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MALDI-TOF MS Analysis of a Library of Polymerizable Cyclodextrin Derivatives

ABSTRACT

Polymerizable cyclodextrin derivatives (PCDs) have been proposed as candidates for use in dental therapeutics (Bowen, 1996; Bowen and Reed, 1997). Here, PCD "libraries" were synthesized by *quasi*-random reactions of 6 moles of methacrylic anhydride plus 6 moles of cyclic glutaric anhydride *per* mole of beta-cyclodextrin (BCD) in solution. BCD has 21 reactive sites on each of its molecules. These proportions were based on probability calculations, which predicted that the products should have a minimum of 2 polymerizable substituents and acidic ligand groups on practically every one of the diverse product molecules. Matrix-assisted laser desorption/ionization (MALDI) time of flight (TOF) mass spectrometry (MS) gave valuable information regarding the masses of molecular ions representing the molecules that made up the PCD libraries. For the MALDI-TOF MS analyses, small samples were analyzed by the successive application of 3 solutions to the sample holder: the matrix in acetone, the products in water, and sodium trifluoroacetate in water. The resulting spectra had > 40 envelopes of mass peaks above background. The ionic-abundance peak heights had *quasi*-Gaussian configurations, with central peaks having masses in the neighborhood of 2000 g/mol (Daltons). Regardless of structural permutations within each peak, the range of these peaks was between about 1500 g/mol and 2900 g/mol. This range of masses was in accord with, but perhaps somewhat more narrow than, that predicted by the statistical method, which was based on equal reactivity of all hydroxyl groups. Analysis by MALDI-TOF MS gave valuable data regarding the masses, structures, and characteristics of the products formed and provided unanticipated information to facilitate improvements in future PCD syntheses.

KEY WORDS: dental, resins, cyclodextrins, MALDI-TOF, spectrometry.

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INTRODUCTION

The long-term objective of this ongoing research project is the preparation and evaluation of surface-active comonomers (SACs) that will facilitate improved adhesive bonding in dentistry. The present study reports the synthesis of a heterogeneous library of many diverse SACs for simultaneous application in a suitable liquid formulation as a candidate for improving adhesion. The availability, economics, and unique characteristics of beta-cyclodextrin (BCD, Fig. 1) suggested the use of BCD for synthesis of such a library. The term "library" refers to a large collection of related but diverse molecules. Libraries of polymerizable cyclodextrin derivatives (PCDs, Fig. 2) have been proposed for use in adhesive bonding compositions (Bowen and Reed, 1997).

Each PCD molecule of this library was designed to have two or more (an average of 6) methacrylate groups to form cross-linked polymers when used in combination with diluent comonomers. Each molecule of this library was also designed to have multiple carboxyl groups (an average of 6) for ionic interactions, and multiple hydroxyl groups for hydrogen bonding with the many highly diverse sites of tooth structures. Contemporary "total-etch" bonding techniques (Fusayama, 1992; Gwinnett *et al.*, 1992) give access to many different kinds of "acceptor sites" for bonding interactions. The simultaneous application of a PCD library mixture (without deconvolution) removes this idea (conception) from the realm of combinatorial chemistry (Burgess, 1997).

There are thousands of references to cyclodextrins and their derivatives in the general literature (Saenger, 1984; Szejtli, 1999). Nonetheless, the authors' searches have not revealed reports by others of PCD libraries or their utilization in dental applications. Therefore, a provisional PCD library was synthesized. Some assurance of its characteristics must be obtained before adhesion testing should be initiated. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) was used for the mass analysis of such complex mixtures of high-molecular-mass compounds (Siuzdak, 1996, p. 11; Wallace *et al.*, 1999). For large libraries where mass resolution of each component is prohibitive, the positions of mass peaks provide useful information regarding the success of the synthesis (Loo *et al.*, 1996). Compared with previous work done with conventional mass spectroscopy (*e.g.*, Farahani *et al.*, 1997, 1998), the MALDI technique (Noble, 1995; Cotter, 1997; Kowalski, 1998) makes it possible for the molecular masses of molecules having large mass-to-charge ratios to be determined with minimal analyte fragmentation.

