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Functional Polymers with Biologically Active Groups

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Functional Polymers with Biologically Active Groups

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ABSTRACT

Synthetic polymers with functional groups, especially where the functional group is a biologically active group, are receiving considerable attention, since such polymeric drugs represent novel drug delivery systems. In this paper methods for the preparation of typical classes of polymers with biologically active groups were explored. As an example of a condensation polymer, primaquine was incorporated in a polymer chain by an allophanate linkage. Primaquine was also substituted on a polyoxyethylene polymer chain as was the N,N-dimethylaminobenzoate group. Oligo(oxyethylene) glycols were endcapped with salicylate or N,N'-dimethylaminobenzoate groups. These functional groups are reversibly linked to polymers and can be removed, for example, by hydrolysis, providing slow and sustained release of the drug. As nondegradable polymers with functional groups, polymers and copolymers of 4-vinylsalicylic acid and 5-vinylsalicylic acid derivatives have been prepared. It was shown clearly that high polymers are active in antibacterial tests, against gram-positive and/or gram-negative bacteria. The activity of poly(4-vinylsalicylic acid) and poly(5-vinylsalicylic acid) was found to be independent of molecular weight. Selective activity has been obtained by preparing copolymers of 4-vinylsalicylic acid or 5-vinylsalicylic acid with methacrylic acid, a comonomer whose homopolymer was inactive.

INTRODUCTION

Synthetic polymers with functional groups of known or potential biological activity have received increasing attention in recent years [1-4]. Polymeric drugs may include all agents which, upon introduction into a living system, cause a physiological response; not only curative but also prophylactic agents are included in this definition. Polymeric materials as biologically active agents have potential advantages and disadvantages, as activity may be related to functional groups and to the polymeric nature of the substances [1]. The most important thrust in the area of biologically active synthetic polymers is the study of materials which may be used outside of the human body. Very little is known about the long-term effects of synthetic polymers in the body, the retention of polymers in tissues, the mechanism of the action of polymeric materials with biological activity, structure/activity relationships, or the metabolic fate of polymers, expecially synthetic polymers.

Polymeric compounds with biological activity are expected to have changed or modified activity and toxicity, compared with the parent compound. Of particular importance is the possibility of prolonged activity, either directly or by sustained release. The activity of polymeric drugs can be influenced by molecular weight or molecular weight distribution. Since some of the most interesting active polymers are actually copolymers, modification of the activity can be visualized by the knowledge of copolymer composition, the distribution of the functional groups along the polymer chain, and the stereochemistry of the polymer. Solubility of the copolymer cannot only be changed by changing polymer molecular weight but also by introducing solubilizing groups by copolymerization or post-reaction. Of particular potential importance is the introduction of hydrophilic and hydrophobic groups and of groups with polyelectrolyte character. Such compositions not only influence the transport properties of the polymers, perhaps through membranes, but also may cause specific accumulation in desired locations. If the materials are either hydrolyzible or otherwise biodegradable, sustained and controlled release of low molecular active compounds at the desired time and location in desired concentration level becomes the essential feature of these materials.

The active group, for example a pharmaceutical, may be a part of the main chain, for example a diamine or a bisphenol which can be allowed to react with dicarboxylic acid derivatives, i. e., bisisocyanates and other bifunctional monomers capable of forming condensation polymers. The active group might be linked to the chain as a pendant group either directly or with a spacer group of specific chain length (Fig. 1). In this case, the materials might be either stable or attached with a hydrolytically or photochemically unstable

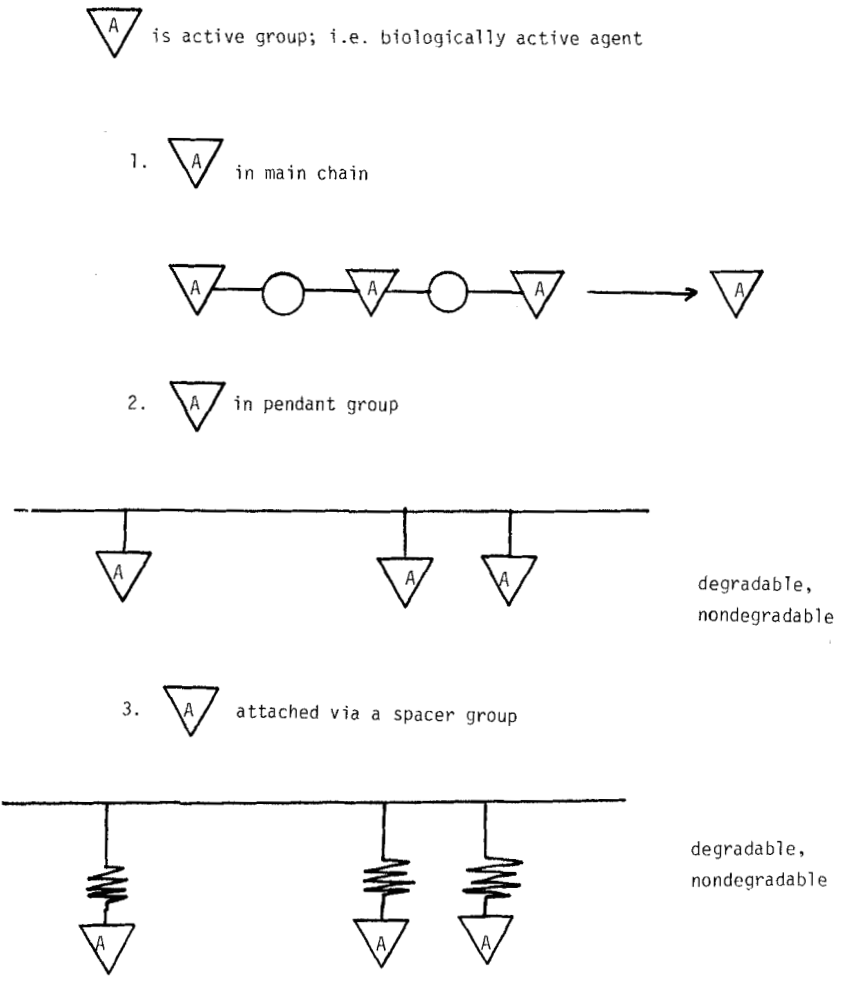


FIG. 1. Functional polymers, general scheme.

linkage to release the small molecule as the biologically active agent (Fig. 2) [5]. The latter concept has become conceptually important because by proper release kinetics, enhanced or prolonged effectiveness of the active agent might be achieved.

The active agent may be at the end of a low molecular weight or moderate molecular weight polymer chain or attached to a high polymer. For the preparation of oligomers, endcapping reactions on

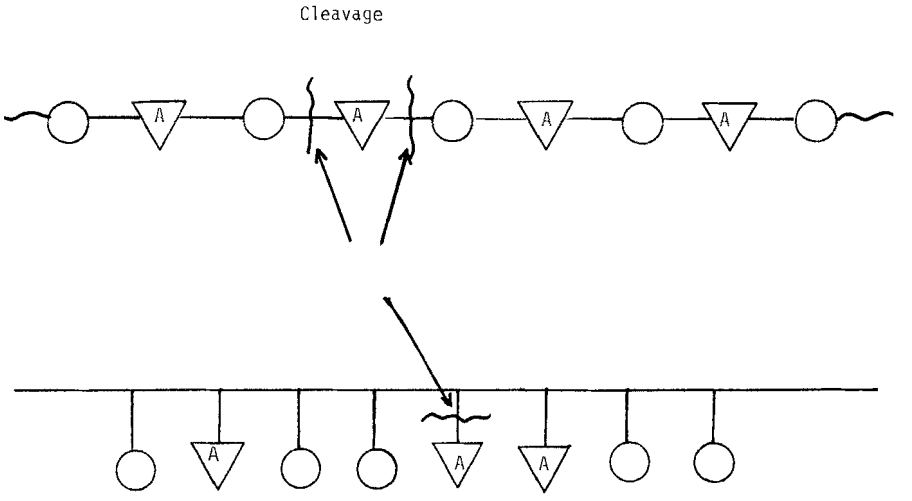


FIG. 2. Functional groups as part of a degradable polymer chain or degradable group attached to main chain.

polymers of low and moderate molecular weight can be achieved by reacting the properly substituted active compound onto the reactive group at the chain ends as, for example, in the case of hydroxyl-terminated polyoxyethylenes or butadiene polymers (Fig. 3).

