Journal of Applied and Natural Science
17(2), 592 - 599 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Preparation of a new liquid dosage from Vitamin D_3 by using pospholipid conjugate

Asmaa Suri Mohammed
Scientific Research Commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Aseel Abdulhussein Obaid
Scientific Research Commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Enas Mohammed Ali Hussein
Scientific Research Commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Nibras Abdulmohsen Akosh
Scientific Research Commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Mustafa Salah Hassan
Scientific Research Commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Huda Abdulrahman Khalaf
Scientific Research Commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Fayhaa Masir Hamza
Scientific Research commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq
Faris Nawfal Abdulrahman
Scientific Research commission /Ministry of Higher Education and Scientific Research, Baghdad-Iraq

*Corresponding author. E-mail: ekhlass838@gmail.com; asmaasuri27@gmail.com

Article Info
https://doi.org/10.31018/jans.v17i2.6267
Received: October 20, 2024
Revised: May 11, 2025
Accepted: May 16. 2025

How to Cite
Asmaa, S. M. *et al.* (2025). Preparation of a new liquid dosage from Vitamin D_3 by using pospholipid conjugate. *Journal of Applied and Natural Science*, 17(2), 592 - 599. https://doi.org/10.31018/jans.v17i2.6267

Abstract
Vitamin D_3 has poor solubility in water, leading to a low dissolution profile, which presents significant biopharmaceutical challenges. To overcome these issues, liposome-conjugation techniques have been researched in recent years. Liposomes can enhance the solubility, stability, and bioavailability of vitamin D_3, improving its therapeutic effectiveness. The study aimed to prepare and evaluate the particles for vitamin D_3 conjugated with phospholipids, focusing on optimizing their formulation, assessing stability, and enhancing bioavailability. Vitamin D_3 conjugated with phospholipids was prepared using the solvent evaporation method in a 1:1 ratio. The conjugation greatly improved the physical properties of the vitamin D_3 in its conjugation and dispersion in a significant way; then the conjugate was characterized by using x-ray diffraction (XRD), fourier transforms infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), optical microscope (Op.M)toconfirm the formation of a complex between vitamin D_3 and phospholipids. The polydispersity index and zeta potential of the prepared vitamin D_3-phosphatidylcholine conjugate were measured as 74.96 and −56.93 mV, respectively, confirming its colloidal stability. These values indicated uniform particle size distribution and strong electrostatic repulsion, preventing aggregation. Additionally, the solubility in water was studied and compared with pure vitamin D_3, demonstrating improved solubility characteristics of the conjugate. This work suggests using liposomal formulations of vitamin D_3-phosphatidylcholine conjugates as an alternative to conventional vitamin D_3 tablets. These formulations can potentially enhance drug absorption and bioavailability, allowing for dose reduction and minimizing side effects. Additionally, improved bioavailability may lead to better patient compliance and therapeutic outcomes, offering a more efficient approach to vitamin D_3 supplementation.

Keywords: Conjugated, Liposomes, Phosphatidylcholine, Solubility, Vitamin D_3

INTRODUCION

Vitamin D_3 is scientifically called Colecalciferol molecular with formula $C_{27}H_{44}O$, molar mass 314.64 g/mol, white needle-like crystals melting point 86.83 ºC.They are a group of fat-soluble secosteroids, which contribute to enhancing the absorption of calcium, magnesium, and phosphate in the intestine, as well as many other biological effects in humans. It is used to treatconditions such as rickets and osteomalacia (Bikle, 2014). Several drug delivery systems have emerged, and liposomal drug delivery systems have been considered one of the emerging technologies (Dhiman *et al.*, 2021). Vitamin D_3 deficiency can have serious health consequences, as evidenced by its impact on severity and recovery following infection with COVID-19. Due to its strong hydrophobic nature, the absorption and subsequent distribution of vitamin D_3 in the body relies on the presence of lipids or proteins. An effective oral formulation of vitamin D_3 must facilitate its penetration into the mucosa and subsequent uptake by competent cells. (Aygun*et al.*, 2020).

Isothermal titration calorimetry and computer simulations indicated that the vitamin D_3 molecule could not exit the hydrophobic environment,suggesting that its absorption occurred primarily through the digestion of the carrier (Aygun*et al.*, 2020) and its deficiency can lead to several diseases. Therefore, effective delivery is essential for effective supplementation. Humans can obtain vitamin D_3 from internal and external sources. The intrinsic sourcerequires exposure to ultraviolet radiation, usually from the sun, which carries a risk of skin cancer; hence, it is best taken orally (Gupta *et al.*, 2018). The Japanese patent relates to an aqueous preparation of active vitamin D_3, where the active vitamin D_3 is made water-soluble using a non-ionic surfactant and stabilized with a specific chelating agent such as citric acid and an antioxidant. This preparation provides a stable, orally or parenterally administered preparation of active vitaminD_3 (Bilezikian*et al.*, 2021).