MATERIALS & METHODS

Methacrylic anhydride and cyclic glutaric anhydride were added to a clear solution of dried BCD in a mixture of aprotic solvents and catalysts. The BCD sample used was courtesy of the American Maize-Products Co. (now Cerestar USA, Inc., Hammond, IN, USA; lot G 6020-42). The BCD as received (167.8 g) was dried in a vacuum oven (about 30 kPa, 110°C, 5 d) and stored at about 23°C in a vacuum desiccator containing indicating Drierite®, yielding 145.6 g of white powder. A

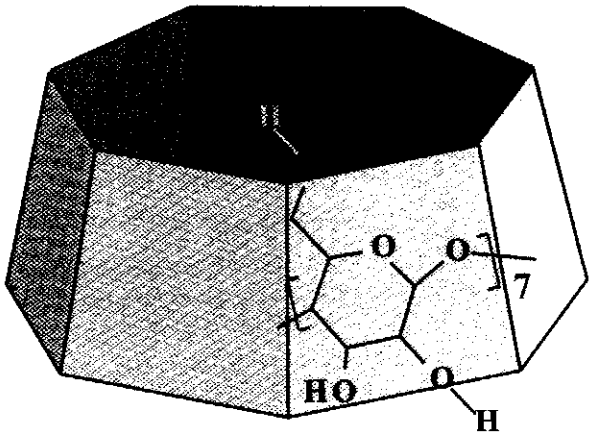

Figure 1. Schematic representation of a beta-cyclodextrin molecule containing 21 reactive hydroxyl groups. The 7 glucose (dextrose) groups are each connected, forming a stable, slightly cone-shaped ring. There is a relatively hydrophobic (organophilic) "host" space in the center of the molecule.

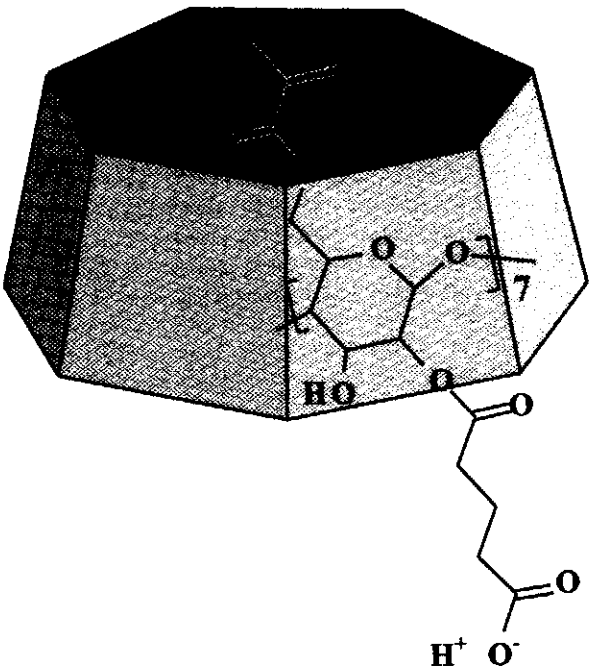

Figure 2. Simplified schematic view of one of the many combinations and permutations (configurations) that are formed in the synthesis of a library of polymerizable cyclodextrin derivatives (PCDs). In the synthesis reported here, only 6 methacrylic anhydride and 6 cyclic glutaric anhydride molecules were actually used for *quasi*-random reactions with each molecule of beta-cyclodextrin. The calculated average molecular mass (if there was 100% of the theoretical yield) for the molecules in the resulting library should be about 2228 g/mol (Da, Daltons, *M*). These PCD molecules also have central organophilic "nests" (host cavities) within which water-insoluble polymerization initiators, comonomers, antimicrobials, etc., might be carried as "passengers" into aqueous (or hydrated) substrates such as etched surfaces of teeth.

solution of 0.0424 g BHT (butylated hydroxytoluene; 2,6-di-*tert*-butyl-4-methyl-phenol) in 144 g of nominally anhydrous pyridine was magnetically stirred in a closed round-bottomed flask. This solution was heated to about 55°C, and 30.0 g (0.0264 mol; 0.555 OH equivalent) of the previously dried BCD were added. The resulting translucent suspension promptly became opaque white and sufficiently high in viscosity to prevent homogenous stirring. The suspension was heated to 94°C without formation of the necessary clear, homogeneous solution and with no apparent drop in viscosity. Therefore, additional aprotic solvents were added, because homogeneous mixing and "*quasi*-random" reactions with the hydroxyl groups of all of the BCD molecules were essential aspects of the protocol.

