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**COENZYME Q10-LOADED FISH OIL-BASED BIGEL SYSTEM : PROBING THE
DELIVERY ACROSS PORCINE SKIN AND POSSIBLE INTERACTION WITH FISH
OIL FATTY ACIDS**

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Running head: Probing the COQ10 delivery across porcine skin

Abstract

Coenzyme Q10 (CoQ10) is a vitamin-like oil-soluble molecule that has anti-oxidant and anti-aging effects. To determine the most optimal CoQ10 delivery vehicle, CoQ10 was solubilised from fish oil and formulated into hydrogel, oleogel, and bigel. Permeability of CoQ10 from each formulation across porcine ear skin was then evaluated. Furthermore, the effects of the omega-3 fatty eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids from fish oil on skin permeation were investigated by means of nuclear magnetic resonance (NMR) and computerised molecular modelling docking experiments. The highest drug permeation was achieved with the bigel formulation that proved to be the most effective vehicle in delivering CoQ10 across the skin membrane due to a combination of its adhesive, viscous, and lipophilic properties. Furthermore, the interactions between CoQ10 and fatty acids revealed by NMR and molecular modelling experiments likely accounted for skin permeability of CoQ10. NMR data showed dose-dependent changes in proton chemical shifts in EPA and DHA. Molecular modelling revealed complex formation and large binding energies between fatty acids and CoQ10. This study advances the knowledge about bigels as drug delivery vehicles and highlights the use of NMR and molecular docking studies for the prediction of the influence of drug–excipient relationships at the molecular level.

Keywords: Nuclear Magnetic Resonance; Molecular Modelling; Eicosapentaenoic acid; Docosahexaenoic acid; Bigel.

Introduction

Coenzyme Q10 (CoQ10, ubiquinone-10), sometimes referred to as vitamin Q, is a vitamin-like oil-soluble molecule. It is a thermolabile lipophilic compound ($\log P > 10$) with a high molecular weight (863 Da) (1, 2). CoQ10 is a popular antioxidant that possesses anti-aging effects (3). Topical application of CoQ10 has been reported to reduce the depth of wrinkles on human skin (2). CoQ10 also protects skin cells from the loss of cellular activity (4). CoQ10 is a component of the electron transport chain in mitochondria of eukaryotic cells that plays an important role in generating adenosine triphosphate and inhibits cell membrane peroxidation (5). It has been also reported to increase the production of basement membrane components, thereby promoting fibroblast proliferation, and to protect cells against oxidative stress (2, 4). Due to its effectiveness after skin application, formulations of CoQ10 as a topical skin care product are now being considered (6). In particular, CoQ10 has been introduced into a liposomal drug delivery system to serve as a dermal antioxidant (2, 5). It has also been formulated into a nanoemulsion (7), a nanostructured lipid carrier (8), and polymer-coated liposomes for topical drug delivery (9).

In dermatology, topical drugs are used to treat skin diseases with the intention to distribute the applied active agent to the affected surrounding tissue. One of the major concerns in topical drug delivery is to achieve permeation of the drug in a controlled manner through the outermost part of the skin, the stratum corneum, to obtain the desired therapeutic effect (10). Topical and, by extension, transdermal delivery of drugs has many advantages as compared to other routes of drug administration (11, 12) because of increased patient compliance, fewer side effects, and immediate cessation of potential toxicity due to ease in washing off the formulation. However, optimal delivery may not always be successfully achieved due to the strong barrier function of the skin, which is attributable mostly to the highly organised structure of the stratum corneum (13, 14). For the purpose of drug delivery across the skin, gels are widely used as vehicles.

Topical gels are unique materials that are rigid and elastic in nature (15) and have a broad range of applications in the cosmetics, medicine, biomaterials, and food industry. Hydrogels are one of the most common types of drug delivery vehicles. They are three-dimensional hydrophilic polymer networks able to absorb large quantities of water (16). However, when it comes to

delivering the drug through stratum corneum, hydrogels lack skin permeability and are less compatible with lipophilic drugs (15). Gels containing oil or non-polar liquids as a dispersion medium are known as organogels or oleogels. Due to lipophilic compatibility with the stratum corneum, edible oils, such as palm oil, fish oil, and coconut oil, have been widely used to prepare oleogels in pharmaceutical and cosmetic products (15, 17, 18). Organogels, which are easy to prepare, enhance drug penetration through the stratum corneum because of their lipophilic nature (15, 18). Bigels, on the other hand, are colloidal mixtures of hydrogels and oleogels that possess the advantages of both hydrogel and oleogel systems (15, 19). Bigels have been reported to be a promising topical and transdermal drug delivery vehicle (19). Bigel formulations share several characteristics with hydrogels, namely cooling effect, enhanced hydration of the stratum corneum, high spreadability, and washability by water upon application to the skin (15). The oleogel portion of bigels consists of fatty acids that are known to aid drug delivery across the skin by increasing the lipophilic nature of the formulations (19, 20).

Fatty acids are frequently used as penetration enhancers in formulations for transdermal and topical delivery of substances (21). Fish oil omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been demonstrated to enhance topical permeation of drugs (22) and have been utilised as drug delivery vehicles (19, 23, 24). These polyunsaturated fatty acids are thought to become incorporated within keratinocytes following topical application to the skin (25). Delivery of these fatty acids together with selected drugs may help to enhance skin permeation. In recent years, nuclear magnetic resonance (NMR) studies and molecular modelling have been used to investigate possible types of interactions between EPA- or DHA- CoQ10 complexes and their influence on CoQ10 permeation through the skin (20, 26). NMR spectra are highly sensitive to the local chemical environment, and NMR has been used previously to probe interactions, where processes such as interactions between drugs and fatty acids are manifested as up- or downfield shifts, depending on the magnitude of shielding/deshielding modulation (26, 27).