There is a study in which the effectiveness of the PhytoSolve formulation is examined in increasing the solubility of vitamin D_3 and improving polycystic ovary syndrome (PCOS) treatment. The PhytoSolve formulacontains a blend of S75 lipids, glycerin, and MCT oil prepared using a sonic probe. The vitamin D_3 loaded in the PhytoSolve formulation was more effective in treating PCOS than in its suspension form (Hakimpour*et al.*, 2023). There is also a study in which nanostructured lipid carriers loaded with vitamin D_3 were prepared. Nanoparticles (NLCs) made from solid fats and oils are a new generation of lipid nanoparticles that demonstrate several advantages compared to traditional lipid nanoparticles utilized in food and beverage fortification and nutrient delivery systems, including liposomes and solid lipid nanoparticles. NLC dispersion loaded with vitamin D_3 was prepared by thermal homogenization using Precirol and Compritol as solid lipids, Miglyol and Octyloctanoate as liquid lipids along with Tween 80, Tween 20, and Poloxamer 407 as surfactants (Mohammadi*et al.*, 2017).

The effectiveness of vitamin D_3 is diminished in some cases due to its poor water solubility, instability, and low bioavailability. Controlling blood,the primary biological role of vitamin D_3 is to maintain calcium and phosphorus levels within a narrow range, which is essential for proper bone mineralization. Additionally, research suggests that vitamin D_3 plays a constructive involvement inpancreatic function, fetal growth, neurological and immune function. D_3 is provided through the skin after sun exposure and diet at a suggested daily dose of 400-200 IU (Ge *et al.*, 2017) micrograms for an adult. Numerous studies suggest vitamin D_3 deficiency is prevalent in many countries globally. Hydrophobic vitamins, such as D_3, are not soluble in water-based media (Ge *et al.*, 2017). Insufficient vitamin D_3 intake is a worldwide health issue associated with serious diseases, particularly affecting individuals with darker skin pigmentation, those suffering from malnutrition, malabsorption syndromes, obesity and the elderly (Gupta *et al.*, 2018).

The present study aimed to improve the solubility and chemical stability of vitamin D_3 in combination with phosphatidylcholine (VitD_3-PC) and evaluate it in comparison with vitamin D_3.

MATERIALS AND METHODS

The reagents and solvents used were analytical grades and commercially available Sigma-Aldrich, Germany. Phosphatidylcholine from soybeans and pure vitamin D_3 were purchased from Maclin Company, China.

Preparation of vitamin D_3-phosphatidylcholine conjugate

The solvent evaporation method was used to prepare vitamin D_3-phosphatidylcholine conjugate VitD_3-PC. Vitamin D_3 and phosphatidylcholine (PC) were placed in a beaker with a 1:1 80 mg and 163 mg PC molar ratio of and dissolved in 27 ml dichloromethane. The mixture was magnetically stirred for 3 hours at 35 ^{o}C. The solvent was eliminated under reduced pressure using a rotary evaporator, and a thin film resulting from vitamin D_3 - PC was formed and then placed in a desiccator (Guo *et al.*, 2014).

Diagnostics vitamin D_3-phosphatidylcholine

X-ray diffraction (XRD)

A room temperature, an X-ray diffractometer (XRD-6000 Shimadzu, Japan) was applied for VitD_3 and VitD_3 -PC. The X-ray generator operates with a radiation source of copper with a wavelength of 1.5406 ºA, a

voltage difference of 40kV, and an electric current of 30mA **.**

Optical microscope

Optical microscope device type (NIKON ECLIPSE ME600), Japan, was used. The Op.M study gives information about the shape of the particle, the surface properties of the particle and the particle size (average diameter). For Vit D_3-PC, the dried product was used.

Scanning Electron Microscopy (SEM)

The surface characteristic of Vit D_3–PC was observed under SEM.

Fourier transforms infrared spectroscopy (FT-IR)

The pure vitamin D_3, PC and VitD_3-PC were analyzed using FTIR spectrophotometer - ABB/SPECTROLAB MB3000, UK. Samples were compressed withKBr into discs scanned from 4000 to 400 cm^{-1}.

Zeta potential

The polydispersity index, drug and lipid dispersion determination Brookhaven Instruments type of sample liquid was examined for the polydispersity index. The sample from each dispersionseparately was diluted with phosphate buffer saline (PB) pH 7.4, and for the polydispersity index using a dynamic light scattering (DLS) instrument. The zeta potential, which reflects the surface charge of the particles, was measured with the same instrument, employing a cell fitted with two electrodes.

