

Towards an understanding of adsorption behaviour in non-aqueous systems: adsorption of poly(vinyl pyrrolidone) and poly(ethylene glycol) onto silica from 2H, 3H-perfluoropentane

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Abstract

The adsorption behaviour of low molecular weight poly(ethylene glycol) (PEG 600) and poly(vinyl pyrrolidone) (PVP K25) to silica particles has been investigated at room temperature (21°C) in the partially fluorinated solvent 2H,3H-perfluoropentane (HPFP). PVP (adsorbed amount, $\Gamma = 12 \text{ mg g}^{-1}$) was found to adsorb more strongly than PEG ($\Gamma = 4 \text{ mg g}^{-1}$). Both of these values were higher than observed in water. In a further distinction to the aqueous case, where PVP displaces PEG from the interface, no competitive adsorption effects were observed between these two polymers in HPFP, with the adsorbed amounts of each polymer being unchanged by the presence of the other. The stability of silica suspensions in HPFP was primarily dependent on the presence of PVP; PEG/silica systems were unstable, but PVP/silica and PEG/PVP/silica systems formed stable suspensions. All suspensions were destabilized by the addition of small (0.15 wt%) amounts of water. The observations made in this work would point to a flocculation phenomenon due to the addition of water, and not Ostwald ripening. The mechanism of this destabilization is likely to be water acting as a flocculation bridge between particles.

Introduction

The formation of stable particle dispersions are essential to the success of a wide range of commercial products from inks, paints and foodstuffs to cosmetic and pharmaceutical formulations. Polymers and surfactants are used routinely to stabilize aqueous dispersions, and these systems have been studied widely (Fleer et al 1993). However, for many applications the solvent required is not water. Previous reports have focussed mainly on water–solvent mixtures i.e. systems where organic solvents such as acetone or ethanol are added to a water-based solution to displace the adsorbed polymers (Cohen Stuart 1982, 1984a; Kawaguchi & Sakakida 1990; Nelson et al 2002). Fewer studies have been reported on adsorption in non-aqueous media. Kawaguchi et al (1989) described the adsorption behaviour of poly(ethylene oxide) onto silica particles from carbon tetrachloride, and found an equilibrium adsorbed amount of approximately 1.2 mg m^{-2} . There is little published work on adsorption from fluorinated media, yet these remain an important class of solvents, especially in the pharmaceutical arena. Products such as topical gels, inhalation drug-delivery or blue blood substitutes (Yokoyama et al 1983; Smith 1995; Courier et al 2002; Riess 2002; Stein & Stefely 2003) rely on the formation of stable dispersions of drug particles in partially fluorinated solvents.

We have investigated polymer/particle combinations designed as model systems of such pharmaceutical formulations to develop a basic understanding of their stability. We have considered the adsorption of two typical polymer excipients, poly(ethylene glycol) and poly(vinyl pyrrolidone) onto micron sized silica particles from a partially fluorinated hydrofluoroalkane (HFA) solvent. In particular, we aimed to address the mechanism of stabilization in these systems, and investigated synergistic effects between excipients. We also considered the mechanism of water destabilization observed in HFA suspensions.

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Materials and Methods

Materials

Poly(ethylene glycol) (600 g mol^{-1} ; PEG 600), and poly(vinyl pyrrolidone) ($25\,000\text{--}35\,000 \text{ g mol}^{-1}$; PVP K25) were purchased from Aldrich. The polymers were dried by heating at 70°C to constant weight and stored in a desiccator before use. Unless otherwise mentioned in the text, 2H, 3H-perfluoropentane (HPFP; Apollo Scientific, 98% pure) was cleaned by shaking with acidic and basic alumina, dried over molecular sieves and filtered before use. Micronized silica, average diameter $3 \mu\text{m}$, was purchased from Aldrich and used as received. All water used for cleaning and solution preparation was obtained from a Millipore purification unit. All other chemicals were of analytical grade and used as received.

Adsorption isotherms

The methods traditionally used to investigate polymer conformation at particle surfaces and in this manner include dynamic light scattering, NMR, SANS and EPR (Cohen Stuart et al 1982; van der Beek et al 1991; Nelson et al 2002); however, the use of non-aqueous and in particular volatile fluorinated solvents makes these types of experiment much less straightforward. The use of large particles means that suspensions are mostly unstable, which precludes the use of solvent relaxation measurements to investigate the adsorbed amounts. Hence, in the first instance we have relied on wet chemistry methods. The adsorbed amounts were determined using the depletion method (Fleer et al 1993). After polymer solutions were equilibrated with a known mass of silica, the particles and any adsorbed polymer were separated by centrifugation. The polymer concentration in the supernatant was then determined spectroscopically.

