IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 4 Ser. II (Jul – Aug 2019), PP 30-35
www.Iosrjournals.Org

Preparation of Dacarbazine Lipid Nanocapsule Based Hydrogel as an Efficient Delivery System for Melanoma

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Abstract: *Although dacarbazine is the gold therapy for melanoma, its systemic use is usually associated with many limitations. These include poor aqueous solubility, short half-life and toxicity. Herein, we explored the potential of lipid nanocapsule (LNC) based hydrogel as an efficient nanocarrier for transdermal delivery of dacarbazine. The proposed system was fabricated in two consecutive steps firstly,dacarbazine LNC was prepared using labrafac and solutol HS15 in different ratios and characterized regarding the particle size, zeta, entrapment efficiency(EE %) and morphological architecture by transmission electron microscope. Secondly, the optimized dacarbazine LNC was incorporated in carbopol 940 hydrogel to improve its transdermal application. The viscosity, in vitro drug release as well as the in vitro cytotoxicity were also conducted. Depending on labrafac to solutol ratio, dacarbazine LNC were successfully prepared with particle size values < 100 nm and dacarbazine EE % ranged from 75.36 ±4.14 to 93.69 ±3.18. Incorporating theoptimized LNC into hydrogel controlled dacarbazine release with minimum burst release. Both dacarbazine LNC and LNC based hydrogel was able to improve cytotoxicity against melanoma cells with respective IC50 of 0.2868 and 0.3052 mg/mL if compared to dacarbazine solution with IC50 0.5176 mg/mL. These results indicated the possibility of dacarbazine LNC based hydrogel for transdermal delivery of melanoma.*

Keywords: *cytotoxicity, dacarbazine, hydrogel, lipid nanocapsule, melanoma*

Date of Submission: 18-07-2019 Date of acceptance: 03-08-2019

I. Introduction

Unfortunately, melanoma is the most aggressive type of skin cancer and associated with poor patient prognosis and about 80% of skin cancer death [1]. Moreover, melanoma is the second relevant type of cancers worldwide with high resistance rate to conventional chemotherapy [2]. Dacarbazine is the gold standard chemotherapy for the treatment of melanoma [3]. Dacarbazine is an alkylating agent that chelates the cancerous cell DNA and consequently destroy them. Nevertheless, dacarbazine efficiency could be diminished by several obstacles as its poor solubility, short half-life, myelosuppression and light sensitivity [4].

Dacarbazine limitations are thought to be overwhelmed by using nanocarriers. Among them, lipid nanocapsule (LNC) is a biocompatible lipid nanocarrier composed of oil core of medium-chain triglyceride enclosed with pegylated surfactant and phospholipid [5]. They have many advantages as small particle size range from (20-100 nm), high physical stability, and ease of fabrication by phase inversion technique with no need for any organic solvent [6]. Moreover, LNC could prolong drug release, protect the encapsulated payload from degradation, increase drug absorption and bioavailability [5].

Hydrogel is semisolid preparation which could be of natural or synthetic origin have a great potential in biomedical applications as tissue regeneration, localized drug delivery and controlled drug release systems [7]. Hydrogel is a 3-Dporous matrix with high water content and swelling degree [8]. The incorporation of lipid nanocarriers into the hydrogel is thought to improve the mechanical properties of the hydrogel as well as prolong drug release [9]. Therefore, the aim of this study was to incorporate dacarbazine LNC into carbopol940 hydrogel as an efficient delivery system for melanoma. The LNC was prepared using different ratios of oil and surfactant to obtain a particle with lowest particle size and highest drug entrapment efficiency. The optimized dacarbazine LNC was incorporated into hydrogel and characterized regarding viscosity, *in vitro* drug release as well as *in vitro* cytotoxicity against B16 melanoma cells.

