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Functional Polymers. XXI. Activity of Low Molecular Weight and Polymeric Salicylic Acid Derivatives

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ABSTRACT

Several structural isomers of vinylsalicylic acid and the corresponding methyl vinylsalicylates have been synthesized in this laboratory previously and have been polymerized and copolymerized with methacrylic acid and with methyl methacrylate. The monomeric as well as the polymeric compounds have been tested for their antimicrobial and cytotoxic properties. This article also deals with the sunscreening properties of some polymeric salicylic acid derivatives and their possible use as the effective ultraviolet-absorbing agent in skin protection formulations. The results show that making homopolymers and especially copolymers is a way of increasing one property and decreasing another, making new compounds with more specific properties.

INTRODUCTION

In recent years, increasing attention has been paid to the role of polymers as drugs [1, 2]. Of particular interest has been the antimicrobial activity of polymers [1, 3]. This paper deals with the antimicrobial and cytotoxic properties of salicylic acid (SA), vinylsalicylic acids, and polymers and copolymers obtained from the latter. Several structural isomers of vinylsalicylic acid (VSA) and their derivatives, especially the methyl vinylsalicylates, have recently been synthesized in this laboratory and have been polymerized and copolymerized with methacrylic acid (MAA) and with methyl methacrylate (MMA) [4-8]. The monomers as well as the polymers have now been tested for their antimicrobial and cytotoxic properties.

Photoinduced reactions have been studied extensively and their effect in plastics was reviewed recently [9]. The interaction of UV radiation with pigments of the human skin and consequent physiological changes were also recently reviewed [10]; it was noted that such interactions can be harmful and therefore sunscreens have been and are being developed. Compounds suitable for this purpose must have high extinction coefficients in the appropriate range of the electromagnetic spectrum (290 to 320 nm), be stable against photodegradation, and must be compatible with the intended application. Among the most successfully used sunscreen compounds are derivatives of p-aminobenzoic acid, SA, and 2,4-dihydroxybenzophenone (DHBP). This article deals with the sunscreening properties of some polymeric salicylic acid derivatives and their possible use as the effective ultraviolet-absorbing agent in skin protection formulations. The requirements placed on such agents are not only that they are efficient absorbers in the erythema range (290 to 320 nm), but also that they have a certain degree of permanence on the skin, a feature associated with the solubility and skin-binding properties of a compound. Additionally, of course, the compounds must not be toxic in the mode of application. We [11] have

discussed these points in detail and suggest that the use of polymeric, relatively high molecular weight compounds as skin protective sunscreens might have several advantages over low molecular compounds. Permanence on the surface of the skin and skin-binding properties might especially be better. At the same time, due to their polymeric nature, the possibility for skin penetration by such compounds is reduced and thus the chance for the occurrence of undesirable toxic and/or allergic reactions is diminished.

EXPERIMENTAL PART

Materials

Monomers and polymers derivatives of vinylsalicylic acid have been synthesized in our laboratory. The monomers include salicylic acid (SA), 3-vinylsalicylic acid (3VSA), 4-vinylsalicylic acid (4VSA), 5-vinylsalicylic acid (5BSA), 3-ethylsalicylic acid (3ESA), 4-ethylethylsalicylic acid (4ESA), 3-ethylacetylsalicylic acid (3EASA), methyl 3-ethyl salicylate (3ESAM), methyl 4-ethyl salicylate (4ESAM), 5-vinylacetylsalicylic acid (5VASA), and 4-vinylacetylsalicylic acid (4VASA). The homopolymers are poly(3-vinylsalicylic acid) (p3VSA), poly(4-vinylsalicylic acid) (p4VSA), poly(5-vinylsalicylic acid) (p5VSA), and poly(5-vinylacetylsalicylic acid) (p5VASA). The copolymers are methacrylic acid/3-vinylsalicylic acid copolymer (MAA/3VSA), methacrylic acid/4-vinylsalicylic acid copolymer (MAA/4VSA), methacrylic acid/5-vinylsalicylic acid copolymer (MAA/5VSA), methacrylic acid/3-vinylmethylsalicylate copolymer (MAA/3VSAM), methyl methacrylate/3-vinylsalicylic acid copolymer (MMA/3VSA), and methyl methacrylate/3-vinylmethylsalicylate copolymer (MMA/3VSAM). For comparison, three commercial sunscreen formulations were tested, namely, 2-hydroxy-4-methoxy-5-sulfobenzophenone (SBP), SPECRASORB #284 (American Cyanamide Co., Ltd.); 2,4-dihydroxybenzophenone (DHBP), Uvinol 400 (General Aniline and Film Corp.); and phenyl salicylate (PSA), pure grade reagent (Tokyo Kasei Kogyo Co., Ltd.).

Measurements

Cidal Concentration for Microbial Activity

A. Preparation of Sample Solution. All tested compounds were dissolved in ethanol except p3VSA which was insoluble in H_2O methanol, ethanol, DMF, DMSO, and acetone; therefore, p3VSA was suspended in water for testing. SA dissolved in ethanol or suspended in water was used as the control for comparison.

B. Media. For the biocide/organism culture, nutrient broth (Difco Labs.) was prepared and sterilized before innoculation with

Staphylococcus aureus (S. aureus) (gram positive bacteria) and Pseudomonas aeruginosa (P. aeruginosa) (gram negative bacteria). Mineral salts with dextrose solution were used for the growth of Aspergillus niger (A. niger) (fungus). (3 g NH_4NO_3, 2 g KH_2PO_4, 0.05 g $MgSO_4/H_2O$, 0.5 g KCl, 10 g dextrose, 15 g agar, 1000 g H_2O). For the plate counts of viable cells, Trypticase Soy Agar c Lethicin and Polysorbate 80 (preservative inhibitor) were used for the bacteria (S aureus and P. aeruginosa) and Sabour and Dextrose agar for the fungi (A. niger).

