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# Overlapping Receptor-Based Pathogenic Cascades in Degenerative Disease: Implications Ranging from Tumor Targeting to Aging and Dementia Therapeutics

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**Abstract:** Previous research has already shown that apolipoprotein (apo)A-I is adsorbed from the bloodstream onto the surface of certain colloidal lipid particles after the intravenous injection of such colloidal nanocarriers. As a result, various blood–brain barrier (BBB) scavenger receptors are targeted by these (apoA-I-coated) colloidal nanocarriers. This targeted molecular interaction is mediated/facilitated by the adsorbed apoA-I, which is then followed by receptor-mediated endocytosis and subsequent transcytosis of the nanocarrier particles across the BBB. A multifunctional combination therapy is obtained by adding the appropriate drug(s) to these biomimetic (lipid cubic phase) nanocarriers. This therapeutic targets specific cell-surface scavenger receptors, primarily class B type I (SR-BI), and crosses the blood–brain barrier. The lipid contents of artificial biomimetic (nanoemulsion) nanocarrier particles and of naturally occurring high-density lipoproteins (HDL) have been shown to be similar, which enables these nanocarrier particles to partially imitate or simulate the known heterogeneity (i.e., subpopulations or subspecies) of HDL particles. Hence, colloidal drug nanocarriers have the potential to be used in the biomedical treatment of complicated medical conditions including dementia, as well as certain elements of aging. Widespread inflammation and oxidative stress—two processes that include several pathophysiological cascades—are brought on by dementia risk factors. More recent studies suggest that proinflammatory cytokines may be released in response to a prolonged inflammatory stimulus in the gut, for example through serum amyloid A (SAA). Therefore, pharmacologically targeting a major SAA receptor implicated in the SAA-mediated cell signaling processes that cause aging and/or cognitive decline, and ultimately Alzheimer's disease or (late-onset) dementia, could be an effective preventive and therapeutic approach.

**Keywords:** aging; Alzheimer's disease; calcium dyshomeostasis; dementia; drug targeting; inflammation; nanocarrier; oxidative stress; pathogenic cascades; tumors



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## 1. Background

There has been a long-standing interest in employing nanoparticles as a formulation technique for poorly (water-)soluble pharmaceuticals, as indicated in a number of reviews published over the previous 20 years (e.g., [1–4]). Furthermore, when administered intravenously, nanoparticles can be engineered to offer passive or even active targeting for the distribution of drugs (e.g., [1,3]). Petri et al. [5] have found, for instance, that surfactant-coated nanoparticles significantly increase the anticancer efficacy of doxorubicin against intracranial glioblastoma in rats. According to these authors, the (plasma) apoA-I molecules, already is anchored (i.e., adsorbed) onto the surfactant-coated nanoparticles, interact with especially the "scavenger receptor class B type I" (SR-BI) at the blood–brain barrier (BBB), and hence may be the cause of the significantly enhanced antitumor effect [5].

The presented findings agree with observations described elsewhere (e.g., [1]) regarding a few (protein-free) parenteral lipid nanoemulsions, such as the Filmix® nanoemulsion, and other "actively targeted" lipid nanoemulsions. In particular, it has been determined that the SR-BI receptor is the most likely receptor to play a primary role in the ligand–receptor interaction of some of these (surface-active lipid) nanoemulsions at specific target

cells, which includes a large number of cancers [2]. To be more exact, the results indicated above are consistent with earlier findings that have been published (see [1]), which demonstrate that SR-BI is the main potential receptor that promotes the improved endocytosis of "lipid-coated microbubble/nanoparticle-derived" (LCM/ND) nanoemulsions into various target cells. These nanoemulsions primarily contain dispersed lipid cubic-phase nanoparticles. Furthermore, the self-assembled lipid "nanoparticle structure" itself appears to be successfully utilized as the "active" targeting ligand in this specific nanoparticle drug-delivery method (after injection into the bloodstream).

