Erciyes Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 38, Sayı 2 , 2022

Erciyes University
Journal of Institue Of Science and Technology
Volume 38, Issue 2, 2022

Infrared Spectroscopic and Calorimetric Studies of the Interaction of Cholecalciferol with Sphingomyelin Model Membranes

Ipek Sahin *[1]

*[1]Department of Physics, Faculty of Science, Ege University, 35100, Bornova, Izmir, Turkey

(Alınış / Received: 26.05.2022, Kabul / Accepted: 29.07.2022, Online Yayınlanma / Published Online: 23.08.2022)

Keywords
Cholecalciferol,
Model Membrane,
FTIR,
DSC

Abstract: Cholecalciferol is a steroid hormone produced in the skin under sunlight and when obtained from dietary foods. In this study, Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) were used first time in order to investigate the interactions between cholecalciferol and sphingomyelin (SM) multilamellar vesicles (MLVs) depending on vitamin concentration and temperature. The present results showed the main phase transition temperature to decrease, the system was disordered, and the system's dynamics increased both in the gel and liquid crystal phases when cholecalciferol was added to pure SM MLVs. For the head group region, hydrogen bonding was also observed after treatment with cholecalciferol.

Kolekalsiferolün Sfingomyelin Model Membranları ile Etkileşiminin Kızılötesi Spektroskopik ve Kalorimetrik Çalışmaları

Anahtar Kelimeler
Kolekalsiferol,
Model Membran,
FTIR,
DSC

Öz: Bu çalışmada, kolekalsiferol ve sfingomyelin (SM) çok katmanlı veziküller (MLV'ler) arasındaki etkileşimleri, vitamin konsantrasyonuna ve sıcaklığa bağlı olarak araştırmak için ilk kez Fourier transform kızılötesi (FTIR) spektroskopisi ve diferansiyel taramalı kalorimetri (DSC) kullanılmıştır. Mevcut sonuçlar, saf SM MLV'lere kolekalsiferol eklendiğinde ana faz geçiş sıcaklığının düştüğünü, sistemin düzensiz olduğunu ve sistem dinamiğinin hem jel hem de sıvı kristal fazlarda arttığını göstermiştir. Baş grup bölgesi için, kolekalsiferol ile etkileşim sonrası hidrojen bağı da gözlenmiştir.

*Corresponding Author, email: ipek.sahin@ege.edu.tr

1. Introduction

As a precursor hormone, the skin produces cholecalciferol (vitamin D3) when exposed to sunlight, which is converted to calcitriol in the body [1]. This is critical for bone health and development as well as preserving calcium levels [2]. Vitamin D deficiency can be prevented or treated with dietary supplements containing cholecalciferol. Diabetes, heart disease, and chronic kidney disease (CKD) may all be exacerbated or even caused by a lack of adequate vitamin D in the body. Vitamin D intake and health outcomes such as cancer prevention and improved immunity have been linked by several researchers [3, 4]. Others have suggested that it may help prevent diabetes and pre-eclampsia during pregnancy, as well as reduce inflammation. [4-6]. Promoting the monoamine's synthesis, neuroprotection and neurotrophic effects [7-11, 12], as well as antioxidant and anti-inflammatory properties [13-15], are among cholecalciferol's primary effects. Vitamin D could effect the development or increase of chronic diseases by influencing oxidative stress [16]. Although cholecalciferol has been shown to have beneficial effects in clinical and preclinical studies, the mechanisms underlying this vitamin's effect are still a mystery. Sphingomyelin, one of the basic structural components of biological cell membranes, plays many important roles and it is a frequently used lipid in model membrane studies [17-19]. Therefore, this study may be important to contribute to the interaction of cholecalciferol with membranes.

Determining the effect of cholecalciferol on membranes may provide an understanding of underlying mechanisms that contribute to its positive benefits in this regard. Despite its significance, only few researches have been carried out to survey the molecular influence of cholecalciferol on membranes [20-24]. They have lately documented the inconsistent effects of cholecalciferol on phase behavior.

Cholecalciferol effect on the lipid order, lipid phase transition, and the polar group's hydration states of zwitterionic dipalmitoyl phosphatidylcholine (DPPC) MLVs's in relation to the temperature and various cholecalciferol concentrations was previously described in our study [25]. It was only this study to be able to thoroughly examine the model membranes's structural and functional parameters; such as lipid phase transitions, the head group's hydration states and membrane order and dynamics. Using FTIR and DSC techniques, SM MLVs including low and high concentrations of cholecalciferol were examined as a temperature and concentration functions compared to pure SM membranes. Contributing to the knowledge of cholecalciferol's action mechanism is significant since it may help interpret prior biological system research and lead to new treatment methods in the future.

2. Material and Method

Sigma (St. Louis, MO, USA) provided cholecalciferol and SM, which were utilized in the absence of any further purification.

According to Severcan et al. [26], pure SM MLVs were prepared for FTIR measurements. In a round-bottomed flask, 5 mg of phospholipid were dissolved in chloroform to make SM MLVs. It was possible to dry a lipid film using nitrogen flux evaporation and a Christ LT-105 spin vac for at least two hours. To hydrate the film, 25 µl of pH 7.4 PBS buffer solution was added. Liposomes were created by whirling the mixture for about 20 minutes at a time above the gel-liquid phase transition. In order to make liposomes containing cholecalciferol, we first made a stock solution of the vitamin in ethanol and then transferred it to a round-bottomed flask. Nitrogen stream was used to eliminate the excess ethanol and the same round-bottomed flask was used to dissolve 5 mg of SM in chloroform. It was then followed by the same procedure used to make pure SM liposomes. FTIR studies were carried out using sample amounts of 20 µl on a CaF_2 window with a cell thickness of 12 µm. Infrared spectra were gathered using the PerkinElmer Frontier FTIR spectrometer. At a resolution of 2 cm^{-1}, 50 scans were taken to calculate the average interferogram. The digital controller (Specac) was used to regulate the temperature increase. Before beginning data collection, each temperature was held for five minutes. There were 2 °C gaps between 25 and 65 °C for the research.
Analysis of the spectra was provided using PerkinElmer Spectrum software. spectrum of the air was saved as the background spectrum and automatically subtracted from the sample spectra. Band positions were measured relative to the center of gravity and bandwidth measurements were calculated from eighty percent of the height of the respective bands. Specific FTIR analyzes were performed from the subtracted spectra.

