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Polysorbate 80 UV/vis spectral and chromatographic characteristics – defining boundary conditions for use of the surfactant in dissolution analysis

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Abstract

Polysorbate 80 is used in the pharmaceutical industry as an additive to enhance the solubility of non-polar compounds in formulation design and during dissolution analysis. In this paper we present the spectroscopic and chromatographic characteristics for a series of commercially available sources of this non-ionic surfactant. The large UV/vis absorbance and broad chromatographic elution of Polysorbate 80 often makes it difficult to accurately quantitate pharmaceutically active compounds in solutions where the surfactant is present. Boundary conditions have been established where analytical interferences can be avoided in spectrophotometric analysis by choice of analysis wavelength and solution concentrations. Chromatographic method development is also presented enabling the removal of Polysorbate interference in instances where spectroscopic interference is too great.

Keywords: Polysorbate 80; UV/vis spectra; Reversed-phase chromatography; Dissolution analysis; Pharmaceutical analysis; Chromatographic interference

1. Introduction

Polysorbate 80, a polymeric non-ionic surfactant, is commonly used for applications in the chemical, cosmetic, food, environmental, and pharmaceutical industries [1–13]. These uses typically involve the ability of the surfactant to stabilize emulsions or to increase the aqueous solubility of hydrophobic compounds. For the pharmaceutical industry one particularly important use is in dissolution analysis, which is necessary to ensure proper drug release performance in formulations. In the many instances where non-polar active pharmaceutical ingredients (APIs) suffer from poor solubility across the physiologically relevant pH range (1.0–7.5), surfactant dissolution media are needed to aid in total solubilization of API from a single dosage unit. It is generally recognized that the FDA allows the addition of surfactants such as Polysorbate 80 and sodium dodecyl sulfate to achieve solubility goals, while the Japanese regulatory agency states that Polysorbate 80 is the preferred surfactant and is allowed up to 5% (w/w) [14]. Furthermore, the use of enzymes to combat pellicle formation in gelatin capsules generally requires the utilization of non-ionic detergents, such as Polysorbate 80. However, along with these favorable solubilization characteristics, Polysorbate 80 also possesses significant spectroscopic and chromatographic properties making it difficult to qualitatively analyze API in solutions where the surfactant is present.

Large Polysorbate 80 UV/vis absorbance over the 200–300 nm range in solutions relevant to dissolution analysis typically causes interference with pharmaceutically active compounds because these organic molecules often contain $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, or $\sigma \rightarrow \pi^*$ chromophores (electronic transitions typically taking place at, or below 300 nm). This spectral overlap can preclude the practical use of simple UV/vis spectrophotometry for rapid quantification of drug release from formulation during dissolution analysis because relevant Polysorbate 80 solutions (0.01–5.0%) alone are greater than two absorbance units at many wavelengths. Furthermore, when applying HPLC/UV separa-

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tion of API from Polysorbate 80 is made difficult due to an extremely broad/polymeric elution pattern of Polysorbate 80 over a wide selection of reverse-phase columns and conditions. These analytical liabilities can often limit the usefulness of the surfactant to the investigator.

The goal of this paper is to give the pharmaceutical development scientist an understanding of surfactant concentration and wavelength boundary conditions where spectroscopic interference in dissolution analysis is minimized. Second, we present a chromatographic approach to remove Polysorbate 80 interference when these spectroscopic interferences cannot be avoided due to experimental circumstances mentioned above. Towards this goal the UV/vis spectrophotometric and chromatographic properties of 0.01–5.0% (w/w) aqueous Polysorbate 80 solutions commonly explored for dissolution media have been investigated. Four common commercial brands of Polysorbate 80 were analyzed and their UV characterization is presented. HPLC method development was performed with several reverse-phase column chemistries and silica pore sizes.

2. Experimental

2.1. Materials and reagents

Polysorbate 80 was purchased from TCI, Aldrich, NOF, and Acros. Water, acetonitrile, phosphoric acid, and methanol were purchased from Fisher Scientific and were all HPLC Grade or better.

2.2. Instrumental

UV/vis spectra were collected with a HP8453 spectrophotometer using a quartz 1-cm pathlength cell. All Polysorbate 80 spectra were taken in 70/30 acetonitrile/H_2O versus 70/30 acetonitrile/H_2O blank. All HPLC measurements were performed with a HP 1100 series instrument with HP G1314A photodiode array UV/vis detector (1 cm pathlength cell).

Gradient HPLC analysis of Polysorbate 80 was performed using the method conditions found in Table 1 with UV detection at 220 nm, ambient column temperature, and a flow rate of 1.0 mL/min with an injection volume of 20 μL. Gradient analysis was performed with the following columns: YMC pack C4 (3 μm, 4.6 mm × 150 mm), Zorbax SB-C8 (3.5 μm, 4.6 mm × 150 mm, Agilent), Hypersil C1 SAS (3 μm, 4.6 mm × 150 mm, Phenomenex), Platinum EPS C18 (3 μm, 4.6 mm × 150 mm, Alltech), Polaris C8 Ether (5 μm, 4.6 mm × 150 mm, Metachem), Zorbax SB-C18 (3.5 μm, 4.6 mm × 150 mm, Agilent), Zorbax SB Phenyl (3.5 μm, 4.6 mm × 150 mm, Agilent). The following columns were used for large pore size experiments: Nucleosil C18 (100 Å, 5 μm, 4.6 mm × 150 mm, Phenomenex), Nucleosil C18 (500 Å, 7 μm, 4.6 mm × 150 mm, Phenomenex), Nucleosil C18 (7000 Å, 7 μm, 4.6 mm × 150 mm, Phenomenex).