II. Materials and Method

1. Materials

Dacarbazine, Solutol® HS15, triethylamine, dimethyl sulfoxide (DMSO), RPMI, FBS, penicillin, streptomycin and L-glutamine were purchased from Sigma-Aldrich. Epikuron 200® was supplied from Cargill (Minneapolis, MN, USA). Labrafac was a kind gift from Gattefossé S.A. (Saint-Priest, France). Sodium chloride

was purchased from Adwia, El-Nasr Pharmaceutical Co., Egypt.Carbopol940 was purchased from Lubrizol Advanced Materials, Inc, Ohio, USA.

2. Method

2.1. Preparation of dacarbazine lipid nanocapsule

Lipid nanocapsule (LNC) were fabricated by adopting the phase inversion technique [10]. Concisely, dacarbazine (10% w/w) was accurately weighed and dissolved in the different oil-surfactant mixture andepikuron 200® (1.5% w/w). The oil-surfactant mixture was composed of labrafac: solutol HS15 in different ratios namely, 1:1, 1:2 and 1:4 w/w respectively (Table 1). The proposed mixture was diluted with equivolume of distilled water (containing 1.75% w/w sodium chloride). Three thermal-cooling cycles, each for between 70 and 40 °C under continuous magnetic stirring (500 rpm). During the last cooling step, cold water (with temperature 4 °C) was added to the formed blend to favor the dacarbazine LNC formation.

2.2. *In vitro* characterization of the prepared dacarbazine lipid nanocapsule

2.2.1. Particle size, size distribution and zeta potential

The particle size, size distribution measured as polydispersity index (PDI) of the prepared NPs and zeta potential of the prepared dacarbazine LNC were measured by dynamic light scattering technique using Zeta sizer, (Malvern Instruments Ltd., UK).Accordingly, different dacarbazine LNC was diluted with deionized water (1: 10v/v) then transferred to a standard quartz cuvette in a thermostatically controlled chamber at 25ºC using an angle of 90°.

2.2.2. Entrapment efficiency

The entrapment efficiency (EE %) was determined indirectly by measuring the unentrapped amount of dacarbazine. Briefly, 1 mL of different dacarbazine LNC was placed in Ultra centrifugal filters (Amicon, cut-off 10 KDa) then centrifuged at 15000 rpm for 45 min[11]. The unentrapped drug was determined in the separated filtrate using UV-spectroscopy at 323 nm. The EE% was calculated using the following equation:

$$\mathbf{EE\%}=\frac{\mathbf{Totalamountofdacarbazineadded-amountoffreedacarbazineinthefilterate}}{\mathbf{Totalamountofdacarbazineadded}}\times\mathbf{100} \quad \text{Eq. (1)}$$

2.2.3. Transmission electron microscope (TEM)

Dacarbazine LNC was visualized on a carbon-coated copper grid after negative staining by 2% phosphotungstic acid using TEM (Jeol, JEM-1230, Japan) [10].

3. Preparation and characterization of dacarbazine lipid nanocapsule based hydrogel

3.1. Preparation of dacarbazine lipid nanocapsule based hydrogel

Drug loaded LNC based hydrogel was prepared by dispersing the dacarbazine LNC into carbopol940 (1% w/v) under continuous stirring (500 rpm). Afterward, triethylamine (0.5% w/w) was added to neutralize the mixture and form the gel [12].

3.2. *In vitro* characterization ofdacarbazine lipid nanocapsule based hydrogel

3.2.1. Viscosity ofdacarbazine lipid nanocapsule based hydrogel

The viscosity of the prepared dacarbazine LNC based hydrogel was measured by using Brookfield rheometer (Brookfield DV-III ultra-programmable cone and plate rheometer fitted with a spindle number 40 and controlled with Brookfield Rheocalc operating software, U.S.A). In brief, 1 g of the prepared LNC based hydrogel was placed into the rheometer and the viscosity was measured as a function to the shear rate.

3.2.2. *In vitro*dacarbazinerelease

The *in vitro*dacarbazine release from the optimized dacarbazine LNC and dacarbazine LNC based hydrogel was conducted using dialysis method. An aliquot of each formula equivalent to 5 mg dacarbazine was placed in a dialysis bag (cut-off 10-12 KDa) and immersed in100 mL of PBS (pH 7.4) at 37 ±0.1 °C [12]. At

predetermined time intervalsup to 24 h, 1mL of the release medium was withdrawn and the dacarbazine was quantified using UV spectroscopy.

