02.001.
16. Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: An update. *Eur J Pharmacol.* 2015 Aug 15;761:288-97. doi: 10.1016/j.ejphar.2015.05.068.
17. Ye M, Fu S, Pi R, He F. Neuropharmacological and pharmacokinetic properties of berberine: a review of recent research. *J Pharm Pharmacol.* 2009 Jul;61(7):831-7. doi: 10.1211/jpp/61.07.0001.
18. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin HS. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology*. 2018 Sep 19;16(1):71. doi: 10.1186/s12951-018-0392-8.
19. Lu Y, Yue Z, Xie J, Wang W, Zhu H, Zhang E, Cao Z. Micelles with ultralow critical micelle concentration as carriers for drug delivery. *Nat Biomed Eng.* 2018 May;2(5):318-325. doi: 10.1038/s41551-018-0234-x.
20. Long JA, Rankin BM, Ben-Amotz D. Micelle Structure and Hydrophobic Hydration. *J Am Chem Soc*. 2015 Aug 26;137(33):10809-15. doi: 10.1021/jacs.5b06655.
21. Santos M, Tavares F, Biscaia E. molecular thermodynamics of micellization: micelle size distributions and geometry transitions. *Braz J Chem Eng.* 2016; 33(3): 515-523. doi.org/10.1590/0104-6632.20160333s20150129.
22. Lu Y, Zhang E, Yang J, Cao Z. Strategies to improve micelle stability for drug delivery. *Nano Res.* 2018 Oct;11(10):4985-4998. doi: 10.1007/s12274-018-2152-3.
23. Yang T, Li W, Duan X, et al. Preparation of Two Types of Polymeric Micelles Based on Poly(β-L-Malic Acid) for Antitumor Drug Delivery. *PLoS One*. 2016;11(9):e0162607. Published 2016 Sep 20. doi:10.1371/journal.pone.0162607
24. Hanafy NAN, El-Kemary M, Leporatti S. Micelles Structure Development as a Strategy to Improve Smart Cancer Therapy. *Cancers (Basel).* 2018;10(7):238. Published 2018 Jul 20. doi:10.3390/cancers10070238
25. Mandal A, Bisht R, Rupental L, Mitra A. Polymeric micelles for ocular drug delivery: From structural frameworks to recent preclinical studies. *J Control Release*. 2017; 248: 96–116, doi: 10.1016/j.jconrel.2017.01.012.
26. Adrion AC, Nakamura J, Shea D, Aitken MD. Screening Nonionic Surfactants for Enhanced Biodegradation of Polycyclic Aromatic Hydrocarbons Remaining in Soil After Conventional Bio-

logical Treatment. *Environ Sci Technol.* 2016 Apr 5;50(7):3838-45. doi: 10.1021/acs.est.5b05243.
27. Lv S, Wu Y, Cai K, He H, Li Y, Lan M, Chen X, Cheng J, Yin L. High Drug Loading and Sub-Quantitative Loading Efficiency of Polymeric Micelles Driven by Donor-Receptor Coordination Interactions. *J Am Chem Soc.* 2018 Jan 31;140(4):1235-1238. doi: 10.1021/jacs.7b12776.
28. Szymczyk K, Szaniawska M, Taraba A. Micellar Parameters of Aqueous Solutions of Tween 20 and 60 at Different Temperatures: Volumetric and Viscometric Study. *Colloids Interfaces.* 2018; 2: 34, doi:10.3390/colloids2030034.
29. Ai X, Zhong L, Niu H, He Zh. Thin-film hydration preparation method and stability test of DOX-loaded disulfide-linked polyethylene glycol 5000-lysine-di-tocopherol succinate nanomicelles. *Asian J Pharm Sci.* 2014; 9(5): 244-250, doi.org/10.1080/10837450.2017.1330345.
30. Rojsanga P, Gritsanapan W, Suntornsuk L. Determination of berberine content in the stem extracts of Coscinium fenestratum by TLC densitometry. *Med Princ Pract.* 2006;15(5):373-8. doi: 10.1159/000094272. PMID: 16888396.
31. Ebrahimi Nik M, Malaekeh-Nikouei B, Amin M, Hatamipour M, Teymouri M, Sadeghnia H.R, et al. Liposomal formulation of Galbanic acid improved therapeutic efcacy of pegylated liposomal Doxorubicin in mouse colon carcinoma. *Sci Rep*. 2019;9:9527. doi.org/10.1038/s41598-019-45974-7.
32. Hatamipour M, Sahebkar A, Alavizadeh SH, Dorri M, Jaafari MR. Novel nanomicelle formulation to enhance bioavailability and stability of curcuminoids. *Iran J Basic Med Sci*. 2019 Mar;22(3):282-289. doi: 10.22038/ijbms.2019.32873.7852.
33. Deepak Sh, Dipika M, Gilphy Ph, Ravish R, Shanu B, Manisha S, et al. Formulation and Optimization of Polymeric Nanoparticles for Intranasal Delivery of Lorazepam Using Box-Behnken Design: In Vitro and In Vivo Evaluation. *BioMed Res Inter*. 2014, 3:156010. doi: 10.1155/2014/156010.
34. Bahari L.A. Hamishehkar H. The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. *Adv Pharm Bull.* 2016; 6 (2): 143. doi: 10.15171/apb.2016.021.
35. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics.* 2018 May 18;10(2):57. doi: 10.3390/pharmaceutics10020057.
36. Mantha S, Qi Sh, Barz M, Schemid F. How ill-defined constituents produce well-defined nanoparticles: Effect of polymer dispersity on the uniformity of copolymeric micelles. 2019;3(2), doi.org/10.1103/PhysRevMaterials.3.026002.
37. Maherani B, Wattraint O. Liposomal structure: A comparative study on light scattering and chromatography techniques. *J Dispers Sci Technol.* 2017;38:1633-9, doi.org/10.1080/01932691.2016.1269651.
38. International Standard ISO22412 Particle Size Analysis-Dynamic Light Scattering, International Organisation for Standardisation (ISO) 2008.
39. Ford J.L: Particle Size Analysis in Pharmaceutics and Other Industries: Theory and Practice. Ellis Horwood, England, 1993.