The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 412-420

Production and Characterization of Nanostructured -Lipid Carriers as Hormones PGF2αand PMSG

Buthina A. Abdullaha[1], Mohanad A. Al-Bayati[2]
[1,2] Physiology and Pharmacology Department, Veterinary Medicine Collage, University of Baghdad, Iraq.
Corresponding author: Buthina A.Abdullaha, **Mobile:** 09647701715129, E-**Mail:** buthinahameed@tu.edu.iq

ABSTRACT

Background: PGF2α-PMSG is one of the hormones used to increase production and reproduction, but there are some side effects regarding its use and the length of its effect. Thus, improvement of the action of hormones and reduction of their side effects by reformulation and optimization of PGF2α-PMSG based on the nano-lipid delivery system became the target.
Objective: This study aimed to reformulate and improve PGF2α-PMSG based on the nano-lipid delivery system.
Materials and methods: In this study, Nano-Lipid Carriers (NLCs) contained fatty acids to obtain nanoparticles with small particle sizes. The hormone Nano lipid was prepared by solvent diffusion method, and has the characters and properties of Nano-lipid.
The results: The results were optimized nanoparticle has a size of Nano-lipid Carrying PGF2α range between 50-166.6 nm and Nano-lipid Carrying PMSG 85.7-300 nm. The entrapment efficiency of hormone SNL PMSG was 79.04 ± 4.96 and SNL PgF2α was 84.11 ± 5.55. The results of characterization for the SLNs under transmission electron microscopy were generally round and uniform in shape. FTIR and XRD analyzers indicate that the hormone is properly laden within the amorphous nanostructure. It also proved that Nano-lipid formula has high stability at pH and Osmo-tolerance. The Nano-Lipid structure PGF2α and PMSG formulas were represented ʎ max 450 nm in UV-visible**.**
Conclusion: The present study showed that the Nanostructured -Lipid Carriers had the ability in producing high-efficiency of PGF2α and PMSG hormones.
Keywords: Nano-lipid preparation**,** PGF2α, PMSG.

INTRODUCTION

Nanotechnology is the branch of technology that is used in practical application [(1)]. Nano-materials differ from bulk materials in a number of ways, including their high surface energy, extremely large surface area, and ability to customize their functions for varied applications [(2)]. Nano-delivery systems have the potency for simultaneous objective and diagnostic and therapeutic action through nanotechnology-based delivery systems that have the potential to drug permeability, solubility, and early elimination issues associated with small molecules and biological materials [(3, 4)].

So targeted delivery systems and regenerative medicine supported by nanotechnology have the possibility to play a central role in future therapy [(5, 6)].

Nano-particles are classified according to their size, shape, and physical and chemical properties. There are carbon nanoparticles, metallic nano-particles, ceramic nano-particles, polymeric nanoparticles, and lipid nanoparticles [(7)]. Lipid nanoparticles are generally spherical, with a diameter of 10 to 100 nm. It contains lipophilic and soluble particles. Its structure consists of a solid core made of lipids matrix, and by means of surfactants and emulsifiers the outer core is stabilized [(8)]. The rapid development of science regarding the ability to produce nanoparticles of uniform shape, structure, and size has revolutionized pharmaceutical science.
With their size-dependent properties, nanoparticles will offer the potential for new and advanced treatments. It also increased the ability to incorporate drugs into Nano-carriers for rapid drug delivery that can be used in a wide variety of therapeutic goals [(9)].

Structure Nano-Lipid (SNL) has good chemical stability, this is what the researchers confirm. It has developed broad prospects for its use, and features of the NLC structure that allow the inclusion of natural bioactive lipids into the matrix of NLC that help creation of high-performance drug carriers. Many drugs are available for stimulating as well as for superovulation such as hormones and medicinal and pharmaceuticals for pharmacological use [(10)].

The aim of the study was to reformulate and improve PGF2α-PMSG based on the nano-lipid delivery system.

MATERIAL AND METHOD

Preparation and standardization of Nano-Lipid PGF2α-PMSG

Nano lipid was prepared by solvent diffusion method [(11)]. The lipid phase comprised of two forms:

1-Lipid form: The lipid status formed by stearic acid 100 mg dissolved by glycerin monostearate 100 mg and dispersing 800 rpm with castor oil (2 ml) to form lipid dispersion by vortex 1500 rpm for 30 minutes.

2-Dissolving form: The forming lipid phase was dissolved in phosphatidic acid 100 mg and the amount of loaded and dispersed hormone requirements was 800 rpm

Received: 20/07/2022
Accepted: 22/09/2022

for 30 min. The dissolved form is then added to the lipid form and dispersed at 800 rpm for 1 hour, and placed in cooling overnight at 8 °C until used. Before use, mix by swirling at 800 rpm for 30 minutes. Correspondence, shape, and size of shell Nano-lipid were inspected and integrated with parametric maneuvers [12].

Micrograph: Structural Nano-Lipid size

a. Light micrograph: The constructional Nano Lipid hormones patch was formed and tested under a microscope with an optical filter at an oil immersion magnificent scale and screening of the created Nano-lipid structure of hormones distribution had been searched [13].

b. Electron microscope scan and transmission: The stocked construction Nano lipid hormone 0.2% was exam sample for scan and transmission by electron microscope. Samples were examined at applied research foundation (BPC-Analysis Center)in Baghdad .

