Gel Formulation Containing Mixed Surfactant and Lipids Associating with Carboplatin

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The interaction of amphiphilic molecules such as lipids and surfactants with the hydrophilic drug carboplatin was investigated to identify suitable self-assembling components for a potential gel-based delivery formulation. ^{1}H-NMR Studies in sodium bis(2-ethylhexyl) sulfosuccinate (aerosol-OT, AOT)-based reverse micelles show that carboplatin associates and at least partially penetrates the surfactant interface. *Langmuir* monolayers formed by dipalmitoyl(phosphatidyl)choline are penetrated by carboplatin. Carboplatin was found to also penetrate the more rigid monolayers containing cholesterol. A combined mixed surfactant gel formulation containing carboplatin and cholesterol for lymphatic tissue targeting was investigated for the intracavitary treatment of cancer. This formulation consists of a blend of the surfactants lecithin and AOT (1:3 ratio), an oil phase of isopropyl myristate, and an aqueous component. The phases of the system were defined within a pseudo-ternary phase diagram. At low oil content, this formulation produces a gel-like system over a wide range of H_2O content. The carboplatin release from the formulation displays a prolonged discharge with a rate three to five times slower than that of the control. Rheological properties of the formulation exhibit pseudoplastic behavior. Microemulsion and *Langmuir* monolayer studies support the interactions between carboplatin and amphiphilic components used in this formulation. To target delivery of carboplatin, two formulations containing cholesterol were characterized. These two formulations with cholesterol showed that, although cholesterol does little to alter the phases in the pseudo-ternary system or to increase the initial release of the drug, it contributes significantly to the structure of the formulation under physiological temperature, as well as increases the rate of steady-state discharge of carboplatin.

1. Introduction. – Platinum-based anticancer drugs are among the most successful anticancer drugs, and since their discovery many new Pt-based drugs have been introduced [1], some of which, including carboplatin, have been successful in the clinic for multiple cancers [2]. Breast cancer is a particularly devastating disease that continues to take the lives of millions every year [3] and projections suggest that this illness will be particularly prevalent in areas of the globe where surgery coupled by adjuvant chemotherapy, followed by radiation therapy, is not possible. Since routine

radiation facilities are not available in developing countries [4], alternative approaches are needed. Given the potency of the Pt-based drugs combating metastatic tumors [5–8], development of a method for which a vehicle of a slow released carboplatin targeting lymphatic tissue could provide attractive and alternative approaches for treatment [9–11]. Considering the success of cisplatin [12] and several other drugs, new formulations developed with these drugs have been granted increased patent life [13].

Carboplatin is water soluble, but is known to slowly hydrolyze in aqueous solution [14–16]. A polar monoanionic vanadium complex is found to readily penetrate interfaces [17] and reside in the hydrophobic environment of microemulsions prepared from the synthetic surfactant Aerosol-OT (sodium bis(2-ethylhexyl sulfosuccinate; abbreviated AOT; *Fig. 1*), which has very favorable properties [18][19] and is used in many industrial applications [20]. Carboplatin (*Fig. 1*) as a polar and neutral molecule is also likely to associate with the amphiphilic AOT, providing a hydrophobic environment for carboplatin. In the studies described here, we investigated the interaction of carboplatin with AOT and natural lipids, and used this information to develop a gel-formulation vehicle for carboplatin for its intracavitary administration following surgical removal of the tumor.

AOT is a non-toxic synthetic surfactant that forms well-known aggregates such as micelles, reverse micelles, or other more complex structures depending on the specific conditions [21–23]. Suspensions of AOT and organic solvent added in small amounts of H_2O form particles in dynamic suspensions with a known size defined by $w_0 = ([H_2O]/[AOT])$, which, when assumed to be spherical, is directly proportional to the average reverse micellar radius (*Fig. 1*) [24][25]. This system is well-understood, and provides an initial verification for the concept that these types of hydrophobic surfactants and lipids would serve well to wrap the carboplatin drug. To further confirm the concept, a similar system was investigated using a dipalmitoyl(phosphatidyl)choline (also referred to as 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DPPC)-based *Langmuir* monolayer system (*Fig. 1*). Monolayers generated by DPPC lipids are particularly well-behaved and give well-defined isotherms that can readily be used to investigate interactions between the drug and the lipid interface [26–28]. A change in the molecular area of the lipids at the air–H_2O interface in the presence of the drug indicates that the lipids and the drug are interacting. Additionally, a change in the slope of the surface pressure–molecular area curve provides further insight into the strength of the interactions [29]. Combining these studies provides fundamental information supporting a concept of developing a gel-based vehicle for anticancer Pt-based drugs using a combination of natural and synthetic surfactants for the treatment of breast cancer and consequent metastatic lesions.

A wide range of polymer-based gels, many of which are used in topical products for drug administration, are currently used in the clinic, such as *Renagel*® and *Welchol*® [30]. Although the most common approaches involved a range of different polymers, limitations exist when gel-based products are to be delivered intracavitarily [31]. Sterile formulations necessary for intracavitary administration products that conform to a limit test for endotoxins are required. Since cellulosic, acrylic acid, and other polymer-based gels are difficult or impossible to sterilize as powders and cannot be sterile-filtered once combined with water, alternative approaches are needed [32]. As a result, polymeric gels are generally terminally sterilized after final packaging by use of

