

2005


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Surfactant properties of low molecular weight phospholipids

Abstract

Surface tensions, critical micelle concentrations (CMCs), contact angles on hydrophobic polyethylene, and foaming characteristics of phosphatidic acids, phosphatidylcholines, phosphatidylethanolamines, and phosphatidylglycerols were measured to determine their suitability as substitutes for traditional surfactants. These phospholipids have fatty acid chains of 5 to 12 carbon atoms, a range over which they are soluble at room temperature. Their surface tensions decrease with increasing concentrations until their CMCs are reached, above which their plateau surface tensions are as low as 21 mN/m, indicating excellent surface activities. In general, plateau surface tensions decrease with increasing chain length within each phospholipid type. The classical relationship for \ln CMC vs. chain length is followed with slopes typical of anionic surfactants for phosphatidic acids and phosphatidylglycerols and resembling zwitterionic surfactants for phosphatidylcholines and phosphatidylethanolamines, consistent with the charge on the hydrophilic group. The wetting capabilities of aqueous solutions on polyethylene are good and foam heights and stabilities are high, the latter two properties being comparable to traditional anionic (sodium dodecylsulfate) and nonionic (octylphenol polyethoxylate) surfactants. Some anomalies are observed regarding the effect of chain length on wetting and foaming, probably due to the depletion effect. Many phospholipids slowly degrade in aqueous solution. We conclude that short-chain phospholipids exhibit excellent surfactant properties and may be useful in many applications.

Keywords

Critical micelle concentration, foaming, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phospholipid, surface tension, surfactant, wettability

Disciplines

Biochemical and Biomolecular Engineering | Biological Engineering | Chemical Engineering

Comments

This is a post-print of an article from *Journal of Surfactants and Detergents*, 8, no. 1 (2005): 65–72, doi: [10.1007/s11743-005-0332-8](https://doi.org/10.1007/s11743-005-0332-8).

Surfactant Properties of Low Molecular Weight Phospholipids

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Running title: PHOSPHOLIPID SURFACTANT PROPERTIES

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Abbreviations: CMC, critical micelle concentration; n , number of carbon atoms in each phospholipid fatty acid chain; OP(EO) _{x} , *t*-octylphenolpolyethoxylate of x mol ethylene oxide/mol octylphenol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; SDS, sodium dodecylsulfate.

ABSTRACT: We measured surface tensions, critical micelle concentrations (CMCs), contact angles on hydrophobic polyethylene, and foaming characteristics of phosphatidic acids, phosphatidylcholines, phosphatidylethanolamines, and phosphatidylglycerols to determine their suitability as substitutes for traditional surfactants. These phospholipids have fatty acid chains of five to twelve carbon atoms, a range over which they are soluble at room temperature. Their surface tensions decrease with increasing concentrations until their CMCs are reached, above which their plateau surface tensions are as low as 21 mN/m, indicating excellent surface activities. In general, plateau surface tensions decrease with increasing chain length within each phospholipid type. The classical \ln CMC versus chain length relationship is followed, with slopes typical of anionic surfactants for phosphatidic acids and phosphatidylglycerols and to those of zwitterionic surfactants for phosphatidylcholines and phosphatidylethanolamines, consistent with the charge on the hydrophilic group. Wettabilities of aqueous solutions on polyethylene are good and foam heights and stabilities are high, the latter two properties being comparable to traditional anionic (sodium dodecylsulfate) and nonionic (octylphenol polyethoxylate) surfactants. Some anomalies were observed on effect of chain length on wetting and foaming, probably due to the depletion effect. Many phospholipids slowly degrade in aqueous solution. We conclude that short-chain phospholipids exhibit excellent surfactant properties and could be useful in many applications.

KEY WORDS: Critical micelle concentration, foaming, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phospholipid, surface tension, surfactant, wettability.

Phospholipids (PLs) have been extensively studied, since they form bilayer biological membranes (1–4). In general, biological amphiphiles with long fatty acid chains can self-assemble to form liquid crystals or flat bilayer membranes (so-called liposomes) (5). Aqueous solutions of PLs with other types of surfactants can form either mixed liposomes or mixed micelles, depending on their compositions (5,6). However, synthetic PLs containing short fatty acid or linear alkyl chains can be soluble in water and can self-orient themselves as spherical micelles at concentrations higher than their critical micelle concentrations (CMCs) without other added surfactants (6,7). If the chain length (n) is too short, micelles do not form, and if it is too long, solubility limits are reached before they form. Longer hydrophobic PLs form bilayer cell membranes and have received much attention (6).

Given the very extensive literature on high molecular weight PLs, it is rather surprising that very few references present the physical properties of PLs having n in the range where surfactant properties (micellization, foaming, solubilization, wetting, and emulsification) are observed. Micelle formation by phosphatidylcholines (PCs) has been discussed in some papers (6–9).

The incentive for this work was the prospect that longer-chain PLs from vegetable oil degumming can yield shorter-chain PLs by transesterification of the existing fatty acid residues, and these could then be used as surfactants for commercial and industrial applications. Transesterification can be accomplished either under acid catalysis if n of both chains is to be the same, by catalysis with phospholipase A₁ if the chain attached to the *sn*-1 position is to be exchanged, or by catalysis with phospholipase A₂ if the chain attached to the *sn*-2 position is to be exchanged. In all cases the shorter-chain fatty acid must be present in great excess to force the reaction to near-completion.

Therefore, we measured surface tension reduction, micelle formation, wetting on hydrophobic plastic surfaces, and foaming of aqueous solutions of synthetic saturated diacyl phosphatidic acids (PAs), PCs, phosphatidylethanolamines (PEs), and phosphatidylglycerols (PGs), where n was the same for both chains but varied from run to run (Fig. 1).

EXPERIMENTAL

Materials. PAs (sodium 1,2-diacyl-*sn*-glycero-3-phosphates), PCs (1,2-diacyl-*sn*-glycero-3-phosphocholines), PEs (1,2-diacyl-*sn*-glycero-3-phosphoethanolamines), and PGs (sodium 1,2-diacyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)s) of $5 \leq n \leq 12$ and of $> 98\%$ purity were purchased from Avanti Polar Lipids (Alabaster, AL) and Sigma Chemical (St. Louis, MO) and used without further purification. Sodium dodecylsulfate (SDS) of 99% purity came from Sigma. Polydisperse commercial branched *t*-octylphenolpolyethoxylate with an average of 10 mol ethylene oxide/mol octylphenol [OP(EO)₁₀] (trade name Igepal CA 630) was purchased from Rhodia (Cranbury, NJ) and was used as the nonionic surfactant. All water was distilled.

Surface tension and CMC. Equilibrium surface tensions of different concentrations of unbuffered aqueous PL solutions at neutral pH and 30°C were measured with a Wilhelmy plate tensiometer (Model K-10, Krüss, Hamburg, Germany). Degradation of PLs at concentrations below their CMCs in aqueous solutions was investigated by measuring surface tension over time. A sharp change in the plot of $\ln [\text{PL}]$ vs. surface tension defines the CMC (10). The surface tension is generally constant above the CMC. When the surface tension changes mildly with concentration above the CMC, the plateau surface tension is defined as that at the CMC.

