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RESEARCH ARTICLE

Anticancer Potential of Novel Nanoemulgel Formulations in Melanoma

Selin Seda TİMUR[°*]

Anticancer Potential of Novel Nanoemulgel Formulations in Melanoma

SUMMARY

Melanoma is classified as one of the most common cancers with an increasing incidence rate, and the conventional treatment options that come with undesirable effects decrease the life quality of patients. Herein, Carbopol-based nanoemulgel formulations for local delivery were designed as an alternative therapy option for systemic administration. Topical drug delivery systems containing Oxaliplatin, a cisplatin derivative anticancer drug used treating colorectal cancers, were evaluated for their potential for melanoma treatment. Nanoemulgel formulations with particle size under 300 nm were prepared and characterized regarding droplet size, zeta potential, and liquid crystal formation. The viscosity, as a critical attribute for topical drug delivery systems, was also evaluated, and the pseudoplastic behavior of these novel drug delivery systems was confirmed. The controlled drug release pattern was shown with in vitro drug release studies with a significant difference from oxaliplatin when applied in solution. In vitro cell viability evaluation with L929 mouse fibroblast cell line confirmed the biocompatibility of prepared formulations, and the anticancer effect of novel nanoemulgel formulations was presented in B16-F10 mouse melanoma cell line. In conclusion, the anticancer potential of Oxaliplatin nanoemulgels was shown in vitro as a therapy option for melanoma, and the advantages of emulsion and gel-based drug delivery systems were combined in a nanotechnology platform for effective and patient-friendly application of an anticancer therapy for melanoma.

Key Words: *Nanoemulsion, nanoemulgel, melanoma, cancer, drug delivery systems.*

Yeni Nanoemüljel Formülasyonlarının Melanomada Antikanser Potansiyeli

ÖZ

Melanoma görülme sıklığı giderek artan kanser türleri arasında yer almakta ve istenmeyen etkilerle gelen konvansiyonel tedavi seçenekleri hastaların yaşam kalitelerini düşürmektedir. Bu çalışmada, sistemik uygulama için alternatif bir tedavi seçeneği olarak, lokal uygulanan Carbopol bazlı nanoemüljel formülasyonları tasarlanmıştır. Kolorektal kanserlerin tedavisinde kullanılan, sisplatin türevi bir antikanser ilaç olan Oksaliplatin içeren topikal ilaç taşıyıcı sistemlerin melanoma tedavisindeki potansiyeli değerlendirilmiştir. Çalışma sonunda partikül boyutu 300 nm'nin altında olan nanoemüljel formülasyonları elde edilmiş ve formülasyonların damlacık boyutu, zeta potansiyeli ve sıvı kristal oluşumu değerlendirilmiştir. Topikal ilaç taşıyıcı sistemler için kritik bir özellik olarak viskozite değerlendirmesi yürütülmüş ve söz konusu yeni ilaç taşıyıcı sistemlerin psödoplastik davranışı gösterilmiştir. In vitro ilaç salım çalışmaları, hazırlanan formülasyonların çözelti halinde uygulanan oksaliplatine kıyasla önemli bir farkla kontrollü ilaç salım davranışı gösterdiğini doğrulamıştır. Hazırlanan formülasyonların biyouyumluluğu L929 fare fibroblast hücre hattı ile gerçekleştirilen in vitro hücre canlılığı değerlendirmesi ile gösterilmiş ve nanoemülgel formülasyonlarının antikanser etkisi, B16-F10 fare melanoma hücre hattında doğrulanmıştır. Sonuç olarak bu çalışmada melanoma için bir tedavi seçeneği olarak Oksaliplatin nanoemüljellerin in vitro antikanser potansiyeli gösterilmiştir ve melanoma tedavisi için emülsiyon ve jel bazlı ilaç taşıyıcı sistemlerin üstünlükleri nanoteknoloji temeli bir yaklaşımla kombine halde hastaya sunularak etkili ve hasta uyuncunu artıracak bir uygulama sunulmuştur.

Anahtar Kelimeler: *Nanoemülsiyon, nanoemüljel, melanoma, kanser, ilaç taşıyıcı sistemler*

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INTRODUCTION

Cancer is still one of the leading health problems causing death despite current advances in therapy. Skin cancer is listed as the 5th most common type of cancer, with a prevalence of an estimated 1,413,976 people in the United States in 2020 (NIH, 2023), and melanoma is the most aggressive type of skin cancer, which is responsible for the most deaths (Liu, Das, Liu, & Huang, 2018; NIH, 2023; Song, Liu, Chen, & Zhang, 2021). A rapid increase in the incidence of melanoma between women has been reported recently, showing faster rates than for any other cancer site (Cronin et al., 2022; Song et al., 2021). Although the death rates are decreased for melanoma as reported, early detection still plays an essential role in the prognosis, due to the high risk of metastasis to distant organs in late stages (Cronin et al., 2022; Liu et al., 2018).

Together with the standard treatment including, surgery, chemotherapy, and radiotherapy, immunotherapy and targeted therapies have become alternatives for treating melanoma, since the side effects caused by conventional chemotherapy are inevitable (Liu et al., 2018). In addition, the impact of skin diseases on the physical and mental health of the patients and the economic burden caused by the incidence of the disease make the novel technologies worthwhile alternatives for treatment (Qu, Geng, Liu, & Zhu, 2022).