Table 1
Method conditions for Polysorbate 80 gradient HPLC analysis

Mobile phase A (0.1% phosphoric acid, %)	Mobile phase B (acetonitrile, %)	Time (min)
90	10	0
90	10	5
10	90	45
10	90	95

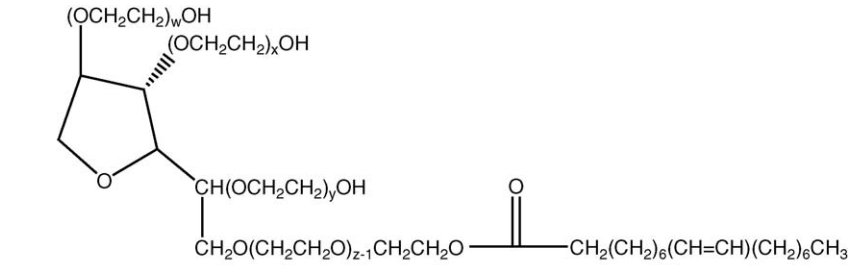
Isocratic HPLC analysis was performed with a mobile phase consisting of 45% (0.1%) phosphoric acid and 55% acetonitrile (presented in Fig. 9). Injections were 50 μL with detection at 245 nm using a Waters Symmetry C18 (5 μm, 4.6 mm × 150 mm) column, at ambient column temperature. For the data presented in Figs. 11–13 (varying organic% in each set of experiments) the isocratic conditions are as described in the text with 50 μL injections and detection at 210 and 245 nm (see individual experiments in Section 3) using a Waters Symmetry C18 (5 μm, 4.6 mm × 150 mm).

3. Results

3.1. UV/vis spectra

During this investigation we analyzed the UV/vis spectra (0.01–5.0% (w/w) aqueous solutions) of four commercially available brands of Polysorbate 80 including TCI, Aldrich, Acros, and NOF. The molecular structure of Polysorbate 80 is shown in Fig. 1. The spectra of TCI product is shown in Fig. 2 for solutions in a 1 cm pathlength cell and absorbance maxima are found at 195, 234, 270, and 282 nm with a small shoulder peak at 310 nm that can be seen on the most concentrated samples. The absorbance tail extends into the visible region (>400 nm). This spectra is surprising given the molecular structure and led us to investigate other commercial sources. While the Polysorbate 80 spectral features remained the same from brand to brand. A comparison of TCI, Acros, NOF, and Aldrich Polysorbate 80 solutions at selected concentrations are shown in Figs. 3 and 4. In order to quantify these differences the molar absorptivity constant, ε (L mol^{-1} cm^{-1}), values for the different vendor's Polysorbate 80 at several λ_{max} are shown in Table 2. We report ε from the 0.01% (w/w) concentration absorbance results for all but NOF where values were calculated with the 1% (w/w) solution results. TCI ε values tended to be lower than Aldrich and Acros and were most different at the 234 nm maxima where the difference was over four-fold. Towards the higher wavelengths and at the 195 nm maxima the values were more reproducible from brand to brand. NOF values were extraordinarily low (typically 2–10× lower than the other three sources) in comparison to the other brands and to the eye the surfactant appears colorless.

Theoretically Polysorbate 80 is a fully saturated molecule (Fig. 1) with only one carbon–carbon double bond (C=C, λ_{max} $[\pi \rightarrow \pi^*] \sim 195$ nm, $\varepsilon \sim 11{,}000$ [15]), an alkyl ester group (RCO_2R', λ_{max} $[n \rightarrow \pi^*] \sim 195$–$210$ nm, $\varepsilon \sim 40$–100 [15]), and poly(ethylene glycol) chains (ROR, $\lambda_{max} \sim [n \rightarrow \pi^*]$ 180–185 nm, $\varepsilon \sim 3000$ [15,16a]). The ε in the references were collected in non-polar organic solvents and therefore are used as a reference of general intensity for the moieties. Given these chromophores the high wavelength (220–340 nm) spectral features of Polysorbate 80 are not consistent with the proposed



Where W + X + Y + Z = 20

Fig. 1. Molecular structure of Polysorbate 80.

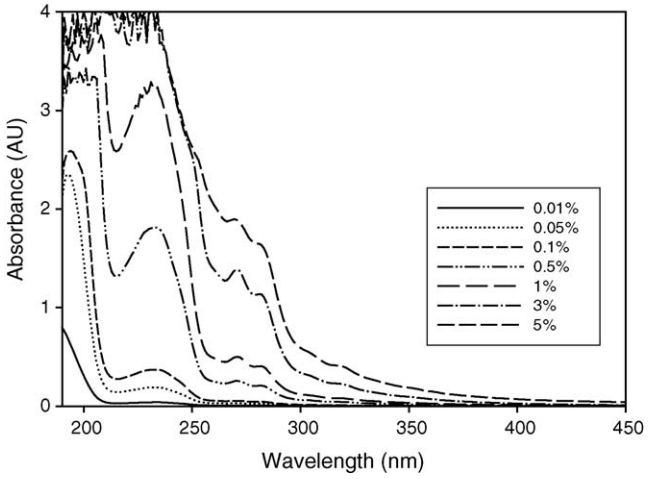


Fig. 2. UV/vis spectra of TCI Polysorbate 80 ((w/w)% solutions).