High molecular weight polymers are often advantageous and can be functionalized in a number of different ways. The simplest way is the use of an already formed polymer with reactive groups, where the reactive groups are substituted with desired functional groups (Fig. 4). Advantages of such polymer reactions are that the molecular weight and molecular weight distribution of the polymer have already been established-although polymer degradation has to be carefully avoided; if copolymers are used for the substitution reactions the amount of groups is limited and their distribution and run number are already established in the copolymer. Additional advantages of reactions on polymers include the possibility of introducing several different functional groups which might provide not only the biologically active group but might also include a solubilizing and compatibilizing anchoring groups, groups with hydrophilic and hydrophobic portions in the same macromolecule.

If the same functionality is used for all the reactions, it requires that the chemical reactions involved are of the same type, as for example, nucleophilic displacement reactions. Reactions on polymers, however, have inherent problems; the reactions have to be carried out under mild conditions (near room temperature), and the yield from

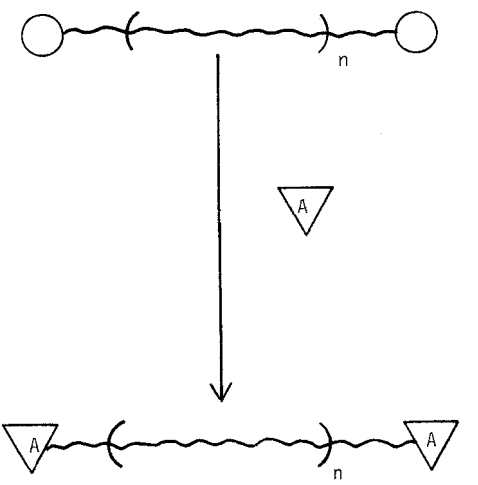


FIG. 3. End-capping of oligomers.

all these reactions must be quantitative. Any "error" in any of these reactions will result in its retention in the polymer chain. Examples of side reactions may be dehydrohalogenation instead of nucleophilic displacement of chloride as, for example, in polyepichlorohydrin, or simple hydrolysis to the acid if an ester or an acid chloride are used for the desired substitution reactions. Other undesirable side reactions might result in color formation or crosslinking.

It must also be realized that during the reaction the solubility of the initial polymer may be changed and precipitation of the final product may occur; a more favorable example is when precipitated polymer dissolves during the reaction.

One of the limitations of the reactions on polymers is the fact that the reactivity of a functional group may be unfavorable when it is directly attached to the main chain or has severe hindrance by neighboring side groups. This effect is normally explained by the generally accepted fact that $k_1 > k_2 > k_3$ for substitution, which causes a reaction not to go to completion. This can be overcome by spacing the reactive group several carbon atoms from the main chain or by spacing the reactive groups more than the usual two carbon atoms along the polymer chain as, for example, in an ethylene oxide backbone chain. Backbone chain stability becomes a problem in such cases, however.

The other approach to the synthesis of polymers with functional groups is the preparation of monomers with polymerizable functional groups attached for subsequent polymerization. This approach also

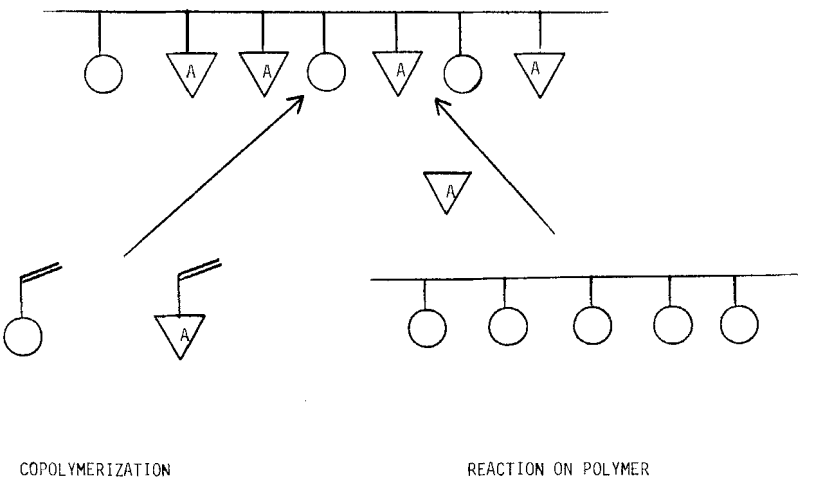


FIG. 4. Introduction of functional groups by copolymerization or reaction on polymers.

has the advantage that monomers may be highly purified and may be polymerized and copolymerized with any number of desirable comonomers. The reaction usually gives, if successful, the polymers in good yield and purity. Functional monomers with a polymerizable vinyl group usually polymerize readily as they often belong to the category of styrenes.

Disadvantages of this approach could also be significant. Sometimes the functional monomer cannot be synthesized or it can only be made by a cumbersome multistep synthesis ultimately giving very low yield of the material. It may not be possible to purify the monomer or it may not polymerize or copolymerize with the desirable comonomer. Copolymerization of monomers with spacer groups, unless they are acrylates or methacrylates, is sometimes difficult to achieve, as in the case of terminally substituted α-olefins.

High monomer purity for polymerization must also be achievable to obtain reasonable molecular weight; high molecular weight is not always necessary. Although in principle an attractive approach, the polymerization of functional monomers to polymers of optimum molecular weight and molecular weight distribution and of desirable sequence distribution and compositional homogeneity may be achieved with difficulty.

In this paper we discuss incorporation of biologically active material in the main chain as in the case of primaquine, a potent antimalarial agent, endcapping of hydroxyl-terminated poly(ethylene

oxide), substitution on side groups of a polymer chain, as exemplified by polyepichlorohydrin, or poly(methacrylic acid) and finally the synthesis and polymerization of 4-vinylsalicylic acid and 5-vinylsalicylic acid derivatives. Since some of the latter derivatives showed unusual and highly encouraging antibacterial activity, the test results will also be discussed.