As a result of emerging technologies, novel drug delivery systems have become popular for treating various cancers, providing effective alternative tools for the patients. Nano-sized drug delivery tools including liposomes and liposome-like vesicles (Feng et al., 2022; Rauca et al., 2021; Tran, Watts, & Robertson, 2009b; Villares et al., 2008), polymeric nanoparticles (Hu et al., 2021; Xiong et al., 2022), dendrimers (Thuy et al., 2022; Wang, Mo, Wei, & Shi, 2013) as well as inorganic nanoparticles (Wang et al., 2018; Zhang et al., 2019) have been investigated recently for the treatment of melanoma (Song et al., 2021). Among these, liposomes have played an essential role in discovering of their potential as topical delivery tools (Tran, Watts, & Robertson, 2009a). In addition, transdermal drug delivery systems have been investigated recently in melanoma therapy for enhanced skin penetration and targeting abilities (Chen et al., 2023; Qu et al., 2022).

Topical drug delivery for treating skin disorders has many advantages, including prevention of first-pass metabolism, sustained drug release, local and systemic drug delivery, and patient compliance. However, low skin penetration could be the bottleneck for these tools (Hua, 2015). At this point, lipid-based drug delivery systems are rapidly gaining attention for their permeation-enhancing properties for the treatment of skin disorders with higher solubility and bioavailability, and emulsion-based colloidal systems are one of the most popular and effective options for the delivery of various drug molecules (Alam et al., 2023; Jain et al., 2021). Another effective and popular tool for topical delivery is gel-based drug delivery systems, which provide ease of application together with high rates of bioadhesion. Gel-based drug delivery is commonly preferred for effectively treating skin cancers (Slavkova, Tzankov, Popova, & Voycheva, 2023).

Oxaliplatin is a platinum derivative, which was discovered for its anticancer effect as an alternative to cisplatin without nephrotoxicity (Alcindor & Beauger, 2011; Lutzky et al., 2006). Although activity against lung cancer, breast cancer, melanoma, and hepatoma was also noted in early clinical studies, oxaliplatin has been mostly used for the treatment of colorectal cancer (Alcindor & Beauger, 2011). Recently, the *in vitro* use of this molecule in a melanoma cell line was found effective, and a clinical trial was conducted for potential treatment (Mohammed & Retsas, 2000).

Herein, a nanoemulgel system containing oxaliplatin was designed for the treatment of melanoma, and *in vitro* characterization of these formulations was conducted. These novel nanoemulgel systems could enhance the drug concentration after local administration to the site, thus allowing effective treatment while reducing systemic toxicity.

MATERIALS AND METHODS

Materials

Peceol™, Gelucire® 44/14, Labrafac™ lipophile WL1349, Plurol oleique and Transcutol®HP were kind gifts from Gattefosse, France. Propylene glycol, Tween 80 and TPGS were purchased from Sigma-Aldrich, Germany. DMEM, Tripsin-EDTA, Penicillin-Streptomycin and Fetal Bovine Serum were purchased from Cegrogen Biotech, Germany. All other chemicals were analytical grade, unless otherwise stated.

Methods

Preparation of nanoemulsions

Nanoemulsion formulations were prepared using ternary phase diagrams. TPGS, Tween 80 and Labrafil® M1944C was used as surfactants, Labrafac™ lipophile WL1349 and Plurol oleique as oil phase and Transcutol®HP and Propylene glycol as cosurfactants to determine optimum excipient combinations for nanoemulsion formation. The oil, surfactant and cosurfactants were weighed in screw-capped borosilicate vials and heated up to 5 °C above the melting point of solid excipients under constant stirring. Pre-concentrates of nanoemulsions were kept overnight at room temperature to reach equilibrium before characterization studies and diluted with phosphate- buffered saline (1:10) (Timur & Gursoy, 2020; Timur et al., 2019).

Preparation of nanoemulgels

Carbopol 940 was used as a gel-base for prepared emulgel formulations. Gel formulations were prepared at a concentration of 0.5% using either phosphate-buffered saline (pH 7.4) for blank gel formulations or oxaliplatin solution at 2 mg/mL. Briefly, Carbopol 940 was dispersed in aqueous media and kept overnight under constant stirring. The pre-concentrates of nanoemulsions were mixed with polymer dispersion, and triethanolamine was added for gelation to obtain nanoemulgels (Patel et al., 2021).

Characterization of formulations

Droplet size and zeta potential

The size distribution and zeta potential of prepared formulations were determined by dynamic light scattering method using Standard Operating Procedures (SOP) established for measurement based on the refractive index and external phase viscosities of the formulation components (Zetasizer Nano ZS, Malvern Instruments Ltd, Worcestershire, UK).

All formulations were assessed in terms of organoleptic properties for physical stability, such as phase separation, creaming flocculation, etc. Formulations were also examined under the polarized light microscope (Leica DM EP, Germany) for liquid crystal formation.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analyses were performed under a nitrogen atmosphere (50 ml/min) from 25 °C to 300 °C at a rate of 10 °C/min using indium as the standard. (DSC Q 100, TA Instruments, USA). The results were analyzed using Universal Analysis Software.

Viscosity measurement

The viscosity of blank and oxaliplatin-containing nanoemulgel formulations was measured using Brookfield DV-2T Viscometer (Essex, UK) at 25°C with CP-40 and CP-52 spindles.