C. Experimental Methods. Stock solutions from the dissolved salicylic acid derivatives were prepared and appropriate amounts transferred to sterilized test tubes. 0.5 mL of a 24-h stock culture of each bacterium was pipetted into 500 mL of sterile nutrient broth. 10 mL of this diluted culture broth was immediately transferred to each of the dilution test tubes. For the fungi, 10 mL of the test media was poured into an agar slant with growing A. niger. The growing fungus was loosened with a sterile swab and the broth was poured back into the flask containing the test media. The average of the inoculum size was for S. aureus 5.4×10^5 bacteria for P. aeruginosa 6.3×10^6 bacteria, and for A. niger 5×10^4 fungi. Following inoculation, the tubes were incubated at 37°C. At 1 h, 6 h, 24 h, 48 h, and 1 week, plate counts were taken for the polymeric and low molecular weight SA derivatives. A 0.1-mL sample from each test tube was placed in a Petri dish where liquid agar was added (TSALP and Saboraurd's). The plates were then incubated and examined for growth. For the low molecular weight group, growth was observed visually after 24 h and plate counts were taken at 48 h.

The tubes were reincubated with the appropriate organism at Day 6 and a final evaluation was determined after 1 week for the polymeric and low molecular weight SA derivatives.

Growth Inhibition Tests

An agar-plate test was performed on some compounds by placing a small amount of dry polymer on an agar plate previously innoculated with S. aureus and Escherichia coli (E. coli). If the compound had antibacterial activity, a zone of growth inhibition resulted. The size of the zone of growth inhibition was regarded as a qualitative measure of the antibacterial potency of the test substance.

Cytotoxic Tests

A. Preparation of Sample Solution. In a test tube, 10 mg of a sample was dissolved in 0.5 mL of DMSO, to which 0.5 mL of water was added. The mixture in a stoppered test tube was autoclaved at 105°C for 5 min, to which 4.0 mL of conc Eagle MEM solution (the concentration was twice as high as the regular solution) was added. Then the solution was brought to pH 7.2 with aq $NaHCO_3$, and then diluted with water to a volume of 10 mL. The standard Eagle MEM solution containing 1000 ppm of the sample was thus prepared, which was

further diluted with Eagle MEM solution (containing 5% DMSO) to provide test solutions having concentrations of 500, 250, 100, and 50 ppm of the sample.

B. Cell Strain. The cell strain used in the present test was the normal human skin origin XX-male (JTC-17). The growth medium was an Eagle MEM solution containing 20% (v/v) calf serum.

C. Experimental Methods. The proliferating cell was treated with trypsin to prepare a single cell suspension, which was diluted with growth medium to a concentration of 1×10^3 cell/mL. Then 1 mL of the diluted suspension was placed in a Petri dish (52 mm, disposable dish of plastics), to which 5 mL of growth medium was added and incubated in a CO_2 incubator (5% CO_2 in air) at 37°C for 2 d.

After 2-d cultivation, the medium of each dish was removed and the attached cells were washed twice with Eagle MEM solution to which 2.0 mL of the sample solution of sunscreening agent was added. Then the cells were incubated at 37°C in a CO_2 incubator for 1 h. Finally, from each dish a sample solution was removed and incubated with 5 mL fresh growth medium for 7 d. After the final incubation, the cell was fixed by 10% neutral formalin solution and stained with Giemsa solution. The number of colonies was counted.

The cytotoxicity was expressed by the relative plating efficiency (RPE) which was given as follows:

$$\text{RPE} = \frac{\text{no. of colonies with agent}}{\text{no. of colonies without agent}} \times 100$$

(A reference experiment was carried out in the same way without the addition of the agent.)

Light Stability Tests

Light stability was tested by determining the optical density of the compounds in solutions of DMSO (0.004 or 0.02% w/v) before and after irradiation. A 1-kW xenon lamp (Type VX2-1000 HK-0) fitted with a filter (Toshiba UV-29) was used as the light source. The solutions were placed in quartz test tubes (d = 8 mm, ϕ = 120 mm) and irradiated for 6 h at a dose rate of 619 erg/cm^2·s (290 to 320 nm). The dose rate was determined with an Optical Radiation Measurement System 740 A, Optronic Laboratories, Inc., U.S.A. A Shimadzu double-beam spectrophotometer model UV-210A, Shimadzu Seisakusho Ltd., was used to measure the optical densities. Measurements were recorded before irradiation and after 4 and 6 h of irradiation.

Sunscreening Efficiency Tests

To test for sunscreening efficiency, the absorption spectra of the samples in solutions of DMSO (0.0004 or 0.02% w/v) were recorded. The spectra were measured with a Shimadzu UV-V1S Digital Double

Beam Spectrophotometer UV-210A. The optical density A at 290 to 320 nm, the erythema range, was used to evaluate sunscreening efficiency. It was then calculated for a theoretical concentration of 1% w/v at λ_{max} (A 1% (λ_{max})). Additionally, the average value of the optical density over the range of 290 to 320 nm was also calculated and tabulated ($\bar{A}$ 1% ($\lambda_{290-320}$)). For these calculations the optical densities were determined at 10 nm intervals between 290 and 320 nm, and averaged.

