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RESEARCH PROJECT INITIATION

Date: 23 May 1973

Project Title: "Chemical Interaction of Model Organic Compounds with Biosurfaces"

Project No: G-33-664 & E-19-615

Principal Investigators: Dr. C. L. Liotta & Dr. J. D. Muzzy

Sponsor: Public Health Service

Agreement Period: From 1 April 1973 Until 31 May 1973

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Amount: $3,940 $2,450 - G-33-664
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Sponsor Contact Person (s):

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RESEARCH PROJECT TERMINATION

Date: ~~December 7, 1973~~

Project Title: "Chemical Interaction of Modal Organic Compounds with Biosurfaces"

Project No: G-33-664 co project with E-19-615

Principal Investigator: Dr. C. L. Liotta & Dr. J. D. Muzzy

Sponsor: Public Health Service

Effective Termination Date: ~~May 31, 1973~~

Clearance of Accounting Charges: ~~December 31, 1973~~

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Other ~~G-32-604~~

Dr. J. W. Crenshaw

SUMMARY REPORTS

Project Title: "Chemical Interaction of Model Organic Compounds with Biosurfaces"

Principal Investigators: Drs. C. L. Liotta and J. D. Muzzy

G-33-664 E-19-615

Introduction

Many attempts have been made to modify the surface characteristics of natural teeth and skin by the formation of chemically bonded coatings. Graft polymerization has been successfully carried out by Rao[1,2,3,4], Brauer[5,6,7], and others by the use of ceric ammonium nitrate activation on the collagen surface in the presence of monomers. The surface characteristics of the polymer grafted collagen were varied from hydrophilic to hydrophobic by selection of the monomers grafted. A true chemical graft was proved by hydrolysis of the collagen backbone and dinitrophenylation[3].

Reactions of aldehydes with pendant amine functions on tooth collagen results in a diminished activity by proteolytic enzymes[8,9,10]. When 5-hydroxymethylfurfural is reacted with collagen it becomes resistant to attack by collangenase[10]. The adduct of N-phenylglycine and glycidyl methacrylate bonds to enamel, dentin and fluoroapatite. Bowen found bond strengths of 55, 26.7 and 41 kgf/cm^2 to enamel, dentin and fluoroapatite respectively.

Use of tri-n-butyl borane as an initiator results in excellent adhesiveness on teeth of 190 to 400 kg/cm shear strength according to Masuhara[12,13]. Clinical tests with V-shaped cavity preparations filled with tri-n-butyl borane initiated resins showed 94.5% retention after 23 months[14]. Tri-n-butyl borane is unstable in air but, its complex with ethylene diamine is stable until activated with p-toluene sulfonyl chloride[15].

Polyurethane liners have been bonded to bovine teeth after an acid etch to yield 91 kgf/cm^2 butt joint strengths by the Gillette Research Institute. Microleakage by fluorescent dye penetration was run with 0° - 37° - 70°C. cycling with good results by Stark[16].

Acid etching alone has been found effective in enhancing bonding by composite restorative material to enamel and dentine. Buonocore[17]

water washes and hydrolysis with 1N HCl. The hydrolysate yielded a UV with maximum absorption at 264 nm. An attempt to calculate the extinction coefficient assuming ε_{247} (6180) for the FMMP was unsuccessful. The amount of FMMP in the washes was greater than originally used in the reaction according to the calculation. Therefore, the extinction coefficient of FMMP in the basic (pH 9.3) wash solutions must be greater than 6180 or other reactions occurred. The peptide linkages may have been broken at pH 9.3 after 16 hours at 50°C. Further experiments are required to determine the course of the purine reaction under basic conditions.