Dissolution

Assays quantification of released vitamin D_3 was performed by Ultraviolet–visible (UV–Vis) spectrophotometry, Shimadzu type UV-Vis spectrophotometer (1650PC) measurements were conducted with the SPECORD 50 single beam spectrophotometer, which features deuterium UV and halogen vis lamps, along with a detector a measuring within the 190-1100nm range. Absorbance values were recorded using samples in a 1 cm quartz cuvette cell.

Solubility

Prepare three saturated of pure VitD_3 and VitD_3-PC in 0.1 N hydrochloric acid (HCl) solution, buffer solution pH of 7 and deionized water were prepared as separate mediums by adding excess amounts of VitD_3 and VitD_3-PC into the volumetric flasks, each containing 25 ml of the respective solution. The flasks were subsequently positioned on a magnetic stirrer for mixing at 25°C for 24 hours, followed by 10 minutes of sonication in a bath sonicator to create the necessary stress conditions and duration for effectively producing a saturated solution. Solubility can be measured through this procedure. The absorption was determined through UV -visible spectrophotometry at the selected λmax and converted into concentrations using VitD_3 calibration curves.

N-octanol/water Partition coefficient

To determine the oil/water partition coefficient of the prepared Vit.D_3-PC, an excess amount of Vit.D_3-PC to solution was prepared using equal volumes of n-octanol and water, 12.5 ml each, themixture was stirred at 25 °C for 24 hours. Following this, the two layers were carefully separated, and the Vitamin D_3 concentration in each layer was determined using a UV spectrophotometer.

RESULTS AND DISCUSSION

In the present study, Vitamin D_3-phosphatidylcholine conjugates were prepared by the solvent evaporation method, adding phosphatidylcholine to vitamin D_3, and the preparation was done with mixing mole ratios 1:1. The collected data are presented in Tables 1,2 and 3 and Figs. 1,2,3,4,5,6,7.

Characterization of Vitamin D_3-PC Conjugate

The suggested vitamin D_3-phosphatidylcholine structure is shown in Fig. 1.

The conjugation of the drug with phosphatidylcholine (PC) mainly occurs via hydrogen bonding between the hydroxyl (OH) group of vitamin D_3 and the phosphate polar head of PC, occurring in a 1:1 ratio.Vitamin D_3, which contains an active hydrogen atom (-OH), can interact with phospholipid molecules through van der Waals forces or hydrogen bonding (Khan *et al* ., 2013). The pharmaceutical-phospholipid complex can form liposomes and increase drug loading into lipid-based delivery systems (Mukherjee *et al* ., 2009). Drugs are colloidal vesicular dispersion containing lipid-bound drugs that aggregate in one or more layers to form vesicles (Kapoor *et al* ., 2018). Vitamin D_3 was chosen as a model pharmaceutical because it contains OH group in its structure in addition to its poor solubility and bioavailability (Glowka*et al* ., 2019).