RESULTS AND DISCUSSION

Toxicity

The antimicrobial activities of the polymeric and low molecular weight salicylic acid derivatives are shown in Tables 1 and 2. All monomeric compounds, including SA, required a high minimum concentration to provide cidal activity. SA was best at 333 ppm. Also, the polymeric compounds of salicylic acid derivatives required a high minimum concentration to provide ideal activity, but the results indicated that the polymers retained biocidal activity. In some cases they were more active than the vinyl monomer or its ethyl precursors. The polymers were also more specific in their activity. p5VSA has more cidal activity against S. aureus than against P. aeruginosa or A. niger. p4VSA is more active against P. aeruginosa as compared to the other two microorganisms, and is more active than either 4VSA or 4ESA. p3VSA was not soluble in any solvent we tried, and thus the results observed, obtained from water dispersions of p3VSA, are not exactly comparable with the vinyl monomers and ethyl precursors.

Tables 1 and 2 also show the differences in activities of the compounds in the growth inhibition zone test. Most of the compounds were more cidaly effective toward bacteria than toward fungi. The vinylsalicylic acid derivatives were more effective than the ethyl salicylic acid derivatives against E. coli. 3VSA seems to be most effective, in general. The homopolymers had no growth inhibition zone except p5VSA, perhaps because of low solubility. The copolymers, on the other hand, showed activity to special bacterias but not to fungi. MAA/5VSA and MAA/3VSA were very effective against P. aeurginosa, more than any of the monomers or their ethyl precursors, and also against S. aureus, but not at all against E. coli.

Tables 1 and 2 give, as a comparison, earlier results from tests of antibacterial activity [7]. These results showed more activity of the homopolymers. Of special interest is p5VASA which was very active against S. aureus but inactive against E. coli. The copolymer MAA/5VSA showed similar results. On the other hand, the monomers were active to both bacteria and fungi.

TABLE 1. Antimicrobial Activity of Vinyl- and Ethylsalicylic Acid Derivatives[a]

Compound	Conc (ppm) of cidal compound			Growth inhibition zone (mm)				Antibacterial activity[b]	
	S.a.	P.a.	A.n.	S.a.	E.c.	P.a.	A.f.	S.a.	E.c.
Salicylic acid	300	300	300						
5-Vinylsalicylic acid	>1000	1000	1000	4	6	3	2	XXX	XXX
5-Vinylacetylsalicylic acid								XXX	XXX
4-Vinylsalicylic acid	>1000	1000	>1000	3	6	3	2	XXX	XXX
4-Vinylacetylsalicylic acid								X	XXX
4-Ethylsalicylic acid	>1000	>1000	>1000	3	2	5	4	XXX	XXX
3-Vinylsalicylic acid	>1000	1000	>1000	4	4	7	4		
3-Ethylsalicylic acid	>1000	1000	1000	0	2	4	2		
3-Ethylacetylsalicylic acid				3	2	5	1		

[a] S.a. = Staphylococcus aureus
P.a. = Pseudomonas aeuroginosa
E.c. = Escherichia coli
A.n. = Aspergillus niger
A.f. = Aspergillus falvus

[b] XXX = very active, XX = active, X = highly active, 0 = inactive.

TABLE 2. Antimicrobial Activity of Polymers of Salicylic Acid Derivatives[a]

Compound	Conc (ppm) of cidal compounds			Growth inhibition zone (mm)				Antibacterial activity	
	S.a.	P.a.	A.n.	S.a.	E.c.	P.a.	A.f.	S.a.	E.c.
Poly(5-vinylsalicylic acid)	700	>1000	>1000	0	0	1	0	XX	XX
Poly(5-vinylacetyl-salicylic acid)								XXX	0
Poly(4-vinylsalicylic acid)	900	600	>1000	0	0	0	0	X	X
Poly(3-vinylsalicylic acid)				0	0	0	0		
Methacrylic acid/5-vinylsalicylic acid copolymer (85/15)				3	0	8	0	XX	0
Methacrylic acid/4-vinylsalicylic acid copolymer (65/35)								XXX	X
Methacrylic acid/3-vinylsalicylic acid copolymer (96/4)				5	0	10	0		

[a]See Table 1 for keys to abbreviations and symbols.

The observed differences in the specificity show that these polymeric materials indeed exhibited significant antibacterial activity, independent of the monomer.

Tables 3 and 4 and Figs. 1 and 2 give the results from the cytotoxicity tests.

Homopolymers showed toxicity at a concentration as low as 100 ppm whereas a copolymer (MMA/3VSA) was almost nontoxic at a concentration as high as 1000 ppm. The toxicities of copolymers were at the same level as those of conventional sunscreening agents in commercial cosmetic products. The toxicities of monomers lie between those of homopolymers and copolymers. The toxicity values (RPE) of monomer agents, however, may have been underestimated because of their low solubilities.

The cytotoxicities of sunscreening agents were tested in two series of experiments, and the two series showed some differences. The most obvious difference is for p5VSA. It was less toxic in the first run but here a shrinkage of the cytoplasm was observed.

Table 5 shows the difference in activity based on the physical state. If the SA was dissolved in ethanol before innoculation, it was active against both bacteria and fungi, but if a water suspension was used, the activity decreased dramatically due to lack of solubility. There is undoubtedly a need for optimization of the right conditions for the application of our samples, and it is expected that their activity could be increased.

Table 6 demonstrates the time dependence of the bacteriocidal activity of p5VSA and compares it to SA. Up to 24 h cidal activity was increasingly observed with p5VSA, after which dramatic bacteria growth was observed. SA retained cidal activity throughout the testing time frame.

Light Stability Tests

With the exception of p5VSA, all of the polymeric compounds retained between 85 and 90% of their original optical density after 6 h of irradiation (see Tables 7 and 8). p5VSA was only partly soluble in DMSO and retained only about 75% of its original optical density under these conditions. The monomeric precursor compounds 3ESAM and 4ESAM were less stable toward UV light and retained only 70 and 60% of their respective optical densities after 6 h of irradiation. Incorporation of the light-absorbing SA derivatives into a polymeric chain improved their light stability considerably. In the range tested, all the polymeric absorbers with the exception of p5VSA had light stabilities which are comparable to those of the commercially used products. After irradiation times of more than 6 h, all the samples showed some degree of discoloration. Figures 3, 4, and 5 show the changes of UV spectra of the samples during irradiation. It is obvious from Fig. 3 that the monomer 3ESAM showed a greater change during irradiation than the homopolymer p3VSA (Fig. 4) or the copolymer MAA/3VSA (Fig. 5).