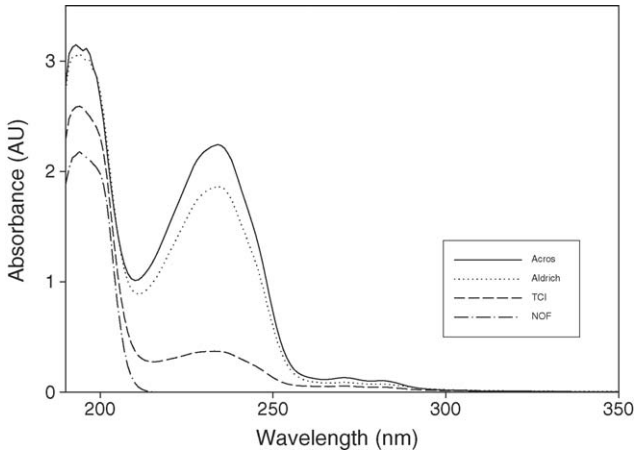


Fig. 4. UV/vis spectra of four commercially available Polysorbate 80 brands (0.1%, w/w solution).

molecular structure. While considerable differences are noted at the higher wavelengths, ε values at 195 nm are more consistent between the different brands of Polysorbate 80. The molar absorptivity calculated for the 195 nm chromophore, ~5000, in Table 2 is reasonably consistent with C=C λ_{max} and ε literature estimates. The ε value consistency amongst the varied sources of Polysorbate 80 suggests that it can be attributed predominantly to the known surfactant alkene, which is certainly present in all vendor's materials. In contrast the high variability of the ε values across the varied sources of Polysorbate 80 at $\lambda > 210$ nm suggests that these transitions are not readily attributable to the core molecular structure, but rather to impurities. The assignment of spectral features at $\lambda > 210$ nm to impurities is further supported by information supplied by NOF, a specialty supplier of Polysorbate 80 [17]. The reference states that purification process removes conjugated fatty acid impurities before ester formation in the synthesis. Spectra of the NOF surfactant is shown in Figs. 3 and 4 showing only the proposed C=C features at low wavelengths. Therefore it is likely that the higher wavelength absorbance results from extended π-conjugation in impurity hydrocarbon chains.

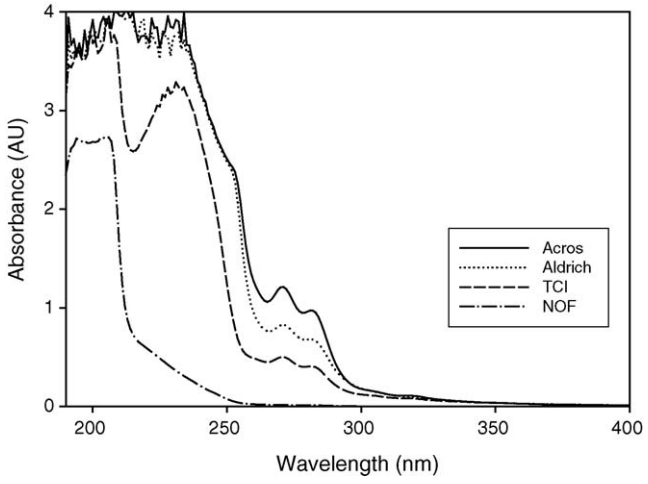


Fig. 3. UV/vis spectra of four commercially available Polysorbate 80 brands (1%, w/w solution).

Table 2
Calculated molar absorptivity constants for Polysorbate 80 at various λ_{max}

Polysorbate 80 source	ε (L mol^{-1} cm^{-1})			
	195 nm	234 nm	270 nm	282 nm
NOF	2727	44	9	6
ACROS	4935	3006	212	181
Aldrich	5065	2469	146	122
TCI	4805	508	99	91

A second potential contributor to the absorbance at $\lambda > 210$ nm may be the product of processing at high temperatures, which causes some extent of oxidation as noted in patent literature [18]. The resultant formation of peroxy radicals ($RCOO^{\bullet}$) and organic hydroperoxides (RCOOH) from processing can further degrade, forming conjugated alkenes and α,β-unsaturated carbonyl groups, eventually leading to the prevalent yellow–orange coloration of the surfactant (abs >400 nm). We find this unlikely as in our experience with these surfactants the higher wavelength UV absorbance tends to decrease when stressed at elevated temperatures, which is more consistent with the loss of present colored impurities (e.g., $C{=}C{-}C{=}C + ROO^{\bullet} \rightarrow ROO{-}C{-}C^{\bullet}{-}C{=}C$) than some on-going oxidation process generating new chromophores. Regardless of either scenario, it is not unreasonable to expect that the spectral characteristics of Polysorbate 80 is dependent on the manufacturing processing method used and therefore does vary between manufacturers (vida infra).

Analysis of Polysorbate 80 solutions with reverse-phase HPLC with photodiode arrays showed that while several peaks are chromatographically resolved the UV/vis spectra are similar for each peak (resembling spectra in Figs. 2–4). This indicates that the high wavelength absorbing chromophores are distributed throughout the polydisperse polymeric surfactant, which is consistent with either proposal (surfactant color resulting from impurities or due to oxidation reactions) proposed in the previous two paragraphs. As a note Nair et al. developed a rapid HPLC–ELSD (evaporative light scattering detection) method to eliminate the spectral uncertainties associated with the surfactant discussed above and to allow for accurate quantitation of Polysorbate 80 [18] in formulation development efforts.