In-vitro Release Studies

The release of oxaliplatin from prepared formulations was evaluated using the dialysis bag method (Hua, 2014). A regenerated cellulose dialysis membrane (12-14 kDa, 16 mm diameter, Spectra/Por 4) was soaked into PBS (pH 7.4) overnight before the experiment. 1 mL of samples from each formulation in triplicates were placed into dialysis bags, and 20 mL of PBS (pH 7.4) was used as release medium. The samples were kept at 100 rpm. After 0.5 mL of samples were withdrawn at 30 mins, 1 h, 2 h, 3 h, and 4 h, the release medium was replaced with fresh media. The sink condition was maintained during the experiments.

The samples were analyzed using a validated isocratic HPLC method (Breno Noronha Matos, 2015). Briefly, a reverse phase C18 column (GL Sciences, InertSustain 5µm, 4.6x 150 mm) and a mobile phase consisted of aqueous acid solution (0.01 M phosphoric acid) : acetonitrile (95 : 5 v/v) mixture was used. The flow rate was set to 1 mL/min with an injection volume of 20 µL. The drug content was detected using a UV detector at 255 nm. The method was validated according to ICH guideline Q2 (R1):Validation of Analytical Procedures: Text and Methodology.

Cell culture and colorimetric viability assay

L-929 mouse fibroblast cell line (ATCC®, NCTC clone 929) and B16-F10 mouse melanoma cell line (ATCC® CCL-6475™) were used for the cytotoxicity evaluations of prepared formulations. Cells were cultured in DMEM (High Glucose, 4 mM L-glutamine, and sodium pyruvate) supplemented with 10 % fetal bovine serum and 0.5 % penicillin-streptomycin (10 000 U/10 000 mg/mL). The cells were seeded in 96-well plates at a concentration of $1x10^4$ cells/mL and kept overnight before the administration of the formulations. Cell viability was assessed for 24 h and 48 h using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as previously described for both cell lines (Timur & Gursoy, 2020; Timur et al., 2019).

Briefly, 72 µg/mL, 18 µg/mL, 1.8 µg/mL, and 0.18 µg/mL oxaliplatin solution or formulation containing an equivalent amount of oxaliplatin were applied to cells. The effect of dilution on the formulations was also assessed. The cells were treated with oxaliplatin solution, blank formulations, or oxaliplatin-containing formulations for predetermined time intervals, and MTT was added to wells at a final concentration of 1 mg/mL. DMSO was added to dissolve formazan crystals formed following 4 h of incubation. The absorbance was measured at 570 nm using an ELISA plate reader (VersaMax™ Molecular Devices, USA).

Statistical analysis

The statistical analyses were performed using Prism 7 software. The results were analyzed using the analysis of variance (ANOVA) test. The groups with statistical differences were further evaluated by multiple comparison tests.

RESULTS AND DISCUSSION

Characterization of formulations

Droplet size and zeta potential

The droplet sizes of prepared formulations were evaluated using the DLS method. Different types and ratios of surfactant, cosolvent, and oil were prepared, and size distributions of these formulations were assessed. Ternary phase diagrams were constructed, and formulation areas were determined. After selecting representative formulations from each diagram, physical stability was evaluated for 14 days (Table 1), and T2 and T5 combinations were found to be suitable for further analysis. The ternary phase diagrams of these combinations are presented in Figure 1.

During 14 days of evaluation (Table 1), particle size distribution of formulations with high PDI values also tended to have physical stability problems, such as phase separation or coalescence. The PDI values of all formulations were increased after 14 days. T2 and T5 combinations, referred to as F2 and F5 hereon, presented droplet size under 300 nm and PDI values under 0.5 after 14 days of stability assessment. After selecting one representative nanoemulsion formulation from each diagram, nanoemulgels were prepared and evaluated in terms of particle size and zeta potential.

The zeta potential values of emulsion formulations were close to zero due to nonionic surfactant components (Table 1). During the assessment, no liquid crystal formation was observed for any formulation.

The size of F5 nanoemulgels containing oxaliplatin was found to be smaller than that of F2 formulation, with a narrower size distribution showing PDI values under 0.3. The size of F2 nanoemulgel was 494.1±12.56 nm with a PDI value of 0.47±0.05, whereas F5 nanomulgels showed 175.5±5.47 nm particles with PDI values of 0.28±0.02 (Table 2).

The zeta potential of nanoemulgel formulations was found to be decreased with the incorporation of the polymer and the drug into emulsion formulations. The zeta potential values were -45.57±1.04 and -44.50±1.95 for F2 and F5 nanoemulgel formulations, respectively. The zeta potential values were in accordance with the literature, confirming the anionic nature of Carbopol gel (Mohammed et al., 2018) and oxaliplatin (Wang et al., 2011).