4. *In vitro* cytotoxicity

B16 melanoma cells were cultured in RPMI media containing FBS (10% v/v), L-glutamine (1%), penicillin (50 U/mL) and streptomycin (50 µg/mL). Cells were seeded into 96-well plate at a density of 7K cells/ well for 24 h. The cells were incubated with a serial concentration of dacarbazine LNC, dacarbazine LNC based hydrogel and Dacarbazine solution ranged from 0.01-10 mg/mL. To investigate the possible toxicity of the proposed vehicles, cells were incubated with the same dilutions of drug-free LNC and LNC based hydrogel. *In vitro* cytotoxicity was estimated by MTT assay after 48h of incubation. Briefly, media was replaced by MTT solution (120 µl) then cells were incubated for 4 h at 37°C and 5% CO_2. Afterward, the formed crystals were dissolved in DMSO (200 µl) then the absorbance was measured at 570 nm by plate reader (FLUO star OPTIMA, BMG Labtech).The results were expressed as the percentage cell survival which was calculated using the following equation:

$$\text{Cell survival (\%)} = \frac{\text{A570 nm of treated cells}}{\text{A570 nm of untreated control cells}} * 100 \quad \text{Eq. (2)}$$

5. Statistical analysis

All experiments were conducted in triplicates and the results are the mean ± standard deviation (SD). Student-t-test was used to compare between two variables where ANOVA followed by Tukey HSD test was applied to compare between groups. The difference was defined as significant at p-value < 0.05.

III. Results and discussion

1. Preparation of dacarbazine lipid nanocapsule

Different dacarbazine LNC were prepared using the phase inversion method. The thermal-cooling cycles used in the fabrication of LNC. Above the phase inversion temperature (70 °C), the mixture appeared as milky dispersion indicating the formation of water in oil microemulsion. Upon cooling below the phase inversion temperature (40 °C), the blend converted to clear due to the formation of oil in water microemulsion. Generally, sodium chloride is an essential additive in the preparation of LNC as it could decrease the phase inversion temperature to the available range [13, 14]. Moreover, the surfactant Epikuron is phosphatidylcholine imparted the nanocarrier biocompatibility and improved the capsular shell rigidity [15]. Solutol HS15 is a mixture of PEG 660 and PEG 660 hydroxystearate was responsible for the stability and stealth properties of LNC [16, 17]. Finally, the lipid core is composed of labrafac which inferred the LNC bulkiness [18].

2. *In vitro* characterization of the prepared dacarbazine lipid nanocapsule

2.1. Particle size and size distribution

Table (1) shows the prepared dacarbazine LNC had a particle size less than 100nm which could be attributed to the high amount of surfactants (solutol and Epikuron). Dacarbazine LNC particle size was significantly decreased by increasing the surfactant amount ($p< 0.05$). This could be attributed to the ability of solutol as a surfactant to decrease the oil surface tension and subsequently the droplet size [10]. It is to be noted that particle size is one of the most crucial parameters that affect nanocarriers' drug release as well as their fate in the body [4]**.** All the prepared LNC had PDI values less than 0.2 indicated the formation of the homogenous monodisperse system [19]].

2.2. Zeta potential

Irrespective to the LNC composition, all the prepared dacarbazine LNC showed a zeta potential in the range -10.45 ±1.54 to -13.45 ±1.78. The negative charge was due to the anionic nature of phosphatidylcholine [20]. Although the prepared LNC had a slight negative zeta potential, the presence of solutol as a PEG derivative improved the particle stability.

2.3. Encapsulation efficiency (%)

Dacarbazine LNC had a high EE % ranged from 75.36±4.14 to 93.69±3.18.One of the most important features of LNC is its ability to encapsulate high drug percentage [12]. The concomitant significant increase in

dacarbazine EE % with surfactant amount could be due to the ability of solutol to improve dacarbazine solubility in the oil core.