Determination of entrapment loading and efficiency:

The percentage of entrapped hormones from Nano-lipid was measured after complete loading of PMSG and PGF2α. The methodological protocol was done according to **Cabral *et al.*** [14]. The entrapping efficiency and loading were calculated using the following equations:

EE %= Total amount-free amount (supernatant) / Total amount× 100

DL% = (Total amount of content-Amount of free content) / (Total amount of lipid) × 100

pH tolerance:

The pH tolerance of Nano-Lipid hormone formula was estimated by spectrophotometer at weave length 460 nm according to **Rahul *et al.*** [15].

Osmolarity to Nano-lipid tolerance:

Tolerance osmolarity was determined by dilution assay method [16]. 0.1ml from standard loading SNL suspension was added to tubes containing different concentrations of NaCl (0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.25 mg\ml, 1.5 mg/ml, 1.75 mg/m, and 2.25 mg/ml) were prepared. After 1 hour incubation at 37 ºC the tubes were measured and the SNL count remaining in each tube was calculated by spectrophotometer.

Fourier transformed infrared (FTIR) spectroscopy:

Fourier transformed infrared (FTIR) was used to evaluate the physical and chemical interaction of lipid and conventional Nanostructural hormones. An FTIR spectroscopy study was conducted to check the compatibility between the conventional and lipid separately. The FTIR was determined by spectrophotometer for at a wavelength from 4000 to 400 cm^{-1} [17].

X-ray diffraction:

XRD analysis of the crystallization status of conventional and SNL hormones was performed using an X-ray diffractometer scanning through a 2θ diffraction angle to assess the structural Nano-lipid hormones and conventional hormones' final structural form homogeneously [18].

Ethical Consideration:

The ethics were adopted on the ACUC protocol "Guide for the Care and Use of Laboratory Animals in research and teaching" for handling and management of mice in college of Veterinary medicine, University of Baghdad, and Ethical Craft Pharmacological Dosing and Cure Manoeuvres.

Statistical Analysis

Data were collected, tabulated and statistically analyzed. Quantitative data were presented in the form of mean and standard deviation, while qualitative data were presented as numbers and percentages. Graphs were developed using Microsoft Excel 2010 software. The statistical significance was set at P-value of less than 0.05.

RESULT

Characterization of Nano-Lipid:

1-Light microscope

The light micrograph exhibited globular like shape of structure Nano-lipid of PGF2α and PMSG in loaded phase. The different magnification and the optical field were aggregated form in different size derived unite in configuration and composition of general pattern as shown in figure (1).

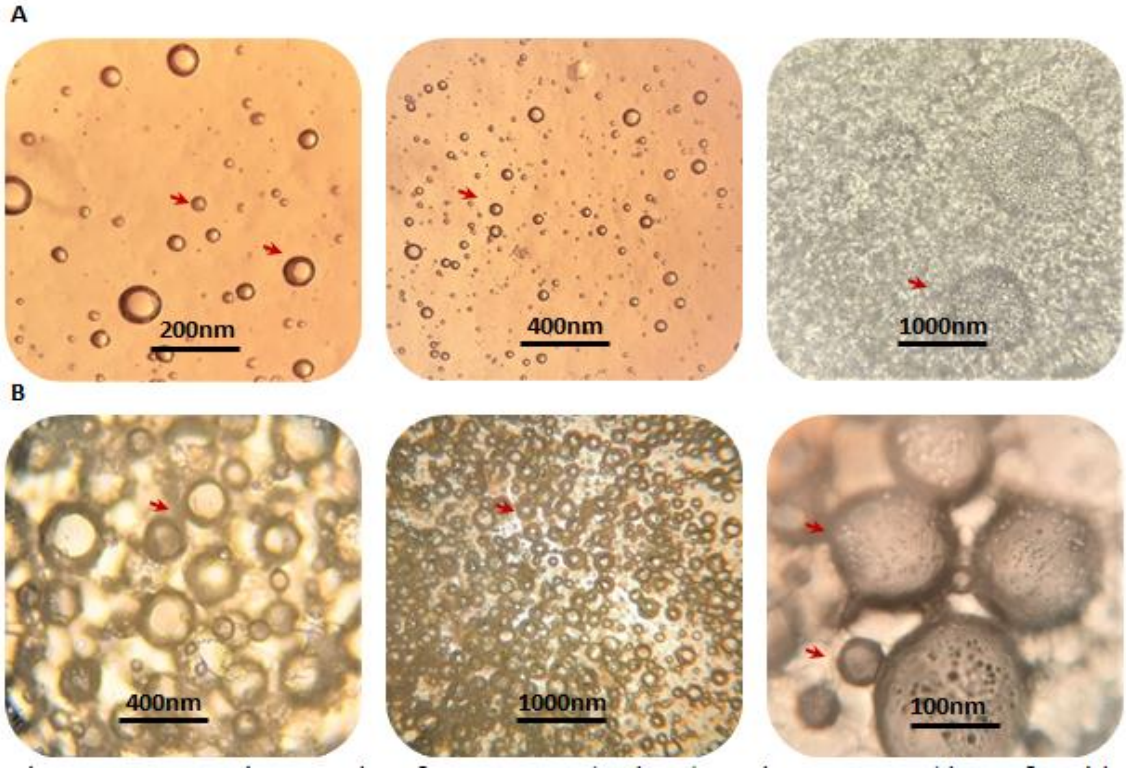
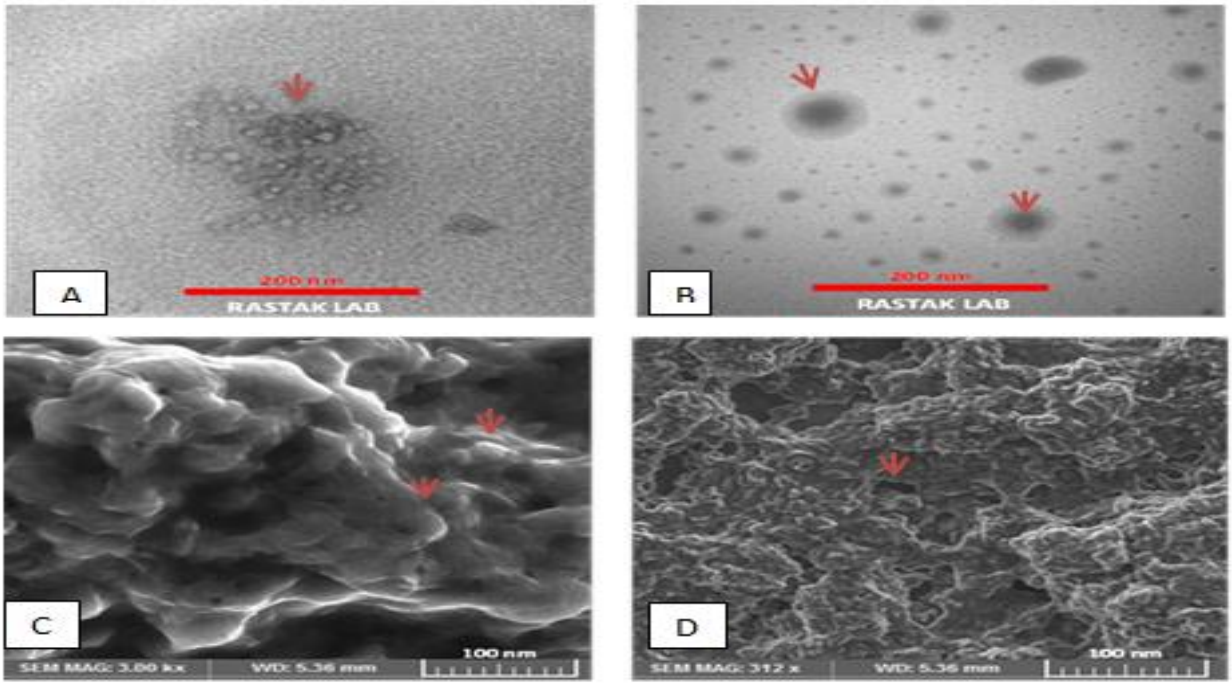