Sample preparation

To accurately prepare the low concentration polymer solutions required, PEG or PVP was added as a concentrated stock solution in ethanol to a pre-weighed vial. The solvent was evaporated by leaving the sample in a warm oven for a couple of hours, and HPFP added to give the desired polymer concentration. Vials were then shaken for 2–4 h to dissolve the polymer. To this solution in HPFP, a pre-weighed amount of silica was added. The vial was then tumbled for 24 to 48 h to equilibrate the system. Due to the low solubility of PVP K25 in the solvent it was important that the PVP concentration after adsorption was kept below the 1000 ppm solubility limit (Paul et al 2005), otherwise any insoluble polymer would be filtered out with the particles and assumed to have adsorbed, leading to falsely high adsorbed amounts being obtained. After equilibration, the samples were filtered into a pre-weighed vial using a $0.8\text{-}\mu\text{m}$ cellulose filter (Whatman). The mass of filtrate was recorded and the solvent allowed to evaporate by gentle heating in an oven. A known quantity of water was added to replace the solvent and the samples

shaken to dissolve the polymer. The final concentrations for each polymer were determined by UV spectroscopy, either directly (PVP) or using a complexation method (PEG) as described below. Using a spectroscopic method has the advantage that the absorbance (whether measured directly or indirectly) is independent of polymer molecular weight, and hence polydispersity does not affect the results. Measurements were carried out using 10-mm path-length quartz cells, at room temperature ($\sim 22^\circ\text{C}$).

Validation

A series of validation experiments were carried out with a PVP K25 solution at a fixed starting concentration of 400 ppm (below the solubility limit), to which various amounts of silica were added. Otherwise the procedure was as described above. To check for reproducibility, triplicate experiments were run at various fixed polymer and silica concentrations using high polymer:particle ratios to ensure measurements were at the plateau of the isotherm. Control experiments with zero wt% silica were performed also.

Statistical analysis

Mean and standard deviation values were calculated for the adsorbed amounts and equilibrium polymer concentrations. Equilibrium adsorbed amounts and standard deviations obtained from full adsorption isotherm experiments were calculated as an average of data in the plateau region. Differences between the various treatments were determined using the Kruskal–Wallis test in association with an appropriate post hoc test ($P < 0.05$ denoting significance).

Determination of PVP concentrations

The absorbance of PVP K25 solutions in water was measured at 196 nm using water as the reference. A calibration plot of UV abs_{196} vs polymer concentration was prepared (Chaimberg & Cohen 1991) and was found to be linear up to 40 ppm PVP. The aqueous polymer solutions from the adsorption samples were diluted to give a measured absorbance at 196 nm below 2.0, corresponding to a PVP concentration between 0 and 40 ppm. Taking into account any necessary dilutions, the equilibrium concentration of polymer in the original HPFP-filtrate was calculated, and this value used to determine the adsorbed amount (mg polymer per g particle) at each polymer concentration.

Determination of PEG concentrations

Since PEG 600 has no chromophore in the UV range, a complexation method was used to measure PEG concentrations. These were determined with reference to a calibration plot prepared by complexation of a series of PEG standards with molybdophosphoric acid, according to the method of Nuyssink & Koopal (1982). Molybdophosphoric Acid Reagent (MPR) 250 mL was prepared by dissolving 0.5 g molybdophosphoric acid hydrate

($\text{H}_3\text{M}_0_{10}\text{PO}_{32}\cdot 24\text{H}_2\text{O}$), 0.5 g barium chloride and concentrated hydrochloric acid (approx. 1.5 mL) in water. A 2-mL sample of PEG solution (in water) was added to 2 mL MPR in a dry centrifuge tube, shaken gently and allowed to stand at 25°C for 15 min. In addition, a blank solution was prepared using 2 mL water instead of the PEG solution. The samples were then centrifuged (15 min at 3 K rev min⁻¹), and 1 mL supernatant was made up to 50 mL with water in a volumetric flask. The UV absorbance of these solutions at 216 nm was measured using pure water as the reference. The difference in absorbance at 216 nm for standard PEG solutions (up to 400 ppm) and the water blank analysed as above was used to prepare a calibration plot of $\Delta_{\text{abs}(216)}$ vs polymer concentration. With reference to this calibration plot, the final polymer PEG concentrations were determined from the difference in absorbance $\Delta_{\text{abs}(216)}$ between the PEG solution and the blank after reaction with MPR in the same way. To use the molybdophosphoric acid method the polymer concentrations in the aqueous solutions to be analysed must be between approximately 10 and 300 ppm.