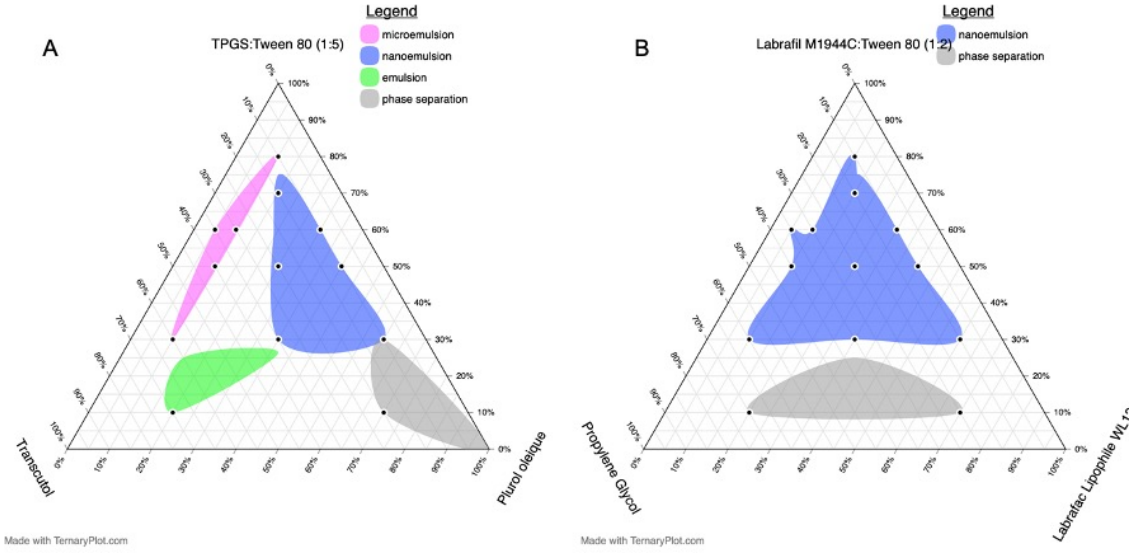

Figure 1. Ternary phase diagrams of A.T2 and B. T5 mixtures.

Table 1. The size and PDI values of prepared emulsions during stability evaluation (n=3).

	Day 1						Day 7						Day 14					
	Size (d.nm)		PDI		Zeta Potential (mV)		Size		PDI		Zeta Potential (mV)		Size		PDI		Zeta Potential (mV)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T1	38.2	6.9	0.125	0.023	-5.23	1.91	397.3	138.6	0.380	0.118	-1.76	0.31	193.4	145.9	0.350	0.049	-3.95	1.63
T2	153.4	60.6	0.233	0.070	2.04	0.88	127.8	15.4	0.141	0.020	1.02	0.29	249.2	39.5	0.305	0.041	1.28	0.53
T3	1163.2	368.6	1.000	0.000	0.05	0.87	Phase separation											
T4	259.4	5.3	0.389	0.017	0.16	0.48	Phase separation											
T5	261.0	5.0	0.409	0.041	1.23	1.89	262.3	22.9	0.298	0.089	-1.19	0.26	419.7	4.2	0.422	0.014	-1.34	0.90
T6	370.6	23.7	0.520	0.098	0.16	1.07	392.0	12.0	0.564	0.043	-1.19	0.59	604.0	20.5	0.597	0.035	-1.12	0.72
T7	1216.0	117.8	1.000	0.000	-0.85	0.50	Phase separation											
T8	1194.0	167.3	0.972	0.048	-0.95	2.08	Phase separation											
T9	867.1	66.4	0.884	0.105	-0.74	0.29	Phase separation											
T10	1265.0	245.5	0.958	0.060	-0.21	0.42	Phase separation											

Table 2. The characterization results of F2 and F5 nanoemulgel formulations (n=3).

	Size (d.nm)		PDI		Zeta Potential (mV)	
	Mean	SD	Mean	SD	Mean	SD
F2	494.10	12.56	0.47	0.06	-45.57	1.04
F5	175.47	5.47	0.28	0.02	-44.50	1.95

Differential scanning calorimetry

The thermal properties of oxaliplatin solution, blank Carbopol gel, and nanoemulgel formulations were analyzed using Differential Scanning Calorimetry. The DSC thermogram of prepared nanoemulgel formulations is presented in Figure 2. The evaporation of water from formulations was observed as a sharp endothermic peak for all formulations, starting at around 70 °C to 120 °C.

The oxaliplatin solution showed an endothermic peak at 73.27 °C, while no peak was observed at the same temperature for blank gel and oxaliplatin-loaded nanoemulgels, indicating the incorporation of the drug into gel formulations. The absence of the exothermic peak has proven the amorphous state of the formulation components, implying no degradation.

The thermal stability of the F2 formulation was found to be slightly higher for F2 nanoemulgels from F5 with a shift from 114.79 °C to 121.01 °C. The results also showed that the thermal stability of oxaliplatin could be strengthened by the incorporation of the drug in gel formulation rather than in solution, since the endothermic peak shifted from 73.27 °C to 121.01 °C and 114.79 °C for F2 and F5 nanoemulgels, respectively (Liu et al., 2017).