Hence, 32.0 g of 1-methyl-2-pyrrolidinone (NMP) were added but did not give perceptible solubility improvement over the same temperature range. When 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was also added (34.0 g) at 80°C, the suspension immediately became a clear, very light yellow solution. As it cooled to about 63°C, 25.1 mL of methacrylic anhydride (26.0 g) were added dropwise to the rapidly stirred solution. There was a prompt exothermic reaction. This elevated temperature was maintained by slight adjustments in the dropping rate during the 17 min of addition. About 2 hrs after the methacrylic anhydride had been added, 19.0 g of cyclic glutaric anhydride, dissolved in 30 g of chloroform, were added dropwise over a 20-minute period at 57°C, with a similar exothermic response. The clear amber solution that formed appeared to remain the same while it was stirred in the closed flask for many days at about 23°C. Toluene was added dropwise while the mixture was stirred. Two phases separated, and after the dense resin phase settled, the upper, toluene-rich phase was decanted. The remaining resin was thinned to a fluid consistency by addition of anhydrous ethanol, and then the toluene precipitation procedure was repeated. After the resulting "dense phase" was diluted again with ethanol, numerous aliquots of this solution were withdrawn successively over an extended period. Different methods were evaluated for the removal of residual solvents and byproducts from the PCD library within each of these samples.

The method evaluated for the aliquot (10 mL) that was analyzed in this report was as follows. The rationale was to retain the PCD product library members within a dialysis membrane that had a stated molecular mass cut-off value of 1000 g/mol (Daltons, Da, *M*). The product library should range above 1135.01 g/mol, which is the formula weight of BCD. It was hoped that the residual byproducts, amine catalysts, and solvents would diffuse into the surrounding aqueous poly(acrylic acid) solution that was in volumetric excess. Therefore, this portion was placed in a dialysis tube (Spectra/Por® membrane, MWCO: 1000) and then rotated in a dilute (mass fraction about 0.013) poly(acrylic acid) aqueous solution (1088 g). The dialysis was followed by use of a freezing-point osmometer (model 5004, "μ Osmette"®, Precision Systems, Inc., Sudbury, MA, USA). The osmolality in the external chamber increased from about 6 mmol/kg to 60 mmol/kg. The pH in the external chamber rose from about 3 to about 4. The solution within the dialysis tube separated into an upper liquid emulsion and a lower semisolid phase. These phases were separated, dried with anhydrous calcium sulfate (Indicating Drierite®) in a partial vacuum, and comminuted with glass rods into clear glassy particles. The fine, off-white powder derived from the upper phase was designated "D-1". The similar powder derived from the lower semisolid phase was designated "D-2".

To gain information about the D-1 and D-2 reaction products, we analyzed samples by MALDI-TOF MS. The spectra were obtained with a REFLEX II™ MALDI-TOF mass spectrometer (Bruker Daltonics, Inc., Billerica, MA, USA). In MALDI, the large molecules to be analyzed are preferably mixed in a common solvent with an excess of matrix material. The solution of large "analyte" molecules and matrix is then allowed to crystallize as a thin layer on

the surface of a sample holder. Compounds containing ions of sodium, potassium, or other elements are sometimes incorporated into the preparation of the sample mixture to facilitate the ionization and analysis of the analytes (Wong and Chan, 1997). Fig. 3, which is not to scale, shows a simplified schematic of the analytic method. The sample holder is repeatedly moved to subject many regions of the sample layer to brief pulses of a narrow laser beam. The selected matrix compound absorbs and becomes excited by the laser pulses, which cause expansion in the vacuum chamber to supersonic velocity and entrainment of the analyte molecules. During excitation of the matrix by the laser pulse, charge-transfer or proton-transfer reactions can occur, generating protonated and free-radical products that ionize the molecules of interest (Hillenkamp *et al.*, 1991). The analytes in the present case are reaction products or tightly associated complexes of PCD library members. A strong electrical field accelerates these analyte ions of interest toward a detector. Because of differences in their mass-to-charge ratios, smaller ions are accelerated more than are larger ions.

