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# Interaction of 8-Hydroxyquinoline Sulfate with Components Present in a Tuberculin PPD Solution I

## Binding of 8-Hydroxyquinoline by Polysorbate 80

By S. LANDI and H. R. HELD

**This article deals with the interaction between the preservative 8-hydroxyquinoline sulfate (8-HQS) and the surface-active agent polysorbate 80. In buffered solution (pH 7.38) 8-HQS is dissociated to 8-hydroxyquinoline (8-HQ) and $H_2SO_4$, and it is the base 8-HQ that forms a reversible association with polysorbate 80. The degree of binding of 8-HQ to polysorbate 80 was shown to be a function of the concentration of the nonionic surface-active agent. Polysorbate 80 at low concentration (about 0.005 per cent) has practically no effect on the concentration of 8-HQ in a buffered solution (pH 7.38) as used for preparing dilutions of tuberculin PPD for the intracutaneous method (Mantoux test). 8-HQS, in a buffered solution of pH 3, does not interact with polysorbate 80.**

8-HYDROXYQUINOLINE sulfate (8-HQS)[1] is added to tuberculin PPD solutions as an antimicrobial agent. In a previous report (1) the authors described how 8-HQS disappears from these solutions when dispensed in glass vials stoppered with rubber closures and showed that most of the loss of 8-HQS from the solution was caused by sorption of 8-HQ by the rubber closures.

Numerous investigators have shown, by using solubility studies and equilibrium dialysis, that binding of preservatives (3–9) or pharmaceuticals (10–14) with polysorbate 80[2] or other macromolecules takes place.

Tuberculin PPD solutions are used intracutaneously for diagnostic purposes in tuberculosis-prevention programs. Such solutions contain 0.01% 8-HQS added as a preservative and 0.005% polysorbate 80 as a stabilizing agent (2). It was, therefore, of interest to find out if some binding between 8-HQS and polysorbate 80 takes place in the buffer used to prepare these solutions.

The solubility method (3) and the equilibrium dialysis method (3) were used by the authors to determine the compatibility of polysorbate 80 and 8-HQS.

Received September 8, 1965, from the Connaught Medical Research Laboratories, University of Toronto, Toronto, Ontario, Canada.
Accepted for publication October 19, 1965.

[1] Marketed as Chinosol.

[2] Marketed as Tween 80 by Atlas Chemical Industries, Wilmington, Del.

## MATERIALS AND METHODS

**Reagents.**—8-Hydroxyquinoline sulfate (8-HQS)[1] (Eastman Organic Chemicals, 1776), 8-hydroxyquinoline (8-HQ) (Fisher Scientific Co., 0-261), polysorbate 80 (polyoxyethylene 20 sorbitan monooleate).[2]

**Buffer Solution (pH 7.38).**—Isotonic phosphate buffered solution (2), pH 7.38 (1.45 Gm. of $KH_2PO_4$, 7.60 Gm. of $Na_2HPO_4$, and 4.8 Gm. of NaCl in 1050 ml.). This buffer is also used as diluent for the preparation of tuberculin PPD solutions (Mantoux).

**Buffer Solution (pH 3.0).**—McIlvaine's buffered solution (15) was prepared by mixing 79.5 ml. of 0.1 *M* citric acid solution and 20.5 ml. of 0.2 *M* $Na_2HPO_4$ solution.

**Dialysis Membranes.**—Thin nylon membrane,[3] as recommended by Patel and Kostenbauder (3), was used for dialysis of 8-HQ. A seamless regenerated cellulose tubing, size identity 36/32 (obtained from the Visking Division of Union Carbide, Canada, Ltd.) was used for the dialysis of 8-HQS and of 8-HQ.

**Determination of 8-HQ and 8-HQS.**—8-HQS, dissolved in buffered solution (pH 7.38), is dissociated in 8-HQ and $H_2SO_4$. The distinct absorption maximum of 8-HQ in the ultraviolet region at 240 mμ lends itself well to the quantitative deter-

[3] Supplied through the courtesy of Youngs Rubber Corp., New York, N. Y.

mination of 8-HQ and herewith to the determination of 8-HQS. The 8-HQS concentration of a solution was determined by measuring its absorbance at 240 mμ after dilution with buffer of pH 7.38, containing 0.005% polysorbate 80 and comparing it with the absorbance at the same wavelength of a standard solution. This method was described previously (1).