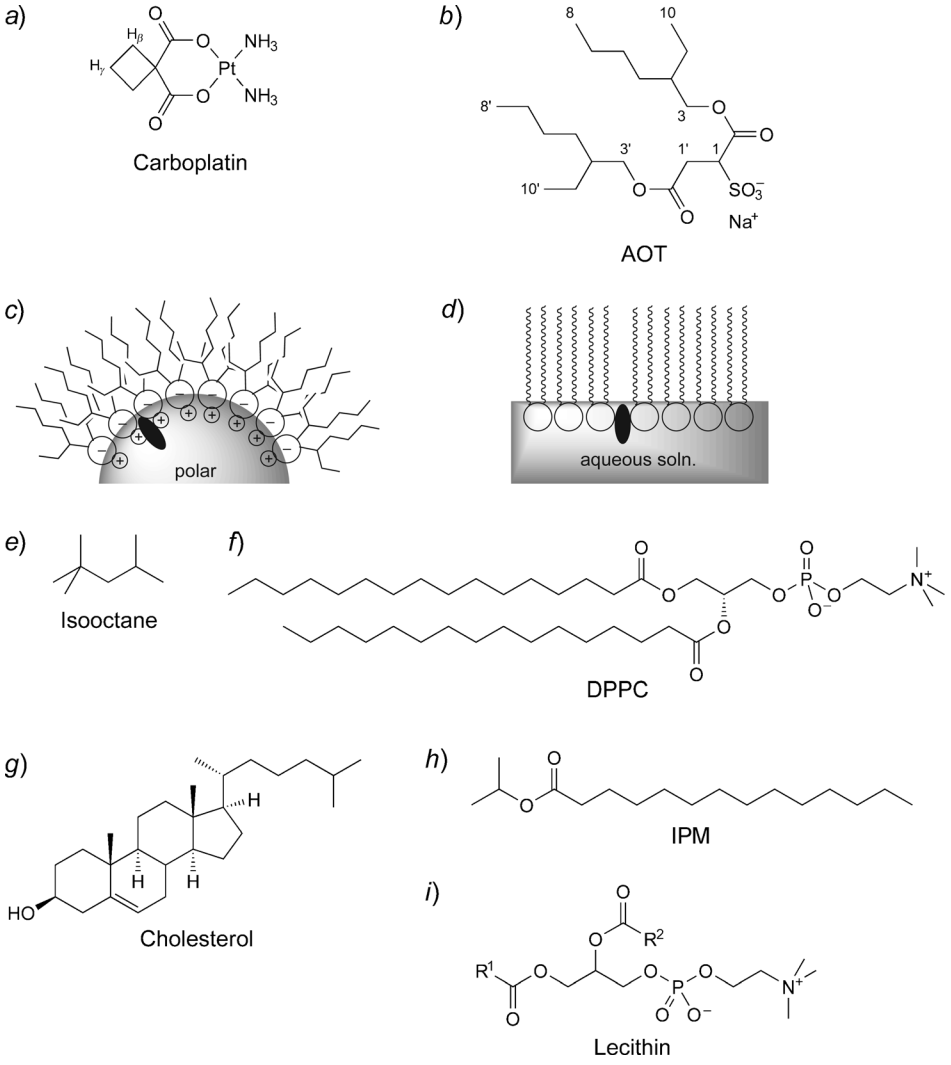

Fig. 1. *Structures of* a) *diammine(1,1-cyclobutanedicarboxylato)platinum(II)* (carboplatin) *and* b) *sodium bis(2-ethylhexyl) sulfosuccinate* (AOT). *Schematic drawing of possible carboplatin location* c) *in AOT/isooctane/H_2O interface, and* d) *in air/1,2-dipalmitoyl*-sn-*glycero-3-phosphocholine* (DPPC)*/H_2O and air/cholesterol/H_2O interfaces.* Carboplatin is shown as a full ellipse. *Structures of* e) *2,2,4-trimethylpentane* (isooctane), f) *DPPC,* g) *cholesterol,* h) *isopropyl myristate* (IPM), *and* i) *the major component of lecithin.*

gamma (γ)-irradiation. However, γ-irradiation always causes significant decreases in the molecular weight of the polymer and often results in the degradation of the active drug. Therefore the application of polymer gels for intracavitary administration is limited to particularly resilient drugs. This fundamental problem of polymeric gels could be overcome by using a surfactant-based gel, and some approaches such as the

matrix-swelling-controlled-release systems have been developed [33][34]. It was the aim of this work to identify a surfactant-based gel system that could be sterile-filtered and transformed from a fluid to a gel with the addition of water. An implantable system, allowing the direct drug action combined with a gradual release system for chemotherapeutic drugs, would provide an alternative for a localized treatment option as well as possible preemptive post-surgical treatment [35].

A range of natural and synthetic surfactants have been used for drug delivery with the preparation of the gamut of microemulsions and emulsions [36][37]. Natural lipids including triglycerides, sphingolipids, and phospholipids have been widely used for preparation of formulation vehicles for anticancer [38][39] and anti-inflammatory agents [40][41]. Such vehicles are currently employed in drugs such as *Neoral* (cyclosporine) [42] and *Lipoplatin* (cisplatin) [39]. AOT has very favorable properties [18][19] and is used in many industrial applications [20]. The gelling properties of AOT are particularly favorable for preparation of gels [43] and, when combined with natural lipids, these systems have potential to surpass the properties of known formulations. Preliminary animal studies [44] confirm that these microemulsions can successfully deliver active drug to the animal after administration.

Since most chemotherapeutic agents are toxic at the level needed for effective treatment [45], the ability to target a drug would significantly decrease the systemic toxicity of the agent [35][46]. Increasing interest in surfactant-based drug-delivery formulations over the past few decades underline the potential benefits and decreased toxicity of such vehicles [47][48]. Thus, incorporation of targeting agents in formulation can significantly enhance how the drugs are tolerated in the patients. Targeting of anticancer drugs is particularly critical considering the toxicity of these agents. Cholesterol has been reported to effectively target the lymphatic tissue and has been used in several drugs investigated in clinical trials [49][50]. Potential formulations for use in the clinic contain increased cholesterol concentrations presumably because of increased drug-delivery potential to the lymphatic tissue [51]. Currently, one of these products, *Lipoplatin*, is being used in the clinics [39]. The vehicle described in the work was targeted for intracavitary administration following surgical removal of the tumor, and cholesterol was added as a biocompatible targeting agent.