TABLE 3. Cytotoxic Test of Low Molecular Weight Compounds

	RPE values[a]				
Compound/ppm	50	100	250	500	1000
Methyl 3-ethylsalicylate		75 ± 2.8	43 ± 2.1	26 ± 0.7	<0.15
Methyl 4-ethylsalicylate	74 ± 2.8	68 ± 0	76 ± 0	16 ± 2.8	
2-Hydroxy-4-methoxy-5, 5-sulfobenzophenone			80 ± 21	81 ± 27.6	94 ± 1.4
			89 ± 7.1	87 ± 4.2	82 ± 4.2
2,4-Dihydroxybenzophenone	84 ± 5.7	84 ± 19.1	83 ± 0.7		

[a] $\text{RPE} = \frac{\text{no. of colonies with agent}}{\text{no. of colonies without agent}} \times 100$

TEST 4. Cytotoxic Test of Polymer Compounds

Compound/ppm	RPE values				
	50	100	250	500	1000
Poly(5-vinylsalicylic acid)		47 ± 0.7	42 ± 9.2	81 ± 1.4	<0.25
	18 ± 1.4	20 ± 6.4	16 ± 1.4	11 ± 2.1	
Poly(4-vinylsalicylic acid)	35 ± 15.6	12 ± 4.2	3.2 ± 1.4		<0.13
Poly(3-vinylsalicylic acid)		47 ± 0	2.7 ± 0.7	<0.15	<0.15
Methacrylic acid/5-vinylsalicylic acid copolymer (85/15)		96 ± 16.3	101 ± 1.4	70 ± 4.9	23 ± 2.8
Methacrylic acid/3-vinylsalicylic acid copolymer (96/4)		72 ± 12.7	79 ± 6.4	79 ± 7.1	86 ± 1.3
Methacrylic acid/3-vinylmethyl-salicylic copolymer (86/14)		85 ± 4.2	93 ± 7.1	25 ± 2.1	0.7 ± 0

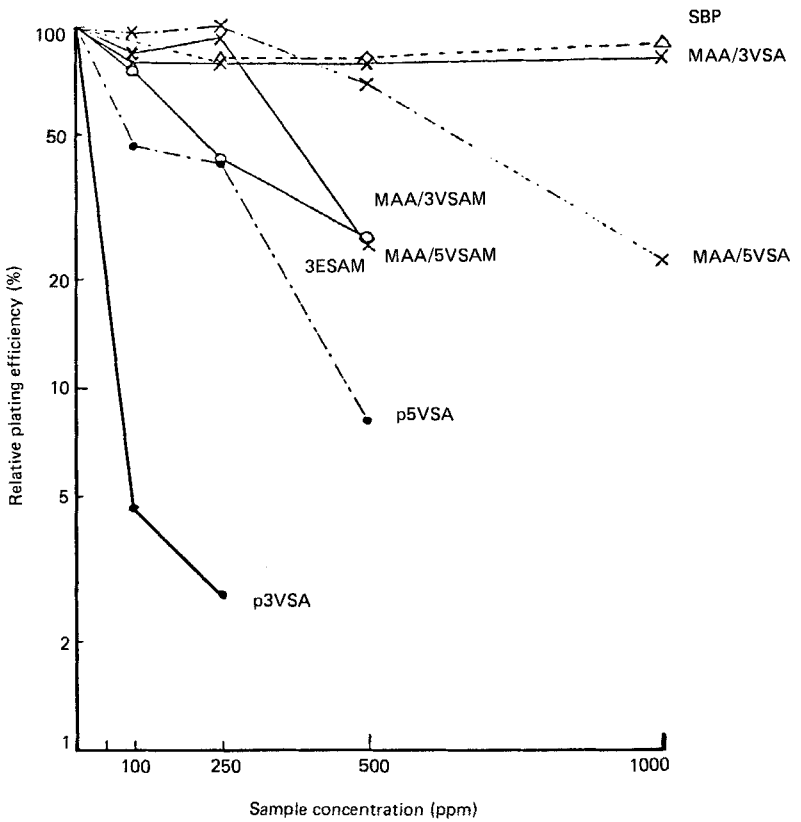

FIG. 1. Cytotoxicities of monomers methyl 3-ethylsalicylate (3ESAM), 2-hydroxy-4-methoxy-5-sulfobenzophenone (SBP); the homopolymers poly(5-vinylsalicylic acid (p5VSA), poly(3-vinylsalicylic acid) (p3VSA); the copolymers methacrylic acid/5-vinylsalicylic acid (MAA/5VSA), methacrylic acid/3-vinylsalicylic acid (MAA/3VSA), methacrylic acid/methyl 5-vinylsalicylic acid (MAA/5VSAM), and methacrylic acid/methyl 3-vinylsalicylic acid (MAA/3VSAM) (RPE values).