Thin films for DSC measurements were made by hydrating 2 grams of phospholipid in 50 µl of phosphate buffer, just as was done for the infrared investigation. 1°C/min heating rate was applied to a DSC instrument from TA Instruments Inc. (TA Instruments Inc is located in New Castle, Delaware in the United States).

3. Results

Two non-invasive methods, FTIR spectroscopy and DSC, were used to evaluate the interaction of cholecalciferol with SM vesicles for the first time in this investigation. These approaches, which have been frequently employed in model membrane research [26-28], enabled us to gather precise information about the effects of agents on phospholipid liposomes depending on the concentration and temperature.

First, the results obtained with the DSC technique were examined. This technique gave information about the calorimetric phase transitions of the system. The parameters considered were the temperature (T_m) of the main phase transition.

Fig. 1 shows DSC curves for SM MLVs in the absence and presence of cholecalciferol. The transition peak for pure SM MLVs is around ~40 °C, which represents to the well-known transition temperature (T_m) from gel to liquid crystal phase. As seen in the figure, the addition of different cholecalciferol concentrations caused T_m values to change to lower degrees. Furthermore, the main DSC curve widened and became less intense.

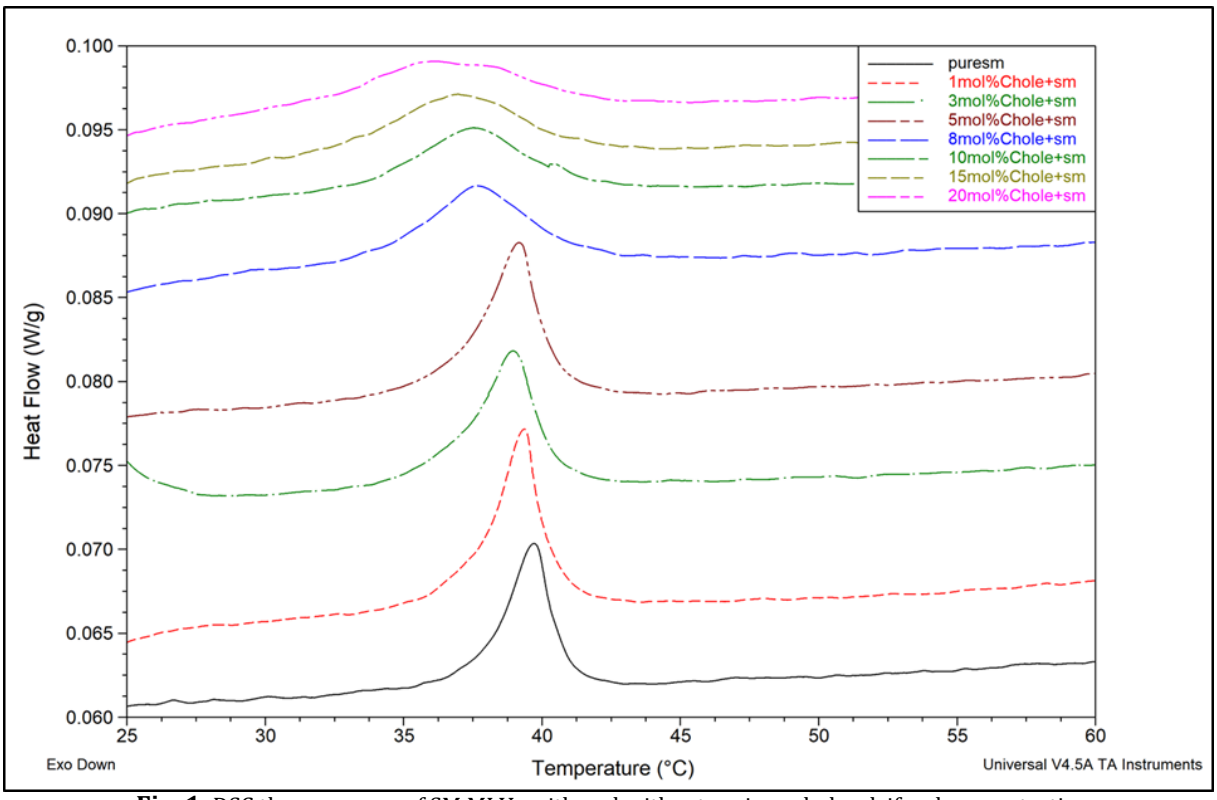

Fig. 1. DSC thermograms of SM MLVs with and without various cholecalciferol concentrations.

Another technique used in the study was FTIR. The results obtained with the FTIR technique provided information about the molecular arrangement and dynamics of the system, and the position of the molecule in the membrane. CH_2 antisymmetric stretching (at ~2920 cm^{-1}) and PO_2^- double antisymmetric stretching bands (at ~1230 cm^{-1}) were investigated in FTIR spectral analysis (Fig. 2).