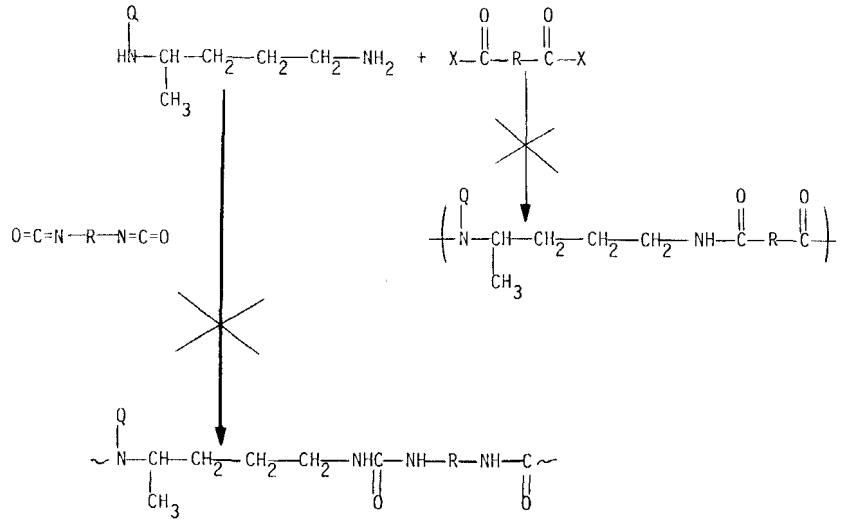
FUNCTIONAL GROUPS IN THE POLYMER MAIN CHAIN

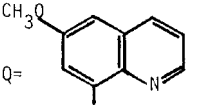
Polymers with biologically active groups in the main chain are considered desirable as they may be hydrolyzed to the active drug and a small innocuous molecule. Prime candidates for the polymer structure in these categories are polyamides, polyesters, and polyurethanes.

We have selected primaquine, a potent antimalarial drug [6-8], as a typical example of a drug that could be introduced into the polymer main chain through its amine function. It was expected that this compound, a diamine, would readily form polyamides by some of the well established methods. However, primaquine is not only a diamine with an aliphatic primary amino group but also has a second amine function which is a hindered aromatic amino-group.

Attempted direct synthesis of a polyamide by solution and interfacial polymerization techniques using acid chlorides such as isophthaloyl, sebacyl, or oxalyl chloride, were ineffective [Eq. (1)]. As a consequence, a two-step polymerization was considered a possibility. First, the more reactive primary amine group was blocked by Schiff base formation [9] with acetone or by the formation of a phthalimide [Eq. (2)]. This objective was readily accomplished; however, the second step, the condensation of the aromatic amino group with a very reactive difunctional monomer could not be accomplished under any circumstances. The original plan had been to prepare the A-B-A monomer blocked at the reactive A end, to remove the blocking group and carry out a normal condensation type polymerization of the unhindered primary aliphatic amines of the A-B-A with a diacid chloride [10, 11]. This technique of regular copolyamide formation has been very successfully used for the preparation of regular copolyoxamides.

The isopropylidene, n-propylidine, and phthalimido derivatives of the primary aliphatic amino group of primaquine could be synthesized in high yields and obtained in crystalline and pure form but the linking of two primaquine units through the aromatic amino groups was not possible. A-B-A type compounds containing two molecules of primaquine per monomer unit have been prepared [Eq. (3)]. When free primaquine was allowed to react with oxalyl or sebacyl chloride,



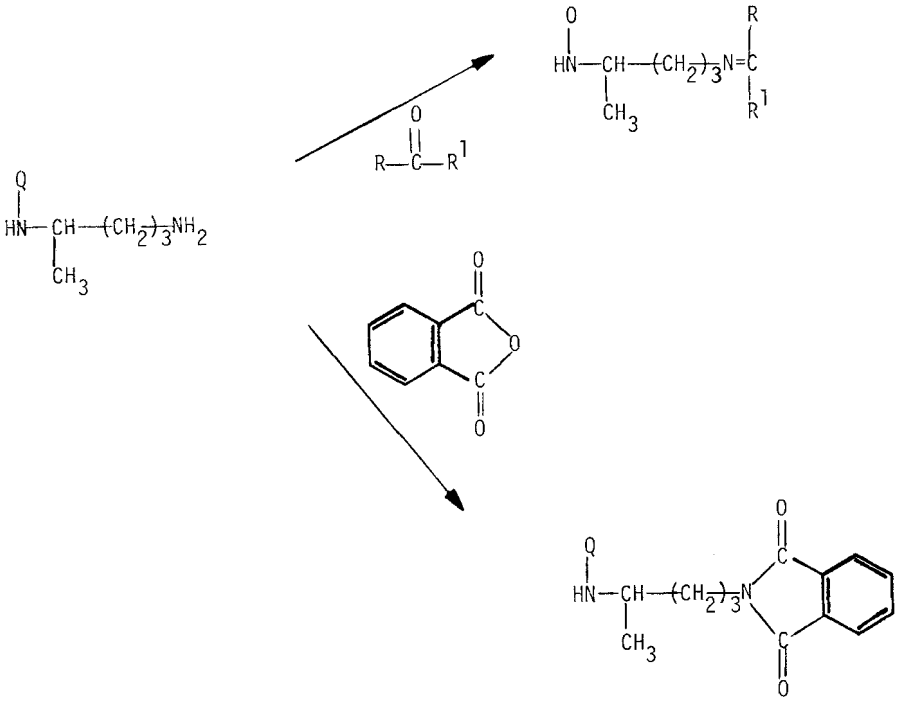


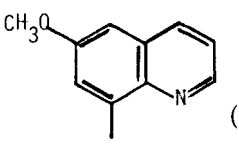
(1)

both the oxamide and sebacamide have been isolated, but linkage is through the aliphatic primary amino group, leaving the completely unreactive aromatic amino group free.

Polymers of low molecular weight containing primaquine in the main polymer chain have been obtained by reacting primaquine with hexamethylene diisocyanate (HMDI) or methylene di-p-phenyl diisocyanate (MDI) or with a 1:1 mixture of these two diisocyanates.

Under normal conditions, from the reaction of a diamine and a diisocyanate, one would expect the formation of a polyurea. All indications show that only one amino group, the aliphatic primary amino group, has been reacted in this reaction sequence and that a polymer was obtained with allophanate linkages [Eq. (4)]. Analysis and spectral evidence also indicated that primaquine and HMDI or MDI or a mixture of HMDI and MDI was present in the polymer but only a low molecular weight polymer was isolated. MDI or HMDI did not react



Q = 

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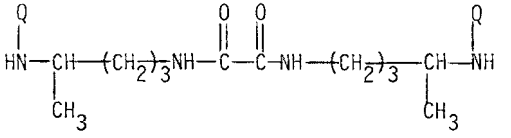

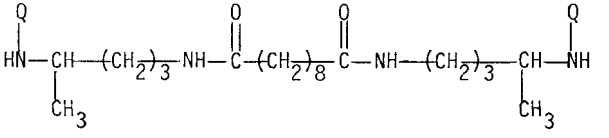

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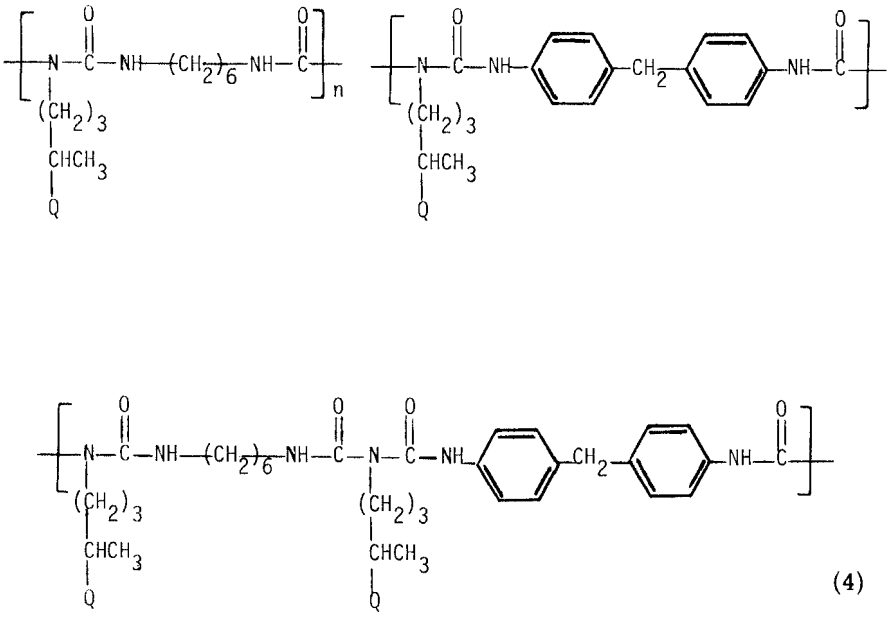


(4)

with protected primaquine, but free primaquine reacted with one mole of phenyl isocyanate to give the phenyl urea of the primary amino group.