Results and Discussion

Adsorption of PVP K25

Figure 1 shows the adsorption isotherm for PVP K25 adsorbing to silica, plotted in the conventional form of adsorbed amount (Γ) mg polymer per g particles (mg g^{-1}) as a function of equilibrium polymer concentration. The general shape of the isotherm was consistent with a high affinity isotherm (Fleer et al 1993). Due to the high degree of scatter in the plateau region it was difficult to obtain the equilibrium adsorbed amount to a high degree of precision. However, the data presented were the cumulative results of several experiments, adding various amounts of polymer at a fixed silica concentration, and adding various amounts of silica at a fixed polymer concentration. From the cumulative results an adsorbed amount of

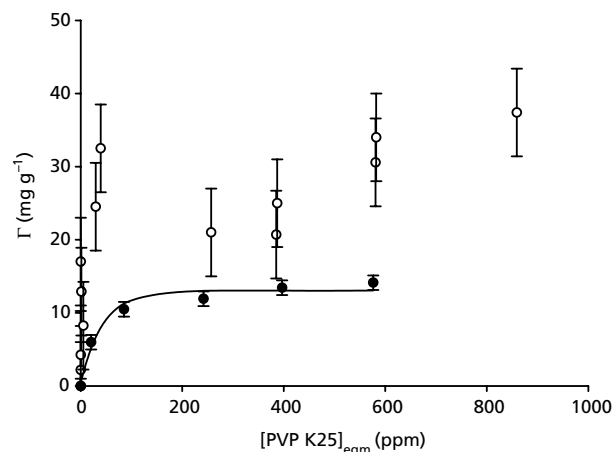


Figure 1 Adsorption isotherm for PVP K25 onto silica in HPFP at 20°C. Open symbols, before drying the solvent. Closed symbols, with dry HPFP. Line is an exponential fit to the data.

$\Gamma = 30 \pm 5 \text{ mg g}^{-1}$ was obtained. Even given the generous margin of error, this Γ value was higher than those found in aqueous solution, where adsorbed amounts from PEG on silica were typically $0.1\text{--}1 \text{ mg m}^{-2}$ (Cohen Stuart et al 1982). To check that the high adsorbed amounts were not due to particles themselves, the adsorbed amount for PVP was measured for these particular silica particles in water. The equilibrium adsorbed amount was found to be approximately 1.2 mg g^{-1} , so the difference in adsorbed amount was not attributable to the surface character of the particles.

Addition of PEG 600 to the system (equal amounts of PEG or PVP were added to the starting solutions) resulted in a significant decrease in Γ to approximately 15 mg g^{-1} (Figure 2). Two repeat experiments were carried out over the entire polymer concentration range, and both sets of data are presented. There was an excellent agreement in equilibrium adsorbed amounts between the two experiments. At face value, this decrease in PVP suggested PEG adsorbed preferentially at the surface. In the aqueous case, however, PVP is known to displace PEG from the silica/water interface (Cohen Stuart et al 1984b; van der Beek 1991; Nelson et al 2002). The drop in the adsorbed amount for PVP K25 when PEG 600 was introduced was therefore unexpected. One possible reason was a kinetic effect due to faster diffusion of the smaller PEG molecule and therefore preferential adsorption to the surface. To investigate this further, the adsorption behaviour of PEG was investigated.

Adsorption of PEG 600

Adsorption isotherm for PEG 600 alone is shown in Figure 3 and that for PEG 600 with PVP K25 in Figure 2, considered over the same concentration range as the PVP data. The equilibrium adsorbed amounts for PEG 600 remained approximately constant with and without PVP; Γ_{eqm} approximately 3 and 4 mg g^{-1} , respectively. There was no evidence that PVP K25 displaced PEG 600