Contact angle. Polyethylene zip bags from Fisher Scientific (Houston, TX) were cut into 2.5-cm-square sheets. Their inner surfaces were used as wetted surfaces without additional treatment. Specimens were prepared by attaching these sheets to glass slides by using a drop of water as the adhesive and then placing the composites on a stand located in the middle of an acrylic box controlled at 30°C by heating tape. Sample droplets of 40, 60, and 80 μL were placed on each sheet with a 100- μL syringe to measure advancing contact angles. High-resolution zoom pictures of the drops were taken with a Nikon (Melville, NY) Coolpix 995 digital camera with a macro lens after the drops settled for 1 min to attain equilibrium. The contact angles between liquid droplet and substrate were estimated by drawing tangents to the drop at the point of contact with the surface and measuring the angles between the horizontal reference lines and the tangent lines with

Adobe PhotoShop, version 6.0 (Fig. 2).

Foaming. Because of high PL cost, a foaming study apparatus was developed requiring only a small solution volume. Many different foam tests are used for various applications (11), so there is no universally accepted protocol. Here a 10-mL aqueous PL solution was carefully poured into a 25-mL, 25 mm o.d. Pyrex glass test tube, avoiding foam formation, and the initial solution height was recorded. A 20-mL graduated gas syringe was connected by a polyethylene tube to a 5-mm i.d. glass tube inserted through the bottom of the test tube, and 20 mL of air was inserted into the solution manually at 1 mL/s to generate foam. The total height of foam and solution was measured at intervals for 20 min.

RESULTS AND DISCUSSION

PL degradation. Many of the PLs studied here degrade at neutral pH and 30°C; for example, the equilibrium surface tension of 100 ppm PC-5 (PC of $n = 5$) decreased from 63.1 mN/m to 57.5 mN/m in two weeks, while that of 100 ppm PG-6 went from 64.4 mN/m to 34.1 mN/m over the same time. Therefore studies requiring long equilibration times such as solubilization could not be conducted.

At neutral pH, PLs are hydrolyzed mainly by alkaline attack on the two carboxyl ester bonds; the two fatty acid chains are removed at equal rates (12). The two phosphate ester bonds are not attacked except under prolonged heating (13). Hydrolysis rate is proportional to hydroxide ion concentration. Hydrolysis rate is only slightly affected by n , being more affected by the identity of the head group (12). Energies of activation are between 40 and 80 kJ/mol (12,14). Hydrolysis rate increases linearly with buffer concentration and varies with buffer type (14).

PL instability in water over extended times would limit use in some applications, for example, in concentrated consumer liquid detergents with long shelf lives. But, some applications, like powdered laundry detergents, involve a short exposure of surfactants to water.

Surface tension and CMC. The relationship between surface tension and concentrations of PLs of varying n is shown in Fig. 3. CMCs and plateau surface tensions for each PL appear in

Table 1. CMCs of PCs compare well with values from two other investigations (15.4 mM vs. 13 mM and 13.7 mM for PC-6, 1.78 mM vs. 1.6 mM and 1.6 mM for PC-7, and 0.16 mM vs. 0.16 mM and 0.23 mM for PC-8 (7,9). The CMC of SDS, a commercial anionic surfactant, is 8.32 mM (10,15), while the CMC of (OP(EO)₁₀), a commercial nonionic surfactant, is 0.33 mM (16). The similarity of the CMCs of low molecular weight PLs to those of commonly used commercial surfactants suggests in the absence of further tests that they may be useful for solubilizing organic solutes.

The well-known linear free-energy relationship (10,17) between \ln CMC and n of each of the four PL types is shown in Fig. 4. The slopes of straight lines on this plot are the free energies of micellization per methylene group ($\Delta G_m/\Delta n$) (10). Values of $\Delta G_m/\Delta n$ are -1.03 ± 0.10 kJ/mol for PA, -1.83 ± 0.11 kJ/mol for PC, -1.86 ± 0.16 kJ/mol for PE, and -1.19 ± 0.10 kJ/mol for PG, where the ranges are standard errors. Values for PA and PG are similar to those expected for anionic surfactants (-1.0 to -1.4 kJ/mol), while those for PC and PE are similar to those for zwitterionic surfactants (-2.1 to -2.2 kJ/mol) (10).

Plateau surface tension. The plateau surface tension (surface tension at the CMC) for each PL is shown in Table 1. The values vary from 21 to 34 mN/m, with 9 of 15 PL surfactants having plateau surface tensions ≤ 25 mN/m. Rosen and Dahanayake state “hydrocarbon-chain surfactants can reduce the equilibrium surface tension of water to about 25 dyn/cm (mN/m); fluorocarbon-chain surfactants to about 15 dyn/cm (mN/m); and silicone-chain (polymethylsiloxane) surfactants to about 20 dyn/cm (mN/m), at a minimum” (18). The plateau surface tensions of SDS and OP(EO)₁₀ are 38.3 ± 1 mN/m (12) and 30.1 mN/m (16), respectively. The remarkably low surface tensions of the PLs approach those of other surfactants with hydrophobic tail groups such as fluorocarbons and silicones, but PLs have the advantage of being biodegradable. A relevant observation is that lung surfactants are PLs (19,20), which can have extremely low surface tensions (<10 mN/m) under dynamic conditions with optimal pulsation times corresponding to physiological pulsations.

Contact angles. The ability of surfactants to allow aqueous solutions to wet hydrophobic

surfaces is an important practical function. Wettability is quantified by the contact angle (θ) of a droplet of aqueous solution on a hydrophobic surface such as polyethylene (Fig. 2). Advancing contact angles, where the liquid is added to the surface and advances over it, are most commonly measured and are reported here.

The value of θ is dictated by the interfacial tensions of the three interfaces that meet at the droplet contact rim (Fig. 2), as shown by the Young equation (10,17): $\gamma_{LV} \cos \theta = \gamma_{SV} - \gamma_{SL}$, where γ_{LV} is the liquid-vapor surface tension, γ_{SV} is the solid-vapor interfacial tension, and γ_{SL} is the solid-liquid interfacial tension. The value of γ_{SV} is approximately constant, since the liquid does not contact solid exposed to vapor. As surfactant concentration increases below the CMC, γ_{LV} decreases, surfactant adsorption on the plastic increases, and γ_{SL} decreases in general. Above the CMC, adsorption on the solid reaches a plateau (17) and γ_{LV} should also become constant. From the Young equation, θ should decrease with increasing surfactant concentration until it becomes constant above the CMC (15).