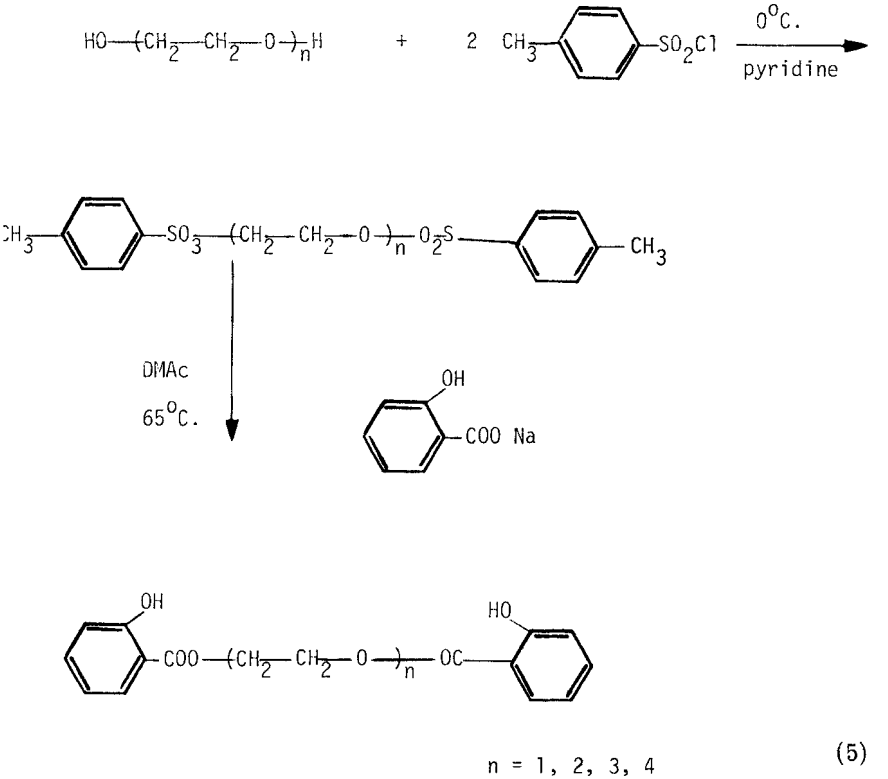
REACTIONS ON OLIGOMERS AND POLYMERS

Reactions on oligomers and polymers are qualitatively similar; however, solubility of the starting material and the final reaction product may require different reaction conditions. Sometimes in reactions of oligomers or polymers the reactive group must be further activated in order to guarantee that the desired reaction can be carried out under mild conditions. However, the preparation of a more reactive derivative often suffers from the normal problem of reactions of polymers in that side reactions prevent quantitative substitution.

Reactions which are carried out on both oligomers and high molecular weight polymers are essentially esterification reactions; the formation of an ester bond which could ultimately be hydrolyzed. Reactions on very low weight molecular material was carried out along the traditional lines for the preparation of N,N'-dimethyl-p-aminobenzoates with ethylene glycol and oligomeric poly(ethylene

glycols) [6, 12]. A sodium methoxide-catalyzed ester exchange reaction of oligo(oxyethylene) glycol with methyl N,N'-dimethyl-p-aminobenzoate in refluxing benzene was most efficiently used with molecular sieves to bind the methanol. Other reactions, namely the reaction of the glycols with N,N'-dimethyl-p-aminobenzoyl chloride in the presence of pyridine or the reaction of potassium N,N'-dimethyl-p-aminobenzoate in DMAc with the di-p-toluene sulfonates of the glycols or with 1,2-dibromoethane showed no particular advantages. These methods required that the terminal hydroxyl groups of the glycols were transformed into an activated group.

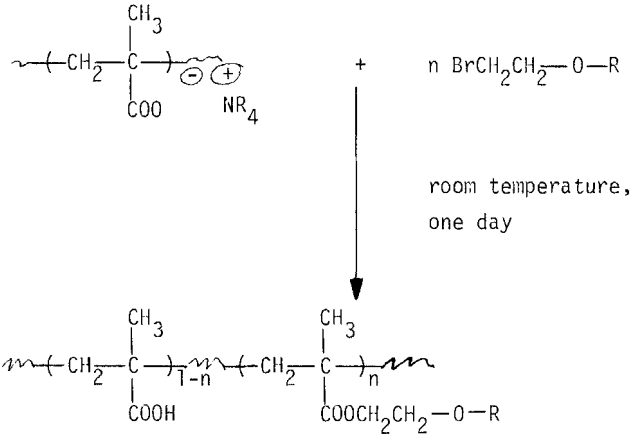
The disalicylates of ethylene glycol and oligo(oxyethylene) glycols were prepared by a displacement reaction of the di-p-toluene sulfonates in DMAc for 5 hr at 75°C or for 20 hr at 65°C with sodium salicylate [Eq. (5)]. The progress of these displacement reactions was studied carefully by ^{1}H-NMR spectroscopy.



n = 1, 2, 3, 4 (5)

Disalicylates of some oligo(oxyethylene) glycols could also be obtained by direct esterifications of the glycol with salicylic acid and p-toluene sulfonic acid as the catalyst in refluxing toluene. These substitutions were about twenty times slower than the previous reactions.

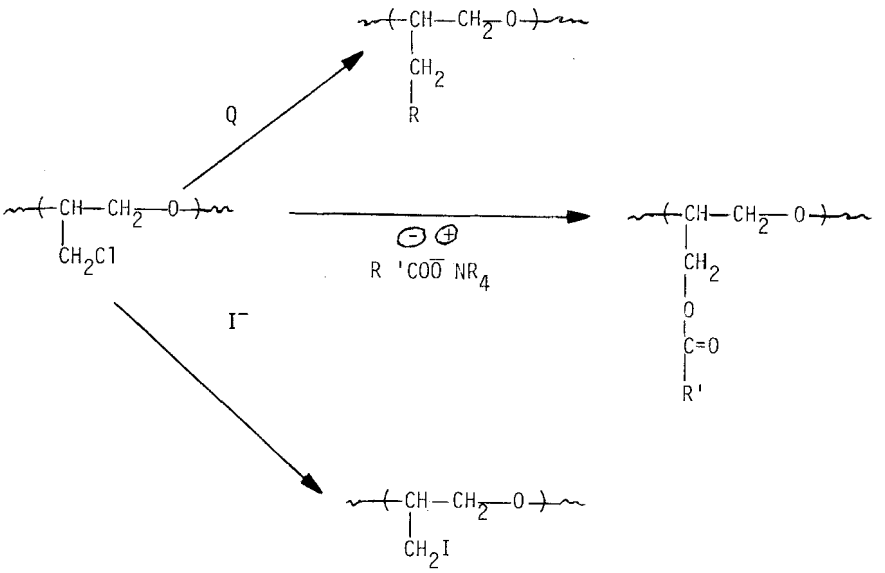
For the displacement reactions of reactive groups on polymers, one specific reaction, the reaction of tetraalkylammonium salts of carboxylic acids with aliphatic halides such as chlorides and bromides in DMAc [13], was investigated. This reaction had recently been developed for low molecular weight materials and has been shown to give very high degrees of substitution, e. g., poly(n-butyl methacrylate) could be readily obtained from the tetraethylammonium salt of poly-(methacrylic acid). Reactions have been carried out where the carboxylic acid function was part of the polymer chain as in the case of poly(methacrylic acid) tetraalkylammonium salts [Eq. (6)] and the