Table (1): *In vitro* characters of the prepared dacarbazine lipid nanocapsule

Formula code	Oil: surfactant	Particle size (nm)	PDI	Zeta potential (mV)	EE %
F1	1:1	71.99±5.78	0.11±0.005	-12.34±1.54	75.36±4.14
F2	1:2	45.61±2.54	0.15±0.007	-10.45±1.54	84.24±2.04
F3	1:4	23.54±1.51	0.09±0.002	-13.45±1.78	93.69±3.18

From the above results, formula F3 with the lowest particle size and maximum dacarbazine EE% was selected for further studies.

2.4. Transmission electron microscope

Figure (1) revels the appearance of dacarbazine LNC as a spherical non aggregated particles with a particle size 20-30 nm which is consistent with the DLS determinations.

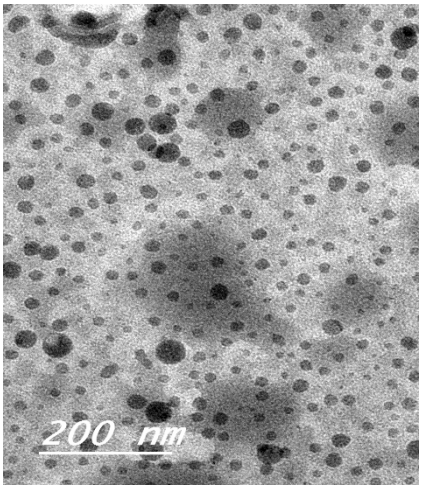

Figure (1): Transmission electron micrograph of dacarbazine lipid nanocapsule (F3).

3. *In vitro* characterization of the prepared dacarbazine lipid nanocapsule based hydrogel

3.1. Viscosity ofdacarbazine lipid nanocapsule based hydrogel

The viscosity of the proposed dacarbazine LNC based hydrogel as a function to shear rate could be depicted from figure 2. The obtained rheological results indicated that dacarbazine LNC based hydrogel is a pseudoplastic system where the viscosity is inversely proportional to the shear rate. In dermatological systems, pseudoplastic flow is preferred over other systems as it is administered under medium shear force where the required flow and spreadability could be achieved [12].

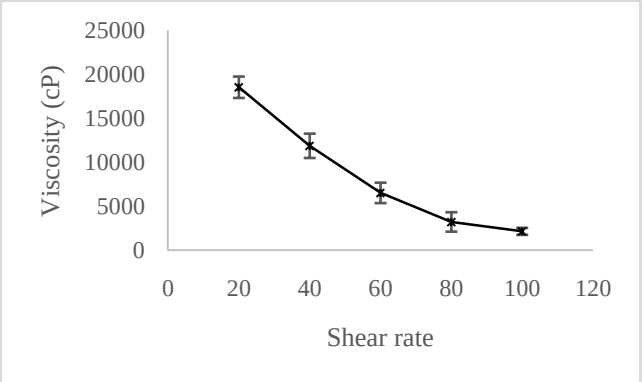

Figure (2): The viscosity of dacarbazine lipid nanocapsule based hydrogel as a function of shear rate. Results are the mean of three replicates ±SD.

3.2. *In vitro* dacarbazine release

Figure (3) demonstrates the *in vitro*release of dacarbazine from LNC and LNC based hydrogel. The percentage of dacarbazine released over 24 h was significantly higher in LNC than LNC based hydrogel (p<

0.05). Moreover, incorporation of LNC into hydrogel decreased dacarbazine burst release. This could be attributed to the high viscosity imparted by carbopol as a gelling agent [21].Increasing viscosity could be considered as one of the simplest techniques to prolong drug release as well as increasing contact time with skin [22]. Moreover, the carboxylic groups of carbopol940 become ionizable at pH 7.4 that promote gel swelling forming network structure which sustains the drug release [23].