In this work, ^{1}H-NMR spectroscopy was used to characterize the interaction of carboplatin with a hydrophobic surfactant to document the inherent stability of such interactions, despite the carboplatin solubility in H_2O. In addition, the interaction of carboplatin with a DPPC lipid monolayer was demonstrated, supporting the studies obtained with the AOT model system. We also investigated the effect of cholesterol on the *Langmuir* monolayers, and found that carboplatin is also interacting with cholesterol. The combined affinity of carboplatin for both AOT and phospholipids was used to design a non-toxic mixed surfactant–lipid-based gel vehicle for intracavitary administration of carboplatin in sites after tumor removal. The phases of the formulation with and without cholesterol (*Fig. 1*) were characterized. We demonstrated that both high (2%) and low (0.2%) cholesterol concentration in the gel has limited effects on the gelling properties of the vehicle.

Polarized light microscopy was used to confirm the nature of the phases. The rheological characterization showed that the surfactant-based gel has a range of physical properties, and that addition of cholesterol enhances the structure of the gel at

physiological temperatures. The gel preparation significantly prolonged release of the drug in the absence or presence of cholesterol. Combining these studies provided data demonstrating the inherent association between carboplatin and hydrophobic surfactants and lipids. We did observe a small, but consistent effect on drug release of cholesterol, which offers a potential modulation of the administration rates for cases where the site of the tumor limits surgical options. We present the characterization of an AOT and lecithin (*Fig. 1*) surfactant blend system forming the basis of an implantable surfactant-based gel system for chemotherapeutic action containing a targeting agent.

2. Results and Discussion. – *Probing the Interaction of Carboplatin with AOT/Isooctane Microemulsion by Using ^{1}H-NMR Spectroscopy.* The nature of interaction of carboplatin with a simple AOT/isooctane interface was investigated to document that drug molecule is not simply located in the aqueous phase. Various additives in AOT/isooctane microemulsions were previously investigated by ^{1}H-NMR spectroscopy [17][52]. Carboplatin (*Fig. 1*) was incorporated into AOT/isooctane microemulsions by loading up to 2.3 mg of the drug per 1 ml of microemulsion. Formulations were prepared from 0.5M NaAOT solution in isooctane and 0.04M carboplatin aqueous stock solution. Information on the location of the drug molecule in the microemulsion was obtained by monitoring the H-atoms on the cyclobutane ring in carboplatin that are not overlapping with signals from the AOT molecules using ^{1}H-NMR spectroscopy.

In *Fig. 2*, the spectra of 40 mM carboplatin stock solution in H_2O at pH 7.1 (*a*) and in AOT reverse micelles (*b* and *c*) are shown. Cyclobutane resonances of carboplatin in aqueous solution appear at 1.89 ppm (H_γ) and 2.87 ppm ($H_{\alpha,\beta}$), and the broad ammine resonance at 4.20 ppm (NH_3), in agreement with literature [14][53][54]. In reverse micellar solutions, NH_3 and H_γ signals overlap with AOT H-atom signals, however; $H_{\alpha,\beta}$ signal can be resolved (*Fig. 2*, *b* and *c*). A small downfield chemical shift of $H_{\alpha,\beta}$-atoms in microemulsion (2.92 ± 0.01 ppm) suggest that carboplatin is associating with the AOT polar interface near the AOT polar headgroups, in agreement with downfield chemical shift reported for $[VO_2(dipic)]^-$ in AOT/isooctane microemulsions [55]. An upfield chemical shift in the past has been interpreted to indicate molecule penetration deep into the non-polar surfactant region, whereas no change in chemical shift suggests that molecule is residing in the H_2O pool [56][57]. Carboplatin uptake into the non-polar isooctane region was ruled out, since drug solubility in isooctane is below the limit of detection, even after extensive sonication with isooctane (spectrum not shown). Since $H_{\alpha,\beta}$ resonance was not affected by the H_2O pool size (*Fig. 2*, *b* and *c*), the observed effects were not attributed to changes in micropolarity [58].

A small but detectable change in the ^3J(H,H) coupling was observed, when carboplatin was transferred from aqueous solution ($^3J(H,H) = 8.1 \pm 0.1$ Hz) to the microemulsion ($^3J(H,H) = 7.3 \pm 0.1$ Hz) system. Changes in coupling constants in cyclobutane rings of a 1 Hz magnitude correspond to a change of *ca.* 10° in the dihedral angle reflecting the conformational change in ring puckering [59]. The decreasing coupling constant presumably indicates changes in the cyclobutane ring conformation due to interaction with the interface. Our results showed that carboplatin was associating with the AOT interface, supporting the expectation that formulations containing AOT will associate with carboplatin.

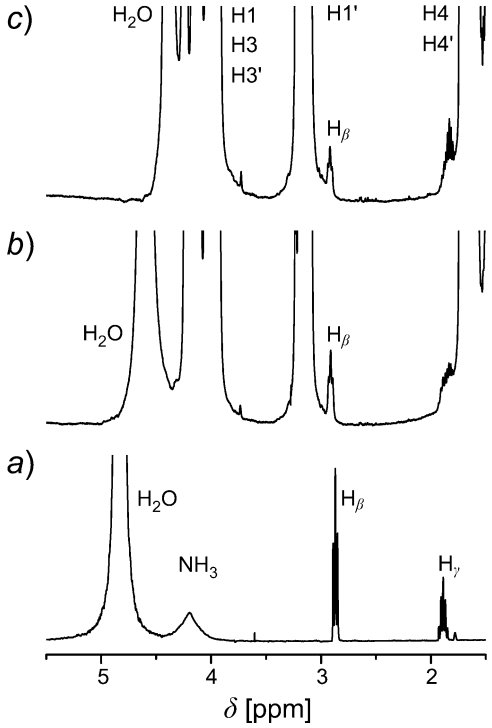

Fig. 2. *^{1}H-NMR Spectra* (400 MHz) *of* a) *0.04*M *aqueous stock solution of carboplatin at pH 7.1, and* b) *and* c) *spectra of carboplatin loaded in AOT/isooctane reverse micelles.* Microemulsion solutions were prepared from *b*) 0.856 ml of 0.50M AOT in isooctane and 144 µl of 0.040M aqueous carboplatin stock solution ($\omega_0 = 20$), and *c*) 0.928 ml and 72 µl of AOT, and carboplatin stock solution ($\omega_0 = 10$).

