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Nano-Liposomes of Crude Soy Lecithin are Effective for Cleaning Fuel-Contaminated Sands and Soils

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Abstract

Large multilamellar lipid vesicles (MLV) and nano-liposomes (small unilamellar vesicles-SUV) made of crude soy lecithin were prepared, characterized and compared for their ability to remove crude oil and jet fuel contaminants from sand and soil samples. The SUV formulation was able to remove 85% of the contaminants, and was significantly the most effective formulation when compared to the other tested lipid products. Raising temperature (from RT to 50°C) and exposure to ultrasonic irradiation further improved SUV cleaning efficiency. The SUV used are highly stable in high salinity concentration (up to 32% W/V NaCl). The crude soy SUV reduces surface tension of the water, in agreement with previous results on highly significant reduction of oil/water interfacial tension (IFT).

These properties make crude lecithin-based SUV a good candidate for the physical cleaning of oil-contaminated soil as well as for accelerating bioremediation of such soil. The use of liposomes composed of such crude lecithin has major advantages: the lecithin is available in quantity at a reasonable cost; fabrication of such SUV on a large scale is straightforward and, most important, such liposomes are biocompatible and environmentally friendly.

Keywords: Small unilamellar vesicles (SUV); Lecithin; Oil-contamination; Biosurfactant; Surface tension

Introduction

Liposomes are known mainly for their medical applications, as is evident from the ~15 liposomal products approved for clinical use [1]. However, liposomes also have many nonmedical applications, as was demonstrated in the four volumes of Handbook of Nonmedical Application of Liposomes [2]. A few of these nonmedical applications are in "Cleantech" for cleaning the environment. We have previously published that liposomes can be used for physically removing oil from contaminated water [3], and for enhancing bioremediation of oil-contaminated soil [4]. In this work we describe an expansion of the use of liposomes for cleaning oil contaminants from soil. The method shown here is a rapid physical cleaning method, using the liposomes as a "washing agent". This rapid method can be used prior to bioremediation, and is expected to improve bioremediation efficiency.

Petroleum contamination of water, soil and sand is one of the most common environmental hazards [5]. Petroleum compounds are highly toxic, causing both immediate and long-term damage to humans, fauna and flora, as well as causing substantial damage to the environment and to groundwater, thereby endangering drinking water reserves [6,7]. In many countries use of land for building and any kind of development is not allowed until the level of contamination is below a threshold of 45 mg/kg [8]. Various methods have been proposed based on physical, chemical and biological techniques for cleaning soils or sands of oil contamination [9]. Most kinds of oil are not soluble in water; to clean the contamination it is necessary to use additives that enable and enhance wetting of the oil [10]. Surfactants that reduce the surface tension enable wetting of the oil and can lead to an effective cleaning process [11]. Gatt et al. [3] found that addition of liposomes to oil spills on water causes an immediate change in the physical state of the oil spreading, from a thin layer of oil that covers a large surface area, to condensed oil droplets, which are easier to remove by skimmers and similar techniques. We have previously shown that nano-liposomes (small unilamellar vesicles, abbreviated SUV) enable wetting of the oil and therefore increase bioremediation [4]. Bioremediation has been found to be environmentally friendly but is a relatively slow process, and not always successful, especially when treating high-concentration oil spills, which have low bioavailability [12,13]. Bioremediation was widely used in cleaning the *Exxon Valdez* oil spill. Long-term follow-up studies showed that the bioremediation was effective in most cases, but a few patches of oil remained even more than 20 years after the spill [12]. This finding indicates that improved or new methods need to be considered.

In this work we tested the feasibility of using liposomes for a rapid method of washing oil-contaminated soils. Such washing of highly contaminated soils and sands can also be a method used prior to the bioremediation to accelerate it. Washing techniques of contaminated soils are widely used in Europe and have recently shown promising results [13]. Washing with biosurfactants may in addition enhance the bioavailability of the oil for bioremediation [11,13].