In every case the percent of basic amino acid reacted was calculated as summarized in Table II. This data when compared to the percent FMMP reacted and the reaction pH strongly suggests that the pH has a strong influence on the reaction. At pH 7.8 all of the available FMMP reacted accounting for the 11.6% of the available basic amino acids. At pH 7.0 only 40 to 50% of the FMMP reacted suggesting that only certain side chains are available for reaction at this pH. Table IV lists the basic amino acids in human dentin with the pK of the pendant basic group in a peptide structure and the percent of basic amino acid in collagen. Comparison of the percent of histidine present in dentin collagen (7.75%) with the calculated percent amino acids reacted in Table II at pH (7.82% for collagen, 7.87% for crushed tooth) gives suprisingly close correlation. As the pH is increased to 7.8 all of the FMMP may be used up in reaction with histidine (pK 6.0) and hydroxylysine (pK 9.67). The results of the FMMP reaction at pH 10.8 was clouded by ambiguous results.

Another major factor in completion of the reaction was the solvent system used. The reaction was not favored in absolute alcohol but went to completion when the ethanol was removed and water was used as the solvent. Reactions of FMMP with collagen and crushed teeth in water went only about halfway to completion. This suggests that, a mixture or sequence of ethanol and water may be effective solvents for a high conversion reaction of FMMP with tooth collagen.

Conclusion

Evidence of a reaction of 6-fluoro-9-methoxymethylpurine (FMMP) with a bovine tooth collagen and crushed bovine teeth was obtained by a shift in the ultraviolet spectrum. The FMMP probably reacts with the basic side chains on the collagen. The reaction is favored in the presence of water and probably depends on the reaction pH and the solvent system. Further optimization of the reaction requires further study.

used an 85% phosphoric acid treatment to increase the adhesion of enamel to acrylic filling material. Improved bonding has also been demonstrated with citric acid, lactic acid, hydrochloric acid and sodium salts of EDTA. Brauer[18] showed, with SEM the effect of etching the tooth surface with acids to increase the surface area available and open tubules for mechanical ingress of resin. It was concluded that improved adhesion to acid treated teeth was not chemical but strictly mechanical as a result of interlocking of the cured resin.

Most of the attempts at improved adhesion to tooth surfaces by grafting or other chemical bonding have been conducted under conditions too stringent or toxic for the oral environment. The 6-fluoro-9-alkoxymethylpurines proposed by Liotta and Muzzy should have low toxicity but good chemical activity by a nucleophilic displacement reaction with tooth structure. In addition the fluoride ion evolved in the displacement can stabilize the hydroxyapatite at the reaction site. The object of the work reported here is to uncover evidence of chemical reaction of 6-fluoro-9-methoxymethylpurine FMMP with tooth structure.

Experimental

Preparation of Collagen From Bovine Dentin

A large Bovine molar tooth was preserved in Normal Saline solution immediately after extraction. The tooth was refrigerated in the solution at 10°C. to retard degradation. The tooth was sectioned at the enamel cementum junction with an Exacto hand saw. The pulp and residual soft tissues were removed. Both sections of the tooth were immersed in 100 ml of a 15% solution of disodium ethylenediamine tetraacetic acid (Na_2EDTA) in deionized water neutralized to pH 7.1 with normal sodium hydroxide solution. The treatment was continued at 37°C. until constant weight was achieved after five days. A 45% weight loss was observed after four days in the neutral EDTA. At this point the dentin separated from the enamel due to shrinkage. The enamel was easily removed and discarded with a dental tool. The dentin was soft like a sponge and contained considerable entrained water. The dentin derived material was washed with neutral deionized water. This material was defined dentin collagen without drying to maintain its structure.

Reaction of Ethanol Solution of 6-Fluoro-9-Methoxymethyl purine (FMMP) with Tooth Collagen

Treated 0.1344 gm (wet) dentin collagen from a powdered Bovine molar with 50 ml of 0.001M (0.0091 grams) 6-Fluoro-9-methoxy-methylpurine (FMMP) in absolute alcohol stirred for 28 hours at room temperature. The ethanol was evaporated with a stream of dry nitrogen in eight hours.

