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The trip of a drug inside the body: From a lipid-based nanocarrier to a target cell

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ABSTRACT

To date, enormous investigations have been conducted to enhance medicines' target-oriented delivery to improve their therapeutic index. In this regard, lipid-based carrier system might have been regarded as prime delivery systems that are very close to the naturally cell-derived vesicles used for biomolecular communication among cells from occasionally remote tissues. Upon examination of the literature, we found a chasm between groups of investigations in drug pharmaceutics and thought that maybe holistic research could provide better information with respect to drug delivery inside the body, especially when they are going to be injected directly into the bloodstream for systemic distribution. While a collection of older research in most cases dealt with the determination of drug partition coefficient between the aqueous and cell membrane compartments, the link has been overlooked in newer investigations that were mostly focused on drug formulation optimization and their association with particle biodistribution. This gap in the literature motivated us to present the current opinion paper, in which drug physicochemical properties like drug lipophilicity/hydrophilicity is considered as an important element in designing drug-carrying liposomes or micelles. How a hypothetical high throughput cell-embedded chromatographic technique might help to investigate a nanocarrier tissue distribution and to design 'multi-epitope grafted lipid-based drug carrier systems' are discussed. Whenever we would need support for our opinions, we have provided analogy from hydrophobic biomolecules like cholesterol, steroid hormones, and sex hormones and encouraged readers to consider our principle hypothesis: If these molecules could reach their targets far away from the site of production, then a large list of hydrophobic drugs could be delivered to their targets using the same principles.

1. Introduction

To date, there has been intensive research on lipid-based drug delivery systems to improve various desired responses and tissue targeting; many of them effective and successful with in vitro settings, but few have found their way into clinical testing [1–4]. The chasm between the bench outcomes and bedside results lies in the fact that in vitro experiments would have been really too simple to give a true picture of the conditions influencing drug distribution in the body [4,5]. This is the main justification that many researchers have pinpointed [5–7]. However, how we could bridge the gap by using more sophisticated experimental setting is the matter that most researchers have attempted to explain in their own way [5,7]. Once a drug is introduced into a physiological system, it would be affected by various mechanical, physicochemical and biological factors that determine the drug's distribution [5]. Encapsulating the drug into lipid-based assemblies like liposomes has shown to thoroughly change the drug distribution, as the lipid membrane of the lipid-based nanoparticles would become the direct barrier interacting with the biological system [4,6]. To this end, how much a physiological medium interacts with the lipid membranes of the lipid-based drug delivery systems has been frequently investigated in the literature [4,6,8,9]. These include the examination of physicochemical stability and release rate of drug from the nanoparticle in media like phosphate-buffered saline (PBS), PBS plus a few serum proteins, cell culture media, cell culture media plus a percentage of serum, the whole serum, and 3-D cell culture when the drug

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is supposed to be injected to the blood circulation. On the other hand, how the molecule's inherent relative water solubility would affect its partitioning into various tissues is another concept found mostly in other papers [10–13]. How these concepts usher the distinguishing profiles of the nanoparticulated drug as compared to the drug alone would have remained to be explored.

When a drug is encapsulated or entrapped in a large nanoparticle, it remains in the blood circulation following intravenous injection for a considerably extended period of time as opposed to the free drug. The reason lies in the fact that the microvascular system in many tissues, including in the glomerulus of kidneys, are impermeable to the large nanoparticles. The studies have shown that the glomerular filtration occurs for the macromolecules with the hydrodynamic diameter below 6 nm. This means that many artificial nanocarriers, including lipid-based carriers, have remained in the circulation until they get into tissues with fenestrated blood capillaries (i.e. hepatic and splenic tissues, inflamed tissues, and cancer tumors). So the first alteration that affects the drug distribution is the inherent physical barrier against the large nanoparticle.

Furthermore, it is well discussed in the literature that once a nano-sized particle, including lipid-based particles (i.e. liposome, micelles, etc.) has been introduced into a physiological fluid, it is then altered by many biomolecular components including proteins [14–16]. The incremental adsorption of proteins and other molecules onto the nanoparticles develops a new layer around the particle known as the “biomolecular corona” or “protein corona”. This confers a new identity to the particle, known as the biological identity of the nanoparticle, which is different from its original synthetic origin. The biological identity of a nanoparticle is dynamic. With time, some proteins in the biomolecular corona are replaced with other proteins developing the biological identity of the nanoparticles as it travels in the body. The mentioned scenario is currently fueling many investigations that are being undertaken and it applied to non-lipid-based nanoparticles (e.g. crystalline nanoparticles, carbon-based nanoparticle, metal-based nanoparticle, etc.) as well. But the basic concept of the drug partitioning into the lipid-based vehicle as opposed to cells has been overlooked, which may elucidate drug dislocation from the vehicle to cells [15–17]. According to the drug partitioning concept, there are three competing factors in determining the biodistribution of drug, when it is injected in the form of a lipid-based nanoparticle: i) the drug tendency to escape from the vehicle to the more energetically favorable position/s in cells and tissues which is closely related to the drug chemical structure ii) the drug tendency to elicit the vehicle lipid membrane-cell membrane coalescence or the vehicle membrane-serum proteins interaction, iii) and the relationship of lipid composition of the vehicle with the rate of the vehicle-cell lipid exchange and drug transition. The more involving players (isotonic media, cells, serum proteins, solid 3D cell culture) in an experimental setting using the physiological media, the more realistic the outcome, while it would become more difficult to explain these factors.

This prompt us to address and develop the concept in the current opinion paper. Some previous investigations will be presented concerning drug partitioning into tissues and liposomal membranes and the potential of these investigations will be discussed in uncovering drug distribution if they come together. In this regard, how encapsulating the drug into a lipid-based nanoparticle, particularly of interest liposomes, will play a key role in the determination of drug destination inside the body will be also discussed. How a drug might be involved in the liposome/protein and liposome/cell membrane interactions and how drug inherent nature could be exploited in the design of drug-loaded lipid-based vehicles will be also discussed.

2. Partition coefficient: a useful broad concept

Partition coefficient (PC) is a useful general concept in diverse fields, defining the relative distribution of a given compound into usually either two different media or matrix [13,18–20]. PC confers useful information in quantitative structure-activity relationship (QSAR) studies, which could be applied in diverse fields including toxicology, pharmaceutics, food and environment sciences and structural biochemistry [21–24]. From the simple determination of relative partitioning of various drugs between n-octanol and water to relative distribution of inhaled anesthetics or hazardous compounds between gas and water compartments and the ratio of drug concentration in tissue to the concentration of the drug in blood, all were attempted to explain the pharmacokinetic profile and pharmacodynamics of a given drug [19,20,25]. It is the most important driving factor for different concentration of drug in various tissues following drug administration via a specific route and for the drug-receptor dynamic interaction, which is translated to the “final effect”.

To begin with, PC is regarded as an important factor in developing physiologically based pharmacokinetic models [20,26]. Primarily, the PC of a drug was being calculated from the in vivo data [19,27,28]. However, it had still been highly interesting to determine what parameters are involved in and how much they carry weight with a drug tissue distribution. The first effective attempt to theoretically determine these parameters might have been made by Lin, Sugiyama, Awazu and Hanano [19], where the researchers incubated the tissue-homogenates and plasma samples of a non-medicated rat in Tris-HCl buffer along with a model drug 4-ethoxy benzamide at 4 °C. They developed their pharmacokinetic model based on the drug association or dissociation constant for each of the tested tissues and checked up the veracity of their models against the models from the in vivo experiments. Later on in [20], Poulin and Krishnan developed a much more sophisticated algorithm to predict tissue: blood partition coefficients (PC_{tb}) of some organic chemicals from the n-octanol: water partition coefficient data (S_o and S_w, which indicates solubility in oil and water, respectively). They used the water content (W_t and W_b, where t and b denotes tissue and blood, respectively), neutral lipid content (N_t and N_b), phospholipid content (P_t and P_b) of the blood and tissues in their equation, where the tissue: blood partition coefficient of a chemical was calculated by dividing their concentration in tissue by the sum of their concentration in erythrocytes and plasma (Eq. 1). In this process, the partitioning into tissue was described as an additive function of the partitioning of a chemical into neutral lipids, phospholipids and water. The solubility of chemicals in tissue or blood neutral lipids and water was approximated by their solubility in n-octanol and water, respectively. The solubility in tissue or blood phospholipids was estimated as a fractional additive function of the solubility in n-octanol and water. Based on the neutral lipid, phospholipid and water fractional content of the tissues and the chemical differential partitioning into n-octanol and water, the authors presented an algorithm capable of predicting the chemical tissue: blood PC. These models are able to predict the biodistribution feature of a drug; however, they are unable to do so for a drug trapped in a lipid-based carrier.

$$PC_{tb} = \frac{[S_o \times N_t] + [(S_w \times 0.7P_t) + (S_o \times 0.3P_t)] + [S_w \times W_t]}{[S_o \times N_b] + [(S_w \times 0.7P_b) + (S_o \times 0.3P_b)] + [S_w \times W_b]} \quad (1)$$

When a drug or a biological molecule (like cholesterol, miRNA, etc.) is formulated into a lipid-based nanoassembly, the above-mentioned algorithm would first seem useless in predicting the blood and tissue distribution of the cargo drug as a set of additional parameters must get involved in the equation. These include the size and surface charge of the carrier, steric interaction between the carrier's lipids and serum proteins or cell surface-protein, the level of the biologically built-up layer of proteins around the carrier, and physicochemical stability of the carrier in a physiological medium. Although these parameters have been investigated pretty well, the part in which the cargo might play in affecting the mentioned parameters has poorly understood. In fact, the same concept of partition coefficient would be helpful in a different way, where the hydrophilicity/lipophilicity of the carried molecule would determine their partitioning in aqueous and lipid membrane

spaces of nanocarriers like micelles, solid-lipid nanoparticles and liposomes [13]. In this regard, the spontaneous diffusion of the cargo from the nanocarrier membrane to cell membranes and the cargo-induced vehicle/cell interaction would also determine their tissue distribution and blood profile. In the following, we will explain this concept first by looking into cholesterol distribution in cell membranes.