(6)

aliphatic halide was part of the functional group containing compound; the aliphatic halide could also be part of a polymer chain as in the case of polyepichlorohydrin which was reacted with nucleophiles where the nucleophile was a carboxylate, or an amino group which was a functional compound [Eq. (7)].

In order to increase the reactivity of the substrate polymers, we have also prepared polyepiiodohydrin at a degree of substitution of 93% by allowing polyepichlorohydrin to react with KI in 2-butanone [14] (the Finkelstein reaction). This reaction, however, was accompanied by a severe molecular weight reduction as the iodide anion was capable of causing displacement reaction on the ether oxygen link of the backbone chain. As a direct consequence, the DP of



(7)

polyepichlorohydrin was sharply reduced, particularly when the displacement reaction was carried out to a high conversion.

In the course of our study on polyepichlorohydrin displacement reactions, primaquine was also allowed to react with polyepichlorohydrin in DMSO and gave substitutions in accordance to the amount of primaquine added. Substitution degrees of 25, 50, and 100% could be achieved. The reaction of primaquine was entirely on the aliphatic amino group; no noticeable molecular weight reduction or crosslinking was observed under these conditions. It has become clear that the spacer inherent in the primaquine structure and the flexibility of the polyether chain contributed to the ease of substitution.

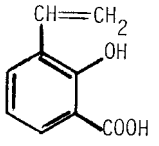
Displacement reactions on polyepichlorohydrin with tetraethylammonium N,N'-dimethyl-p-aminobenzoate were carried out in DMF at 55°C. After 1 day, more than 90% displacement of the chloride to the poly(glycidyl-N,N-dimethyl-p-aminobenzoate) was achieved. With DMSO at 50°C, a similar result has been obtained. It was, however, found that some insoluble material was present, possibly due to crosslinking by quaternization of the dimethylamino group of the N,N-dimethyl-p-aminobenzoate with unreacted epichlorohydrin units. When highly substituted, the polymer is apparently not as sensitive to quaternization; this crosslinking reaction was particularly observed upon drying of partially substituted epichlorohydrin units.

The use of the tetraalkylammonium salt rather than the alkali metal salt is critical, as the reactivity of the carboxylate is determined by the solvation of the specific tetraalkylammonium salt. Tetraalkylammonium salts with short alkyl groups gave low yields, and when the alkyl chain became too long and the tetraalkyl ammonium cation very hydrophobic, a reduced yield was obtained. The tetraethyl- and tetrabutylammonium salts seem to be solvated strongly by DMAc and optimum yields were obtained with these cations.

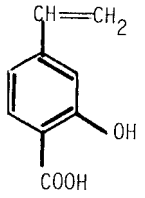
Poly(tetrabutylammonium methacrylate) was allowed to react with 4-(2-bromoethoxy)-2-hydroxybenzophenone at room temperature in DMAc in one day and a copolymer of methacrylic acid and 2-hydroxy-4-(2-methacryloxy ethoxy)-benzophenone which contained nearly 30 mole % of the hydroxybenzophenone unit was obtained [12]. Poly(tetrabutylammonium methacrylate) reacted with butyl bromide and gave pure poly(butyl methacrylate) which was identical with poly(n-butyl methacrylate) obtained by polymerization of butyl methacrylate, although the polymer obtained by reaction of the tetrabutylammonium salt of poly(methacrylic acid) had a very small amount of nitrogen left in the polymer. The displacement reaction on poly(tetrabutylammonium methacrylate) does not have to be carried out with completely dehydrated polymer, but the water content must be less than 1/2 mole per monomer unit.

PREPARATION AND POLYMERIZATION OF FUNCTIONAL MONOMERS

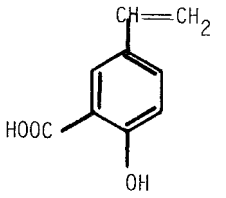
Vinylsalicylic acid derivatives have been chosen to demonstrate the usefulness of the preparation of functional monomers and their homo- and copolymerization as one of the approaches to functional polymers. Of the four isomeric vinylsalicylic acids, three are relatively easily prepared, the 3-vinyl-,4-vinyl-, and 5-vinylsalicylic acids. Salicylic acid derivatives have been known as useful UV absorbers, complexing agents and as drugs and it was expected that



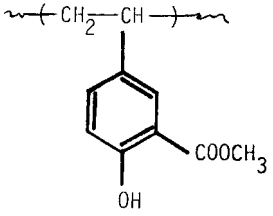
3-Vinylsalicylic acid



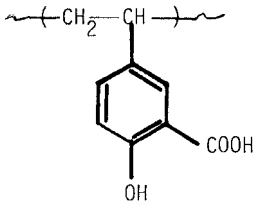
4-Vinylsalicylic acid



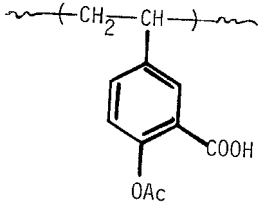
5-Vinylsalicylic acid



UV absorber



Complexing agent



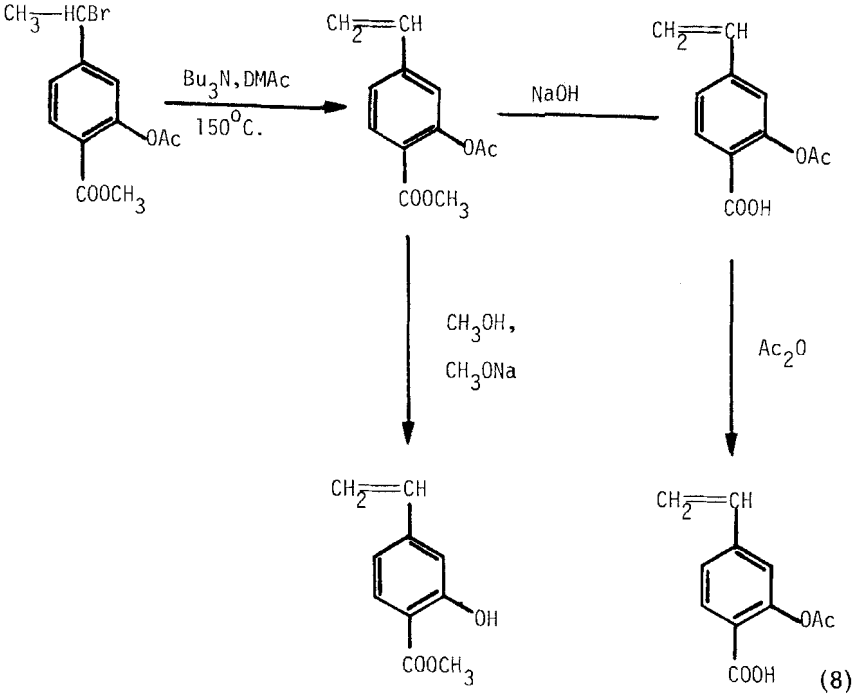
Polymeric drug

they would also be of interest in their polymeric form with possible enhanced usefulness.

