Complexation Between Hexylresorcinol and Polyvinylpyrrolidone and Its Microbiological Evaluation

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Abstract □ An investigation of the role of polyvinylpyrrolidone (PVP) as a stabilizer for hexylresorcinol in a compressed tablet was conducted and the effect of PVP on the microbiological activity of hexylresorcinol was investigated. The presence of PVP in the tablet was shown to be responsible for the color stability of the hexylresorcinol. A molecular interaction between hexylresorcinol and PVP, of unusually strong complexing tendency, was identified by the solubility isotherm method and by IR spectroscopy. The stoichiometry of the complex was calculated to be one hexylresorcinol molecule per vinylpyrrolidone repeating unit. The antimicrobial activity of hexylresorcinol was evaluated by two methods using both Gram-positive and Gram-negative bacteria, and was found to be reduced in the presence of PVP. This reduction in activity was apparently due to the molecular interaction between hexylresorcinol and PVP and, therefore, indicates a need for the biological evaluation of complexes whenever their formation is suspected.

Keyphrases □ Hexylresorcinol-polyvinylpyrrolidone—complexation □ Stability, hexylresorcinol—PVP effect □ Antimicrobial activity, hexylresorcinol—PVP complexation effect

Hexylresorcinol, an alkylated dihydroxybenzene, is subject to the oxidative decomposition characteristic of phenols, both as a solid and in aqueous solution. The observation that compressed tablets, which contained hexylresorcinol and polyvinylpyrrolidone (PVP), did not discolor after 19 weeks at 50° suggested an investigation of the role of PVP as a stabilizer and also suggested an investigation of the effect of PVP on the microbiological activity of hexylresorcinol.

EXPERIMENTAL

Influence of Polyvinylpyrrolidone on Hexylresorcinol Stability in Compressed Tablets—The influence of PVP on hexylresorcinol stability in compressed tablets was investigated by observing the effect of heat on the physical stability of tablets containing hexylresorcinol made with and without PVP. Where present, the PVP was added as a 7.5% w/v alcoholic solution during granulation; the ratio of PVP to hexylresorcinol was 15 mg. to 5.25 mg. The tablets were packaged in amber glass bottles and stored at 50°, 37° and room temperature.

Complexing of Hexylresorcinol with Polyvinylpyrrolidone—The method of Klotz *et al.* was employed to determine the complexing of hexylresorcinol with PVP (1). It consisted of bringing two solutions, one containing hexylresorcinol and the other PVP, into equilibrium across a semipermeable membrane. The membrane was chosen to permit the hexylresorcinol molecules to pass freely through it and achieve the same concentration in both solution phases. At the same time, the membrane was impermeable toward the PVP macromolecules. Therefore, any increase in the hexylresorcinol concentration on the PVP side of the barrier was attributed to complex formation.

Cellulose dialyzing bags[1] were filled with 10 ml. of varying concentrations (0 to 0.3% w/v) of PVP solutions. The bags were immersed in 20 ml. of 0.025% w/v hexylresorcinol solution. The glass jars containing these solutions were mechanically shaken overnight at room temperature (approximately 23°). The following morning, the bags were removed from the glass jars and the remaining external solutions were analyzed for hexylresorcinol by measuring the absorbance at the maximum near 279 mμ using a Beckman model DU spectrophotometer. Six hours of mechanical shaking were adequate for attainment of hexylresorcinol equilibrium.

Microbiological Evaluation—The PVP-free and PVP-containing tablets were evaluated for antimicrobial activity of hexylresorcinol by two methods, using *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Proteus vulgaris*. These organisms were selected because each had a reported sensitivity to hexylresorcinol and because they represented both Gram-positive and Gram-negative bacteria.