The aim of this study was to develop a suitable gel formulation for topical delivery of CoQ10 on the skin. Furthermore, the ability of delivering CoQ10 with the bigel formulation was evaluated in skin permeation experiments. Lastly, we investigated the interactions between fish oil fatty

acids (EPA and DHA) and CoQ10 using proton NMR. Molecular models were constructed to predict the possible geometrical parameters of EPA- or DHA-CoQ10 complexes and their binding energies to assess the potential influence of fatty acids on CoQ10 permeation. The chemical structures of all the compounds included in this study are given in **Fig 1**.

Materials and Methods

Materials

Fish oil from menhaden (crude source of omega-3 fatty acids), *cis*-eicosapentaenoic acid analytical standard, *cis*-docosahexaenoic acid analytical standard, cetrimide, and butylated hydroxyanisole (BHA) were purchased from Sigma-Aldrich (USA). CoQ10 powder was purchased from Beijing Wisapple Biotech Co., Ltd (China). Methanol and deuterated chloroform were purchased from Cambridge Isotope Laboratories (USA), and beeswax was obtained from R&M Chemicals (Selangor, Malaysia). Benzalkonium chloride and carbopol 940 were purchased from ACROS (New Jersey, USA), triethanolamine was obtained from Friendemann Schmidt (Parkwood, WA, Australia), and porcine ears were obtained from an abattoir in Ipoh, Malaysia.

Preparation of formulations

The formulations (hydrogel, oleogel, and bigel) were prepared according to the method described in our earlier studies (19, 20). To prepare hydrogels, ingredients (**Table 1**) were weighed and dispersed in deionised water under continuous mechanical stirring at 500 rpm (Ultraturrax ®IKA T25 basic, Germany) for 2 h at room temperature. Benzalkonium chloride was also added to the aqueous phase as a preservative and carbopol as a gelling agent. Subsequently, the mixture was neutralised by adding a corresponding amount of triethanolamine at a speed of 1000 rpm (Ultraturrax ®IKA T25 basic, Germany) for 15 min.

The oleogel was prepared by adding CoQ10, beeswax, and butylated hydroxyanisole to fish oil and the mixture was dissolved under continuous magnetic stirring at 300 rpm (Daihan Scientific,

South Korea) at 70 °C for 15 min. CoQ10 hydrogel (CH) and CoQ10 oleogel (CO) were stored in an incubator at 25 °C for 24 h before being used for the preparation of bigels. After the storage period, bigel formulations (CB) were prepared by adding the prepared hydrogel to the oleogel at a 50:50 ratio (W/W) followed by homogenizing the mixture by mechanical stirring at 800 rpm. All prepared formulations were then stored at 5 ± 2 °C. Each formulation consisted of 5% CoQ10 as the active ingredient. The CoQ10 content was determined by using a UV-1800 spectrophotometer (Shimadzu, Japan) and analysed at a wavelength of $\lambda = 275$ nm. The content of CoQ10 in each formulation is given in **Table 2**.

Preparation of the skin model

Porcine skin was used as model membrane in permeation studies due to similar thickness of its stratum corneum to human membrane (28, 29). Porcine ears were obtained before the carcass was exposed to steam cleaning or any high-temperature cleaning procedure to ensure the integrity of skin barrier (29). The ears were washed carefully under running water and the hair was shaved using an electric clipper (Aesculap, Melsungen, Germany). Precaution was taken to prevent any skin abrasion during shaving. The dorsal portion of the ears was removed from the underlying cartilage using surgical scalpel. The skin was then cut into 2×2 cm sections, and each skin sample was wrapped with aluminium foil to be stored at -20 °C and used within a month (22, 30). The dorsal portion of the skin was used for the permeation studies.

***In vitro* permeation study**

In vitro permeation was performed using Franz diffusion cells (PermeaGear, Inc., Hellertown, PA, U.S.A.) with an average volume of 3.5 mL and a diffusional area of 0.95 cm². Skin samples were mounted in Franz cells with the stratum corneum facing the donor compartment and clamped into position. Receptor chambers were filled with the receptor solution (30 mg/mL cetrimide and 0.05% BHA) (17, 22, 24, 25, 31) and maintained at 37 °C under continuous stirring at 300 rpm. The donor compartment was equilibrated with water for 1 h to facilitate skin hydration, which was then removed and replaced by an infinite dose (1 g) of the formulation.

Samples were periodically (1, 2, 4, 6, 8, 12, and 24 h) taken from the receptor phase and analysed using a UV-1601 spectrophotometer at a wavelength of 275 nm (32-34). The rate of drug flux ($\text{mg}/\text{cm}^2/\text{h}$) was obtained from the plot gradient (slope of permeation curve).

Skin content analysis

After 24 h of the experiment, the skin mounted on Franz cell was carefully removed and the remaining formulation that adhered to the skin surface was scraped off using a spatula. To determine the amount of CoQ10 retained by the skin, skin sections were soaked in methanol to extract CoQ10. The skin soaked in methanol was mechanically stirred overnight in a water bath (BW-20G, Lab Companion, USA), followed by ultracentrifugation (Beckman Coulter, CA, USA) for 20 min (12,000 rpm at 10 °C) and sonication for 2 h at room temperature (20, 30). The resulting solutions were filtered with a 0.45 μm polytetrafluoroethylene membrane filter and the amount of CoQ10 was determined by using a UV-1601 spectrophotometer at a wavelength of 275 nm and calculated against the calibration curve.