Taking cholesterol as an example might help to explain the above-proposed concept with drugs as well. The cholesterol, a major lipid membrane component of various vertebrate cells, has shown to exhibit heterogeneous membrane distribution in cellular membranes due to the differential affinity of cholesterol to various phospholipids [13]. It was shown that cholesterol-enriched domains (or rafts) in the plasma membrane are rich in sphingolipids, which has been reported to have the highest affinity toward cholesterol [29–31]. On the other hand, no cholesterol is present in the intracellular membranes of mitochondria and endoplasmic reticulum (ER) as their membranes composed mainly of lipids with a low affinity toward cholesterol like phosphatidylethanolamines (PE) [31]. In other words, the differential affinity of various lipids to cholesterol would drive the lateral non-homogenous partitioning of the cholesterol across the plasma membrane and signifies its membrane-stabilizing function [32]. These data were obtained by tracking the cholesterol transition between artificial model vesicles of different lipid composition [13]. As a matter of fact, one could conclude that a hydrophobic molecule like cholesterol would be transported between the cellular membranes of different tissues according to the specific tissues' cell membrane affinity to the naturally-made cholesterol vesicles (i.e. chylomicrons, HDL, LDL, etc.). Similar inter-membrane transit would be imagined for a drug in a lipid-based nanoparticle, where the difference in the lipid composition between the liposome, for instance, and a cell is hypothesized to affect the rate of drug transport from the vehicle to the cell as with the case of cholesterol. In this regard, variation in the lipid composition and protein content of different tissues would also further affect the rate of drug transition from the lipid-based vehicle to these tissues, which is evident from the heterogeneous distribution of a drug to tissues following administration of the liposomal drug [33–35]. Although this heterogeneous distribution of the particles is putatively associated to the level of tissue vascularization and variation in the inter-endothelial cell gap size of the endothelial vascular system in tissues [36–38], the spontaneous transfer of a molecule from the particle to the cell membrane would be a valuable subject to come under investigation. For instance, a collection of useful information might be retrieved if the amount of a liposomal drug's uptake by various cells were measured, where each is representative of a particular tissue. In this regard, the varied pre-incubation periods of the liposomes with plasma proteins could be combined with the incubation periods with the thawing frozen cells previously harvested from various tissues. Using fluorescent tracking dyes, the level of liposomal-cell interaction-mediated drug transfer can be measured in the cells and an enormous collection of data could be obtained via automation and high-throughput techniques in a single experimental setting. Maybe the incubation of a liposomal drug with the cells would offer more detailed information with regard to the drug tissue distribution of a nanoformulated drug than their incubation with a limited collection of cells, where the rate of the liposomal drug removal by the cells could be assessed using the hypothetical quantification techniques that are going to be described in the section “Smart multi-epitope-grafted lipid-based carrier systems” (Fig. 1). Adopting these approaches, the PC of a given molecule or drug in the whole system of the “liposome versus various tissue cells” presumed highly likely to be determined. The information achieved through this process help to gain insight into similarities and differences among various tissues, through which a set of target-oriented drug delivery system, each suitable for a specific tissue, could be achieved.

The other important issue must be taken into account is the proportion of drug and cell in an in vitro setting. For instance, a liposomal drug in bloodstream faces enormous amounts of diverse interacting proteins, which affects its subsequent encounter with the massive proportion of the blood cells. In other words, whatever the “molecular corona” made change in the membrane of the liposomal drug, it would exert an influence over the driving forces that lead to liposomal assimilation into the blood cells. It has been reported that there is about 5×10^6 blood cell per volume (ml). A drug-cell association study with 1×10^4 cells/ml would be probably an under-estimation of the real setting and biomolecular corona effect. Similar fractional content of the phospholipid, neutral lipid and water must be present in in vitro conditions as in vivo environment if a researcher would intend to predict correctly the behavior of the nanoencapsulated drug supposed to be administered via intravenous route. For instance, if a drug or a liposomal drug formulation is supposed to be administered intravenously to reach the final destination, like an inflamed tissue, it would be of high significance to calculate first how much of the drug or the liposomal drug proportion would be assimilated into the blood cells before reaching the target tissue. Similarly, how much of the remaining would interact with the endothelial layer of vasculature, which is quite ubiquitous in the body. As a result, in vitro drug release profile, liposomal membrane stability, interaction with plasma proteins and the blood cells, which has been doing as prerequisite steps to predict the biodistribution profile for these formulations, should consider these factors in the experiments to provide the optimum prediction.

3. Membrane-mediated determination of drug partition coefficient, a key for contemplation

The traditional approach to determine the drug-membrane partition coefficient in isotropic biphasic solvent systems like n-octanol/water, alkanes/water, chloroform/water, dibutyl ether/water, etc. falls short of optimum drug lipophilicity prediction as these systems fail to present all forces involved in drug lipophilicity [39]. They fail to mimic the hydrophobic core and charged, polar surface of phospholipids present in biomembranes, where there are hydrophobic, H-bond, dipole-dipole and electrostatic interactions between drug and membrane [13]. The partition coefficient supplies useful information about quantitative structure-activity relationship studies as it allows us to predict drug absorption, distribution, metabolism and elimination processes in association with the drug physicochemical properties [13]. It would be, therefore, of great interest to seek for more optimum approach to determine drug-membrane partition coefficient. The accuracy of the prediction depends greatly on the chosen biomimetic model and on how the model is close to the real setting. In this regard, anisotropic membrane-like chromatographic systems, including Immobilized Artificial Membrane (IAM) chromatography, Immobilized Liposome Chromatography (ILC), and Liposome Electrokinetic Chromatography (LEKC) are regarded as more advanced systems in predicting correctly the drug-cell membrane partition coefficient and the structurally-dependent pharmacokinetic profile [39]. Moreover, in the biphasic solvent system, physical phase separation is required to determine the concentration of drug in each phase, which is a laborious and erroneous way as the phase separation might disturb the equilibrium state between the two phases. Whereas the mentioned biomimetic membrane models with the help of the chromatographic techniques determine drug-membrane partition coefficient with ease in most cases. On the other hand, fluorescence-quenching techniques, UV–Vis derivative spectrophotometry are able to determine partition coefficient in liposome or micelle models without the need for phase separation, where the permeation of drug from water to the biomembrane is associated with a change in some drug-related absorption parameters like maximum wavelength or molar absorptivity [40,41].

The technique is precise and logical as the drug structure comprising hydrophobic (e.g. aliphatic C—C chains) and hydrophilic parts (e.g. functional groups of O—H, N—H, etc.) would influence their membrane partitioning. Once a drug is used in the form of a lipid-based carrier system, however, additional parameters including drug/lipid

