# Highlight on lipids and its use for covalent and non-covalent conjugations

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## Abstract:

Lipids are organic fatty or waxy compounds which are used to make nanocarriers that are promising for drug delivery. When lipids associated covalently (lipid-drug conjugate LDC) or non- covalently (drug-lipid complex) to drugs to form Lipid Drug Conjugates (LDC). Most common types of lipids used for drug conjugation are fatty acids, glycerides, steroids, and phospholipids. Conjugation with lipids may change the properties of the drug and significantly increase the drug lipophilicity. Lipid-drug conjugation could improve the delivery of drugs by the lymphatic system, enhance bioavailability of oral administered drugs, improve drug targeting in tumor diseases, enhance the loading of drugs into some delivery carriers, increase drug stability, and many others. Lipid-drug conjugates can be prepared through different strategies for conjugation and by chemical linkers depending on the chemical structure of both drugs and lipids, careful selection of lipids and drug are necessary in designing the lipid-drug conjugate to achieve maximum benefits.

**Key words:** Assay, (HPLC) High performance liquid chromatography, (AUP) Area under the peak, Ampicillin Trihydrate (AMP. Tri. H).

## تسليط الضوء على فعالية الدهون ومعقد الدواء الدهني على سلوك مقترناتها

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### الخلاصة:

الدهون هي مركبات دهنية أو شمعية عضوية تم استخدامها لصنع ناقلات نانوية واعدة لتوصيل الدواء. عندما ترتبط الدهون بطريقة تساهمية أو غير تساهمية بالأدوية ، فإنها تشكل ما يسمى بمركبات الأدوية الدهنية. أكثر أنواع الدهون شيوعًا المستخدمة في الاقتران الدوائي هي الأحماض الدهنية ، الجلسريدات ، الستيرويدات ، والفوسفوليبيدات. الاقتران بالدهون له فوائد عديدة ، فهو سيغير خصائص الدواء ويزيد بشكل كبير من حساسية الدواء للدهون. يمكن أن يؤدي اقتران الأدوية الدهنية إلى تحسين توصيل الدواء عن طريق الجهاز اللمفاوي ، وتعزيز التوافر البيولوجي عن طريق الفم ، وتحسين استهداف الأورام ، وتحسين تحميل الدواء في ناقلات التسليم ، وزيادة استقرار الدواء ، والعديد من الفوائد الأخرى. يمكن تحضير مقترنات الأدوية الدهنية من خلال العديد من استراتيجيات الاقتران والروابط الكيميائية اعتمادًا على التركيب الكيميائي للأدوية والدهون ، كما أن الاختيار الدقيق للدهون والأدوية ضروري في تصميم اقتران الأدوية الدهنية لتحقيق أقصى فائدة.

**الكلمات المفتاحية:** الدهون، الدواء المقترن مع الدهون، محبة للدهون، الروابط الكيميائية.

## Introduction:

Lipids are organic compounds that are insoluble in polar organic solvents and soluble in non-polar solvents, they are essentially waxy or fatty compounds [1]. Lipids were used to make nanocarriers that are promising for drug delivery. As excipients, lipids possess typical properties such as, superiority in biodegradation (as they are generally derived from natural sources) and high potential to pass the barrier of gastrointestinal tract [2]. Lipids can be linked covalently or non- covalently to drugs to form Lipid Drug Conjugates (LDC), various types of lipid derivatives such as, glyceride, fatty acids, phospholipids, and sterols can be used for this purpose. Lipid Drug Conjugates are developed to overcome the drug delivery problems such as drug targeting, bioavailability, and to reduce drug toxicity [3]. These drug conjugates can improve bioavailability and hence therapeutic efficacy of the drugs by enhancing the drug penetration through the physiological membrane [4]. Lipid-drug conjugates can be prepared through different strategies for conjugation and by chemical linkers depending on the chemical structure of both drugs and lipids. The aim of this study involves an overview on lipids and its capability to form complex and conjugate with drugs and its contribution on the physic-chemical properties of the drug.