Nuclear magnetic resonance

Proton NMR studies were carried out on a Bruker AscendTM 400 spectrometer. NMR was operating at 400 MHz and 25 °C to determine any interaction between fish oil (pure *cis*-EPA and *cis*-DHA) and CoQ10. Mixtures of fatty acid and CoQ10 at different ratios (75:25, 50:50, 25:75) were prepared using analytical grade *cis*-EPA and *cis*-DHA. Constant volumes of saturated solutions in deuterated chloroform (CDCl_3) were added into NMR tubes. The proton NMR spectra were obtained and the chemical shifts were investigated.

Molecular modelling

Computerised molecular modelling of CoQ10 and fatty acids (*cis*-EPA and *cis*-DHA) was conducted to elucidate possible binding interactions between fish oil and CoQ10. The 2D and 3D structures of the individual drug molecules as well as of EPA and DHA fatty acid compounds

were built with ChemBioOffice[®] 2008 (PerkinElmer, Inc., Waltham, MA, USA). The minimisation using CHARM energy protocol section in Discovery Studio[®] 3.1 (Accelrys, Inc., San Diego, CA, USA) had been established in our laboratory (35, 36). The calculations were performed using 1,000 steps of the Steepest Descent with RMS gradient tolerance of 3, followed by conjugate gradient minimisation with the 2000 of maximum number of cycles for the minimization and 0.1 kcal/(mol x Å⁰) of minimization RMS Gradient. Docking studies were carried out with CDOCKER protocol section in Discovery Studio[®] 3.1. Complexes between CoQ10 and EPA or DHA were constructed by bringing together optimised structures and re-optimizing them using the same method. The conformational energy landscape of these complexes was explored by randomly altering the mutual orientation of CoQ10 and fatty acid as well as the dihedral angles of any rotatable bonds. This was followed by full energy minimisation. During the docking process, the fatty acids as macromolecules were held rigid, whereas the CoQ10 was allowed to flex during the refinement. The CoQ10 was heated to 700 K in 2,000 steps, and the cooling temperature was set to 300 K in 5,000 steps. Grid extension was set automatically based on individual fatty acids. Finally, 500 random CoQ10 and EPA or DHA conformation poses were ranked according to their -CDOCKER interaction energy, and the predicted binding interactions were analysed.

Statistical analysis

Drug flux and skin content data were analysed by using one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey's multiple comparison tests. Non-parametric comparisons were performed by the Kruskal-Wallis test followed by the Dunn's multiple comparison tests. All statistical analyses were conducted by GraphPad Prism[®] version 5 (GraphPad software, USA) with a significance level of $\alpha = 0.05$ ($P < 0.05$).

Results and discussion

***In vitro* permeation study**

During *in vitro* permeation studies, we observed that CB formulation exhibited significantly higher ($P < 0.05$, *post hoc* Tukey's test) cumulative permeation and drug flux than CO or CH formulations (**Fig 2**). CB cumulatively released $0.514 \pm 0.072 \text{ mg/cm}^2$, which was significantly higher than the amount released by CO ($0.351 \pm 0.034 \text{ mg/cm}^2$) or CH ($0.350 \pm 0.04 \text{ mg/cm}^2$) formulations. CB was also associated with a significantly higher drug flux ($0.0198 \pm 0.003 \text{ mg/cm}^2/\text{h}$) than that associated with CO ($0.0140 \pm 0.001 \text{ mg/cm}^2/\text{h}$) or CH ($0.0130 \pm 0.001 \text{ mg/cm}^2/\text{h}$) formulations. The results are also shown in **Table 2**.

The reason for the higher CoQ10 permeation from CB formulation as compared to permeation from CH and CO may be in the combined effect of carbopol hydrogel and fish oil oleogel. This synergistic effect is likely explained by the adhesiveness of carbopol in bigels, which should increase the contact time with the skin, whereas the fatty acids EPA or DHA probably promote higher CoQ10 permeation by interrupting and disrupting the tightly packed lipids in the stratum corneum, leading to increased skin fluidity and, subsequently, reduced skin barrier resistance (19, 37). The strength of binding interactions between CoQ10 and fatty acids may correlate with drug flux. To examine binding interactions between fatty acids and CoQ10, we performed molecular docking studies. It has been shown previously that compounds that have strong binding interactions with fatty acids may permeate at a higher rate as a whole unit (25, 26).

Skin content analysis

Skin content analysis was performed to estimate the content of the drug retained in the skin. The amount of CoQ10 extracted from the skin 24 h after the exposure to each formulation is shown in **Fig 3**. The highest amount of CoQ10 was recovered from the skin that was exposed to CB ($0.153 \pm 0.027 \text{ mg}$), whereas amounts recovered after the use of CH ($0.123 \pm 0.007 \text{ mg}$) or CO ($0.132 \pm 0.033 \text{ mg}$) formulations were lower ($P < 0.05$, *post hoc* Tukey's test). The presence of fish oil in oleogel (CO) and bigel (CB) formulations may have influenced the CoQ10 permeation by interrupting and disrupting the tightly packed lipids in stratum corneum which will lead to increase in skin fluidity and subsequently reduce skin barrier resistance (37). There also might be an influence of fish oil fatty acids (EPA and DHA) which helped CO and CB formulations to

perform better in terms of CoQ10 permeation through stratum corneum and availability of drug inside the skin as compared to CH.