Data from Table 2 and Figs. 2–4 can be used for guidance in developing UV/vis spectrophotometric assays for dissolution analysis with Polysorbate 80 containing media. The data shows that in order to minimize interference from Polysorbate 80, higher detection wavelengths and lower surfactant concentrations are clearly preferred. One can estimate the interference/bias expected if an API and Polysorbate 80 spectra overlap at desired analysis wavelengths by either calculating absorbances using molar absorptivity, or by inspection of the absorbance in the spectra shown. Analysis of 5% TCI solutions (Fig. 2) shows that by 290 and 250 nm the solution absorbance has risen to above 1 and 2 AU, respectively, and the absorbance is even larger for the Acros and Aldrich brands. Even low wavelength absorbance from intermediate Polysorbate 80 concentrations (i.e. 1%), depending on vendor, can elevate to nearly 1 AU around 250 nm (Fig. 3). Limiting straight forward spectrophotometric analysis in these cases will be the deviations from Beer's law at absorbance $\gg 1$ in these solution concentrations possibly leading to non-linear calibrations with multiple standards to ensure accuracy [19]. Using Polysorbate 80 solutions with absorbances considerably above 1 AU as blank solutions for instrument zeroing/background subtraction can present precision errors as spectrophotometers are taken out of prime response range ($A = 0.1$–0.7) where detector dark current and amplifier noise cause more error as fewer photons actually hit the detector [19]. These effects should be considered by the analyst during method development.

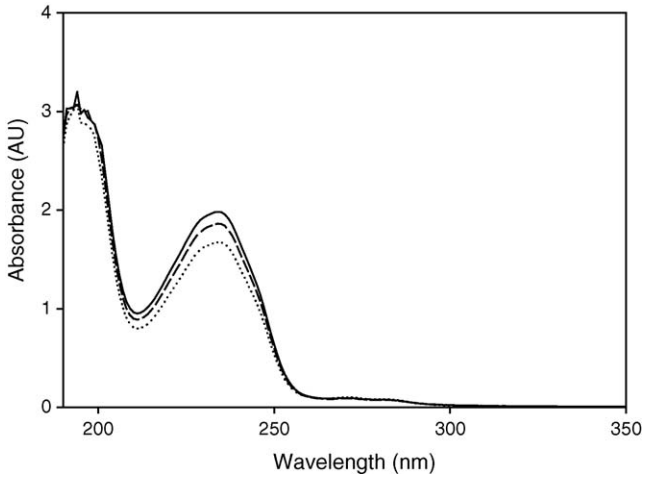


Fig. 5. UV/vis spectra of three lots of TCI Polysorbate 80 (0.1%, w/w solution).

The potential errors due to the highly absorbing nature of Polysorbate 80 considerably narrow the wavelengths available to the analyst. Higher wavelength analysis is clearly less subject to the previously mentioned concerns and will more likely allow for suitable direct spectrophotometric analysis. In light of the previous discussions we feel *general* boundary conditions for dissolution media analysis can be stated as follows: wavelengths of approximately 300 nm or higher are necessary for Polysorbate 80 concentrations of 3–5% (w/w), while for intermediate concentrations, 0.1–1% (w/w), wavelengths as low as 260 nm are acceptable. For low Polysorbate 80 solution concentrations such as 0.01–0.05% (w/w) Polysorbate 80 solution absorbance is low enough at most wavelengths that few problems should arise. For lower concentration Polysorbate 80 solutions (<3%) a local minima in absorbance occurs near 210 nm that could also be useful in reducing interference in comparison to the 234 nm local maxima.

The extent of lot-to-lot variation from a single vendor was addressed by analyzing three lots of Aldrich sourced Polysorbate 80. Upon visual inspection it was clear that each lot was differently colored and also produced spectra with different UV absorbance levels (~0.3 AU at 234 nm—Fig. 5). Due to the lack of inter- and intra-vendor reproducibility the properties of the Polysorbate 80 lot being used and potential problems that may arise from a different future lot should be understood.

The importance of using the lowest absorbing surfactant available to enable spectrophotometric analysis is clear. In dissolution analysis where active solubility constraints require high levels of Polysorbate 80 to achieve suitable dissolution profiles and API spectral properties demand low wavelengths for analysis it is likely that HPLC separation of the components will be necessary.

3.2. Polysorbate 80 chromatographic characteristics

In order to gain a better understanding of Polysorbate 80 chromatographic characteristics we studied the surfactant using

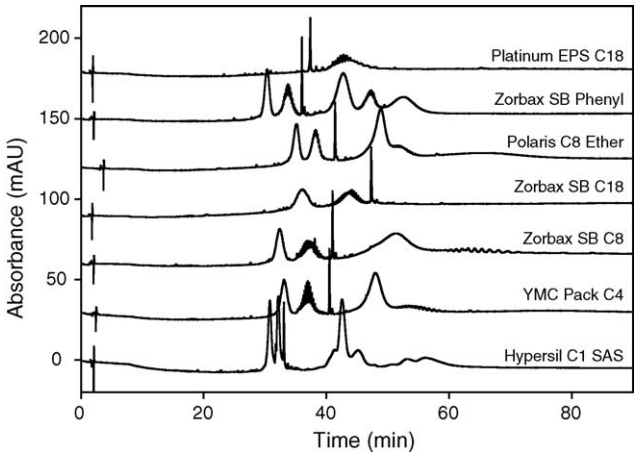


Fig. 6. Polysorbate 80 chromatographic elution patterns.

several different column chemistries and silica pore sizes. Fig. 6 shows the gradient elution profile of a 1% (w/w) Aldrich Polysorbate 80 solution (20 μL injection) on C18, C18-EPS, C8, C8-ether, C4, C1, and phenyl columns. All columns were of the same length, approximate particle size, and approximate silica pore size (3–5 μm, 50–100 Å pore size, 4.6 mm × 150 mm columns). The individual column dimensions and method information are given in Section 2. The method used a 45-min linear gradient as described in Table 1 with UV detection at 220 nm. The shallow gradient was used to allow for maximum resolution of Polysorbate 80 components with the 50 min isocratic portion at 90% ACN ensuring elution over the experimental time frame. Given the different column chemistries the similarity of the chromatography was striking as neither chemistry nor chain length appeared to have a significant effect. In general the early elution profiles (<30 min) were featureless except for occasional low level peaks (<5 mAU height) that may be either impurities or low MW Polysorbate 80 (possibly polydispersity in the PEG chains). At 30 min (∼70% organic in mobile phase) the major surfactant peaks began to elute from all columns with the gradient conditions with the final peak field eluting by 70 min (90% organic in mobile phase). The differences in the first eluting Polysorbate 80 peak retention times are considerably smaller than one would expect for a typical small molecule being analyzed on such a wide selection of columns.

