Cloud Point of Nonionic Surfactants: Modulation with Pharmaceutical Excipients

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Purpose. To determine the cloud point of a variety of nonionic surfactants and to search for means to raise the surfactant cloud point in liquid formulations.

Methods. Cloud points of nonionic surfactants were determined visually in a water bath. Organic compounds, many of which have been used as pharmaceutical excipients, were tested initially for effect on the cloud point of poloxamine 908. Four effective cloud point boosters (CPBs) from different structural classes were further tested on additional surfactants.

Results. A number of compounds can raise the cloud point of nonionic surfactants. These cloud point boosters are classified into two categories: nonionic and ionic. The nonionic CPBs include poly(ethylene glycols), propylene glycol, methanol, ethanol, isopropanol, and 2-hydroxypropyl-β-cyclodextrin. They are effective at molar concentrations. The ionic CPBs include anionic and cationic surfactants, charged phospholipids, long chain fatty acids, and bile salts. They are effective at millimolar concentrations.

Conclusions. The cloud point of nonionic surfactants used in liquid formulations can be modulated through the proper choice of excipient.

KEY WORDS: nonionic surfactants; cloud point; cloud point boosters; poloxamers; poloxamines; liquid formulation.

INTRODUCTION

Nonionic surfactants with poly(ethylene oxide) (PEO) chain(s) as the hydrophilic moiety are used frequently in pharmaceutical formulations. Some of them are effective solubility enhancers for poorly soluble drugs and some are strong dispersants and colloid stabilizers used in emulsion and suspension dosage forms. A unique property of this class of nonionic surfactant is the display of cloud point, the temperature above which the surfactant phase-separates and precipitates out of solution. A study of cloud points of a variety of nonionic surfactants has shown them to increase with the hydrophile-lipophile balance (HLB) (1). A number of studies have been reported on the effects of organic and inorganic additives on the cloud point (2–8). Among them, Schott and coworkers have studied inorganic salts extensively, separating the effect on cloud point into contributions from the cation and anion (3,4,6,7). They noted that the effect of anions followed the order of the Hofmeister series, i.e., anions that break the water structure, such as I^{-1}, $[Fe(CN)_5NO]^{-2}$, and SCN^{-1}, boost the cloud point whereas those that promote the water structure or bind water molecules extensively, such as OH^{-1}, F^{-1}, Cl^{-1}, SO_4^{-2}, and PO_4^{-3}, suppress the cloud point (6,7). For cations, they proposed that most divalent and trivalent cations, together with H^{+1}, Ag^{+1}, and Li^{+1}, boost the cloud point by forming complexes with the ether groups of the PEO chain. On the other hand, Na^{+1}, K^{+1}, Cs^{+1}, and NH_4^{+1} do not form such complexes and suppress the cloud point by dehydration (6,7).

In developing liquid formulations containing a nonionic surfactant, the cloud point of the surfactant can be a key parameter for consideration. Phase-separation of the surfactant under elevated temperatures, such as that encountered in the steam sterilization of parenteral products, often leads to content heterogeneity. In the case of dispersed dosage forms, phase separation of the surfactant can result in physical instability of the dispersion (9–11). Conversely, a surfactant phase change could be utilized beneficially in pharmaceutical formulations. A case in point is the gelation of poloxamer solutions and its potential applications in the controlled-release of drugs through various routes of delivery (12–15). In modulating the cloud point, it appears easier to lower it than to raise it; many common salts, including sodium chloride, are very effective cloud point suppressors. A few salts, such as those containing I^{-1}, $[Fe(CN)_5NO]^{-2}$, and SCN^{-1} anions, can raise the cloud point (6,7). However, they are likely to be toxic and unsuitable for use in pharmaceutical formulations. Furthermore, the presence of an electrolyte at high concentrations can weaken the electrostatic stabilization of colloids and is detrimental to the physical stability of dispersed dosage forms. We studied various organic compounds, particularly those suitable as pharmaceutical excipients, that can raise the cloud point and prevent the surfactant phase separation in liquid formulations at high temperatures. In addition, we examined two variables, the concentration and purity of the surfactant, for effect on the cloud point. The block copolymer poloxamine 908 was used as a model surfactant in the initial probe. Four of the effective cloud point boosters (CPBs) identified were subsequently tested on nonionic surfactants of various structures. The results of these studies are reported in this paper. A subsequent paper (11) describes the physical stability of a submicron size crystalline particle (nanocrystal) suspension under steam sterilization conditions with a focus on the role of the surfactant cloud point and the stabilization effect of the CPBs identified in this paper.