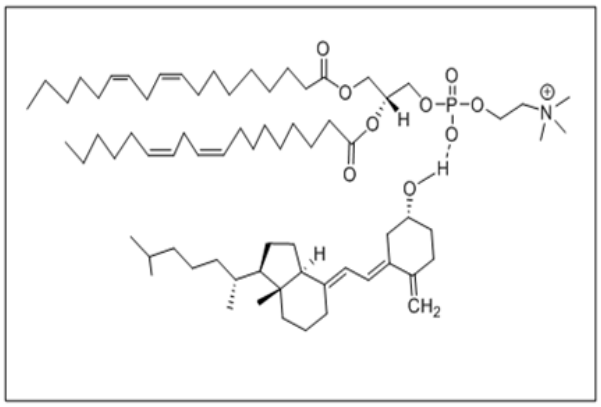

Fig. 1. *Structure of vitamin D_3-phosphati*dylcholine

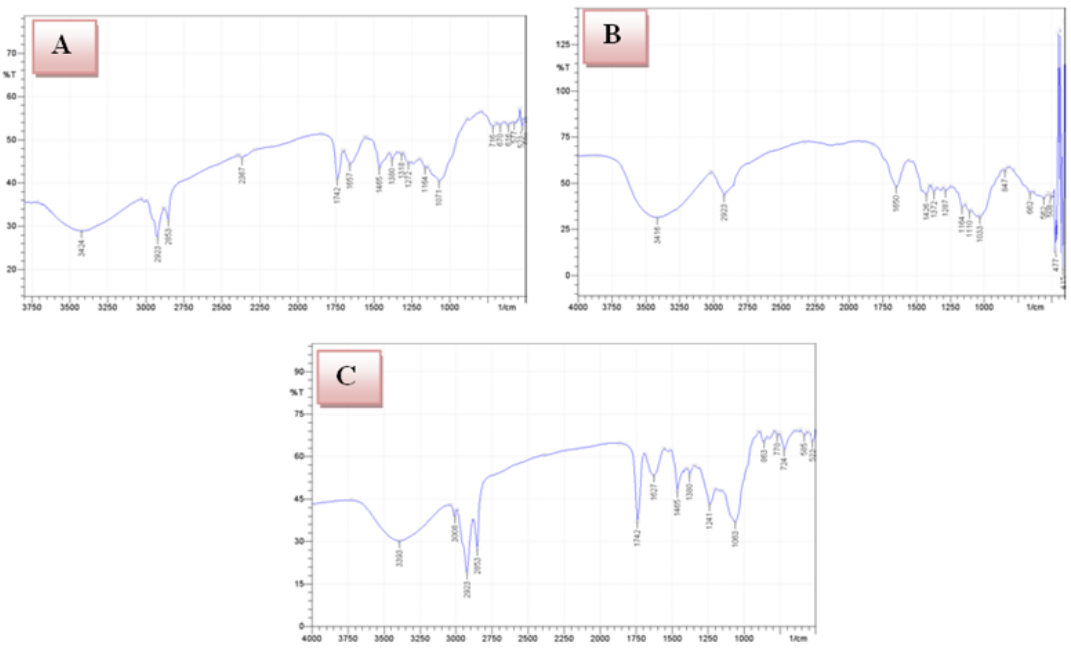

Fig. 2. *A-FTIR spectrum of VitD$_3$-PC, B- FTIR spectrum of VitD$_3$, C- FTIR spectrum of PC*

There were some significant changes in the FT-IR spectra of VitD$_3$, PC, and VitD$_3$-PC in Fig.s2,A, 2B, and 2C in the spectrum of VitD$_3$-PC. The absorption peak of OH extended 3424 cm^{-1} was a significantly less intense band than the OH absorption peak in VitD$_3$. The OH bending band at 1426 cm□¹ and the C=O stretching band at 1650 cm□¹ in VitD$_3$ were shifted to higher wave numbers, appearing at 1465 cm□¹ and 1657 cm□¹, respectively. In the VitD$_3$-PC compound, the PC compound's P=O stretching band at 1241 cm□¹ moved and combined with the VitD$_3$ absorption band at 1287 cm□¹ to form a new peak at 1272 cm□¹. These findings imply that the polar phosphate portion of PC and the OH group of vitamin D$_3$ interact.

The surface morphology of Vit.D$_3$-PC was observed using an Optical microscope and Scanning Electron Microscopy (SEM). The image showed the surface morphology of VitD$_3$-PC in Fig.s 3A and 3B, which also shows the size distribution of liposomes containing vitamin D$_3$.

The surface morphology of VitD$_3$-PC showed a lack of crystals and the appearance of the drug that formed a complex with PC, which hides the crystallization of the drug and causes multiple changes (Mukherjee *et al* ., 2009).

Table 1. Particle size (average diameter) using optical microscopy

Sample	Radius µm	Diameter µm
VitD$_3$	14	28
PC	10	20
VitD$_3$-PC	34	68

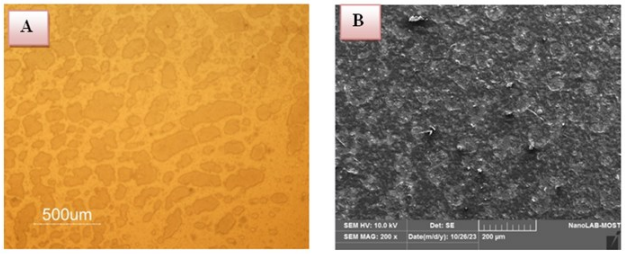

Fig. 3. *A-Image of the surface of Vit.D$_3$-PC using optical microscopy morphology, B-Image of the surface morphology of Vit.D$_3$-PC using Scanning Electron Microscopy (SEM*

Scanning Electron Microscopy (SEM)

The surface morphology of Vit.D$_3$-PC was observed using an optical microscope and Scanning Electron Microscopy (SEM) . The image shows the surface morphology of VitD$_3$-PC in Fig. 5 and Fig. 6 . The surface morphology of VitD$_3$-PC showed a lack of crystals and the appearance of the drug that formed a complex with PC, which hides the crystallization of the drug and causes multiple changes(Mukherjee *et al* ., 2009). Fig. 5 also shows the size distribution of liposomes containing vitamin D$_3$.