Each vendor's material was analyzed with the Zorbax SB Phenyl column and each showed the same profile seen in Fig. 6 only differing in signal intensity due to the previously mentioned molar absorptivity values. The result indicates that in general the retention characteristics of the polymeric surfactant is basically unchanged by the small impurity or oxidative degradation differences that we postulate give rise to changes in spectra.

Organic composition of the mobile phase is the predominant driver for retention characteristics for the surfactant. We report the percentage of organic in the mobile phase when major Polysorbate 80 elution begins as a convenient marker of organic strength, and will use this value to guide isocratic HPLC work. Some differences in Polysorbate 80 elution pattern do appear after the onset of significant elution but are of little use for practical method development aimed to resolve an API from the surfactant. These differences and their implications are discussed further later in the text. Table 3 shows the % organic in mobile phase associated with the onset of major Polysorbate 80 peaks for each column chemistry. The onset of elution over the seven columns evaluated was between 61 and 75% with an average of 67% (R.S.D. = 7%). A separate experiment showed that when MeOH was substituted for ACN similar results were found indicating that retention was not effected by the use of a hydrogen bonding solvent. Raising column temperature to 40 °C did not significantly affect the retention characteristics either.

Table 3
Mobile phase composition for Polysorbate 80 elution

Column type	Retention time of first peak (min)	ACN in mobile phase (%)
Zorbax SB C18	36.2	72
Zorbax SB C8	32.5	65
YMC Pack C4	33.1	66
Hypersil C1	30.8	62
Zorbax SB phenyl	30.4	61
Platinum EPS	35.2	75
Polaris C8 ether	37.4	70
Nucleosil 100 Å	37.1	74
Nucleosil 500 Å	36.4	73
Nucleosil 4000 Å	31.3	63

As a second parameter we explored silica pores size as a potential means to generate modified Polysorbate 80 retention characteristics. It was hypothesized that a large Polysorbate 80 hydrodynamic radius may not allow access to the inner pore structure of the relatively small 50 Å silica resulting in limited interaction with the majority of column stationary phase (e.g. the eluate would only see the exclusion volume of the column). The pore size of the individual column dimensions are given in Section 2. Applying the same gradient used in the previous experiments, little change in retention time was observed with the 100, 500, 4000 Å silica pore C18 columns. Again, the % of organic in the mobile phase was the driver for Polysorbate 80 (Aldrich) elution. The chromatograms for the large pore size columns are shown in Fig. 7 (220 nm, 20 μL injection, 1%, w/w

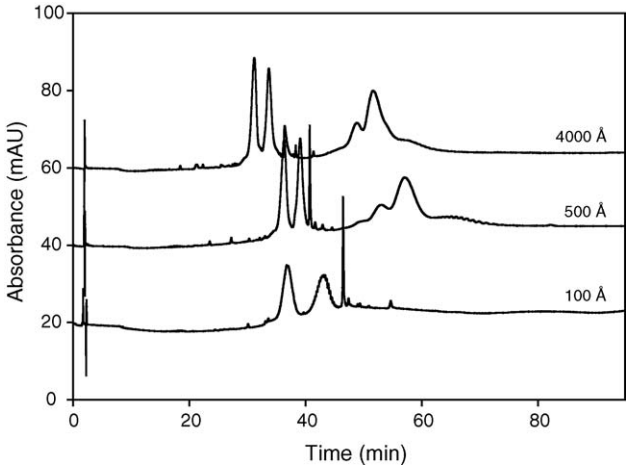


Fig. 7. Chromatography of Polysorbate 80 with 100, 500, and 4000 Å pore size C18 columns.

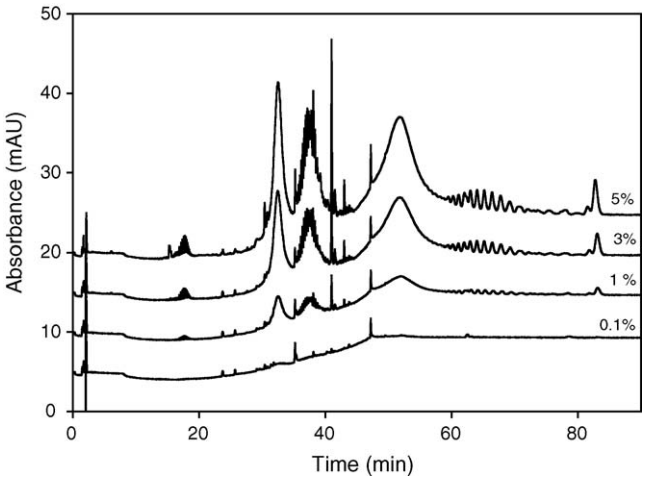


Fig. 8. Polysorbate 80 (TCI) chromatograms at several concentrations.

