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In vitro lipid digestion models

Kethavath Bhagya * and M. Sunitha Reddy

Department of Pharmaceutics, Centre for Pharmaceutical Sciences, JNTUH UCEST, JNTUH Hyderabad, Telangana-500085, India.

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Abstract

The models are evaluated in terms of their suitability to assess lipid based drug delivery systems, and their ability to produce *In vitro - in vivo* correlations (*IVIVCs*). While the pH-stat lipolysis model is by far the most commonly utilized *In vitro digestion* model in relation to characterizing Lipid based drug delivery Presently, no single *In vitro digestion* model exists which is able to predict the *in vivo* performance of various methods. However, a recent study has shown the potential of combined lipid digestion-permeation models as well as specific digestion models. Lipolysis test is to assess the drug solubility in conditions that mimic the fasted intestinal tract in term of volume, pH, enzymes, and temperature, bile acids. Human GI digestion includes two main processes occurring simultaneously, i.e. mechanical and enzymatic digestion. The digestion processes begin in the mouth and continue through the GI tract to the large intestine. In the mouth, ingested food is masticated, mechanically broken down and mixed with saliva. This review mainly useful for lipid based in-vitro digestion and lipolysis models for lipid-based drug delivery systems.

Keywords: *In vitro digestion;* Lipolysis; *IVIVC;* Bioavailability; Solubility; Enzymes; Lipids

1. Introduction

The understanding and controlling the digestibility of lipids within the human gastrointestinal (GI) tract[1-3] The pharmaceutical industry is using this knowledge to design lipid-based delivery systems that either increase the bioavailability of highly lipophilic drugs The food industry is using a similar approach to design food-grade delivery systems to encapsulate, protect, and release bioactive lipid components, with the aim of either enhancing their bioavailability or controlling their delivery of drugs.[4-9]There are a number of bioactive lipid components that may benefit from encapsulation within this type of delivery system, including Ω-3 fatty acids, conjugated linoleic acid, butyrate, phytosterols, carotenoids, antioxidants, coenzyme Q, and vitamins A and D.[10–14] The availability of effective delivery systems for lipophilic bioactive components could lead to the creation of functional foods specifically designed to maintain human health. Functional foods could increase the digestibility of lipids in individuals with their health conditions that impair the normal digestive process.[15] Functional foods control the human satiety, satiation, and hunger by controlling the rate and extent of lipid digestion in different regions of the GI tract.[16–20] For example, recent studies show that emulsions that remain stable to gravitational separation in the stomach and which have a delayed digestion in the small intestine can stimulate the release of gut hormones that induce satiety and reduce food intake.[21-22] Functional foods could be designed to deliver bioactive components to specific locations within the GI tract where they can exhibit their functional attributes, e.g., anticancer components could be released in the colon. An understanding of the basic physicochemical and physiological processes that occur as an emulsified lipid passes through the human gastrointestinal (GI) tract is required to develop effective *In vitro* models that accurately simulate lipid digestion. After ingestion, emulsified lipids experience a complex series of physical and chemical changes as they pass through the mouth, stomach, small intestine, and large intestine, which affect their ability to be absorbed.

*Corresponding author: Kethavath Bhagya

2. Composition on the pH-stat method

One of the most important factors affecting the digestion rate determined using the pH-stat method is the composition of the simulated small intestinal fluid (SSIF) used. Previous researchers have used various SSIF compositions when carrying out *In vitro* lipid digestion studies using the pH stat method. In addition, a number of workers have examined the influence of specific SSIF components on the rate and extent of lipid digestion. Ideally, the conditions used should closely simulate those found in the human GI tract. [23- 24]

2.1. Lipase and other enzymes

Pancreatic lipase is the key component in any *In vitro* model designed to simulate *lipid digestion* within the small intestine. Consequently, it is important to use an appropriate type and concentration of pancreatic lipase in the pH-stat method. Previous researchers have used various types and forms of lipase in SSIF, including *Pancreatin,* pancreatic lipases, and non-pancreatic lipases. Pancreatin is a complex mixture of digestive enzymes (*lipase, protease, amylase,* etc.) and other biological components produced by the exocrine cells of the pancreas, which is typically obtained from animal sources, such as pigs or cows. The chemical composition and functional performance of *Pancreatin* (and consequently its enzyme activity) varies depending on its biological origin, isolation, and purification procedures, and hence there are often considerable batch-to-batch and supplier-to-supplier variations. [25]