Droplet volume (40, 60, and 80 μL) has no significant effect on θ for all PLs at various concentrations, conforming to theory, so average values are reported. Values of θ for PL solutions are shown in Fig. 5 and are summarized for $n = 8$ in Fig. 6, along with those of model synthetic anionic (SDS) and nonionic ($\text{OP}(\text{EO})_{10}$) surfactants for comparison. Values of θ for PC-8 and PE-8 are less than those of SDS and $\text{OP}(\text{EO})_{10}$ at all relative concentrations studied, while those of PA-8 are lower at high concentrations, showing their potential as commercial wetting agents. However, as a class, the PLs do not exhibit better wetting characteristics than typical commercial hydrocarbon surfactants.

Values of θ for PC-5 through PC-8, PE-6 and PE-8, SDS, and $\text{OP}(\text{EO})_{10}$ become almost or completely constant above their CMCs, as expected (Figs. 5B,5C,6). Slope changes occur at the CMC for PA-8 and PA-10, although level regions do not occur (Fig. 5A), while θ values for PG-6, PG-8, and PG-10 show no discontinuity at their CMCs (Fig. 5D). A partial explanation is that surface tensions of PG-6 and PG-8 increase above their CMCs (Fig. 3D).

For all of these PLs (except for PE-6 and PE-8 at very low concentrations), θ decreases as n

increases, yielding better wetting agents. However, for all four PL classes, this behavior is reversed at high values of n , as with PA-12, PC-10, PE-10, and PG-12, giving almost no reduction of θ below 90° at any concentration. Although this should occur when PL solubility is exceeded at high values of n , all the low molecular weight PLs studied here are water-soluble at 30°C . A possible explanation is the depletion effect (15). This is caused by surfactant loss from the bulk solution due to adsorption at the liquid/solid interface, which can be significant at very low bulk concentrations and can cause overestimations of quasi-equilibrium contact angles. This loss can be estimated from the wetted surface area and the area of the surfactant head group. If complete bilayer adsorption occurs, as much as 30% of surfactant can be lost. The fractional loss can be higher with increasing n due to greater adsorption on the hydrophobic surface.

In general, good wetting agents have low γ_{LV} , but from the Young equation, γ_{SL} also affects θ . In wetting, the importance of surfactant adsorption effects on γ_{SL} is often overlooked (21). For example, Rosen and Dahanayake (18) point out that fluorinated surfactants do not improve wetting of aqueous solutions on hydrocarbon surfaces as much as silicone surfactants, despite the lower air/water surface tensions of the former, due to its lower adsorption at the solid/water interface. From the results here, it is reasonable to conclude that PL surfactants do not adsorb on the polyethylene surface as well as SDS or OP(EO)₁₀, explaining why ultralow surface tensions do not result in ultralow contact angles.

Foaming. Foam is an important surfactant characteristic (11,22). It can be produced by shaking, beating, stirring, and air injection. The Ross-Miles method is generally used to reproducibly measure surfactant foaming properties (17). However, we were unable to apply this method because the required volume (250 mL) of tested solution was too high. Therefore, foam was generated by direct injection of ambient air into solutions of PLs and the commercially used surfactants SDS and OP(EO)₁₀. Foamability, a measure of foam volume after it is produced, and foam stability, a measure of change of foam volume upon aging, were measured. High relative foam heights (H_i/H_o), where H_i is height of foam and solution at an arbitrary time and H_o is the initial solution height, indicate better foamabilities. $H_i/H_o = 1$ indicates no foaming. A rapid decrease of

H_i/H_o means low foam stability. The results, using three replications, were reproducible.

Values of H_i/H_o of all four PL types studied here increase with concentration to their CMCs or beyond, as expected (10) and as shown for PC-8 (Fig. 7). Since the maximal foam height generally occurs near the CMC, the effect of n on foaming was measured only at that point for PLs other than PC-8 (Fig. 8). All four PL types become more powerful foaming agents with increasing n until a certain limit, reached by PA-12, PC-8, PE-10, and PG-10. At low n , there is insufficient cohesiveness of the liquid lamellae to enclose gas in the foam. At high n , surfactants become less water-soluble, resulting in high film rigidity or low film elasticity and cell breakage.

Values of H_i/H_o for PL-8s, SDS, and OP(EO)₁₀ at their CMCs appear in Fig. 9. The zwitterionic surfactants PC-8 and PE-8 give the highest H_i/H_o values, followed by the nonionic surfactant OP(EO)₁₀ and the anionic surfactants PG-8, PA-8, and SDS, respectively. It is surprising that SDS exhibits poorer foamability and foam stability than OP(EO)₁₀ since anionic surfactants generally exhibit better foaming than nonionic surfactants. From the Ross-Miles foam test, for example, SDS shows an initial foam height of 155 mm vs. 135 mm for OP(EO)₁₀ and foam heights after 5 min are 100 mm vs. 30 mm, respectively, for these well-characterized surfactants (21). So, the relatively poorer foaming of SDS compared to OP(EO)₁₀ must be due to the dynamic nature of the test used here. So, without trying to overinterpret the results, we conclude that the PL surfactants exhibit foaming behavior typical of commercial surfactants.

Values of H_i/H_o of PC-8 and most other PLs at higher concentrations decrease modestly with time (Figs. 7–9), indicating their good foam stabilities. There is no clear relationship between foam stability and n .

The properties measured in this work indicate that low molecular weight PLs are promising surfactants. As a group they have very low plateau surface tensions, while at certain values of n they exhibit good wettabilities and foaming. Their foam stabilities are high. It is surprising that these properties have not been investigated earlier. It is hoped that this study will lead to the further use of these PLs.

ACKNOWLEDGMENTS

Financial support for this work was provided by the U.S. Department of Agriculture through the Biotechnology Byproducts Consortium, a grouping of Iowa State University and the University of Iowa. In addition, support was received from the sponsors of the Institute for Applied Surfactant Research at the University of Oklahoma, including Akzo Nobel, Clorox, Conoco/Phillips, Church and Dwight, Dial, Ecolab, Halliburton, Huntsman, Kerr-McGee, Oxiteno, Procter & Gamble, Shell Chemical, and Unilever.

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TABLE 1
Effect of Fatty Acid Chain Length on CMC (mM) and Plateau Surface
Tension (parentheses) (mN/m)

<i>n</i>	PA		PC		PE		PG	
5	—	—	59.5	(34)	—	—	—	—
6	—	—	15.4	(31)	12.1	(24)	10.8	(31)
7	—	—	1.78	(27)	—	—	—	—
8	1.55	(23)	0.16	(25)	0.17	(23)	1.04	(25)
10	0.28	(25)	0.009	(24)	0.007	(26)	0.059	(25)
12	0.025	(21)	—	—	—	—	0.011	(26)

FIGURE CAPTIONS

Fig. 1. PL: General PL structure. PA, PC, PE, PG: Replacements of X in individual PL structures.

Fig. 2. The contact angle of a droplet on a surface.

Fig. 3. Effect of n and concentration on PL surface tensions. (A) PA, (B) PC, (C) PE, (D) PG.

Fig. 4. Effect of n on CMCs of different PLs.