It should be noted that the artificial biomimetic (LCM/ND nanoemulsion) nanocarrier particles can mimic or partially replicate the known heterogeneity, viz. subpopulations or subspecies, of high-density lipoprotein (HDL) particles because of the documented similarities in lipid composition between naturally occurring HDL and these nanocarrier particles [1]. Colloidal drug nanocarriers have the potential to be used in the biomedical treatment of complex medical conditions such as dementia (see below). In conclusion, the aforementioned explanation or illustration of active targeting through ligand-receptor binding aligns nicely with the previous findings [1] about lipoprotein-receptor-mediated drug delivery to tumor cells via targeted LCM/ND nanoemulsions. In particular, among all lipoprotein receptors, the surface-membrane receptor known as "scavenger receptor class B type I" (or SR-BI) became the most likely prospective target because of its recognized multiligand capabilities (cf. [1,2]). Dissociation of the drug from the lipid nanoemulsion particle might occur intracellularly, at the cell surface, or at the (near) extracellular space once the drug binds to the target cell-surface receptors [1]. Furthermore, there is ample evidence of the multiligand capacity of SR-BI (in rodents), also referred to as CLA-1 (in humans), documented elsewhere (cf. [1,2,6–10]). [SR-BI is well known and long-studied for its binding characteristics with HDL in mammals.

## 2. Cardiovascular Risk Factors, Inflammation, Oxidative Stress, and Serum Amyloid A (SAA) Inflammatory Effects

Endothelial cell activation is often a result of brain tissue hypoxia caused by the cardiovascular risk factors associated with aging and dementia. Reactive oxygen species (ROS) and proinflammatory proteins are produced as a consequence, and when these factors combine, they can cause oxidative stress and systemic inflammation—each of which can compromise the integrity of the blood–brain barrier [11,12] (Note: in Alzheimer's disease, oxidative stress and inflammation are closely related with each other. The participation of inflammatory factors in signaling processes, which are important mediators of oxidative stress and neuroinflammation and lead to neurodegeneration, is modulated by the redox status. In the Alzheimer's-disease brain, the ensuing cellular damage encourages further neuroinflammation [11,13]).

While the preceding paragraph acknowledges that oxidative stress and widespread inflammation are often caused by dementia risk factors (e.g., [14]), it is also true that these processes can have biological effects beyond the increased calcium load in brain tissue and neurodegeneration. Pathophysiological cascades linked to a variety of diseases, including aging, are involved in both oxidative stress and inflammation [11,15]. When comparing individuals with Alzheimer's disease to those who are aging normally, the Alzheimer's patients exhibit a greater frequency and progression of cerebral vascular atherosclerosis and various other cardiovascular (or "inflamm-aging" type) disorders. In addition, changes in arterial elasticity provide a simple explanation for the apparent pathogenic connection of such "inflamm-aging" disorders to dementia and the accompanying brain microvascular damage. One potential treatment approach to postpone dementia could entail the use of lipid nanocarriers, or biobased nanoemulsion technology, to deliver drugs locally. These carriers would target a key serum amyloid A (SAA) receptor that is implicated in specific proinflammatory cell signaling pathways mediated by SAA. Furthermore, by adding drug molecules to these lipid nanocarriers, one can obtain a "combination therapeutic" that targets multiple cell types simultaneously (through cell-surface scavenger receptors); any

targeted cell type may be connected to different dementia or aging pathophysiological cascades, providing for multifunctional local drug delivery in vivo [16].

Moreover, HDL (and ApoA-I) interaction with the SR-BI receptor was discovered to be reduced in Alzheimer's disease patients, according to Khalil et al. [17]. Their experimental findings also suggested that these patients had elevated levels of oxidative stress [11,17]. The authors concluded that their clinical trial shows, for the first time, that HDL functionality is decreased in Alzheimer's disease, and that the oxidative stress and inflammation associated with Alzheimer's disease may be the cause of this modification [17]. This finding is in line with previous research (cf. [18]) that identified SR-BI on astrocytes and vascular smooth muscle cells in the brains of Alzheimer's patients, and that this scavenger receptor has been shown to mediate microglia adherence to aggregated Aβ. Additionally, these authors report that SR-BI controls Aβ-related pathologies and modulates the perivascular macrophage response [18]. Finally, it was discovered by Fung et al. [19] that SR-BI mediates HDL uptake and transcytosis across the brain's microvascular endothelial cells (i.e., across the BBB). Further, according to these researchers, modifying HDL transcytosis across the blood–brain barrier to enhance plasma apoA-I delivery may, in turn, facilitate the increased transport of therapeutic drugs in association with "HDL-like synthetic particles" across the BBB to treat neurodegenerative illnesses like Alzheimer's disease [19].