Optical microscopic characterization

The shape of the particles was studied by optical microscopy, and then the particle size (average diameter) was calculated because the particle size is very important because it affects the solubility, stability, drug

Table 2. Zeta potential and Mobility for VitD$_3$ and VitD$_3$-PC

sample	Zeta Potential (mV)	Mobility (µ/s)/(V/cm)
VitD$_3$	74.96	5.16
PC- VitD$_3$	56.93-	3.92-

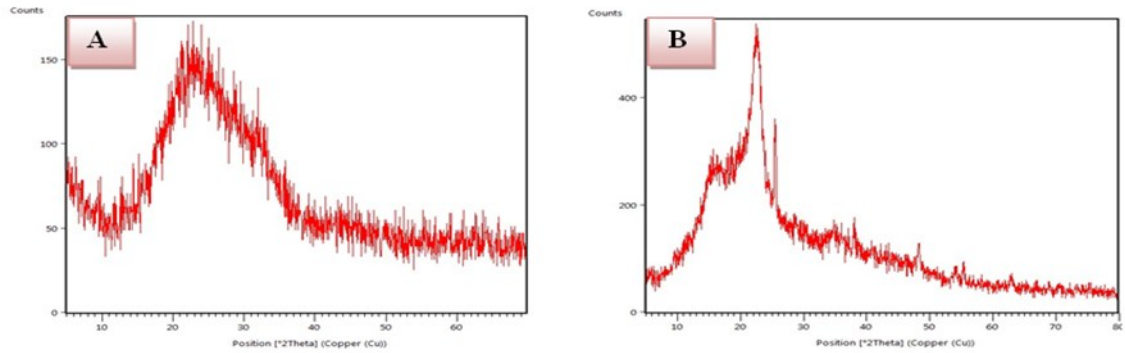

Fig. 4. *Showing: A-XRD of VitD_3; B-XRD of VitD_3-PC*

release rate and the effectiveness of VitD_3-PC to deliver the targeted drug. An increase in the average particle size and size distribution was observed for VitD_3-PC formulations. Fitting the experimental data shows that the average liposome size of 68 micrometers was determined based on measurements for each sample. The results showed a difference in the average size of VitD_3 and between VitD_3-PC and PC. This can be explained by the fact that the conjugation between VitD_3 and phospholipids leads to particles with a larger molecular weight. Therefore, greater liposome volume is generated when paired in compared to pure VitD_3 and PC (Beg *et al* ., 2016).

Fig. 4A shows X-ray diffraction of the crystalline nature of Vit.D_3, while Fig. 4B for the compound VitD_3-PC shows a lack of crystalline appearance of the drug that formed a complex with PC that hides the crystalline of the drug.

The polydispersity, the index (PDI), zeta potential and surface dispersion of pure and liposomal pharmaceutical vitamin D_3 were used. The results in Table 2 show that the mobility value is 5.16 and -3.92 (μ/s)/(V/cm) for vitamin D_3 and VitD_3. -PC particle distribution, accordingly, both compounds have uniform dispersions, but the VitD_3-PC dispersion gives a bell-shaped peak, indicating better uniform distribution.

The zeta potential of the drugs and the liposomes was 74.96 and 56.93 mV, respectively. This variation could be attributed to the differing molecular structures of the drug and the liposomes. The negative charge primarily arises from phosphatidylcholine, resulting in a negative surface charge for the particles. This helps reduce particle aggregation and thus results instable dispersion (Danaei*et al* ., 2018).

Fig.s 5 and 6 indicate the zeta potential and give information about the electric charge on the particle surface, indicating the stability of VitD_3-PC. For better stability, zeta voltage checking is required.

Techniques are fast and relatively inexpensive, so using spectrophotometry is a good option. Absorbance readings were obtained using samples in a quartz cell. The following procedures were performed in the tests: choosing the wavelength at which vitamin D_3 provided the highest absorption, which is 280 nm, preparing a standard solution of vitamin D_3 in absolute ethyl alcohol and a calibration curve for vitamin D_3 as shown in Fig. 7, using serial dilutions in the linear range from 0 to 1000.mg.mL^{-1}, vitamin D_3 concentration as a function of absorbance (Asmaa *et al* ., 2023).

Solubility study

A solubility assessment was conducted for VitD_3-PC, comparing its performance with pure VitD_3 across various solvents. These included 0.1 N HCl solution at pH 1.2, phosphate-buffered solutions at pH 7 and 7.4, as well as deionized water, as summarized in Table 3. Results indicated that VitD_3-PC exhibited significantly higher solubility ($p < 0.05$) in all tested media. In particular, its solubility was approximately 10 times greater than that of VitD_3 in 0.1 N HCl solution with a pH of 1.2, exhibits solubility approximately five times greater than that of PB solutions at pH 7 and 7.4 and in deionized water. This significant increase in solubility is attributed to the amorphous structure of the polar head of PC, which further contributes to the drug's enhanced solubility. These differences in dissolution can be attributed to the dispersion of the vesicular structure, where the PC polar head groups are essential for reducing surface tension, resulting in a fast and improved dissolution rate compared to the pure drug. (Semalty*et al* ., 2014). In addition, VitD_3 conjugation with phosphatidylcholine (PC) resulted in an amorphous form of the drug, which significantly improved its dissolution ($p<0.05$) (Qin *et al* ., 2018).