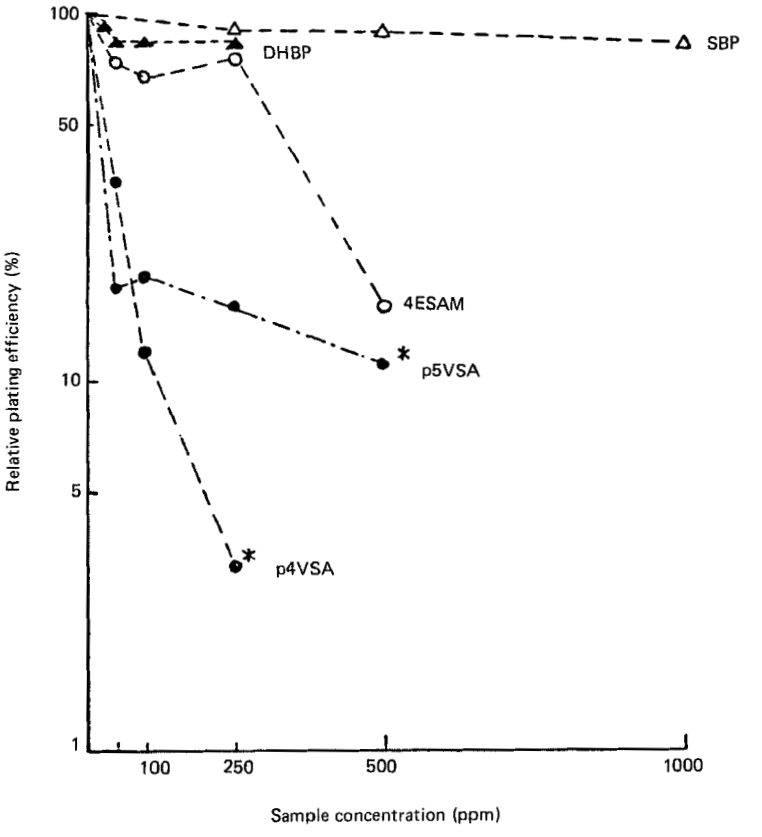

FIG. 2. Cytotoxicities of the monomers methyl 4-ethylsalicylate (4ESAM), 2-hydroxy-4-methoxy-5-sulfobenzophenone (SBP), and 2,4-dihydroxybenzophenone (DHBP); and the homopolymers poly(5-vinylsalicylic acid (p5VSA) and poly(4-vinylsalicylic acid) (p4VSA) (RPE values).

TABLE 5. Organisms Remaining after Contact with Salicylic Acid. A Comparison between Salicylic Acid, Ethanol Predissolved Sample Compared to a Direct Water Dispersion[a]

Ethanol predissolved						Water dispersion				
Final conc (ppm)	1 h	6 h	24 h	48 h	1 w	Final conc (ppm)	1 h	4 h	24 h	1 w
				Staphylococcus aureus						
900	0	0	0	0	0	830	TNTC	TNTC	TNTC	TNTC
600	25	0	0	0	0	553	TNTC	TNTC	TNTC	TNTC
300	TNTC*	TNTC	0	0	0	277	TNTC	TNTC	TNTC	TNTC
150	TNTC	TNTC	TNTC	0	0	173	TNTC	TNTC	TNTC	TNTC
90	TNTC	TNTC	TNTC	TNTC	TNTC	104	TNTC	TNTC	TNTC	TNTC
60	TNTC	TNTC	TNTC	TNTC	TNTC	69.2	TNTC	TNTC	TNTC	TNTC
30	TNTC	TNTC	TNTC	TNTC	TNTC	35	TNTC	TNTC	TNTC	TNTC

				Pseudomonas aeruginosa						
900	25	0	0	0	0	830	TNTC	TNTC	TNTC	TNTC
600	175	TNTC	0	0	0	553	TNTC	TNTC	TNTC	TNTC
300	TNTC	TNTC	TNTC	TNTC	TNTC	277	TNTC	TNTC	TNTC	TNTC
150	TNTC	TNTC	TNTC	TNTC	TNTC	173	TNTC	TNTC	TNTC	TNTC
90	TNTC	TNTC	TNTC	TNTC	TNTC	104	TNTC	TNTC	TNTC	TNTC
60	TNTC	TNTC	TNTC	TNTC	TNTC	69.2	TNTC	TNTC	TNTC	TNTC
30	TNTC	TNTC	TNTC	TNTC	TNTC	35	TNTC	TNTC	TNTC	TNTC
				Aspergillus niger						
900	0	0	0	0	0	830	TNTC	TNTC	TNTC	TNTC
600	0	0	0	0	0	553	0	0	0	0
300	0	0	0	0	0	277	87	53	TNTC	TNTC
150	TNTC	TNTC	14	2	TNTC	173	TNTC	TNTC	TNTC	TNTC
90	TNTC	TNTC	TNTC	TNTC	TNTC	104	TNTC	TNTC	TNTC	TNTC
60	TNTC	TNTC	TNTC	TNTC	TNTC	69.2	TNTC	TNTC	TNTC	TNTC
30	TNTC	TNTC	TNTC	TNTC	TNTC	35	TNTC	TNTC	TNTC	TNTC

TABLE 6. The Time Dependence in Activity. Organisms Remaining after Contact with Poly(5-vinylsalicylic acid) Compared to Salicylic Acid

Poly(5-vinylsalicylic acid)						Salicylic acid					
Final conc (ppm)	1 h	6 h	24 h	48 h	1 w	Final conc (ppm)	1 h	6 h	24 h	48 h	1 w
					Staphylococcus aureus						
1000	TNTC	45	0	0	0	1000	0	0	0	0	0
667	TNTC	75	3	0	TNTC	667	0	0	0	0	0
333	TNTC	450	400	TNTC	TNTC	333	TNTC	TNTC	22	0	0
167	TNTC	TNTC	45	TNTC	TNTC	167	TNTC	TNTC	TNTC	TNTC	TNTC
100	TNTC	TNTC	TNTC	TNTC	TNTC	100	TNTC	TNTC	TNTC	TNTC	TNTC