### Lipids used for conjugation:

### Fatty acids:

The molecule of Fatty acids composed from a hydrocarbon chain that is aliphatic in nature with a terminal end of monocarboxylic acid moiety. The hydrocarbon chain exists in two forms, one of them is saturated that has no double bonds while the other one is unsaturated as it contains one or more double bonds at different positions [5]

The monocarboxylic acid moiety of the fatty acid can be conjugated with a drug having an amine or hydroxyl group to form amide or ester bond as shown in figure 1**.** Several types of fatty acids were used for the drug conjugation, such as docosahexaenoic acid (DHA), stearic acid, squalenoic acid, and palmitic acid. Stearic acid through its carboxyl group has been used for conjugation with Decitabine that has hydroxyl and amino group to increase the permeability of the drug along with its protection from chemical degradation [5, 6].

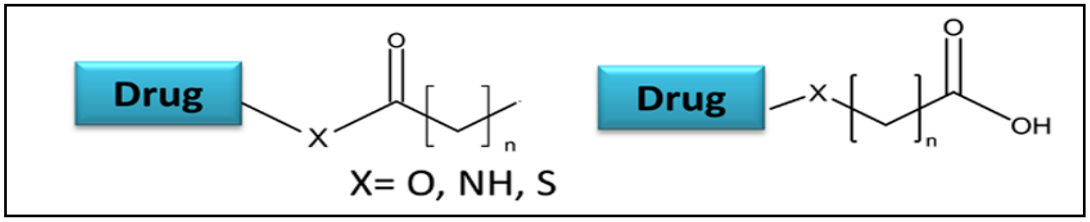


**Figure (1): Fatty acids-drug conjugate [7]**

### Triglycerides:

Triglyceride (TG) is a glycerol moiety connected to three fatty acids through ester bond. The classification of TGs depends on the length of the fatty acid's hydrocarbon chain, they are classified into three categories of short, medium, and long chain [5]. Triglycerides were used to synthesize lipid-drug conjugates, the modification of the drug pharmacokinetic behavior that could be occur through TGs conjugation is the major benefit [8].

A strategy developed by scientists is to substitute one of the three groups of fatty acyl with a drug molecule typically at position 2 (figure 2 and 3) to take the benefit of TG metabolism pathway as TG sn-1 and sn-3 positioned fatty acids will be selectively hydrolyzed at the intestinal lumen leaving the sn-2 monoglyceride to

be absorbed naturally, this pathway can be used for drug targeting to different organs and also to overcome the difficulties of drug delivery [9]. For instance, naproxin the nonsteroidal anti-inflammatory drug (NSAID) conjugated to the sn-2 position of TG exhibited improved absorption through lymphatic system [10].