The $H_2SO_4$ liberated from 8-HQS was determined by precipitation and weighing as $BaSO_4$.

**Determination of Polysorbate 80.**—Polysorbate 80 shows an absorption maximum in the ultraviolet region at 230 mμ. The degree of absorption is too weak to be used for very low concentrations of polysorbate 80 or for higher concentrations of polysorbate 80 in the presence of strong U.V. absorbers like phenolic preservatives, etc. However, for pure aqueous solutions of polysorbate 80, this absorption at 230 mμ can be conveniently used for its determination. The authors determined the polysorbate 80 content by diluting 1 ml. of a pure aqueous solution of polysorbate 80 with 19 ml. of a buffered solution (pH 7.38), and comparing its absorbance at 230 mμ with the absorbance at the same wavelength of a standard solution of polysorbate 80.

**Solubility Method.**—The solubility of 8-HQS or 8-HQ in polysorbate 80 solutions was evaluated by placing into glass-stoppered weighing bottles 10 ml. of buffered solution containing various amounts of polysorbate 80 and an excess of either 8-HQS (200 mg. of a mixture, consisting of equivalent amounts of 8-HQS and $NaHCO_3$) or of 8-HQ (140 mg.). The bottles were agitated for 3 hr. at 28° then the contents were filtered through Whatman No. 1 filter paper and the 8-HQS or 8-HQ in solution determined spectrophotometrically.

**Dialysis Method at pH 7.38.**—The dialysis membrane was used in the form of small nylon bags. Into each bag was placed 10 ml. of a buffered solution containing different concentrations of polysorbate 80. Each nylon bag was then placed in a glass-stoppered weighing bottle, containing 10 ml. of buffered solution and 200 mg. of a mixture consisting of equivalent amounts of 8-HQS and $NaHCO_3$. In another experiment the weighing bottles contained 10 ml. of a 0.02% solution of 8-HQS in the same buffer. Each bottle was then stoppered tightly while the end of the nylon bag was protruding to the outside of the bottle, thus providing also a tight closure for the nylon bag. The bottles were then agitated for 4 days at 28°. After this period of time, an equilibrium between the inner and outer solution was reached for each concentration of polysorbate 80 employed, and samples were taken from the inside and outside of the nylon bags to determine the concentration of 8-HQ.

The same type of dialysis as described above was carried out for 2 days using a seamless cellulose tubing instead of the nylon bags.

**Dialysis Method at pH 3.**—Similar experiments as described were carried out in a solution of approximately pH 3 using as dialysis membrane a seamless cellulose tubing. Into each tubing was placed 10 ml. of an aqueous solution of different concentrations of polysorbate 80. The glass-stoppered weighing bottles contained in one experiment 10 ml. of a 0.2% solution of 8-HQS in $H_2O$ (final pH 3.2), and in another experiment the weighing bottles contained 10 ml. of a 0.02% solution of 8-HQS in buffer (pH 3.0). The bottles were agitated for 2 days at 28°.

## RESULTS AND DISCUSSION

**Interaction between Polysorbate 80 and 8-HQ at pH 7.38.**—When 8-HQS is dissolved in buffered solution (pH 7.38), it is dissociated in 8-HQ and $H_2SO_4$. This is evidenced from the fact that 8-HQ can be completely extracted with diethylether from this buffered solution into which 8-HQS was added. Further evidence that 8-HQ is present in a buffered solution (pH 7.38) was obtained in a previous article (1), where it was shown that rubber absorbed 8-HQ only while the $H_2SO_4$ remains entirely in the aqueous solution. Dialysis experiments to be described will show that it is 8-HQ that interacts with polysorbate 80 and not 8-HQS. Nevertheless, since in the literature dealing with tuberculin the concentration of this preservative is given as percentage of 8-HQS, the authors have in the present investigation expressed all measurements in 8-HQS equivalents.