Fig. 5. Effect of n and concentration relative to CMC on PL contact angles. (A) PA, (B) PC, (C) PE, (D) PG.

Fig. 6. Effect of concentration relative to CMC on contact angles of different surfactants.

Fig. 7. Effect of concentration relative to CMC and elapsed time on PC-8 relative foam heights.

Fig. 8. Effect of n and elapsed time on relative foam heights of PLs at their CMCs. (A) PA, (B) PC, (C) PE, (D) PG.

Fig. 9. Effect of elapsed time on relative foam heights of different surfactants at their CMCs.

Figure 1

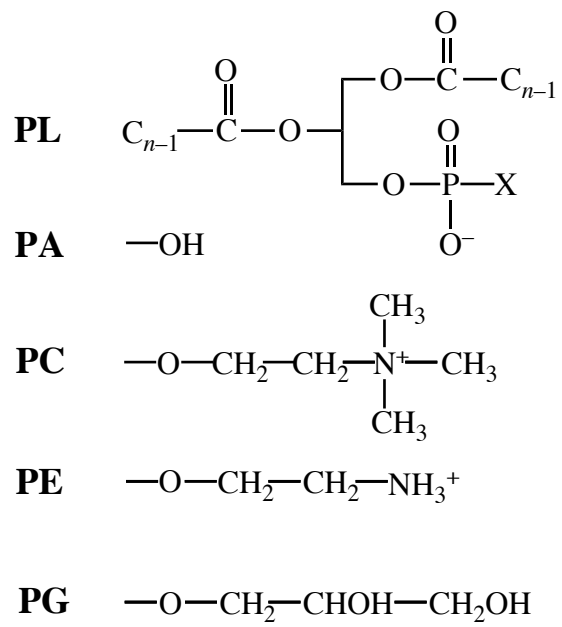


Figure 2

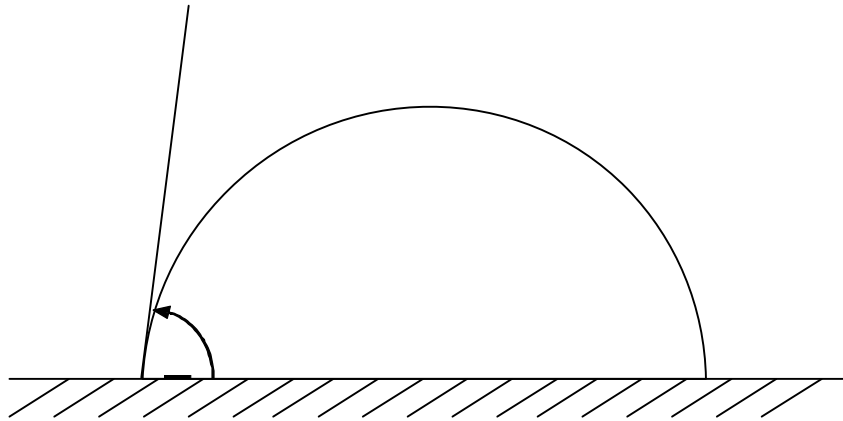


Figure 3

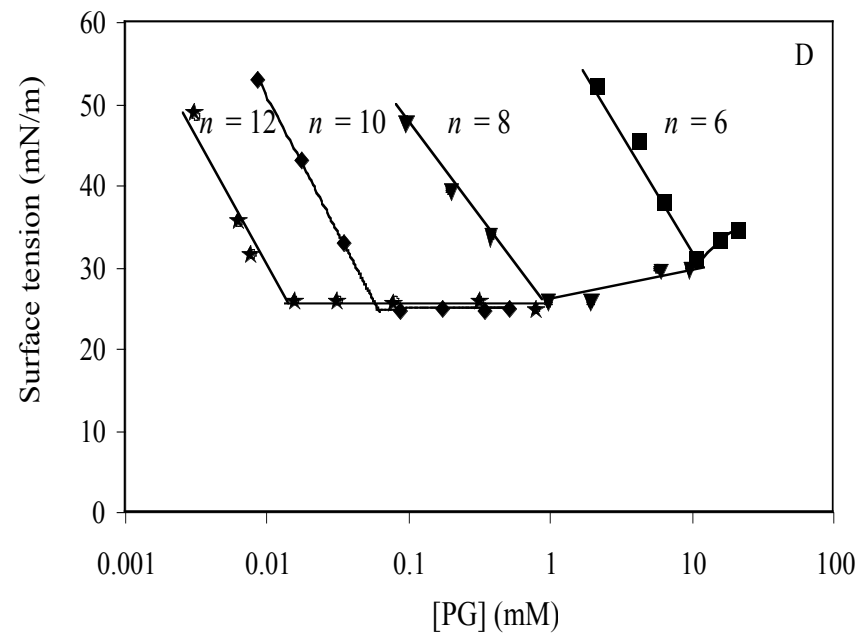
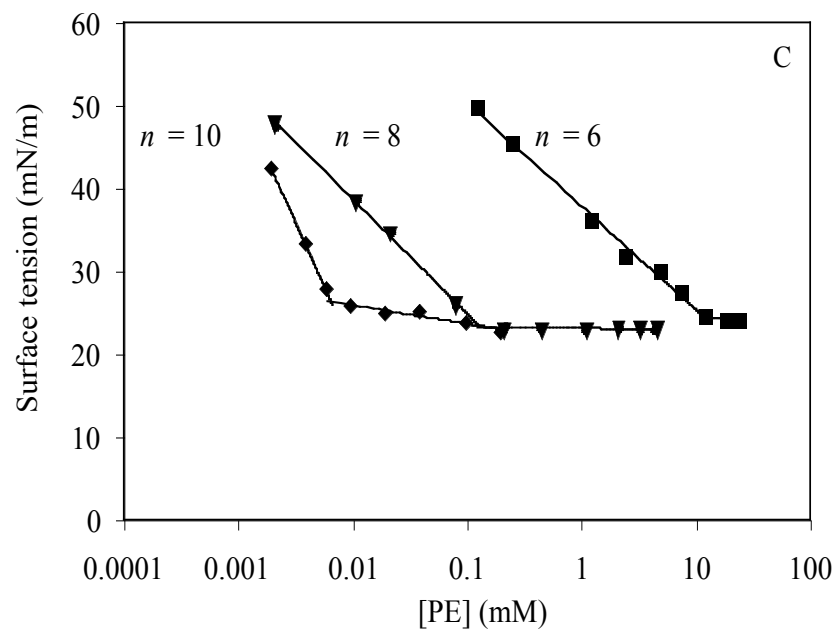
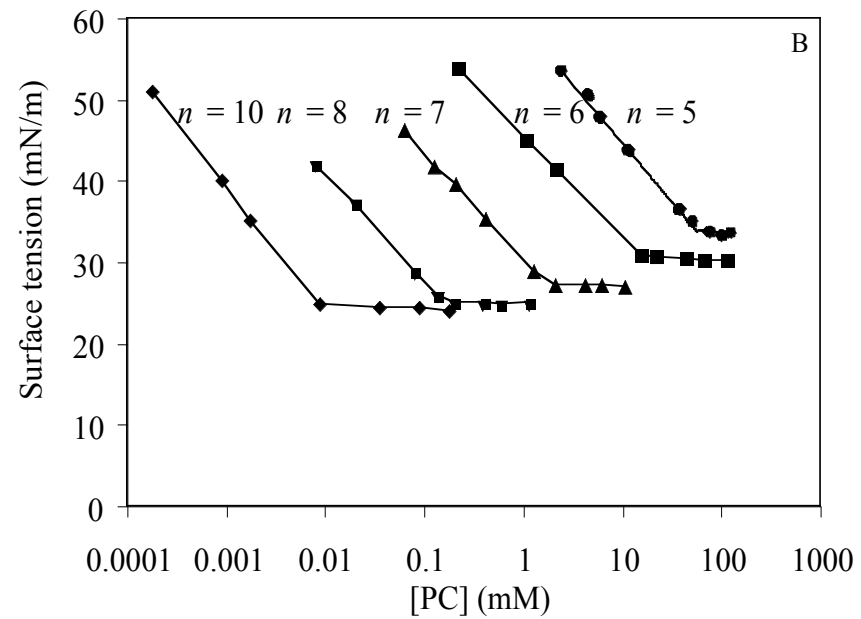
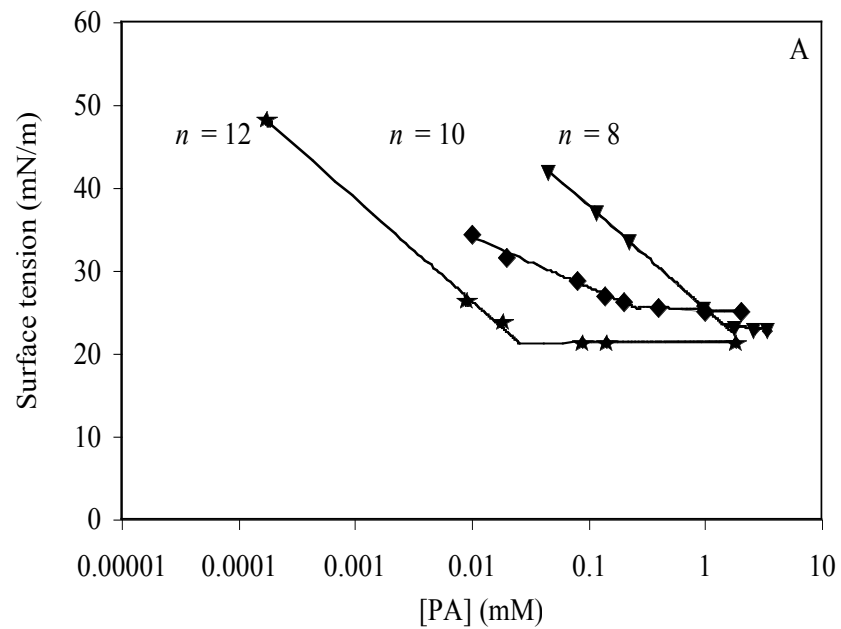