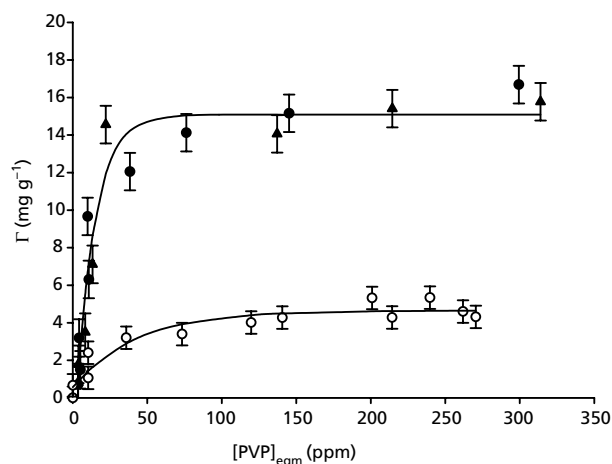


Figure 2 Adsorption of PVP K25 with PEG 600. Closed symbols, PVP (two sets of data); open symbols, PEG.

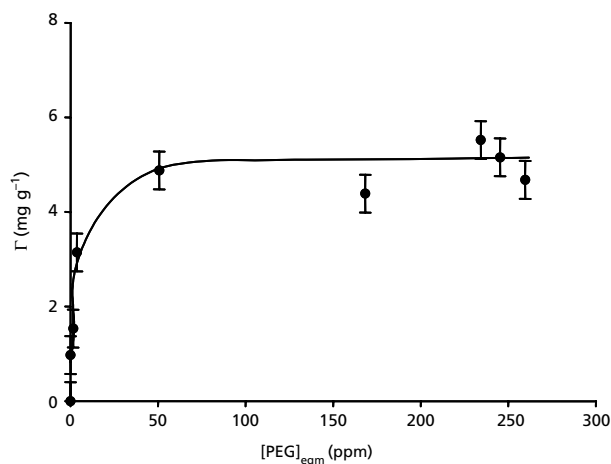


Figure 3 Adsorption isotherm for PEG 600 onto silica in HPFP at 20°C.

in these fluorinated systems. Co-adsorption of PVP and PEG had been observed in aqueous systems by Nelson et al (2002), who used NMR solvent relaxation measurements to study the adsorption of PVP ($7 \times 10^5 \text{ g mol}^{-1}$) and PEG ($4 \times 10^6 \text{ g mol}^{-1}$) onto silica (16 nm diameter Ludox HS30, Dupont) from water. In general, they found PVP displaced PEG at the silica/water interface, and coexistence at the surface only occurred for systems where there was excess silica surface after adsorption of all the PVP, i.e. the PVP concentration was insufficient to reach Γ_{eqm} . However, in our study, there appeared to be coexistence of the polymers on the particle surface independent of the relative particle/polymer concentrations. If insufficient time was allowed for the system to fully equilibrate, this may explain the drop in PVP adsorbed amount when PEG was added. To test this hypothesis, a series of experiments were carried out at a single polymer concentration (i.e. a single point on the isotherm was re-measured); these experiments allowed for much longer equilibration times (up to two weeks), and pre-adsorption of PEG 600 or PVP K25 before adding the second polymer. No difference in the measured adsorbed amounts was obtained compared with the control experiment in which both polymers were added together. Average results are shown in Figure 4. These confirmed that the change in the measured adsorbed amount for PVP with added PEG was not attributable to non-equilibrium effects.

It is known (Cohen Stuart et al 1982) that polymer adsorption is highly dependent on the strength of polymer/solvent interactions. As the quality of the solvent for a particular polymer decreases, an increase in adsorbed amount is expected, and this may go some way to explaining the high PVP adsorbed amounts observed in this study. An explanation for the decrease in PVP K25 adsorption on addition of PEG 600 may be found from studies of the phase behaviour (Paul et al 2005). The solubility of PVP K25 was found to increase upon the addition of PEG 600. This would result in a lower driving force for adsorption of PVP K25 to the silica surface as it becomes more soluble in the solvent.

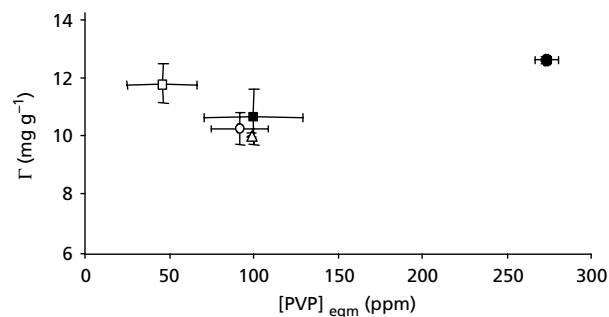


Figure 4 Single point measurements of equilibrium adsorbed amounts of PVP K25. PEG and PVP present (○), PVP only (◆), pre-adsorb PEG (△), pre-adsorb PVP (■), pre-adsorb PEG, repeated with different starting concentration (□). Each data point is an average of three identical experiments. Error bars are standard deviations.