In the study of n-Octanol/Water partition coefficient, the approach is only applicable to active compounds that are moderately hydrophobic (log P<5). The amount of hydrophobic compounds is usually quantified by the log octanol/water partition coefficient; compounds cannot

Table 3. Solubility examined in various solvents

Solvent	Con. VitD_3 μg /mL	Con. VitD_3-PC μg /mL
HCl 0.1N	140	710
Buffer pH 7.4	200	1350
Deionized water	410	1200

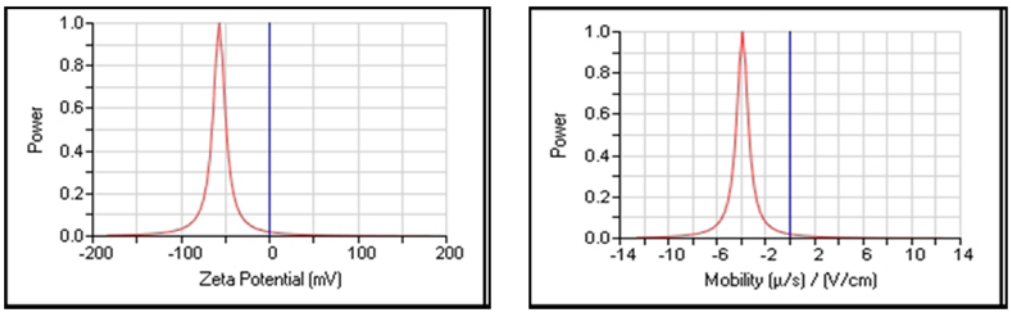

Fig. 5. *Showing Zeta potential and mobility for vitamin D_3 set the zeta potential*

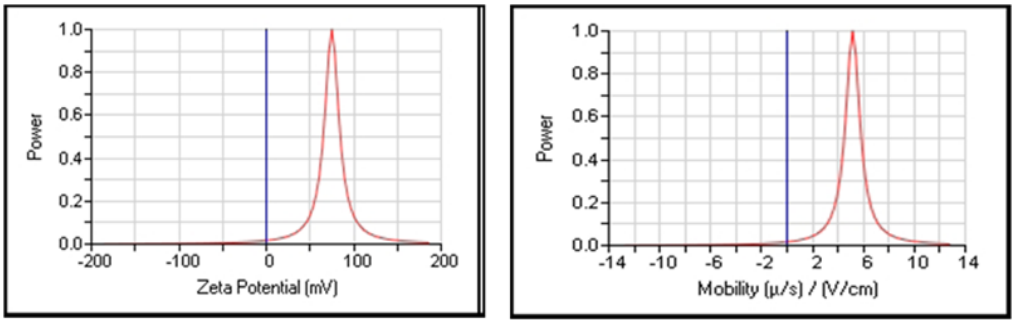

Fig. 6.*Showing Zeta potential and mobility for vitamin D_3*

targeted delivery. This study demonstrated that the main binding site for phosphatidylcholine is a phosphorylated group that combines with a model drug containing one hydroxyl group ($VitD_3$) in a 1:1 ratio. Such a complex leads to the transfer of $VitD_3$ from the crystalline form to the amorphous form, significantly improving its solubility and partition coefficient. The prepared $VitD_3$-PC liposomes showed higher dissolution than pure $VitD_3$ with a much more rigid morphology.

The drug dispersion also gave higher drug release at pH 7.4,different from the marketed $VitD_3$, which can be a good alternative for enhancing drug bioavailability, lowering the dose, and improving patient compliance. Incorporation of lipophilic and hydrophilic drugs offers several advantages, including the absence of carrier-induced biotoxicity, avoiding the use of organic solvents, enabling controlled drug release and targeted delivery, enhancing drug stability, and posing no challenges for large-scale manufacturing. The $VitD_3$-PC dispersion showed a higher solubility level than pure $VitD_3$ due to the crystal structure of the drug form of $VitD_3$.