Surfactants are molecules that due to their ability to reduce surface tension can increase dispersibility and bioavailability of oil. Different natural and synthetic surfactants are being used for washing oil-contaminated soils [14]. Liposomes made from lecithin have the advantage of being more biodegradable than most synthetic surfactants and less toxic [15]. We suggest the use of liposomes as a source for biosurfactants. Liposomes are vesicles composed of amphiphiles that self-assemble due to their polar head and a nonpolar tails [3,16]. This unique "regional" separation results in certain physicochemical properties that force these molecules to self-assemble. The driving force is the second law of thermodynamics and its related hydrophobic effect [17]. Amphiphiles, due to their hydrophobic domain self-assemble, at a certain critical concentration (critical aggregation concentration, CAC). Lecithin like the one used in this study has a CAC in the range of 10^{-10}M [18]. The exact type of the assembly is dependent on the ratio between the cross-section of its hydrophobic and hydrophilic domain which is defined as the "packing parameter" of the amphiphile [17,19]. Individual amphiphiles or amphiphile mixtures that have a packing parameter or "additive

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packing parameter" in the range of 0.74 to 1.0 will form stable liposomes [20-22]. Many, but not all, naturally occurring phospholipids are "liposome-forming lipids" [19]. We confirm previous observations that liposomes reduce the surface tension of the water in which they are present. This is the result of the amphiphiles which built the liposomes being released from the liposomes and spreading as a monomolecular layer at the interface between the high-dielectric-constant polar water and the low-dielectric-constant nonpolar oil. This spontaneous spreading can be measured from changes in tension at the air/water (A/W) interface and the interfacial surface tension for the oil/water (O/W), interface, reaching an equilibrium described as the "equilibrium spreading pressure" [23]. These phospholipids at the A/W or O/W interface enable wetting the oil contaminating the water or the soil [3]. Once phospholipid molecules from the A/W interface interact with the oil phase, some liposomes are dissociated and release additional phospholipids, or alternatively, phospholipid molecules are desorbed from the liposomes; in both cases the liposomes act as a reservoir for the phospholipids that will interact with the oil contaminant and wet it. Thereby these liposomes facilitate the cleaning of the contaminating oil [4]. The wetting process occurs when the phospholipid molecules (that originate from the liposomes) "coat" the oil, with their tails directed/inserted to/in the oil phase and their heads interacting with the aqueous environment [3]. Wetting the oil increases the efficiency of cleaning the oil contamination both by physical and biological (bioremediation) means [10].

In this work we demonstrate that small-unilamellar liposomes (SUV) composed of crude lecithin can be used as bio surfactants for rapid washing of soil and sand contaminated by crude oil and jet fuel.

Material and Methods

Soil and sand source

The samples were a kind gift from Dr. Yoseffa Ben-Asher, Israel Petroleum Institute. The loam and silt were from a field in Israel accidently contaminated by crude oil. Sand was accidently contaminated by jet fuel, Israel.

Lipids

Liposomes were prepared from S-45 soy crude lecithin containing these phospholipids: 45-50% soybean phosphatidylcholines (PCs), 10-18% phosphatidylethanolamine (PE) and a 4% lyso PCs. Typical fatty acid composition of S-45: 58-65% linoleic acid, 12-17% palmitic acid, 8-12% oleic acid, and other minor fatty acids. All PCs used in this study were obtained from Lipoid GmbH Ludwigshafen, Germany.

Hexane

Hexane, 99.5% pure was purchased from Sigma-Aldrich.

Liposome preparation

An SUV dispersion made of crude S-45 at 10% (W/V) lipids was prepared by hydrating the lipids using sterile double distilled water (DDW), in two steps. Firstly the dry lipids were hydrated in DDW to form MLV at 10% (W/V) lipids using a high-shearing homogenizer (Polytron, Kinetica, GmbH, Littan-Luzern, Switzerland). In the second step the MLV were downsized to form nano-liposomes (SUV) using a high-pressure one-step homogenizer, Rannie Mini Lab 8.30 H, at a pressure of 10,000 psi [4,24]. Liposomes were sterilized by filtration via a 0.22-µm-pore-size filter (Millipore).

Liposome size distribution analysis

SUV were tested for size distribution by dynamic light scattering (DLS) using a Coulter N4SD sub-micron particle size analyzer at 90 degrees (Coulter Electronics, Luton, UK). The majority of the SUV population (>95%) was in the range of 30-60 nm. MLV size distribution was measured by laser diffraction particle size analysis (Beckman Coulter LS I3 320 l), which combines multi-wave light diffraction and polarization intensity differential scattering (PIDS polarization of the light). This combination allows determining broad size distributions in the range of 40 nm to 2 mm [24].