Nuclear magnetic resonance

Next, NMR experiments were conducted to analyse possible interactions between fatty acids and CoQ10 and their influence on each other. Mixtures of EPA-CoQ10 and DHA-CoQ10 were analysed in NMR and their chemical shifts were monitored. EPA-CoQ10 mixture had a dose-dependent upfield chemical shift at the region of 2.33–2.40 ppm of NMR spectra, which represented the acyl groups of fatty acids (-OCO-CH₂, -CH₂-). This slight dose-dependent upfield chemical shift was in the region of carbon 20-protons of the EPA structure (**Fig 4a**). In CoQ10-DHA mixtures, there were chemical shifts of the protons in C1 and C2 of CoQ10 and in C1 of DHA chain. DHA-CoQ10 mixtures displayed a downfield chemical shift at the region of 4.00–3.97 ppm (**Fig 4b**) of spectra, which represented the -OCH₃ group of CoQ10 in its aromatic ring. It also displayed a downfield shift at 1.00–0.93 ppm (**Fig 4c**), which was likely associated with DHA acyl groups. It is clear that the addition of EPA and DHA to CoQ10 resulted in slight chemical shifts in the signals of protons in EPA, DHA, and CoQ10 structures. DHA appeared to have a direct dose-dependent influence on chemical shifts of protons (-OCH₃) of CoQ10 located in the aromatic ring. This chemical shift pattern may be due to weak interactions between protons of CoQ10 and fatty acids. Omega-3 fatty acids such as EPA and DHA enhance transport of drug molecules across the stratum corneum by a variety of mechanisms, e.g., by increasing the lipophilic environment of the skin, partitioning into the lipid bilayers and disrupting their ordered domains, improving drug partitioning into the stratum corneum and forming lipophilic complexes with the drugs (20, 38-40). It is now well established that such permeation enhancers can have two possible modes of action that improve topical bioavailability of a drug (41). The first, known as the “push” effect, is to increase the solubility of the solute in the formulation and hence, its concentration gradient in solution. The second, the “pull” effect, is related to the flux of the enhancer itself into and through the skin, which can induce structural transformations of the skin barrier. This effect reduces the diffusional resistance of the barrier and promotes transdermal delivery of pharmacological substances. The chemical shifts in observed in NMR experiments correspond to these “push” and “pull” phenomena.

Molecular Modelling

To predict the influence of fatty acids and to interpret NMR results, docking experiments were performed to determine the relationships between the fatty acids and CoQ10. Different geometrical models of EPA-CoQ10 and DHA-CoQ10 were assembled together to highlight the presence of any interaction that could influence permeation. Many geometric complexes exhibited different interactions of EPA-CoQ10 and DHA-CoQ10. It was observed that C48, C53, and C59 in the CoQ10 structure form weak interactions of about 3.52–4.17 Å with C20 in the EPA chain (**Fig 5a**). This $-\text{CH}_3\cdots\text{H}_3\text{C}$ interaction may be described as a hydrophobic interaction. There is another interaction found in the complexes of CoQ10 and EPA (**Fig 5b**): the aromatic ring of CoQ10 forms a weak π -alkyl interaction of about 4.32–4.57 Å with C20 in the EPA chain (aromatic ring $\cdots\text{CH}-$). The protons in C1 and C2 of CoQ10 form a strong hydrogen bond with a carboxylic group in the EPA chain (2.40–2.96 Å). This strong interaction may be due to low steric hindrance of free fatty acids (42) that allow the latter to contact tighter with CoQ10 protons and hence, exert a greater effect. The carboxylic group in EPA has lone pairs of electrons on oxygen. These lone pairs of electrons contribute to the formation of another strong interaction (π -lone pair): between the aromatic ring of CoQ10 and a carboxylic group in EPA chain of about 2.88 Å (**Fig 5c**). Weak hydrophobic interactions, weak π -alkyl interactions, hydrogen bonds and π -lone pair interactions between CoQ10 and EPA model predict that EPA might influence CoQ10 permeation. There were also complexes between CoQ10 and DHA (**Fig 6a, b**). Weak hydrophobic interactions ($-\text{CH}_3\cdots\text{H}_3\text{C}$) between C4, C18, C48, C58, and C59 of CoQ10 with C1 of DHA chain (3.79–4.44 Å) were also observed. Overall, CoQ10 docked with EPA exhibited a favourable $-\text{CDOCKER}$ interaction energy of 26.59 kcal/mol and CoQ10 docked with DHA had $-\text{CDOCKER}$ interaction energy of 19.53 kcal/mol. These interaction energies can be considered as strong binding energies. According to CDOCKER protocol, the value of $-\text{CDOCKER}$ interaction energy is commonly reported as a positive value. The higher the value of $-\text{CDOCKER}$ energy is, the stronger is receptor-ligand interaction (43). Therefore, it can be said that CoQ10 has a better receptor-ligand interaction with EPA than DHA. These strong binding energies may be due to strong hydrogen bonds and π -lone pair interactions between CoQ10 and fatty acids. These interactions likely contribute to the overall stability of CoQ10-fatty acids

complexes and facilitate their permeation across the skin barrier as a whole unit (25). Thus, EPA and DHA may influence CoQ10 permeation through the skin not only by increasing its lipophilic environment, but also by forming lipophilic complexes with CoQ10 molecules as has been indicated in molecular modelling docking studies (20, 26). Due to fatty acid-CoQ10 complex formation, fatty acids (EPA and DHA) likely promote CoQ10 permeation through the skin via the “push and pull” mechanism.

Conclusion

CB formulation allowed for higher drug permeation than did CO and CH formulations. Bigel formulation proved to be effective in delivering CoQ10 across the skin membrane due to a combination of adhesiveness, viscosity, and lipophilic nature. Bigel formulation allowed a close contact of CoQ10 with the biological membrane and enhanced local concentration gradient, exhibiting properties of a superior topical drug delivery vehicle. NMR and docking experiments demonstrated that the omega-3 fatty acids EPA and DHA may also have positively affected CoQ10 permeation. NMR data demonstrated a clear pattern of chemical shifts of protons in CoQ10, EPA or DHA. Both EPA and DHA formed complexes with CoQ10 that improved its permeation through the skin via the “pull” and “push” mechanisms. The magnitude of chemical shifts in NMR experiments appeared to be proportional to the ratio of fatty acids (EPA and DHA). These results also correlated with data from docking experiments as CoQ10 expressed strong binding affinity towards EPA and DHA. It is possible that binding energy between fatty acids and CoQ10 influences drug permeation flux. Higher binding energy and number of interactions between the CoQ10 and fatty acids may have resulted in higher drug permeation.