In Method I, aqueous suspensions of each test sample were prepared to contain the equivalent of 1.2 mg. of hexylresorcinol per ml. The test samples were: hexylresorcinol crystals (the control), hexylresorcinol tablets without PVP, and hexylresorcinol tablets with PVP. One-half milliliter of a 24-hr. culture[2] of *S. aureus* FDA 209 ATCC 6538 in standard broth medium[3] was added to 5 ml. of each test sample. After 1 min. a 4-mm. loopful of inoculated test solution was transferred to a tube of standard broth medium. The inoculated broth was incubated at 37° for 48 hr., after which time the tubes were examined. Growth in the subculture indicated that the organism had not been killed; no growth in the subculture indicated the organism had been killed.

In Method II, a cup plate test, aqueous suspensions of each test sample were prepared to contain the equivalent of 1 and 0.25 mg. hexylresorcinol per ml. The test samples were the same as those listed above. Test cultures of *S. aureus* Smith and *P. vulgaris* 1810 were grown overnight at 37° in brain heart infusion broth,[4] except for *S. pyogenes* C-203 for which horse serum was added to the broth to a final concentration of 10%. The overnight cultures were diluted to a final concentration of 10^{-3} in brain–heart infusion agar, again adding serum for *S. pyogenes* C-203. Each plate received 10 ml. of seeded agar. Two cups were used for each of the dilutions tested.

RESULTS AND DISCUSSION

Influence of Polyvinylpyrrolidone on Hexylresorcinol Stability in Compressed Tablets—After 1 week at room temperature, 37 and 50°, the tablets without PVP were darker in color than the tablets containing PVP. After 1 month under the same temperature conditions, the tablets without PVP appeared as mottled, brownish-pink tablets whereas the PVP-containing tablets remained white. Placebo tablets, containing neither hexylresorcinol nor PVP, did not discolor. These observations indicated that the discoloration of the hexylresorcinol in the compressed tablets was prevented through the addition of PVP and thus suggested that PVP acted as a stabilizer for hexylresorcinol, possibly through complex formation.

Complexing of Hexylresorcinol with Polyvinylpyrrolidone—Figure 1 shows that hexylresorcinol complexes with PVP. The results are expressed according to the method of Higuchi and Kuramoto (2). If hexylresorcinol had not complexed with PVP, the hexylresorcinol concentrations would be equal on each side of the membrane. The total hexylresorcinol concentration and the concentration of unbound hexylresorcinol thus would be equal and K would be 1. However, if hexylresorcinol complexed with PVP, the hexylresorcinol concentrations would be unequal on each side of the membrane. The concentration of unbound hexylresorcinol

[1] Arthur H. Thomas Company, Philadelphia, Pa.

[2] This culture was standardized so that after 5 min. a 1:75 phenol solution did inhibit growth whereas a 1:80 phenol solution did not inhibit growth.

[3] Contains beef extract, peptone, sodium chloride, and distilled water.

[4] Baltimore Biological Laboratories, Inc., Baltimore, Maryland.

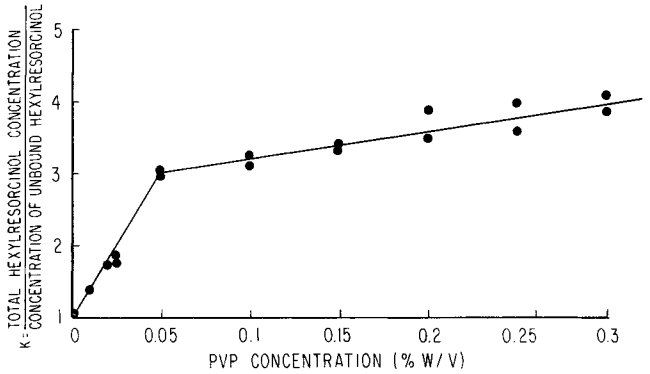

Figure 1—*Complexing between hexylresorcinol and PVP.*

would be less than the total hexylresorcinol concentration and K would be greater than 1.

Complex formation between PVP and a number of pharmaceutical substances was studied by Higuchi and Kuramoto (2, 3). The greatest K value reported by them was 1.2 for sulfathiazole at 0.05% PVP. Since the authors observed a K value of 3.0 for hexylresorcinol at 0.05% PVP, it appears that hexylresorcinol showed an unusually strong complexing tendency with PVP.