Polysorbate 80 solution). Note that the 100 Å pore size C18 column here (Nucleosil) showed slightly different chromatography as the 100 Å pore size C18 column in the initial gradient experiment (Zorbax), however the onset of major Polysorbate 80 elution is again at ~70% organic as before. Also notable and further supporting this analysis was data from a non-porous silica column that showed elution at the same % organic mobile phase and chromatography similar to the other column chemistries (data not shown).

Fig. 8 shows 0.1–5.0% aqueous solutions of TCI Polysorbate 80 (20 μL injection, detection at 220 nm) on a Waters Symmetry C18 (5 μm, 4.6 mm × 150 mm) column. The magnitude of the chromatographic interference clearly evolves and is problematic by the 1% level. For higher Polysorbate 80 concentrations only early in the chromatography, less than 30 min, could API be quantitated free from interference. The most important aspect of the chromatograms is the noted ability to retain the majority of Polysorbate 80 until late in the gradient, which can be seen most dramatically with the 5% (w/w) Polysorbate 80 injection of Fig. 8 and in the previous figures. While no control over the surfactant elution has been achieved, the reproducibility of elution pattern on various columns allows for a constant in method development. Method development efforts should be spent finding conditions where API (and degradates if designed to be useful as a stability indicating method for Polysorbate 80 containing drug product analysis) will elute before the onset of major surfactant elution rather than attempting to modify the relatively insensitive surfactant retention. As in the UV/vis spectrometry discussion, choice of low absorbing surfactant will aid in reducing Polysorbate 80 peaks. Clearly, interference problems will be exacerbated by injecting larger volumes of Polysorbate 80 dissolution media on columns.

3.3. Polysorbate 80 build up on-column

The Zorbax SB C18 and Platinum EPS C18 column data from Fig. 6 show two major peak fields, while the C8, C4, C1, and phenyl columns yield five to eight peaks. All but the Hypersil C1 and Platinum EPS chromatograms have similar peak shapes that include two first eluting broad peaks (similar to polymeric size exclusion peaks) typically followed by a sharp peak, followed by a field of smaller peaks that eventually become small ripples in the baseline. The lack of some peak fields from the Platinum EPS, YMC Pack C4, Polaris C8 Ether, Zebrax SB C8, and Zorbax SB C18 columns indicate a higher level of Polysorbate 80 retention. In order to determine if Polysorbate 80 is fully removed from the columns during these gradient runs we compared peak integration of these injections versus injections of Polysorbate 80 solutions where the column was removed and flow went directly to the UV cell. Dilutions of TCI Polysorbate 80 solutions for the direct-to-detector injections were made to yield similar detector response to the individual peak groups seen in Figs. 6–8 chromatography (~40 mAU height, ~10,000 mAUs area). To establish that the direct to detector injections with only a union were reasonable for use as a "standard" to quantitate Polysorbate 80 recovery from the columns, we determined response linearity over a range of solution concentrations. A stock 1.0% Polysorbate 80 solution was diluted to three levels (0.2, 0.1, and 0.05%) and response (area count) from these three lower levels yielded a R^2 value of 0.9998. The 1.0% solution did not show a linear response in relation with the lower concentrations. Comparison to column injections for recovery of Polysorbate 80 was therefore made with the 0.1% solution. Summation of the single elution peak (no column) area in comparison to the summation of all peaks found in the gradient runs showed that for the Polaris C8 column approximately 30% of the Polysorbate 80 is retained on the column and that approximately 60% remains on the Zorbax SB C18 column. When running Polysorbate 80 injections on a HPLC system it is noted that when flow is stopped and restarted, surfactant elution occurs in the form of broad peaks over the first few minutes of a run giving way to a smooth baseline. This will occur with or without injection of a blank solution indicating that the elution is not simply Polysorbate 80 being washed from the injection loop hardware (carryover). The reason that new material elutes during these instruments restarts and not during the 90% organic portion of the gradient is unclear, but ultimately means that with multiple washings a majority of the surfactant can be removed from columns. A series of over 200 injections of a Polysorbate 80 solution spiked with caffeine on a C8 column showed no change in caffeine retention under the gradient conditions (system restarted with new mobile phase and washed with 100% organic for 1 h after every 13 injections). After the analysis performed for this paper with the Zorbax SB C18 column, approximately 1000 injections with appropriate washings, we saw no reduction in column efficiency using a standard caffeine HPLC method (4 min 85/15 acetonitrile/H_2O (0.1% acetic acid) isocratic method with detection at 272 nm at ambient temperatures). While it is clear that surfactant stays on the column from injection to injection at a first approximation (with the assumption that caffeine retention is affected by similar factors as other potential drugs) there appears to be no significant effect on chromatography, as long as frequent washings with high organic mobile phase occur (vide supra). This assessment has been confirmed by analysis of one other compound, which has less than 1 μg/mL aqueous solubility across the physiologi-

cally relevant pH range and is therefore strongly differentiating from caffeine. Gradient HPLC analysis (1.5 mL/min, 10–90% MeOH over 15 min on a Vydac C18 (250 mm × 4.6 mm, 5 μm, 300 A column, UV detection at 220 nm) of solutions containing up to 8% Polysorbate 80 and 0.02% active compound gave reproducible results in terms of retention time and assay accuracy over approximately 250 sample injections over a several month period. The compound was easily resolved from Polysorbate 80 eluting approximately 3 min before the major Polysorbate 80 peaks.