Two isomeric vinylsalicylic acids were prepared by two different routes which are frequently used for the synthesis of styrenes. 4-Vinylsalicylic acid was prepared from ethyl phenol as the starting material; the last steps were bromination of the ethyl group followed by dehydrobromination. The 5-vinylsalicylic acid derivatives were prepared from methyl salicylate; the last step was dehydration of the secondary alcohol. The last route, the classic technique for the preparation of styrenes, gave purer monomer, as the purification of the 4-vinylsalicylic acid derivatives prepared by dehydrobromination was more cumbersome.

For the synthesis of 4-vinylsalicylic acid derivatives [15], 3-ethylphenol was carbonated by a modification of the Kolbe-Schmitt reaction at 1,000 psi and 175°C to give 4-ethylsalicylic acid in 80% yield. Crude 4-ethylsalicylic acid could be directly esterified with methanol and sulfuric acid and gave a 72% yield of methyl 4-ethylsalicylate. This product was acetylated with acetic anhydride and sulfuric acid to methyl 4-ethylacetylsalicylate in 97% yield. This acetylation was necessary as it had been found earlier in a related system that bromination of the methylene carbon atom of the side group does not occur unless the phenolic OH group is protected by an acetyl group, but ring bromination proceeds exclusively [16]. Benzylic bromination of methyl

4-ethylacetylsalicylate proceeded smoothly and gave the desired benzylic bromide in quantitative yields when a stoichiometric quantity or a slight access of N-bromosuccinimide was used. Methyl 4-(1-bromoethyl)acetylsalicylate was dehydrobrominated in 62% yield with tri-n-butylamine in DMAc at 150°C. Chromatography on acidic alumina followed by distillation was necessary to obtain a pure product. Methyl 4-vinylacetylsalicylate was converted to methyl 4-vinylsalicylate in over 80% yield by treatment with sodium methoxide in methanol. Methyl 4-vinylacetylsalicylate was saponified with aqueous sodium hydroxide to 4-vinylsalicylic acid which was obtained by sublimation in 60% yield as white needles with a melting point of 130°C [Eq. (8)]. Visual determination of the melting point of this compound (and that of 4-vinylacetylsalicylic acid) could not be carried out as polymerization occurred below the melting point of the sample. This behavior



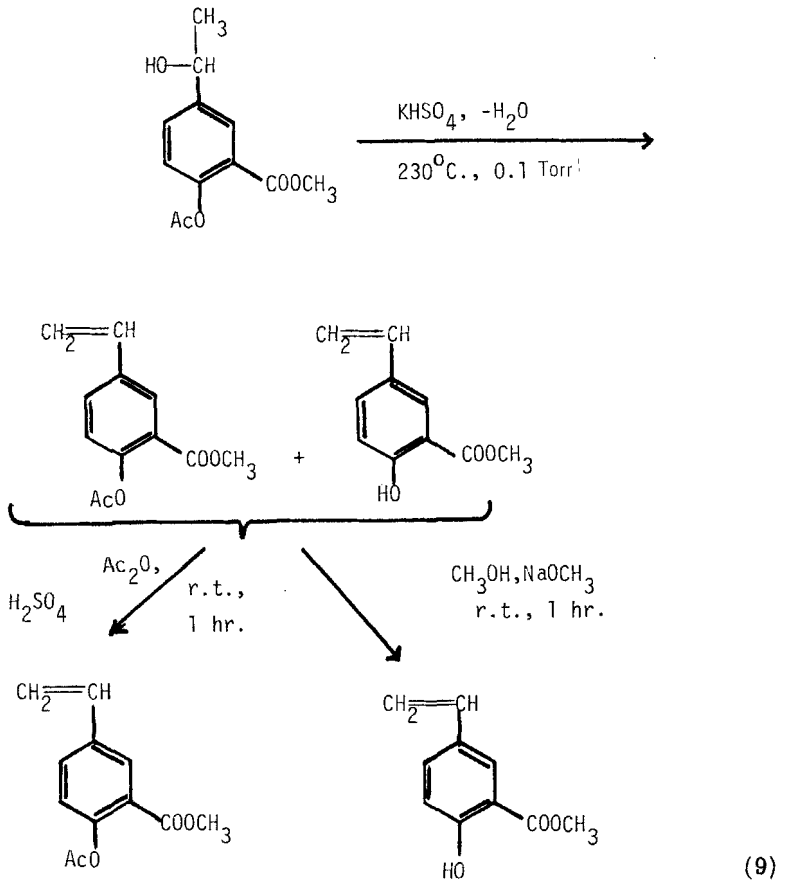
(8)

was confirmed by DSC studies of the melting and polymerization behavior of these compounds. 4-Vinylsalicylic acid could be acetylated in acetic anhydride and sulfuric acid to 4-vinylacetylsalicylic acid, melting point 124°C.

5-Vinylsalicylic acid derivatives were prepared [16-18] by a slightly different route. Methyl salicylate was acetylated with acetic anhydride and sulfuric acid to methyl acetylsalicylate which was rearranged to methyl 5-acetylsalicylate. The same compound could be directly obtained by Friedel-Crafts acetylation at lower temperatures which prevented the formation of the isomeric methyl 3-acetylsalicylate, a reaction product at higher temperatures. The acetyl group in the 5 position had to be reduced with sodium borohydride, but direct reduction formed insoluble salts. As a consequence, the phenolic -OH group was protected by acetylation, and methyl 5-acetylacetylsalicylate was then reduced in about 90% yield with sodium borohydride in ethanol to give methyl 5-(1-hydroxyethyl)acetylsalicylate. This compound was dehydrated by dropping it onto $KHSO_4$ at 222°C/0.1 Torr; this temperature and pressure combination guaranteed an essentially quantitative dehydration and quick removal of the vinyl compound [Eq. (9)]. Some material was lost during the operation, probably because polymerization occurred in the flask on the surface of the dehydrating agent. The material which distilled over was actually a mixture of methyl 5-vinylacetylsalicylate and methyl 5-vinylsalicylate; some hydrolysis apparently occurred during the dehydration. The ratio of the two compounds depends much on the geometry of the flask and the rate of addition and subsequent removal of the vinyl compounds. Typically a 2:1 ratio of these compounds has been obtained. The fact that a mixture of products was produced in this reaction was not a significant inconvenience as the mixture could be easily transformed into one or the other pure compounds.