Probing the Interaction of Carboplatin with DPPC and Cholesterol in Langmuir *Monolayers.* Monolayers of DPPC and cholesterol were formed at the air–H_2O interface, and the interactions of these lipid layers with carboplatin were investigated. The surface pressure–area curves of these lipids were determined on H_2O and in the presence of 0.2 mM of carboplatin (*Fig. 3*). The presence of carboplatin increases the molecular area of DPPC (*Fig. 3*,*a*) consistent with carboplatin penetration into the lipid interface. The initial steeper slope in the surface pressure–area curve in the presence of carboplatin shows that the DPPC molecules become more rigid, supporting the interaction between DPPC and carboplatin. However, the later part of the curve, the steeper slope for the DPPC, compared to the DPPC–carboplatin suggest that the carboplatin is increasing the fluidity of the DPPC layer, consistent with at least partial carboplatin penetration into the lipid layer. Both effects indicate that carboplatin is interacting with the DPPC molecules and monolayer.

Self-assembly of cholesterol is also strongly affected by carboplatin in the subphase, as shown in *Fig. 3*,*b*. Cholesterol forms a very rigid monolayer on an aqueous subphase (*Fig. 3*, *b*), and the very steep slope in the surface pressure–area curve reflects the low compressibility of cholesterol layer. The drug dramatically increases the surface area consistent with carboplatin penetration. In the presence of carboplatin, the isotherm

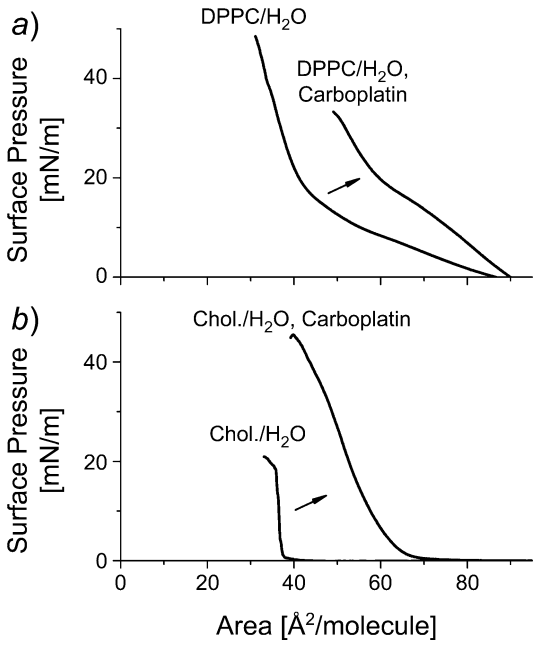

Fig. 3. *Surface pressure–area isotherms of* a) *DPPC and* b) *cholesterol spread on H_2O and on 0.20 mM of aqueous carboplatin subphases at 25°*

(*Fig. 3*,*b*) indicates that cholesterol self-assembled structures are more compressible, consistent with significant interaction between carboplatin and cholesterol, and with carboplatin disrupting *Van der Waals* interactions and the H-bonding in cholesterol. Thus, our data indicate that carboplatin is interacting with both the DPPC and cholesterol interfaces.

The combination of ^{1}H-NMR and *Langmuir* monolayer results suggest that carboplatin will non-covalently interact with AOT, DPPC, and cholesterol interfaces. These results provide impetus for further studies of drug-delivery formulations based on these bio-compatible components.

Phase Behavior of Isopropyl Myristate (IPM)*/(AOT/Lecithin 3:1)/H_2O Formulation.* The formulations of IPM (*Fig. 1*) with and without cholesterol (oil), AOT/lecithin 3:1 mixture (surfactant), and H_2O were examined. The formation of one- and two-phase systems were characterized visually, by polarized light microscopy and rheology. In *Fig. 4*, we show the pseudo-ternary phase diagrams with composition regions where optically transparent liquid (TL), opaque liquid (OL), and gel (G) are observed. The region with large oil and surfactant amount, and low amount of H_2O exhibited optical transparency, a characteristic of reverse microemulsions (L_2). A smaller region, transparent liquid phase with high H_2O content, and low oil and surfactant contents, most likely represents the region of micellar (L_1) aggregates. The remaining regions of opaque liquids were identified as unstable emulsions, transition phases, and mixtures of liquid and gel phases. The gel phase formed in mixtures with 10% or less of oil content and 20% and more of H_2O. This region of gelling remained, for the most part, optically