Figure 4

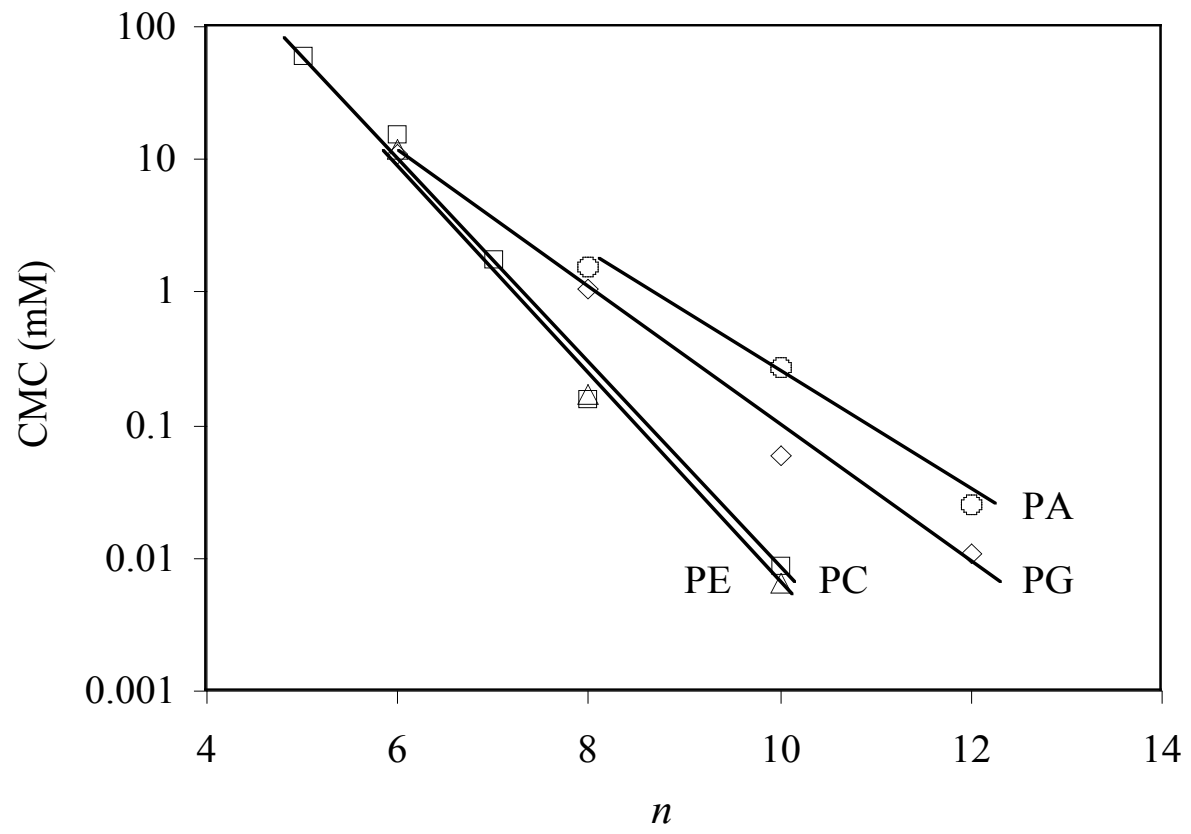


Figure 5

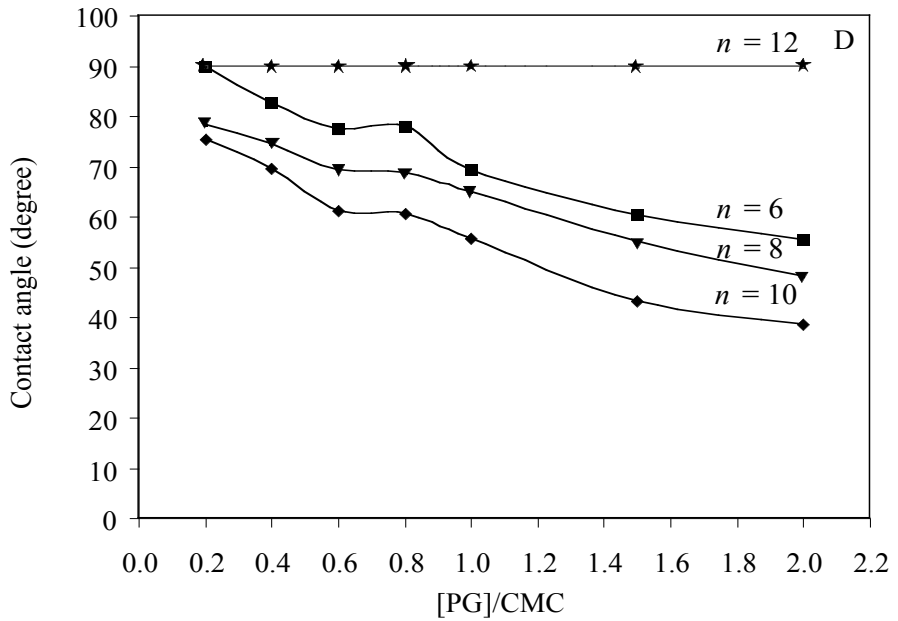
