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Research Article

Adsorption and Degradation of Doxorubicin from Aqueous Solution in Polypropylene Containers

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Abstract. The purpose of this study was to examine doxorubicin adsorption in polypropylene containers as a function of pH and drug concentration based on anecdotal evidence of such adsorption. Doxorubicin loss was first examined in high-performance liquid chromatography (HPLC) glass inserts by UV absorbance to determine appropriate pH and time durations for subsequent analysis. Doxorubicin loss was then investigated in polypropylene microcentrifuge tubes at different pH values and starting drug concentration at 37°C over 48 h using HPLC with fluorescent detection. Doxorubicin concentrations was essentially constant in HPLC glass inserts at pH4.8 up to 12 h but declined 5% at pH7.4 by 3 h. The percent doxorubicin adsorption was calculated in polypropylene microcentrifuge tubes from extrapolations to zero time and was the least at pH4.8, but increased with pH values 6.5 and 7.4, and decreased with drug concentration to reach a maximum adsorption of 45% in 2.0 μg/mL at pH7.4 and 37°C. Degradation rate constants, ranging from 0.0021 to 0.019 h^{-1}, also increased with pH in these studies. Determinations of low amounts of doxorubicin in polypropylene containers at pH7.4 may be underestimated if adsorption and degradation issues are not taken into account.

KEY WORDS: adsorption; analysis; chemical stability; doxorubicin; glass; HPLC; polypropylene.

INTRODUCTION

Doxorubicin is widely used for treatment of various solid tumors (1) and it is frequently used for research and development of drug delivery systems. Its usefulness, however, is hampered by its well-known complications of degradation and adsorption. Several thorough reports (2–6) describe its degradation dependence on pH, buffer, and light. Additional studies describe its adsorption to glass, siliconized glass, polyethylene, polytetrafluoroethylene, polyvinylchloride, and cellulose dialysis membranes (5–8), but it is reported to have little or no adsorption to polypropylene (6,7). Consequently, polypropylene containers are often used with aqueous solutions of this drug to minimize its loss from solution. However, there is anecdotal evidence of its adsorption in these containers. In this lab, doxorubicin staining has been observed on polypropylene volumetric flasks. Based on the possibility of meaningful doxorubicin adsorption to polypropylene, this study was conducted to examine doxorubicin adsorption in polypropylene containers as a function of pH and drug concentration.

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MATERIALS AND METHODS

Stock solutions of 1.0 mg/mL doxorubicin HCl (Bristol-Myers Squibb, New York, New York) in water were stored at −80°C in polypropylene microcentrifuge tubes covered with aluminum foil to protect them from light. Doxorubicin-buffered solutions were prepared from stock solutions by diluting with 0.03 M potassium phosphate buffer containing 0.12 M sodium chloride at pH values of 4.8, 6.5, and 7.4. Polypropylene materials were used to prepare sample solutions which were covered with aluminum foil during experiments.

Doxorubicin-buffered solutions at pH values of 7.4 and 4.8 were examined in glass high-performance liquid chromatography (HPLC) vial inserts (National Scientific Co., Rockwood, Tennessee) within capped vials at room temperature in the HPLC sample chamber. Injection times were staggered to maintain the appropriate sample replicate times (n=4). Independent sets were used for each time point. Measurements at each pH were conducted in one run with injections of a standard doxorubicin solution before and after the sample injections to confirm peak area reproducibility (RSD< 1.5%). The change of doxorubicin peak area with time was monitored up to 180 min at both pH values with 25 μg/mL, as well as up to 720 min at pH4.8 with 5.0 μg/mL.

Doxorubicin-buffered solutions at pH values of 4.8, 6.5, and 7.4 were evaluated for adsorption and degradation at 37°C in polypropylene microcentrifuge tubes (Eppendorf, cat. no. 022363352, Hauppauge, New York). Four independent samples of 200 μL doxorubicin buffer solutions were used at each time point. Samples of 50 μL were collected

and diluted with pH4.8 buffer for HPLC analysis. Polypropylene pipette tips and microcentrifuge tubes were used for these dilutions. Calibration plots (see below) were prepared in the same manner.