2.2. Calcium and other minerals

A number of *In vitro digestion* studies have highlighted the important role that calcium plays in determining the rate and extent of *lipid digestion* using the *pH-stat* method. [26-28] Calcium ions may impact the lipid digestion process due to a number of different physicochemical mechanisms.

- **Enzyme activity:** Calcium is believed to be a necessary cofactor for the proper functioning of *pancreatic lipase* [29-30]
- **Removal of FFA from droplet surfaces:** *Lipase digestion* of emulsified lipids can be inhibited by accumulation of long-chain fatty acids (LCFA) at the droplet surfaces, since this restricts the access of the lipase to the triacylglycerol. Calcium is known to precipitate these LCFA, thereby removing them from the lipid droplet surface and allowing the lipase to access the emulsified lipids. Consequently, calcium ions are able to increase the rate and extent of *lipolysis* by this mechanism. Reduced digestibility of flocculated droplets. Calcium ions are highly effective at causing droplet flocculation in emulsions containing lipid droplets coated with anionic emulsifiers. It is more difficult for the lipase molecules to reach the surfaces of the lipid droplets trapped in the centre of a floc, which may slowdown the lipid digestion rate in flocculated emulsions.
- **Reduced digestibility due to gel formation:** Calcium ions may promote the gelation of certain types of biopolymers (such as alginate and pectin), which can lead to the formation of hydrogel matrices that trap lipid droplets and inhibit the diffusion of lipase to the droplet surfaces.
- **Reduced bioavailability of precipitated FFA:** Studies have shown that insoluble soaps are formed between calcium ions and LCFA, which can reduce the *bioavailability* of these fatty acid digestion products.

2.3. Bile

Bile is another key component in any SSIF used simulates *lipid digestion* in the small intestine. typically, researchers use either bile extract or one or more individual bile acids in their simulated small intestinal fluids. Bile extract is a complex mixture of various kinds of molecules typically found in the GI tract (such as *bile acids, phospholipids, and minerals*),and may therefore more accurately reflect *in vivo* conditions than individual bile acids, however it tends to be more variable and inconsistent in composition and performance. In addition, bile extract often contains insoluble matter, which can interfere with the analysis of lipid droplets and other colloidal structures in digestion media, and therefore it should be filtered before use.

2.4. pH

The pH of the small intestine depends on a number of factors and typically varies from one location to another. In the stomach, the droplets are surrounded by a highly acidic environment (pH 1 to 3), but when they enter the duodenum the pH is increased to around neutral (pH 5.8–6.5) due to secretion of sodium bicarbonate. Nevertheless, studies with human subjects have shown that there may be large variations in duodenum pH depending on the individual involved, and the type and amount of food consumed. In particular, the pH of the stomach contents may increase appreciably after *ingestion* of a food, before gradually returning to the fasting pH level. As the food passes along the small intestine the pH

tends to increase to around pH 7 to 7.5. The pH used in an In vitro digestion model may influence the results for a number of different reasons:

- **Enzyme activity:** Pancreatic lipase has an optimum pH where it exhibits its maximum rate of lipid digestion. It has been reported that pancreatic lipase has a maximum activity around pH but that it also has good activity at pH values around neutral.[31]
- **FFA ionization:** The ionization of free fatty acids depends on solution pH relative to their pKa value. In aqueous solution, the ''true'' pKa of FFAs has been reported to be around 4.7 to 4.9 depending on their chain length, and so they should be predominantly in their ionized anionic form around neutral pH.