MATERIALS AND METHODS

Materials

Poly(ethylene glycols) (PEGs) from Baker (Phillipsburg, NJ) and Union Carbide (Danbury, CT) were used. 2-Hydroxypropyl-β-cyclodextrin (HPBCD) was from American Maize (Hammond, IN). Sodium dioctyl sulfosuccinate (DOSS), sodium dodecylsulfate (SDS), sodium dodecylbenzenesulfonate (SDBS), cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), alkyl and fatty acids, taurocholate (TC), and taurodeoxycholate (TDC) were purchased from Sigma (St Louis, MO). The phospholipids used included cardiolipin, dimyristoylphosphatidyl glycerol (DMPG), 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS),

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dipalmitoyl-sn-glycerol-3-phosphatidic acid (DPPA), 1,2-distearoyl-sn-phosphoethanolamine (DSPE), 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) and were purchased from Avanti Polar Lipids (Alabaster, AL). Poloxamers (Pluronics), poloxamines (Tetronics), alkyl phenol poly(ethylene oxides) (Iconols), and cremophors were from BASF (Parsippany, NJ). The alkyl ether poly(ethylene oxides) (BRIJs), alkylate poly-(ethylene oxides) (MYRJs) and polysorbates (Tweens) were from ICI (Wilmington, DE). *p*-(1,1,3,3-Tetramethylbutyl)phenol poly(ethylene oxide) polymer (Tyloxapol) was from Sterling Organics (Rensselaer, NY). All other chemicals used were reagent grade. The acetate, citrate, and phosphate buffers were prepared from the sodium salts and the tromethamine buffer was prepared from the chloride salt.

Determination of Surfactant Cloud Points

Surfactant cloud points were measured in a constant temperature bath. The bath was filled with PEG 400 to reach temperatures above 100°C. Surfactants were dissolved in water at 1% (w/v) concentration, and 5 ml aliquots were filled in 10-ml glass vials. The vials were covered with rubber stoppers, sealed with aluminum caps, and immersed partially in the bath fluid. Around the temperature of interest, the bath temperature was raised in 0.2°C increments, each followed by a 3 to 5 min equilibration period. At the end of the equilibration period, the vials were lifted out of the bath fluid momentarily and inspected visually for cloudiness. The sample temperature was determined with a Fluke 52 digital thermometer, its thermocouple was inserted through the rubber stopper of a similarly prepared vial containing PEG 400. The digital readings were calibrated against an ASTM-designated glass thermometer.

The phase separation of a nonionic surfactant at its cloud point is known to take place within a narrow temperature range. Typically, we observed the solution turning slightly blue and translucent, then completely turbid within one degree. Among the surfactants examined here, only the two polysorbates exhibited broad transitions and remained translucent for about 5 degrees before turning turbid. The temperature at the first sign of turbidness was taken as the cloud point. On cooling, the phase-separated surfactant re-dissolved immediately. We cycled the sample temperature around the cloud point at least twice to confirm the result.

HPLC Analysis of Poloxamine 908

Size exclusion HPLC (SE-HPLC) was conducted on a Waters 840 system (Milford, MA). A Toso Haas TSK G2000 SWXL column (7.8 × 300 mm) was used. Elution was at a rate of 1 ml/min using a mobile phase of 20% methanol and 24 m*M* NaCl. The analytes were detected by a refractive index detector. Nominal average molecular weights of the parent and impurity peaks were determined from SE-HPLC using poly(ethylene glycol) molecular weight standards from Polymer Laboratories (Amherst, MA) and a Waters GPC software.