Petroleum extraction

Petroleum extraction was performed as described previously [4]. To enable comparison of the amount of residual oil in the samples, all sand and soil samples were dried prior to oil extraction. After extraction and weighing of residual oil, each sample was analyzed for phosphorus to determine that no liposomal phospholipids were extracted [4].

Sample rinsing from oil contaminants

Selected cleaning media included: water alone, dry lecithin (S-45) in water, S-45 MLV, or S-45 SUV. A volume of 150 ml of the cleaning medium was added to 30 g of dried (for 5 days at RT) soil or sand. The suspended sand or soil sample was mixed for 15 min using a rotary shaker at 70 rpm. Then the suspended mixture was centrifuged at 3000 rpm for 15 min in order to allow the sample to precipitate, and the liquid phase was removed from the sample. Samples were then assessed for residual oil by weighing the oil extracted.

Treatment by ultrasonic-irradiation (sonication)

Sonication of oil-contaminated samples mixed with the liposomes was performed in an bath for 20 min at 50°C (Transsonic 460/H, 35 khz, Elma, Bergweis, Austria).

Surface tension measurements

Effect of the SUV nano-liposomes on surface tension of water at 22°C was measured by Paz Oil Company Ltd. (Haifa, Israel) using the du Nouy method [25].

Salinity tolerance

Salinity tolerance was measured by mixing small volumes of the liposomes at specified concentrations of salinity. Stability of the liposomes was checked after 24 h storage at RT by analyzing the size distribution.

Results

Rinsing silt using various lecithin based formulations

Cleaning jet-fuel-contaminated silt was tested by rinsing with various forms of lecithin. It has been shown that lecithin is environmentally compatible and improves the rinsing of oil [26]. The crude soy lecithin is defined by the percentage of phosphatidylcholines (PCs), scaled 10-100% (S-10 to S-100). The higher the PC content, the more expensive is the lipid. For our application it is necessary to find the cheapest lipid that is effective. Therefore we compared lecithin preparations varying in their PC contents (S-10, S-30, S-45 and S-100). Lecithin preparations with PC content lower than S-45 are not efficient (data not shown); thus we proceeded with the S-45, which is both relatively cheap and highly effective, as shown in Figure 1. SUV were also more efficient than MLV for the cleaning of the soil (Figure 1).

Ultrasonic irradiation further improves nano-liposomes' rinsing capacity of different oil-contaminated sand and soil samples

Washing sand and soil with SUV was next tested on three different samples: loam, sand and silt. The loam was highly contaminated with crude oil; silt and sand were contaminated with jet fuel. All samples were rinsed with water or with nano-liposomes (SUV-45,0.5% W/V). Ultrasonic irradiation at 50°C was tested to see if it improves the washing outcome. Our results show clearly (Table 1) that sonication of the samples wetted with SUV S-45 improves the cleaning for all samples tested, reaching 97% removal of the oil in the contaminated sand. These results are in a good agreement with previously reported data [27].

MLV and SUV liposomes are stable in various salt concentrations

Maximal stabilities of MLV and SUV dispersions were measured, by mixing small volumes of the liposomes at specified concentrations of salinity (Table 2). Stability was checked after 24 h.

The results showed that maximal salinity stability tolerance of MLV was reached at 15% NaCl whereas SUV showed maximal salinity stability tolerance up to a concentration of 32% NaCl. Stability in high salinity concentration may be of great importance, as oil-contaminated areas such as oceans, water reserves, soils, and beach sands may have high salt concentrations that inhibit the natural bioremediation [28]. Stability in high salinity may also be an advantage in the long-term natural nontoxic preservation of liposomes [29,30].

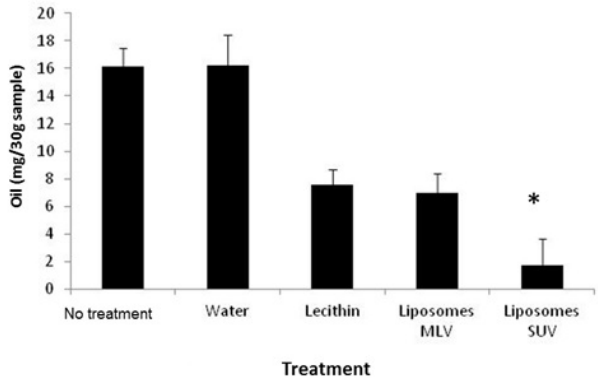

Figure 1: Nano-liposomes (SUV) are efficient for rinsing jet-fuel-contaminated silt. Residual oil was measured after rinsing jet fuel contaminated silt with lecithin, MLV and SUV liposomes, and was compared to rinsing with water (negative control) *$p<0.05$.