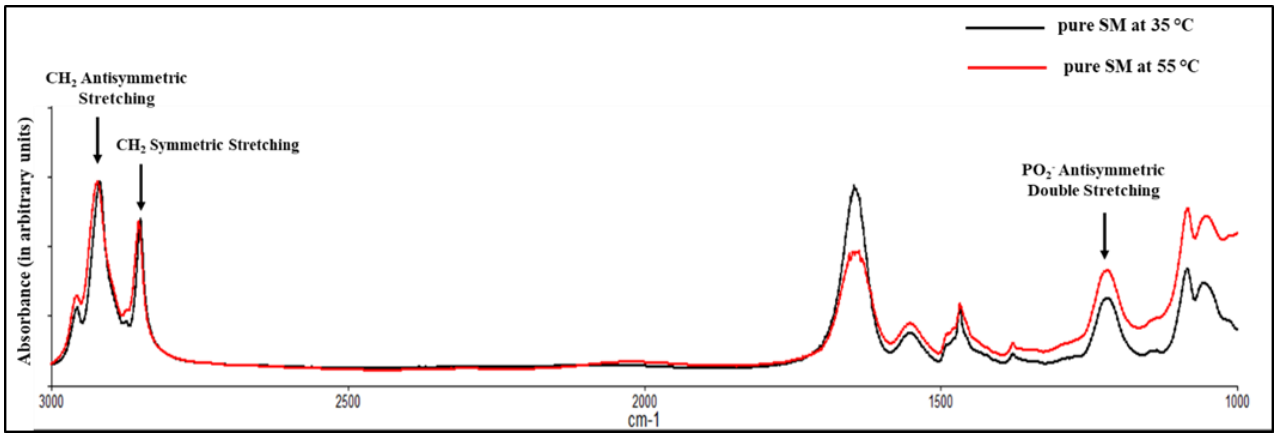

Fig. 2. Pure SM infrared spectra after representative Fourier transformations at 35 and 50 °C.

When the temperature-dependent values of the CH_2 antisymmetric stretching vibration wavenumbers in Fig. 3 were examined for pure SM liposome; it was observed that the transition from the gel phase to the liquid crystal phase occurred at about 40°C. The addition of low and high concentrations of cholecalciferol to pure SM liposomes caused the phase transition curve to become progressively broader. In addition, increasing concentrations of cholecalciferol caused a decrease in T_m values. These results obtained using the FTIR technique are in agreement with the DSC results. Addition of cholecalciferol to pure SM liposomes increased the wavenumber both in the gel (at temperatures below the phase transition) phase and in the liquid crystal (at temperatures above the phase transition) phase. An increment in peak positions (wavenumber) meant a raise in the number of gauche conformers in the system or a decrease in the acyl chain (hydrocarbon chain) order of the system [26, 28].

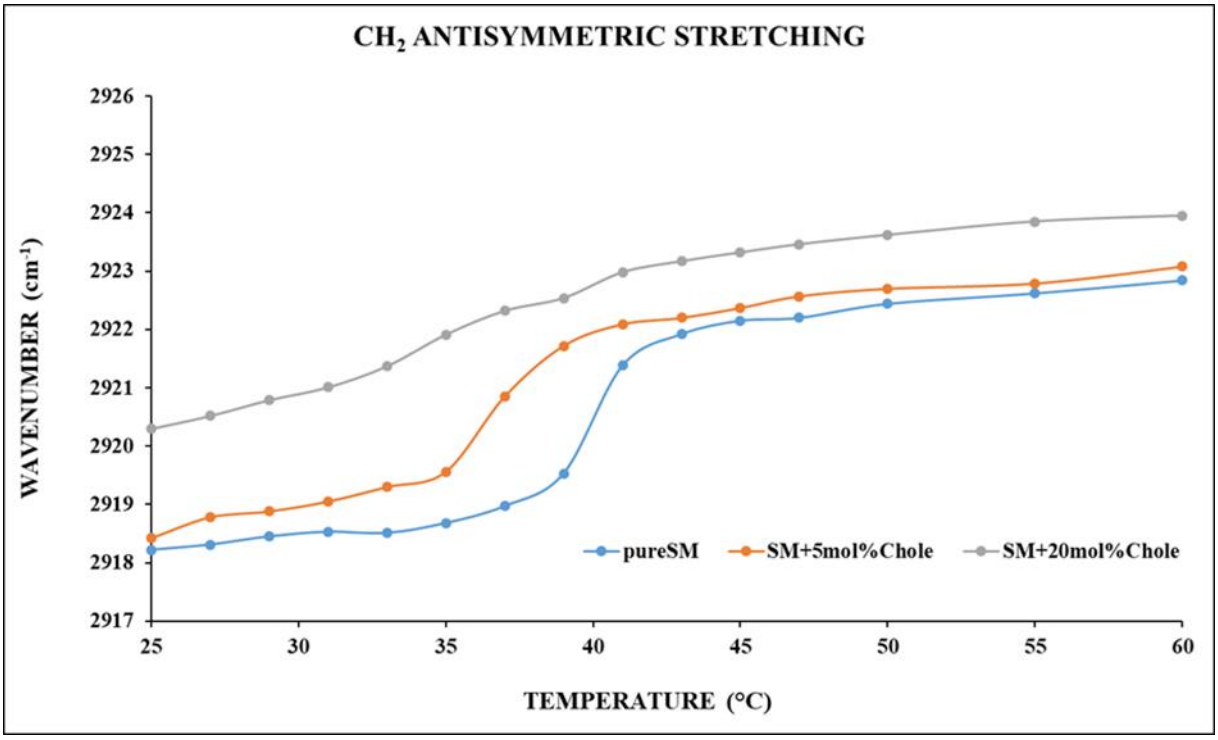

Fig. 3. The temperature and cholecalciferol concentration dependent changes in the wavenumber values of the CH_2 antisymmetric stretching band positions of SM MLVs.

When the temperature-dependent values of the bandwidth of the CH_2 antisymmetric stretching vibration in Fig. 4 were examined; in both gel and liquid crystal phase, addition of cholecalciferol to SM liposomes increased the bandwidth values. The increase in bandwidth values compared to those of pure SM meant that the membrane fluidity increased due to the increase in the freedom of motion of the lipid acyl chains [26, 28].