2.5. Ionic composition

There may be considerable variations in the type and concentration of ions surrounding the lipids droplets, which may impact the electrostatic interactions in the system through electrostatic screening or binding effects. For example, long chain fatty acids may precipitate in the presence of calcium ions, thereby removing them from the lipid droplet surface (which facilitates further digestion), but which may also reduce their subsequent absorption due to calcium soap formation. Sufficiently high concentrations of monovalent and multivalent counter-ions can promote extensive flocculation in emulsions containing electrically charged droplets, which may restrict the access of lipase to the oil–water interface and slowdown digestion. Certain types of mineral ions are capable of promoting the gelation of biopolymers within the GI tract, which would affect the ability of digestive enzymes to reach any entrapped lipid droplets. For example, alginate or pectin form Strong gels if there are sufficiently high levels of free calcium ions present in solution. [32]

2.6. Enzyme activity

There are various kinds of enzymes in the mouth, stomach, small intestine and colon that can digest food components, such as *lipids (lipases), proteins (proteases), starch (amylases) and dietary fibers (glycosidase)*. The ability of these enzymes to interact with their specific substrates within a food may impact lipid digestibility and the absorption of encapsulated lipophilic components. Enzyme accessibility to a substrate may be influenced by physical barriers between the encapsulated substrate and the surrounding aqueous phase where the digestive enzymes are normally located. Lipid digestion may not be initiated until the lipid droplets are released from their original emulsifier coatings or from any matrices that they are encapsulated in. The rate of lipid digestion may be decreased by coating lipid droplets with a dietary fiber layer or by embedding them within dietary fiber particles. Similarly, it may be necessary for protein or starch coatings or particles to be digested by *proteases or amylases* before the lipase can act on the lipids. Enzyme activity may also be influenced by any food components that can bind to them, either specifically or non-specifically. For example, some foods contain natural enzyme inhibitors, such as peptides from soybeans that inhibit proteases and polyphenols from fruits and vegetables that inhibit lipases. [33]

2.7. Surface active components

There are a variety of endogenous (*e.g. proteins, peptides, phospholipids, and bile salts*), exogenous (*e.g., surfactants, proteins*), and internally generated (*e.g., lipid and protein digestion products*) surface active substances present within the aqueous phase surrounding the lipid droplets. [34] These substances compete with the surface active substances already present at the oil–water interface, potentially leading to changes in interfacial composition and properties.

2.8. Flow profiles and mechanical forces

The encapsulated lipids may be exposed to various kinds of forces and flow profiles during their passage through the human body. These processes mix the various components together, breakdown structures (*lipid droplets, protein particles, hydro-gel matrices etc.*), and transport materials from one location to another. It is therefore important to simulate or model these flow profiles in *In vitro digestion models*.

2.9. Other factors

Various other factors should also be considered when designing an appropriate simulated small intestinal fluid for *pH-stat* studies. Lipids are normally consumed by humans in a diet that contains a range of other components, including dietary fibers, proteins, carbohydrates, minerals. [35] Many of these components may interfere with the digestion process, either decreasing or increasing the rate and extent of digestion. For example, dietary fibers may interact with the lipid digestion process through a variety of physicochemical mechanisms depending on their molecular characteristics.

3. *In vitro* lipid tests

A number of *In vitro* approaches are commonly used to study the lipid digestion. Some of these approaches focus on one particular region of the gastrointestinal tract; whereas others utilize a number of sequential steps to more accurately mimic the entire digestion process. *In vitro* digestion models can therefore be conveniently characterized as the following:

3.1. Single Step Models

One particular region of the GI tract is simulated, e.g., the mouth, stomach, small intestine. The *pH-stat* method is an example of this type of model, which only simulates digestion in the small intestine.

3.2. Multiple Step Models

Two or more regions of the GI tract are simulated, e.g., mouth, stomach, small intestine, and colon. Multiple step *In vitro* digestion models designed to simulate the entire human GI tract.