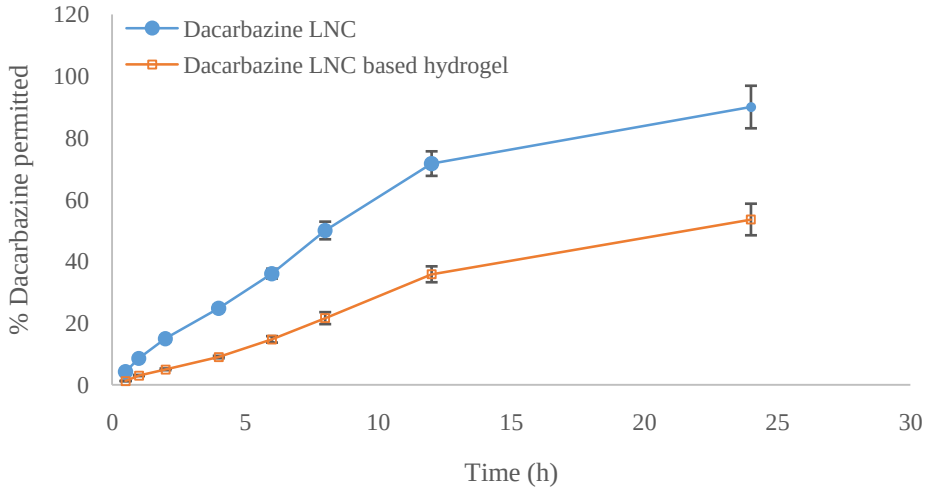

Figure (3): *In vitro* dacarbazine release from lipid nanocapsule and lipid nanocapsule based hydrogel in PBS pH 7.4. Results are the mean of three replicates± SD.

4. *In vitro* cytotoxicity

The B16 cell viability as a function of different dacarbazine concentrations from either LNC, LNC based hydrogel after 72 h incubation could be depicted from figure (4). High cell viability could be observed after incubation of B16 with either plain LNC or LNC based hydrogel with respective IC_{50} of 3.808 and 5.296 mg/mL eliminating the toxicity of the vehicles. Contrarily, a dose-dependent decrease in cell viability could be observed after incubation with all dacarbazine formulae. The calculated dacarbazine IC50 values were 0.2868, 0.3052 and 0.5176 mg/mL for dacarbazine LNC, dacarbazine LNC based hydrogel and dacarbazine solution respectively. The cytotoxicity of LNC and LNC based hydrogel is ≈ 1.7 fold of dacarbazine solution. Moreover, the incorporation of dacarbazine LNC into hydrogel did not interfere with its efficiency against melanoma cells. The improved cytotoxicity of both dacarbazine LNC or LNC based hydrogel could be attributed to the increased lipophilicity of LNC over aqueous solution that improves drug penetration into the tumour cells [24, 25]. In addition, the high amount of surfactants (solutol and epikroun) with their well-reported penetration enhancement activity could improve dacarbazine uptake and subsequently improved its efficiency [26]. Finally, the low particle size of the prepared dacarbazine LNC (23.54±1.51 nm) is considered ideal for the endocytosis process which facilitates drug penetration and accumulation into the tumour cells [27].