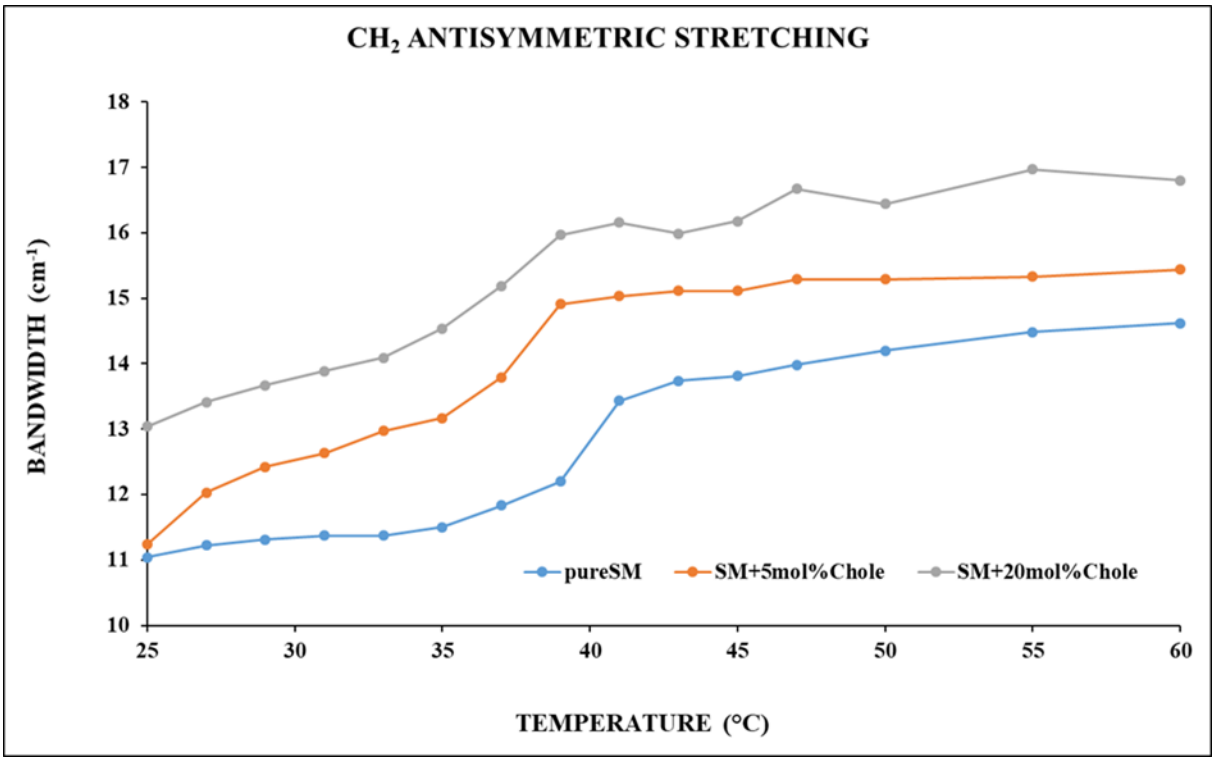

Fig. 4. The temperature and cholecalciferol concentration dependent changes in the bandwidth values of the CH_2 antisymmetric stretching band positions of SM MLVs.

When the temperature-dependent variation of PO_2^- antisymmetric stretching vibration wavenumber in Fig. 5 was examined, a decrease was observed in both phases compared to pure SM values with varying cholecalciferol concentrations. This change indicated strong hydrogen bonds between cholecalciferol and phosphate groups of phospholipids.

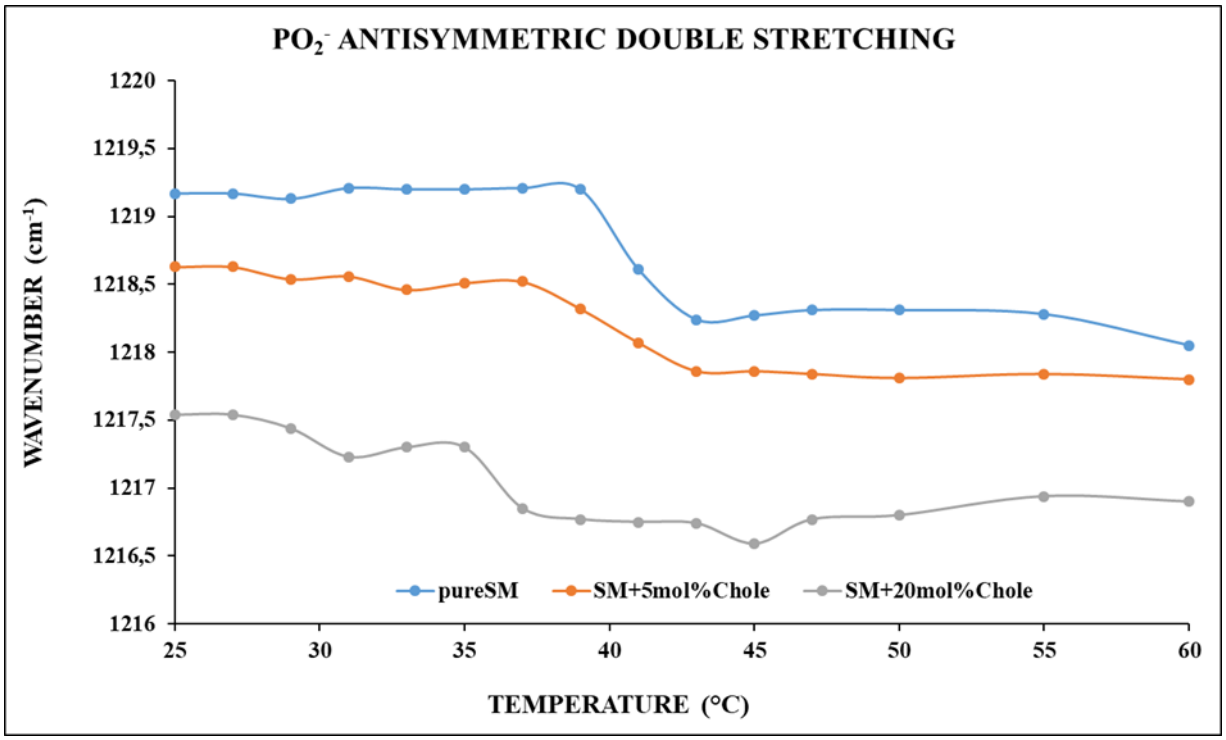

Fig. 5. The temperature and cholecalciferol concentration dependent changes in the wavenumber values of the PO_2^- antisymmetric double stretching band positions of SM MLVs.