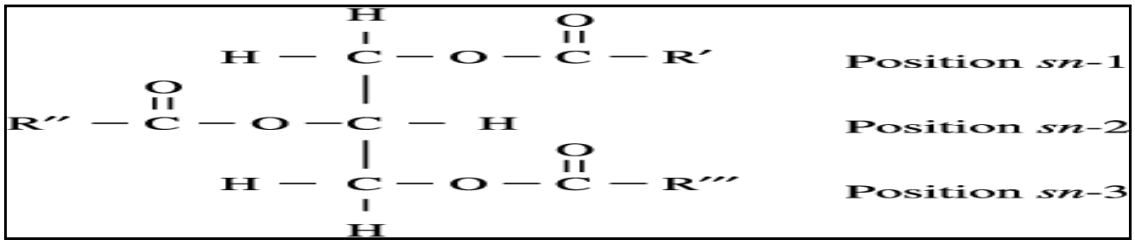


**Figure (2): Stereospecific numbering (sn) system of the glycerol molecule [11]**

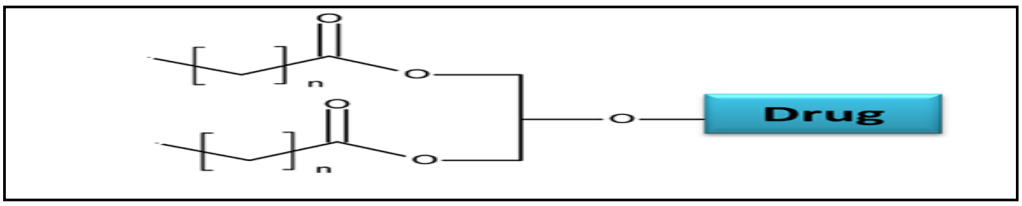


**Figure (3): Triglycerides-drug conjugate [7]**

**Steroids:**

Steroids are common 4-ringed structures. Common examples of steroids are cholesterol and derivatives of cholic acid. Steroids were used for conjugation with drugs, the hydroxyl group of the steroid ring is the primary location for conjugation (figure4). Drug conjugation with cholesterol provides benefits such as, enhanced tumor targeting, better cellular uptake, and side effect reduction [12].

Drugs conjugated with cholesterol are increasingly used to target ovarian cancer as the ovarian tissues are rich in cholesterol which is used for sex hormones synthesis. For instance, cholesterol conjugate of phosphotyrosine revealed promising efficacy and selectivity against platinum-resistant ovarian cancer cells [13].

Another type of steroids is lithocholic acid (LCA) which can be utilized for conjugation through the three hydroxyl groups of it, tamoxifen has been conjugated with LCA by covalent attachment of tamoxifen amine group with LCA molecule. Tamoxifen-lithocholic acid conjugate revealed superior anticancer activity than that of free tamoxifen because lipid can drug interaction with cells [14].

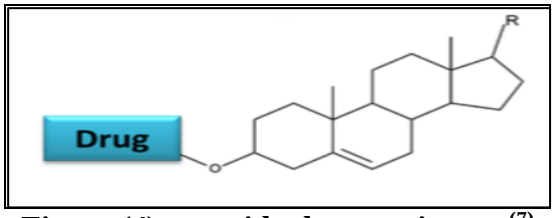


**Figure (4): steroids-drug conjugate [7]**

**Phospholipids:**

Phospholipids (PLs) are molecules that have a hydrophilic head of phosphate moiety connected to two hydrophobic chains of fatty acids through an alcohol or glycerol molecule. Consequently, PLs are considered as amphiphilic molecules [15].

The unique properties of PLs are superior biocompatibility and amphiphilicity which made them suitable and important pharmaceutical excipients that have been applied in a variety of DDS, such as, improvement of bioavailability, modifying the drug release, transport through lymphatic system, and reduced side effects of drugs. Moreover, PLs act as surfactants, solubilizer, and permeation enhancer. Due

to PLs amphiphilicity, it has unique characteristics such as wetting, emulsifying, and self-assembly [16].

Phospholipids can be conjugated with drugs in two ways: linkage with the phosphate polar head or utilizing position 2 of the glycerol backbone for attachment as shown in (figures 5 and 6). Such conjugates can be used for liposomes formation or improve the loading of drugs into lipid-based delivery systems, also, it is reported that gastrointestinal safety of non-steroidal anti-inflammatory drugs (NSAIDs) improved upon complexation with phospholipid. Gemcitabine hydroxyl group has been used as a site for conjugation with the phosphate group of a phospholipid [17, 18]. A list of various lipid-drug conjugates is shown in table (1).