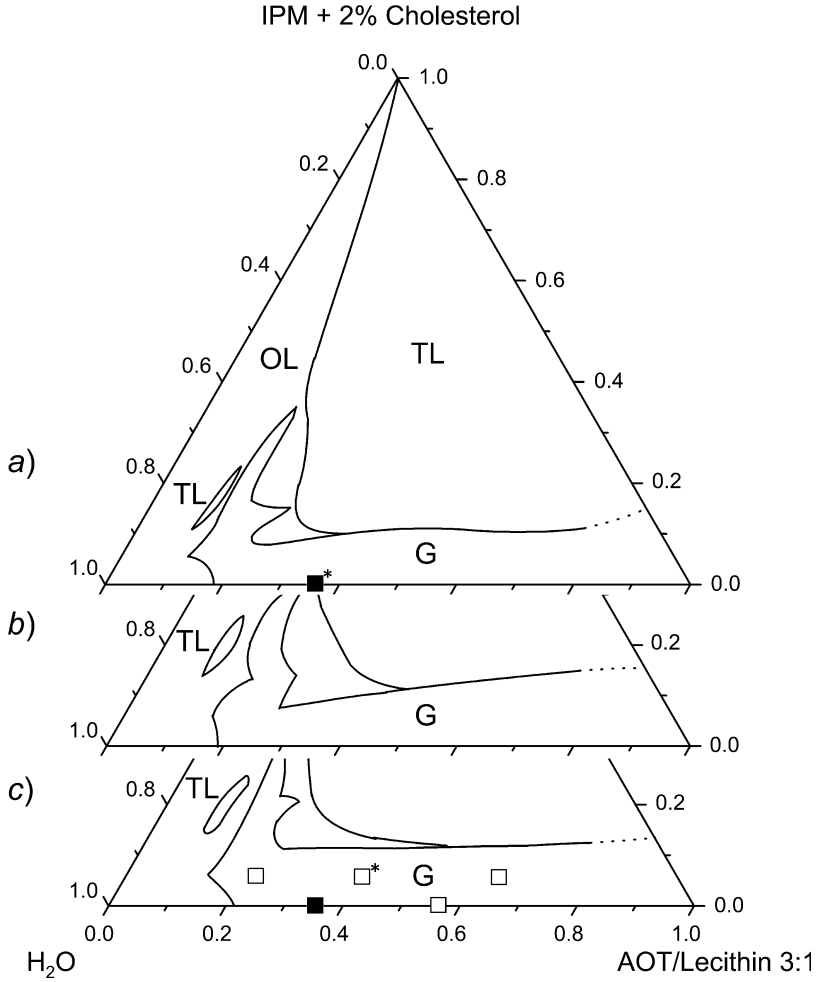

Fig. 4. a) *Pseudo-ternary phase diagram of formulation of H_2O, IPM with 2% of cholesterol, and surfactant* (AOT/lecithin 3:1). Two slices of pseudo-ternary diagrams with cholesterol amounts of *b*) 0.2% and *c*) 0% in mixture with IPM are shown. Phase regions, denoted as transparent liquid (TL), opaque liquid (OL), and gel (G) were identified from samples prepared in triplicates and incubated at 25° for 24 h. Selected samples were characterized by polarized light microscopy (*) and rheology (□). Systems shown as filled-in squares were used in carboplatin release studies.

clear in the cholesterol-free or parent formulation. A distinct variation in appearance, viscosity, and character was observed within the gelling region.

The addition of cholesterol at low (0.2%; *Fig. 4*,*b*) and high (2%; *Fig. 4*,*a*) concentrations has a limited effect on the phase boundaries, with a slight extension of the gel formation region towards the high H_2O content. The (AOT/lecitin)/IPM/H_2O system shows similar phase regions as the AOT/isooctane/H_2O [60] and other surfactant systems reported previously [61][62]. The region of gel formation is

commonly observed in compositions with high surfactant and/or high aqueous content [63][64].

Polarized light microscopy images (*Fig. 5*) under tenfold magnification are shown for formulations marked in *Fig. 4* by the asterisks. In *Fig. 5*,*a*, we show a representative photograph of a birefringent sample with composition of 35% of AOT/lecithin 3:1 and 65% of H_2O, documenting the structural mesophase order, typical for a gel [65]. Addition of 2% of cholesterol to the formulation resulted in the cholesterol crystallization as shown in the image of formulation with 2% of cholesterol, 34.4% of AOT/lecithin 3:1, and 63.7% of H_2O (*Fig. 5*,*b*). The planar crystals observed in *Fig. 5* are characteristic for cholesterol [66], and randomly shaped structures are likely cholesterol and surfactant aggregates. Aggregates of cholesterol have previously been reported to form in AOT solutions with cholesterol in isooctane [67]. Summarizing, we have documented gel-phase formation in the 3:1 mixture of AOT and lecithin surfactant blend.

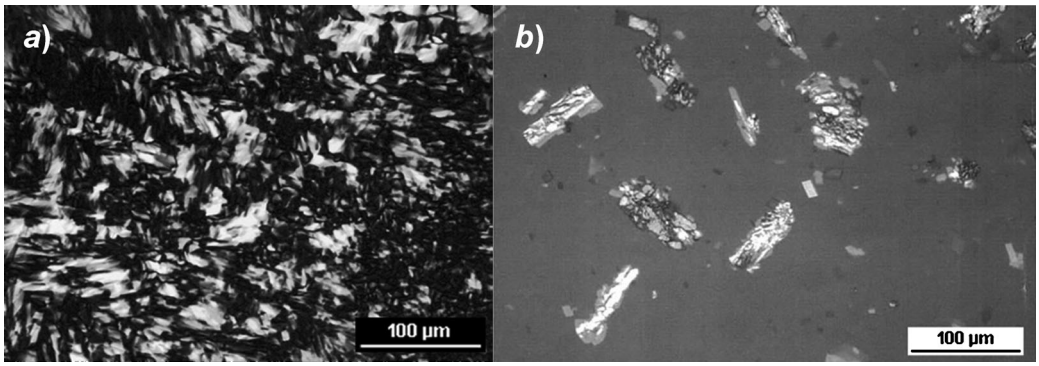

Fig. 5. *Polarized light microscopy image at 10× magnification of* a) *40% of AOT/lecithin 3:1, 5% of IPM, and 55% of H_2O system and* b) *2% of cholesterol, 34.4% of AOT/lecithin 3:1, and 63.7% of H_2O.* Image *b* was taken 4 weeks after sample preparation. The thin plate-like structures are crystals of cholesterol, whereas large irregular crystals are likely cholesterol and AOT aggregates.

Phase behavior for the (AOT/lecithin)/IPM/H_2O system meets all of our requirements for an intracavity delivery system. Compositions formulated in the transparent liquid phase readily flow through a 0.2-µ sterile filter. IPM to AOT/lecithin 3:1 transparent liquid ratios ranging from 75:25 to 20:80 can be aseptically blended with sterile H_2O to form surfactant-based gels. The resulting gels can incorporate up to 80% of H_2O and contain as little as 15% of AOT. This concentration level of AOT will be tolerated after intracavity administration, based on rat studies using the 35% of surfactant blend, 0% of IPM, and 65% of H_2O which contained 26.25% of AOT [44].