4. Discussion and Conclusion

In this study, physical interactions of cholecalciferol with model membranes consisting of sphingolipids, namely its effects on membrane phase transition, membrane arrangement and membrane dynamics, and thermal changes, depending on temperature and concentration, were investigated for the first time using DSC and FTIR techniques.

Glycerophospholipids, sphingolipids, and cholesterol make up the vast majority of biological membranes. An important factor in agent-membrane interactions is the structural differences between the two types of phospholipids (sphingo- and glycerophospholipids), such as variations in the head group, acyl-chain composition, or unsaturation.

One of the most ubiquitous phospholipids, sphingomyelins (SMs) found in plasma membranes' external leaflet, generally include a combination of molecular species with various fatty acyl chain moieties [29,30]. Furthermore, it is, in certain situations, the predominant phospholipid. For instance, the phospholipid content of the outer layer in the intestinal brush border membrane is ~75% SM in and ~25% phosphatidylcholine (PC) [31]. Despite the fact that both DPPC used in our previous study [25] and SM have the same zwitterionic phosphocholine polar head group their hydrophobic backbones are distinct. SM includes both hydrogen bond accepting and donating groups, while PC solely comprises hydrogen bond accepting ones. All these differences change the interfacial characteristics and hydrogen bonding properties [29]. Both DPPC and SM are completely saturated lipids with a wide range of physical and chemical characteristics. Using liposomes as model cell membranes, we investigated the molecular interactions between cholecalciferol and SM in this research.

The effect of cholecalciferol on SM liposome phase transitions was studied using DSC in this work. DSC is a technique that measures heat capacity as a function of temperature at constant pressure and and detects lipid phase transitions with peaks in the heat capacity profile. In the context of phase transitions in membrane bilayers, DSC is a thermodynamic approach for studying these in the presence and absence of agent molecules. It's been frequently employed to look into the thermal changes that occur when an agent is incorporated into membrane

bilayers. The major phase transition in DSC thermograms is the transition from gel to liquid crystalline form. The interaction between agents and acyl chains of phospholipids is shown by variations in phase transition behavior [32,33]. The transition from gel to liquid-crystalline phase is known to be "cooperative". In “cooperative” transitions, molecules become organized and have the ability to act together. That is, molecules cooperate with each other to move to another phase where they have better freedom of movement [33,34]. Furthermore, the bandwidths of the main transition thermograms provide information on the conversion's cooperativity [34,35].

According to Fig. 1, when the phase transition curve of the SM liposome was examined, it was seen that the pre transition was not observed. Many thermotropic studies, like present, found no pretransition in SM liposomes [36-38]. Cholecalciferol caused a decrease and widening of the intensity of the phase transition curve of the SM, as well as a shift of the phase transition temperature to lower values. This effect could be explained by cholecalciferol interacting with SM liposome hydrocarbon chains, reducing van der Waals interactions among lipid molecules and therefore cooperativity [39, 40]. The lack of cooperativity and broadening of the transition curve suggested that cholecalciferol was inserted into the C2–C8 part of the fatty acyl chains (cooperativity region) [41]. In the presence of the agent, a decrease in T_m values represents the instability in the phospholipid bilayer [42], which is consistent with the FTIR results. The present obtained result are consistent with our previous DPPC MLVs study [25], as well as other research examining the interaction between vitamin D2 or vitamin D3 and model membrane systems prepared using DPPC, DMPC, DSPC and DPPE phospholipids [43-45].

Most bioactive compounds have been shown to alter membrane lipid characteristics to achieve their desired effects. Flexibility (lipid acyl chain order) and dynamics properties of membrane can be altered by bioactive molecules. They can also modify the forces that exist between the head group and the hydrocarbon domain [46,47]. FTIR spectroscopy, a vibrational spectroscopic technique, can detect these types of molecular changes. The structure and organization of phospholipid bilayers can be studied using FTIR spectroscopy, a powerful but low-cost spectroscopic technique. All of the phospholipid molecule's regions can be analyzed simultaneously using this method without the need for extrinsic probes to be introduced.

Aspects of the head group, interfacial and acyl chains regions of lipid molecules were all studied using FTIR spectroscopy to detect even the smallest changes in their structure and function [25, 26, 32, 48]. Furthermore, in addition to contributing to the knowledge of membrane dynamics, the present study intended to look at the impact of cholecalciferol on membrane acyl chain order and head group hydration state, which have never been studied before.

Analyzing changes in peak position or peak width in different regions can reveal the knowledge of various physicochemical processes occurring in systems. Carbon-hydrogen vibrations produce the CH_2 antisymmetric stretching band, which is located at 2920 cm^{-1} and is highly sensitive to conformational changes. The trans/gauche acyl chain ratio is also a factor in its response [49]. Since the properties of the IR-active bands resulting from the fundamental vibrations of the hydrocarbon chain C-H groups depend on the trans/gauche rotamer content of the hydrocarbon chains, these spectral parameters can also be used to obtain structural information about the conformational arrangement of the lipid hydrocarbon chain [50]. C-H stretching band positions analysis was used to investigate the system's phase transition behaviour and order/disorder state. The rise in the quantity of gauche conformers corresponds to the shifts to higher wavenumbers [51]. In addition, the change in bandwidth values of the CH_2 stretching bands ensure information about the system's dynamics or fluidity [26, 43].