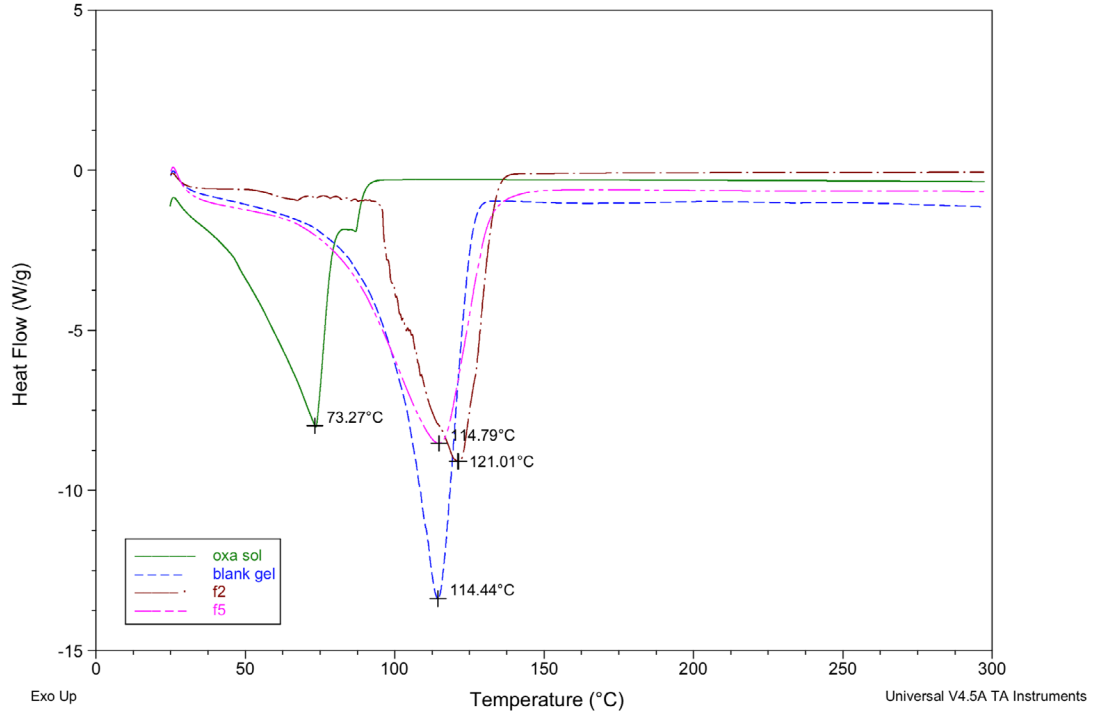

Figure 2. Differential scanning calorimetry (DSC) thermograms for drug solution, blank Carbopol gel and drug-containing nanoemulgel formulations.

Viscosity measurement

The viscosity of prepared nanoemulgels was measured under increasing shear rates, and the results confirmed the pseudoplastic behavior of samples, showing decreasing viscosity with increasing shear rate. The viscosities of blank nanoemulgel formulations were found to be between 23.74±1.56 to 54.07±2.86 cP and 21.91±2.36 to 49.22±4.86 cP, while oxaliplatin-loaded gels showed viscosity levels between 573.0±87.68 to 2560.33±476 cP and 291.03±29.54 to1323.0±177.45 for F2 and F5 formulations, respectively. The addition of the drug substance was observed to increase the viscosity of the formulation. The viscosity values of blank formulations were found to be similar ($p>0.1$), while significantly higher viscosity values were determined for F2 formulations than F5 at 10 rpm and 20 rpm (Figure 3, $p<0.0001$ and $p=0.0012$, respectively).

The pseudoplastic behavior of prepared Carbopol-based gels was confirmed with the viscosity measurements (Islam, Rodriguez-Hornedo, Ciotti, & Ackermann, 2004), and the viscosity of the prepared formulations was found to be significantly higher than blank gel formulations.

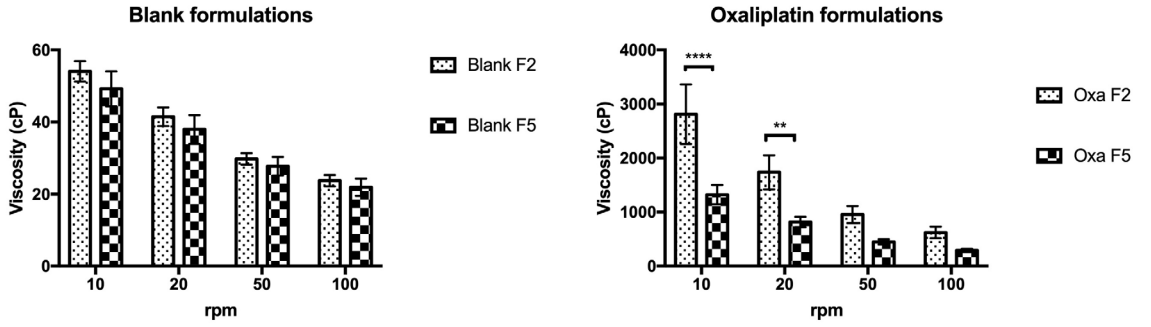

Figure 3. The viscosity results of blank and oxaliplatin-loaded formulations. The viscosity values of blank formulations were found to be similar ($p>0.1$), while significantly higher viscosity values were determined for F2 formulations than F5 at 10 rpm and 20 rpm ($p<0.001$) (n=3).

The viscosity is a critical attribute for topical drug delivery systems, since optimum viscosity is sufficient to prolong skin retention time (Lin, Lin, Sun, Liu, & Wu, 2020). Here, the viscosity of oxaliplatin nanoemulgel formulations was found in accordance with the literature, and suitable for the ease of application (Binder, Mazal, Petz, Klang, & Valenta, 2019; Azeran et al., 2017).