Highly hydrophobic vehicles cannot enter the body's internal cells without assistance. This is well illustrated by cholesterol absorption and biodistribution, which requires transporters above log P> 7 with complex molecules in the structure of lipoproteins, specific receptors, and intricate cell-level redistribution systems. Vitamin D_3 is a hydrophobic molecule on which numerous immunological and metabolic functions depend (Guo *et al* ., 2014). A study of the oil/water partition coefficient (Co/Cw) was utilized as an indicator of fat content in the study conducted on the prepared $VitD_3$-PC. As illustrated in Table 3, $VitD_3$-PC scored a significantly higher ($p < 0.05$) log Co/Cw value (log P) than $VitD_3$ alone. This enhancement in the lipophilic complex is likely due to the hydrophilic groups encapsulating vitamin D_3 through the conjugation between $VitD_3$ and PC. (Lavelli*et al* ., 2021).

An aqueous preparation of vitamin D_3 was prepared; vitamin D_3 was made water-soluble using phosphatidylcholine. This preparation provides a stable preparation that can be administered orally or parenterally for vitamin D_3

Conclusion

Liposomes have been widely used over the past decade in biomedicine for their potential as targeted delivery systems. This study demonstrated that the main binding site for phosphatidylcholine is a phosphorous group that combines with $VitD_3$, containing one hydrox-