The position of the CH_2 antisymmetric stretching peaks of SM MLVs changes depending on cholecalciferol concentrations and temperature, as seen in Fig. 3. T_m for pure SM is shown in the figure, with a sharp increase at ~40 °C. These substantial changes reveal a transition from gel phase to liquid crystalline, as well as a cooperative variance from trans to gauche conformation [52]. Any changes in the morphology of the main phase transition caused by cholecalciferol provide information on the molecule's location in the phospholipid liposomes [46]. When a molecule enters the hydrophobic section of the membrane, it disrupts densely packed hydrocarbon chains by reducing van der Waals forces between phospholipid chains, causing the phase transition curve to expand [26,46,48]. Cholecalciferol changed the form of the main phase transition curve and T_m values, as seen in Fig. 3. The wavenumber values of the CH_2 antisymmetric stretching band rose as cholecalciferol concentration increased, suggesting a reduction in the order of SM membranes or an increase in the number of gauche conformations in gel and liquid crystalline phases. [43, 46, 48]. In our previous study, we found that cholecalciferol and DPPC MLVs interacted in the same way [25].

Membrane dynamics are critical for the normal functioning of biological membranes, as well as various cellular activities and disease situations [53,54]. Moreover, fluidity is particularly crucial for bioactive agent activities especially in terms of their effects on membrane permeability. The temperature-dependent bandwidth alterations of the CH_2 antisymmetric stretching mode of SM MLVs, as a function of low and high cholecalciferol concentrations,

are shown in Fig. 4. As seen in Fig. 4, the increase in bandwidth values with increasing concentrations of cholecalciferol added to SM MLVs indicated that cholecalciferol also increased the membrane dynamics. Cholecalciferol molecules may disrupt the SM liposomes' tight packing of acyl chains and the strong hydrophobic interactions between lipid molecules, explaining the disordering and destabilizing effects. Even at high concentrations, the cholecalciferol molecules may interact more strongly with the phospholipid acyl chain than with each other. This could explain why the cooperative transition is lost. The membrane may become disordered and unstable as a result of the strong interaction between SM and cholecalciferol molecules.

The wavenumber alterations of the position of the PO_2^- antisymmetric stretching mode were analyzed to investigate the interaction of cholecalciferol with the head group of SM liposomes. SMs do not have a glycerol backbone, unlike triglycerides or other phospholipids, and instead have a fatty acid, a long chain sphingosine backbone and a phosphocholine head group [55]. As a result, the FTIR spectra do not show C=O stretching bands (~1735 cm^{-1}) for SM MLVs. The phosphate (PO_2^-) band is extremely sensitive to hydration changes [56]. In the PO_2^- antisymmetric stretching mode, a decrease in wavenumber indicates increased hydrogen bonding strength, whereas an increase indicates dehydration [26]. Fig. 5 shows the shifts in this band's position as a function of temperature. In the gel and liquid crystalline phases, the location of this band shifted dramatically to lower wavenumber values, and decreased wavenumbers compared to pure SM in the presence of cholecalciferol resulted in an increment in hydrogen bond strength in the polar area of the lipids. Many of sphingomyelin's distinguishing structural and dynamic features in bilayers are due to its capacity to establish intramolecular and intermolecular hydrogen bonds. The OH group dominates hydrogen interactions, forming connections with water molecules or cholecalciferol's hydroxyl groups [57].

To summarize, the current research discovered that cholecalciferol absorbed into lipid liposomes interacted efficaciously with them and caused changes in their physico-chemical characteristics for the first time. Furthermore, by expanding our earlier analysis [25] to the interactions of cholecalciferol with SM, the present study aimed to provide a better understanding of the influence of cholecalciferol on the key components of biological lipid assemblies consisting of SM. Moreover, the information obtained at the molecular level about the interactions of membrane components and bioactive compounds such as cholecalciferol can give an idea about the construction of the structure of vitamin-based drugs and the prediction of the expected biological effects.

References

[1] Coulston, A.M., Boushey, C., Ferruzzi, M., Nutrition in the Prevention and Treatment of Disease, Academic Press. p. 818. ISBN 9780123918840. Archived from the original on 30 December 2016. Retrieved 29 December 2016.

[2] Norman, A.W., From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health, The American Journal of Clinical Nutrition, 88(2)(2008), 491S–499S.

[3] Plum, L.A., & DeLuca, H.F., Vitamin D, disease and therapeutic opportunities, Nat Rev Drug Discov, 9(2010), 941–955.

[4] Hoeck, A.D., & Pall, M.L,. Will vitamin D supplementation ameliorate diseases characterized by chronic inflammation and fatigue?, Med Hypotheses, 76(2011), 208–213.

[5] Holick, M.F., Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis, Am J Clin Nutr, 79(2004), 362–371.

[6] Tagliaferri, S., Porri, D., De Giuseppe, R., Manuelli, M., Alessio, F., and Cena, H., The controversial role of vitamin D as an antioxidant: results from randomised controlled trials, Nutrition Research Reviews, 32(1)(2019), 99 – 105.

[7] Chabas, J.F., Stephan, D., Marqueste, T., Garcia, S., Lavaut, M.N., Nguyen, C., Legre, R., Khrestchatisky, M., Decherchi, P., Feron, F., Cholecalciferol (vitamin D_3) improves myelination and recovery after nerve injury, PLoS One, 8(2013), e65034.

[8] Hua, F., Reiss, J.I., Tang, H., Wang, J., Fowler, X., Sayeed, I., Stein, D.G., Progesterone and low-dose vitamin D hormone treatment enhances sparing of memory following traumatic brain injury, Horm. Behav., 61(2012), 642-651.

[9] Longoni, A., Kolling, J., dos Santos, T.M., dos Santos, J.P., da Silva, J.S., Pettenuzzo, L., Gonçalves, C.A., de Assis, A.M., Quincozes-Santos, A., Wyse, A.T.S., 1,25-Dihydroxyvitamin D3 exerts neuroprotective effects in an ex vivo model of mild hyperhomocysteinemia, Int. J. Dev. Neurosci., 48(2016), 71-79.

[10] Tang, H., Hua, F., Wang, J., Sayeed, I., Wang, X., Chen, Z., Yousuf, S., Atif, F., Stein, D.G., Progesterone and vitamin D: improvement after traumatic brain injury in middle-aged rats, Horm. Behav., 64(2013), 527-538.