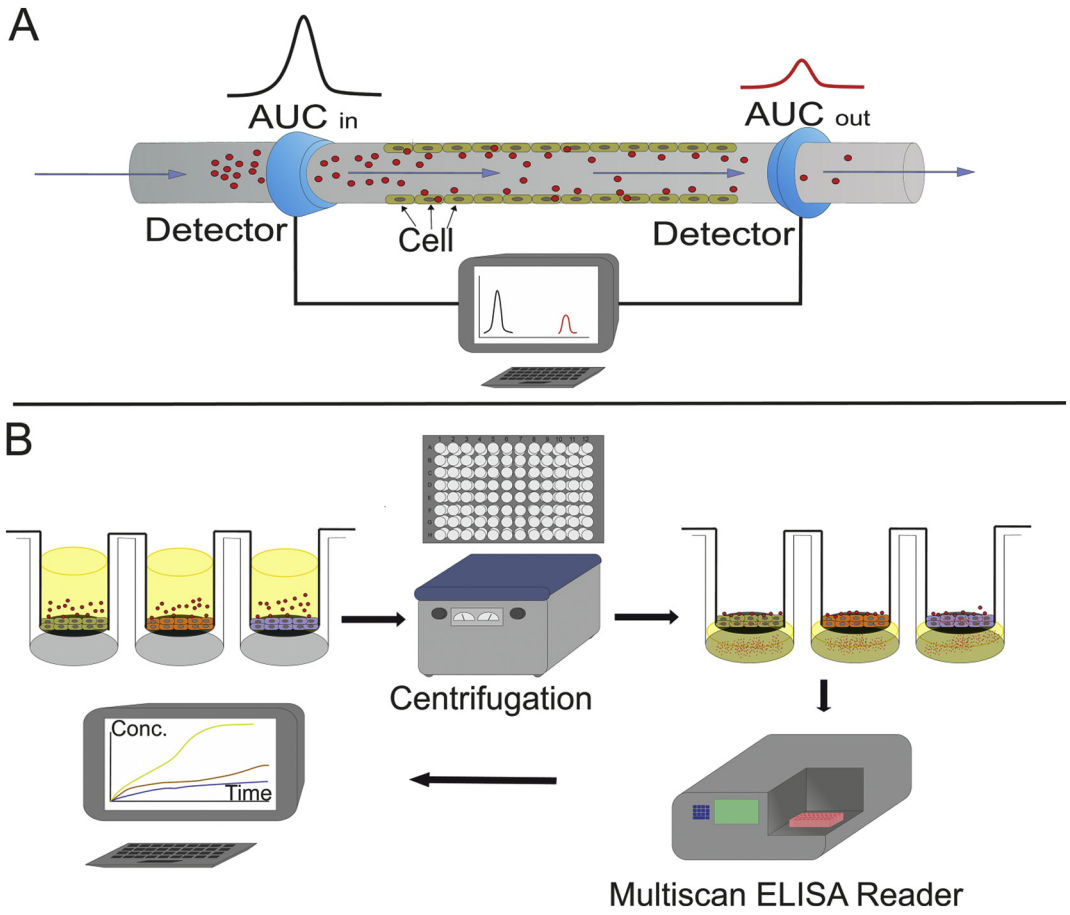

Fig. 1. Cell-trapping matrix-based analysis of lipid-based nanocarrier interaction with various kinds of cells derived from primary tissues. The cell-embedded beads could be packed into a column, where a slow flow of an aqueous medium acts as the mobile phase (A). The dynamic particle-cell adsorption/desorption could restrict the particle movement through the column to keep going with the flow (red curve in the computer screen, A). Moreover, the area under the curve (AUC_{out}) of drug concentration of the post-column would be decreased in comparison with those (AUC_{in}) of the pre-column, which indicated the level of drug assimilation by the cells. The cell-trapping matrix of various cell kinds could be also placed onto the overlaying chambers of a hypothetical two-chamber 96-well plate, where the time-course cellular uptake of a nanoformulated drug could be achieved following centrifugation at several time points and measurement of drug concentration in the below chambers (B).

mole fraction and gel-to-liquid phase transition temperature of the lipid membrane must come under close scrutiny. For instance, with a hydrophobic drug, it has been shown that the molar ratio of drug to total lipid of the carrier system could alter the partition coefficient of drug between water and lipid membrane and within the membrane [42], it could affect the gel-to-crystalline liquid phase transition temperature of the membrane as well as the temperature at which the phase transition begins and ends.

For a drug to be escorted by a lipid-based nanocarrier to reach a target cell, it must overcome multiple obstacles, which is along their way from the site of administration to the target tissue. For instance, oral administration of a drug has been reported to face the multiple challenges of the drug denaturation, chemical alteration, and agglomeration in the gastrointestinal tract, low affinity for the epithelial brush cells, and first-pass metabolism in the liver, depending on the chemical structure of the drug, which reduces the ultimate drug dose afterward. In many cases, the best nanocarrier provides a physically protective barrier around the drug cargo so that it could aid with passing drug through such obstacles. In the hepatic tissue, the protective lipid layer of the carrier provides an energetic barrier that limits the carrier/hepatic cell interaction, carrier/blood cell interaction and the resulting drug delivery. However, the very nanocarrier faces a dilemma when it is to deliver the drug to the target cell. This is highly desirable to design a carrier system responsive to the target tissue condition, where the specific multiple protein-protein interactions between the lipid carrier and the target cell catalyze the cargo delivery to the cell. It would be energetically favorable for the nanocarrier to interact only with the target cell and for the drug to pass from the nanocarrier to the membrane of the cell if there were significant levels of facilitation helped with the “specific protein-protein interaction” within the target site. Thus, it would be important to study the thermodynamics of such interactions between various lipid-based carriers having multiple protein epitopes and different cells to obtain nanocarrier systems with desirable net free energy for drug transition to the target cells. The next-generation of the lipid-based carrier system would probably focus more on the interactions of the “protein-protein” between the carrier and the cells and would be more than studying the lipid membrane interactions. A carrier that its dynamic nature as it travels in the body is also studied.

To elaborate on this idea more, we could find the analogy in the body. Consider the way cholesterol and testosterone, two fat-loving biological molecules, travel throughout the blood and reach their targets in the body. To this date, the efficient drug-carrier system for hydrophobic medicines would have yet been a big challenge where the drug was going to injected directly into the bloodstream [43–45]. For the cholesterol, however, it is much resolved. The thermodynamically favorable condition provided in the form of the low-density lipoproteins (LDL) allows cholesterol distribution to literally all peripheral tissues since this trip is feasible and it necessitates little energy consumption [46]. The LDL particle includes all fat molecules with cholesterol, phospholipids, and triglycerides dominant and with Apo B. On the

other hand, for reverse cholesterol transport, from the peripheral tissues to the liver site, the process needs energy; otherwise, the cell membrane of the tissue would have been the eternal, final destination of cholesterol. With the help of ABCA1 (ATP-binding cassette transporter), Apo A-1, and cholesterol esterification, cholesterol is removed from the tissues. Cholesterol esterification by lecithin-cholesterol acyltransferase (LCAT) turns the cholesterol to a more hydrophobic molecule that would facilitate reverse cholesterol transition from the cell membrane to high-density lipoprotein (HDL) and sequestration in HDL [47,48]. Likewise, for the nanocarrier system to deliver a given drug to a target cell, such specific facilitating functions of the proteins at the target site should be exploited. In the following, we will develop this concept further and propose how these smart carrier systems could be attained.

4. Smart multi-epitope-grafted lipid-based carrier systems

Considering that about 50% of a cell is composed of proteins, on average, the function of proteins in the above-mentioned phenomenon could not be overlooked [49]. The next-generation of nanocarrier system for drug delivery should consider designing protein or epitope-grafted smart nanocarriers with thermodynamically enhanced cell-specific interaction. In this system, a variety of proteins, epitopes, and ligands would contribute to enhanced differential interaction of nanocarrier system with the target cell, where a “cumulative effect” is imagined upon introduction of each epitope to the carrier in every step for a group of cells. For instance, imagine cholesterol, estrogen, glucose, α-fetoprotein, and human epidermal growth factor-2 (HER-2) as candidates that could be exploited in a multi-epitope-grafted drug delivery system for breast cancerous tumors. They all have been reported to be highly drawn by the cancer cells for their rapid growth and progression. The cancer cells overexpress the relevant receptors to meet their needs for growth. Having cholesterol and estrogen in the lipid composition could enhance the uptake of the carrier and the drug, which is supposed to be transported by the carrier. The introduction of the associated epitopes for estrogen receptors, glucose transporter 1 (GLUT1), α-fetoprotein receptor and HER-2 in every step of the formulation design could also enhance more and more the uptake of the carrier by the cancer cells, which is defined as “accumulative effect” that a smart multi-epitope-grafted lipid-based carrier system could offer. The more interaction between the proteins from the cell and the carrier, the further advancement in the specific cancer cell- carrier interaction thermodynamically [50]. In fact, the function of the membrane lipids would become subordinate to the function of the proteins for drug transition from a carrier to a target cell in the next-generation of the lipid-based carrier systems.

To this end, an enormous collection of the mentioned candidates would have to be screened for the optimum selection that exhibits specific interaction with the target cells. The task is laborious and time-consuming to fulfill, which requires devising high throughput and high-screening methods, miniaturized tests, and computational approaches to come to our aid. In the high-throughput method, the cells extracted from tissues could be immobilized onto a supporting matrix for 3D cell growth development in a column, where a mixture of nanocarriers, each containing different fluorescent dyes and epitope, could be passed through (Fig. 1A). High-screening techniques in which a whole collection of various cell types could be placed in a multi-well plate could offer a thorough collection of data regarding a nanocarrier interaction with all the cells (Fig. 1B). On the other hand, the various nanocarriers could be immobilized onto a supporting matrix, on which the cells could be tested for their tendency to take them up. The calculation of these interactions based on Gibbs free energy status of the epitope-receptor interaction and the frequency of the receptors on the surface of various cells would be the valuable input data in in silico studies orienting researchers toward optimum combinatorial carriers. Fourier transformed (FT)-based docking programs, including HexServer and ZDOCK, have already paved the way for some calculations, however, they would become more helpful if they were modified based on the empirical cell-carrier interaction data from the single-epitope-grafted lipid-based carriers with the cells [51,52]. These computational tools apply a “receptor-ligand” conformation as the input data without interference from other biomolecules, whereas in the biological milieu, the conformation of the proteins is dynamic and responsive to the surrounding interacting biomolecules that might change the receptor-ligand interaction. Therefore, we assume that a lipid-based carrier could offer an implement to obtain this kind of data, where they then could be utilized in the development of the current computational calculations. The ultimate verification of the mentioned interactions could be assessed using hypothetical cell-embedded substrate in the chromatographic system, where the system would assess the retention time and absorption of the vehicles rather than merely drug. In the following, this concept will be addressed.