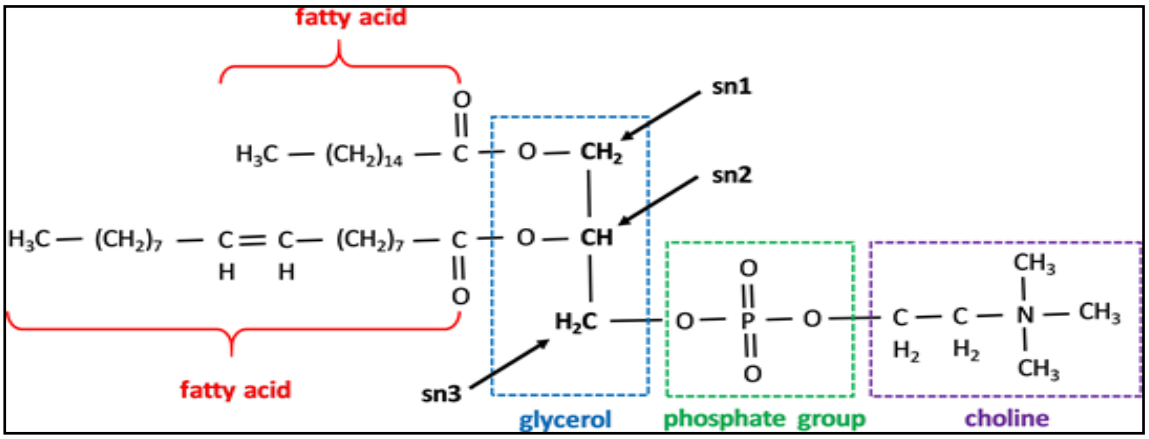


***Figure (5): Phosphatidylcholine structure with stereospecific numbering (sn)*** [19]

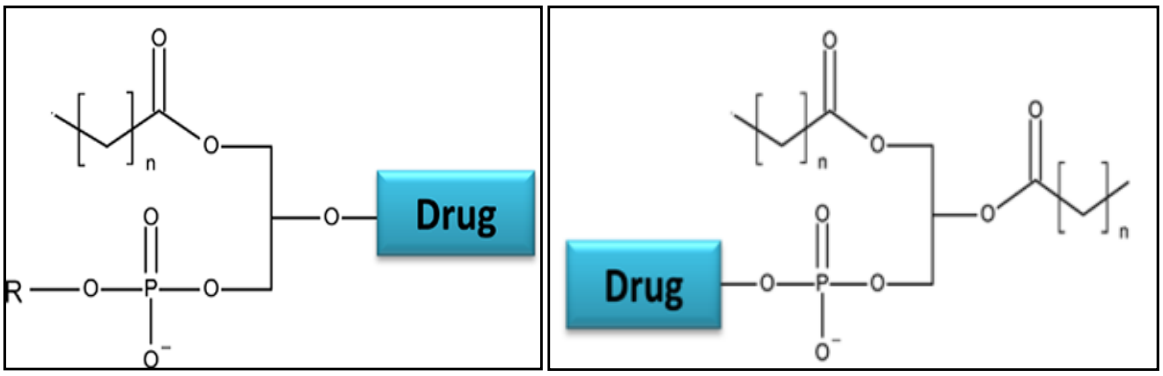


***Figure (6): Phospholipids-drug conjugate*** [7]

**Table (1): List of recent covalently or non-covalently bounded drugs to lipids with their advantages**

| Conjugated drug | Lipid used | Advantages |
|---|---|---|
| 5-aminolevulinic acid | Squalene (fatty acid) | Increase tumor targeting [20] |
| Doxorubicin | Squalene (fatty acid) | Overcome cancer resistance [21] |
| Ibrutinib | Stearic acid (fatty acid) | Better antitumor activity [22] |
| Nicotine | Stearic acid (fatty acid) | Increase drug loading in delivery nanoparticles [23] |
| Combretastatin A4 | Linoleic acid (fatty acid) | Better anticancer activity [24] |
| Amphotericin B | Oleic acid (fatty acid) | Reduce drug toxicity [25] |
| Ciprofloxacin | Oleic acid (fatty acid) | Better apoptosis-inducing effects [26] |
| siRNA | Palmitic acid (fatty acid) | Enhance targeting and potency [27] |
| Camptothecin | Palmitic acid (fatty acid) | High anticancer activity [28] |
| Docetaxel | DHA (fatty acid) | improved inhibition efficacy of lung cancer metastasis [29] |
| Doxorubicin | DHA (fatty acid) | Higher cytotoxic selectivity [30] |
| Dopamine | Eicosapentaenoic acid (fatty acid) | Immune-modulating properties [31] |
| Brevinin 2R | Lauric acid (fatty acid) | Better anitleishmanial activity [32] |
| Artemisinin | Cholesterol (steroid) | Enhanced activities [33] |
| RNAi | Cholesterol (steroid) | Aid in drug delivery [34] |
| Testosterone | Glycerides | Enhance bioavailability [35] |
| Methotrexate | Phospholipids | Colonic targeting [36] |
| Dabigatran | Phospholipids | Higher bioavailability [37] |
| Atorvastatin | Phospholipids | Higher bioavailability [38] |