Due to a number of difficulties with native lipoproteins [2], artificially produced "reconstituted lipoprotein(s)" have been developed as an alternate approach to targeted medication delivery. The expectation that lipoproteins increase their therapeutic efficacy by targeting drug(s) to tumors as well as other target tissues that overexpress lipoprotein receptors, and thus minimize systemic toxicity (by shielding the drug from contact with most normal tissues [20]), is the driving force behind the continued pursuit of such reconstituted lipoproteins. Once the reconstituted lipoprotein nanoparticle enters the bloodstream, a specific receptor–ligand interaction—provided by the particular polypeptide component (apolipoprotein, e.g., apoA-I) of the reconstituted lipoprotein complex—enables the drug to be delivered from the nanoparticle [8,21–23]. Regarding the lipoprotein receptor itself, CLA-1/SR-BI is a good contender since it is frequently shown that, in contrast to normal tissues, many brain tumor cells and neurodegenerative tissues overexpress this receptor (cf. [1,20,21]). The "reconstituted lipoprotein vehicle" was initially made entirely of lipids, but the aoplipoprotein(s)—which are required for targeting—were only obtained following a serum incubation. Similar descriptions of pure lipid nanocarriers that can effectively absorb apolipoprotein(s) when they come into contact with blood plasma can be found in other studies. Williams and Scanu [24], for instance, report that intravenously injected phosphoglyceride liposomes uptake endogenous apolipoprotein A-I (apoA-I); moreover, in vitro, it has been discovered that phosphoglyceride liposomes (cultured with plasma) uptake apoA-I at the expense of HDL [24] (cf. [25]).

It is believed that the situation with LCM/ND nanoemulsions is comparable [1]; namely, the LCM/ND nanoemulsion particle's lipid composition—which consists of different glycerides, cholesterol, and cholesterol esters—resembles the lipid content of a "generic" lipoprotein and, hence, is comparable to the lipids found in a number of plasma lipoproteins [8,19]. Consequently, it is thought that, upon injection into the bloodstream, LCM/ND nanoemulsion(s) will probably pick up (i.e., bind) plasma apolipoprotein(s). In accordance with the aforementioned factors, it was previously suggested that such bound forms of apolipoproteins, including apoA-I, can successfully be identified by their corresponding lipoprotein receptors (which are frequently shown to be overexpressed on the surface membrane of different target cells) ([1]; refer to Section 3 below).

## 3. Gut–Brain Axis, ApoA-I, Amyloidosis, Aging, SAA versus SR-BI Targeting, and Dementia

According to Wang et al. [26], atherosclerosis, hypertension, and vascular amyloidosis are conditions that, in many ways, are consistent with accelerated aging. For amyloidosis, there are only two types of amyloid proteins—apolipoprotein A-I (or apoA-I) and

medin—that are primarily linked to vascular amyloidosis and that can also accumulate at the aorta [26]. Many drug-delivery studies have already made use of apoA-I (see [2] for a recent review). The biobased LCM/ND nanoemulsion type of targeting *vehicle* was employed in these investigations. The primary receptor candidate on the cell surface that can promote the accelerated endocytosis of these colloidal–lipid nanocarrier particles into different target cells—namely, apoA-1-assisted endocytosis—is the SR-BI receptor [1,2,11,16,27,28].