Figure 1 shows that the solubility of 8-HQS or 8-HQ increases linearly with increasing polysorbate 80

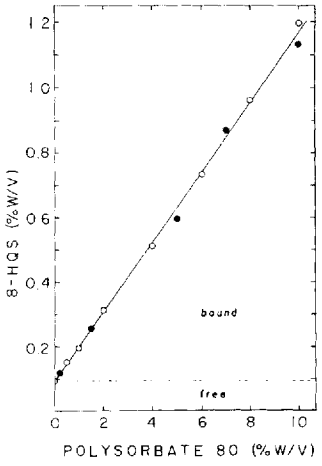


Fig. 1.—Solubility of 8-HQS and of 8-HQ in a buffered solution (pH 7.38, temperature 28°) containing various amounts of polysorbate 80. Key: ○, solubility of 8-HQS; ●, solubility of 8-HQ, plotted in 8-HQS-equivalents.

concentration, and when the solubility of 8-HQ is expressed in 8-HQS equivalents the same slope is obtained for both substances. Because the solubility of free preservative in a buffered solution is constant, as represented by a dashed line in Fig. 1, the increase in solubility of the preservative with increasing polysorbate 80 concentration can be considered to be due to bound preservative. This binding represents a relatively high degree of interaction. Such interaction between polysorbate 80 and other preservatives had been reported previously by several investigators (3–9).

To obtain further evidence of binding between polysorbate 80 and 8-HQ and to obtain data of binding between polysorbate 80 and low concentrations of 8-HQ, the equilibrium dialysis method (3) was used. Figures 2 and 3 show that when the concentration of polysorbate 80 in a buffered solution con-

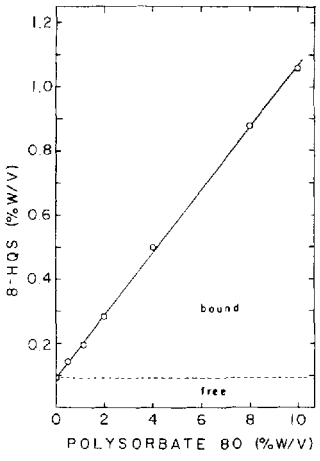


Fig. 2.—Equilibrium dialysis through a nylon bag. Outside the bag was placed a buffered solution (pH 7.38) to which an excess of solid 8-HQS and $NaHCO_3$ was added. Inside the bag was placed a buffered solution (pH 7.38), containing various amounts of polysorbate 80. The total 8-HQ concentration in the dialysis bag is plotted in 8-HQS equivalents *vs.* the polysorbate 80 concentration.

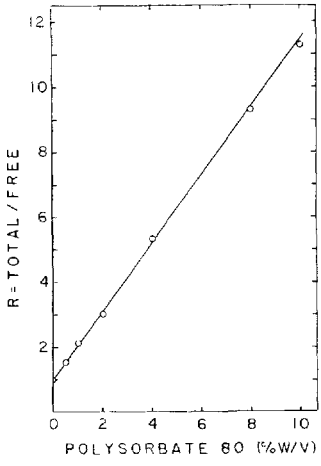


Fig. 3.—Equilibrium dialysis through a nylon bag as in Fig. 2. The binding of 8-HQ (expressed as the ratio $R$ = total/free) is plotted *vs.* the polysorbate 80 concentration.

tained in the nylon bag is increased from 0 to 10% the total concentration of 8-HQ in this solution increases linearly from 0.094 to 1.06%. In this experiment an excess of solid 8-HQS and $NaHCO_3$ was added to the solution outside the dialysis bag. Since at equilibrium the concentration of free preservative is assumed to be approximately the same inside and outside the dialysis bag (12), the increase of 8-HQ with increasing polysorbate 80 concentration (represented by the area above the dashed line in Fig. 2) must correspond very closely to the degree of interaction between polysorbate 80 and 8-HQ. The authors designated this increase in 8-HQ "*bound 8-HQ.*" To test the reversibility of this interaction the contents of the bags were dialyzed against running tap water, and it was found that all 8-HQ (free plus bound) dialyzed out of the bags in approximately 10 days. This shows that the "bound 8-HQ" was reversibly associated to the polysorbate 80.