3.4. Dissolution HPLC method development

While the preceding paragraphs give an overview of the full reverse-phase characteristics of Polysorbate 80, for dissolution analysis short, isocratic methods are preferred. In order to apply the understanding gained in the previous section we analyzed a Merck API dissolved in an aqueous Polysorbate 80 solution to mimic a realistic dissolution analysis using HPLC under isocratic conditions (55% ACN/45% phosphoric acid (0.1%)) with UV detection at 245 nm wavelength, 20 μL injection). The organic percentage was intentionally set at an organic percentage near the nominal 70% organic strength that brought about significant Polysorbate 80 elution in the gradient experiments. The full set chromatographic conditions are listed in Section 2. In order to maximize the interference from surfactant, 5% (w/w) dissolution media solutions (TCI) were used to dissolve the active in the following experiments. Fig. 9 plots every 20 injections for the method and in following the progression across the injections some clear features appear. Note that the entire run is 5 min in comparison to the previous 90-min gradient runs. The first 20 injections look relatively reproducible with only a slight change in active retention time and baseline slope. By the 40th injection the baseline begins to show a variable slope and active retention time has changed by 0.25 min. The change in retention over the run is due to retained Polysorbate 80 that has not been removed fully from the column changing the partitioning environment for the active molecule. Over the rest of the run (injections 41–100) the changes in baseline continue as build-up continues and some surfactant begins to elute from earlier injections. By the 100th injection the retention time has changed by 0.5 min. The point is made that by not rinsing with a high organic mobile phase analytical columns are slowly changed under isocratic conditions by retained Polysorbate 80 and likely will *eventually* cause unacceptable changes in method performance, possibly mid-analysis, jeopardizing analytical results. Even with the eventual retention shift and column build up of Polysorbate 80 on LC columns, this case study clearly identifies an approach to the removal of surfactant interference from chromatographic analysis. We propose that low organic methods be developed (lower than 70%) that retain the surfactant, allowing for selective active analysis, then use high organic rinses to wash off retained material and return the column to original characteristics. As a side note, even with this baseline and retention shifting quantitation of the peak was not effected by the background interference in this case showing only a 0.8% R.S.D. over the 100 injections.

To further investigate the feasibility of low organic methods we studied Polysorbate 80 retention characteristics across mobile phase organic compositions from 20 to 80% without addition of active drug to illustrate expected interferences in isocratic methods. Figs. 10–13 show the elution profile of every 20 injections in a series of 100 injections at 20, 40, 60, and 80% ACN mobile phase to further illustrate the characteristic build-up/elution of Polysorbate 80 (TCI). All injections on a Waters C18 Symmetry column (20 μL) were of 5% (w/w) surfactant solutions with detection at 234 nm for a worst case scenario (high weight % Polysorbate 80 and local maxima absorbance wavelength—see Fig. 3). In Fig. 10, with a 20% ACN mobile phase, complete retention of all Polysorbate 80 occurs over the entire 100 injections, however shift in the void peak indicates that column characteristics are changing. This again shows that Polysorbate 80 can be completely removed from analysis by use of low organic conditions. In Fig. 11 where 40% ACN mobile phase was used the dead volume marker is clearly affected by Polysorbate 80 elution and a sloping baseline is becoming preva-

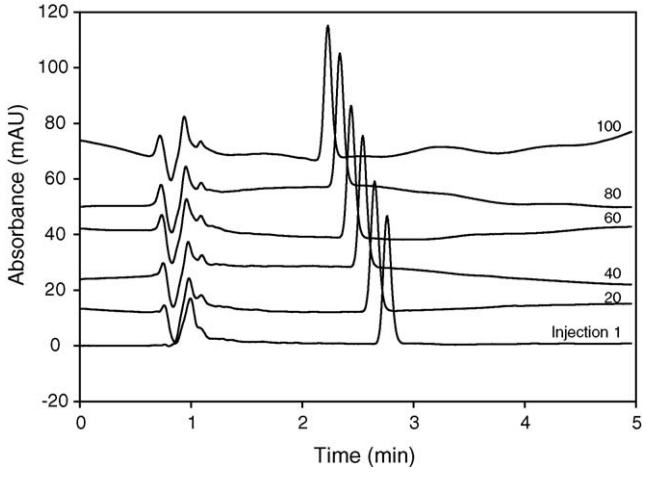


Fig. 9. Isocratic elution of Merck compound (55% ACN/45% 0.1% phosphoric acid mobile phase, Waters Symmetry C18 column) in aqueous TCI Polysorbate 80 (5%, w/w) over 100 injections.

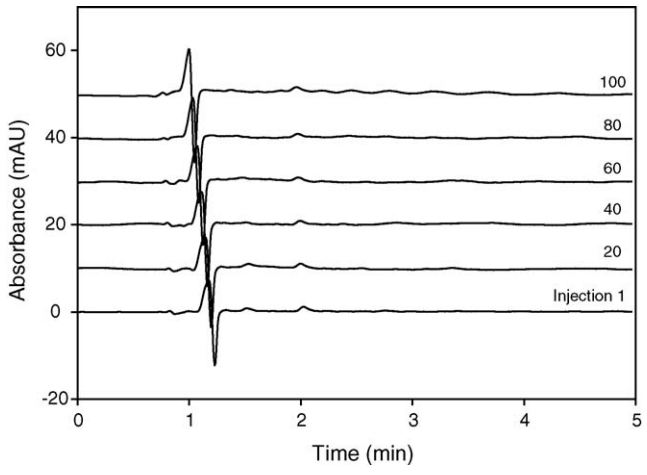


Fig. 10. Isocratic elution of 5% (w/w) TCI Polysorbate 80 (20% ACN/80% 0.1% phosphoric acid mobile phase, Waters Symmetry C18 column) over 100 injections.