A common solvent, or mixture of solvents, in which both matrix and analyte could be combined and applied to the sample holder would have been preferable (Yalcin, 1998). However, such a solvent could not be found, and each of 3 solutions was successively applied by hand and allowed to dry on the surface of the sample holder (probe). These solutions were the matrix, 2,5-dihydroxybenzoic acid (DHB, FW 154.12; 100 mg/mL in acetone), the analyte (the product sample D-1 or D-2; 5 mg/mL in water), and sodium trifluoroacetate (FW 136.00; 5 mg/mL in water). From different areas on the surfaces of the sample holders, numerous spectra were acquired for each of the D-1 and D-2 phases of the PCD library of products.

In this initial MALDI-TOF mass spectrometry of PCD library samples, the loci and range of component masses were not known *a priori*. Therefore, internal calibration standards were not added during sample preparation. Prior external calibration was considered adequate for the purposes of this study. Nonetheless, the numerical values that are presented herein (each rounded to its nearest integer) should be interpreted as only approximations.

RESULTS

As determined by MALDI-TOF MS, the PCD spectra of both portions of the D sample showed more than 40 outstanding mass peaks above background noise and isotopic multiplicities. These peaks ranged between about 1500 and 2900 g/mol, with ionic abundance peak heights (a.i.) having *quasi*-Gaussian distributions. The centers of the distributions of prominent peaks were in the neighborhood of 2000 g/mol. The configurations of peak heights above baseline in the product library, as determined by MALDI-TOF MS, were reasonable approximations of the probability statistic's theoretical predictions. The range of molecular masses appeared to be narrower than would be expected from products formed from molecules having 21 equally reactive hydroxyl groups with no steric hindrance factors.

Numbers equaling those of the enumerated peak values of the MALDI-TOF spectra (Figs. 4 and 5) were found in spreadsheet arrays that were prepared for this purpose. These arrays had cells containing theoretical formula weights for molecular ions of potential members of this PCD product library. The equations used to form the arrays were such that they would indicate masses corresponding to all of the theoretically possible combinations of BCD and polymerizable and ligand substituent groups. Each of these peak mass values could represent a large

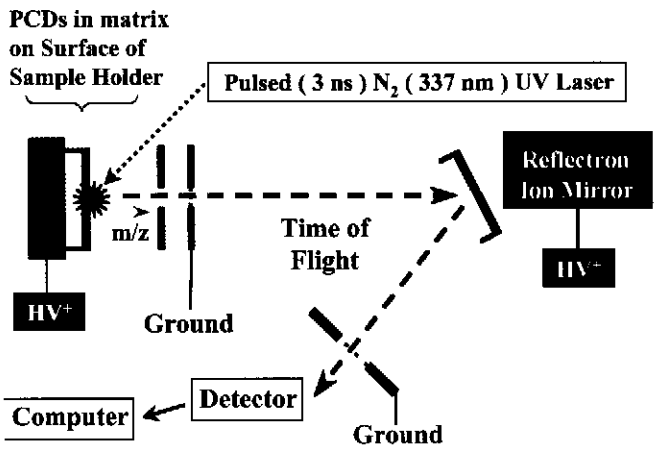

Figure 3. In the MALDI-TOF MS analysis, an ultraviolet laser was used to vaporize the excess matrix molecules containing the embedded molecules of the PCD library. The strong electrical field accelerated the PCD ions toward a detector in proportion to their mass-to-charge ratios (m/z). Smaller ions traveled at higher speeds than did larger ions. The ions were therefore separated during their flights through the field-free region. With appropriate calibration, the equipment's computer program calculated relative mass values from the differential times of the ions' flight to the detector. Thirty-nine shots of 3-ns pulses were used to obtain the enumerated spectrum shown in Fig. 4, and 41 shots were used for that of Fig. 5. The acceleration potential was 25 kV with gridless ion extraction. The effective pathlength, *via* the two-stage gridless reflector, was 3 m to the microchannel detectors.

number of structural permutations of PCD compounds contained in the library. Arrays were prepared and searched for all PCDs ± Na, ± pyridine, ± water, ± 2 (water); PCDs ± Na, ± NMP, ± water, ± 2 (water); PCDs ± Na, ± DBU, ± water, ± 2 (water); PCDs ± Na, ± DHB, ± water, ± 2 (water); etc.