Recent research [29,30] is particularly significant as it indicates that proinflammatory cytokines may be released in response to a persistent inflammatory stimulation in the gut, i.e., through the release of serum amyloid A (SAA). Simultaneously, aging-related or dysfunction-related increased BBB permeability permits these proinflammatory cytokines to penetrate the brain and trigger glia reactivity [29,30]. According to these recent findings and a number of previous research studies, inflammation is crucial to the process of Aβ deposition. As a result, amyloidogenic processes (including Alzheimer's disease) may be lessened by inhibiting inflammatory cascades [31] (cf. [17,32]). The often repeated, and now comprehensive, as well as emphatic, overall research conclusion that chronic neuroinflammation is a common feature of classical neurodegenerative illnesses (including Alzheimer's disease) lends more support to this kind of therapy strategy [33]. Therefore, directing medication(s) specifically towards a key SAA receptor, which is accountable for the SAA-mediated cell signaling events that cause cognitive decline and ultimately Alzheimer's disease or (late-onset) dementia, could be a useful preventive and therapeutic approach.

The function of SR-BI receptors, or their human ortholog CLA-1, as cell-surface SAA receptors that bind, internalize, and mediate SAA-induced proinflammatory effects has already been demonstrated by prior studies [7] (cf. [34]). Baranova et al. [7] also note that CLA-1/SR-BI ligands "efficiently compete" with SAA for CLA-1/SR-BI binding in cell culture. (For example, the fact that both apoA-I and SAA are substrates for SR-BI receptors has previously been reported in the literature (e.g., [35]), thereby indicating that SR-BI may mediate the transport of both these proteins over the BBB). Thus, it should come as no surprise that Robert et al. recently claimed that numerous lines of evidence point to HDL and its main apolipoprotein (apoA-I) having a protective function against Alzheimer's disease [36]. Therefore, the intravenous use of the LCM/ND lipid nanoemulsion vehicle for clinical purposes may well be accompanied by a similar benefit (of its "competitive binding", versus SAA, to SR-BI receptors); more specifically, based on multiple in vivo animal experiments, this (apoA-I-based) nanoemulsion vehicle has already been reported and defined multiple times in the peer-reviewed literature as a targeted, apoA-I-based (SR-BI mediated) drug-delivery agent (cf. [1]). Furthermore, it is possible to create a multifunctional "combination therapeutic" that targets cell-surface SR-BI by incorporating drug molecules into the LCM/ND lipid nanoemulsion formulation. Various cell types, all potentially linked to Alzheimer's disease [1,2] and/or (late-onset) dementia, might be simultaneously searched and better targeted for the localized pharmacological therapy of brain tissue in vivo [1,37] with the help of this (intravenous) colloidal–nanocarrier therapeutic.

## 4. Analysis of Nanocarrier: Formulation, Biophysical Structure, Particle Stability, and Bio-Safety

### *4.1. Formulation*

Salentinig et al. [38] described a (shearing) process for the manufacture of stable dispersions of nanostructured lipid mesophases (consisting primarily of monoglycerides, or "surfactant lipids"). High yields of internally self-assembled, precisely sized nanoemulsion particles have been reported by these authors. According to their research, the dispersed particles with various well-ordered nanostructures are kinetically stabilized systems that remain stable for several months [38]. In this study, the authors go on to say that because of their enormous potential for use in the food, cosmetic, and pharmaceutical industries, these inverse-type liquid–crystalline phase-containing nanoemulsion particles are still attracting a lot of interest [38] (cf. [39–45]). The enhanced surface area provided by these nonlamellar liquid–crystalline nanostructures, which include inverse cubic phases, stems from their

intrinsic nanostructure and is one of their main advantages [46] (cf. [47]). Because of their semirigid periodicity, these nanostructured lipid mesophases are also far more durable than liposomal delivery methods [46]. In addition, one noteworthy characteristic of a small number of lipid-based liquid–crystalline systems is their ability to remain colloidally stable in excess water (e.g., [46,48]). This property enables the predispersion of these systems in blood plasma as submicron particles appropriate for intravenous drug delivery. Monoglycerides [48], cholesterol, and cholesterol esters [49] are examples of polar lipids that are known to display this type of phase behavior. These lipids are repeatedly described as being present in significant amounts in the "particularly preferred form" of LCM/ND nanoemulsion formulations [50,51]. Furthermore, the saturated variant comprises the entire monoglyceride content used in this "preferred form" of the LCM/ND formulations (cf. [50,51]). There is an extra advantage to creating such nanoemulsion formulations solely using saturated monoglyceride. Specifically, the advantage of saturated fatty chains, or saturated acyl groups, is that they do not undergo peroxidation processes—which would reduce the acceptable storage life (cf. [49]) of these "oil-in-water" nanoemulsions.