Figure (1): Micrograph of NLSPGF2α (a, b & c) and NLSPMSG (d, e & f) with spherical shape and glomerulization appearance.

2-Scanning and transmission electron microscope:

The type and morphology of Nano-Lipid hormones formulas electron microscope scan and transmission. A scan depiction of the Nano-Lipid showed fine spherical separated vesicular assemblies. The transmission mode depiction image exhibited small size of the Nano-Lipid (Figure 2).



Figures (2): Scan and transmission electron. A, B Transmission electron. C, D Scan electron.

3- Entrapment efficiency and loading %

The entrapment efficiency and loading % of hormones PGF2α and PMSG are mentioned in table (1) for five patches. The amount percentage of PMSG in the SNL was 79.04, whereas the amount percentage of PGF2α in the SNL was 84.11. The loading efficiency percentage of hormones PMSG and PGF2α were 75.72 and 81.04 respectively. $p \leq 0.05$ denoted significance between hormones.

Table (1): The loaded profile of SNL $PgF_{2\alpha}$ and PMSG and percentile of entrapment values.

SNL hormones	Entrapment %	Loaded %
SNL PMSG	79.04 ± 4.96 a	75.72 ± 83 a
SNL $PgF_{2\alpha}$	84.11 ± 5.55 b	81.04 ± 61 b

n= 5 samples, Data presented ± SE of mean, SNL: Structure Nano-Lipid PGF2α: prostaglandin F2 alpha PMSG: pregnant mare serum gonadotropin.

Nano Size of structure Nano Lipid (SNL) carrying hormones PMSG and $PGF_{2\alpha}$:

The particle size of Nano-metric scale was 50-166.6 nm for SNL PGF2α and for SNL PMSG was 85.7-300 nm as shown in table (2).

Table (2): Size of SNL carrying hormones; PMSG and PGF2α

Type of SNL hormones	Size of Nano-lipid nm	
	Range size	Mean ± SE
Nano-lipid Carrying PGF2α	50-166.6	99.8 ± 17.5 **a**
Nano-lipid Carrying PMSG	85.7-300	222.9 ± 24.9 **b**

n= 5 samples, Data presented ± SE of mean, SNL: structure Nano-Lipid, PGF2α: prostaglandin F2 alpha PMSG: pregnant mare serum gonadotropin.

4- Effect of PH on the Nano-Lipid formulas in *vitro*

The Nano-Lipid absorbance versus pH values was plotted the curve behaviour Nano partials stability derived declined with the reduction of pH at 2 - 6 post-one hour incubation as compared to Zero time of incubation. The Nano-lipid formula was more stable of both Nano-Lipid structure PGF2α and PMSG at pH 7 - 8.5.

The Nano-lipid formula was less stable at the pH 7.8 for Nano-Lipid PGF2α, whereas Nano-Lipid PMSG at pH 5 - 8.5 was less stable as shown in figure (3).