5. Cell-trapping matrix-equipped capillary chromatography

As explained above, the anisotropic membrane partitioning systems are regarded as the more precise and advanced predicting analysis of the drug lipophilicity. In these systems, the membrane would offer all possible interactions that might happen between a drug molecule and a cell in vivo; where on one hand, the polar phospholipid head groups would make electrostatic interactions possible and on the other hand, the acyl chain would provide hydrophobic interaction. Various techniques based on the membrane-mediated partitioning analyzing scenario have been introduced so far and they have proven to be more accurate in calculating pharmacokinetic features [39]. In IAM chromatography, the monolayer membrane is covalently attached to silica beads. In ILC, phospholipid-based liposomes are noncovalently entrapped in gel beads pore (e.g. Superdex 200) as a stationary phase. But the most compositionally versatile and naturally closest biomimetic membrane-based chromatography could be observed in LEKC, where liposomes of various compositions and even red blood cell extract microsomes have been incorporated into the buffer as the pseudo-stationary phase for drug-membrane interaction.

The mentioned membrane-based chromatographic system might be able to be utilized for the determination of nanocarrier-cell membrane interaction, where various live cells could be fixed on an extra-large porous matrix scaffold as the stationary phase (Fig. 1A). The hypothetical cell-trapping matrix-equipped capillary chromatography could have a very slow flow of an isotonic solution, similar to capillary blood flow, where the dynamic absorption/desorption of the nanocarrier would restrict their movement along the column to keep up with the flow. The pre-column and post-column induced difference in the retention time and the area under the curve (AUC) would be translated into nanocarrier-cell interaction in the system. Although the hypothetical column would face many challenges, including homogenous cell dispersion in the matrix, rapid column exhaustion, and collapse, column uniformity between different column preparations, the system could be standardized using membrane-imaging techniques, which would allow assessing the cell density and uniformity in the column. Moreover, a simplified version of the nanocarrier-cell specific interaction-determining system might be able to be developed onto a two-chamber multiple-well cell culture plate (Fig. 1B), in which each well would present a cell membrane composition, probably derived from tissue sources. The above chamber would contain the cells in 3D matrix and the below chamber would contain the fraction of the drug and/or drug-nanocarrier that might have migrated across the matrix. Automation of lipid-based carrier preparation process would allow the production of enormous data on the composition of lipid and proteins in the platform of multi-epitope-grafted lipid-based carriers.

6. The involvement of drug chemical structure in nanocarrier design development

Although special attention has been paid to the lipid composition (variation in lipid head group and acyl chain), surface charge, particle size of the nanocarrier systems in relation with their serum clearance kinetic, serum protein interaction and cell interaction [45,53], little is known about the relationship between the structurally relevant physicochemical properties of the drug and the mentioned features of a lipid-based nanocarrier as the data of the control drug-free liposome could not be found in many experiments. For instance, it has that large unilamellar vesicles of about 100 nm in size have the lowest liposomal clearance within a set of liposomes with similar lipid composition than small unilamellar vesicles and larger multi-lamellar vesicles [54]. Cationic liposomes have the highest serum protein binding affinity (K_B). LUVs containing phosphatidylserine (PS), phosphatidic acid (PA) and cardiolipin (CL) exhibited very fast clearance in circulation, while liposomes containing phosphatidylglycerol (PG) and phosphatidylinositol (PI) circulate for longer periods. Liposomes containing bovine PI with the major acyl chain species being 18:0 have been demonstrated to rapidly remove from the circulation in minutes with K_B of 158 g protein/mol lipid, whereas ones with plant PI with unsaturated acyl chains (18:2) display half-life of 90 min in the circulation with K_B of 27 g protein/mol lipid. Surface coating of liposomes through PEGylation is the most well-known approach to achieve nanoparticles capable of evading reticuloendothelial system [54].

All of these findings have been achieved on the presumption that the nanocarrier membrane would be the limiting barrier in restricting the drug transition to serum proteins and cells. However, how a drug molecule structure would influence nanocarrier-cell interaction is far less studied in the drug delivery field using lipid-based nanocarriers.

7. Dynamically protein-protein-induced conformational change

Particle-biomolecular corona is a dynamic, built-up layer of biomolecules, in particular proteins, surrounding a particle of exogenous origin, when it encounters a physiological medium [4,16]. As a result of protein deposition onto the surface of the nanoparticle, the biological identity of the nanoparticles would progressively develop, which might change their physiological behavior. This concept has been shown to intensively fuel many investigations in the drug delivery field in recent decade; it has been fully discussed as the next-generation of tunable parameters in optimizing target-oriented drug delivery in some reviews and opinion papers [4,5,15–17]; and it could brighten up another concept termed “Dynamically protein-protein-induced conformational change”.

Dynamically protein-protein-induced conformation change is a concept that might explain how exogenous hydrophobic drug molecules or endogenous lipophilic molecules like progesterone, cortisol, estrogen, and androgen travels inside blood circulation and affect their target far away from the origin of secretion (Fig. 2). For instance, it has been stated that about 44% of testosterone and estradiol are tightly bound to sex-hormone binding globulin (SHBG), 55% bound loosely to serum albumin and corticosteroid-binding globulin (CBG), and only 1–2% remains free and biologically active [55,56]. Variation in SHBG reservoir level has also been stated to be connected with some physiological changes and pathological disorders, which indicates the regulatory function of these proteins. However, the questions remain unanswered and that is why 98% of this repertoire should have to be kept up ineffective and protected in connection with these proteins? Why does the body require having such a large collection of these hydrophobic hormones attached to these proteins awaiting to get unleashed and become effective? Would it be economically wise for the body to devote such high energy cost for the production of this group of hormones and their relevant proteins?

The answer might lie in the fact that SHBG, albumin, etc. proteins would not functionally as passive as they might appear, since this is virtually the common strategy for hydrophobic drugs to be prescribed at high saturating dosage to compensate for their vast metabolism and poor delivery to the target tissue [55,57]. In fact, lipophilic hormones would have to be transferred to their target tissues through a multi-step transition from tightly-connected SHBG to loosely-connected albumin and finally to their relevant G-protein coupled receptors on their target cells (Fig. 2). This highly specific transition of steroid hormones would seem to safeguard against non-specific drainage of these lipophilic compounds into other tissues to cause physiological problems. Once a single SHBG and albumin come up together, the albumin would not be sufficiently capable of inducing a transient conformational change in SHBG to allow a hormone transition from SHBG to the albumin; however, it would be able to do it if the SHBG confronted two albumins in its vicinity simultaneously. This would be called “dynamically protein-protein-induced conformational change” that might happen in the case of a lipophilic drug transfer as well. Once the hormone-bound protein reach up to plasma cell receptor, it would probably induce a conformational change in the receptor and a lipid rearrangement around the receptor; however as the transition-facilitating receptor proteins were rare, almost all these interactions with cell membranes would be aborted and only 1–2% of the entire interactions would face the receptor proteins with right orientation that would end up successful pass of the hormone to the cell membrane. In this regard, transient induced exposure of hydrophobic regions in the proteins (the receptor and the carrier protein) would facilitate hormone transition, whereas the hydrophilic shell of the carrier proteins would counteract such inducement unless the relevant receptor got involved in the hormone's transaction.

Indeed, the above-mentioned concept of the protein-mediated hydrophobic molecule delivery has been exploited in the design of the enhanced-specific drug delivery of several hydrophobic medicines, where the robust carrier protein-drug connection thermodynamically makes the drug-to- cell membrane transition unfavorable. This could lead to prolonged drug circulation time and a long trip inside the body [58]. Only the effective contact between the carrier and the specific protein receptor does provide enough interaction to overcome the energetic barrier for the carrier protein conformational change and the drug transition. Single protein exploitation in drug delivery has shown evidence of success in terms of the improvement of pharmacokinetics and pharmacodynamics of several drugs so far. However, the system depends on the successful contact of the carrier protein with the receptor, which rarely occurs and it necessitates the utilization of enormously large reservoir of the “protein carrier-drug” to offer sufficient drug delivery to the target site. In the following, we will complete the concept of multiple protein-protein interactions in the platform of lipid-based drug delivery on the particle-cell contact, where the tendency of drug-to-cell transition would bring the assisting proteins together from the lipid-based carrier and the target cell.