Sample/ Rinsing method	Before rinsing mg	Water RT mg	Water+Sonication 50°C mg	SUV RT mg	SUV+sonication 50°C mg
loam	46	20	19	6	3
silt	25	21	20	13	3
sand	15	14	13	1	0.4

Table 1: Amounts (mg/30 g sample) of oil in loam, sand and silt before and after rinsing with SUV at room temperature (RT) or at 50°C with sonication.

Liposome	Liposome concentration %	Maximal salinity tolerance %
MLV	1	15
MLV	5	15
SUV	1	32
SUV	5	32

Table 2: Maximum salinity tolerance of MLV and SUV liposomes.

Liposomes reduce surface tension of water. Increasing concentrations of S-45 liposomes were added to water, and surface tension was measured. The lecithin has a critical aggregate concentration (CAC) of ~10^{-10} M (18). The concentrations we used are much above this value.

The measurements showed that the liposomes reduce the surface tension of the water (Figure 2) at low lipid concentration (already below 0.1%). As discussed in the Introduction, reduction of surface tension indicates the potential of the liposome-forming amphiphiles to be available to interact with the contaminating oil and thereby improve the capacity to remove it.

Discussion

In this work we demonstrate that lecithin-composed nano-liposomes (SUV) are effective for cleaning oil-contaminated soil and sand (Figure 1, Table 1). We compared washing with water only to washing with three forms of S-45 lecithin: lecithin dispersed in water and two types of lecithin-composed liposomes. The results described in Figure 1 demonstrate that, compared to water, all forms of lecithin reduced the amount of oil in the samples substantially. However, the nano-liposome (SUV) dispersion was significantly ($p<0.05$) the most efficient agent, as by a simple washing technique it reduced ~85% of the contaminating oil.

These findings can be explained by the results showing that lecithin liposomes act as surface-active agents, which reduce A/W surface tension in a concentration-dependent manner having saturation kinetics (Figure 2). These results are in good agreement with previously obtained data on the effect of S-45 soy lipids on the O/W interfacial tension (O/W IFT) [29,30].

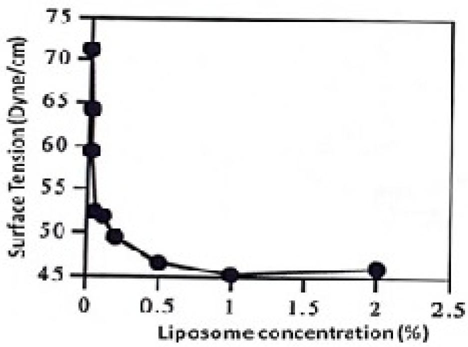

Figure 2: Liposomes reduce surface tension of the water. Surface tension of water was measured at 22°C, with the addition of increasing concentrations of S-45 SUV liposomes (0-2%).

Surface tension relates to a single liquid and gas, while IFT is related to the interface between two immiscible liquids such as oil and water, where there are two forces, one for each liquid. Surface tension is a kind of IFT where the force from a second surface, such as air, is very small or zero. For the A/W interface we measure only the high cohesive forces between water molecules, which result in high surface tension. In order to achieve wetting by water this high surface tension has to be reduced, as was demonstrated using S-45 SUV (Figure 2). By reducing the surface tension at the A/W interface the cohesive forces between water molecules of the surface are reduced, thereby leading to oil wetting. A wetting agent is defined as a substance that reduces surface tension of the liquid. In this respect, the SUV act as a wetting agent of the oil.

Indeed, surfactants, which lower the O/W IFT, were found to increase the oil bioavailability, thus enhancing biodegradation of oi-contaminated soils [31] and predict the surfactant's ability to remove oil from soil by washing [32].

In our previous study, O/W IFT was determined at both 22°C and 60°C using hexadecane as a defined oil contaminant [29]. Under comparable conditions of temperature and concentration, S-45 nano-liposomes (SUV) always reduced the IFT significantly more than S-45 MLV, for example; at 10 mg/ml and 60°C, SUV reduce the IFT to less than 1 millidyne/cm compared with a value of 100 millidyne/cm for MLV.