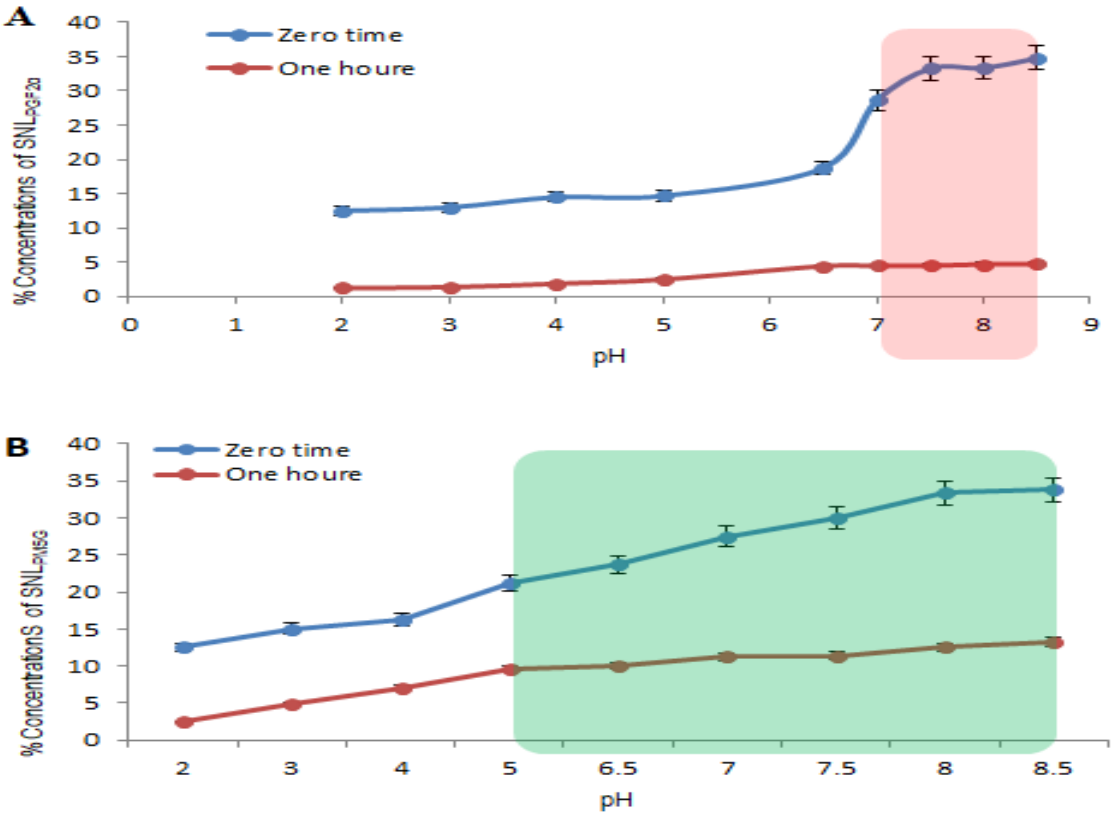
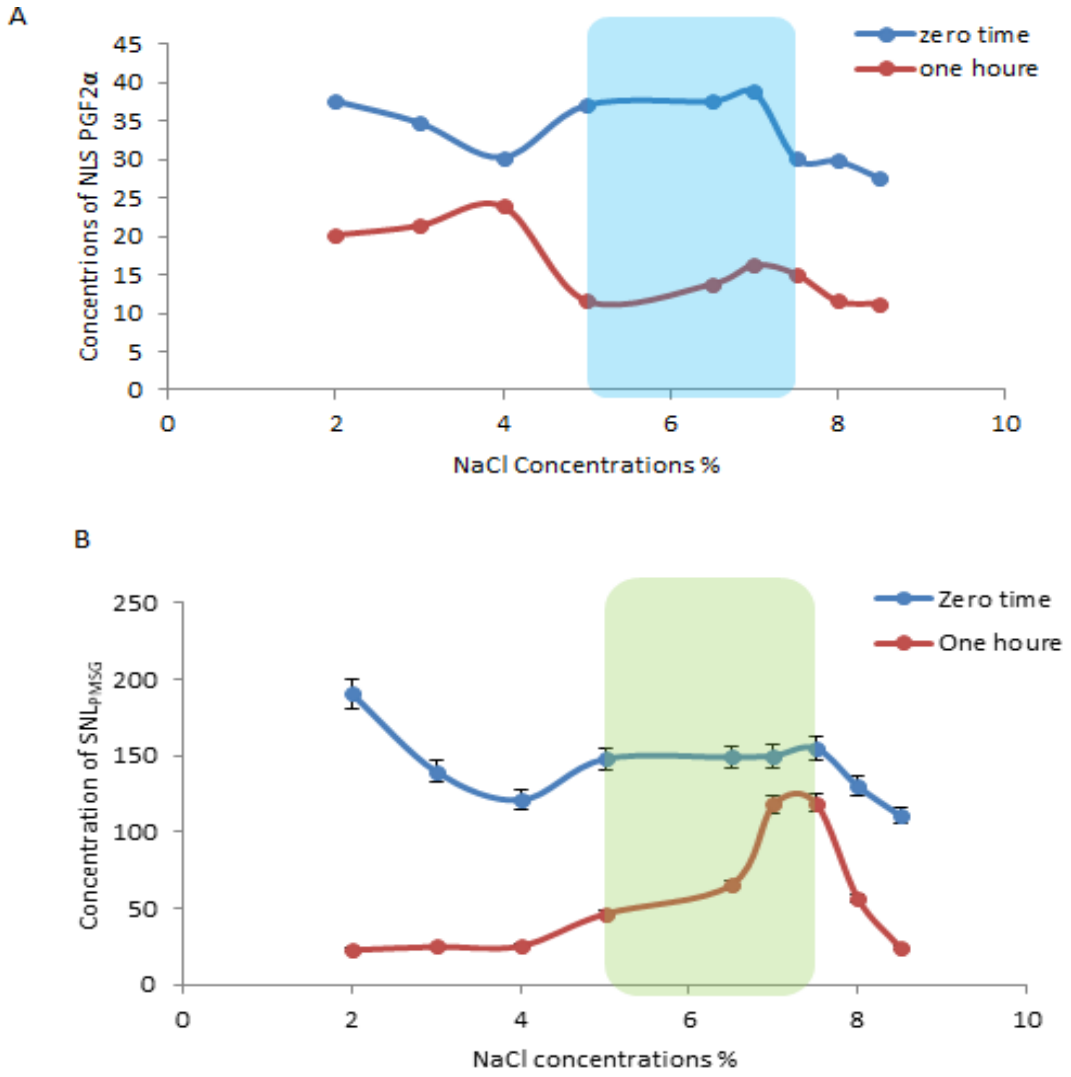