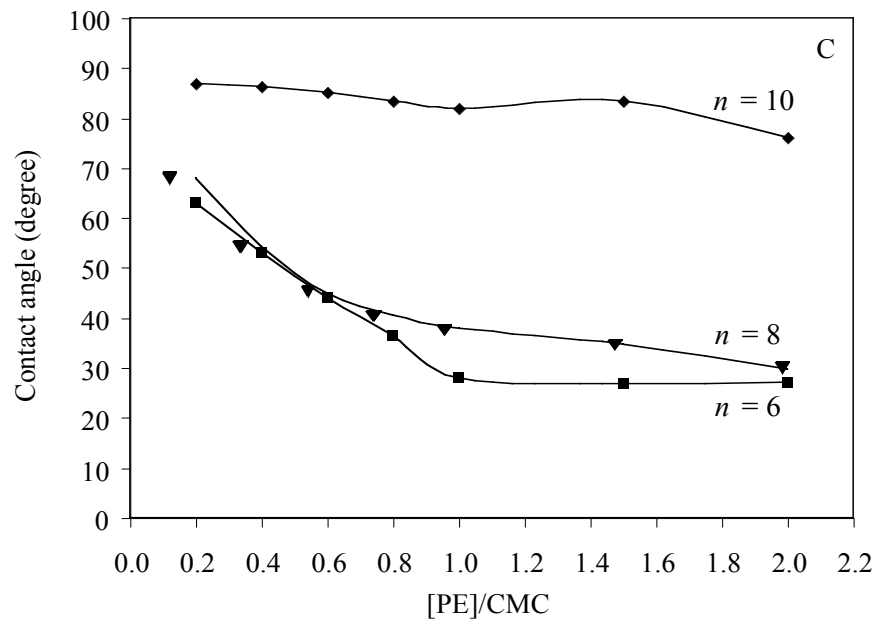
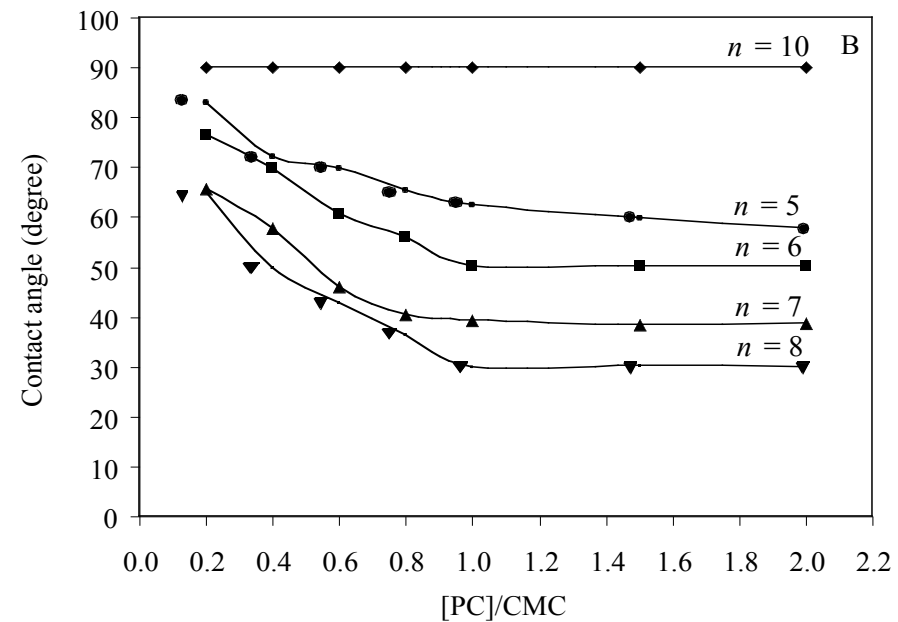
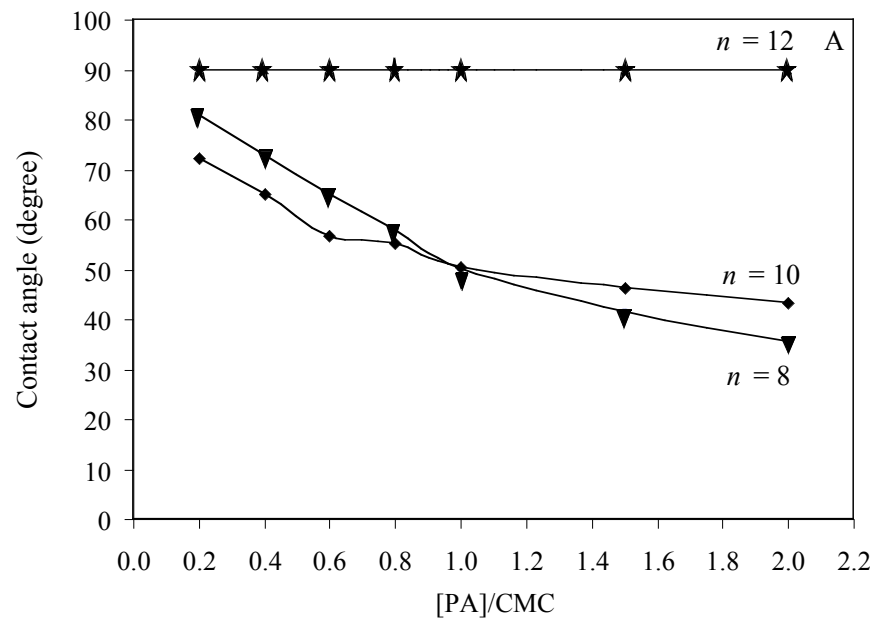