8. Drug-induced lipid rearrangement on particle-cell contact

When a lipid-based nanocarrier approaches the plasma membrane, some counteracting chemical forces would come into effect that would determine the extent of the carrier-membrane-mediated drug transition. The hydrophobic interior of the cell membrane and the lipid carrier would drive them up together, whereas the hydrated layer around the polar exterior (relevant to phospholipid head groups, oligosaccharides, and exposed, charged amino acid residues of the membrane-integrated proteins) on cell membrane would limit the particle effective contacts with the cell membrane. A lipophilic drug compound might induce some lipid rearrangement upon particle-cell membranes contact that would make hydrophobic interactions strong enough for drug transition from the carrier to the cell. This might signal to trigger the cellular phagocytic event as well for the lipid-based carrier invagination by the cell in the end (Fig. 3). The process would be further

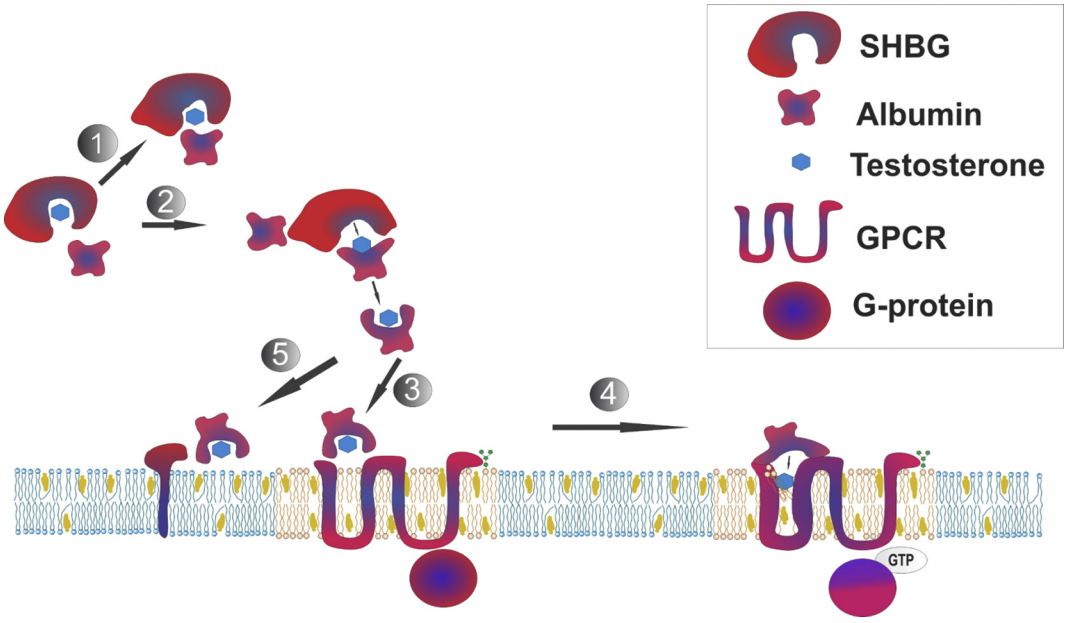

Fig. 2. Conceptualizes a dynamic conformational change in proteins as they approach a poorly-water soluble molecule or other proteins, in which the hydrophobic (shown by the dark blue color in proteins) and hydrophilic (shown by red color) domains of proteins would be transformed if sufficient energy for this transition were provided. The molecule could be of either endogenous (like testosterone) or exogenous (any drug molecule) origin. SHBG tightly surrounds the hydrophobic testosterone, where their contact with single albumin might not be able to cause sufficient conformational changes in both proteins, necessary for drug transport from SHBG to albumin (1). However, it might be able to do it if the SHBG have contact with two proteins simultaneously (2). The loosely-bound testosterone now could be transported by albumin to GPCR on their contact (3), where the approach of the loaded albumin would signal for the protein and the coupled G-protein to reorganize their hydrophobic and hydrophilic domains (4). This cell transportation would be site-specific since the non-specific interaction of albumin with other cell-membrane proteins might not lead to overcome the energetic barrier required for the albumin conformation change and the drug transition (6). SHBG and GPCR stand for sex hormone binding globulin and G-protein-coupled receptor, respectively.

target-oriented and effectively faster than the single protein drug carrier system described in the previous section as the chance of efficient contact between the carrier and the cell improves. Within the system, the first contact between any of the proteins from the carrier with the assisting protein on the cells would cause a delay in desorption of the carrier from the cell in the dynamic sorption/desorption process. This allows for the secondary protein-protein interaction that is recruited together and finally the principle receptor-ligand interaction which ultimate to the drugs transition.

Hydrophobic drug molecule-containing lipid-based nanocarriers usually exhibit particle size physical instability, even particle growth and sedimentation on storage and fast drug transition to any cell on injection to the bloodstream. The evidence for this assumption might be related to liposome studies, in which cholesterol transition from the

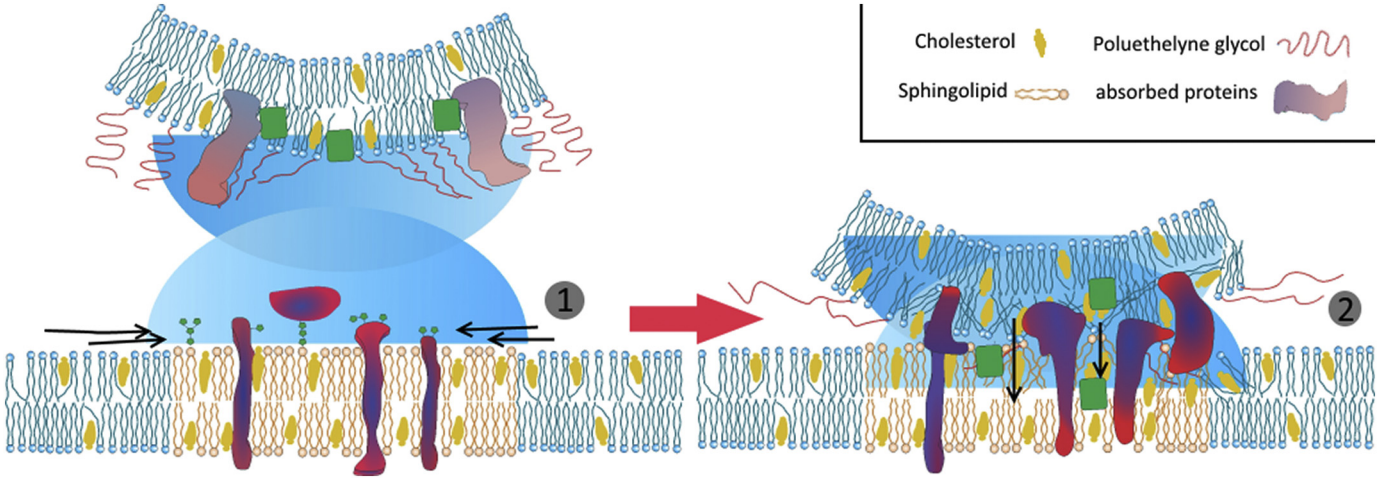

Fig. 3. conceptualizes the dynamic protein-protein-induced conformational change and lipid rearrangement. When a liposome containing drug (green rectangles) comes up to a cell membrane, a hypothetical region of hydrophobic and hydrophilic interactions would face off (shown with light blue semi-circles) with each other, in which poorly-water soluble drugs might play a major role. It might affect the level of protein corona around the liposome; it might add up to hydrophobic interactions between liposome and cell membrane; and as a result, it might cause a conformational change in both liposome-grafted proteins and cell-integrated proteins (their hydrophilic and hydrophobic domains are shown with red and blue, respectively). Moreover, upon the approach of liposome to the cell membrane, the phospholipids and cholesterol would be laterally rearranged across the cell membrane (shown with yellow color), where cholesterol and sphingolipids might be concentrated in these rafts and move the membrane proteins along as well (1). This might cause phospholipid membrane transition, necessary protein conformational change, and drug transition from the liposome membrane to the plasma membrane.

liposome to cell membrane has been defined to cause liposomal membrane instability. To overcome these problems, decrease in drug/lipid ratio, increase in the molar fraction of PEG-lipids and addition of lipids with longer hydrophobic tails into formulation might produce a nanoparticle with decreased drug inter-particle transition and/or reduced drug-to-cell transition. However, to this date, not as many reports with respect to successful targeted delivery of highly hydrophobic drugs could be found as there is for hydrophilic drug-containing nanoparticles [59–61]. A high concentration of drug delivery to the target tissue could be achieved along with extended periods of drug serum retention if the drug were encapsulated into the interior aqueous medium of liposomes.

As explained above, however, lipophilic compounds of endogenous origin such as cholesterol and steroid hormones could travel a long way inside blood circulation to reach their targets with the aid of protein-integrated lipid-carriers (LDL, HDL, etc. for cholesterol) and globular proteins, respectively. For a highly hydrophobic drug, we hypothesize that multiple-epitope-grafted lipid-based carrier system might hold the key for such drug patrolling toward the target site. When a lipid-carrier approached a cell membrane, the drug would diffuse to the cell membrane to reach a hypothetical steady-state. In this regard, as like for "cholesterol-lipid raft and cholesterol transfer" example, the drug might induce somehow a transient lipid rearrangement and even conformational changes in plasma membrane-integrated proteins, potentiating hydrophobic interactions between the cell membrane and the carrier. The succession of the protein-protein interaction and the type of proteins involved determines whether or not the drug would transfer. A hypothetical protein-integrated lipid-based drug carrier might give rise to a system thermodynamically stable in blood circulation with really slow drug serum clearance kinetics, while the protein-protein specific interaction between the target cells and the carrier would catalyze target-oriented particle delivery in the end. To this end, the smart multi-epitope-grafted carrier systems along with the testing methods of cell-trapping matrix-equipped experimental methods might help to acquire the knowledge.