Figure (3): Effect series value of PH on different Nano-Lipid Hormones formulation (A) Nano-Lipid PGF2α and (B) Nano-Lipid PMSG.

5- Osmo-tolerance of structure Nano-Lipid of PGF2α and PMSG *in vitro*

The osmo-tolerance in NaCl gradient tonicity of the Nano-Lipid structure PGF2α and PMSG formulas was represented ʎ max 450 nm in UV-Visible. The result of NaCl concentration between 5-7.5 % of NaCl gradient for Nano-Lipid PGF2α and Nano-Lipid PMSG (Figure 4).



Figures (4): Effect of tonicity series of NaCl solution on Nano-Lipid Hormones formulation (A) Nano-Lipid PGF2α and (B) Nano-Lipid PMSG

6- X-ray diffraction (XRD):

X-ray diffraction was used to determine the geometric scattering of X-ray crystalyed planes for assessing degree of crystallinity of Nanoparticles. The results of XRD showed the disorderly peaks of 2θ between 20-30 (Figure 5 A). Also, figure (5 B) showed the clear peaks at 2θ of 20-30 indicating a crystalline, which showed SNLPG execrated diffraction pattern that displayed a relatively sharp peaks indicating that it is a crystallization structure. However, the diffraction peaks for SNLPMSG was broader and less intense than that of SNL PG.

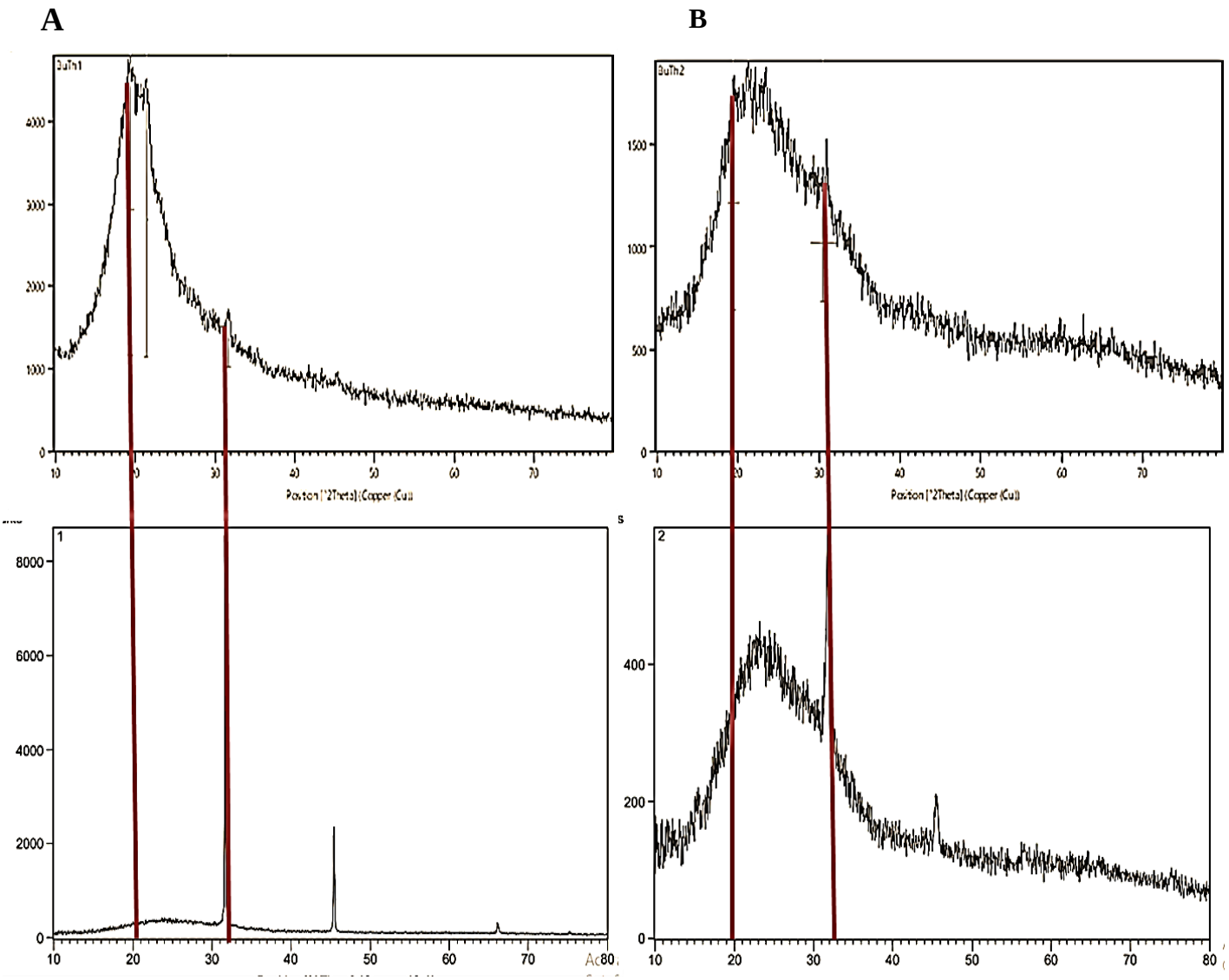


Figure (5 a): The XRD patterns for (A) SNLP-PGF2α (B). Conventional PGF2α, the red line indicated the hormone group peaks structural Nano lipid PGF2α .