Doxorubicin samples were analyzed (9) using a Shimadzu HPLC equipped with a C_{18} column. The mobile phase was 75:25 buffer solution/acetonitrile, with the buffer solution composed of 10 mM sodium phosphate buffer with 10 mM triethylamine and adjusted to pH3.5. A UV detector at 300 nm was used for the insert measurements. A fluorescence detector with excitation and emission wavelengths of 475 and 550 nm was used for the adsorption and degradation measurements. Injection volume was 45 μL, and the flow rate was 1 mL/min. Samples were bracketed and determined by two 5-point daily calibration plots at pH4.8. The correlation coefficients of calibration plots were greater than 0.99 and a typical intra-day accuracy of standards in these plots ranged from 98 to 102%.

SigmaStat for Windows Version 3.5 was used for statistical analysis with a significance level at 0.05 using a one-way ANOVA, Tukey's post hoc test, and a *t* test.

RESULTS

Doxorubicin-buffered solutions at near isotonicity were used for this investigation to represent conditions of formulation and drug release studies. The pH values selected correspond to regions of interest for drug delivery: an intracellular lysosomal pH4.8, an intratumor interstitial pH6.5, and circulation pH7.4. Peak areas of doxorubicin from solutions in glass HPLC inserts were first compared at pH values of 7.4 and 4.8 to identify a suitable pH and duration for subsequent HPLC analysis. Peak areas were used instead of concentrations to avoid potential complications of adsorption on the inserts under study and on inserts used in calibration plots. The change of peak area up to 180 min at pH values of 7.4 and 4.8 is shown beginning at 40 min in Fig. 1. Earlier times were not used because of poor reproducibility of peak areas of doxorubicin standard solutions. The plot indicates a 5% decline in doxorubicin concentration at pH7.4 but is essentially constant at pH4.8 over this period. Doxorubicin concentration remained constant at pH4.8 up to 12 h (data not shown). The 25- and 5.0-μg/mL concentrations examined at pH4.8 cover most of the experimental concentrations and represent a five-fold range.

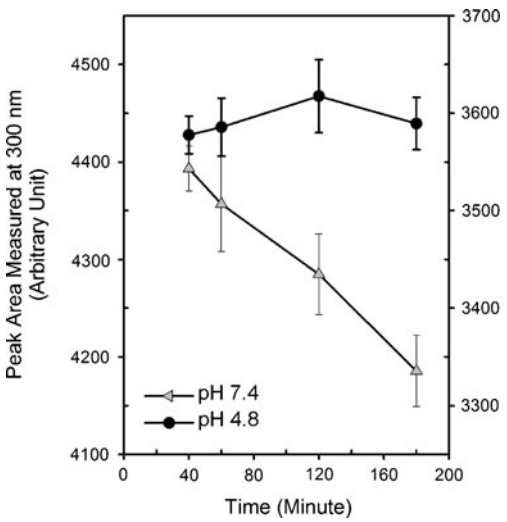

Fig. 1. Change of HPLC peak area of doxorubicin solution over time at pH values of 7.4 (*right axis*) and 4.8 (*left axis*) at room temperature in glass HPLC vial inserts. Each value represents the mean±SD (n=4). Doxorubicin concentration is 25 μg/mL in 0.03 M potassium phosphate buffer with 0.12 M sodium chloride

The amounts of doxorubicin remaining in solution from 3 to 48 h at 37°C in polypropylene microcentrifuge tubes are shown in Fig. 2a, b, and c at pH values of 4.8, 6.5, and 7.4, respectively, for various starting concentrations. While at pH 4.8, there is little change with time; there is a trend of decreasing concentration with time for pH values 6.5 and 7.4. Also, there is a trend of decreasing first time points with decreasing starting concentration. At pH6.5, the values at the first time point of 3 h follow this trend but it is unclear if the absence of this trend for all time points at this pH is due to accurate measurement or experimental artifact.