Given the protein's role in transmitting different cargo, which is achieved in the context of a lipid-carrier, the part that is played by the lipid-based assembly could not be ignored. In the following, three different types of lipid-based carrier system and their potential for drug delivery will be discussed. These are micelles [62], liposomes [63], and lipid-emulsions [64]. I will discuss the simplified concept of transition between lipid monomer, micelle and liposome constructions [65,66]. I feel that this is a prerequisite knowledge to the interpretation of abilities and behavior of the lipid-based carrier in a physiological system like the bloodstream. I will discuss that the system is also an important factor involved in the determination of the behavior of a lipid-based carrier, which I feel that it is overlooked in many of the studies.

9. The transition of emulsifier from water to lipid assembly transition is determined thermodynamically

In a ternary phase system composed of an emulsifier (surfactant), lipid and water, a reciprocal transition of the emulsifier from water to lipid-assemblies. i. e. micelles, liposomes, large multilamellar vesicles, occurs, which is governed thermodynamically by the system, the lipid monomer structure, emulsifier structure, and emulsifier concentration [66].

Being hydrophobic at one end and hydrophilic at another, lipid molecules and similar artificial polymers are forced by the system to be gathered and arranged in the form of anisotropic structures, like micelles and liposomes [62,65]. This spontaneous arrangement happens in the system to reach a low free energy thermodynamically favorable state [67]. As the biological system is governed mainly by the polar water molecules, the behavior of the lipid-based carriers simplistically is studied in aqueous media as a simulant of a physiological medium, like blood [68,69], gastrointestinal fluid [70], etc. [69]. In fact, the tendency of the water-based system for maximum water-water polar interaction and low-free energy steady state drives the lipid molecules and their artificial equivalents to come together and form lipid assemblies like micelles, liposomes, and emulsions [65]. Alteration of the system by the addition of a salt results in a system with a new free-energy state, which potentiates the drive for the recruitment of the hydrophobic tails of the remaining lipid monomers to form lipid assemblies along with the emulsifier. In other words, the added free energy resulted from ionic interaction in the system pushes the lipid monomers to come together more strongly and hide their hydrophobic tails by the formation of lipid-assemblies. Similarly, the concept of the addition of salt has already been extrapolated to proteins with inner hydrophobic regions and outer hydrophilic areas as a technique to precipitate proteins (salting out protein precipitation technique) [71], in which the addition of extra salt to a water-based system enhances the driving forces for proteins aggregation and precipitation to reach a thermodynamic balance. Therefore, the lipid monomer/lipid assembly transition should be studied in an isotonic, ionic media similar to body fluids. Temperature input to the system as it increases the solubility of water have an opposite effect in terms of reciprocal transition of emulsifier to lipid-assemblies [66].

Besides the system-related factors, the lipid monomer structure, emulsifier structure and their concentration are also important factors in the determination of the proportion of the emulsifier monomer-to-lipid assembly, which is highly regarded and studied in the design of micelle and liposome drug delivery systems [62,72,73]. Natural surfactants with small hydrophobic tails tend to transit from monomer form to micelle at high lipid concentration, meaning that a large proportion of them are monomer at thermodynamic steady state [62]. In contrast, artificial emulsifiers with big hydrophobic and hydrophilic areas tend to form micelles at comparably low concentration of their monomers [62]. Use of block copolymer with extremely large hydrophobic and hydrophilic areas as compared to natural surfactants is based on the fact that these artificial emulsifier/lipid mixtures reach a thermodynamic steady state with an extremely higher proportion of the lipids in the form of micelles [62]. In other words, these structures with great hydrophobic tails are highly unfavorable to be in the monomer form and exposed to water molecules; therefore, a higher proportion of them tend to come together and form stable micelles at low concentrations of their monomers. As compared to natural micelles composed of surfactants, block copolymers form stable micelles and entrap high content of hydrophobic drugs as they can accommodate the drug molecules in their big hydrophobic region of the micelles. Following injection to the bloodstream and dilution, the block copolymers remain in the form of micelle, keeping the drug inside, and carry it for longer distances in the bloodstream, while a micelle made of natural surfactant with small hydrophobic and hydrophilic region are disintegrated, transit to the monomer form, and lead to premature delivery of the drug within the vicinity of the injection site [62,74–76].

The system-directed aspect of the transition of emulsifier to lipid assembly is not considered as opposed to the monomer-to-assembly transition directed by the lipid structure [62,77,78]. A large number of investigations are focused on the lipid structure/emulsifier-directed one and it seems that the influences of the system are overlooked in many of them [62,66,69,77,78]. In fact, the field term hydrophilic-lipophilic balance (HLB) is defined with the focus on the lipid structure role in the mentioned lipid monomer-to-micelle transition scenario [66], which is not helpful in most part. For instance, HLB, which is defined as a measure of the degree to which a molecule is hydrophilic or lipophilic, determined by calculating the molecular mass of the hydrophobic portion of the molecule (M_h) to the molecular mass of the whole molecule (M) according to the Eq. 2 [79].

$$HLB = 20^{*}\frac{M_h}{M} \tag{2}$$

It is helpful in explaining the molecule behavior in a standard

system of water/oil at 25 °C. Whereas it fails to predict the behavior of the lipid-assembly in a system composed of the different system-related players, i.e. lipid type and concentration, salt concentration and body temperature [65,66]. In fact, As opposed to HLB, calculating the system characteristics is a more pragmatic approach to predict the type and behavior of the lipid-assembly formed, which will be discussed later. With the bloodstream composed of many fluent proteins and blood cells, the interaction of the lipid-assemblies with serum protein and blood cells would also play a significant role in determining the behavior of these assemblies and their transformation from one form to another. Thanks to Prof. Steven Abbot website, I found a robust evidence for such a claim and refer the readers to the concept of Hydrophilic-lipophilic difference (HLD) [80], in which the system-related factors are also integrated with the determination of the proportion of lipid monomer and lipid assemblies at thermodynamic steady state of the system. According to HLD, increase in salinity and temperature (the factors related to the system), decreases and increases the HLD value, respectively; and determines how much of a lipid is supposed to be in the form of monomer and lipid-assembly to minimize the HLD value. Therefore, to acquire a better understanding of the emulsifier/lipid/water system, the system-related factors should be included in the study of the lipid-based assembly.

10. The reciprocal micelle-to-vesicle transition is governed thermodynamically by the emulsifier concentration

With regard to critical micelle concentration (CMC) term, there is a misinterpretation of the phenomenon as well, which is also discussed in Prof. Steven Abbot website [81]. Once an emulsifier/lipid assembly system is placed in a physiological media, the system-related factors will come into effect. First of all, surfactant monomers are distributed from the water compartment to the water/hydrophobic surfaces and come close to form micelles, which is defined by CMC within the specific system. CMC is defined as the lowest critical concentration of a surfactant capable of producing micelle structures and it is the result of the surfactant tendency to be at the water/hydrophobic regions and micellar aggregation number. It is the maximum impact of the surfactant to the system, beyond which no further impact of water tension reduction is observed (phase 1). With respect to emulsifiers with HLB lower than surfactants, the same distribution profile of the emulsifier to water/hydrophobic regions and emerged micelles would exist. As explained above, the system also affects the behavior of the surfactant/emulsifier in addition to the surfactant/emulsifier molecule structure. It means that in determining CMC of a surfactant/emulsifier, the system characteristic should be taken into account. Therefore, it is not the CMC, but the thermodynamic appropriate state must be achieved in a system (phase 1), for an emulsifier/lipid assembly starts to form (Fig. 4A). The remaining surfactants/emulsifiers are partitioned between lipid monomers and water to form mixed micelle structures (phase 2). Given the packing number (P) of a surfactant ($P < .5$) and phospholipid ($P = 1$), which could be depicted as the ratio of hydrophobic-to-hydrophilic surface area, it could create different lipid-based assemblies at different surfactant/lipid mole ratios [66]. A high molar ratio of a surfactant/emulsifier-to-lipid monomer provides a sufficient curvature to form small spherical surfactant/lipid mixed micelles. With the decrease of the surfactant/emulsifier-to-lipid mole ratio, the micelles elongated forming disk-shape micelles and then the vesicles appear upon the micelle folding and rim-to-rim merging [65,66]. Within this phase, various micelle structures and vesicles form and coexist, creating a population of particles with heterologous size distribution (phase 3, coexistence region) and the phenomenon happens when the packing number of the net surfactant/lipid is between 0.5 and 1. With decreasing the surfactant/emulsifier-to-lipid mole ratio further, the population of the mixed micelles is reduced until they disappeared, leaving only vesicles (phase 4). The inclusion of an emulsifier is an indispensable factor in order to have stable lipid-assembly structures. Otherwise, the lipid-assembly coalesce and form extremely big lamellar lipid structures and totally separated from the water phase.

The scenario that the decrease in surfactant concentration causes micelles merging to create larger vesicles is exploited in the reconstitution of liposomes and proteoliposomes, in which micelles-containing proteins merge to the larger vesicles in the coexistence region phase by surfactant removal from the system [72,73].