Figure (5 b): The XRD patterns for (A) SNLP-PMSG (B). Conventional PMSG the Red line indicated the hormone peaks of groups structural Nano lipid PMSG.

7- Fourier Transform Infrared (FTIR):

Concerning molecular structures and interactions, Fourier transform infrared spectroscopy (Figure 6 and table 3) showed the functional group and its quantified frequencies for the conventional hormones and SNL hormones [adopted from the reference *(17)].

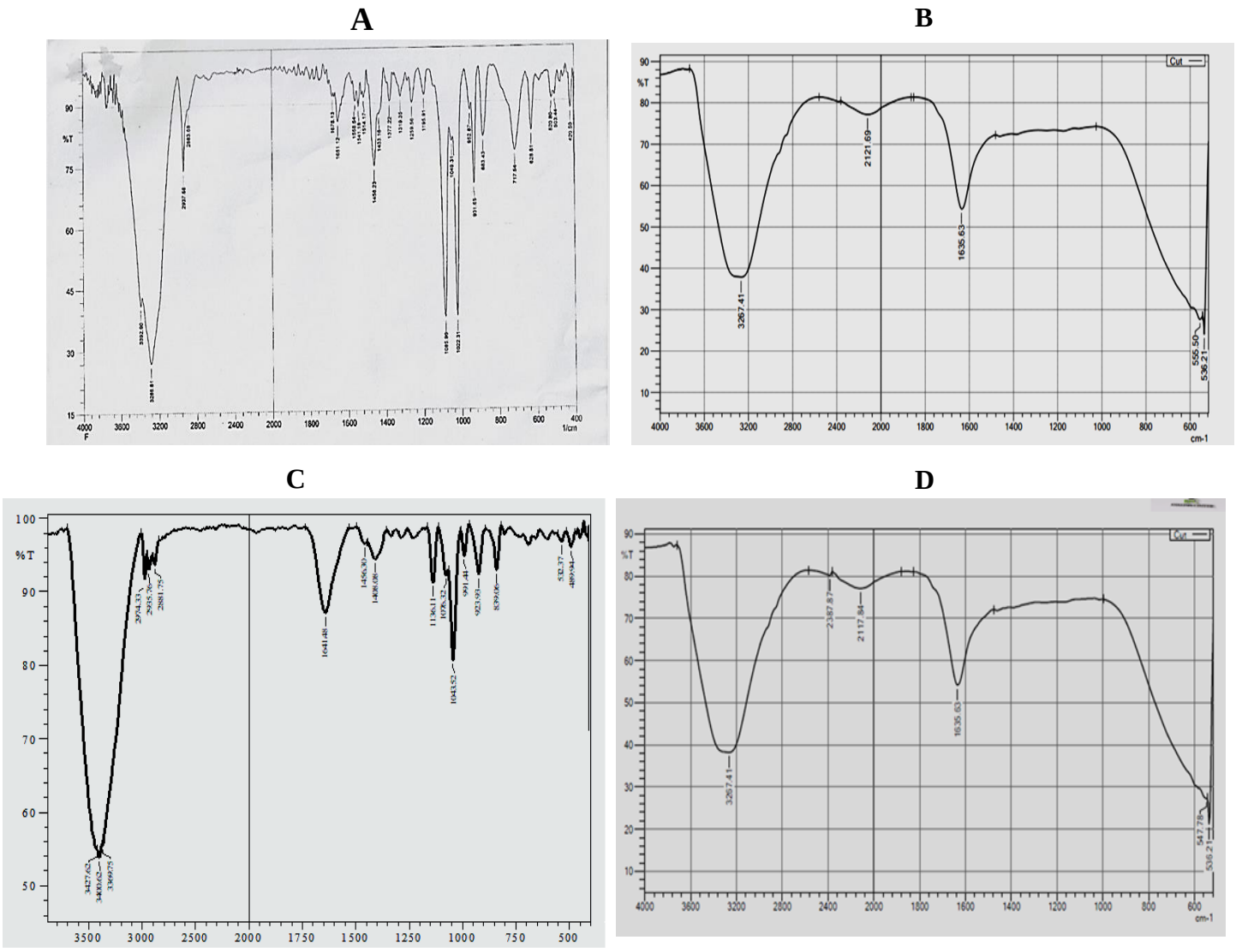


Figure (6): The functional group in Fourier-transform infrared spectroscopy FTIR scheme of the hormones. A- Conventional hormone PMSG ,B-Nano-Lipid PMSGH, C- Conventional hormone PGF2α, D- hormone, Nano-Lipid PGF2α.