Two consecutive positive slopes are shown in Fig. 1. This multislope solubility isotherm indicates that a complex molecular interaction has occurred, which can possibly be explained by a change of the spacial orientation of the PVP polymer as hexylresorcinol molecules attach to it. Lach and Pauli (4) have reported similar interactions between several pharmaceuticals and Schardinger dextrins.

The method of Klotz *et al.* was used to determine the maximum number of bound hexylresorcinol molecules per PVP molecule and is illustrated in Fig. 2 (1). The reciprocal of the value of the intercept at the y-axis is the maximum number of bound hexylresorcinol molecules per PVP molecule and was calculated to be 417 hexylresorcinol molecules. Since there are 360 vinylpyrrolidone repeating units per PVP molecule, the number of bound hexylresorcinol molecules was calculated to be 1.16 per vinylpyrrolidone repeating unit. Presumably, 1 is the number of bound hexylresorcinol molecules per vinylpyrrolidone repeating unit.

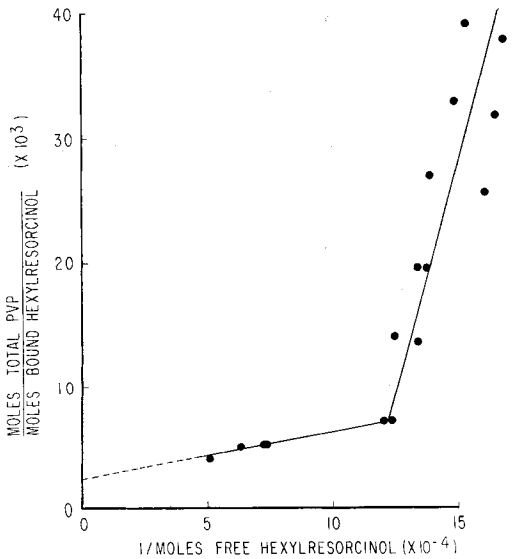

Figure 2—*Determination of the maximum number of bound hexylresorcinol molecules per PVP molecule.*

Table I—Microbiological Evaluation—Method II

Test Sample	Hexylresorcinol Concn., mg./ml.	Average Inhibition Zone (mm.) *S. aureus*	*Strep. pyogenes*	*P. vulgaris*
Crystals	1.00	21	16	14
	0.25	15	10	8
Tablet without PVP	1.00	19	15	12
	0.25	13	9	8
Tablet with PVP	1.00	11	8	8
	0.25	8	8	0

An examination of the IR spectrum of the hexylresorcinol-PVP complex, which was isolated as a white solid, revealed a shift in the frequency of the hydroxyl band from 3420 to near 3270 $cm.^{-1}$. It is reasonable to expect the hydroxyl groups of the hexylresorcinol molecule to be involved in the complex formation, and so the observed shifts in the hydroxyl frequency is acceptable.

Microbiological Evaluation—The results of the microbiological evaluation by Method I are summarized as follows. Growth was not observed in the subcultures containing test samples free of PVP, namely hexylresorcinol crystals and the hexylresorcinol tablet without PVP. However, growth was observed in the subcultures containing the test sample with PVP, namely the hexylresorcinol tablet with PVP.

The results of the microbiological evaluation by Method II are summarized in Table I. The crystalline hexylresorcinol test samples showed greater inhibition zones than either of the tablet test samples for all conditions except the PVP-free tablet with *P. vulgaris* at 0.25 mg./ml. The presence of insoluble tableting adjuvants in the tablet test samples probably hindered diffusion from the cup, which was reflected as smaller zones of inhibition. In all cases, the tablet test samples with PVP showed smaller inhibition zones than the tablet test samples without PVP. The inhibition zone was reduced from 11 to 100%, the average being 45%.

These results indicated that the antimicrobial activity of hexylresorcinol was reduced in the presence of PVP. This reduction in activity was apparently due to the molecular interaction between hexylresorcinol and PVP, and therefore indicates a need for the biological evaluation of complexes whenever their formation is suspected.

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