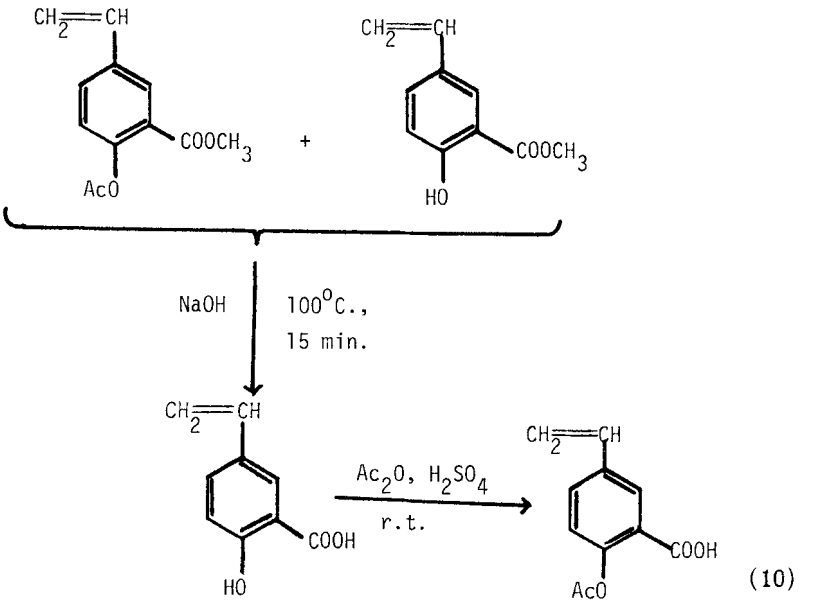
Acetylation with acetic anhydride and sulfuric acid gave methyl 5-vinylacetylsalicylate in good yield while treatment of the mixture in methanol with sodium methoxide gave an excellent yield of methyl 5-vinylsalicylate; both compounds can be easily purified by distillation. The mixture of methyl 5-vinylsalicylate and methyl 5-vinylacetylsalicylate obtained directly from the dehydration reaction could also be simply hydrolyzed with sodium hydroxide at 100°C and gave an essentially 100% yield of 5-vinylsalicylic acid [Eq. (10]. This product, however, was impure, as 5-vinylsalicylic acid polymerizes very readily; usually a yield of 50-60% of pure 5-vinylsalicylic acid can be isolated by sublimation. The remaining products were oligomers of 5-vinylsalicylic acid. 5-Vinylsalicylic acid was readily acetylated to 5-vinylacetylsalicylic acid.

Bulk polymerization of methyl 4-vinylsalicylate at 60°C with AIBN as initiator gave a 72% yield of poly(methyl 4-vinylsalicylate) of an inherent viscosity of 2.6 dl/g. Poly(4-vinylsalicylic acid) was obtained by solution polymerization of the monomer in DMF with AIBN [Eq. (11)]. The inherent viscosities of polymer obtained in a number of polymerizations ranged from 0.5 dl/g to 2.2 dl/g depending on the initiator concentration and was directly related to the initiator concentration. Some interference occurred in the polymerization in DMF



(9)

as the solvent, as in some samples nitrogen remained in the polymer. The polymers could however be purified by dissolving the polymers in aqueous sodium hydroxide and reprecipitation of the polymers by acidification. A complication arose when 4-vinylacetylsalicylic acid was polymerized in DMSO: a crosslinked gel, which could be dissolved in sodium hydroxide, was obtained [Eq. (12)]. When the polymerization was studied in more detail it was found that during the polymerization acetic acid was liberated indicating that crosslinking had occurred via polyester formation. As a consequence, the polymerization of 4-vinylacetylsalicylic acid was carried out in acetic acid as the solvent and gave in good yield a completely soluble polymer of 4-vinylacetylsalicylic acid.



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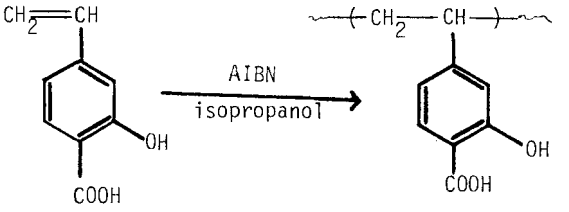
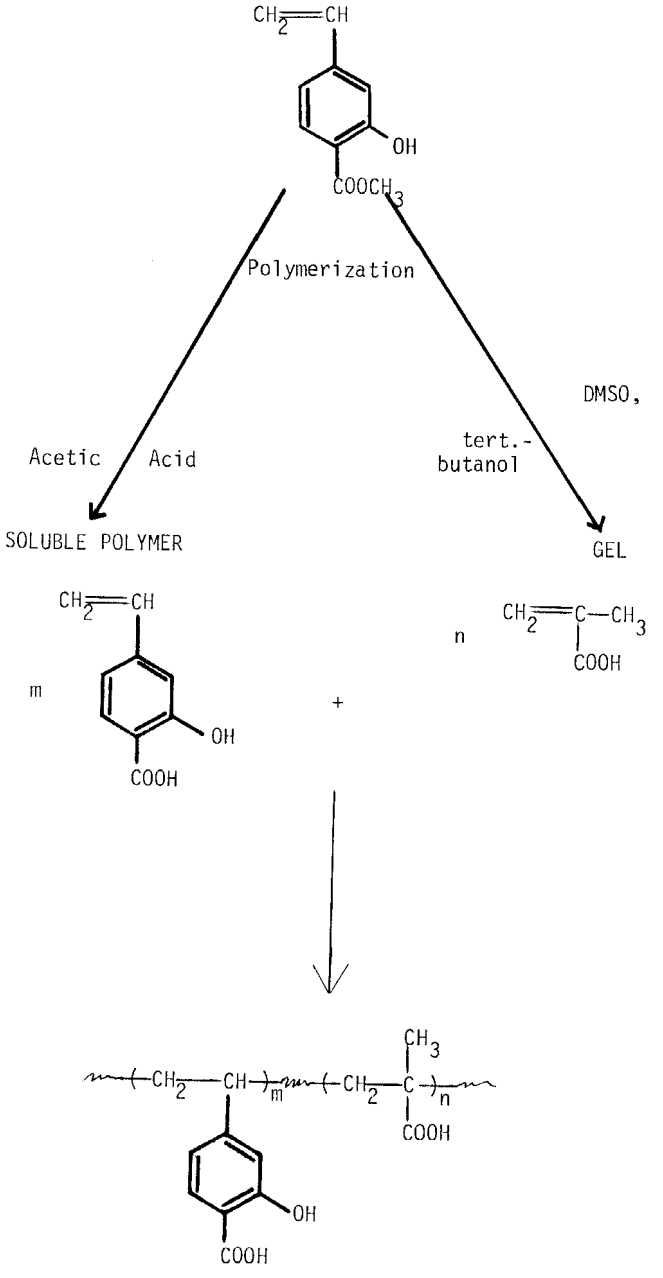


(11)

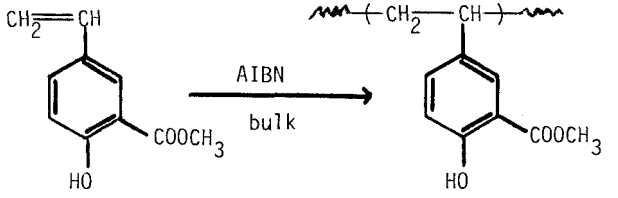
4-Vinylsalicylic acid was copolymerized with methacrylic acid in a feed ratio of about 1:1 and produced a copolymer in nearly 90% yield. The copolymer contained about a 2:1 molar ratio of methacrylic acid and 4-vinylsalicylic acid [Eq. (13)] and had an inherent viscosity of 1.73 dl/g after a day at 60°C in DMSO. As expected, poly(4-vinylsalicylic acid) was found to be an excellent complexing agent for copper (II) salts. When an aqueous solution of cupric acetate was added to an acetone solution of the polymeric acid, a light green polymer complex precipitated as the solutions were mixed and gave a compound which had 90% stoichiometric quantity of copper complexed. It has previously been found that suspensions of poly(5-acrylamidosalicylic acid) can complex essentially 100% of the theoretical amount of copper at a pH of 5.6.