Hydrolysis of FMMP - Ethanol Treated Collagen

The dried residue from the treatment of tooth collagen with ethanolic FMMP was hydrolyzed in 100 ml of 3N HCl at reflux for five hours. A brown residue resulted which was filtered and neutralized to pH 6.8 with normal sodium hydroxide solution and the precipitate formed was filtered and analyzed by x-ray.

UV Analysis of FMMP - Ethanol Treated Collagen Hydrolyzate

UV analysis of the solution obtained by hydrolysis of FMMP ethanol solution treated powdered collagen yielded a broad absorption at 247 nm. This absorption was presumed to be due to the presence of unreacted FMMP and therefore no evidence of reaction product of the FMMP with collagen was formed.

Reaction of FMMP First in Ethanol Then in Water Solution with Tooth Collagen

Treated 0.9636 grams (wet) collagen prepared from unground Bovine dentin with 50 ml of 0.001N FMMP solution in ethanol 16 hours. The collagen shrank and hardened in the ethanol. The ethanol was evaporated under vacuum on a Rotovap. To the dry residue was

added 15 ml of deionized water pH 7.8. The mixture was rotated at 50°C. for 20 hours. The collagen was soft and spongy as before the treatment. The water was evaporated with a water pump.

Hydrolysis of FMMP (1) Ethanol (2) Water Treated Collagen

The residue was washed with deionized water then refluxed in 1N HCl for three hours. The resulting 120 ml of solution was filtered but not neutralized.

UV Analysis of FMMP (1) Ethanol (2) Water Treated Collagen Hydrolyzate

A 10% dilution of the hydrolysis solution of FMMP (1) ethanol (2) water treated collagen gave a new absorbance at 268 nm with 65% transmittance. Calculation of Extinction Coefficient: Assuming complete reaction of FMMP 0.050 liter x 0.001 mole/liter = 5×10^{-5} moles FMMP:

$$\varepsilon \text{ (268 nm)} = \frac{\log \frac{100}{\%T}}{C} = \frac{\log \frac{100}{65}}{\frac{5\times10^{-5}}{0.120\times10}} = 4480$$

Reaction of FMMP - Water Solution with Tooth Collagen

Treated 0.7076 grams (wet) collagen with 0.009 grams FMMP in ten ml of neutral deionized water in a 50 ml flask on the Rotovap. The reaction was carried out for 20 hours at 50°C. The water was evaporated under vaccum. The residue was extracted and washed with deionized water to remove unreacted FMMP. The extract and wash solutions were set aside for a UV analysis material balance.

Hydrolysis of FMMP - Water Treated Collagen

The washed residue from the FMMP water treatment of collagen was refluxed in 120 ml of 1N HCl for three hours. The resulting solution was filtered and analyzed by UV as shown in Table I. ε_{264} (5020)

Control A - Hydrochloric Acid Reflux Bovine Tooth

To 0.3514 grams of Bovine tooth 60 ml of 1N HCl was added. The mixture was refluxed for 3 hours filtered and analyzed by UV. No interference was noted in the 230 to 340 nm region.

Control B - Hydrochloric Acid Reflux on FMMP

A solution of 0.0009 gm FMMP in 12 ml of 1N HCl was refluxed for three hours and analyzed by UV. An ε_{247} (8040) was found.

Reaction of FMMP - Water Solution with Crushed Bovine Tooth

A fresh bovine tooth crown was crushed under deionized water to yield 1.6440 grams material. The crushed tooth was treated in a

Rotovap with 0.0090 gms FMMP in 10 ml. pH 7.0 deionized water for 20 hours at 50°C. The water was evaporated under vacuum. The residue was extracted and washed with deionized water to remove unreacted FMMP. The residue was then hydrolyzed with 60 ml of 1N HCl in three hours. The hydrolysis solution was analyzed by UV and absorbed at 264 nm (4750).