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Conflict of interest

The authors have no conflict of interest to report.

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Table Caption

Table 1: Composition of CoQ10 loaded hydrogels, oleogel and bigel formulations.

Table 2: Content uniformity, cumulative amount of CoQ10 permeated (mg/cm^2) and drug flux ($\text{mg}/\text{cm}^2/\text{h}$) of CoQ10 formulations using porcine skin (Data expressed as mean \pm S.D).

Table 1: Composition of CoQ10 loaded hydrogels, oleogel and bigel formulations

Ingredients (g)	CH (Hydrogel)	CO (Oleogel)	CB (Bigel)
Coenzyme Q10	5	5	CB Bigel was prepared by homogenizing 50:50 ratio (W/W) of CH and CO.
Carbopol	2	-	
Benzalkonium chloride	0.05	-	
Triethanolamine	0.5	-	
Bees wax	-	10	
Butylated Hydroxianisole	-	0.5	
Deionized water	100 (q.s)	-	
Fish oil	-	100 (q.s)	

Table 2: Content uniformity, cumulative amount of CoQ10 permeated (mg/cm²) and drug flux (mg/cm²/h) of CoQ10 formulations using porcine skin (Data expressed as mean ± S.D).

Formulation	Content Uniformity (%)	Cumulative amount of CoQ10 permeated (mg/cm ²)	Drug flux (mg/cm ² /h)
CB	99.8	0.514 ± 0.072	0.0198 ± 0.003
CO	99.5	0.351 ± 0.034	0.0140 ± 0.001
CH	99.2	0.350 ± 0.040	0.0130 ± 0.001

Figure Caption

Figure 1: Chemical structures of Coenzyme Q10, Eicosapentaenoic acid and Docosahexaenoic acid.

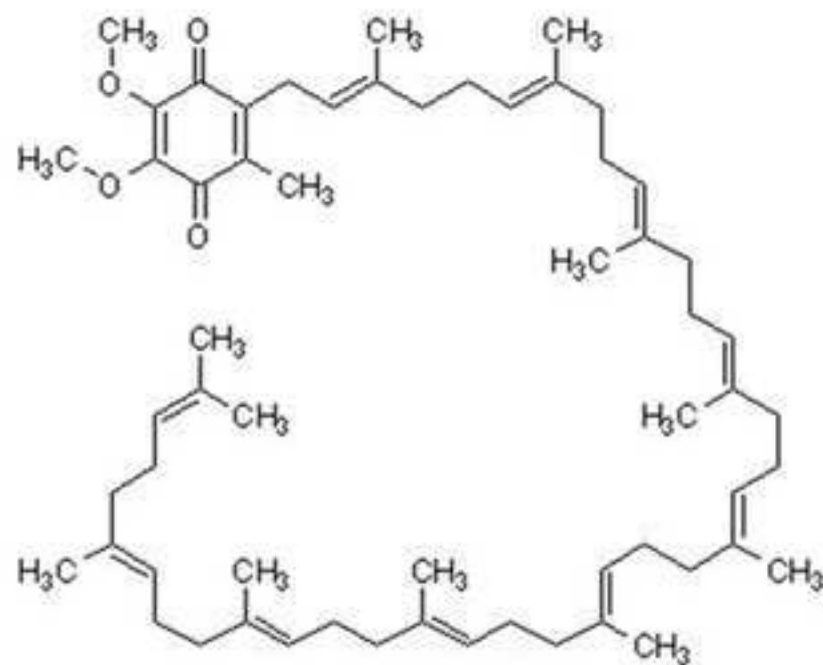
Figure 2: *In vitro* cumulative permeation of CoQ10 loaded bigel (CB), hydrogel (CH) and oleogel (CO) formulation (mean \pm SD, n=3, P<0.05, one-way ANOVA).

Figure 3: CoQ10 extracted from porcine skin after 24 hours of permeation study (mean \pm SD, n=3, P<0.05, one-way ANOVA).

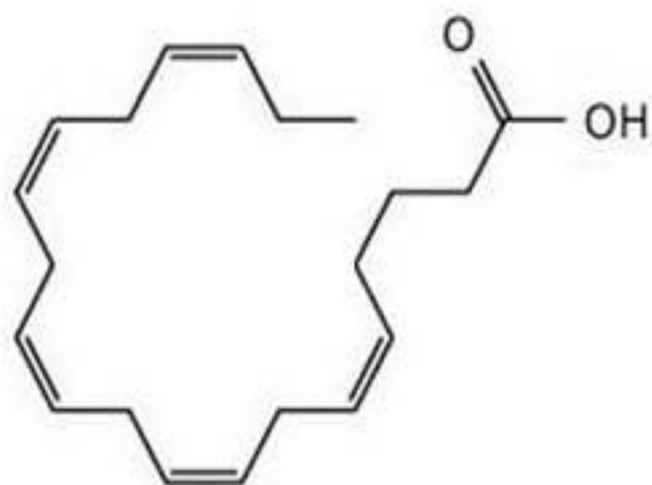
Figure 4: Fatty acids and CoQ10 mixtures showed chemical shifts in NMR spectrum of (a) EPA-CoQ10 and (b and c) DHA-CoQ10 (blue: 75%; green: 50%; red: 25% of fatty acid).