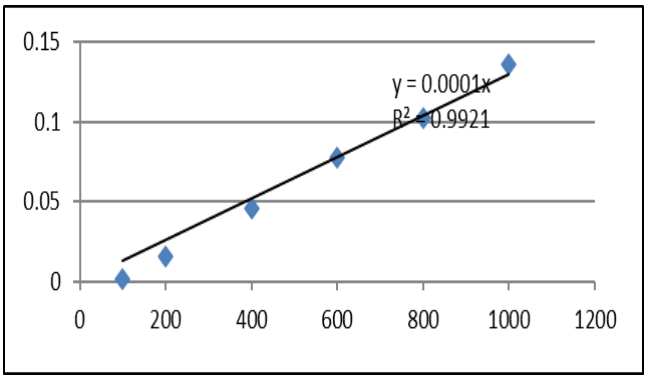

Fig. 7. *Calibration curve for vitamin D_3 determination Spectrophotometric*

yl group in a 1:1 ratio. Such a complex leads to the transfer of $VitD_3$ from the crystalline form to the amorphous form, significantly improving its solubility and partition coefficient. The $VitD_3$-PC liposomes prepared showed higher dissolution than pure $VitD_3$ with a much more rigid morphology. The $VitD_3$-PC dispersion also gave higher $VitD_3$-PC release at pH 7.4 , which is different from the marketed $VitD_3$, which can be a good alternative for enhancing drug bioavailability, lowering the dose, and improving patient compliance. Incorporation of lipophilic and hydrophilic drugs offers several advantages, including the absence of carrier biotoxicity, the elimination of the need for organic solvents, the potential for controlled drug release and targeted delivery, improved drug stability, and the feasibility of large-scale production without significant challenges.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Asmaa, S.M., Eman, W.A., Nebras, A.A., Ruaa, M.A., Huda, A.K., Fyhaa, M.H., & Ali, E. M. (2023). Evaluation of a New Spectrophotometric Method of Amlodipine in Pharmaceutical Formulation by Molecular Spectrum Using Iron (II) Iron (III). Iraqi *Journal of Science and Technology*, 13(1) 1-9.
2. Aygun, H. (2020). Vitamin D can prevent COVID-19 infection-induced multiple organ damage. *Naunyn-schmiedeberg's Archives of Pharmacology*, 393(7), 1157-1160. doi: 10.1007/s00210-020-01911-4. Epub 2020 May 25. PMID: 32451597; PMCID: PMC7246956.
3. Beg, S., Raza, K., Kumar, R., Chadha, R., Katare, O. P., & Singh, B. (2016). Improved intestinal lymphatic drug targeting via phospholipid complex-loaded nanolipospheres of rosuvastatin calcium. *RSC Advances*, 6(10), 8173-8187.
4. Bikle, D. D. (2014). Vitamin D metabolism, mechanism of action, and clinical applications. *Chemistry &Biology*, 21 (3), 319-329. doi: 10.1016/j.chembiol. 12.016. Epub 2014 Feb 13. PMID: 24529992; PMCID: PMC3968073.
5. Bilezikian, J. P., Formenti, A. M., Adler, R. A., Binkley, N., Bouillon, R., Lazaretti-Castro, M., ... & Giustina, A. (2021). Vitamin D: dosing, levels, form, and route of administration: does one approach fit all? *Reviews in Endocrine and Metabolic Disorders,* 22(4), 1201-1218. doi: 10.1007/s11154-021-09693-7. Epub 2021 Dec 23. PMID: 34940947; PMCID: PMC8696970.
6. Danaei, M. R. M. M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., ... &Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics,* 10(2), 57. doi: 10.3390/pharmaceutics10020057. PMID: 29783687; PMCID: PMC6027495.
7. Dhiman, N., Awasthi, R., Sharma, B., Kharkwal, H., & Kulkarni, G. T. (2021). Lipid nanoparticles as carriers for bioactive delivery. *Frontiers in Chemistry,* 9, 580118. doi: 10.3389/fchem.2021.580118. PMID: 33981670; PMCID: PMC8107723.
8. Ge, L., He, X., Zhang, Y., Zhang, Y., Chai, F., Jiang, L., ... & Zheng, C. (2018). A dabigatran etexilate phospholipid complex nanoemulsion system for further oral bioavailability by reducing drug-leakage in the gastrointestinal tract. *Nanomedicine: Nanotechnology, Biology and Medicine*, 14(4), 1455-1464.
9. Glowka, E., Stasiak, J., & Lulek, J. (2019). Drug delivery systems for vitamin D supplementation and therapy. *Pharmaceutics*, 11(7), 347. doi: 10.3390/pharmaceutics11070347. PMID: 31323777; PMCID: PMC6680748.
10. Guo, B., Liu, H., Li, Y., Zhao, J., Yang, D., Wang, X., & Zhang, T. (2014). Application of phospholipid complex technique to improve the dissolution and pharmacokinetic of probucol by solvent-evaporation and co-grinding methods. *International Journal of Pharmaceutics,* 474(1-2), 50-56. doi: 10.1016/j.ijpharm.2014.08.006. Epub 2014 Aug 7. PMID: 25108049.
11. Gupta, R., Behera, C., Paudwal, G., Rawat, N., Baldi, A., & Gupta, P. N. (2019). Recent advances in formulation strategies for efficient delivery of vitamin D. *AAPS Pharmscitech*, 20, 1-12. doi: 10.1208/s12249-018-1231-9. PMID: 30560516.
12. Hakimpour, S., Govahi, A., Eghbali, S., Shabani, R., Mehdizadeh, R., Ajdary, M., & Mehdizadeh, M. (2023). The Effect of Vitamin D_3 Supplemented with PhytoSolve on Genes Involved in Implantation in an NMRI Mice Model of Polycystic Ovary Syndrome: An Experimental Study. *JBRA Assisted Reproduction,* 27(4), 694. doi: 10.5935/1518-0557.20230064. Epub ahead of print. PMID: 37962973; PMCID: PMC10718550.
13. Kapoor, B., Gupta, R., Singh, S. K., Gulati, M., & Singh, S. (2018). Prodrugs, phospholipids and vesicular delivery-An effective triumvirate of pharmacosomes. *Advances in Colloid and Interface Science,* 253, 35-65. doi: 10.1016/j.cis.2018.01.003. Epub 2018 Feb 7. PMID: 29454464.
14. Khan, J., Alexander, A., Saraf, S., & Saraf, S. (2013). Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of plant actives. *Journal of controlled Release,* 168(1), 50-60. doi: 10.1016/j.jconrel.2013.02.025. Epub 2013 Mar 6. PMID: 23474031.
15. Lavelli, V., D'Incecco, P., & Pellegrino, L. (2021). Vitamin D incorporation in foods: Formulation strategies, stability, and bioaccessibility as affected by the food matrix. *Foods*, 10(9), 1989. doi: 10.3390/foods10091989. PMID: 34574096; PMCID: PMC8467460.
16. Mohammadi, M., Pezeshki, A., Abbasi, M. M., Ghanbarzadeh, B., &Hamishehkar, H. (2017). Vitamin D_3-loaded nanostructured lipid carriers as a potential approach for fortifying food beverages; in vitro and in vivo evaluation. *Advanced Pharmaceutical Bulletin*, 7(1), 61. doi: 10.15171/apb.2017.008. Epub 2017 Apr 13. PMID: 28507938; PMCID: PMC5426735.
17. Mukherjee, S., Ray, S., & Thakur, R. S. (2009). Solid lipid nanoparticles: a modern formulation approach in drug delivery system. *Indian Journal of Pharmaceutical Sciences,* 71(4), 349. doi: 10.4103/0250-474X.57282. PMID: 20502539; PMCID: PMC2865805.
18. Qin, L., Niu, Y., Wang, Y., & Chen, X. (2018). Combina-

tion of phospholipid complex and submicron emulsion techniques for improving oral bioavailability and therapeutic efficacy of water-insoluble drug. *Molecular Pharmaceutics*, 15(3), 1238-1247. doi: 10.1021/acs.mo lpharmaceut.7b01061. Epub 2018 Feb 12. PMID: 29412674.

19. Semalty, M., Badoni, P., Singh, D., &Semalty, A. (2014). Modulation of solubilityand dissolution of furosemide by preparation of phospholipid complex. *Drug Development and Therapeutics,* 5(2), 172. DOI:10.4103/WKMP-0090.139641.