Reaction of FMMP - Water Solution with Collagen at pH 10.8

Treated 0.7000 gm of tooth collagen with 0.0090 gm FMMP in 10 ml pH 10.8 for 20 hours at 50°C. in the Rotovap. The water was evaporated under vacuum. The residue was extracted and was with a total volume of 50 ml of neutral deionized water. A final washing with 25 ml of neutral deionized water was carried out. The powder was hydrolyzed in 120 ml of 1N HCl for three hours and then a UV was run. UV analysis showed maximum absorption at 264 nm.

Discussion of Results

Reaction of FMMP in Ethanol on Collagen

The hydrolyzed reaction product of FMMP in ethanol (λ max 247) with collagen from fresh bovine teeth gave a broad UV absorption with a maximum at 247 nm. Examination of the solution on which the UV was run with a light beam showed no Tyndall effect. Thus, the peak broadness was not due to a light scatter by a suspension, but may be attributed to the presence of some FMMP - collagen reaction product in the presence of a large excess of FMMP.

Reaction of FMMP - (1) Ethanol, (2) Water on Collagen

A more definitive result was obtained when the reaction of FMMP with collagen was carried out first in ethanol and then after evaporation of the ethanol in deionized water at pH 7.8. After the reaction product was washed with water to remove unreacted FMMP, it was hydrolyzed in refluxing 1N HCl. The UV spectra of the hydrolysis solution showed a shift in the maximum absorption, from 247 nm (6180) to approximately 268 nm (4540) as shown on Table I. This shift indicates that the FMMP reacted with the collagen. The extinction coefficient was calculated assuming complete reaction of the FMMP. Inspection of subsequently determined extinction coefficients determined for similar reactions gave an average ε_{264} (4820) indicating that the assumption of complete reaction by FMMP was valid. During the reaction of the bulk collagen in alcohol the collagen shrank and hardened, but expanded and softened again in water. This indicates that the collagen structure is more open to reaction at the side groups in water than in ethanol. Based on this lead the remaining experiments were run in water.

Reaction of FMMP in Water on Collagen

When FMMP was reacted with tooth collagen in pH 7.0 deionized water, washed with water and hydrolyzed in 1N HCl the UV absorption maximum 264 nm (5020) was observed. The extinction coefficient was calculated by measuring the amount of unreacted FMMP in the wash solution.

Reaction of FMMP in Water on Crushed Tooth

The reaction of FMMP on a crushed bovine tooth in water at pH 7.0 followed by water washes and hydrolysis with 1N HCl yielded a UV absorption maximum at 264 nm (4750). The extinction coefficient was calculated by measuring the amount of unreacted FMMP in the wash solutions.

Reaction of FMMP in Water on Collagen at pH 10.8

FMMP was reacted with collagen in water at pH 10.8 followed by

water washes and hydrolysis with 1N HCl. The hydrolysate yielded a UV with maximum absorption at 264 nm. An attempt to calculate the extinction coefficient assuming ε_{247} (6180) for the FMMP was unsuccessful. The amount of FMMP in the washes was greater than originally used in the reaction according to the calculation. Therefore, the extinction coefficient of FMMP in the basic (pH 9.3) wash solutions must be greater than 6180 or other reactions occurred. The peptide linkages may have been broken at pH 9.3 after 16 hours at 50°C. Further experiments are required to determine the course of the purine reaction under basic conditions.

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Evidence of a reaction of 6-fluoro-9-methoxymethylpurine (FMMP) with a bovine tooth collagen and crushed bovine teeth was obtained by a shift in the ultraviolet spectrum. The FMMP probably reacts with the basic side chains on the collagen. The reaction is favored in the presence of water and probably depends on the reaction pH and the solvent system. Further optimization of the reaction requires further study.