Figure 2 confirms the results of solubility studies (Fig. 1) and also shows that the concentration of free 8-HQ is independent of the polysorbate 80 concentration when enough preservative is present to form a 8-HQ saturated solution. The free 8-HQ is represented by a dashed line in Figs. 1 and 2. It follows that the ratio between total 8-HQ (bound plus free) and free 8-HQ also increases linearly with increasing polysorbate 80 concentration (Fig. 3). This linearity facilitates the calculation of this ratio for any other concentration of polysorbate 80. Therefore, the results in Table I reveal that, when a concentration of 0.005% polysorbate 80 is present in a buffered solution containing an added amount of 0.094 to 1.06% total 8-HQS, there should be approximately 99.5% of free 8-HQ present. Thus, the loss of free 8-HQS caused by the interaction of 8-HQ with polysorbate 80 should be approximately 0.5%. In order to verify these results for lower concentrations of total 8-HQS (0.0096 to 0.0181%), the dialysis method (3) was also employed by using a concentration of 0.02% 8-HQS in the solution outside the nylon bag. Table II shows that the ratios between total and free 8-HQS are of the same order as those measured for more concentrated solutions of 8-HQS (0.094 to 1.06%, Table I), and that the loss of free 8-HQS caused by the presence of 0.005% polysorbate 80 should also be approximately 0.5%. Therefore, it is obvious that the reversible interaction between 0.005% polysorbate 80 and the 8-HQ liberated from 0.01% 8-HQS, in the buffer solution, as used for the preparation of tuberculin PPD solutions (2, 16), will have practically no effect on the concentration of free 8-HQ and presumably on its antimicrobial activity. That tuberculin PPD solutions containing these concentrations of polysorbate 80 and 8-HQS exhibit antimicrobial activity has been recently demonstrated by Pivnick *et al.* (17).

Further evidence that 8-HQ, and not 8-HQS, interacts with polysorbate 80 was obtained by using a cellulose instead of a nylon membrane. While 8-HQ dialyzed readily through the nylon membrane and reached equilibrium after 4 days, only traces of $H_2SO_4$ liberated from 8-HQS had passed through the nylon membrane (0.002% w/v inside as compared to 0.35% w/v outside the bag, Table I). It was further ascertained that sulfates like 8-HQS or $Na_2SO_4$ in buffered solution (pH 7.38) dialyze very slowly, if at all, through a nylon membrane. However, the seamless cellulose tubing was found to be readily permeable to 8-HQ as well as to 8-HQS and $Na_2SO_4$. Although the cellulose tubing was found to be to some extent permeable to polysorbate 80 (3), as in the case of the nylon membrane, most of the polysorbate 80 was kept inside the bag after 2 days, dialysis. However, considerable water was drawn inside the cellulose tubing from the outside solution changing the concentration of polysorbate 80. For this reason all the concentrations inside ($C_i$) and outside ($C_0$) the cellulose tubing were determined after equilibrium was reached. In fact, Table III shows that the binding of 8-HQ by polysorbate 80 is of the same order as previously determined using a

TABLE I.—DEGREE OF BINDING[a] OF 8-HQ BY POLYSORBATE 80 CALCULATED FROM INCREASE OF 8-HQ CONCENTRATION DUE TO PRESENCE OF POLYSORBATE 80 (DIALYSIS METHOD AT pH 7.38, USING A NYLON DIALYSIS BAG)

| Concn. Inside Nylon Bag: Polysorbate 80 | Concn. Inside Nylon Bag: Total 8-HQ Expressed as 8-HQS Equiv. | Concn. Inside Nylon Bag: Total $H_2SO_4$ | Binding of 8-HQ, Expressed as: Ratio of Total/Free | Binding of 8-HQ, Expressed as: % of Total 8-HQ |
|---|---|---|---|---|
| % w/v | % w/v | % w/v | ... | % |
| 10 | 1.06 | 0.002[c] | 11.28 | 91.14 |
| 8 | 0.876 | 0.002[c] | 9.32 | 89.27 |
| 5 | 0.58[b] | ... | 6.2[b] | 83.87[b] |
| 4 | 0.500 | ... | 5.32 | 81.20 |
| 2 | 0.283 | ... | 3.01 | 66.78 |
| 1 | 0.194 | ... | 2.06 | 51.46 |
| 0.5 | 0.146 | ... | 1.553 | 35.61 |
| 0.1 | 0.104[b] | ... | 1.104[b] | 9.42[b] |
| 0.01 | 0.09498[b] | ... | 1.0104[b] | 1.03[b] |
| 0.005 | 0.09449[b] | ... | 1.0052[b] | 0.52[b] |
| 0 | 0.094 | ... | 1 | 0 |

[a] After reaching equilibrium. (All concentrations of 8-HQ are expressed in 8-HQS equivalents.) [b] Calculated from slope of curve (total/free *vs.* concentration of polysorbate 80). [c] Total $H_2SO_4$ liberated from an excess of 8-HQS added to the buffered solution (pH 7.38) outside of the nylon bag was 0.35% w/v.