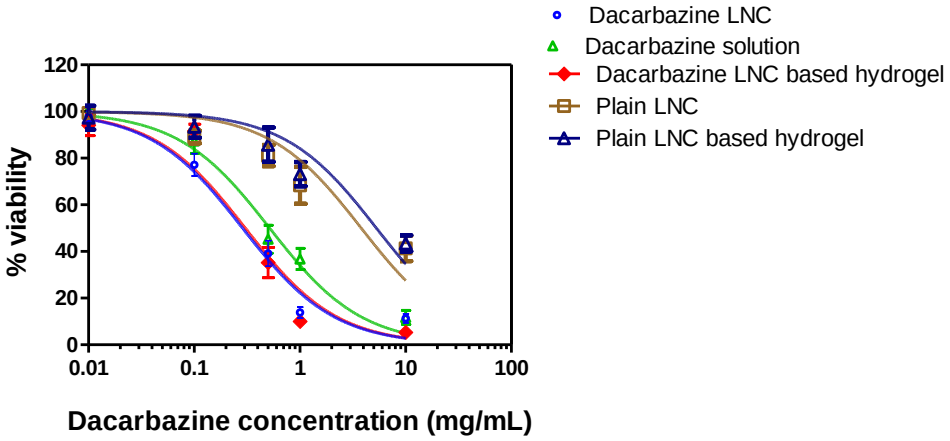

Figure (4): *In vitro* dacarbazine cytotoxicity from lipid nanocapsule and lipid nanocapsule based hydrogel compared to the solution after 72 h. Results are the mean of three replicates± SD.

IV Conclusion

This study investigates LNC based hydrogel as an efficient carrier for dacarbazine for melanoma. To sum up, dacarbazine LNC was prepared using the phase inversion technique by combining different ratios of Labrafac (as oil phase) and solutol HS15 (surfactant). Increasing the oil: surfactant ratio increased the particle size and decreased the EE %. However regardless of the oil: surfactant ratio, the obtained particle size was < 100 nm with relatively high dacarbazine EE % over 75%. Loading dacarbazine LNC into hydrogel controlled the *in Vitro* drug release where ≈ 53% dacarbazine released over 24 h. The obtained LNC based hydrogel exhibited pseudoplastic flow which is suitable for the dermatological application. Moreover, either loading dacarbazine in LNC or LNC based hydrogel improved drug cytotoxicity by 1.7 fold over the free dacarbazine solution. These results provided a rationale for further *in vivo* therapeutic and toxicological studies of the elected system.

References

[1]. Mishra H, Mishra PK, Iqbal Z, Jaggi M, Madaan A, Bhuyan K, Gupta N, Gupta N, Vats K, Verma R, Talegaonkar S. Co-Delivery of Eugenol and Dacarbazine by Hyaluronic Acid-Coated Liposomes for Targeted Inhibition of Survivin in Treatment of Resistant Metastatic Melanoma. Pharmaceutics,11, 2019, pii: E163.

[2]. Baharara J, Amini E, Nikdel N, Salek-Abdollahi F. The Cytotoxicity of Dacarbazine Potentiated by Sea Cucumber Saponin in Resistant B16F10 Melanoma Cells through Apoptosis Induction. Avicenna J Med Biotechnol. 8, 2016, 112-9.

[3]. DominguesB, LopesJM, SoaresP, PópuloH. Melanoma treatment in review. Immunotargets Ther. 7, 2018, 35–49.

[4]. HafeezA, KazmiI. Dacarbazine nanoparticle topical delivery system for the treatment of melanoma. Sci Rep. 7, 2017, 16517.

[5]. VarshosazJ, HajhashemiV, SoltanzadehS. Lipid Nanocapsule-Based Gels for Enhancement of Transdermal Delivery of Ketorolac Tromethamine. Journal of Drug Delivery. Article ID 571272, 2011.

[6]. HuynhNT, PassiraniC, SaulnierP, BenoitJP, Lipid nanocapsules: a new platform for nanomedicine.InternationalJournal of Pharmaceutics,379, 2009, 201–209.

[7]. VashistA, GuptaYK, AhmadS, Recent advances in hydrogel based drug delivery systems for the human body. J. Mater. Chem. B 2, 2014, 147–166.

[8]. BiondiM, BorzacchielloA, MayolL, AmbrosioL. Nanoparticle-Integrated Hydrogels as Multifunctional Composite Materials for Biomedical Applications. Gels 1, 2015, 162-178.

[9]. BassoJ, MirandaA, NunesS, CovaT, SousaJ, VitorinoC, PaisA, Hydrogel-Based Drug Delivery Nanosystems for the Treatment of Brain Tumors. Gels. 2018; 4(3): pii: E62.

[10]. Safwat S, Hathout RM, Ishak RA, Mortada ND. Augmented simvastatin cytotoxicity using optimized lipid nanocapsules: a potential for breast cancer treatment. J Liposome Res. 27, 2017, 1-10.