Separation of Poloxamine 908 from Lower Molecular Weight Impurities

Commercially obtained poloxamine 908 (Tetronic T908) showed significant amounts of lower molecular weight impurities. The impurities were removed by diafiltration of poloxamine 908 solution against water with an Osmonics 192-T HNO2 polysulfone spiral-wound membrane (Minnetonka, MN) having a nominal molecular weight cutoff of 15 to 25 K. The lower molecular weight species found in poloxamine 908 were obtained by passing a 5% poloxamine 908 solution three times through an Amicon YM-10 Diaflo ultrafilter (Danvers, MA) which retained poloxamine 908.

Differential Spectroscopy to Detect OP-10 Binding to HPBCD

The interaction of OP-10 surfactant with HPBCD was probed by differential spectroscopy using a Hewlett-Packard 8453 UV-Vis spectrophotometer. The samples were prepared in triplicate and contained 0.02% OP-10 and 0.8% HPBCD. Two references were also prepared in triplicate, one contained 0.02% OP-10 and the other 0.8% HPBCD. UV spectra were recorded from 250 to 300 nm. The difference spectrum was calculated by subtracting the two reference spectra from the sample spectrum.

RESULTS

Effects of Poloxamine 908 Purity and Concentration on Cloud Point

When analyzed by SE-HPLC, the block copolymer surfactant poloxamine 908 showed a parent peak with a nominal average molecular weight of 22.6 kD and an impurity peak with a nominal average molecular weight of 6.8 kD (Fig. 1). The percent area of the impurity peak varied from lot to lot and had ranged from 13 to 20%. Figure 1 also shows the chromatograms of a purified poloxamine 908 and isolated lower molecular weight impurities.

Three lots of poloxamine 908 with 80, 92, and 96% purities by SE-HPLC were prepared. They showed the same cloud point of 111 ± 1°C. The isolated lower molecular weight species (bottom chromatogram in Fig. 1) showed a cloud point of 117 ± 1°C.

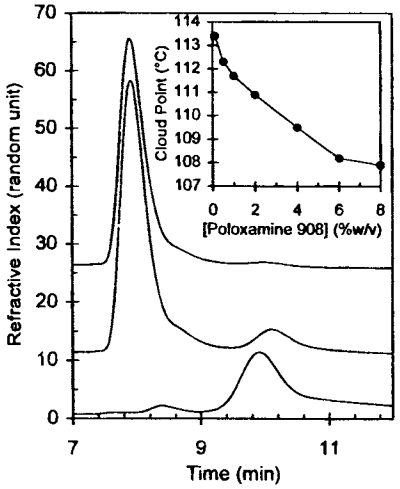

Fig. 1. Size exclusion HPLC chromatograms of a purified poloxamine 908 (top), a commercial poloxamine 908 (middle), and isolated lower molecular weight impurities of poloxamine 908 (bottom). The inset shows the dependence of the cloud point of poloxamine 908 on its concentration.

At 1% concentration, poloxamine 908 showed a cloud point of about 111°C. The cloud point changed very little with the surfactant concentration, decreasing about 5°C as the concentration increased from 0.1 to 8% (Fig. 1, inset). All cloud points reported in the remainder of this paper were measured with 1% (w/v) surfactant.

Poloxamine 908 and Nonionic Cloud Point Boosters

A number of nonionic compounds were tested against poloxamine 908 as cloud point boosters. Those we identified effective fell into three structural classes: polyglycols, alcohols/polyalcohols, and cyclodextrins.

Polyglycols

Three poly(ethylene glycols) with different molecular weights were tested and the results are plotted in Fig. 2. PEGs at the 1 to 10% (w/v) concentration range raised the cloud point of poloxamine 908. The effectiveness of PEGs as CPBs, measured by the change in cloud point per unit concentration of PEG, decreased with increasing molecular weight.

Alcohols and Polyalcohols

Methanol, ethanol, *n*-propanol, *i*-propanol, propylene glycol, and glycerol were tested and the results are plotted in Fig. 3. Among the monoalcohols, methanol, ethanol and *i*-propanol were effective cloud point boosters whereas *n*-propanol was only slightly effective. Among the polyalcohols tested, propylene glycol was a very effective cloud point booster whereas glycerol was only slightly effective. Two six-carbon polyalcohols, mannitol and sorbitol, were tested at 10% concentration and they lowered the cloud point of poloxamine 908 slightly from 111 to 107°C (data not plotted).