[11] Tang, H., Hua, F., Wang, J., Yousuf, S., Atif, F., Sayeed, I., Stein, D.G., Progesterone and vitamin D combination therapy modulates inflammatory response after traumatic brain injury, Brain Inj., 29(2015), 1165-1174.

[12] Jiang, P., Zhang, L.H., Cai, H.L., De Li, H., Liu, Y.P., Tang, M.M., Dang, R.L., Zhu, W.Y., Xue, Y., He, X., Neurochemical effects of chronic administration of calcitriol in rats, Nutrients, 6(2014), 6048-6059.

[13] Camargo, A., Dalmagro, A.P., Rikel, L., da Silva, E.B., Simão da Silva, K.A.B., Zeni, A.L.B., Cholecalciferol counteracts depressive-like behavior and oxidative stress induced by repeated corticosterone treatment in mice, Eur. J. Pharmacol., 833(2018), 451-461.

[14] Souza, S.V.S., da Rosa, P.B., Neis, V.B., Moreira, J.D., Rodrigues, A.L.S., Moretti, M., Effects of cholecalciferol on behavior and production of reactive oxygen species in female mice subjected to corticosterone-induced model of depression, Naunyn Schmiedeberg's Arch. Pharmacol., 393(2020), 111-120.

[15] Yamini, P., Ray, R.S., Chopra, K., Vitamin D3 attenuates cognitive deficits and neuroinflammatory responses in ICV-STZ induced sporadic Alzheimer's disease, Inflammopharmacology, 26(2018), 39-55.

[16] Mokhtari, Z., Hekmatdoost, A., Nourian, M., Antioxidant efficacy of vitamin D, Journal of Parathyroid Disease, 5(1)(2017), 11–16.

[17] Siani P., Donadoni E., Ferraro L., Re F., Di Valentin C., Molecular dynamics simulations of doxorubicin in sphingomyelin-based lipid membranes, Biochimica et Biophysica Acta (BBA) – Biomembranes, 1864(1)(2022), 183763.

[18] Leitea N. B., Martins D. B., Alvares D. S., dos Santos Cabrera M. P., Quercetin induces lipid domain-dependent permeability, Chemistry and Physics of Lipids, 242(2022), 105160.

[19] Dahley C., Garessus E. D. G., Ebert A., Goss Kai-Uwe, Impact of cholesterol and sphingomyelin on intrinsic membrane permeability, Biochimica et Biophysica Acta (BBA) – Biomembranes, 1864(9)(2022), 183953.

[20] Castelli, F., Gurrieri, S., Raudino, A., Cambria, A., Effect of cholecalcipherol on thermotropic behaviour of phosphatidylethanolamine and its N-methyl derivatives, Chem. Phys. Lipids, 48(1–2)(1988), 69–76.

[21] Merz, K., Sternberg, B., Incorporation of vitamin D3-derivatives in liposomes of different lipid types, J. Drug Target, 2(5)(1994), 411–417.

[22] Elgavish, A., Rifkind, J., Sacktor, B., In vitro effects of vitamin D3 on the phospholipids of isolated renal brush border membranes, J. Membr. Biol., 72(1-2)(1983), 85–91.

[23] Kamal, A., Pal, A., Raghunathan, V.A., Modulated phases of lipid membranes induced by sterol derivatives, Soft Matter, 8(2012), 11600–11603.

[24] Bondar, O.P., Rowe, E.S., Differential scanning calorimetric study of the effect of vitamin D3 on the thermotropic phase behavior of lipids model systems, Biochim. Biophys. Acta, 1240(1995), 125–132.

[25] Sahin, I., Cholecalciferol has strong effect on the order and dynamics of DPPC membranes: A combined Fourier transform infrared spectroscopy and differential scanning calorimetry study, Vibrational Spectroscopy, 113(2021), 103207.

[26] Severcan, F., Sahin, I., Kazancı, N., Melatonin strongly interacts with zwitterionic model membranes—evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry, Biochim. Biophys. Acta, 1668(2005), 215–222.

[27] Biruss, B., Dietl, R., Valenta, C., The influence of selected steroid hormones on the physicochemical behaviour of DPPC liposomes, Chem. Phys. Lipids, 148(2007), 84-90.

[28] Villalain, J., Arranda, F.J., Gomez-Fernandez, J.C., Calorimetric and infrared spectroscopic studies of the interaction of a-tocopherol and a-tocopheryl acetate with phospholipid vesicles, Eur. J. Biochem., 158(1986), 141-147.

[29] Nyholm, T., Nylund, M., Söderholm, A., and Slotte, J. P., Properties of Palmitoyl Phosphatidylcholine, Sphingomyelin, and Dihydrosphingomyelin Bilayer Membranes as Reported by Different Fluorescent Reporter Molecules, Biophys J., 84(2)(2003), 987–997.

[30] Niemelä, P.S., Hyvönen, M.T., Vattulainen, I., Influence of chain length and unsaturation on sphingomyelin bilayers, Biophys J., 90(3)(2006), 851-63.

[31] Vénien, C., and Le Grimellec, C., Phospholipid asymmetry in renal brush-border membranes, Biochim. Biophys. Acta., 942(1988), 159–168.

[32] Sariisik, E., Kocak, M., Kucuk Baloglu, F., Severcan, F., Interaction of the cholesterol reducing agent simvastatin with zwitterionic DPPC and charged DPPG phospholipid membranes, Biochim. Biophys. Acta Biomembr., 1861(2019), 810–818.

[33] Zhang, Y.P., Lewis, R.N., Hodges, R.S., McElhaney, R.N., Interaction of a peptide model of a hydrophobic transmembrane alpha-helical segment of a membrane protein with phosphatidylethanolamine bilayers: differential scanning calorimetric and Fourier transform infrared spectroscopic studies, Biophys. J., 68(1995), 847–857.