(12)

(13)

Methyl 5-vinylsalicylate polymerized in bulk or in solution with AIBN as the initiator, to give polymers in nearly 80% yield with an inherent viscosity as high as 2.5 dl/g [Eqs. (14)-(17)] [17]. Methyl 5-vinylacetylsalicylate polymerized in benzene solution to poly(methyl 5-vinylacetylsalicylate) of somewhat lower molecular weight. 5-Vinylsalicylic acid was polymerized in isopropanol at 60°C for one day with AIBN and gave a nearly 80% yield of polymer with an inherent viscosity



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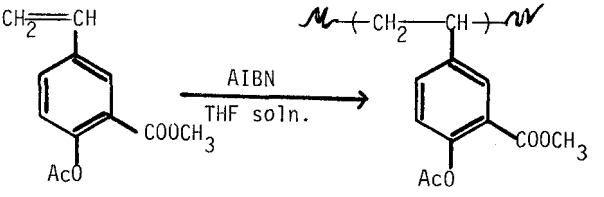

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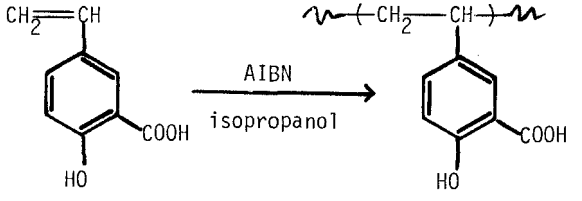

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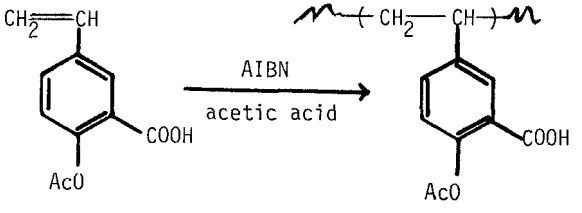

(17)

of 0.6 dl/g. Solvents for these polymerizations must be carefully selected in order to have appropriate solubility of the monomers as well as solubility of the corresponding polymers. Copolymerization of methacrylic acid with 12 mole % of 5-vinylsalicylic acid was successfully carried out in isopropanol and gave a polymer glass of high molecular weight. 5-Vinylacetylsalicylic acid in acetic acid gave a good yield of soluble poly(5-vinylacetylsalicylic acid).

Intermediates in the synthesis of the monomers, homopolymers, and copolymers of 4-vinylsalicylic acid and 5-vinylsalicylic acid were selected for preliminary testing of antibacterial activities against gram-positive and gram-negative bacteria [18].

ANTIBACTERIAL ACTIVITY OF VINYLSALICYLIC ACID DERIVATIVES

Twenty-three monomeric and polymeric derivatives of the vinylsalicylic acids prepared in our laboratory were subjected to preliminary studies of antibacterial activity through the courtesy of L. G. Donaruma of the New Mexico Institute of Mining and Technology. Activities against Escherichia coli and vs. Staphylococcus aureus were determined by the agar-plate test. In this test, the sample compound is applied to cultured bacteria, and, if the compound has antibacterial activity, a zone of growth inhibition results. The size of the zone of growth inhibition may be regarded as a qualitative measure of the antibacterial potency of the test substance. Results of these tests are summarized in Tables 1 and 2, in which the activity against each bacterial strain is listed for each of the active substances.

It is apparent from these data that many of the new vinylsalicylic acid derivatives exhibit substantial antibacterial activity, against both the gram-negative E. coli and the gram-positive S. aureus. The significance of testing against two bacterial strains stems from the differences in the structures of the cell walls of the strains. Since an antibacterial agent must first interact with the cell wall, it is important to understand the influence of cell wall structure on antibacterial activity.

If the data are examined more closely, several additional important conclusions may be reached. The first of these concerns the relative specificities of the monomeric and polymeric agents. If, for example, the activity of 5-vinylacetylsalicylic acid is compared with that of its homopolymer (Table 1), it is seen that the homopolymer shows specificity against E. coli, while the monomer is nonspecific. This may have implications in the design of new, specific antibacterial agents, but it also speaks directly to a possible criticism of these test results. Although all of the polymeric salicylic acid derivatives were carefully purified, the presence of unreacted

TABLE 1. Antibacterial Activity of 5-Vinylsalicylic Acid Derivatives

Compound	Antibacterial activity[a]	
	E. coli	S. aureus
5-Vinylsalicylic acid	XXX	XXX
5-Vinylacetylsalicylic acid	XXX	XXX
Poly(5-vinylsalicylic acid)		
η_{inh} = 0.1 dl/g	XX	XX
η_{inh} = 0.6 dl/g	XX	XX
Poly(5-vinylacetylsalicylic acid), η_{inh} = 0.5 dl/g	XXX	0
Methacrylic acid/5-vinylsalicylic acid copolymer (85/15), η_{inh} = 2.7 dl/g	XX	0

[a]XXX = very active; XX = active, X = slightly active, 0 = inactive.

TABLE 2. Antibacterial Activity of 4-Vinylsalicylic Acid Derivatives

Compound	Antibacterial activity[a]	
	E. coli	S. aureus
4-Ethylsalicylic acid	XXX	XXX
4-Vinylsalicylic acid	XXX	XXX
4-Vinylacetylsalicylic acid	X	XXX
Poly(4-vinylsalicylic acid), η_{inh} = 0.4-2.2 dl/g	X	X
Methacrylic acid/4-vinylsalicylic acid copolymer (65/35), η_{inh} = 1.7 dl/g	XXX	X

[a]XXX = very active; XX = active; X = slightly active; 0 = inactive.

monomer in these samples could not be ruled out. In view of the high potency of the monomers in these tests, it might be tempting to ascribe the activity of the polymers to a "leaching" of unreacted monomer. Should this be the case, however, the specificities of monomer and polymer must be the same, since one is actually observing the behavior of the monomer in each case. The observed differences in the specificity show that these polymeric materials do indeed exhibit significant antibacterial activity, independent of that of the monomer.

A second observation concerns the influence of molecular weight on antibacterial activity. Five samples of poly(4-vinylsalicylic acid) spanning a range of 0.4 to 2.2 dl/g in inherent viscosity were examined in these tests (Table 2). All of the polymers showed slight activity against both strains, but the activity was essentially independent of molecular weight. The same result was found for the homopolymers of 5-vinylsalicylic acid (Table 1).

Finally, these data allow some encouraging speculation concerning the possibility of controlling the biological activity of synthetic polymers through changes in polymer structure and properties. The data in Table 2 indicate that 4-vinylsalicylic acid is a potent, but nonspecific, antibacterial agent, and that homopolymerization of the compound still does not give a specific agent. However, if this same monomer is copolymerized with methacrylic acid, substantial specificity vs. E. coli is induced. The same observation may be made for the 5-vinylsalicylic acid derivatives in Table 1. Since the specificity of the copolymer differs from that of the monomer in each case, the activity of the copolymer cannot be ascribed to leaching of monomer. The possibility that the methacrylic acid units may be responsible for the activity may also be discarded, since copolymers of methyl 5-vinylsalicylate with methacrylic acid are inactive in these tests (data not shown). Thus it has been demonstrated that the specificity of a given biological agent may be altered through copolymerization with the appropriate comonomer. The implications of this observation in the design of new biological agents of high specificity are manifold.

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