[11]. Dimer FA, Pohlmann AR, Guterres SS. Characterization of rheology and release profiles of olanzapine-loaded lipid-core nanocapsules in thermosensitive hydrogel. J NanosciNanotechnol. 13, 2013, 8144-8153.

[12]. Pereira RL, Leites FI, Paese K, Sponchiado RM, Michalowski CB, Guterres SS, Schapoval EE. Hydrogel containing adapalene- and dapsone-loaded lipid-core nanocapsules for cutaneous application: development, characterization, in vitro irritation and permeation studies. Drug Dev Ind Pharm. 42, 2016, 2001-2008.

[13]. Heurtault B, Saulnier P, Pech B, Proust JE, Benoit JP. A novel phase inversionbased process for the preparation of lipid nanocarriers. Pharm Res 19, 2002, 875–880.

[14]. Heurtault B, Saulnier P, Pech B, Benoit JP, Proust JE. Interfacial stability of lipid nanocapsules. Colloids and Surf B Biointerfaces 30, 2003, 225–235.

[15]. Vonarbourg A, Saulnier P, Passirani C, Benoit JP. Electrokinetic properties of noncharged lipid nanocapsules: influence of the dipolar distribution at the interface. Electrophoresis 26, 2005, 2066–2075.

[16]. Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates-a review. J Control Rel 128, 2008, 185–99.

[17]. Anton N, Gayet P, Benoit JP, Saulnier P. Nano-emulsions and nanocapsules by the PIT method: an investigation on the role of the temperature cycling on the emulsion phase inversion. Int J Pharm 2007, 344, 44–52.

[18]. Heurtault B, Saulnier P, Pech B, Venier-Julienne MC, Proust JE, Phan-Tan-Luu R, BenoîtJP.The influence of lipid nanocapsule composition on their size distribution. Eur J Pharm Sci 18, 2003, 55–61.

[19]. Izquierdo P, Feng J, Esquena J, Tadros TF, Dederen JC, Garcia MJ, Azemar N, Solans C. The influence of surfactant mixing ratio on nano-emulsion formation by the pit method. J Colloid Interface Sci 285, 2005, 388-394.

[20]. Hallan SS, Kaur P, Kaur V, Mishra N, Vaidya B. Lipid polymer hybrid as emerging tool in nanocarriers for oral drug delivery. Artif Cells NanomedBiotechnol. 44, 2016, 334-349.

[21]. Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. Nat Rev Mater. 1, 2016, pii: 16071.

[22]. Ian P. Harrison, FabrizioSpada. Hydrogels for Atopic Dermatitis and Wound Management: A Superior Drug Delivery Vehicle. Pharmaceutics. 10, 2018, 71.

[23]. Qindeel M, Ahmed N, Sabir F, Khan S, Ur-Rehman A. Development of novel pH-sensitive nanoparticles loaded hydrogel for transdermal drug delivery. Drug Dev Ind Pharm. 45. 2019, 629-641.

[24]. Karasulu HY, Karabulut B, Göker E, Güneri T, Gabor F, 2007. Controlled release of methotrexate from w/o microemulsion and its in vitro antitumor activity. Drug Deliv14, 2007, 225-233.

[25]. Hwang TL, Fang CL, Chen CH, Fang JY, 2009. Permeation enhancer-containing water-in-oil nanoemulsions as carriers for intravesical cisplatin delivery. Pharm Res 26, 2009; 2314-2323.

[26]. Som I, Bhatia K, Yasir M. Status of surfactants as penetration enhancers in transdermal drug delivery. J Pharm BioalliedSci 4, 2012, 2-9.

[27]. Zhang S, Li J, Lykotrafitis G, Bao G, Suresh S. Size-dependent endocytosis of nanoparticles. Adv Mater 21, 2009, 419-424.

Hend Mohamed Abdel-Bar. " Preparation of Dacarbazine Lipid Nanocapsule Based Hydrogel as an Efficient Delivery System for Melanoma."IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.4 (2019): 30-35.