4. *In vitro* digestion models

Digestion models are attractive and simple, *In vitro* release is not suitable to predict the *in vivo* performance of Lipid based formulations because of the inconsistency in achieving *In vitro in vivo correlation (IVIVC)*. The primary drawback of the test is the lack of mimicking the complex *in vivo* digestion of lipid based formulations and micelle solubilization. Accordingly, *in vitro* lipolysis is more suitable for assessing the fate of LBFs by mimicking the intestinal lipid digestion process shown in Fig-1. To obtain a strong *IVIVC*, it is very difficult to simulate the physiological conditions that present in the human *GI tract*, such as *pH, enzymes, transit times, and mixing*. However, none of the currently available models can simulate all of these complex multistage processes owing to technical challenges. [36]

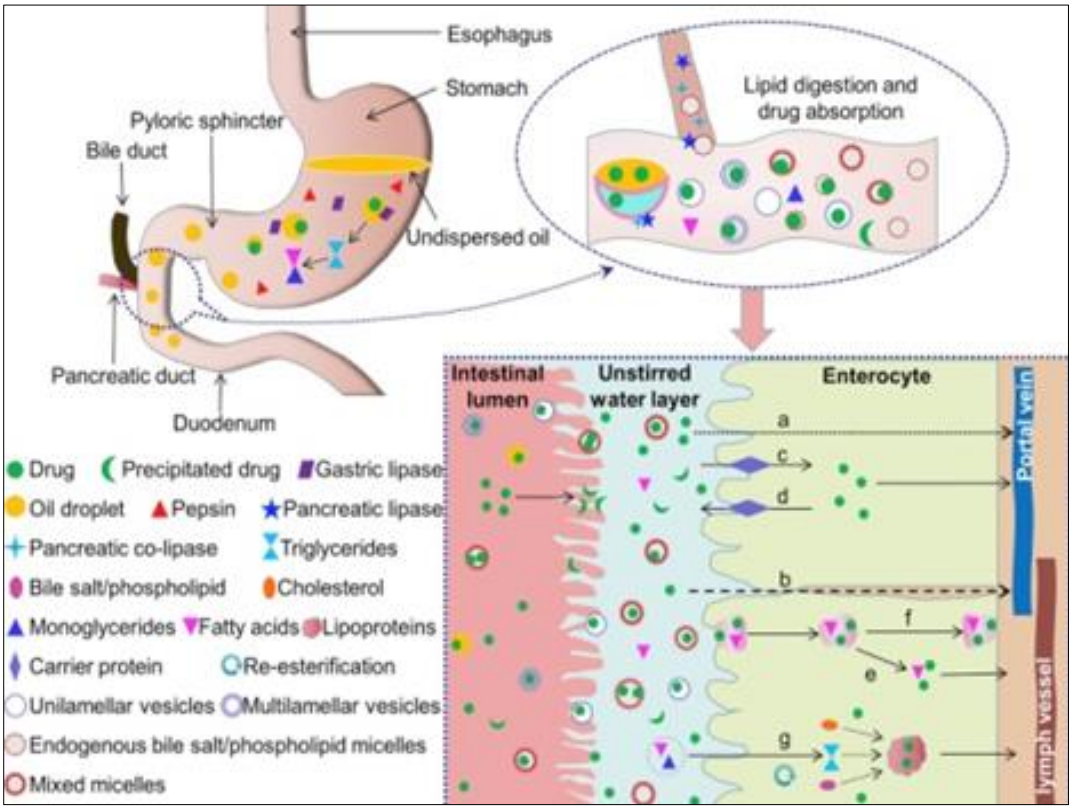

Figure 1 Illustration of gastrointestinal lipid digestion

4.1. pH- stat method

The *pH-stat* method is an analytical tool widely used in pharmaceutical and food research for the *In vitro* characterization of lipid digestion under simulated small intestinal conditions. [37] It is based on measurements of the amount of free fatty acids released from lipids, usually triacylglycerol, after lipase addition at *pH* values close to neutral. The sample to be analyzed is placed within a temperature-controlled reaction chamber that contains simulated small intestinal fluid (SSIF)The SSIF should contain appropriate levels of the major digestive components are known to cause lipid digestion, such as lipase, co-lipase, bile salts, phospholipids, and mineral ions.. The concentration of alkali(NaOH) added to the digestion cell to neutralize the FFAs produced by lipid digestion, and thereby maintain the pH at the initial pre-set value (e.g., pH 7.0), is recorded versus time The *pH-stat* method is relatively simple and rapid to carry out and enables comparison of different systems under similar experimental conditions. This technique can therefore be used to rapidly screen the impact of different physicochemical factors expected to affect lipid digestion. Recently, a simple mathematical model was developed to describe the FFA versus time profiles obtained by the *pH stat method*. The percentage of total free fatty acids released (F) as a function of time (t) measured by the *pH-stat method* is characterized by the following equation:

$$\emptyset=\emptyset_{max}\,(1-(1+3kMt\div 2d_0\rho_0)-^2)$$

Here, ∅ max provides a measure of the total extent of digestion (i.e., the maximum percentage of the total FFA present that is released at the end of the reaction), k provides a measure of the rate of digestion (i.e., mmols of FFA released per unit droplet surface area per unit time), d_0 is the initial droplet diameter, ρ_0 is an oil droplet density, M is the molar mass of the oil. A *pH stat* profile can then be characterized in terms of just two parameters: ∅max and k, which can be determined by finding the values which give the best fit between the experimental data and the mathematical model.

4.2. One-compartment intestinal digestion model

The experimental procedure mainly contains the thermo stated vessel (generally, at 37 °C), and stirrer, a pH electrode, and a titrator. LBFs are dispersed in a medium mimicking fasted- or fed-state intestinal digestive fluid. Initiation of lipid digestion by addition of lipase and co-lipase leads to the liberation of fatty acids, causing a drop in the pH consequently. The pH variation is measured by the electrode, while the released fatty acids are automatically titrated with sodium hydroxide using the titrator. The extent of the digestion can be indirectly quantified using the rate of the addition of sodium hydroxide based on its stoichiometric reaction with fatty acids. Samples can be taken during the digestion process and ultra-centrifuged to obtain three distinct phases, namely, an oil phase containing undigested lipids, a micelle phase containing a solubilized drug in colloidal structures, and a pellet phase comprising the precipitated drug. Quantification of the drug amounts in each phase enables prediction of the solubilizing capability of the formulation to co-formulated drugs in the GI tract shown in fig-2. Furthermore, the solubilized amount of the drug in the micelle phase can be correlated with the *in vivo* PK parameters. [38]