Tables 1 and 2 exemplify some of the likely PCD masses and substituents of ionic members detected and illustrated in Figs. 4 and 5. The mass values (g/mol) used in the equations that formed the spreadsheet arrays and for the "passengers" (components of the *quasi*-molecular ions that are adducts, salts, and/or host-guest complexes) referred to in Tables 1 and 2 were: Na (sodium), 22.99; pyridine, 79.10; NMP (1-methyl-2-pyrrolidinone), 99.13; water, 18.02; DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), 154.24; and DHB (2,5-dihydroxybenzoic acid), 154.12.

From study of the data obtained in the sampling of the compositions, it appeared that the mean masses of the library components sampled here were somewhat lower than predicted. Although 6 mol of methacrylic anhydride plus 6 mol of cyclic glutaric anhydride *per* mol of BCD were used in the synthesis, the analysis suggested that, on average, the product molecules had more methacrylate groups than glutarate substituents. There was a small rise above baseline in the mass region that corresponded to minor but detectable molecular ions of PCD dimers. The 8 peaks that were enumerated in this region had masses that matched predicted values for dimer ions.

DISCUSSION

In the preparation of this PCD library, the numbers of methacrylic anhydride reagent molecules added *per* molecule of BCD were based on probability calculations (Lechner, 1996). Because quantitative reactivity and steric hindrance weighting

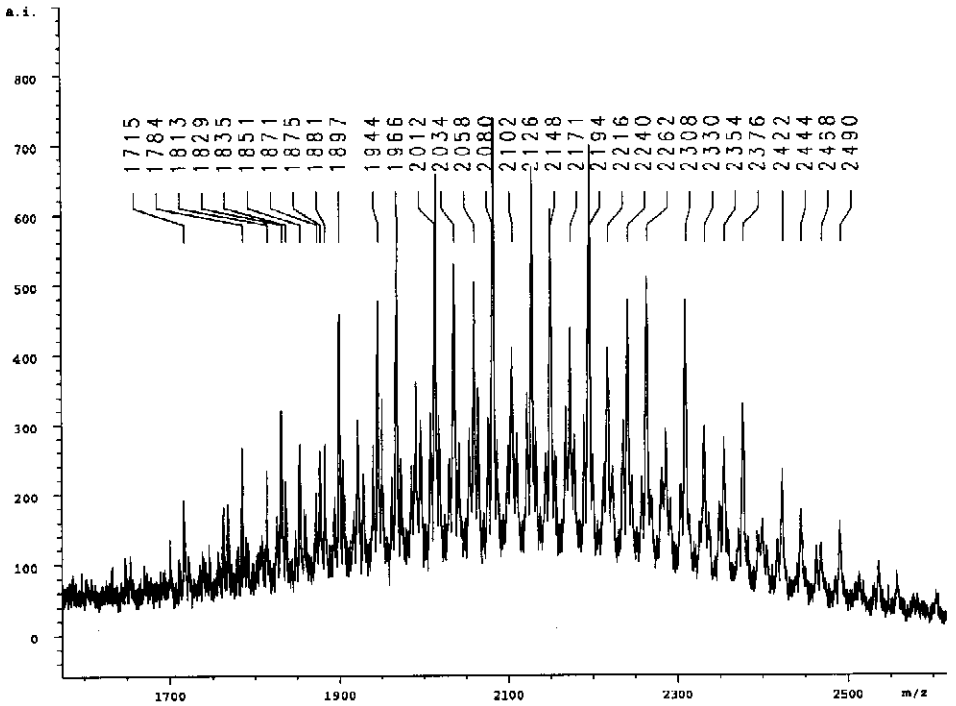

Figure 4. A representative MALDI-TOF MS plot obtained from a sample of the D-1 phase. The peaks indicate approximate masses of ions having one positive charge. The relative abundance of detected ions (a.i.) is plotted against the mass-to-charge ratio (m/z). Shown here are mass values of some of the major peaks, as enumerated by the instrumentation software. The numbers are interpreted as representative approximations of masses of some PCDs (plus "passengers") in this library. Each major peak may represent combinations and many permutations of molecular configurations. Table 1 gives some of the provisional constituents of cations detected during this scan.