Figure 5: (a) Molecular model of EPA and CoQ10 showing alkyl interactions, (b) possible π -alkyl interaction and π -lone pair interactions between aromatic ring EPA-CoQ10, (c) EPA-CoQ10 showing possible hydrogen bonds.

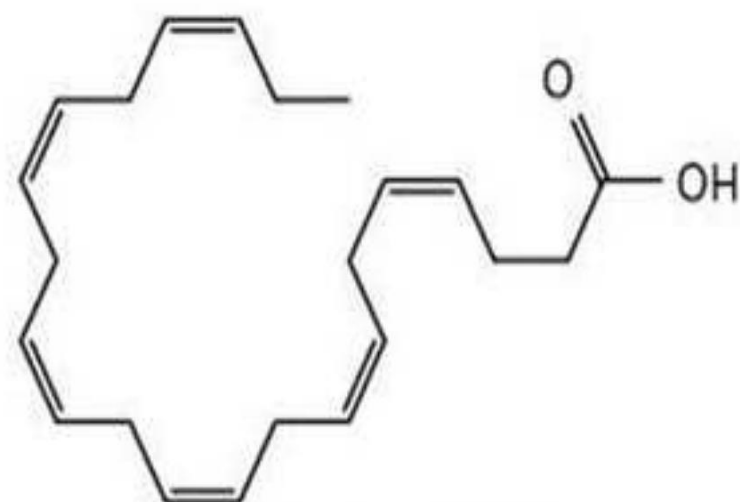
Figure 6: (a-b) DHA and CoQ10 showing the possible interactions between the structure of drug and long of chain of fatty acid.