| Valproic acid | Phospholipids | Higher bioavailability (39) |
| Diclofenac | Phospholipids | Improved solubility and reduced GI toxicity [40] |
| Curcumin | Phospholipids | Better hepatoprotective activity [41] |
| Diminazinediaceturate | Oleic acid | High drug loading [42] |
| Tamoxifen | Phospholipids | Higher bioavailability [43] |
| Erlotinib | Phospholipids | Higher cytotoxicity [44] |
| Clarithromycin | Phospholipids | Good stability [45] |

**Conjugation strategies:**

The conjugation of lipid with drug can take place through different methods via covalent or non-covalent interactions between the drug and lipid functional groups.

**Lipid-drug conjugates through covalent bonds without spacer:**

Drug and lipid functional groups can be conjugated directly through one of the following:

**Ester bonds:**

Ester bonds are commonly used for lipid-drug conjugate formation. This bond formation can occur by the reaction between the lipid carboxylic acid group with the drug hydroxyl group as displayed in figure (7). Drugs that have carboxylic groups may form ester bond with hydroxyl groups of lipids [46].

Ester bond hydrolysis to free the active drugs occurs through the help of enzymes (for example esterase). Paclitaxel (as an example) had been linked with lipids through an ester bond [47].

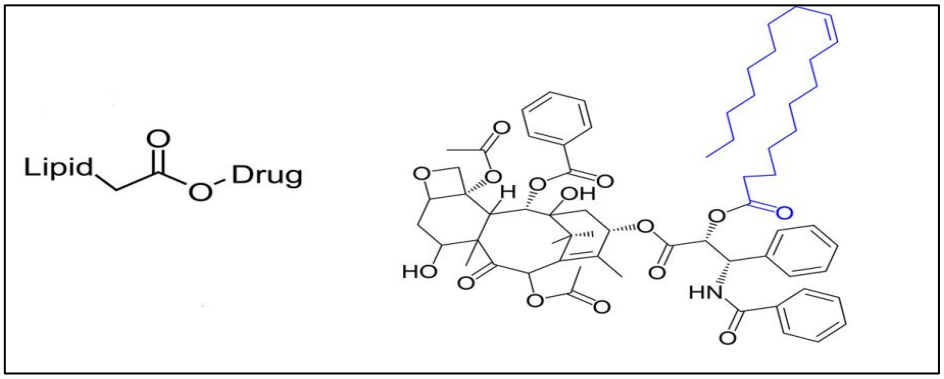


**Figure (7): Conjugation of paclitaxel with oleic acid via an ester bond [7]**

**Amide bonds:**

Amide bonds can be used for conjugation of drugs with lipids. This amide conjugation is made by the chemical reaction (carbodiimide coupling) between an amine group of a drug and a lipid carboxylic end (figure 8). After amide bond cleavage by enzymes in the body, the inactive prodrug will be converted to the active drug. Doxorubicin is an example of a drug bonded to lipids through an amide bond [48].