Rheological Characterization of Formulation Gels. Rheological properties of IPM with and without cholesterol (oil), AOT/lecithin 3:1 (surfactant), and H_2O formulations were characterized to determine gel response to the mechanical stress. In *Fig. 6*, we show shear stress curves with increasing shear rate for formulations of 35% of AOT/lecithin blend 3:1 and 65% of H_2O, and 2% of cholesterol, 34.4% of AOT/lecithin 3:1, and 63.7% of H_2O at 25° (*Fig. 6*,*a*) and 37° (*Fig. 6*,*b*). These gels exhibit hysteresis in shear curves with counterclockwise profile, suggesting the ordering in gel structure

upon increasing shear rate. The significantly smaller hysteresis at physiological temperature implies smaller restructurization of the gel than at room temperature. The flow curves at 25° (*Fig. 6,a*) indicate that addition of cholesterol has no significant effect on the rheological behavior of the gel. At 37° (*Fig. 6,b*), however, cholesterol modifies the gel structure, as documented by significantly increased hysteresis loop. Gels with high surfactant and oil content, such as 65% of AOT/lecithin 3 : 1, 5% of IPM, and 30% of H_2O exhibited the clockwise hysteresis profile, suggesting structural degradation with increasing shear rate. Increasing amount of H_2O in the gel produced soft gels with the hysteresis loop inversion during the sheare rate ramp procedure (data not shown).

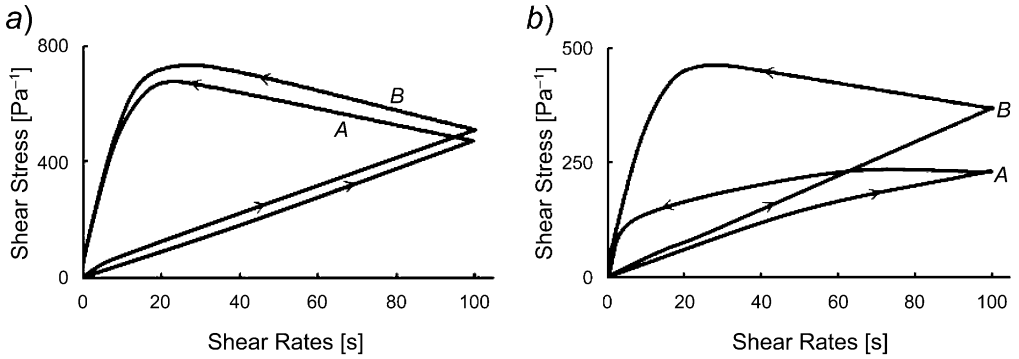

Fig. 6. *Hysteresis loops of shear stress* vs. *shear rate profiles of two formulations, the parent formulation of 35% of AOT/lecithin 3 :1 and 65% H_2O* (*Curve A*), *and 2% of cholesterol, 34.4% of AOT/lecithin 3 :1, and 63.7% of H_2O formulation* (*Curve B*) *at both 25°* (*a*) *and 37°* (*b*).

We conclude that, at physiologically relevant temperatures, cholesterol-enriched gel maintains its structure with shearing more readily than in the formulation without the cholesterol, and formulation's ability to retain its structure under a range of conditions would be beneficial for intracavitary delivery systems. The formulation of 35% of AOT/lecithin blend 3 : 1 and 65% of H_2O also exhibits a definitive shear thinning (pseudoplastic) behavior throughout the examined shear rates (*Fig. 7*). This behavior is also observed in the formulation of 2% of cholesterol, 34.4% of AOT/lecithin 3 : 1, and 63.7% of H_2O at ambient and physiological temperatures (*Fig. 7*). Surprisingly, gel saturation with cholesterol had only limited impact on its dynamic properties. The pseudoplastic behavior of a gel is beneficial for its potential application *in vivo*, warranting formulation administration *via* a sterile syringe. Intracavity gels can be placed at the surgical site by a canula or piece of flexible sterile tubing. For greatest versatility, the gel needs to flow through a relatively narrow gauge opening, perhaps as small as an 18-gauge canula (inner diameter of 0.838 mm). As depicted in *Fig. 7*, these gels are of relatively low viscosity and tend to thin upon experiencing the shear of application. This will allow for intracavity placement of the gel even after completion of a minimally invasive procedure.

In vitro *Release of Carboplatin from AOT/Lecithin/H_2O in PECF Buffer.* A clear formulation that is a single-phase gel with defined yet pliable structure was chosen for

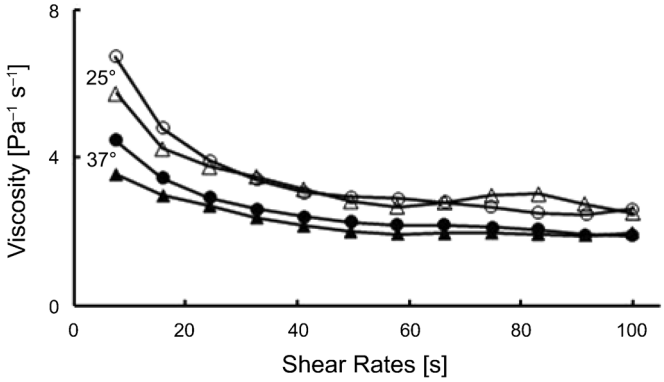

Fig. 7. *Formulation viscosity at 25°* (open symbols) *and 37°* (full symbols) *for 35% of AOT/lecithin 3 : 1 and 65% H_2O formulation* (circles), *and the 2% of cholesterol, 34.4% of AOT/lecithin 3 : 1, and 63.7% of H_2O formulation* (triangles). The data were taken while increasing the shear rate from 0 to 100 s^{-1} in 120 s.