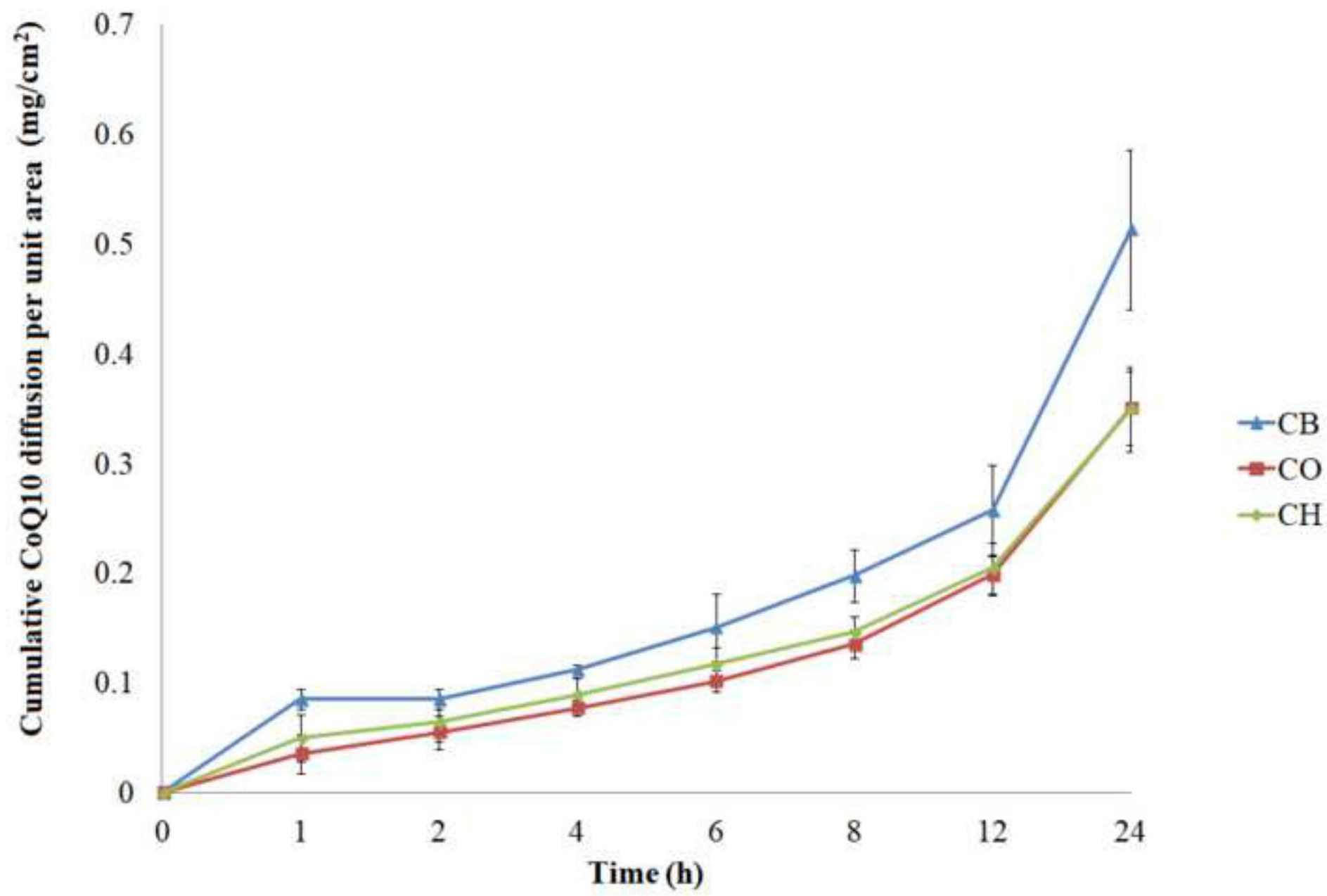
Coenzyme Q10



Eicosapentaenoic acid



Docosahexaenoic acid



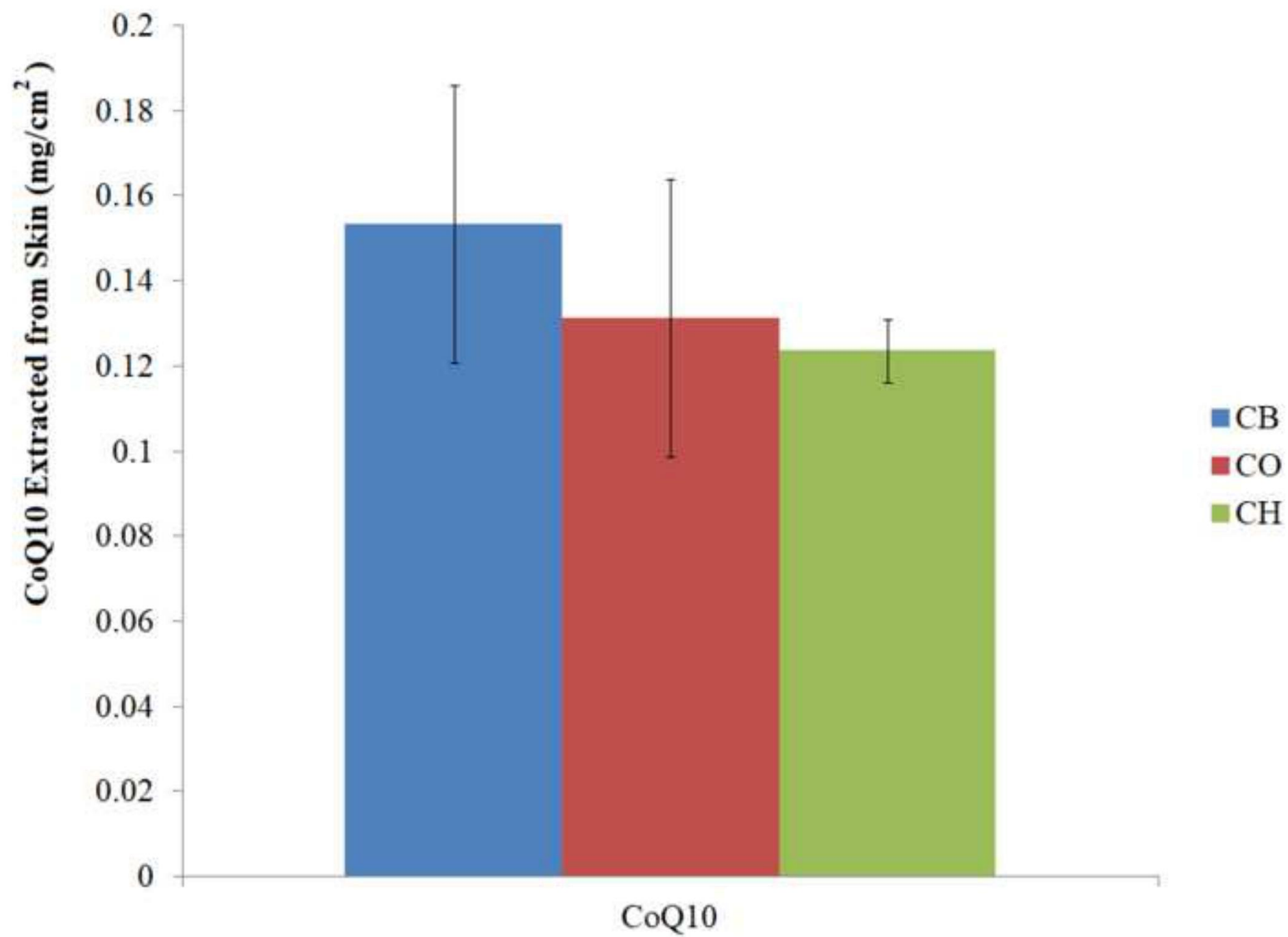