In-vitro Release Studies

Oxaliplatin solution, Carbopol gel (0.5 %) containing oxaliplatin, F2 and F5 nanoemulgel formulations were evaluated for *in vitro* drug release behaviors. All the gel groups, either containing nanoemulsions or not, have shown significantly lower drug release than oxaliplatin solution for the predetermined time points (Figure 4). The drug release from F2 nanoemulgel was found to be significantly higher than F5 formulation after 30 mins for all time points ($p<0.0001$). In contrast, no significant difference was found between Carbopol gel and F2 formulation after 30 mins of drug release. The amount of oxaliplatin released from F5 formulation was the lowest among all groups ($p<0.0001$).

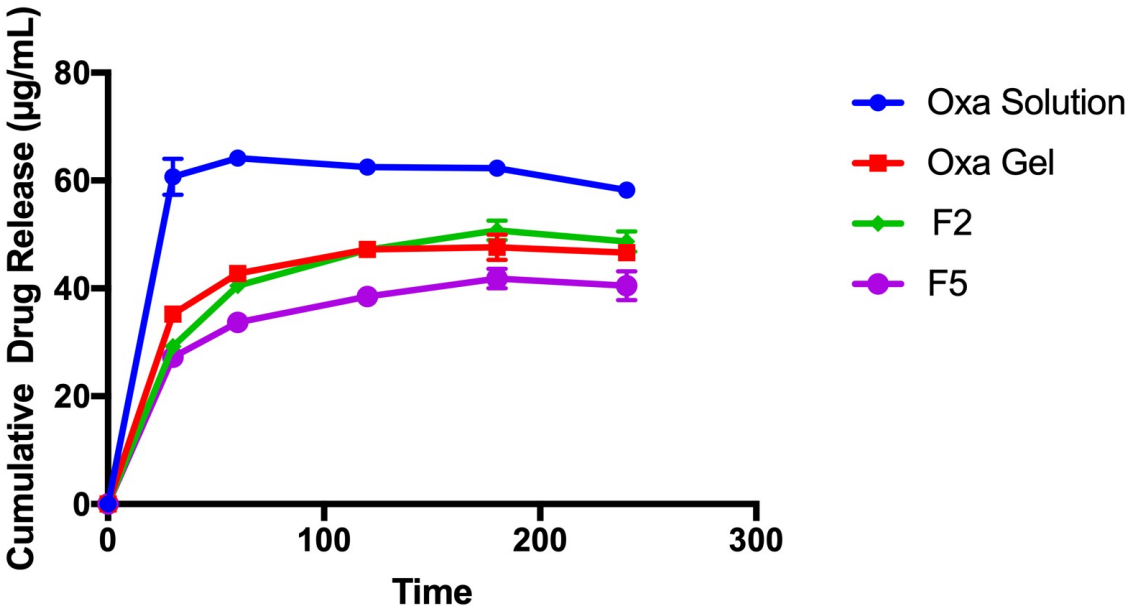

Figure 4. *In vitro* release results were represented as cumulative drug release (µg/mL) ± SD (n=3).

The drug release from the formulations containing Carbopol polymer was found to be slower than drug solution as the crosslink of Carbopol polymer matrices were reported to slow drug release. This could also be attributed to the higher viscosity of prepared gels, causing longer diffusional paths when compared to solution (Shaik & Sekaran, 2021). Although the nanoemulsion effect was not observed after four hours of drug release for prepared formulations, the higher drug release from F2 nanoemulgel formulation could be attributed to the smaller droplets of the aforementioned formulation in nanoemulsion form (Table 1), due to the larger surface area causing the higher dissolution rates (Sandri et al., 2014; Hua, 2019). In addition, the higher amount of drug release could be explained by the higher solubility enhancing capacity of surfactant composition due to the existence of TPGS in F2 formulation. Although the surfactant concentrations of the two formulations are the same, the composition ratio and the HLB values of the surfactant mixtures are different. Thus, higher permeability results obtained for F2 formulation could be attributed to higher HLB value, which promotes enhanced dissolution, and permeability accordingly (Chakraborty, Shukla, Jain, Mishra, & Singh, 2009; Mahdi et al., 2011; Sotthivirat et al., 2020; Umbreit & Strominger, 1973; Varma & Panchagnula, 2005).

Cell culture and colorimetric viability assay

The cytotoxic effect of blank formulations was assessed using L929 mouse fibroblast cell line for 48 h. L929 fibroblast cell line is commonly used for the biological evaluation of formulations in terms of *in vitro* cytotoxicity. L929 cell line is mainly used for the biocompatibility evaluation of medical devices as indicated in ISO standard 10993-5, and the cytotoxic effect is defined as more than 30 % reduction in cell viability (ISO, 2009).

The results showed that both F2 and F5 formulation was biocompatible after 1:1000 dilution, showing more than 75 % cell viability. For the F5 formulation, the cell viability values were close to the control group for 24h and 48 h of incubation. Although the F2 formulation showed decreased cell viability compared to the F5 formulation, both blank formulations were evaluated as suitable for application after 1:1000 dilution. The difference between F2 and F5 was significant except for the most concentrated sample for 24 h of

incubation ($p<0.0001$), while no significant difference in cell viability was observed for 1:25 and 1:10000 dilution in 48h ($p<0.01$) (Figure 5). The higher cytotoxicity trend observed with the F2 formulation could be attributed to the TPGS composition of the surfactant mixture used in this formulation. Although TPGS is commonly utilized for its solubilization and permeabilization effect in drug delivery system formulations, it is also reported to induce cell death (Neophytou, Constantinou, Papageorgis, & Constantinou, 2014).