drug-release studies. The formulation with a weight-% composition of 35% of surfactant blend (AOT/lecithin 3 : 1) and 65% of H_2O was chosen for further characterization for carboplatin release studies. Carboplatin release profile (*Fig. 8*) was determined from the 2% of cholesterol, 34.4% of AOT/lecithin 3 : 1, and 63.7% of H_2O formulation, and from the parent formulation, without cholesterol (35% of surfactant blend (AOT/lecithin 3 : 1) and 65% H_2O) at 37° and pH 7.7 through a semi-permeable membrane into the pseudo-extracellular fluid (PECF), mimicking wound fluid [68]. Drug release from these vehicles was compared to an aqueous control solution without oil and surfactant. The release of the drug from the aqueous solution follows the *Higuchi* model [69] ($C_{drug} = kt^n$, $n = 0.53 \pm 0.08$) during the first 8 h with gradual transition into zero-order release. Drug-release half-life time from aqueous control solution is 3.2 ± 0.2 h. Carboplatin release from gels is significantly longer as shown in *Fig. 8*. After initial burst with 13–17% of drug release in 0.5 h, likely originating from drug leaching out of a swollen gel, the transition into zero-order regime is observed for both 2% cholesterol and cholesterol-free parent formulations. Zero-order release suggests that drug leaching is accompanied by gel swelling and/or degradation [70]. Surprisingly, the addition of cholesterol to IPM reduced the carboplatin half-life from 55 ± 8 h in parent formulation to 28.4 ± 1.5 h in the 2% cholesterol formulation. Although drug release *in vivo* is likely to be even slower, we conclude that, depending on the dosage regiment, the drug-release rate can be attenuated by the variation of cholesterol amount in the gel.

Interestingly, at physiological temperature, the cholesterol-enhanced gel displayed initial burst and release of drug slightly greater than the parent formulation without cholesterol. This is in contrast to the anticipated ordering effect reported by cholesterol in other systems [71]. Cholesterol has been shown to decrease the thermal sensitivity of a gel by decreasing the distance between polar head groups [72], and such interactions could deter the mechanic properties of a gel. It is the fluctuating traits that allow for the flexibility of gel to swell in an aqueous environment [63]. By decreasing the ability of

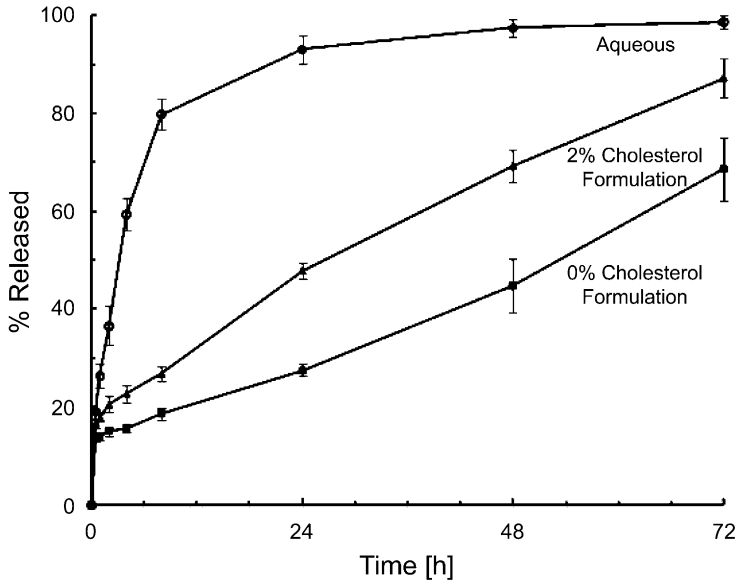

Fig. 8. *Percentage of the carboplatin release over time from the 10 mg/ml aqueous solution* (●)*, 35% of AOT/lecithin 3 : 1 and 65% H_2O gel* (■)*, and 2% of cholesterol, 34.4% of AOT/lecithin 3 : 1, and 63.7% of H_2O gel* (▲). Both aqueous and gel samples were loaded into dialysis vials and allowed to diffuse into wound fluid (PECF) [68] solution. Error bars indicate standard deviations of three measurements.

formulation to swell, the gel initially degraded at an accelerated rate releasing a higher amount of drug more quickly.

In summary, the drug release from the gels with and without cholesterol follows the trends observed in matrix-swelling-controlled-release systems [33]. When the gel formulations are placed in a largely aqueous environment, the influx of this aqueous component induces swelling and increases the aqueous solvent content within the formulation. Initially, there is an acute burst of drug release on the outer regions of the gel, then the solvent volume increases within the gel enabling the drug to diffuse through the degrading surfactant structure into the external environment, prolonging the therapy [35]. This pattern of discharge permits for acute treatment at the site, as well as a prolonged after-treatment. However, unlike implantable release systems [35][73], no second invasive procedures are necessary, because the gel degrades in the physiological environment.

3. Conclusions. – Carboplatin is a polar molecule that has inherent affinity for hydrophobic environments, and, as a result, it interacts with lipid interfaces. In this report we described studies that characterize this interaction, and then we continued to capitalize on the association, and developed a surfactant-based delivery formulation, using the desirable properties of the synthetic surfactant and the natural lipid. The objective was to generate a stable gel system capable to carry drug upon intracavitary administration. The pseudo-ternary phase diagram of the AOT/lecithin/IPM/H_2O system investigated has many characteristics advantageous for a successful drug-

delivery vehicle. Phase diagrams were constructed to describe the regions of gel formation in the presence and absence of cholesterol. The addition of cholesterol to the formulations produced minimal impact on the regions of phase diagram. Having a reservoir of cholesterol may be important for cholesterol to exert its targeting properties *in vivo* [51]. The rheological properties for the chosen formulation show that the structural stability of the system is significantly increased at physiological temperatures. The cholesterol-enriched gels maintained an increased ability at physiological temperatures in comparison to the cholesterol-free formulations demonstrating that these gels will be more resilient at physiological conditions. The release profile of a formulation containing 10 mg/g carboplatin with 2% cholesterol depicts an initial spike in drug release, providing for rapid treatment to the insertion site. Following this, an approximately linear release trend is observed through 72 h. The addition of cholesterol increased the burst release, thus providing an initial higher drug level, establishing treatment parameters in addition to its targeting function. The combination of a natural lipid (lecithin) and surfactant (AOT) is found to produce a formulation that can effectively be used for intracavitary administration of anticancer drugs.

D. C. C. and *D. R. W.* thank the Colorado State University, Cancer Supercluster, for funding this project. We thank *Jacqueline Harding* for preliminary work on these systems. We also thank *TOLMAR Inc.* (Fort Collins, CO) for access to the equipment to carry out the rheological studies.