Figure 6

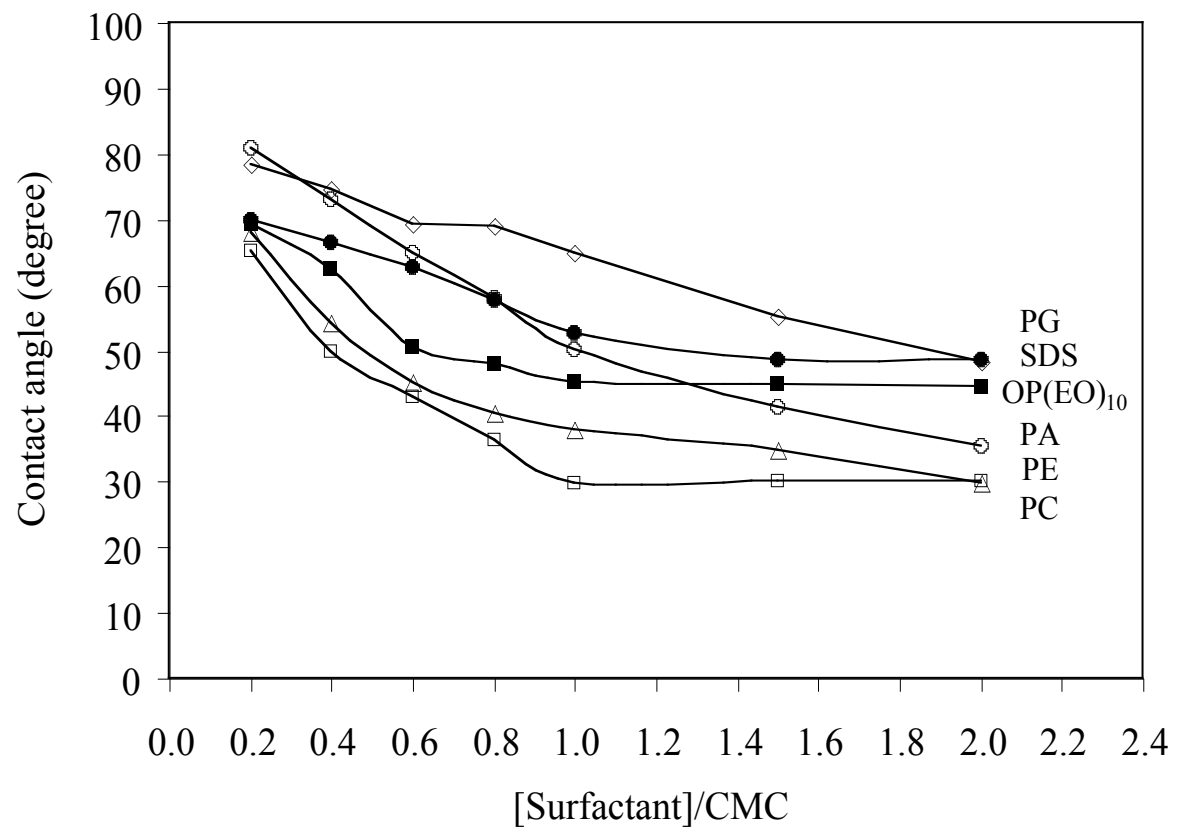


Figure 7

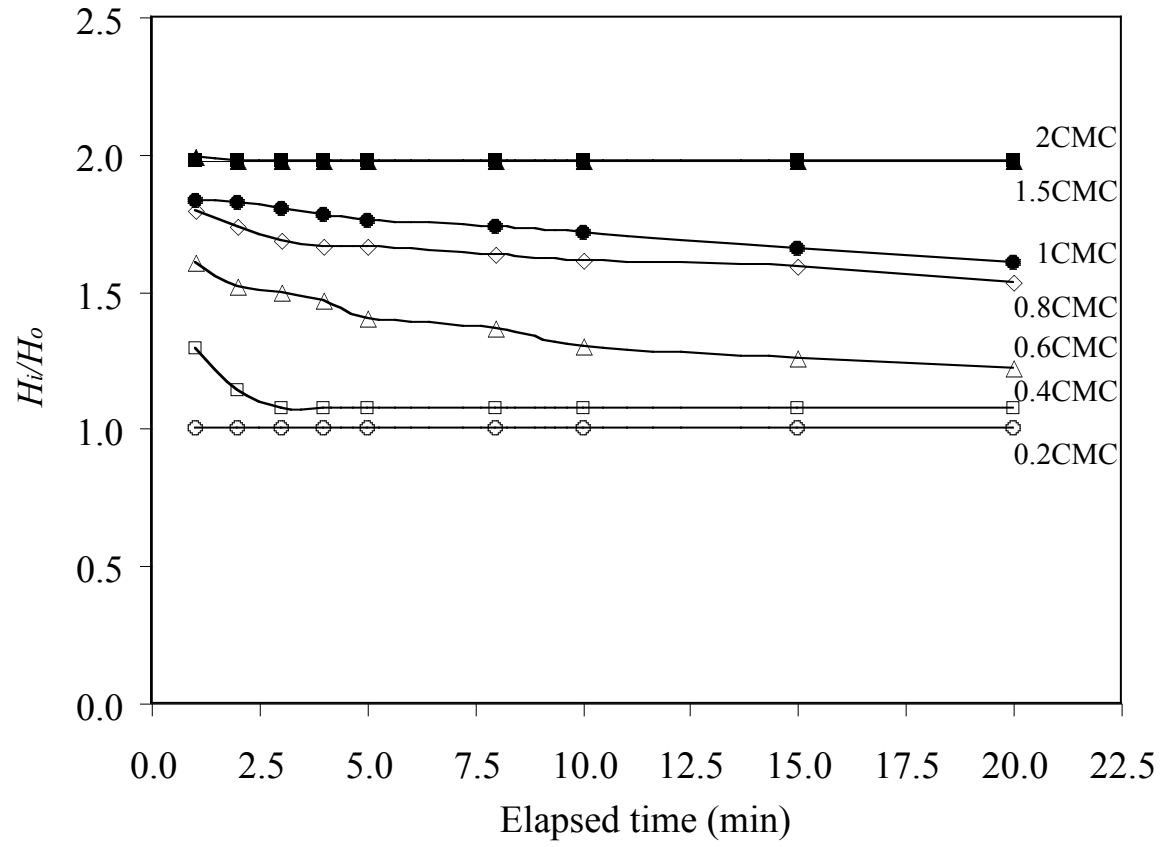


Figure 8

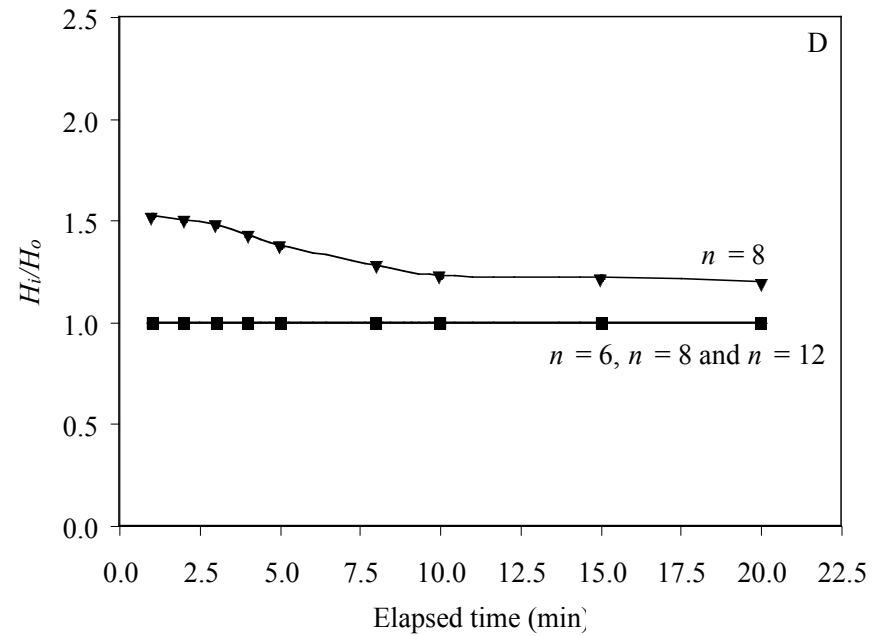