TABLE II.—DEGREE OF BINDING[a] OF 8-HQ BY POLYSORBATE 80 CALCULATED FROM RATIO $C_i/C_0$ (DIALYSIS METHOD AT pH 7.38, USING NYLON DIALYSIS BAG)

| Concn. in Nylon Bag: Polysorbate 80 | Concn. in Nylon Bag: 8-HQ: Total $C_i$ | Concn. in Nylon Bag: 8-HQ: Free $C_0$ | Binding of 8-HQ, Expressed as: Ratio of Total/Free | Binding of 8-HQ, Expressed as: % of Total 8-HQ |
|---|---|---|---|---|
| % w/v | % w/v | % w/v | ... | % |
| 10 | 0.0181 | 0.00154 | 11.75 | 91.5 |
| 5 | 0.0167 | 0.00276 | 6.05 | 83.5 |
| 2 | 0.0147 | 0.00482 | 3.06 | 67.3 |
| 1 | 0.0135 | 0.00688 | 1.96 | 49.0 |
| 0.5 | 0.0125 | 0.00764 | 1.64 | 38.9 |
| 0.1 | ... | ... | 1.104[b] | 9.42[b] |
| 0.01 | ... | ... | 1.0104[b] | 1.03[b] |
| 0.005 | ... | ... | 1.0052[b] | 0.52[b] |
| 0 | 0.0096 | 0.0096 | 1 | 0 |

[a] After reaching equilibrium. (All concentrations of 8-HQ are expressed in 8-HQS equivalents.) [b] Calculated from slope of curve (ratio $C_i/C_0$ *vs.* concentration of polysorbate 80). $C_i$ = concentration $C_i$ inside nylon bag (representing total 8-HQ inside nylon bag). $C_0$ = concentration $C_0$ outside nylon bag (representing free 8-HQ outside and inside nylon bag).

TABLE III.—DEGREE OF BINDING[a] OF 8-HQ BY POLYSORBATE 80 CALCULATED FROM RATIO $C_i/C_0$ (DIALYSIS METHOD AT pH 7.38, USING SEAMLESS CELLULOSE TUBING)

| Concn. in Soln. Inside ($C_i$): Polysorbate 80 $C_i$ | Concn. in Soln. Inside ($C_i$): 8-HQ $C_i$ | Concn. in Soln. Inside ($C_i$): $H_2SO_4$ $C_i$ | Outside ($C_0$): Polysorbate 80 $C_0$ | Outside ($C_0$): 8-HQ $C_0$ | Outside ($C_0$): $H_2SO_4$ $C_0$ | Retention of Polysorbate 80: Expressed as Ratio $C_i/C_0$ of: Polysorbate 80 $C_i/C_0$ | Degree of Binding of 8-HQS: Expressed as Ratio $C_i/C_0$ of: 8-HQ $C_i/C_0$ | Degree of Binding of 8-HQS: Expressed as Ratio $C_i/C_0$ of: $H_2SO_4$ $C_i/C_0$ |
|---|---|---|---|---|---|---|---|---|
| % w/v | % w/v | % w/v | % w/v | % w/v | % w/v | ... | ... | ... |
| 10 | ... | ... | ... | ... | ... | ... | 11.3[b] | ... |
| 6.30 | 0.70 | 0.21 | 0.115 | 0.099 | 0.21 | 54.8 | 7.07 | 1.00 |
| 5 | ... | ... | ... | ... | ... | ... | 6.2[b] | ... |
| 3.21 | 0.462 | 0.18 | 0.065 | 0.097 | 0.18 | 49.4 | 4.76 | 1.00 |
| 1.82 | p.331 | 0.18 | 0.023 | 0.100 | 0.18 | 79.1 | 3.31 | 1.00 |
| 1 | ... | ... | ... | ... | ... | ... | 2.0[b] | ... |
| 0.005 | ... | ... | ... | ... | ... | ... | 1.005[b] | ... |
| 0 | 0.100 | 0.19 | 0 | 0.100 | 0.19 | ... | 1.00 | 1.00 |