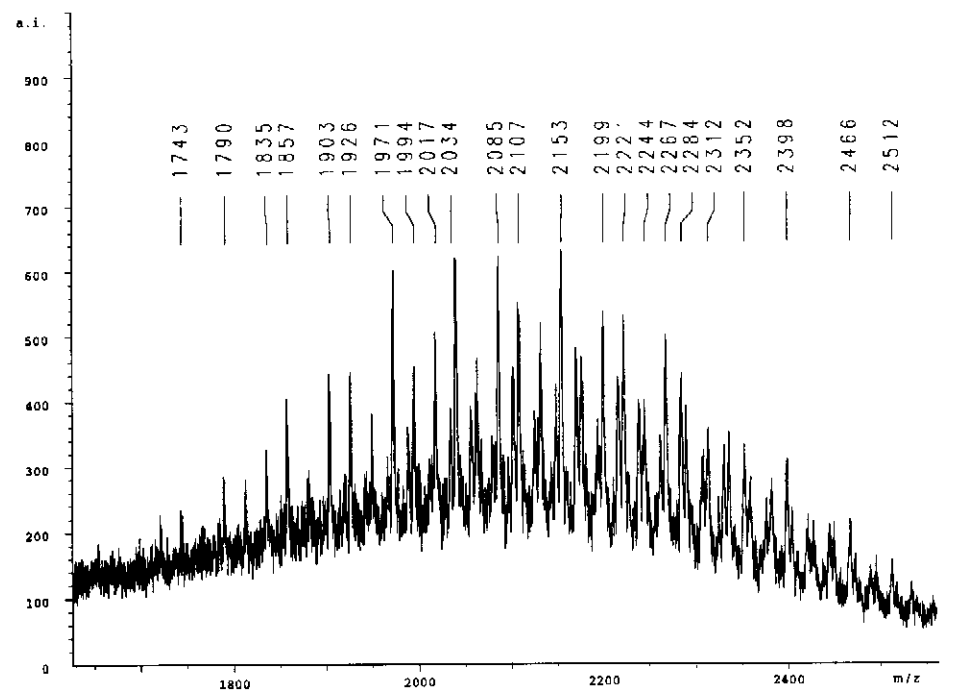

Figure 5. MALDI-TOF MS plot of a sample from the D-2 phase. The enumerated peaks indicate approximate masses of some of the cationic species detected in this scan. The program of the associated instrumentation software provided these mass numbers, which provisionally represent combinations formed in this PCD synthesis. Each peak may represent many permutations of molecular configurations. Table 2 gives constituents that would correspond to cations enumerated here.

factors were not available, these calculations used the simplifying assumption that each of the hydroxyl groups (21 *per* BCD molecule) was equally reactive. The calculations indicated that 5.8 mol of methacrylic anhydride *per* mol of BCD would be needed to ensure, to a high probability (> 0.95), that at least 99% of the product molecules would have 2 or more methacrylate groups bonded to every molecule in the product library. However, the hydroxyl groups on BCD molecules are not all equally reactive. Usually, the 7 primary hydroxyl groups are the most reactive. Next in reactivity are 7 of the secondary hydroxyls (in Fig. 1 or 2, the second carbon atom clockwise from the oxygen atom in the heterocyclic ring). Generally, the least accessible and reactive are the remaining 7 secondary hydroxyl groups (at the 3-position on each heterocyclic ring). Steric hindrance also becomes a factor. These considerations increase the probability that each product molecule would have more than 2 polymerizable groups when 5.8-to-1 molar ratios are used. Impurities, side reactions, and methods of removing solvents and catalysts would influence the proportions of the resultant product libraries.

Therefore, approximately 6 mol of methacrylic anhydride *per* mol of BCD was used, followed by the addition of about 6 mol of cyclic glutaric anhydride *per* mol of the derived BCD. This reaction scheme should lead to a distribution with multiple polymerizable groups and glutaric acid monoester ligand groups on virtually all of the molecules of the product library. These proportions of reagents should yield a library with sufficient carboxyl and residual hydroxyl groups to provide water solubility, enabling its molecules to diffuse through surface hydration and also form multiple bonding interactions with the high diversity of sites on collagen, proteoglycans (Linde, 1985), hydroxyapatite, and/or other substrates. These proportions theoretically yielded a PCD library of thousands of different molecular combinations and permutations to produce

complementary biomimetic shapes and functional groups for docking with the thousands of diverse configurations of receptor sites accessible in demineralized dentin surfaces. Such substrate physicochemical diversity was shown in three-dimensional computer modeling of Type I collagen (Bowen and Reed, 1997). The rationale, therefore, was not to carry out combinatorial chemistry and to analyze for one compound or a very small subset of compounds to interact with one particular biological receptor site. The rationale was to synthesize a prototype library, evaluate it sufficiently to enable subsequent improvements to be made, and then make modifications and adjustments toward the goal of optimization.