The highly effective reduction in IFT and surface tension by S-45 SUV helps to explain the high efficacy of the liposomes both for bioremediation and for rinsing of oil-contaminated soils. The surface tension and IFT results also support the data previously published [2,3], showing that the addition of liposomes, and especially SUV, to the water contaminated with oil prevents the oil from being distributed over a large area of the water. It was suggested that reducing the A/W surface tension can improve the cleaning of the contaminated site [32].

Non-aqueous phase liquids such as petroleum hydrocarbons are trapped in porous media by capillary forces due to high oil/water IFT [33].

An explanation as to why SUV are superior to MLV, although both types of liposomes have the same lipid composition, is that the SUV, which are unilamellar and in the nano size range, provide a much larger exposure of the lipids to the media. Therefore availability of lipids to interact with the contaminating oil is much higher than that of the MLV. In addition SUV, due to "frustration" related to difference in packing of lipid molecules present in the two leaflets of the SUV, show a greater tendency to interact with the contaminating oil [19].

Efficiency, costs, stability and toxicity are important factors when choosing a surfactant. Surfactants are molecules that due to their ability to reduce surface tension can increase dispersibility and bioavailability of oil. Many surfactants which are synthetic detergents are effective in washing the soil, reaching efficiency of~95% of oil removal as described by Urum et al. and Urum and Pekdemir [26,32], which is similar to our soy lecithin SUV, however they may cause damage as they may be toxic to the environment. In the Deep Water Horizon oil spill in the Gulf of Mexico, the oil dispersant Corexit® was widely used [34]. Follow-up on the impacts of using this surfactant showed that it became a factor of toxicity for the marine environment [35], and it may also be a risk for human health. Due to the environmental damage, research efforts have focused on biosurfactants that are biodegradable and environmentally friendly [10].

We have found that the S-45 SUV are stable at high salinity (Table 2) and as previously described [29], stable at high temperature. This may have a great advantage for a "green" bio-surfactant [36,37], as our results (Table 1) show that increasing temperature can further improve rinsing results.

Stability in high salinity may be of great importance, as oil-contaminated areas such as the oceans, water reserves, soils and sands (including beaches) may be salty and thus inhibit natural bioremediation [28]. Stability in high salinity may also be an advantage in the long-term natural nontoxic preservation of liposomes [29,30].

Biosurfactants, such as lecithin, which are friendly to the environment, have been shown to reduce oil contamination [32]. Commercial use of many biosurfactants is still limited due to their high cost [11]. Crude lecithin, a byproduct of the production of edible oil and protein from soy and other plants, is readily available in large quantities and at a reasonable cost. Such lecithin is considered an environmentally friendly, nontoxic, biocompatible, biodegradable substance [24,38]. It also has the major advantage that it is classified as "Generally Recognized as Safe" (GRAS) by the U.S. Food and Drug Administration [24,39]. In this work we have shown that nano-liposomes made of crude lecithin are effective for rinsing oil-contaminated soil and sand. This work expands the possibilities of the environmental use of liposomes, which we have previously shown enhance bioremediation of oil-contaminated soils [4].

Conclusions

We have found that the crude S-45 soy lecithin when present in the form of nano-liposomes is an efficient agent for cleaning oil-contaminated sands and soils. Our results indicate that the small liposomes (SUV) are more efficient in cleaning soil than dry lecithin in water and the larger (MLV) liposomes. We assume that smaller-sized particles have better ability to penetrate between the soils particles, as well as to expose a much larger surface area due to their higher surface-to-volume ratio. This is one of the major advantages of nano-particles that serve as one of the pillars of the uniqueness of the nanotechnology field. Exposure of the S-45 SUV-treated soil to high temperature and/or ultrasound irradiation further improves the cleaning. The SUV reduces surface tension and IFT, resulting in wetting of the oil, which may explain the mechanism of action of these nano-liposomes in the physical process of cleaning oil-contaminated soil, as well in the facilitation of its bioremediation. In addition, the use of nano-liposomes composed of crude lecithin has major advantages: crude lecithin is readily available in large amounts and at a reasonable cost, liposomes composed of such crude lecithin are simple to manufacture at high lipid concentration (which can be stored as a concentrate), and in high salt concentration (which reduces its bio-burden). This solution can be diluted at the cleaning site. Finally, but not least, these S-45 SUV are environmentally friendly.

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