TABLE I

Ultraviolet Spectra Reaction Data Summary

Reaction	Concentration (mole/liter)	%T	λ_{max} (nm)	(ϵ)	pH
FMMP - EtOH	5.0×10^{-5}	65%	247	6180	-
FMMP - EtOH:Collagen L12-40-3	5.32×10^{-5}	broad peak at 247 nm			6.8
FMMP (1) EtOH (2) H_2O: Collagen L12-41-1	4.17×10^{-5} (a)	65 64.3	∿ 268 ∿ 268	4480 4600	1.5
FMMP - H_2O:Collagen L12-48-5	2.03×10^{-5} (b)	79	264	5020	1.5
FMMP:1HNCl L12-48-5	4.08×10^{-5}	47	264	8040	1.5
FMMP - H_2O:Crushed Tooth	3.45×10^{-5}	67 69.25 69.5	264 264 264	5040 4630 4580	7.0
FMMP - H_2O:Collagen at pH 10.8 L12-59-3	2.49×10^{-5} (c)	76.25	264	4820 (c)	1.5

note: (a) assumed complete reaction of FMMP
(b) calculated based on $\epsilon_{247} = 6180$
(c) calculated based on $\epsilon_{264} = 4820$

TABLE II

Percent Theoretical Basic Amino Acid Reaction

	Reaction pH	Percent Reaction Basic Amino Acid	Percent Reaction FMMP
FMMP - (1) EtOH (2) H_2O Collagen L12-41-1	7.8	11.6%	100%
FMMP - H_2O Collagen L12-48-3	7.0	7.82%	49.3%
FMMP - H_2O Crushed Tooth L12-49-3	7.0	7.87%	41.9%
FMMP - H_2O Collagen L12-59-3	10.8	9.58%	60.4%

TABLE III

Amino Acid Composition of Bovine Dentin

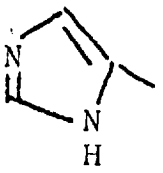
		Residues[19] 1000
Histidine		3.7
Hydroxylysine	$H_2NCH_2CH(OH)CH_2CH_2CH(NH_2)COOH$	13
Lysine	$H_2N(CH_2)_4CH(NH_2)COOH$	19
Arginine	$H_2NC(=NH)NH(CH_2)_3CH(NH_2)COOH$	47
		(82.7)
Tyrosine		3.2
Phenylalanine		16
Glutamic Acid		72
Aspartic Acid		52
Threonine		16
Serine		39
Hydroxyproline		103
Proline		130
Glycine		327
Alanine		112
Valine		22
Methionine		4.1
Leucine		25
Isoleucine		11

TABLE IV

Basic Amino Acids in Human Dentin Collagen

	MW	pk	gms/100gm[20]	moles/gram collagen	
Histidine	155	6.0	1.07	6.90×10^{-5}	7.75%
Hydroxylysine	147	9.67	0.99	6.80×10^{-5}	7.64%
Lysine	132	10.53	3.31	2.50×10^{-4}	28.1%
Arginine	157	12.48	7.9	5.30×10^{-4}	56.51%
			13.27	8.90×10^{-4}	

Calculations

FMMP-(1) EtOH (2) H_2O Treated Collagen L12-41-1

Data: 65% T, ~268 nm, 64.3% T, 120 ml volume, 10% Dilution

Concentration: $\frac{0.001 \text{ mole/liter} \times 0.050 \text{ l.}}{10 \times 0.120 \text{ liters}} = 4.17 \times 10^{-5}$ mole/liter

$$\varepsilon_{268} = \frac{\log \frac{100}{65}}{4.17 \times 10^{-5}} = \frac{0.187}{4.17 \times 10^{-5}} = 4480$$

$$\varepsilon_{268} = \frac{\log \frac{100}{64.3}}{4.17 \times 10^{-5}} = \frac{0.192}{4.17 \times 10^{-5}} = 4600$$

FMMP - H_2O Treated Collagen Wash L12-48-2

Data: 49% T, 247 nm, 50 ml volume, 10% dilution, assume ε_{247} 6180

Concentration FMMP: $\frac{10 \times \log \frac{100}{49}}{6180} = 5.02 \times 10^{-4}$ mole/liter