Saccharides

Three mono- and disaccharides, dextrose, sucrose, and trehalose, were tested at 10% concentration. Similar to mannitol and sorbitol, the mono-and disaccharides lowered the cloud point of poloxamine 908 from 111°C to 107°C.

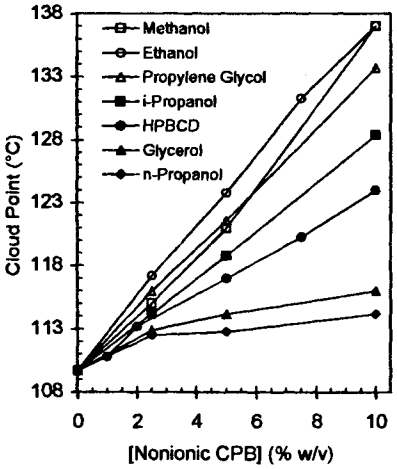

Fig. 3. Effects of nonionic additives on the cloud point of poloxamine 908 at 1% (w/v).

The cyclic polysaccharide 2-hydroxypropyl-β-cyclodextrin (HPBCD) was tested in the concentration range from 1 to 10%. In contrast with the mono- and disaccharides, HPBCD raised the cloud point of poloxamine 908 quite effectively (Fig. 3).

Poloxamine 908 and Ionic Cloud Point Boosters

The effective ionic CPBs we identified fell into three structural classes: (1) ionic surfactants, (2) charged phospholipids, and (3) fatty acids.

Ionic Surfactants

Three anionic surfactants (SDS, DOSS, and SDBS) and two cationic surfactants (CTAB and DTAB) were tested against poloxamine 908 and all were very effective in raising the cloud point. As shown in Fig. 4, the effective concentrations of the ionic surfactants were in the millimolar range. This is in contrast with the molar concentrations required for the nonionic CPBs. Among the two quaternary amine cationic surfactants tested,

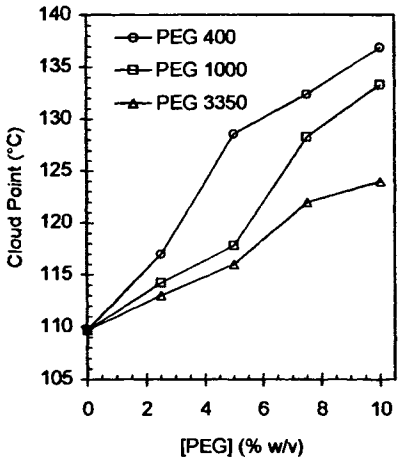

Fig. 2. Effects of PEGs on the cloud point of 1% (w/v) poloxamine 908. The data points are connected by straight lines to show the trend.

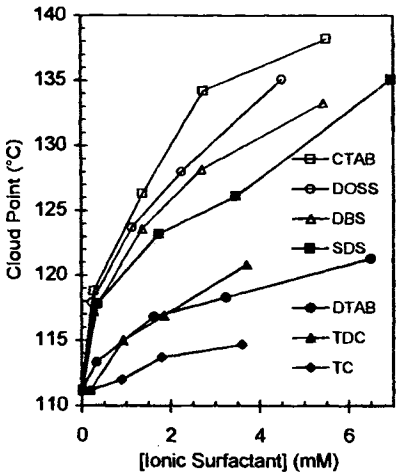

Fig. 4. Effects of ionic surfactants on the cloud point of 1% (w/v) poloxamine 908.

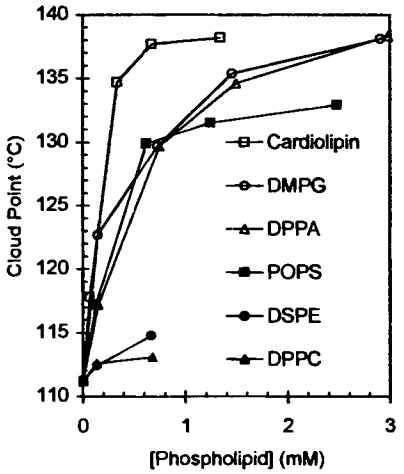

Fig. 5. Effects of phospholipids on the cloud point of poloxamine 908 at 1% (w/v).