Pseudomonas aeruginosa

1000	9	11	14	TNTC	TNTC	1000	0	0	0	0	0
667	105	50	500	TNTC	TNTC	667	0	0	0	0	0
333	TNTC	TNTC	TNTC	TNTC	TNTC	333	0	0	0	0	0
167	TNTC	TNTC	TNTC	TNTC	TNTC	167	TNTC	TNTC	TNTC	TNTC	TNTC
100	TNTC	TNTC	TNTC	TNTC	TNTC	100	TNTC	TNTC	TNTC	TNTC	TNTC

Aspergillus niger

1000	TNTC	TNTC	TNTC	TNTC	TNTC	1000	0	0	0	0	0
667	TNTC	TNTC	TNTC	TNTC	TNTC	667	0	0	0	0	TNTC
333	TNTC	TNTC	TNTC	TNTC	TNTC	333	10	0	0	0	TNTC
167	TNTC	TNTC	TNTC	TNTC	TNTC	167	TNTC	100	6	TNTC	TNTC
100	TNTC	TNTC	TNTC	TNTC	TNTC	100	TNTC	TNTC	TNTC	TNTC	TNTC

TABLE 7. Light Stabilities of Monomeric and Polymeric UV Absorbers

Sunscreening agents	Optical densities of 0.004% DMSO solution[a]		
	Original (before irradiation)	After 4 h irradiation	After 6 h irradiation
Methyl 3-ethylsalicylate	1.03	0.80 (0.78)	0.71 (0.69)
Methyl 4-ethylsalicylate	1.03	0.68 (0.66)	0.59 (0.57)
2-Hydroxy-4-methoxy-5-sulfobenzophenone	1.39	1.24 (0.89)	1.20[c] (0.86)
2,4-Dihydroxybenzophenone	2.40	2.30 (0.96)	2.20[c] (0.92)
Poly(5-vinylsalicylic acid)[b]	0.60	0.49 (0.82)	0.44 (0.73)
Poly(4-vinylsalicylic acid)	1.03	0.92 (0.89)	0.89 (0.86)
Poly(3-vinylsalicylic acid)	0.98	0.90 (0.92)	0.86 (0.88)

[a]The values in parentheses are based on the ratios of the optical densities after irradiation divided by the original optical density (Column 1) of the respective samples.

[b]This sample was partly insoluble in DMSO.

[c]These data were taken after 8 h irradiation.

TABLE 8. Light Stabilities of Copolymeric UV Absorbers[a]

Sunscreening agents	Optical Densities of 0.02% DMSO solution		
	Original (before irradiation)	After 4 h irradiation	After 6 h irradiation
Methacrylic acid/5-vinylsalicylic acid copolymer (85/15)	0.87	0.80 (0.92)	0.76 (0.87)
Methacrylic acid/3-vinylsalicylic acid copolymer (96/4)	1.10	0.99 (0.90)	0.94 (0.87)
Methacrylic acid/methyl 5-vinylsalicylic acid copolymer (80/20)	1.62	1.55 (0.96)	1.42 (0.88)
Methacrylic acid/methyl 3-vinylsalicylate copolymer (88/12)	1.18	1.08 (0.92)	1.04 (0.88)
Methyl methacrylate/3-vinylsalicylic acid copolymer (86/14)	1.18	1.10 (0.93)	1.06 (0.90)
Methyl methacrylate/methyl-3-vinylsalicylate copolymer (79/21)	1.57	1.44 (0.92)	1.40 (0.90)

[a]The values in parentheses are based on the ratios of the original optical density after irradiation divided by the original optical densities of the respective samples.

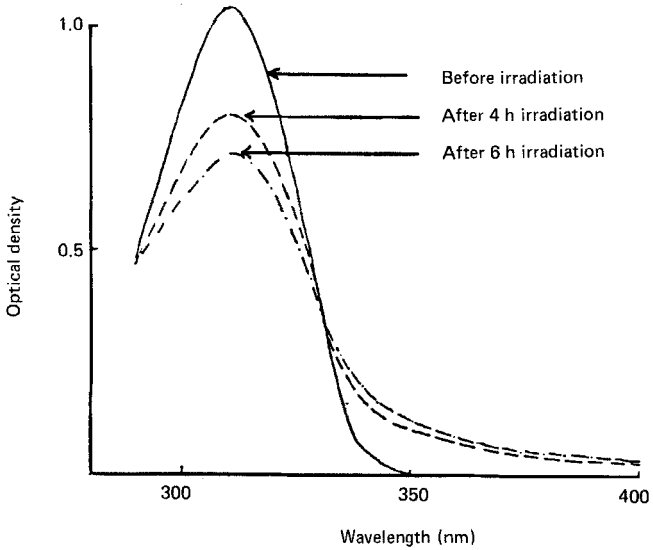

FIG. 3. The change of UV spectra of methyl 3-ethylsalicylate (3ESAM) by irradiation in a 0.004% solution of DMSO.

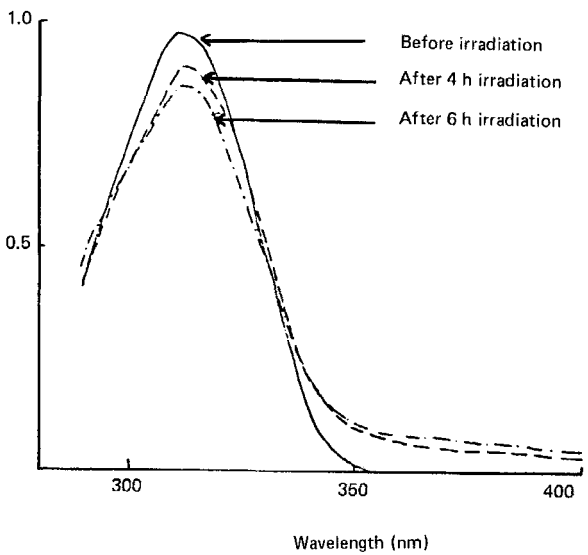

FIG. 4. The change of UV spectra of poly(3-vinylsalicylic acid) (p3VSA) irradiation in a 0.004% solution of DMSO.