Effect of water

Stability studies demonstrated that PVP was essential for suspension stability; samples containing PVP only at $[\text{PVP}] > \Gamma_{\text{eqm}}$ formed stable solutions, whereas samples containing PEG and silica only were not stable. Addition of PVP to these systems caused stabilization of the system, whilst adding PEG to the previously stable PVP had no effect. Addition of water to a concentration of 0.15 wt% (almost four times the solubility limit in HPFP) caused an instant destabilization of the suspension.

Previous phase behaviour studies have identified the sensitivity of these systems to the presence of small amounts of water; PEG 600/HPFP solutions can tolerate small amounts of moisture, PVP K25 solutions are highly susceptible to the presence of water, and the mixed polymer systems phase separate when even small ($< 0.15 \text{ wt}\%$) amounts of water are added (Paul et al 2005). During the adsorption experiments described above the polymers were dried during sample preparation, in the process of evaporating the ethanol, but the solvent used was not dried before use. The maximum solubility of water in HPFP at room temperature was $390 \pm 40 \text{ ppm}$ (Rogueda 2003), sufficient to significantly alter the phase behaviour and therefore any adsorption. The adsorption isotherms were therefore repeated after drying the solvent, data for which is shown in Figure 1. The equilibrium adsorbed amount of PVP K25 thus obtained fell from approximately 30 to 12 mg g^{-1} i.e. to the same value observed in the presence of PEG 600 (for wet or dry systems). This suggested that PEG might have decreased the driving force for PVP adsorption by binding the water and thereby promoting solubility of PVP K25 in the solvent. In previous phase behaviour studies (Paul et al 2005) PVP solutions were destabilized by addition of small quantities of water, due to incompatibility of PVP with the wet solvent. The adsorption behaviour was consistent with the solution phase behaviour, and the higher adsorbed amounts in wet systems were consistent with an increased driving force for adsorption, up to the point of polymer insolubility in the wet solvent leading to precipitation.

This was seen by measuring equilibrium polymer concentrations after addition of water to the system (up to the expected water solubility in HPFP) – in all cases, the polymer concentration measured was zero.

The use of the model system using silica, rather than an organic drug molecule, gives a clearer insight into the likely method of water-induced destabilization of formulations in HFAs. Most drugs have a finite solubility in HFA and water, hence instability may be due to Ostwald ripening of the system and this possibility may not be ruled out. As silica is insoluble in HFA and water, Ostwald ripening is not the cause. It only remains to distinguish between two possibilities: water acting as a bridging agent between particles, bringing about destabilization, and excipient scavenging. In the light of adsorption experiments carried out in the presence of different amounts of water, and the decrease in adsorbed amount observed in the dry systems, the role as bridging agent seems more likely. Addition of water to the system causes the solvent/polymer interactions to become less favourable. This will lead to a collapse of the polymer layer around the particle and therefore destabilization of the suspension.

Conclusions

In model mixed polymer/particle systems comprising PEG/PVP and silica, suspension stability was dependent on adsorption of the larger PVP molecules to the particle (silica) surface and PEG did not play an equally important stabilization role. Adsorbed amounts obtained were significantly higher than those observed in aqueous media and we suggest that this was due to a decrease in the solvent/polymer interaction leading to a higher thermodynamic driving force for adsorption. Unlike the aqueous case, there was no competitive adsorption of PVP and PEG onto silica in HPFP. However, the PVP adsorbed amount was mediated by the presence of PEG, seemingly due to modification of the solvent properties. Hence PEG performed an important indirect role in suspension stability. Adsorbed amounts for PVP decreased on addition of PEG or drying of the system. These results were consistent with the solution phase behaviour of both polymers in HPFP, suggesting that PEG acted as a water scavenger, promoting PVP solubility in HPFP and therefore decreasing PVP adsorption. The sensitivity of these systems to small amounts of water was attributed to enhanced insolubility of the PVP in wet fluorosolvents. Furthermore it may be postulated that the HFA suspension destabilization by water was not due to Ostwald ripening, but a decrease in solvation of the polymer layer.

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