CTAB has a longer alkyl chain than DTAB and was the more effective CPB of the two.

Two bile salts, taurocholate (TC) and taurodeoxycholate (TDC), in the concentration range of 0.2 to 4 m*M* were moderately effective in raising the cloud point of poloxamine 908 with TDC being more effective than TC (Fig. 4).

Phospholipids

Six phospholipids were tested and the results are plotted in Fig. 5. Those phospholipids with a net negative charge (phosphatidyl glycerol, phosphatidic acid and phosphatidyl serine) were very effective cloud point boosters. Conversely, the zwitterionic phospholipids (phosphatidyl choline and phosphatidyl ethanolamine) have no net charge and were ineffective in raising the cloud point.

Fatty Acids

Alkyl and fatty acids from C-6 to C-18 were tested in the millimolar concentration range for effect on the cloud point of poloxamine 908. As shown in Fig. 6, the cloud point boosting effectiveness of fatty acids increased with increasing alkyl chain length. In general, fatty acids with a chain length longer than 12 carbons were quite effective CPBs whereas the shorter ones were only marginally effective.

Buffer Salts

Four pH buffers used in parenteral products, acetate (pH 4), citrate (pH 5), phosphate (pH 7), and tromethamine (pH 8), were tested for effect on the cloud point of poloxamine 908 and the results are plotted in Fig. 7. All four buffers suppressed the cloud point of poloxamine 908. However, the acetate and tromethamine buffers showed much weaker suppressing effects than the citrate and phosphate buffers.

Effects of CPBs on the Cloud Point of Other Surfactants

Two nonionic CPBs (PEG 400 and HPBCD) and two ionic CPBs (DOSS and DMPG) were tested for effect on the cloud point of additional nonionic surfactants including poloxamers, cremophors, alkyl phenol poly(ethylene oxides) (tyloxapol and the Iconol NP and OP series from BASF), polysorbates, MYRJ 52, and BRIJ 35. The data in Table I show that the four CPBs were effective against most of the nonionic surfactants tested with only a few exceptions: (1) PEG 400 was only marginally effective against cremophors and OP-10 and ineffective against polysorbates and (2) HPBCD was ineffective against cremophors, BRIJ 35, and polysorbates.

DISCUSSION

The surfactant cloud point of a liquid formulation is preferably modulated without the use of any additives. We therefore tested the surfactant purity and concentration for effect on the cloud point. Several poloxamers and poloxamines we analyzed by SE-HPLC showed a substantial lower molecular weight impurity peak. The removal of the lower molecular weight impurities from poloxamine 908 did not significantly affect the cloud point. In fact, the isolated lower molecular weight species showed a slightly higher cloud point than the parent compound, suggesting that the former may have a higher HLB value (1). The cloud point of poloxamine 908 also changed relatively

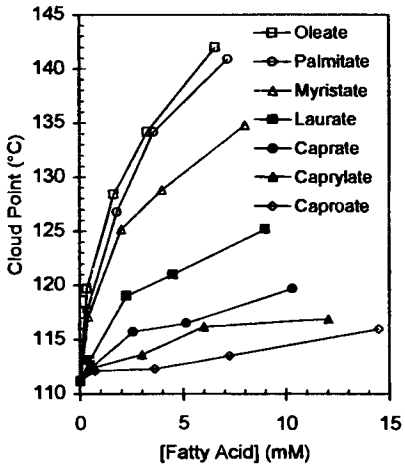

Fig. 6. Effects of fatty acids of various chain lengths on the cloud point of 1% (w/v) poloxamine 908.

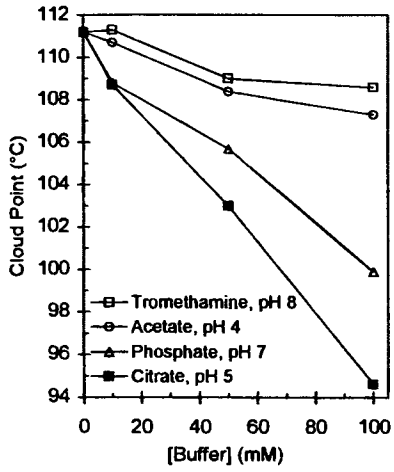

Fig. 7. Effects of four pH buffers on the cloud point of 1% (w/v) poloxamine 908.