In addition, the smaller droplet size of the F2 formulation could cause higher cytotoxicity for this formulation, since cytotoxicity is affected by the size, shape, and composition of the particle, and smaller particles were reported to have higher toxicity when compared to the nanoparticles with the same composition (Napierska et al., 2009; Sohaebuddin, Thevenot, Baker, Eaton, & Tang, 2010).

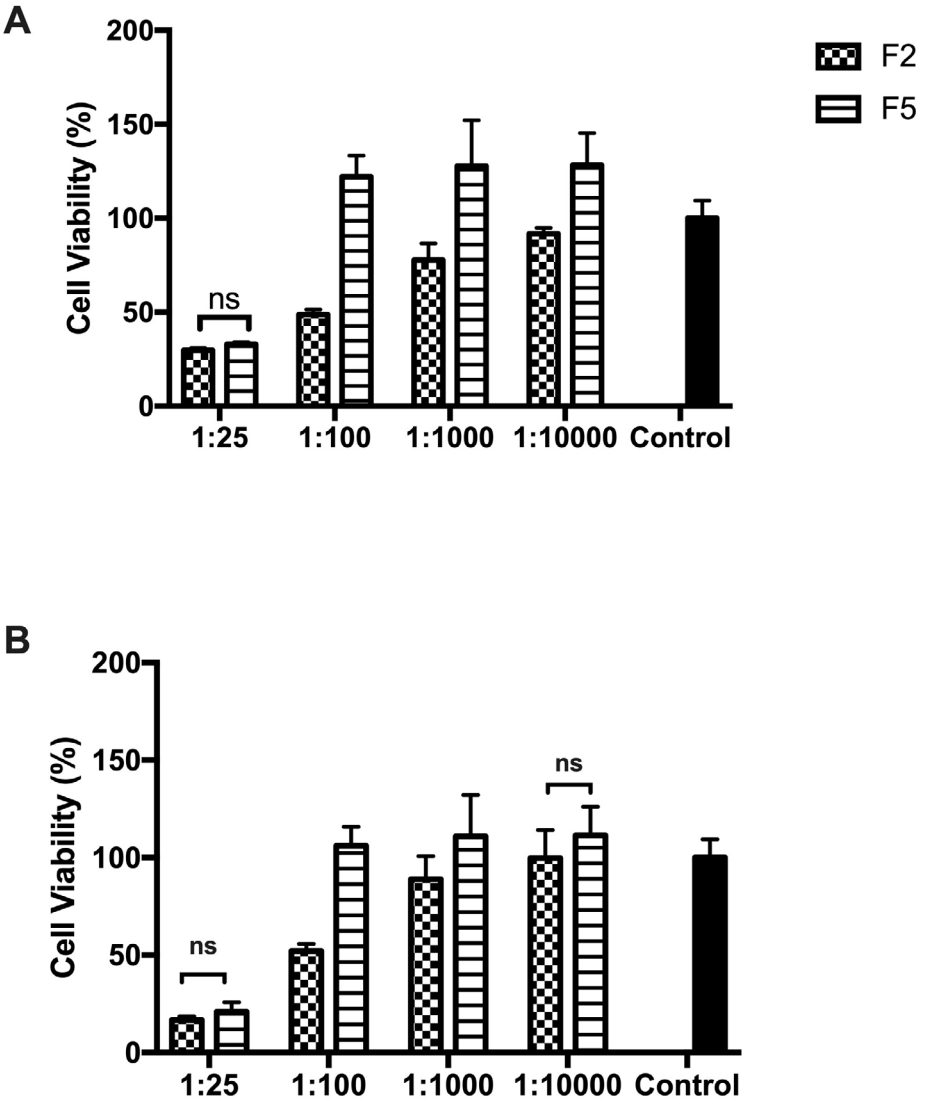

Figure 5. Cell viability assessment after the administration of blank nanoemulgel formulations on L929 cell line for A. 24 h and B. 48 h. Changes in the cell viability (%) were expressed as the percentage of control cells ± SD (n=6) (ns: not significant).

The anticancer activity of prepared formulations was assessed on B16-F10 melanoma cell line for 24 h (Figure 6). The same trend for blank formulations was observed as the F2 formulation decreased the cell viability more than the F5 formulation. F2 formulation loaded with the anticancer drug has significantly decreased the cell viability ($p<0.01$), when compared to the blank formulation, except the highest concentration applied. After 24 h of incubation, a statistically significant difference was also observed for F5 formulations loaded with 1.8 and 18 µg/mL of oxaliplatin compared to blank formulations ($p<0.01$). When the anticancer effect of formulations was compared to the drug solution, the activity was significantly higher for gel formulations than the solution when the drug was applied at the lowest and the highest concentrations ($p<0.001$) for F2 formulation, while a significant difference was only observed at the lowest concentration for F5 ($p<0.001$).