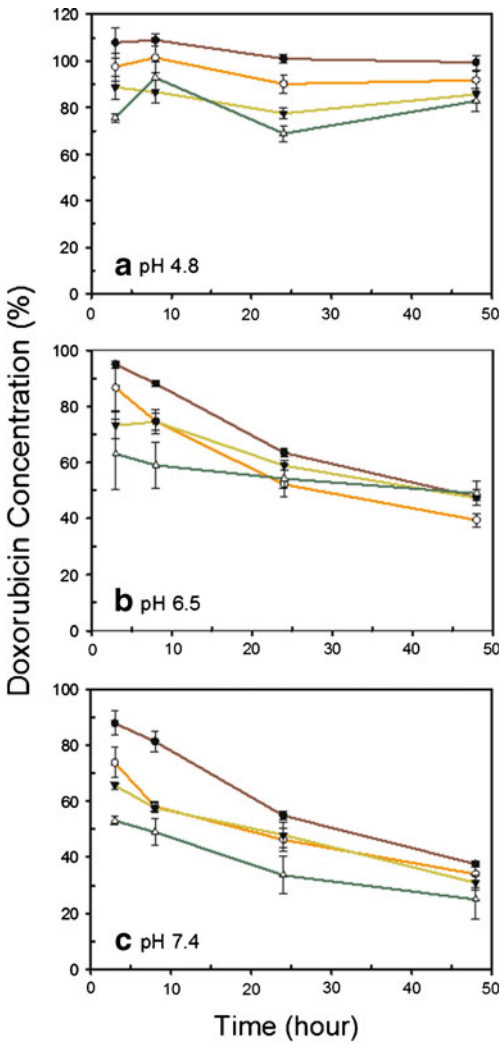

Fig. 2. Doxorubicin remaining in solution in polypropylene microcentrifuge tubes at 37°C over 48 h at pH4.8 **a**, pH6.5 **b**, and pH7.4 **c**. Each value represents the mean±SD (n=4). Doxorubicin is dissolved in 0.03 M potassium phosphate buffer with 0.12 M sodium chloride at various concentrations. Key: *black circle*: 50 μg/mL; *white circle*: 20 μg/mL; *black down-pointing triangle*: 4.5 μg/mL; *white up-pointing triangle*: 2.0 μg/mL

First-order degradation rate constants were determined from plots of the natural logarithms of remaining doxorubicin concentrations as a function of time and are listed in Table I. The rate constants increased as the pH rose from pH4.8 to 7.4 which is consistent with the literature (3–6). The rate constants at the most stable pHof 4.8 are within experimental error and not statistically significant, except for the highest doxorubicin concentration of 50 μg/mL. At the least stable pH of 7.4, there were no statistically significant differences between the rate constants at different doxorubicin concentrations (p=0.70).

These plots were also used to calculate an estimate of doxorubicin adsorbed onto the polypropylene microcentrifuge tubes by extrapolating doxorubicin concentration to time zero (C_0). The difference between C_0 and the starting concentration expressed as a percent of the starting concentration is used as an estimate of percent adsorbed doxorubicin. Adsorption results for pH values 4.8, 6.5, and 7.4 are listed in Table I. Adsorbed doxorubicin was pH dependent with the highest percent adsorption observed at pH7.4. In addition, the percent adsorption increased with decreasing drug concentration, resulting in the highest percent adsorption of 45% at the lowest concentration (2.0 μg/mL) at pH7.4.

DISCUSSION

The measurement of doxorubicin in glass HPLC inserts is shown in Fig. 1. The virtually constant concentration at pH4.8 up to 12 h indicates suitable conditions for HPLC analysis. Any adsorption occurring in the glass inserts at pH4.8 before the constant solution concentration beginning at 40 min would also occur in the glass inserts used for HPLC standard plots, and would therefore be taken into account in determining the solution concentrations in the polypropylene containers. Early injections, however, (less than 40 min) may not be reliable unless reproducibility is confirmed. The low concentration of 5 μg/mL examined at pH4.8 up to 12 h produced only a 3% difference in absorbance values at 40 and 60 min (data not shown) which was not statistically significant. For these experimental conditions, a fivefold range of concentration did not have an influence on the time of doxorubicin adsorption to glass inserts.

Measurement of doxorubicin adsorption based on measurement of loss in solution is a common technique (7,8,10–12). This approach was modified to estimate doxorubicin adsorption for the current study by extrapolation to time zero based on the assumption of maximum adsorption by the first time point of 3 h. The assumption is reasonable but some cautions should be noted. It is reasonable because maximum doxorubicin adsorption from solution onto polymer surfaces has been reported from 6 min to 3 h (7,8,12,13). In addition, the rate constants obtained in this study at pH7.4 (for the greatest adsorption of all pH values) are close to those reported in the literature for similar conditions (3,5). Also at pH7.4, the obtained rate constants have no correlation with initial drug concentration (r^2=0.46) and are not statistically different from each other. Consequently, at pH7.4 there is little evidence of adsorption occurring during measurement of degradation. The results at pH6.5, however, are less clear on this matter. The obtained rate constants are not as close (as noted for pH7.4) to the few reported values for approximately similar conditions (4,5). Also, the obtained rate constants have some statistically significant, as well as non-significant differences from each other. Nevertheless, the values obtained are not unreasonable, and based on the above discussion of little adsorption at pH7.4 during degradation, it appears unlikely that such adsorption occurs during degradation measurement at pH6.5. Finally, the method cannot distinguish between adsorption on to the surface and absorption into the bulk or matrix of the polymer, but the apparent saturation at the first time point suggests that adsorption onto the surface is more likely than absorption.