Table I. Effects of PEG 400, HPBCD, DOSS, and DMPG on Surfactant Cloud Point

	Additive[b]				
	None	PEG400	HPBCD	DOSS	DMPG
Surfactant[a]	Cloud Point (°C)				
Poloxamine 908	111	137	125	135	138
Poloxamer 108	110	125	123	138	146
Poloxamer 188	110	127	127	138	>148
Poloxamer 217	109	122	123	141	133
Poloxamer 238	109	124	124	136	143
Poloxamer 338	109	123	123	135	136
Cremophor EL	72	81	61	138	147
Cremophor RH	93	99	81	139	134
Tyloxapol	96	106	107		
NP-40	117	135	131		
NP-50	116	133	137		
NP-70	114	130	139	134	135
NP-100	112	130	131	129	134
OP-10	67	71	145		
OP-30	114	133	139		
OP-40	114	132	145		
BRIJ-35	117	132	118	148	130
MYRJ-52	102	110	[c]	131	130
Polysorbate 80	92	90	60	127	132
Polysorbate 20	97	96	47	123	123

[a] The nonionic surfactants were at 1% (w/v) concentration.
[b] PEG 400 and HPBCD were at 10% (w/v) whereas DOSS and DMPG were at 0.2% (w/v).
[c] The effect of 10% HPBCD on 1% MYRJ-52 was not measured due to poor solubility.

little within the concentration range from 0.1 to 8% (Fig. 1 inset). Therefore, variation of surfactant concentration and purity provided limited modulation of the cloud point which prompted us to examine additives for effects.

The CPBs listed here are classified into ionic and nonionic because they were effective at different concentration ranges: millimolar for the ionic ones and molar for the nonionic ones. The difference in effective concentrations suggests that the nonionic and ionic CPBs function via different mechanisms. The nonionic surfactants of interest here consist of a hydrophobic tail group, which is usually insoluble in water by itself, and a PEO chain, which is capable of forming hydrogen bonds to water molecules and renders the surfactant amphiphilic and soluble in water. The phase separation of nonionic surfactants at high temperatures is believed to be a result of enhanced intermolecular association driven by hydrophobic interaction between the surfactant tail groups and/or a decreased hydration of the PEO chain (16). The ionic CPBs listed here are amphiphilic in the sense that each contains an apolar group and an ionic group. The apolar portion of the molecule should allow the ionic CPBs to associate with nonionic surfactants through hydrophobic interaction, probably in the form of mixed micelles (17). Such an association will, in effect, turn the nonionic surfactant into an ionic species, and the repulsive force between the electrostatic charges could keep the nonionic surfactant from coalescing and phase separating. The formation of mixed micelles has been suggested earlier as the mechanism of cloud point boosting by ionic surfactants (2,6). Several observations described in this paper further support such a mechanism. First, the ionic CPBs were effective at millimolar concentrations, comparable to that of the nonionic surfactant (Fig. 4–6). Second, of the six phospholipids examined, only those with a net charge were effective CPBs, reflecting the essence of a net charge (Fig. 5). Third, the effectiveness of fatty acids as CPBs increased with increasing alkyl chain length (Fig. 6). Thus, the two key structural features of the ionic CPBs are: a hydrophobic group to allow association with the nonionic surfactant and a net charge to impart electrostatic repulsion to the surfactant molecules.

Unlike the ionic CPBs, the nonionic CPBs were effective only in the molar concentration range (Figs. 2 and 3), suggesting that they interact weakly with nonionic surfactants. A possible mechanism for the nonionic CPBs is through weakening of the hydrophobic interaction between the tail groups of the surfactant. Perturbation of water structure has been invoked in the past to explain the weak non-specific effects of additives on the surfactant cloud point and gelation temperature (6,13). However, direct evidence of structural change of water molecules by cosolvents has been rare and the extent of ordering of water molecules surrounding apolar groups remains controversial (18). A more rigorous and quantitative approach to understanding the effect of the nonionic CPBs is through the measurement of preferential interaction (19). Such an approach is based on thermodynamics and has been used very effectively towards understanding the effects of various solvent additives on the protein solubility and stability (19). Based on the theory of preferential interaction, if a nonionic CPB is preferentially attracted by a nonionic surfactant, the presence of the CPB should lower the chemical potential and enhance the solubility of the surfactant (19). We have measured the preferential interaction of the nonionic surfactant poloxamer 338 in 10% PEG 400 by precision density measurement. The results showed