Table (3): Functional group and its quantified frequencies of conventional and SNL hormones

PMSG	SNLPMSG	PGF2α	SNLPGF2α	Functional group* (17).	
3362.90	3267.91	2974.33	3427.62	4000-2500	O-H, N-H,C-H
3267.61	2121.69	2935.76	3400.62	2500-2000	C≡C, C≡N
2937	1635.68	2881.75	3369.75	2000-1500	C=C,C=O,C=N
2683.55		1641.48			
167.12		1456.30			
1651					

DISCUSSION

The technology of Nano-drugs is of importance within the framework of Nano-drugs branch in Nano-pharmacology, especially the vital Nano-Lipid cells through their ability to carry and maintain the drug as well as their rapid breakdown, which increases drug dealing with lower doses and the ability to cross through different biological barriers, which rise and maximize drug effect and reduce the side effects of the drug by minimizing the dose of the drug [19]. The electron microscopic-sized SNL was denoted in figure (1) and the TEM and SEM in figure (2) as fully formed and direct appearance of hormonal load. Structured lipid nanocarriers, composed of liquid lipids (oils), generated changes in the structure of solid lipids, leading to a crystalline arrangement that prevents drug leakage and provides a higher drug load compared to structural lipid nanocarriers [20]. Also, their properties such as the size of lipid nanoparticles influence their conductivity type. For example, particles smaller than 10 nm can pass directly through cells, while larger particles between 10 nm and 200 nm reach organ openings. Furthermore, the physiological lipid composition of lipid nanoparticles increases better permeability of drug molecules [21].

The present study showed that the high efficiency of PGF2α and PMSG ligands were 79.04 ± 4.96, and 84.11 ± 5.55% respectively, which may be due to structure of Nano-lipid composed of stearic acid, phosphatidic acid, which is well-entrapped in regular form and glycerol with castor oil providing stability and prolonging drug retention in SLN as well as increasing the permeability of drug molecules in the biological environment [22]. Entrapment efficiency, stability, drug-loading capacity, and the controlled release behavior of NLC formulations are also affected by their lipid component and surfactant [23].

pH is one of the most important factors affecting the stability of a product. The drug release profile from SLNs is considerably affected by the pH medium [24]. The current study reported Nano- lipid stability by exposing it to multiple concentrations of acid at different times at zero time and after an hour. We observed that nano-lipid stability at zero was more stable post one hour as well when calculating the number of Nano-lipids, their numbers seemed not affected by different compositions at zero while their numbers seemed to be affected and decreased after an hour and affected by high concentrations of acid. The result may be presumably due to that SNL entrapped hormone approved transit to acidic media after one hour through the electrostatic interaction, which dominate in the SNL entrapped protein hormones. Hydrophilic interaction are important for binding of the SNL with the hydrogen, which occurs between the protein hormone and the phospholipid head group of the lipid bilayer [25]. The enhanced rate of drug emission at low pH may be due to the decreased electrostatic pull among the negatively charged lipid essence and the positively charged doxorubicin [26]. To test the osmotic influence independently on electrostatic challenge interaction, the osmotic pressure variability by the addition of series concentrations of NaCl can penetrate through phospholipids bilayers. Hypoosmotic pressure was achieved by diluting nanolipid at different concentrations of normal saline. The numbers of nanolipid were checked after one hour, which indicated osmotic sporadic or rupture [27]. In the current study the hypoosmolarity produced increasing of particle size after one hour, the peak under hypoosmotic condition was the explanation for this fact where phosphatidylcholine- cholesterol may has fluidity and flexibility, which increases the size of the particles, so that they become large, which leads to osmotic swelling [28].

The current study illustrated in figure (5) the XRD results. The amorphous structure produced from Nano-carriers indicated that the hormones were well encapsulated in NLCs. This also demonstrated the successful placement of the drug in the cavities of the Nano-particles [29]. As a result, some of the hormone peaks did not appear in the NLC spectrum and the visional peaks are less intense, which may be due to the presence of material in the structure of the NLCs such as surfactants and liquid lipids, and this may lead to a strong correlation between drugs for lipids and lipid-matrix [30].

The Fourier Transform Infrared (FTIR) method is an important analytical technique for researchers. This type of analysis is used to characterize samples in shapes, functional groups, and their quantitative frequencies [31]. The current study observed that the band at around 3267 cm-1 could be assigned to O-H stretching vibration of hydroxyl groups. The bands at about 2121 cm-1 could be assigned to C≡C Terminal alkyne and 1635 cm-1 were attributed to organic nitrates (Nitrogen-oxy compounds). Data were adopted from **Sabin** [17].

However, no shifting of the characteristic peaks was observed and these peaks at their positions as shown in their spectra that confirmed no interaction among the components. The aforementioned characteristics ensure that the SNL hormone chemical integrity and the molecular structure are not changed during the preparation process. Thus, FTIR spectroscopy further validated the compatibility between hormones and lipids, surfactants, and co-surfactant even after the formulation.

CONCLUSION

The present study showed the Nanostructured-Lipid Carriers had the ability in producing high-efficiency of PGF2α and PMSG hormones.

Funding: This work was funded by the corresponding author.

Author Contribution: All authors contributed equally.
Conflict of Interest: The author declared no conflict of interest.