Weight of recovered FMMP: $5.02 \times 10^{-4} \times 0.050 \times 182 = 4.56 \times 10^{-3}$ gm

Weight of FMMP Reacted 0.0090 - 0.00456 = 0.00444 gm (49.3%)

FMMP - H_2O Treated Collagen L12-48-3

Data: 79% T, 264 nm, 120 ml volume, 10% dilution, 0.00444 gm FMMP reacted

Concentration: $\frac{0.00444}{182 \times 0.120 \times 10} = 2.03 \times 10^{-5}$ moles/liter

$$\varepsilon_{264} = \frac{\log \frac{100}{79}}{2.03 \times 10^{-5}} = \frac{0.102}{2.03 \times 10^{-5}} = 5020$$

FMMP Refuxed in 1NHCl L12-48-5

Data: 47% T, 247 nm, 12 ml volume, 10% dilution, 0.0009 gm FMMP

Concentration: $\frac{0.0009}{182 \times 0.012 \times 10} = 4.08 \times 10^{-5}$

$$\varepsilon_{247} = \frac{0.328}{4.08 \times 10^{-5}} = 8040 \quad \text{at pH 1.5}$$

FMMP - H_2O Treated Crushed Tooth Wash L12-49-1

Data: 19.5% T, 247 nm, 25 ml volume, 10% dilution, assume $\varepsilon_{247} = 6180$

Concentration FMMP: $10x \log \frac{100}{19.5} \over 6180 = 1.15x10^{-3}$ mole/liter

Weight of recovered FMMP: $1.15x10^{-3}x0.025x182 = 0.00523$ gm

Weight of FMMP reacted: 0.009 - 0.00523 = 0.00377 gm (41.9%)

FMMP - H_2O Treated Crushed Tooth L12-49-3

Data: 67, 69.25, 69.5% T, 264 nm, 60 ml volume, 10% dilution, 0.00377 gm FMMP reacted.

Concentration: $\frac{3.77x10^{-3}}{0.060x10x182} = 3.45x10^{-5}$ mole/liter

(1) $\varepsilon_{264} = \frac{\log \frac{100}{67}}{3.45x10^{-5}} = \frac{0.1738}{3.45x10^{-5}} = 5040$

(2) $\varepsilon_{264} = \frac{\log \frac{100}{69.25}}{3.45x10^{-5}} = \frac{0.1596}{3.45x10^{-5}} = 4630$

(3) $\varepsilon_{264} = \frac{\log \frac{100}{69.5}}{3.45x10^{-5}} = \frac{0.1580}{3.45x10^{-5}} = 4580$

FMMP - H_2O Treated Collagen at pH 10.8 Wash L12-59-1

Data: 12% T, 247 nm, 50 ml volume, 10% dilution

Concentration FMMP: $10x\log \frac{100}{12} \over 6180 = \frac{9.208}{6180} = 1.49x10^{-3}$ mole/liter

Weight FMMP recovered: $1.49x10^{-3}x182x0.050 = 0.0136$ gm FMMP

But original weight of FMMP used was only 0.0090 gm. Therefore, this calculation on the data is not valid.

FMMP - H_2O Treated Collagen at pH 10.8 L12-59-3

Data: 76.25%T, 264 nm, 120 ml, 10% dilution assume $\varepsilon_{264} = 4820$

Concentration FMMP reacted = $10 \times \log \frac{100}{76.25} \div 4820 = 2.49 \times 10^{-4}$ mole/liter

Weight FMMP reacted: $2.49 \times 0.120 \times 10 \times 182 = 0.00544$ gm (60.4%)