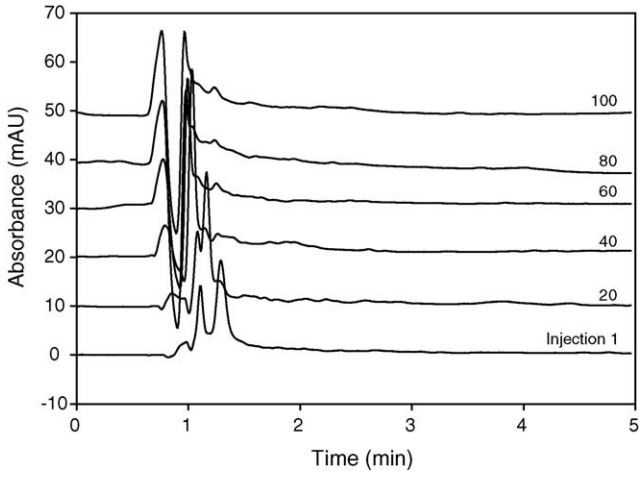


Fig. 11. Isocratic elution of 5% (w/w) TCI Polysorbate 80 (40% ACN/60% 0.1% phosphoric acid mobile phase, Waters Symmetry C18 column) over 100 injections.

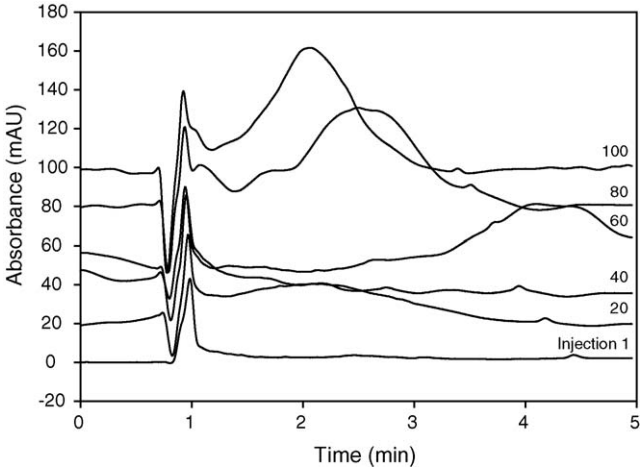


Fig. 12. Isocratic elution of 5% (w/w) TCI Polysorbate 80 (60% ACN/40% 0.1% phosphoric acid mobile phase, Waters Symmetry C18 column) over 100 injections.

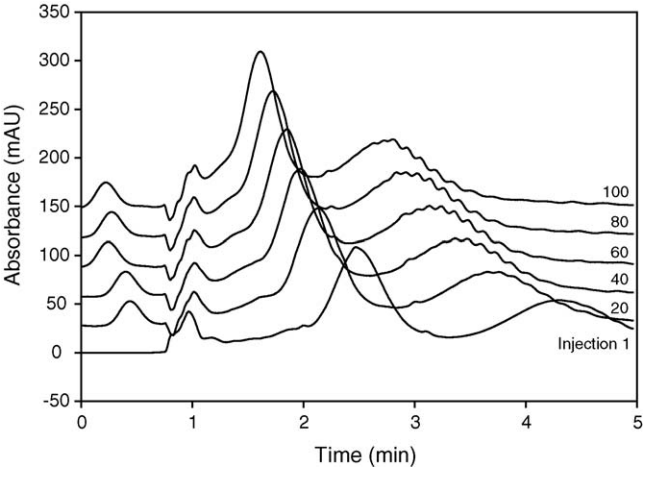


Fig. 13. Isocratic elution of 5% (w/w) TCI Polysorbate 80 (80% ACN/20% 0.1% phosphoric acid mobile phase, Waters Symmetry C18 column) over 100 injections.

lent particularly at early retention times. It is still likely that an active could be quantitated without interference in this case. A marked transition occurs in Figs. 12 and 13, where 60 and 80% ACN mobile phase are used, respectively, which show Polysorbate 80 elution from early numbers of injections. Undesirably large and irreproducible peaks continue to elute throughout the 100 injections. With these conditions dead volume markers do not appear as affected which is consistent with surfactant elution and less column build-up as in the low organic conditions. In the 80% ACN injections it is clear that complete Polysorbate 80 elution has almost been achieved in this short method by the repeated but slightly shifted retention with each set of injections.

The data indicates that organic percentages up to 60% will retain most Polysorbate 80 peaks for at least one injection. Conditions towards 60% organic will tend to elute Polysorbate 80 within 20 injections leading to random baseline slopes, at lower percentages it will take longer to see these effects. In the higher organic conditions Polysorbate 80 peaks, which may cause errors in analysis if co-eluting with active peaks, are likely to be present. Successful dissolution chromatographic method development will include the use of a low organic mobile phase that should be followed by some high organic wash cycle throughout an analysis sequence to ensure no significant surfactant build-up. Use of the method conditions presented in this manuscript should enable accurate, repeatable analysis in high concentration Polysorbate 80 dissolution media.

4. Conclusions

While analysis interferences are dependent on the API in question it has been shown that in general analytical interference in Polysorbate 80 containing dissolution media can be considered manageable at 3–5% (w/w) in UV/vis spectrophotometry where wavelengths greater than 300 nm are used, in 0.1–1% solutions, if wavelengths higher than 260 nm are used, or in 0.05–0.01% solutions at all wavelengths with proper background subtraction. In cases where these boundary conditions cannot be met chromatographic separation are necessary. To best resolve the active from Polysorbate 80 in dissolution media one should use low organic (<40%) to effectively retain the surfactant on the column while active elutes. However it is necessary to wash the column with a high organic content mobile phase to remove the retained material periodically to remove retained Polysorbate 80 that eventually will cause changes in column retention characteristics.

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