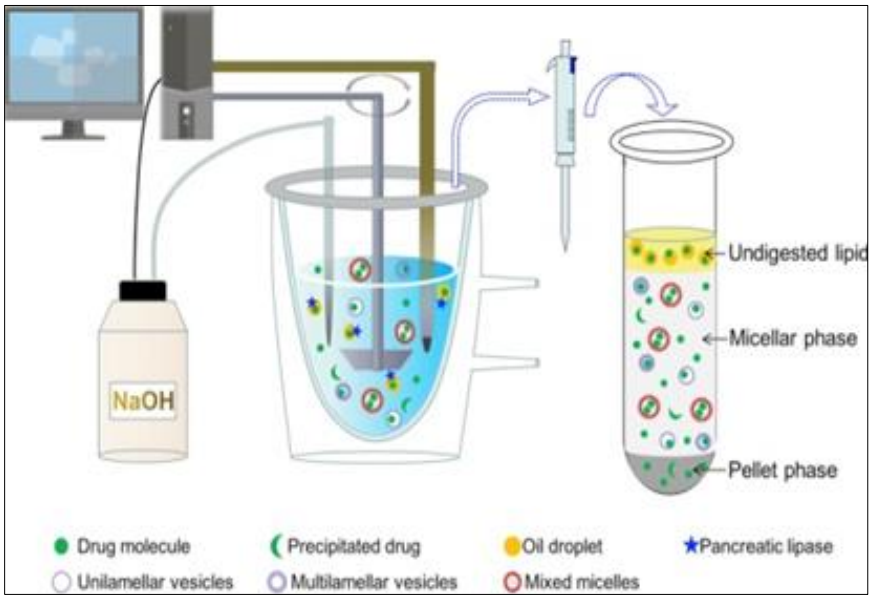

Figure 2 Schematic representation of the one-compartment pH-stat lipolysis model

4.3. GI digestion model

The one-compartment intestinal digestion model is simple and has been widely adopted in the evaluation of LBFs. The rationale of the model is that the intestine is the main site for lipid digestion and drug absorption. However, the model is inadequate for simulating *GI* physiology because it does not consider processes and conditions in the stomach. As mentioned above, lipid digestion in stomach contributes to ~15% of the overall lipid digestion in the *GI tract.* In addition, the effects of gastric emptying and sudden pH changes on the solubilization of co-formulated drugs are ignored. Therefore, GI digestion *pH-stat* models, either two-step one compartment or two-step two compartments, were developed to simulate both gastric and intestinal digestion. In the one-compartment model, the simulated gastric and intestinal digestion is performed in two sequential steps, respectively. LBFs are first dispersed in SGF, and gastric digestion is initiated by adding gastric lipases. After a period of time, the SGF was transferred to a medium similar to the intestinal fluid by addition of a concentrated SIF and pancreatic lipases. During both steps, automatic titration with sodium hydroxide maintains a constant pH, corresponding to the gastric and intestinal pH, respectively. Two individual setups of the *pH-stat model* are used in the two-compartment model to simulate the stomach and small intestine, respectively.[40] SGF and SIF, as well as the corresponding lipases, are respectively added to the two reaction vessels, which are connected by a peristaltic pump shown in Fig-3. During the digestion process, the medium in the gastric compartment is continuously pumped to the intestinal one at a rate mimicking gastric emptying. In this regard, the two-compartment model more closely mimics the *in vivo* conditions than does the one-compartment model.

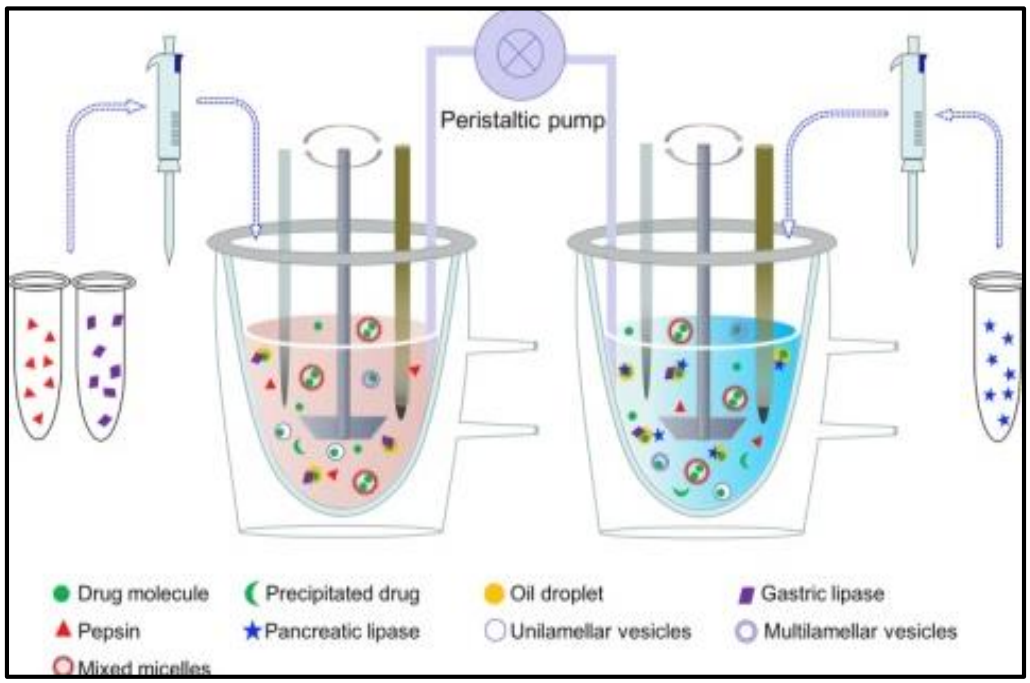

Figure 3 Simulation of the digestion process in the stomach and small intestine by a two –compartment digestion model