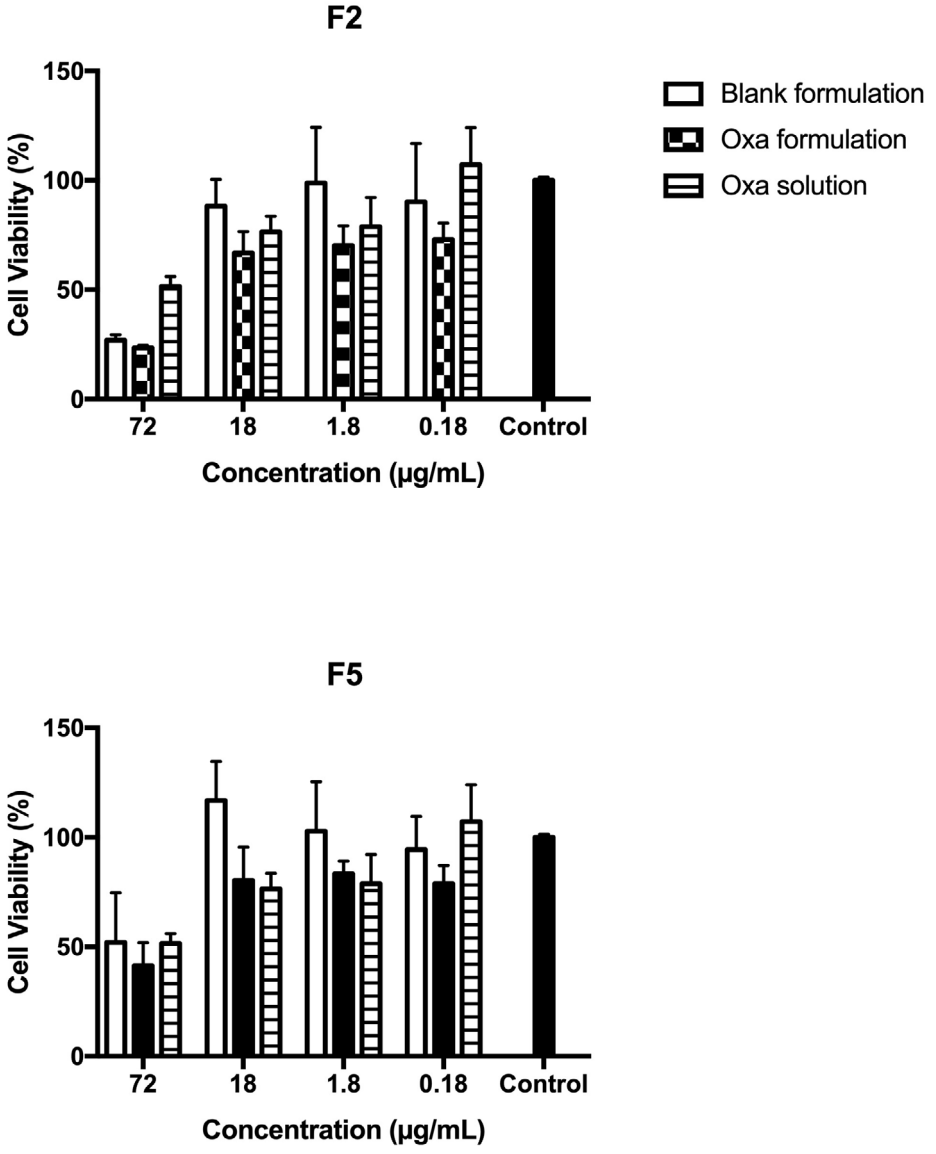

Figure 6. Cell viability assessment after the administration of oxaliplatin formulations on B16-F10 melanoma cell line. Changes in the cell viability (%) were expressed as percentage of control cells ± SD (n=6)

The amount of viable cells after treatment with anticancer drug-loaded formulation has shown that the administration of oxaliplatin in nanoemulgel formulations increased the anticancer activity on the melanoma cell line. This difference was observed more significantly for F2 formulation. These results were expected, when the data was interpreted together with particle size and drug release assessment, since F2 formulation released a higher amount of oxaliplatin at a predetermined time interval, and a higher anticancer effect could also be attributed to the smaller droplet size of F2 nanoemulsions.

All in all, both nanoemulgel formulations were found to be suitable for application with comparable droplet size, viscosity, drug release, and anticancer potency for treating melanoma. These local drug delivery systems could provide alternative treatment options with ease of application and decreased side effects for better patient compliance.

CONCLUSION

Although the mortality rate for melanoma is decreased, as stated in the annual report of the National Cancer Institute, there is still a need for novel approaches for treating melanoma patients, since it is still affecting a large population with increasing incidence (Cronin et al., 2022). Oxaliplatin is an anticancer drug that is primarily used in the treatment of gastric and in breast cancer recently. The application of oxaliplatin in the treatment of melanoma is a new approach to be considered, and recent advances are under investigation, including clinical trials. Herein, we designed a topical drug delivery system for the treatment of melanoma, and the in vitro performance of these novel formulations was evaluated. A promising anticancer effect of nanoemulgel systems was determined, and the potential of these systems for the treatment of melanoma is presented. In light of these findings, the local administration of oxaliplatin via nano drug delivery systems could be considered as an alternative approach for the treatment of melanoma patients together with current therapy tools.

AUTHOR CONTRIBUTION STATEMENT

Design, experimental studies, data interpretation and manuscript writing (SST)

CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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