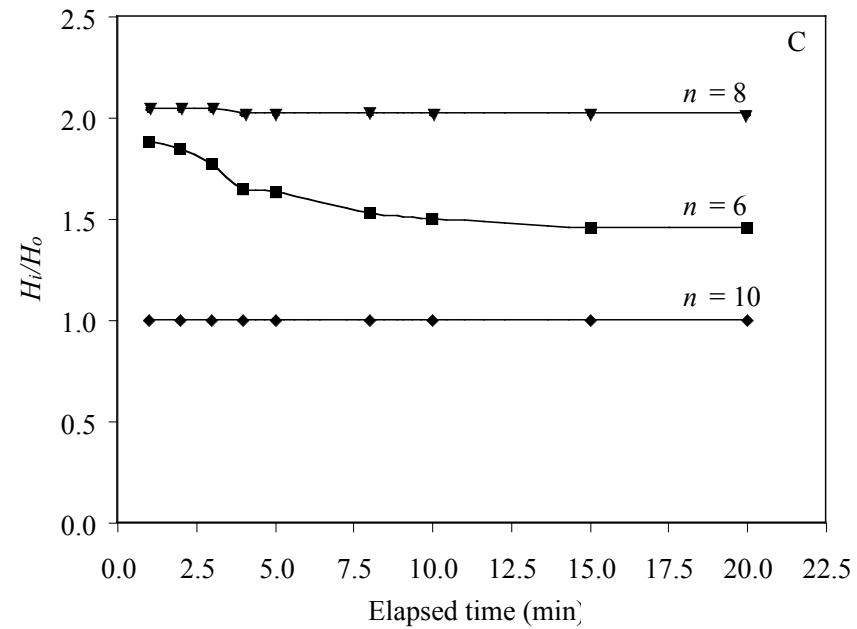
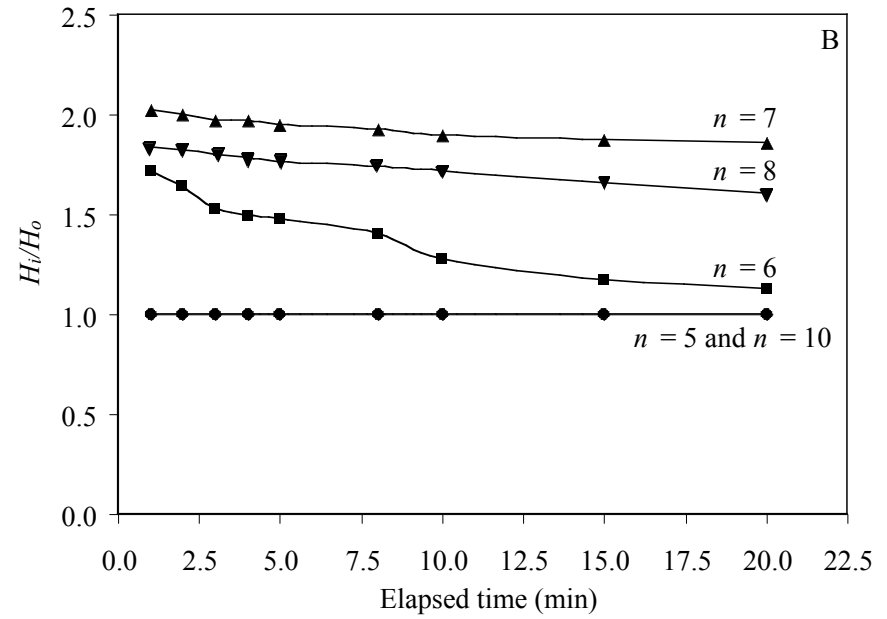
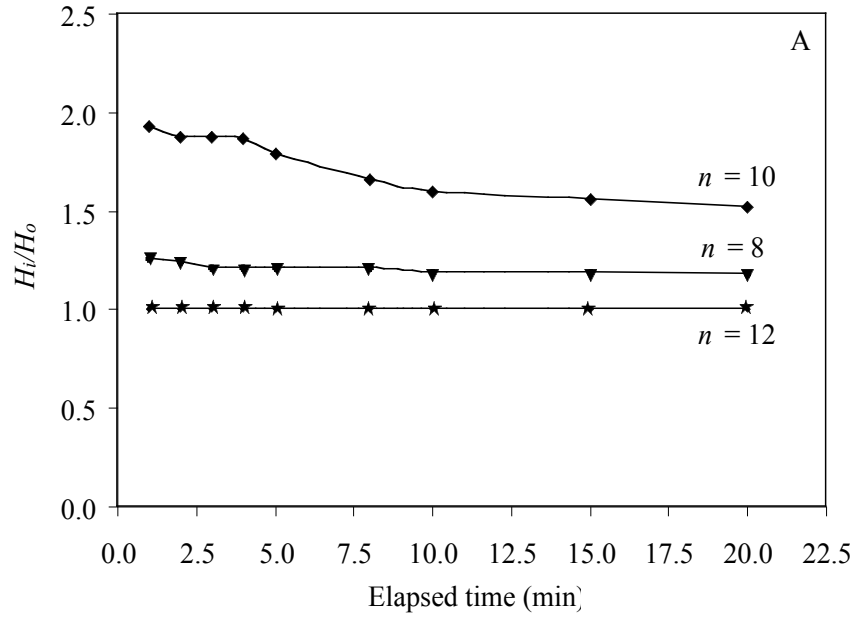


Figure 9