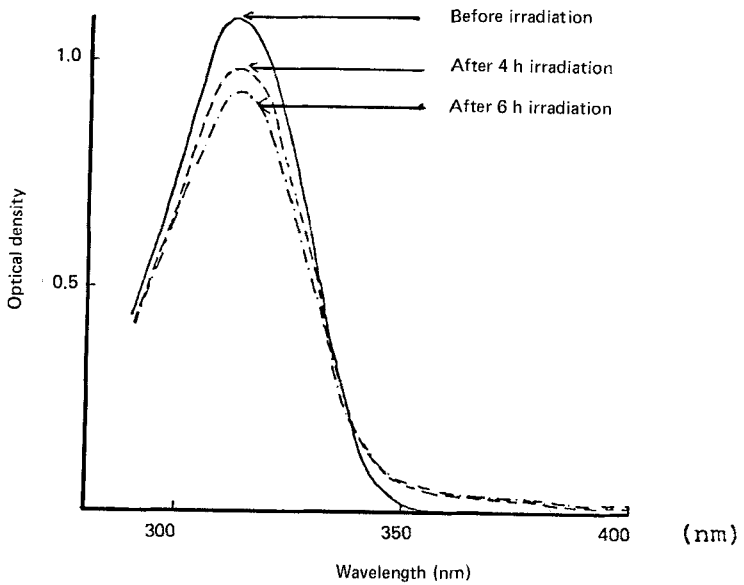

FIG. 5. The change of UV spectra of methacrylic acid/3-vinylsalicylic acid copolymer (MAA/3VSA) in a 0.02% solution of DMSO.

Sunscreening Efficiency

Figures 6 and 7 show UV spectra of the SA derivatives, monomers, polymers, and copolymers. Tables 9 and 10 show the two values of λ_{max} and the optical density at λ_{max} which have been calculated for a concentration of 1% (A 1% (λ_{max})). Tables 9 and 10 also include the average value of the optical densities ($\bar{A}$ 1% ($\lambda_{290-320}$)) in the region of 290 to 320 nm (erythema range) for a 1% concentration.

As seen in Figs. 6 and 7, all the samples have absorptions in the erythema range and the λ_{max} absorption at 304-314 nm. These characteristics of the UV absorption are taken to meet the qualification for the "sunscreening agents." The A 1% (λ_{max}) values of the homopolymers were higher than those of the copolymers. The A 1% (λ_{max}) values of the monomers were comparative to those of the homopolymers. The $\bar{A}$ 1% ($\lambda_{290-320}$) values of the homopolymers, which are taken as a measure of the sunscreening efficiency in the UV erythema range, were 3 to 6 times as high as those of the copolymers. However, the $\bar{A}$

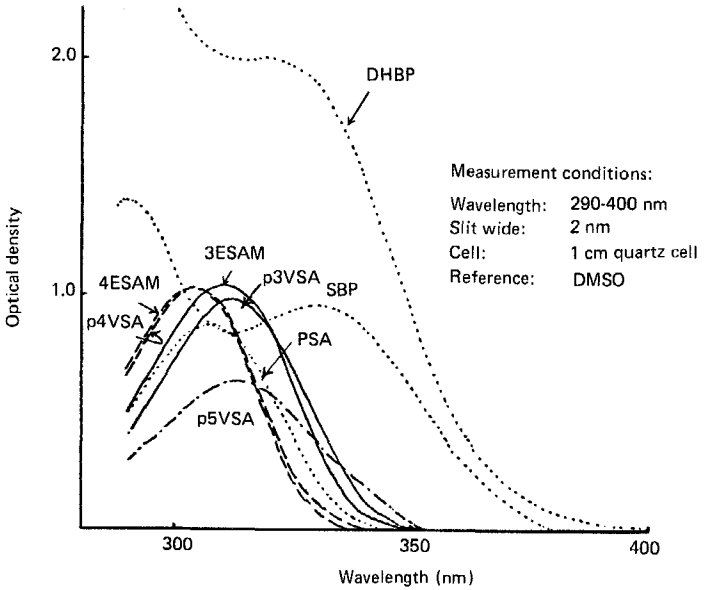

FIG. 6. UV spectra of 0.004% sample in DMSO of the monomers methyl 3-ethylsalicylate (3ESAM), methyl 4-ethylsalicylate (4ESAM), 2-hydroxy-4-methoxy-5-sulfobenzophenone (SBP), 2,4-dihydroxy benzophenone (DHBP), and phenylsalicylate (PSA); and the polymers poly(5-vinylsalicylic acid) (p5VSA), poly(4-vinylsalicylic acid) (p4VSA), and poly(3-vinylsalicylic acid) (p3VSA).

1% ($\lambda_{290-320}$) values of the homopolymers are lower than those of the commercial products. Thus, the $\bar{A}$ 1% ($\lambda_{290-320}$) values of the homopolymers were about a half of that of a commercial product of DHBP, and those of the copolymers were one-tenth of that of the commercial product. Thus, it may be concluded that the linkages of the vinyl groups as they form the homopolymer chain barely influence the light absorbancy of the SA portion of the polymer.