REFERENCE

1. **Bartosz K, Joanna D, Magdalena P, Jarek B (2018):** Applications of Noble Metal-Based Nanoparticles in Medicine. Int J Mol Sci., 19 (12): 4031.
2. **Mansoori G (2005):** Nanotechnology-An Introduction for the Standards Community. J. ASTM Int., 2: 1–22.
3. **Kumar V, Bayda S, Hadla M *et al.* (2016):** Enhanced Chemotherapeutic Behavior of Open-Caged DNA@Doxorubicin Nanostructures for Cancer Cells. Journal Cell Physiology, 231: 106–110
4. **Yang W, Cheng Y, Xu T, Wang X, Wen L (2016):** Targeting cancer cells with biotin dendrimer conjugates. European Journal of Medicinal Chemistry, 44 (2): 862–868.
5. **Yang Z, Marotta F (2012):** Pharmacometabolomics in Drug Discovery and Development: Applications and Challenges Metabolomics. JOM., 2 (5): 1000-122
6. **Iqbal M, Md S, Sahni J *et al.* (2012):** Nanostructured lipid carriers system: Recent advances in drug delivery. Journal of Drug Target, 20: 813–830.
7. **Zebon S, Eesa M, Hussein D (2020):** Efficacy of Nano Composite Porous 3D Scaffold of Crab Shell and Al-Kharit Histological and Radiological for Bone Repair in Vivo. The Iraqi Journal of Veterinary Medicine, 44 (2): 15–24.
8. **Fatima M, Mohanad A (2021)**: The Reproductive Effect of Liposomal Cimetidine on Male Mice. Plant Archives, 21 (1): 757-769.
9. **Averina E, Müller R, Popov D, Radnaeva L (2011):** Physical and chemical stability of nanostructured lipid drug carriers (NLC) based on natural lipids from Baikal region (Siberia, Russia). Pharmazie, 66 (5): 348-56.
10. **Van D, Thi-Thao L, Han-Joo M (2020**): Preparation of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers for Drug Delivery and the Effects of Preparation Parameters of Solvent Injection Method. doi: 10.3390/molecules25204781
11. **Mohanad A, Hashim W, Ferial M (2016):** Estrus Cycle Synchronization and and Superovulation Effect of L Arginine Cefatoxim Perssary (ALCECO®) IN Ewes (PART II), ejbps. Plant archves, 3 (3): 115-124.
12. **Riyam L, Elham M, Thekra M, Hadeel Y (2021):** Synthesis of silver Nanoparticles using –L-Rose flower extract thermodynamic and kinetics studies on the inhibitory effects of Nanoparticles on kinase activity. Iraqi journal of science, 62(8):2486-2500
13. **Zhou P, Yang X, Wang X *et al.* (2020):** A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579 (7798): 270–273.
14. **Cabral E, Zollner R, Santana M (2004):** Preparation and characterization of liposomes entrapping allergenic protein. Braz. J. Chem. Eng., 21 (2): 678-4383.
15. **Rahul N, Ashok C, Vishnu K, Chakrapani M, Prasanna Y (2012):** Formulation and evaluation of chitosan solid lipid nanoparticles of carbamazepine. Lipids in Health and Disease, 11: 72
16. **Asmita S, Prerana V, Sudha R, DesIgn B (2014):** Development and Characterization of liposome Neem Gell. International Journal of Pharma Sciences and Research, 5 (4): 510-523
17. **Sabin J (2006):** Size and Ability of Liposomes: A possible Role of Hydration and Osmotic Gorce. Eur phys J., 20: 401-408.
18. **Coates J (2006):** Interpretation of Infrared Spectra, a Practical Approach, in Encyclopedia of Analytical Chemistry. ed. R. A. Meyers, John Wiley and Sons Ltd., 654-756.
19. **Ahmed T, Wal1 H, Majida A (2019):** Biosynthesis, Characterization and Bioactivity of Selenium Nanoparticles Synthesized by Propolis. The Iraqi Journal of Veterinary Medicine, 43 (1): 197 – 209.
20. **Pandey V, Gajbhiye K, Soni V (2015):** Lactoferrin-Appended Solid Lipid Nanoparticles of Paclitaxel for Effective Management of Bronchogenic Carcinoma. Drug Delivery Journal, 22: 199–205. DOI: 10.3109/10717544.2013.877100.
21. **Huang X, El-Sayed M (2010):** Gold Nanoparticles: Optical Properties and Implementations in Cancer Diagnosis and Photothermal Therapy. J. Adv. Res., 1: 13–28.
22. **Murthy R (2005):** Solid lipid nanoparticles as carriers for anti-cancer drugs to solid tumors. Drug Delivery Journal, 12: 385–392. DOI: 10.1080/10717540590968855
23. **Salvi V, Pawar (2019):** P-Nanostructured lipid carriers (NLC) system: a novel drug targeting carrier. Drug Deliv. Sci. Technol., 51: 255–267.
24. **Mu¨ller R, Radtke M, Wissing S (2002**): Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm., 242: 121–8.
25. **Chen H, Huang W, Chiang W *et al.* (2015):** pH-Responsive therapeutic Solid Lipid nanoparticles for reducing P-glycoprotein-mediated drug efflux of multidrug-resistant cancer cells. Int. J. Nanomed., 10: 5035–5048.
26. **Stefan R, Velichka Y (2021**): Andonova Lipid Nanoparticulate Drug Delivery Systems: Recent Advances in the Treatment of Skin Disorders. Pharmaceuticals, 14: 1083.
27. **Mishra V, Bansal K, Verma A *et al.* (2018).** Solid lipid nanoparticles: emerging colloidal nano-drug delivery system. Pharmaceutics, 10: 191.
28. **Eloy J, Claro M, Petrilli R *et al.* (2014):** Liposomes as carriers of hydrophilic small molecule drugs: strategies to enhance encapsulation and delivery. Colloids Surface B Bio interfaces, 132 (1): 83–88
29. **Adulghani A, Al-Ogedy W (2016):** Synthesis and charactrization multishapes of FE2O4 Nanoparticles by solve-hydrothermalmethod using microwave radation ,.DOI:10.21123/bsj.2016.13.2.0331
30. **Li S, Zhao Y (2017):** Preparation of Melatonin-Loaded Zein Nanoparticles using Supercritical CO2 Antisolvent and in vitro Release Evaluation. International Journal Food, 13: 201.
31. **Fan M, Dai D, Huang B (2012**): Fourier transform infrared spectroscopy for natural fibres. In Fourier transform-materials analysis. InTech., 25: 554