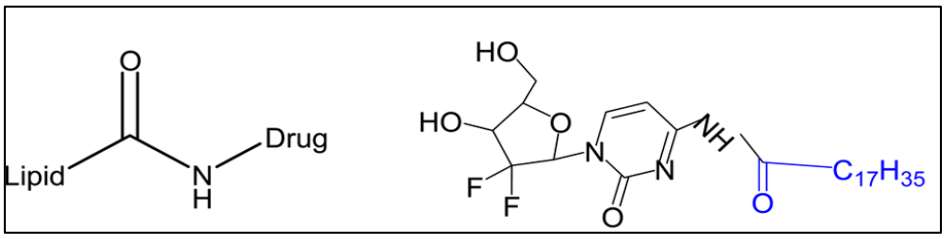


**Figure (8): Conjugation of gemcitabine with stearic acid via an amide bond [7]**

**Hydrazone bonds:**

Lipid-drug conjugates (LDC) formed by hydrazone bonds have a pH-sensitive characteristic. These bonds show little or no decomposition at neutral pH, while it decomposes efficiently at a lower pH. pH-sensitive lipid conjugated with doxorubicin was synthesized using a hydrazone bond (figure 9) [49].

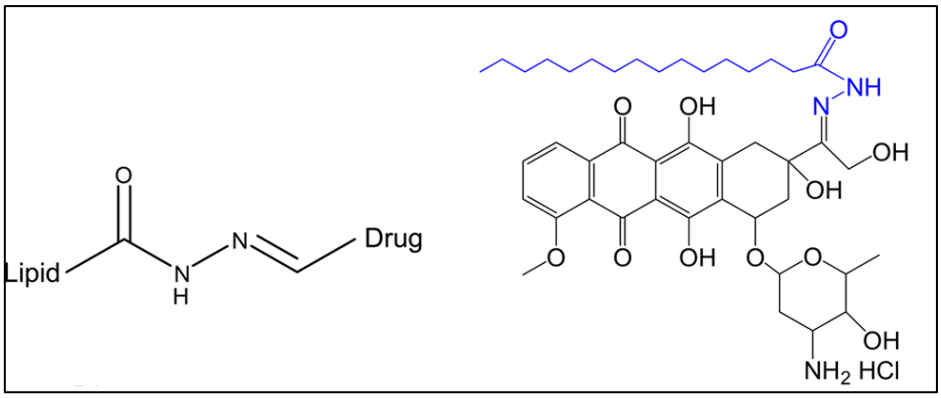


**Figure (9): Conjugation of doxorubicin with palmitic acid via a hydrazone bond (7)**

**Disulfide bonds:**

Lipid conjugates that have disulfide bonds will acquire a unique property wherein the extracellular oxidative environments they remain stable but after cellular uptake where there is reductive intracellular environment, cleavage will occur. A lipophilic derivative of mitomycin C was prepared utilizing this property (figure 10)

that is used for drug-resistant human ovarian carcinoma treatment [50].

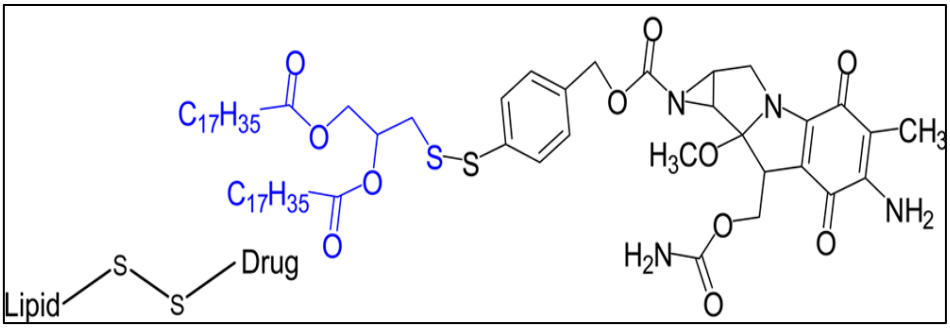


**Figure (10): Conjugation of mitomycin C with lipid through a disulfide bond [7]**

**Lipid drug conjugates with spacer:**

When the drug and the lipid molecules don't have functional groups that can form a bond between them, a spacer molecule can be used to help in conjugation. The spacer moiety (which has alcohol or amine functional group) will first react with the drug, the complex (drug-spacer) is then reacted with activated fatty acid or other lipid moiety to produce a lipid-drug conjugates (Figure 10) [51]. For example, succinic acid (linker) used to ease the conjugation of doxorubicin with different fatty amines [46].