Experimental Part

1. *Materials.* Refined lecithin, isopropyl myristate (IPM; 98% purity), and cholesterol (95% purity) were purchased from *Alfa Aesar* (Ward Hill, MA), and dipalmitoylphosphatidylcholine (DPPC) powder was from *Avanti* (Alabaster, AL). High-purity solvents (hexane, i-PrOH, and isooctane) were used throughout. Carboplatin was purchased from both *Hospira* and *Aldrich*. These materials were used without further purification. Sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was purchased from *Sigma-Aldrich* (St. Louis, MO) and purified as reported in [74] to remove an acidic impurity. Deionized water (*Barnstead E-pure* system) with specific resistivity of 18 MΩ cm was used for sample preparation.

2. *The ^{1}H-NMR Spectroscopy.* The microemulsions of carboplatin in AOT/isooctane were prepared by the injection of the 0.04M carboplatin aq. stock soln. (pH 7.0) into 0.5M Na(AOT) stock in isooctane to yield translucent single-phase solns. The ^{1}H-NMR spectra were acquired at 21° on a *Varian Inova 400* spectrometer operating at 400.131 MHz, using the unlocked mode settings without ^{2}H lock, as reported in [74] for these complex samples, because the addition of ^2H_2O oxide would significantly change the system. Aq. samples were referenced to 0.000 ppm against external DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) in D_2O, and AOT/isooctane/H_2O samples were referenced against isooctane signals (0.905 (Me, *s*), 0.922 (Me, *d*), 1.146 (CH_2, *d*), and 1.676 ppm (CH, *m*)). The chemical-shift reproducibility was within 0.002 ppm.

3. Langmuir *Monolayer Film Preparation and Surface Pressure–Area Isotherms. Langmuir* monolayer films were prepared using a *Kibron μTrough S-LB* (Helsinki, Finland). The subphase (20 ml) consisted of H_2O or a 0.2 mM aq. soln. of carboplatin (dissolved by sonication for 20 min). DPPC and cholesterol were dissolved in hexane/i-PrOH 3:2 to yield 2 mg/ml (DPPC) and 1 mg/ml (cholesterol) before application of *ca.* 19 nmol to the subphase. Monolayers were allowed to equilibrate for 15 min before compression to ensure complete solvent evaporation. Trough barriers were computer controlled to allow for uniform compression of the lipid layer. Surface pressure–area isotherms were taken at 25° and at an average rate of 8 Å^2/molecule/min. The surface pressure was measured with a *Wilhelmy* wire interfaced to a computer.

4. *Microemulsion Preparation and Phase Diagram Construction.* All mixture compositions in this work are given as weight-%. Samples were prepared *via* two different methods depending on the oil

content. Gels with 25% or more of IPM were prepared from one master stock soln. consisting of 25% of IPM and 75% of AOT/lecithin 3 : 1. Two additional stock solns. were prepared by addition of cholesterol to the master stock soln. to yield final concentration of 0.2 and 2% of cholesterol in IPM. The stock solns. were heated up to facilitate dissolution. All three stock solns. were transparent viscous liquids after cooling them to r.t. The 0.25 g of the initial IPM and AOT/lecithin stock soln. was combined with an appropriate amount of IPM, and titrated by incremental addition of 25, 50, and 100 µl of H_2O. Samples with IPM amounts below 25% were prepared individually, directly weighting out AOT, lecithin, IPM, cholesterol, and adding the required volume of H_2O. Qual. visual observations were recorded during the titration series and on samples prepared individually at ambient temp. (23–25°). Selected samples near the phase boundaries were incubated for 24 h at 25° and 37° to ensure the equilibrium conditions and to identify changes in the phase diagram at physiological temp. All samples were stirred for uniformity with a magnetic stir bar. The phase diagrams were constructed with Origin 7 software. Polarized light microscopy images and rheological measurements were conducted on selected samples to characterize phases in the pseudoternary system.

5. *Polarized-Light Microscopy.* Selected samples were studied by polarized light microscopy to determine birefringence in the formulation. Images were taken on *Olympus BK51* polarizing-light microscope interfaced with the Image-Pro Plus (version 6.3) software. All samples were examined at tenfold magnification.

6. *Rheological Characterization of Gels.* Rheological measurements were performed at both $25 \pm 0.2°$ and physiological temp. $37 \pm 0.2°$ with a *Brookfield R/S-CPS+* rheometer equipped with cone and plate spindle geometry. Sample loading ensured negligible shear prior to a measurement. The up-down rate ramp was applied to a sample with shear rate increasing from 0 to 100 s^{-1} in 120 s, and immediately back from 100 to 0 s^{-1} over 120 s. The Rheo 3000 software (version 1.2.1307.1) was used to generate flow curves and viscosity profiles.

7. *Carboplatin-Release Measurements.* Carboplatin-release studies were carried out at 25° and 37° on the 35% surfactant (AOT/lecithin 3 : 1) and 65% H_2O gel formulation. The 2.7–3.0 mg of carboplatin were typically loaded into formulation by using the 10 mg/ml aq. soln. of carboplatin. The amount of the drug was adjusted according to the dosage required for the ongoing animal studies. The 0.50 ml of formulation were placed into dialysis tubes (*Slide-A-Lyzer® MINI* Dialysis Units, 10,000 MWCO, *Thermo Scientific*, Rockford, IL) and immersed into 50 ml of a pseudo-extracellular fluid (PECF), an aq. soln. of 0.12M NaCl, 0.03M KCl, 0.03M NaH_2PO_4, and 0.30M $NaHCO_3$ with pH of 7.7 ± 0.1 [68]. The PECF soln. surrounding the dialysis tube was stirred at 60 rpm with a magnetic stir bar. The 0.5-ml aliquots of aq. PECF soln. were taken and analyzed by atomic absorption spectroscopy (AAS; *SpectrAA 55B* spectrometer, *Varian*, Palo Alto, CA) for the amount of Pt accumulated in soln. Drug-release experiments were conducted in triplicates. Carboplatin absorbtion on the dialysis membrane was negligible, since aq. control soln. released 99% of the drug in 72 h.

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Received March 23, 2011