[34] Arouri, A., Dathe, M., Blume, A., Peptide induced demixing in PG/PE lipid mixtures: a mechanism for the specificity of antimicrobial peptides towards bacterial membranes, Biochim. Biophys. Acta, 1788(2009), 650–659.

[35] Riske, K.A., Barroso, R.P., Vequi-Suplicy, C.C., Germano, R., Henriques, V.B., Lamy, M.T., Lipid bilayer pre-transition as the beginning of the melting process, Biochim. Biophys. Acta, 1788(2009), 954–963.

[36] de Almeida, R.F.M., Fedorov, A., Prieto, M., Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts, Biophys. J., 85(2003), 2406-2416.

[37] Maulik, P.R., Shipley, G.G., N-palmitoyl sphingomyelin bilayers: structure and interactions with cholesterol and dipalmitoylphosphatidylcholine, Biochemistry, 35(1996), 8025-8034.

[38] Arsova, Z., González-Ramírez, E.J., M.Goñi, F., Tristram-Nagle, S., Nagle, J.F., Phase behavior of palmitoyl and egg sphingomyelin, Chemistry and Physics of Lipids, 213(2018), 102-110.

[39] Wrobel, D., Appelhans, D., Signorelli, M., Wiesner, B., Fessas, D., Scheler, U., Voit, B., Maly, J., Interaction study between maltose-modified PPI dendrimers and lipidic model membranes, Biochim. Biophys. Acta Biomembr., 1848(2015), 1490–1501.

[40] Sanchez-Bueno, A., Watanabe, S., Maria Jose, S., Saito, T., Studies of confirmation and interaction of the cytoclohexenone and acetyl group of progesterone with liposomes, J. Steroid Biochem. Mol. Biol., 38(1991), 173–179.

[41] Custódio, J.A., Almeida, L.M., Madeira, V.M.C., The anticancer drug tamoxifen induces changes in the physical properties of model and native membranes, Biochim. Biophys. Acta Biomembr., 1150(1993), 123–129.

[42] Sarpietro, M.G., Accolla, M.L., Cova, A., Prezzavento, O., Castelli, F., Ronsisvalle, S., DSC investigation of the effect of the new sigma ligand PPCC on DMPC lipid membrane, Int. J. Pharm., 469(2014), 88–93.

[43] Toyran, N., Severcan, F., Competitive effect of vitamin D_2 and Ca_2^+ on phospholipid model membranes: an FTIR study, Chem. Phys. Lipids, 123(2003), 165–176.

[44] Bondar, O.P., Rowe, E.S., Differential scanning calorimetric study of the effect of vitamin D3 on the thermotropic phase behavior of lipids model systems, Biochim. Biophys. Acta, 1240(1995), 125–132.

[45] Castelli, F., Gurrieri, S., Raudino, A., Cambria, A., Effect of cholecalcipherol on thermotropic behaviour of phosphatidylethanolamine and its N-methyl derivatives, Chem. Phys. Lipids, 48(1–2)(1988), 69–76.

[46] Turker, S., Wassall, S., Stillwell, W., Severcan, F., Convulsant agent pentylenetetrazol does not alter the structural and dynamical properties of dipalmitoylphosphatidylcholine model membranes, J. Pharm. Biomed. Anal., 54(2011), 379-386.

[47] Baber, J., Ellena, J.F., Cafiso, D.S., Distribution of general anesthetics in phospholipid bilayers determined using 2H NMR and 1H-1H NOE spectroscopy, Biochemistry, 34(1995), 6533-6539.

[48] Aleskndrany, A., Sahin, I., The effects of Levothyroxine on the structure and dynamics of DPPC liposome: FTIR and DSC studies, Biochimica et Biophysica Acta (BBA) – Biomembranes, 1862(6)(2020), 183245.

[49] Do, T.T.T., Dao, U.P.N., Bui, H.T., Nguyen, T.T., Effect of electrostatic interaction between fluoxetine and lipid CrossMark membranes on the partitioning of fluoxetine investigated using second derivative spectrophotometry and FTIR, Chem. Phys. Lipids, 207(2017), 10–23.

[50] Lewis, R. N. A. H., & McElhaney, R. N., Membrane lipid phase transitions and phase organization studied by Fourier transform infrared spectroscopy, Biochimica et Biophysica Acta - Biomembranes, 1828(10)(2013), 2347–2358 pp.

[51] Ergun S., Demir, P., Uzbay, T., Severcan, F., Agomelatine strongly interacts with zwitterionic DPPC and charged DPPG membranes, Biochim. Biophys. Acta Biomembr., 1838(2014), 2798–2806.

[52] Casal, H.L., Mantsch, H.H., Polymorphic phase behaviour of phospholipid membranes studied by infrared spectroscopy, Biochim. Biophys. Acta, 779(1984), 381-401.

[53] Harrison, J.E., Groundwater, P.W., Brain, K.R., Hadgraft, J., Azone® induced fluidity in human stratum corneum. A Fourier transform infrared spectroscopy investigation using the perdeuterated analogue, J. Control. Release, 41(1996), 283-290.

[54] Chen, H., Mendelsohn, R., Rerek, M.E., Moore, D.J., Fourier transform infrared spectroscopy and differential scanning calorimetry studies of fatty acid homogeneous ceramide 2, Biochim. Biophys. Acta, 1468(2000), 293-303.

[55] Fanani, M.L., Maggio, B., The many faces (and phases) of ceramide and sphingomyelin I - single lipids, Biophys Rev., 9(5)(2017), 589–600.

[56] Casal, H.L., Mantsch, H.H., Paltauf, F., Hauser, H., Infrared and 31P-NMR studies of the effect of Li+ and Ca2+ on phosphatidylserines, Biochim. Biophys. Acta, 919(1987), 275–286.

[57] Niemelä, P.S., Hyvönen, M.T., Vattulainen, I., Influence of chain length and unsaturation on sphingomyelin bilayers, Biophys J, 90(3)(2006), 851-63.