Taken together, the surfactant/lipid ratio change plays a critical role in the reciprocal micelle-to-vesicle transition scenario, in which the surfactant distribution to lipid/water interfaces results in the production of varying size of lipid-particles at high surfactant/lipid ratios and decrease in the surfactant/lipid ratio due to either the addition of lipids or reduction of surfactants causes the micelles and vesicles coalesce to reach a thermodynamic balance. In the following, we will discuss that the same scenario is also exploited in the communication of triglyceride throughout the body at enormous amounts of 100 g/day [82].

11. Lipoproteins, the natural emulsion system, are transported using micelle-to-vesicle transition scenario

An emulsion is a system of oil-in-water, in which the oily hydrophobic core composed of lipophilic molecules is surrounded by an external layer of amphiphilic molecules that forms the interfacial phase between the external water phase and the internal oil phase [64]. In contrast to micelles that tends to mix rapidly with other big vesicles and reach a thermodynamic steady state, the emulsions might be slowly mixed as it contains a comparably lower ratio of the surfactant/lipid [66]. Within the body, the elaborated micelle-to-vesicle transition scenario is exploited to absorb the dietary triglycerides from the intestine and transported to all organs for consumption and reverse-transport to the liver through energy consumption from all tissues using combinations of lipoproteins and enzymes [66,82].

The exogenous lipoprotein pathway starts in the intestine with micelles (Fig. 4B). Triglyceride is hydrolyzed to monoacylglycerol and fatty acids; and natural emulsifiers, i.e. bile acids, cholesterol, etc. are added to form micelles [82]. As the micelles tend to acquire fatty acids, it absorbs fatty acids from the intestinal lumen and coalesce with the intestinal plasma membranes, which leads to the transport of the dietary lipids. Triglyceride hydrolysis results in the formation of monoacylglycerol and fatty acids by the intestinal lipases which are more appropriate species than triglyceride to form micelles with the natural emulsifiers, including bile acids, cholesterol, plant sterols, and fat-soluble vitamins. In the intestinal cells, the cholesterol is esterified by the addition of fatty acids, which blocks the cholesterol transport back to the intestinal lumen [82]. Conversion of cholesterol to cholesterol ester could be considered as the conversion of an emulsifier to a non-emulsifier molecule. As a result of reduction of emulsifier and addition of fatty acids to the lipid-assembly in the reticulum endothelium of the intestinal cells, very large vesicle structures are produced with Apo-B48 and high content of lipid, which is called the first lipoprotein, chylomicron. The addition of Apo-B48 is a necessary step for the newly-formed chylomicron to leave the intestinal cell and secreted to the lymph. Once, the chylomicron, big vesicle, entered the circulation, it acts as a reservoir of fatty acids and transfers them to albumin and other tissues. As compared to micelle, it is assumed to have a low tendency to interact with blood cells and merge with them. The assimilation of the chylomicron is achieved through the lipoprotein lipase activity of the target tissues, i.e. muscle and adipose cells, which hydrolyzes triglyceride in the chylomicron to create absorbable species of fatty acids and monoacylglycerol. Therefore, it could be concluded that whenever in the body, the assimilation of lipid species to a cell is needed, emulsifier-like species are generated through enzymatic activity, which are more appropriate species to be taken up by the cells. On the other hand, whenever in the body, their excretion from the cells is needed, the natural emulsifiers are converted to non-emulsifier species, as in the case cholesterol and cholesterol esters.

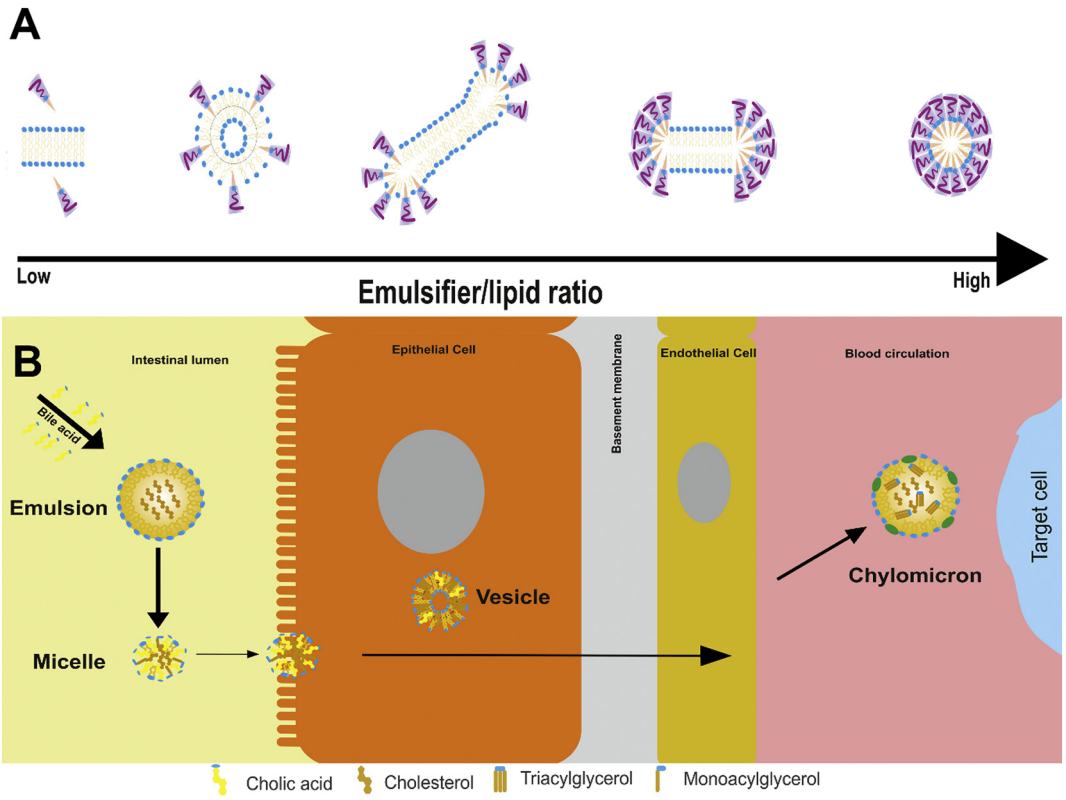

Fig. 4. A simplified view of the emulsifier/lipid ratio role in vesicle-to-micelle transition (A) and in lipid transport within the body (B). As the level of emulsifier increases significant curvature is provided resulting in the respective transformation of the vesicle, disk-shaped or cylinder-shape micelle, spherical micelle mixed lipid-assemblies (take into account that micelles and vesicles phase are mixed together and they are not separate from each other) (A). As micelles are more absorbable lipid-assemblies to merge with cell lipid-membrane, emulsifier-like species, e.g. cholesterol and bile acid components, are secreted to the intestinal lumen resulting in increased emulsifier/lipid ratio suitable for dietary lipid-emulsion to form emulsions and then micelles. Once they reach the intestinal cells, the micelles are converted back to vesicles by enzymatic conversion of cholesterol to non-emulsifier cholesterol ester, to block their reverse-transport to the intestinal lumen. The lipid-emulsion, chylomicron, with low tendency to merge with lipid-membranes, are then stable to patrol lipids in the circulation. They are only transported to the target membrane cells by the enzymatic breakdown of chylomicron triglycerides to fatty acids and monoacylglycerol with an emulsifier-like activity that facilities the chylomicron-cell membrane transition (B).

Moreover, the role of proteins in the targeted delivery of lipid species are of critical importance, whenever crossing a thermodynamic barrier is necessary. The proteins regulate the level of lipid-assemblies-to- cell transition by altering the level of emulsifier-like molecules. A higher proportion of proteins are required throughout the thermodynamically unfavorable pathway of cholesterol and lipid transfer to liver tissue as in the case of high-density lipoprotein (HDL) as compared to the more favorable pathway of cholesterol and lipid transfer to tissues [82].

Taken together, the elaborated scenario could also be concluded to be involved in the transfer of a drug through the circulation, where the alteration of the lipid composition of a lipid-carrier could be tailored to different tissues through the exploitation of a combination of protein-protein interactions. The mentioned point of view may open up a way to escort the drug to unreachable cerebra-pathologic regions using rational combination of epitopes and lipids in the design of a carrier.

12. Differential drug delivery to tissues, a goal of drug delivery system

Drug delivery systems are exploited to alter the pharmacokinetic and biodistribution of a drug [83]. Their aim is usually to provide a sustained drug release profile, restrict the drug delivery to non-target tissues, enhance the drug dose at the target tissue, and increase the drug assimilation by the target cells. In the design and selection of the type of a lipid-based drug delivery carrier, different factors should be taken into account to achieve a successful therapeutic outcome in the body. These could be classified into the factors related to the drug chemical and therapeutic characteristics, the factors related to the carrier physicochemical properties, and the factors related to the histological and cellular features of the target tissue and cells. A thorough review of these factors and their role in drug delivery is presented in the opinion paper of Allen and Cullis [83], and I just briefly explain them from a different perspective.