The majority (by wt. %) of the lipids utilized to make (Filmix®) LCM/ND nanoemulsions are monoglycerides. When exposed to water, monoglycerides collectively demonstrate the capacity to self-assemble into a variety of useful dispersed cubic phases (among other liquid–crystalline phases) [1,52,53]. Two separate classes can be formed from the (lyotropic or solvent-induced) cubic liquid–crystalline phases: bicontinuous cubic phases [54] and micellar or discontinuous (e.g., type Fd3m) cubic phases [55]. A variety of lipid systems can combine to form an interesting lipid cubic phase of the latter category, which is built upon packings of discrete inverse micellar aggregates [55]. The most commonly seen (inverse micellar cubic) structure, according to Seddon et al. [56], is a cubic phase of crystallographic space group Fd3m, which calls for a mixture of several different polar lipids [57,58]. The previously reported LCM/ND lipid nanoemulsion is especially relevant to the dispersed Fd3m cubic phase; in this case, both of the described nanostructures often specifically contain cholesterol, as well as three different types of (saturated) glycerides, i.e., tri-, di-, and monoglycerides [50,51]. Dissociation of the drug from the lipid nanoemulsion particle might occur intracellularly, at the cell surface, or at the (near) extracellular space once the drug binds to the target cell-surface receptors [1].

### *4.2. Biophysical Structure*

The mechanism by which different (biobased) lipids and their mixtures can consistently form self-assembled non-lamellar nanostructures, or lipid cubic phases, is not fully explained by previous reports on colloidal nanocarriers (e.g., [59]). These lipid cubic phases have already been found to function as colloidally stable nanocarriers for drug(s) in excess water (e.g., in blood plasma) [59,60]. This fundamental question about the self-assembly mechanism of lipid cubic phases has a physical–chemical explanation: these constituent (biobased) lipids have a propensity to take on a non-lamellar inverse topology. The hydration of the lipid head group, the length of the acyl chain, and the cholesterol concentration all influence this unique tendency of certain surface-active lipids (see below). According to Schwarz and Gompper's review [61], the reason why the lamellar phase predominates at room temperature is that the lipids themselves have rather bulky hydrocarbon chains (e.g., [61–63]), in contrast to many synthetic surfactants, which often have large head groups and form micelles. Spontaneous curvature can be increased, however, by changing the molecular architecture. For example, in order to prevent Coulombic repulsion between head groups, charged lipids can be replaced with similar ones that solely have nonionic head groups; alternatively, lipids with bulkier chains can be added to promote spontaneous curvature. It is common to refer to lipids with spontaneous curvature as "nonbilayer lipids". Therefore, a cubic phase of inverse spherical micelles is frequently found with the concomitant display of significant spontaneous (negative or inverse) curvature [61]. It is noteworthy that there is a general agreement that amphiphilic lipids, having hydrophilic head groups which are weakly hydrated, aid in the formation of an Fd3m cubic phase (also referred to

as phase $Q^{227}$) ([63]; see also [64]). This physicochemical relationship is especially pertinent to the LCM/ND nanoemulsion formulations that were previously discussed [65]: The basic Filmix® (LCM/ND) nanoemulsion formulation is composed of saturated glycerides and cholesterol (and its ester derivatives) [1]; as a result, each (nonionic) amphiphilic lipid in this lipid combination would only have a weakly hydrated, hydrophilic head group. The earlier preliminary conclusion that the dispersed Fd3m micellar cubic phase reflects the most likely or preferable lipid polymorphic shape adopted by the particles in the LCM/ND nanoemulsions is, therefore, supported by the above facts when taken together [1,65]. In addition, regarding the Fd3m cubic phase (or $Q^{227}$) formation and the acyl chain length of the saturated glycerides in the LCM/ND nanoemulsion formulations, it is helpful to also consider related experimental work using biological amphiphilic lipids with saturated acyl chain lengths ranging from 12 to 16 carbon atoms long [see below]. Because the saturated (nonionic) glycerides in the "particularly preferred" lipid mixture—which is used to create LCM/ND nanoemulsions—have acyl chain lengths of 12 carbons (for example, glycerol monolaurate) and 16 carbons (for example, glycerol tripalmitate), we have focused on this particular range of chain lengths [1,50,51]. Therefore, it is pertinent to discuss a study conducted by Seddon et al. [66] on the phase behaviors of a homologous series of saturated phosphoglyceride/fatty acid mixes with chain lengths of C12 and longer. The phase behaviors were examined as a function of water content using X-ray diffraction and colorimetry. According to these researchers, at all (saturated acyl) chain lengths longer than C12 and at all hydration levels, the lamellar phase is suppressed in these lipid mixtures and is replaced by inverse non-lamellar phases [66].