Calculation of Percent of Basic Amino Acids Reacted

FMMP - (1) EtOH (2) H_2O Treated Collagen L12-41-1

assume $\varepsilon_{264} = 4820$ average $\begin{cases} 5020 \\ 5040 \\ 4630 \\ 4580 \end{cases}$

found 4480 and 4600 $\simeq$ 4820

FMMP completely or 100% reacted

0.001 mole/liter x 0.050 liter = 5×10^{-5} mole reacted

From Table IV Basic amino acid content of dentin is:

8.90×10^{-4} moles/gm

Assuming the weight of collagen is 18% the tooth weight

0.5 x 0.9636 = 0.4818 gm collagen

0.482 x $8.90 \times 10^{-4} = 4.29 \times 10^{-4}$ moles basic AA

$\frac{5 \times 10^{-5} \text{ moles reacted}}{4.29 \times 10^{-4} \text{ moles basic AA}} \times 100 = 11.6\%$ basic AA reacted

FMMP - H_2O Treated Collagen L12-48-3

$\frac{2.44 \times 10^{-3}}{182 \text{ mole/gm}}$ gm FMMP reacted = 2.44×10^{-5} mole FMMP reacted

8.90×10^{-4} mole basic AA/gm collagen x 0.35 gm collagen =

3.12×10^{-4} mole basic AA

$\frac{2.44 \times 10^{-5}}{3.12 \times 10^{-4}} \times 100 = 7.82\%$ basic AA reacted

FFMP - H_2O Treated Crushed Tooth L12-49-3

$\frac{3.77 \times 10^{-3}}{182} = 2.07 \times 10^{-5}$ mole FMMP reacted

Assuming 18% of the crown is collagen

$0.18 \times 1.644 \times 8.90 \times 10^{-4} = 2.63 \times 10^{-4}$ mole basic AA

$\frac{2.07 \times 10^{-5}}{26.3 \times 10^{-5}} \times 100 = 7.87\%$ basic AA reacted

FMMP - H_2O Treated Collagen at pH 10.8 L12-59-3

$\frac{5.44 \times 10^{-3}}{182} = 2.99 \times 10^{-5}$ mole FMMP reacted

$0.35 \times 8.90 \times 10^{-4} = 3.12 \times 10^{-4}$ mole basic AA

$\frac{2.99 \times 10^{-5}}{31.2 \times 10^{-5}} \times 100 = 9.58\%$ basic AA reacted

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THEORY:

This report concerns itself with the bonding of certain groups ot teeth, the point being to investigate which groups may prove best suited to be made into dental adhesives.

Each of groups were in the form of a ring compound so that one could study the chemsorption of each compound directly by use of U.V. spectroscopy. The procedure used (to be described later) had two objectives: 1) to make sure the bonding was chemsorption and not involving any sort of physical absorption and 2) to make sure the groups studied bonded to the teeth directly and that there was no interlocking effects observed. Toward these ends, the concentration was small so that there would be little chance of interlinking and also that one could read them by U.V. spectroscopy.

The problem of whether or not one gets physical or chemical absorption is thought to be resolved by putting the teeth in a water solution for the final U.V. spectra reading.

Methods Used:

In the beginning the approach used to achieve the results found is as follows:

1- make up a water solution of the desired concentration of the compound to be tested. This was done by using the extinction coefficients (1) for the compound as all tests were run on a U.V. spectrophotometer. If the solution was not at the desired strength one made another solution and hence some test compounds ended up with a trial and error method in order to find the desired initial absorbance.

2- After the solution was made up, 2.75 ml. of it was placed in a silica test cell. This silica cell allows the U. V. spectra to pass through it and be unaffected by the cell itself. This 2.75 ml. sample was allowed to stand for at least three hours and then was dried completely by blowing dry nitrogen gas over the cell.

3- After the water had been completely evaporated 0.01 gm. of the dried, powdered teeth was then added to the cell along with 0.2 ml. of an organic solvent. The organic solvent used was 1,2-Dimethoxyethane. The choice of 1,2-DME was based on chemical compatibilityto these reactions.

4- The teeth and the 1,2-DME was allowed to sit for not less than three hours and up to 72 hours to insure steady state has been reached.