Doxorubicin loss was substantial in polypropylene microcentrifuge tubes and was dependent on pH and concentration

Table I. First-Order Degradation Rate Constants and Calculated Adsorption of Doxorubicin at Various pH and Concentrations in Polypropylene Microcentrifuge Tubes

pH	Doxorubicin concentration (μg/mL)	Degradation rate constant[a] (1/h)	Calculated C_0 [a,b] (mg/mL)	Calculated adsorbed doxorubicin[c] (%)
4.8	50	0.0021±0.0007	54.3±1.0	−8.8
	20	0.0019±0.0013[d]	19.8±0.7	1
	4.5	0.0009±0.0020[d]	3.9±0.2	14
	2.0	0.0004±0.0044[d]	1.6±0.2	20
Mean		0.0013		
6.5	50	0.0156±0.0014	49.1±1.9	1.9
	20	0.0172+0.0023	17.2±1.1	14
	4.5	0.0104+0.0012	3.5±0.1	23
	2.0	0.0051±0.0023	1.2±0	38
Mean		0.0121		
7.4	50	0.0193±0.0014	46.2±1.8	7.6
	20	0.0161±0.0025	14.2±1.0	29
	4.5	0.0162±0.0011	3.1±0.1	32
	2.0	0.0178±0.0018	1.1±0.1	45
Mean		0.0174		

In 0.03 M potassium phosphate buffer with 0.12 M sodium chloride at 37C. Data from Fig. 2
[a] Mean±SE (n=4)
[b] Concentration from extrapolation to time zero in semi-log plots of Fig. 2
[c] Calculated from difference between initial doxorubicin concentration and C_0
[d] Not statististically significant from zero

(Fig. 2, Table I). The least adsorption occurs at pH4.8, but only at the higher doxorubicin concentrations. At the intermediate pH6.5, only the highest concentration studied (50 μg/mL) produced a small extent of doxorubicin adsorption (<2%). At physiological pH7.4, adsorption is substantial at most concentrations, with a maximum value of 45% at the lowest concentration. The increase in adsorption with pH is ascribed to an increasing proportion of the unionized doxorubicin specie (glucosamino group pK_a=8.6). While a similar extent of doxorubicin adsorption has been reported in glass at this pH and near concentration (7), no reports were found for such adsorption to polypropylene.

These adsorption results differ from results in two prior reports of little or no adsorption in polypropylene containers. Wood *et al.* (6) reported 1.3% loss from a 2-mg/mL doxorubicin solution in water stored in polypropylene syringes at 4°C. In addition to possible degradation, the 40–1,000-fold higher drug concentration used in the prior study might account for the difference with the current study which shows a decreasing percent adsorption with increasing drug concentration. Tomlinson and Malspeis (7) reported decreasing radioactivity in Hanks solution (essential salts and glucose) from ^{14}C-labeled doxorubicin to describe adsorption to polyethylene and polytetraflouoroethylene containers but not to polypropylene containers. However, the different salt composition, as well as glucose might have interfered with adsorption. Alternatively, it is conceivable that the radioisotope moiety of the drug was chemically degraded at the experimental pH of 7.4 and released after adsorption to indicate the absence of adsorption.

CONCLUSIONS

Assay of doxorubicin using glass HPLC vial inserts is recommended at pH4.8 instead of pH7.4 because of drug loss up to 3 h in the inserts and because of degradation at longer times. Doxorubicin adsorption in polypropylene microcentrifuge tubes shows a trend with increasing pH values of 4.8, 6.5, and 7.4, as well as with decreasing doxorubicin concentrations. Determinations of low amounts of doxorubicin in polypropylene containers at pH7.4 may be underestimated if adsorption and degradation issues are not taken into account.

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