### *4.3. Particle Stability*

With reference to nanocarrier particle stability, the physical characterization of the actual size distribution of the LCM/ND lipid nanoemulsion particles (which mostly represent dispersed lipid cubic-phase nanoparticles; see Figure 1) has previously been discussed in detail [1]. Particle measuring systems (Boulder, CO, USA) produced five distinct optical particle counters that were used to measure the scattered light from such nanoemulsions over various collection angles (In practice, the five counters measured the scattered light over different collection angles using various light sources. The quantity of scattered light depends on the characteristics of the illuminating light, the scattering angle, and the refractive index; hence, the raw data may differ (1) between various counters, (2) during brief data collection periods, and (3) before additional statistical analysis. However, thorough statistical examinations of the generated data verified that the particle concentrations recorded by the five devices were all similar). Nearly 10 billion particles smaller than 0.10 μm were found in each milliliter of the filtered LCM/ND lipid nanoemulsion; over 90% of these particles had a diameter of less than 0.2 μm (refer to Figure 1) [1]. Using the S100 optical particle counter, the influence of concentration on the nanoemulsion size distributions was also ascertained. As a new equilibrium state was reached, we deliberated whether the size distribution might also vary if the total concentration of LCM/ND nanoemulsion material changed (Using the dilution/flow system configured for the S100 equipment, multiple nanoemulsion material dilutions were prepared and then injected into water at varying rates to carry out the experiment. This would enable the attainment of various concentrations in the injecting and final suspensions). The fact that nearly all the measurements were the same suggests that the LCM/ND lipid nanoemulsion was not affected by the various concentration settings in terms of particle size. Furthermore, by utilizing the M65 optical particle counter to measure the nanoemulsion size distribution at various points throughout a 37-day period, the impact of LCM/ND nanoemulsion age on the particle size distribution was ascertained. There was no discernible shift in the size distribution over a minimum of one month [1]. In summary, many analytical approaches were employed to measure the LCM/ND nanoemulsion size distribution in deionized water. Using optical particle counts, the number of particles smaller than 0.1 μm in one

milliliter of this sort of nanoemulsion is approximately 10 billion. Ninety percent or more of the nanoemulsion particles had a diameter of less than 0.2 μm.