[a] After reaching equilibrium. (All concentrations of 8-HQ are expressed in 8-HQS equivalents.) [b] Calculated from slope of curve (ratio $C_i/C_0$ of 8-HQ *vs.* concentration of polysorbate 80.).

nylon membrane. Example: 10% w/v polysorbate 80, binding of 8-HQ 11.28 (Table I) and 11.30 (Table III); 0.005% w/v polysorbate 80, binding of 8-HQ 1.0052 (Table I) and 1.005 (Table III). Furthermore, when the cellulose tubing was used the final concentration of $H_2SO_4$ (liberated from 8-HQS) becomes equal on both sides of the dialysis membrane for all the concentrations of polysorbate 80 employed (Table III). This indicates that only 8-HQ and not 8-HQS interacts with polysorbate 80 because, if an appreciable amount of 8-HQS was present at pH 7.38 and did interact with polysorbate 80, the ratio $C_i/C_0 = 1$ of $H_2SO_4$ (Table III) would become numerically higher than 1.

**Interaction between Polysorbate 80 and 8-HQS at pH 3.**—Although a diluent at this pH is not used for the preparation of tuberculin PPD solutions, it is, nevertheless, useful to describe briefly the results of a study of a possible interaction between polysorbate 80 and 8-HQS at pH 3. It might con-

tribute to the understanding of the basic mechanism between these two substances in the buffer (pH 7.38) previously employed.

8-HQS, dissolved in buffered solution (pH 3), shows no noticeable dissociation. This was evidenced from the fact that 8-HQ cannot be extracted with diethylether from 8-HQS in solution at pH 3 and that, in contrast to what was found previously using a buffered solution (pH 7.38), pure rubber does not absorb 8-HQ from 8-HQS in a buffered solution (pH 3).

Figure 4 shows the result of an equilibrium dialysis experiment in which seamless cellulose tubing

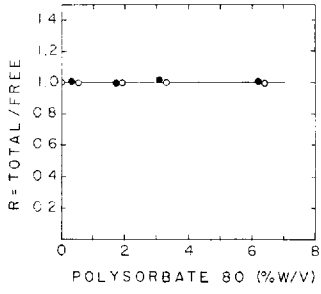


Fig. 4.—Equilibrium dialysis of 8-HQS through seamless cellulose tubing at pH 3. No interaction was detected. Key: O, 0.2% 8-HQS dissolved in $H_2O$ was placed outside the dialysis tubing; ●, 0.02% 8-HQS dissolved in buffered solution (pH 3.0) was placed outside the dialysis tubing.

was used as a dialysis membrane. The ratios of the 8-HQS concentrations inside ($C_i$) and outside ($C_0$) of the tubing were plotted *versus* the polysorbate 80 concentration at equilibrium. The final polysorbate 80 concentrations were determined separately by ultraviolet spectrophotometry. The horizontal line (Fig. 4) indicates that there is no interaction between polysorbate 80 and 8-HQS at pH 3.

**Interaction of 8-HQ with Other Macromolecules.**—The interaction of 8-HQ liberated from 8-HQS with other macromolecules (tuberculoprotein, nucleic acid, and polysaccharide) present in tuberculin PPD solutions is now under investigation and will be reported subsequently.

## SUMMARY

1. The interaction of 8-HQS with polysorbate 80 in buffered solutions of pH 7.38 and of pH 3.0 have been studied by means of the solubility method and the equilibrium dialysis method.

2. 8-HQS dissolved in buffered solution (pH 7.38) is dissociated in 8-HQ and $H_2SO_4$, and it is the base 8-HQ which interacts with polysorbate 80.

3. The degree of binding of 8-HQ by polysorbate 80, expressed as the ratio of total to free 8-HQ, increases linearly with increasing concentration of polysorbate 80.

4. The degree of binding of 8-HQ by polysorbate 80 is the same, regardless of whether 8-HQS or 8-HQ is added to a buffered solution (pH 7.38) containing polysorbate 80.

5. A relatively high degree of interaction has been observed for polysorbate 80 concentrations of 1 to 10%. However, when polysorbate 80 is added at the concentration of 0.005%, as used in tuberculin PPD dilutions for the Mantoux test, practically all 8-HQ (approximately 99.5%) is still present in the free state.

6. The association between 8-HQ and polysorbate 80 is reversible.

7. 8-HQS in a buffered solution of pH 3 does not interact with polysorbate 80.

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