that PEG 400 is preferentially attracted to poloxamer 338 at temperatures near the cloud point. We are currently expanding the measurement to other nonionic CPBs and the results will be published separately.

Among the nonionic CPBs, the PEGs are uniquely interesting because of their higher molecular weights. In addition to the commonly known interacting forces between small molecules, one expects the excluded volume effect to come into play in the interaction between PEGs and nonionic surfactants. The excluded volume effect is derived from steric exclusion between molecules, i.e. two molecules cannot occupy the same space at the same time (20). Such a steric exclusion effect should increase with increasing molecular weight of PEG and has been suggested to cause the decreased solubility of proteins in the PEG-water cosolvent (21). One would therefore expect the steric exclusion effect to favor the phase separation of nonionic surfactants and lower the cloud point. This is consistent with our observed weakening of the cloud point boosting effect of PEGs with increasing molecular weight. At a very high molecular weight, the excluded volume effect could dominate the interaction and turn PEG into a cloud point suppressor. Indeed, a PEO with a molecular weight of 6×10^5 has been reported to suppress the cloud point of a nonionic surfactant (22).

Out of the six polyalcohols and saccharides we tested, only the cyclic polysaccharide HPBCD showed a strong boosting effect on the cloud point of poloxamine 908. The anomaly of HPBCD prompted us to test it further against additional surfactants. The data in Table I show that HPBCD is an effective cloud point booster towards a variety of nonionic surfactants, particularly those with a benzene ring. HPBCD is well known for its ability to form inclusion complexes with hydrophobic molecules and surfactants (23). The association between surfactants and cyclodextrins has been shown to raise the surfactant critical micelle concentrations (23). We conducted a differential spectroscopic study of the nonionic surfactant OP-10 which has an octylphenol tail group. A hyperchromicity of OP-10 at 270 to 290 nm was observed in the presence of HPBCD (data not shown), suggesting the formation of an inclusion complex. Thus, HPBCD is likely to function as a CPB by yet another mechanism, i.e., forming inclusion complexes with the hydrophobic tail group of nonionic surfactants thereby preventing the latter from coalescing and phase-separating at high temperatures.

Many liquid formulations contain a buffer to control the product pH. Among the four representative buffers we tested, the tromethamine (pH 8) and acetate (pH 4) buffers exerted much weaker suppressing effects on the cloud point than the phosphate (pH 7) and citrate (pH 5) buffers. Schott and Royce have reported boosting of surfactant cloud point by acetic acid, citric acid, and phosphoric acid at low pHs where they are mostly protonated, but suppression of cloud point by the sodium salts of these acids (6). In the same report, tromethamine was shown to boost the cloud point whereas tromethamine hydrochloride to suppress the cloud point. Thus, the acetate and tromethamine buffers at pHs near their pK_as are composed of about equal concentrations of a cloud point booster (acetic acid and tromethamine) and a cloud point suppressor (sodium acetate and tromethamine hydrochloride). This could be the reason for the weak cloud point suppressing effects of the two buffers. In contrast, the citrate and phosphate buffers are composed mostly of monobasic and dibasic salts, both of which are strong cloud point suppressors, and, therefore, the strong cloud point suppressing effects of the buffers.

In summary, cloud points of nonionic surfactants were effectively raised by a number of compounds, some of them are suitable for use in liquid pharmaceutical formulations. The ionic CPBs appeared to act by interacting with surfactants through the hydrophobic group and imparting an electrostatic charge. The nonionic CPBs functioned through weak nonspecific interactions with the surfactants. The driving force could be derived from a preferential attraction of the CPBs by the surfactants. Finally, 2-hydroxypropyl-β-cyclodextrin appeared to function by forming inclusion complexes with the surfactants.

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