4.4. Combined models and IVIVC

In vitro lipolysis studies may fail to accurately predict the oral bioavailability of LBFs because the model does not fully represent *in vivo* conditions. As a closed system, this model lacks the absorption sink that is present *in vivo* and may therefore overestimate the precipitation potential. The intra luminal solvation capacity may be damaged because of the altered composition of GI fluids in the process of intestinal digestion, leading to super saturation and consequent drug precipitation. Meanwhile, *in vivo* absorption may lead to a rapid and sufficient drop in the luminal drug concentration to avoid precipitation. The absorption sinks effect works even when the initial super saturation is high, provided that the absorption is fast. In addition to the absorption issue, absorbed drugs may undergo first-pass metabolism. In this case, the *In vitro* lipolysis model may overestimate the solubilization potential. Therefore, combined lipolysis–permeation and digestion–microsomal metabolism models were developed, respectively, to obtain a better *IVIVC.*[41]

4.5. In vitro lipolysis–permeation models

In addition to the solubilization, super saturation, and precipitation of co-formulated drugs during digestion of LBFs, permeation of model drugs is included in the lipolysis–permeation models. The original setup of the model consisted of two separate single compartments. The lipolysis and permeation were performed in a consecutive way. Dispersion and digestion of LBFs were performed in a single compartment, utilizing the regular *pH-stat* lipolysis model. At predetermined intervals, samples were withdrawn and transferred to another compartment for the permeation study. A normal setup of the Trans well system (top to bottom) or using chambers (side by side) can be adopted in this step. However, the absorptive membrane should resemble the intestinal epithelia and withstand the harsh lipolysis conditions, including pancreatic enzymes, diverse surfactants, excipients of LBFs, and digestion. Permeability through the Caco-2 cell (a human colon carcinoma cell line) monolayer represents the gold standard for the evaluation of oral drug absorption. Differentiated Caco-2 cells resemble the epithelium of human intestine, which enables the assessment of drug transport mediated via different pathways, e.g., passive versus active transport and paracellular versus trans-cellular routes. Due to the intolerability of Caco-2 cells to the pancreatic enzymes, immobilized lipase was used in the digestion step and was shown to successfully digest LBFs and be tolerated by cell monolayer. An artificial membrane (*PermeaPad*®) and intestinal rat tissue are used as alternative membranes for Caco-2 cell monolayer [42]. However, the model fails to establish the *IVIVC* for LBFs because of the lack of concurrence of the digestion and permeation.

5. Applications

- LBDDS can be used to deliver various types of drugs from new chemical entities to more recent new developments for proteins and peptides, nucleic acids (DNA, saran), and cellular site-specific delivery.
- The utility of lipid-based formulations to enhance the absorption of poorly water-soluble, lipophilic drugs has been recognized for many years. Lipids are perhaps one of the
- Most versatile excipients classes currently available, providing the formulator with many potential options for improving and controlling the absorption of poorly water-soluble drugs.
- Lipid-based formulations, which are by no means a recent technological innovation, have not only proven their utility for mitigating the poor and variable gastrointestinal absorption of poorly soluble, lipophilic drugs, but also, in many cases, have shown the ability to reduce or eliminate the influence of food on the absorption of these drugs.

6. Conclusion

A variety of *in vitro* models have been developed to understand and predict the *in vivo* performance of LBFs. However, none of the present models are able to mimic fully the overall processes of LBFs occurring *in vivo*, leading to frequent failure in obtaining level A IVIVCs. Great efforts have been made to improve the predictive power of *in vitro* models by closely simulating the gastrointestinal physiology. This article provides an overview of the major physicochemical events that occur during lipid digestion, and reviews a number of in vitro testing methods that have been developed to monitor lipid digestion. In particular, we focused on the pH stat method for simulating lipid digestion in the small intestine, since this method is useful as a rapid screening tool for studying the influence of product composition and structure on lipid digestibility

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors declare no conflict of interest.

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