5- The 1,2-DME is then evaporated with a stream of nitrogen and then 2.75 ml. of distilled water is added to the cell. After the water is added to the cell, the cell is shook and then centrifuged. The cell can now be taken to have its U.V. spectra read in the spectrophotometer.

The range used on the U.V. readings was 3500 to 2000 Å. The high peak observed is measured and a comparison of these peaks yield a relative absorbance.

It was found that due to the time factor and the number of available cells that another method would be highly desirable if it could cut the three days of the above method down to a reasonable time. The reasonable time became one day. The method (denoted the new system) is as follows:

1. Place 0.01 gms. of dentin and enamel (the powdered teeth) in three of the four U.V. cells.
2. Add 0.25 ml. of a solution made up with 1,2-Dimethoxyethane as the solvent.
3. Let this sit for at least three hours.
4. Evaporate the samples, either by using a stream of dry nitrogen gas or by lettin the open test cells sit overnight in a dessicant.
5. Aftre the cell is completely dry add 2.75 ml. of distilled water.
6. Test on the U.V. spectrophotometer.

Preparation of the Teeth:

The teeth used in this experiment were human teeth procured from the Oral Pathology clinic at the Emory University School of Dentistry. The teeth were sorted through to choose the "better" specimens. The specimens were first washed in distilled water. Next, the root portion of the teeth were (1) first cut to a point near the crown and then (2) ground the rest of the way down. The grinding was stopped when the root portion of the tooth was completely ground away. The crown was then washed and brushed to clean it. If any soft organic material was left in or around the crown, the crown was then scraped until the organic material was completely removed. If the material was in the interrior of the crown, it was picked out. After this organic material was removed, the teeth were then etched in a 50% acetic acid solution for three minutes, after which they were thoroughly washed in distilled water. The teeth were then dried and then powdered in a hammer mill. The result was put through a 200 mesh screen. The powder that passed through the screen was used as the teeth for the experimental work.

The powdered teeth that was not used immediately was stored in a .9% NaCl solution in a refrigerator. When this store of teeth was needed the procedure was to 1) drain off the NaCl solution, 2) etch these teeth for three minutes in a 50% acetic acid solution, 3) rinse thoroughly in distilled water and 4) dry with a gentle stream of nitrogen.

RESULTS:

The following table gives the results found.

Compound	Percent Absorbed	
	Old System	New System
N - Phenylglycine	0	0
Benzoic Acid	NR	0
Phthalic Acid	NR	*
Aniline	NR	*
Pyridine	NR	0
Benzonitrile	0	*
6-F,9 MM Purine	53	28

NR - Was not run under the HOH/evap./DME + teeth/HOH system.
* - indeterminate data

CONCLUSION AND RECOMMENDATIONS:

The conclusion is that, at best, the tests performed are indeterminate. The results that were not thrown out give that 6-Fluoro,9-Methoxymethylpurine has the best chemsorption with fifty-three percent (old system of testing). Nothing else was observed to absorb.

The recommendations are as follows:

1- One should learn more about the mechanics of this reaction in order to get a better idea of what happens and thus one can design a better experiment and thus find a better way of testing for chemsorption. The present way has no guarentee of consistant, much less 'correct', results.

2- Given the lack of knowledge about any of the reactions, i.e.- is it concentration dependent? (it appears to be) is it affected by temperature? (probably) is the chemsorption permanent under conditions met in the mouth? (?); one should investigate this, but in order to do it one must at least be able to guess what is happening during the reaction.

3- More co-operation should be given to the person(s) doing the actual data collection. Such things as READY ACESS TO THE LAB must be one of the criteria. Also, as assigned place must be given to that project alone and all other students should not use that bench. Hassels over the pens to the U.V. spectrophotometer, having people 'clean up' an entire quarter's work, etc. should be avoided by judicious forsight and by bestowing the consequences of such actions in the proper place.