On the other hand, it was expected that the optical densities of the copolymers MMA/VSA in these tests would be considerably less than for the homopolymers. Only one part of the copolymer is due to VSA or its derivative. Poly(methacrylic acid) itself does not absorb in the 290 to 320 nm range. It could therefore be assumed that it is only the VSA unit or the unit of its derivative which is responsible for the observed optical density at 313 nm. Recalculation of A for 1% v/w with respect to the VSA unit (or the unit of its derivative) alone in the copolymers would change the value of A 1% (see Table 10). These data do suggest that light screening efficiency per VSA

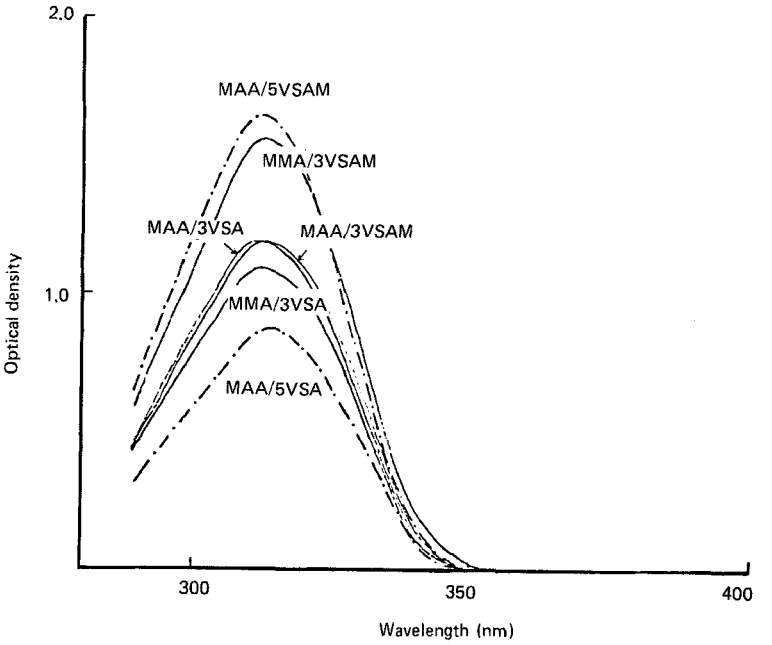

FIG. 7. UV spectra of 0.02% sample in DMSO of copolymers of methacrylic acid/5-vinylsalicylic acid (MAA/5VSA), methacrylic acid/3-vinylsalicylic acid (MAA/3VSA), methacrylic acid/methyl 5-vinylsalicylic acid (MAA/5VSAM), methacrylic acid/methyl 3-vinylsalicylic acid (MAA/3VSAM), methyl methacrylate/3-vinylsalicylic acid (MMA/3VSA), and methyl methacrylate/methyl 3-vinylsalicylate (MMA/3VSAM).

(or its derivative) unit increased as their amount in the polymer chain decreased.

CONCLUSIONS

The results show that use of homopolymers and especially copolymers of biologically active monomers is a way of increasing specificity. For example, as shown in Table 2, the copolymers MAA/3VSA have a very high activity against one bacteria but none to another. It was also found that the cytotoxicity was high for the homopolymers and the monomers but not for the copolymers. MMA/3VSA was almost nontoxic at concentrations as high as 1000 ppm.

TABLE 9. Sunscreening Efficiency for Monomeric and Polymeric UV Absorbers

Sunscreening agents	λ_{max} (nm)	A 1% (λ_{max})[a] (nm)	$\overline{A}$ 1% ($\lambda_{290-320}$)[b] (nm)
Methyl 3-ethylsalicylate	311	260	202
Methyl 4-ethylsalicylate	304	258	192
2-Hydroxy-4-methoxy-5-sulfo-benzophenone	290 (329)[c]	348 (238)	263
2,4-Dihydroxybenzophenone	293 (320)[c]	600 (499)	539
Poly(5-vinylsalicylic acid)[d]	313	158	122
Poly(4-vinylsalicylic acid)	304	260	198
Poly(3-vinylsalicylic acid)	312	245	188

[a]The optical density at λ_{max} for 1% solution.

[b]The average value of the optical densities in the region of 290 to 320 nm.

[c]Since these samples have two absorption peaks, the second peak is shown in the parentheses.

[d]This sample was partly insoluble in DMSO.

TABLE 10. Sunscreening Efficiency for Copolymeric UV Absorbers

	Concentration of comonomer		λ_{max} (nm)[a]	A 1% (λ_{max}) (nm)	A 1% (λ_{max}) calculated for 100% comonomer (nm)	$\bar{A}$ 1% ($\lambda_{290-320}$)[b] (nm)	$\bar{A}$ 1% ($\lambda_{290-320}$) calculated for 100% comonomer (nm)
	mmole%	weight%					
Methacrylic acid/5-vinylsalicylic acid copolymer (85/15)	15	25	314	43.5	174	32	128
Methacrylic acid/3-vinylsalicylic acid copolymer (96/4)	4	7	313	55.0	786	41	586
Methacrylic acid/methyl 5-vinylsalicylate copolymer (80/20)	20	34	313	82.5	243	62	182
Methacrylic acid/methyl 3-vinylsalicylate copolymer (88/12)	12	22	314	60.0	273	44	200
Methyl methacrylate/3-vinylsalicylic acid copolymer (86/14)	14	24	312	59.5	280	45	188
Methyl methacrylate/methyl 3-vinylsalicylate copolymer (79/21)	21	35	314	78.5	224	58	166

[a]The optical density at λ_{max} for 1% solution.

[b]The average value of the optical densities in the region of 290 to 320 nm.

It is important to remember that the activities seem to be very dependent on solution properties (Table 5) and sometimes also are time-dependent (Table 6). The light stabilities of the polymers were similar to the stability of DHBP, which was superior to that of the monomers of the SA derivatives. The values of the absorption efficiencies in the erythema range (290 to 320 nm) of polymers were about one-half of the value of DHBP. The values of copolymers were about one-tenth of the value of DHBP. Recalculation of absorption efficiencies based on the VSA unit (or the units of its derivative) changed the value, and one copolymer (MAA/3VSA) seemed to have an even higher value than DHBP.

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