With respect to the drug chemical structure, the therapeutic and toxic dose of the drug for the potentially accessible different tissues/cells, the drug hydrophilicity/lipophilicity, and the carrier physical structure are the factors that should be considered [25,83]. For instance, for chemotherapeutic agents that affect a broad spectrum of the body tissues and cells, drug delivery carriers that could restrict out-of-target drug release in the circulation and patrol therapeutic dose of the drug to the pathological site such as solid tumors are suitable carriers [53,63,76]. In this regard, for instance, liposomes are not a good candidate for the delivery of non-potent, large, hydrophobic drugs as they would fail to accommodate therapeutic dose of the drug in the limited spaces of the liposome membrane [84,85]. They also fail to protect them from interaction with various blood cells and tissues as they travel in the circulation to reach tumor microenvironment. As a result, a substantially lower dose of the drug would reach cancer cells to pose a therapeutic effect. Instead, lipid-emulsions or liposomes with drug-entrapping agents in their interior would be a more appropriate candidate for the delivery of these drugs as they could accommodate higher content of the drug in their structure [85,86]. For highly potent drugs, like some immunoconjugates with selective interaction with the target

cells, stable large copolymer/lipid mixed micelles could enhance the circulation half-life of the drug and penetrate to deep areas of the target tissue due to their small sizes and natural tendency for cell assimilation [77,83]. A small proportion of these carriers are imagined to be taken up by the cells, especially by different blood cells, hepatic and nephrotic cells, is predicted not to be accompanied by serious consequences, as the dose of the drug agent would be negligible. However, it is highly recommended to examine their toxicity for hyperactivity reactions and accumulative dose through repeated doses [87].

If the point is to decrease the level of cellular drug delivery and enhance the drug circulation half-life, highly stable liposomal structures of average size 100 nm, e.g. PEGylated stealth liposomes, are recommended as these drug carrier systems could be stable in blood for days and could be accumulated to a high level in tumor 1-to-3 days post-injection [35,83]. These liposomes are shown to be accumulated in the tumor through the enhanced permeation and retention (EPR) effect, in which the exclusively leaky vasculature of the tumor allows the extravasation of these nanoparticulated lipid-assemblies to the interstitial spaces of cancer cells [35]. The lack of a lymphatic system in tumor blocks their drainage, leading the drug retention in the tumor tissue. Whereas non-PEGylated liposomes could be exploited when the point is to deliver the drug to mononuclear phagocyte system for therapeutic purposes, e.g. antileishmanial drugs against the parasite that is resident within macrophages [83].

Besides the factors that are explained above, the differential tissue and cellular features of the pathological site are already exploited for the "triggered-delivery of the drug". These include the difference in pH [88], enzymatic activity [89], and cell receptor between a pathological tissue and normal tissue [90]. However, there is critical obstacles for their exploitation that must be taken into account. For instance, pH-sensitive drug delivery systems are designed based on the fact that the tumor interstitial space is more acidic than the surrounding normal tissues and such a difference could be exploited by the design of a pH-sensitive lipid-drug carrier [88]. In this regard, pH-sensitive PEGylated liposomes in which a PEG-lipid with a pH-sensitive hydrolyzable bond is used could lose their PEG chains upon PEG-lipid hydrolysis in acidic pH in vitro. However, an acidic region in a tumor is out of reach of tumor vessels with packed cells. These cells and interstitial areas have low nutrient and gas communication with blood vessels, and for these reasons, they are also out of reach of liposomes with large sizes [91]. For the liposome to become a practical pH-sensitive delivery system, it must first pass the mentioned barrier, which is pretty bleak. With respect to the enzymatic activity and cell-receptor difference, these are more appropriate factors as they could break the thermodynamic barrier of the lipid-assembly for cell assimilation [53]. For instance, use of lipid species that could be converted to the emulsifier-like species upon the enzymatic activity of the target tissue could result to the disintegration of the lipid-assembly, triggered-release of drug and cellular uptake of the carrier. Similarly, multi-epitope lipid-based drug delivery might enhance the cellular uptake of the carrier and drug, in a cell population that overexpresses multiple receptors [50,69,90,92].

13. Conclusive remarks

We tried in the current opinion paper to open up like a panoramic view to researchers who have been devoting their life to drug delivery systems. The concept of drug delivery would require researchers to look it from a broader perspective and to do so; much cooperative work would be needed among researchers in different disciplines, including chemists, biochemists, biologists, software programming engineers, physics experts. We have presented the "triad" of drug physicochemical properties, lipid-based formulation and cell membrane bio-participants in defining the destiny of drug once they were administered alone or in the form of a lipid-nanocarrier.

We discussed how PC of a drug, which was previously applied to predict the biodistribution of the non-formulated drug, could be also applied to predict the tissue distribution of that drug even when it is administered in the form of a lipid-based carrier. We drew an analogy between the cholesterol mechanism of biodistribution and a drug tissue distribution administered in a nano-sized lipid-based carrier. Besides the previously addressed additional parameters that should be utilized in the prediction of a nanoformulated drug (e.g. the level of tissue vascularization, the surface charge and size of the carrier), the propensity of that drug to transit from the lipid-based carrier to the membrane of tissues should be considered. The hypothetical experiments that might contribute to support this concept were proposed. We presented an argument for the significance of the mentioned parameter in the investigation of the biomolecular corona and stated that for the study of the biomolecular corona effect, special attention should be paid to the proportion of the membranes from the cell and the carrier.

The anisotropic membrane-chromatographic systems, which are more precise techniques for the prediction of PC of a drug in vivo (between the membrane part of tissues and aqueous part), could be applied for the design of a carrier system capable of preventing a drug from being rapidly removed. In this regard, it was discussed that a lipid-based carrier system could limit fast drug assimilation by all tissues if there were a thermodynamically energetic barrier for both the drug transition and the carrier-cell interaction. Next, the specific protein-protein interaction between the protein corona of the carrier and the cell receptors could overcome the barrier and facilitate the drug transition. We assume that multiple-epitope-grafted lipid-based carrier system would be probably the next generation of the drug carriers specifically tailored for a given target tissue. A carrier on which multiple biorelevant ligands of estrogen, glucose, α-fetoprotein, and HER-2 exist could significantly interact with breast cancer cells overexpressing the corresponding receptors, where each protein-protein interaction cumulatively enhances the chance for the carrier to be taken up by the cells. As a consequence, enormous groups of protein candidates might be screened for such multi-epitope carriers, in which hypothetical high throughput cell-embedded matrix- experimental methods might help. Moreover, the retrieved data could be utilized in the learning enhancement of the current docking programs for the improved prediction of the "receptor-ligand" interaction in their natural milieu.

We assume that the dynamically protein-protein-induced conformational changes provide a conduit for the transfer of hydrophobic biomolecules to their final destination as the example of "protein-escorted estrogen" was shown. We also assume that the drug itself contributes to the transfer of the carrier to the cell as it might induce lipid-rearrangement in both the cell and the carrier. In this regard, lipid-carrier systems with multiple-epitopes on their surface would probably be faster and more effective in the transition of the drug to the target cells than the single protein-mediated drug transport. The next generation of the drug-delivery systems should probably pay more attention to the potential of the cellular and plasma proteins in the target-oriented delivery systems, where these concepts would be addressed.

With respect to lipid-assemblies, we highly suggest the formulation scientist pay more attention to the elements of the ternary phase water, oil, emulsifier system to achieve an improved perception and prediction of the elements involved in lipid-assemblies formation. The system parameters, e.g. temperature and ionic forces are as important as the type and concentration of emulsifiers and lipids in the determination of the type of lipid-assemblies that are formed. i.e. micelle, liposome, emulsion. In this regard, HLD that takes into account the system parameters is a more accurate explanation of the phenomena involved in lipid-assemblies formation than HLB that focuses on the emulsifier molecular structure.

Among the parameters, the emulsifier/lipid ratio is a determining factor, which is exploited in both formulation field and in the transfer of lipids in the body. High ratios of emulsifier/lipid provide small micelles with high affinity for vesicles and cells, while low ratios provide stable vesicular structures capable of evading cell interaction. The decrease in emulsifier/lipid ratios by surfactant removal is exploited as a

formulation technique for the constitution of proteoliposomes and vesicles with narrowed size distribution. In the body, proteins and enzymatic activity alter the emulsifier/lipid ratio to formulate the desired lipid-assembly through the addition/subtraction of natural emulsifiers, enzymatic conversion of them to non-emulsifier species and vice versa. Whenever the rapid assimilation of lipids by a group of cells is required, high emulsifier-containing lipid assemblies are generated, as with the mixed nutritional lipid/micelles that are easily taken up by the intestinal cells. Whereas for patrolling lipids in the circulation, large stable emulsions with decreased emulsifier/lipid ratio as with chylomicrons are generated. What is really intriguing in the micelle-to-vesicle transition scenario is the design of a hypothetical sophisticated lipid-based drug carrier which could be transited to a more absorbable lipid carrier form on interaction with the target cells. It is potentially achievable through the rational selection of protein-epitopes, lipid species, and lipid ratio and the perception of the target tissue enzymatic activities. It might improve the delivery of various drugs to the pathological sites, such as tumors and brain infarctions. It is assumed that differential enzymatic activity and cell-receptor profile of the pathological tissues like solid tumors could be exploited effectively to enhance the carrier cell uptake if multi-epitope lipid-based drug delivery systems with hydrolyzable lipid components are designed.

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Conflict of interests

None.

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