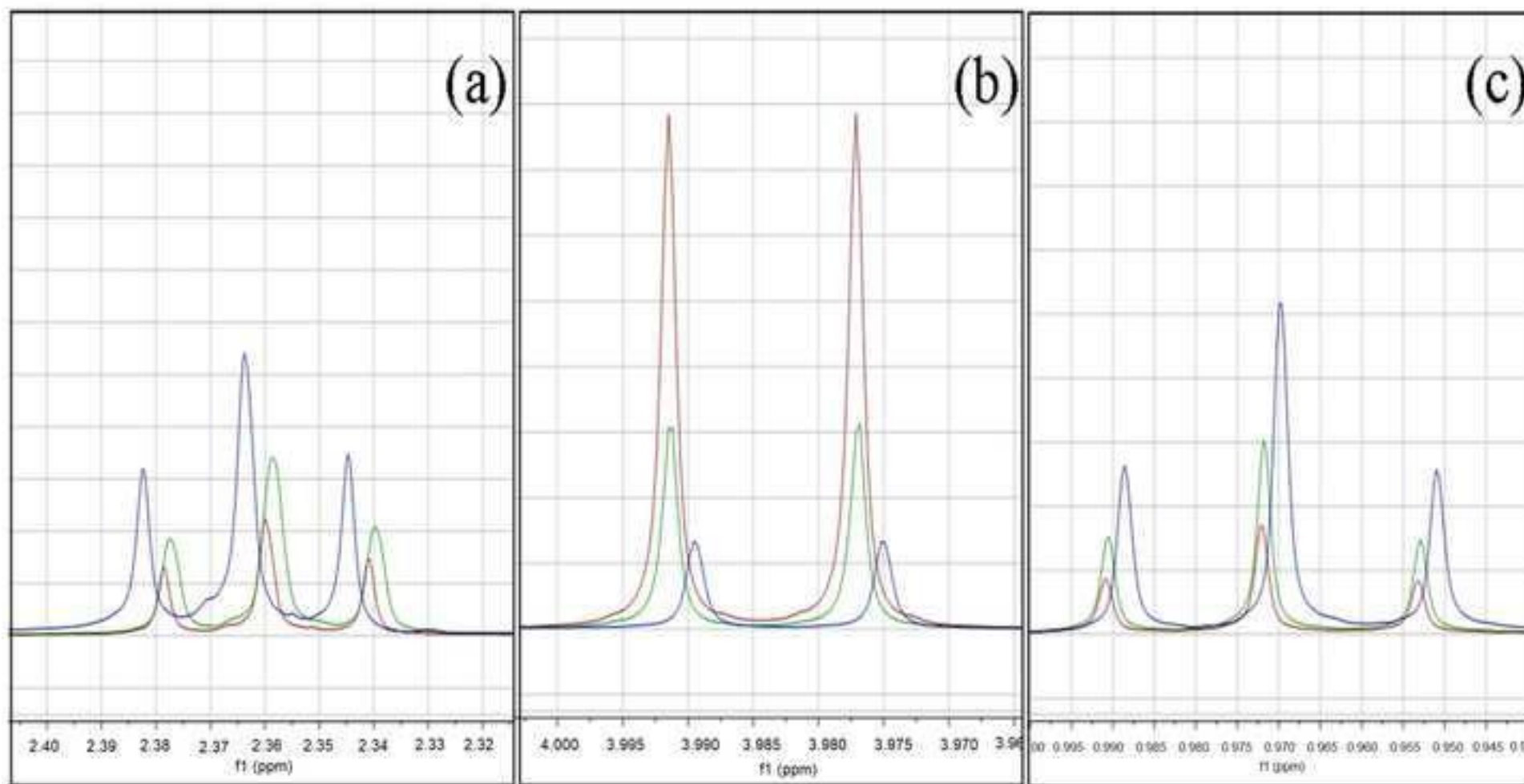
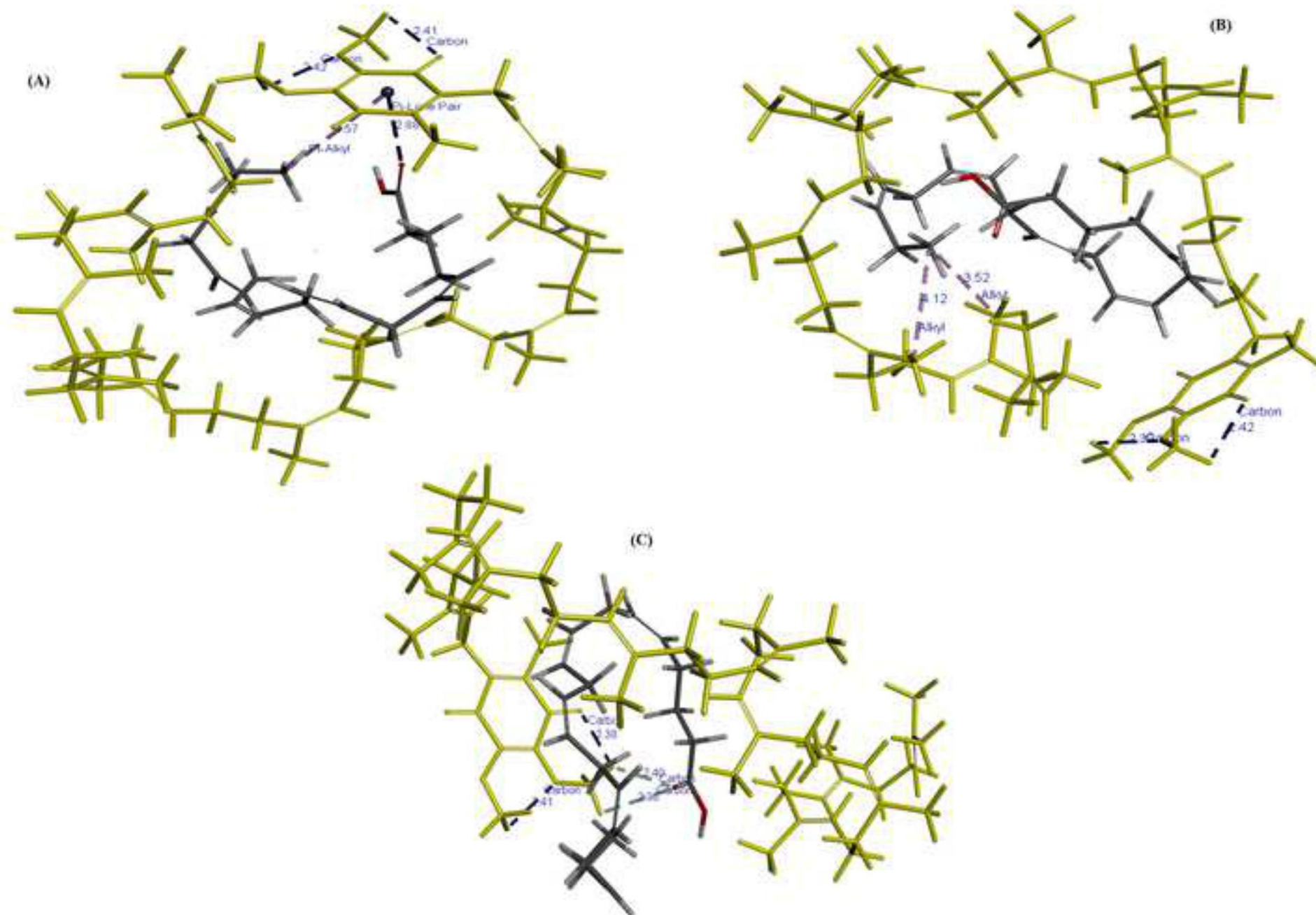


Figure 4





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