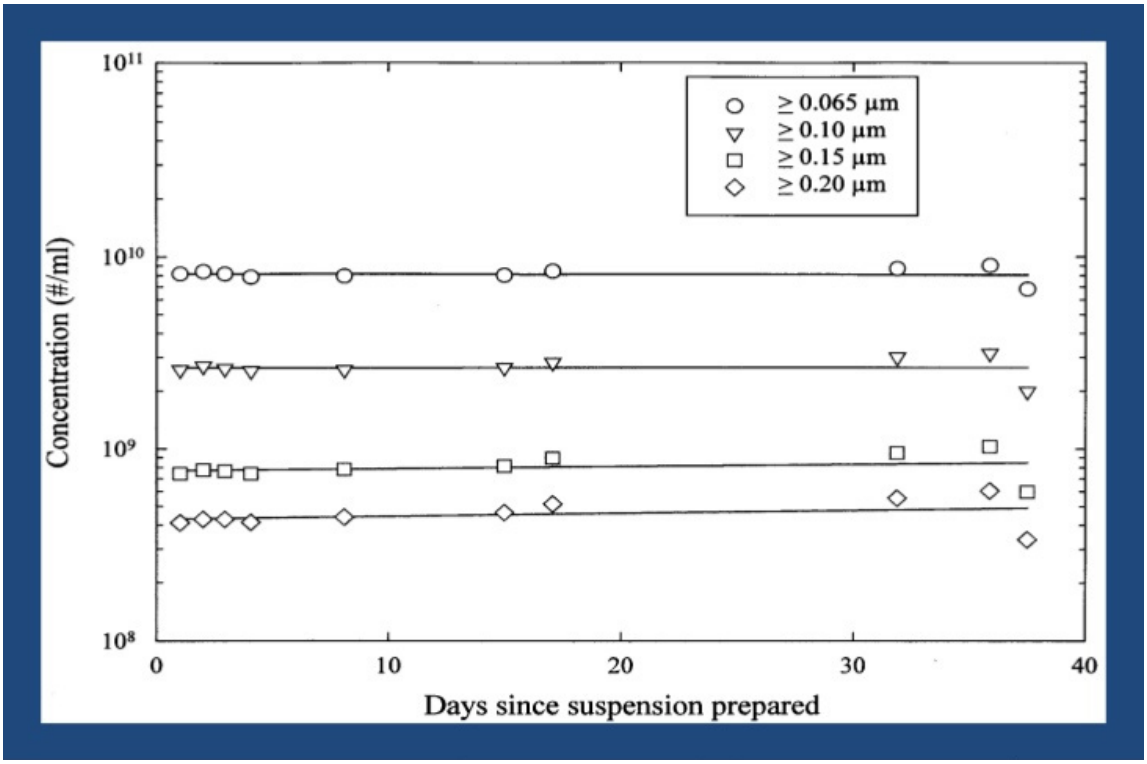


**Figure 1.** LCM/ND nanoemulsion stability over time (Adapted from Ref. [11]).

*4.4. Bio-Safety*

Concerning safety-related issues, there is very little chance of embolus because the LCM/ND lipid nanoemulsion particles were not observed to aggregate or merge into any "superparticle or microbubble-like" structure bigger than 5 μm, either in vitro or in vivo [1]. To be more precise, a previous quality assurance (Q/A) testing of the LCM/ND lipid nanoemulsion involved other particle size analyses, using a Coulter Multisizer for electroimpedance-sensed volumetric sizing. This method consistently produced the following results: more than 99% of the detected nanoemulsion particle population is under 4.5 μm, and all nanoemulsion particles are less than 5.0 μm in diameter when using a Coulter aperture tube with a 50 μm orifice (giving the instrument a nominal particle diameter detection range from ~1.0 to 30.0 μm). Additionally, the Coulter Multisizer's individual nonoptical counting of the LCM/ND lipid nanoemulsion particles—those with diameters greater than or equal to 1.0 μm—repeatedly produced a total concentration estimate of almost $5 \times 10^5$ particles/mL for the nanoemulsion samples. When the LCM/ND nanoemulsion agent is stored at ambient temperature, all the above product parameters hold true for several months, and when the nanoemulsion agent is stored in a refrigerator (not frozen), they hold true for more than a year. Furthermore, using later, more sensitive Coulter Multisizer models (Models II and IIe), the complete particle size analysis (Q/A testing) was repeated on numerous batches of LCM/ND lipid nanoemulsion, and the maximum nanoemulsion particle size remained below 5.0 μm. Nevertheless, data from these more recent Coulter instruments revealed that most of the LCM/ND-lipid-nanoemulsion particle population has a diameter smaller than 1.0 μm (cf. preceding paragr.) [1].