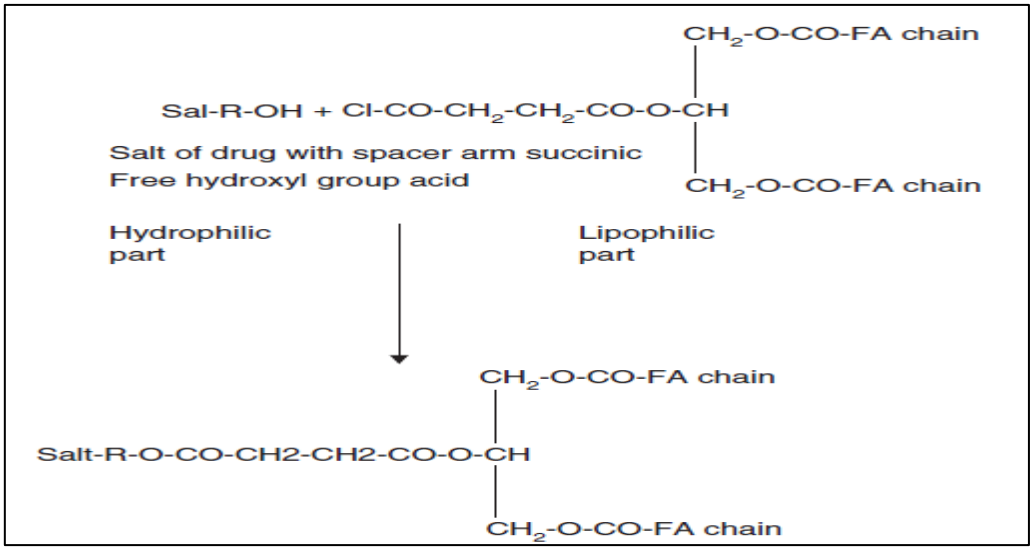


**Figure (10): The formation of LDC via a spacer molecule [52]**

**Lipid-drug conjugates with non-covalent bonds:**

Drug and lipid molecules of oppositely charged groups can develop non-covalent bonds such as, van der Waals and hydrophobic interactions or by forming hydrogen bonds as shown in Figure 11. Phosphatidylcholine had been majorly employed by the researchers to form drug complexes by non-covelent bonds due to its morphology and its ability to form such complexes [53]. Melting and dissolution methods are the most common methods used for the preparation of LDCs with non-covalent bonds. Rifampicin–phospholipid complex is an example of this group in which hydrogen bond formed between rifampicin and phospholipid molecule without formation of new compound [54]. Rosuvastatin is another drug that have been used to form complex with phospholipids through hydrogen bonds [55]. Noncovalent bonds are generally weak, require less energy to break than covalent

bonds and have a transient existence at physiological temperatures (25–37 °C) [56].

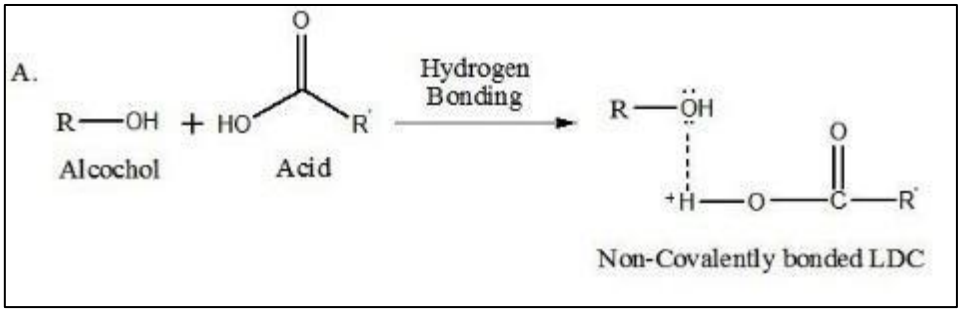


**Figure (11): The formation of lipid-drug complex via non-covalent bond [57]**

**Benefits of lipid-drug conjugates:**

Conjugation with lipids offers variety of desirable characteristics, some of these benefits are:

**Enhancing the bioavailability of oral drugs:**

Conjugation with lipid will enhance the lipophilic nature of the molecule which makes it a good candidate for lymphatic uptake and lymphatic targeting will avoid the first-pass metabolism and hence improve bioavailability. Enhancement in lymphatic drug transport exhibited by mycophenolic acid triglyceride conjugate which demonstrate a great potential for lymphatic targeting [58]. In another study, aspirin bioavailability was reported to be enhanced after complexation with phospholipids. The solubility of aspirin was enhanced after conjugation with phospholipids and accordingly improve the bioavailability [59].

**Targeted drug delivery:**

The aim of drug targeting is to increase the efficiency and specificity of the drug in the relevant tissues and cells after administration, thus increasing treatment efficacy and aid in drug side effects reduction [60]. Specific lipids such as docosahexaenoic acid (DHA) and cholesterol can be used for drug conjugation to enhance tumor targeting since these lipids are aggressively taken up by the tumor cells as a source of energy and to supply biochemical precursors. For instance, DHA conjugation with Docetaxel revealed a superior inhibition efficacy of lung cancer metastasis to bone in the treatment of lung cancer and patients with bone metastasis of lung cancer than the free drug [29].

**Central nervous system (CNS) targeting:**

Drugs conjugated with phospholipids by replacing sn-2 position fatty acid will resemble endogenous PLs [61]. Valproic acid was conjugated to PLs by this approach to enhance the penetration to the CNS as intact conjugate then release the active drug (valproic acid) by the action of phospholipase A2 [62]. Drugs such as GABA have been linked with glycerides to improve CNS drug targeting, the conjugated GABA exhibited a 127-fold increase in the brain penetration index compared to free GABA [57].

**Improve the stability:**

Metabolic instability of some drugs can be prevented by conjugation with lipids. The chemotherapeutic agent phyllotoxin has been conjugated with unsaturated fatty acids and showed improvement in the stability of the drug and better efficacy [63].

**Enhanced drug loading into delivery carriers:**

Drug loading of hydrophilic drugs on delivery carriers are usually low due to drug leakage. When hydrophilic drugs conjugated with lipids it will increase the lipophilicity of parent drug and enhance the drug loading significantly. Also, the conjugation can reduce drug leakage by

enhancing the affinity between drug and lipidic components of carrier. For example, drug loading of paclitaxel increased from 10 to 47% inside nanoparticles after conjugation with behenic acid. Similar results were observed in case of 4-(N)-stearoyl gemcitabine conjugate [64, 65].

**Achieve extended drug release:**

Some lipid-drug conjugates have low water solubility which help in providing extended drug release on administration. Careful selection of lipids is important for controlling the properties of the prepared conjugate such as water solubility and partition coefficient which helps altering the drug release profile. Palmitic acid conjugate of paliperidione (antipsychotic drug) is an FDA-approved drug formulation [66].

## Conclusion:

Drug conjugation with lipids were utilized successfully to enhance the delivery of different drugs. Conjugation with lipids whether covalently or non-covalently will change the properties of the drug. It will significantly increase the lipophilicity. Lipid-drug conjugation could assist the drug delivery by the lymphatic system, enhance oral bioavailability, improve targeting of tumors, increase drug stability, enhanced the loading of drugs into some delivery carriers and many others. Detailed understanding of lipid properties such as digestion and absorption mechanisms with careful selection of lipids and drug are necessary in designing the lipid-drug conjugate to achieve maximum benefits.

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