Acute intravenous toxicity investigations of this (isotonic) LCM/ND nanoemulsion agent in dogs and rabbits were carried out at an independent GLP contractor with regard to in vivo testing (Note that the abbreviation "GLP" denotes that the research carried out by the aforementioned contractor complies with 21 CFR Part 58's Good Laboratory Practices

Regulations, which are intended to be submitted to the U.S. Food and Drug Administration in support of an Investigational New Drug Application (INDA). Thus, it is expected from this statement that there are no GLP regulation deviations that could compromise the study's integrity or quality). It was determined that the acute intravenous LD50 in dogs and rabbits was higher than 4.8 mL/kg. Moreover, at a dose of 4.8 mL/kg, no evidence of gross toxicity or mortality was seen. More precisely, 4.8 mL/kg of LCM/ND lipid nanoemulsion were intravenously given to ten rabbits (five males and five females) in the first trial. During this investigation, signs such as decreased activity and increased respiration were noted. Throughout the study, none of the animals perished. Upon terminal necropsy, one animal's lungs were pale. At the same time of terminal necropsy, none of the remaining animals had any more obvious lesions. In a different trial, four dogs—two male and two female—were given an intravenous injection of LCM/ND lipid nanoemulsion at a dose of 4.8 mL/kg. Throughout the investigation, no clinical symptoms were seen. Not a single animal perished at the 4.8 mL/kg dose level. At the time of the terminal necropsy, no animals had any obvious lesions [1].

Using the same (isotonic) lipid nanoemulsion agent, it was found in other animal (range-finding subchronic intravenous) toxicology studies that the following toxicology results were observed at intravenous doses of 0.14 mL/kg given three times per week for six weeks in rats and, separately, at intravenous doses of 0.48 mL/kg given three times per week for three months in rabbits: serum chemistry, liver functions, hematology, clotting profile, adrenal glands, brain, heart, kidney, liver, lungs, marrow, pituitary, spleen, testes, thyroid, or ureters did not exhibit any abnormalities [1]. Finally, the lipids found in the LCM/ND nanoemulsion agent are similar to those found in the commercial products Liposyn III and Intralipid, with the difference being that the LCM/ND nanoemulsion agent is applied at far lower concentrations and dosages than the two clinical treatments that have been approved for use with humans. Intralipid is a fat emulsion designed for intravenous injection; specifically, the neutral triglycerides, di- and monoglycerides, and/or saturated fatty acids are metabolized by the same metabolic routes. Therefore, when compared to the lipid composition of the intravenous fat-emulsion products (liposyn and intralipid) and their respective (intended clinical) dosages, there are significant safety margins for the LCM/ND nanoemulsion in its intended clinical application for targeted drug delivery therapy in humans.

## 5. Concluding Remarks

Aging is the single most important factor in the development of Alzheimer's disease and associated dementias (ADRD), as it is in many chronic illnesses. Cognitive decline and neurodegenerative changes are noticeable with aging, even in older persons who do not develop dementia throughout the course of their lifetimes [67,68]. This suggests that similar pathophysiological mechanisms may be at play. Recent reviews of clinical and translational investigations by Gonzales et al. [67] have established a connection between biological aging processes and the underlying pathophysiology of ADRD. By focusing on the basic mechanisms that underlie biological aging, it may be possible to mitigate ADRD and age-related cognitive decline in a mostly unexplored way [67]. Significant progress has been achieved in determining the pathophysiological mechanisms underlying biological aging and multisystem organ decline in the area of biology concerning aging [67,69]. For example, oxidative stress and systemic inflammation brought on by cerebral vascular risk factors may jeopardize the blood–brain barrier. More recent studies suggest that proinflammatory cytokines may be released in response to a prolonged inflammatory stimulus in the gut, for example through serum amyloid A (SAA). In the meantime, proinflammatory cytokines can reach the brain, inducing glia reactivity, due to the increased BBB permeability brought on by aging and/or malfunction. Similarly, it has been noted that neuroinflammation following traumatic brain injury is a chronic response to an acute injury and is frequently linked to activated microglia and the release of pro-inflammatory cytokines [33,70]. Young adults who suffer moderate to severe head trauma also have a greater-than-two-fold

increased risk of developing Alzheimer's disease or a related dementia later in life. As a result, early (or even proactive) drug targeting toward a major SAA receptor, implicated in SAA-mediated cell signaling processes leading to normal aging brain alterations [67] and/or dementia [71], may provide an effective preventive and therapeutic strategy.

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