The term "combinatorial" has been objected to (Burgess, 1997) unless the resulting complex library mixture can be deconvoluted to the point where the active component(s) can be identified. Therefore, only the term "library" is used here to represent a very large number of combinations and permutations of related compounds (PCDs) for use together (not deconvoluted) in adhesive formulations. This particular library's preparation methods are not meant to represent an optimized protocol; however, useful information was obtained in this study. The MALDI-TOF MS results showed that heterogeneous libraries of surface-active comonomers, such as PCDs, could be prepared. Subject to adhesion test results with optimized PCD libraries and formulations, improved adhesive bonding is expected to result. Further analytical details gained from elemental analyses, nuclear magnetic resonance spectra, Fourier-transform infrared spectroscopy, solubility (miscibility) tests, and other evaluative procedures will be submitted for publication separately.

The hypothesis that will need to be tested is that non-deconvoluted libraries of PCDs can penetrate and bind selectively as if by "biomimetic recognition" to the diverse physicochemical sites within demineralized and mineralized structures of teeth or other substrates. The remaining unbound PCD molecules and comonomers are expected to form cross-linked polymer, filling the remaining nanospaces, and to copolymerize with other resins and composite materials. PCD surface-active comonomers with multiple carboxylate ligand groups would qualify as "multiple-bonding organic coupling agents" (Bowen, 1974).

Several ways of improving syntheses of PCD libraries and MALDI-TOF MS analyses of them emerged from this pilot study:

(1) Syntheses involving anhydride reagents require control of the water content. Compared with the method described here, more rigorous drying should be used for removal of tightly bound water within crystals of BCD. Water content of dried BCD could be determined by the use of near-infrared spectroscopy (Dickens and Dickens, 1999). Adequate provisions are important to avoid uptake of ubiquitous water during the synthesis. To obtain essentially

Table 1. Examples of PCD Masses and Substituents of Enumerations Shown in the Fig. 4 Spectrum

g/mol (Daltons)		BCD Substituents			
PCD Ion[a]	Peak Mass[b]	Methacrylate	Glutarate	Na	Other "Passengers"
1636	1715	4	2	0	Pyridine
1682	1784	3	3	1	Pyridine
1636	1813	4	2	1	DBU or DHB
1750	1829	4	3	0	Pyridine
1658	1835	6	1	1	DBU or DHB
1772	1851	6	2	0	Pyridine
1772	1871	6	2	0	NMP
1796	1875	3	4	0	Pyridine
1704	1881	5	2	1	DBU or DHB
1818	1897	5	3	0	Pyridine
1842	1944	2	5	1	Pyridine
1864	1966	4	4	1	Pyridine
1910	2012	3	5	1	Pyridine
1932	2034	5	4	1	Pyridine
1886	2058	6	3	0	DBU or DHB + water
1956	2058	2	6	1	Pyridine
1978	2080	4	5	1	Pyridine
2000	2102	6	4	1	Pyridine
1954	2126	7	3	0	DBU or DHB + water
2024	2126	3	6	1	Pyridine
2046	2148	5	5	1	Pyridine
1976	2171	9	2	1	DBU or DHB + water
2022	2194	8	3	0	DBU or DHB + water
2114	2216	6	5	1	Pyridine
2068	2240	7	4	0	DBU or DHB + water
2160	2262	5	6	1	Pyridine
2136	2308	8	4	0	DBU or DHB + water
2158	2330	10	3	0	DBU or DHB + water
2228	**2330**	**6**	**6**	1	Pyridine
2182	2354	7	5	0	DBU or DHB + water
2252	2354	3	8	1	Pyridine
2204	2376	9	4	0	DBU or DHB + water
2250	2422	8	5	0	DBU or DHB + water
2320	2422	4	8	1	Pyridine
2272	2444	10	4	0	DBU or DHB + water
2296	2468	7	6	0	DBU or DHB + water
2366	2468	3	9	1	Pyridine
2318	2490	9	5	0	DBU or DHB + water
2388	2490	5	8	1	Pyridine
Mean = 1958	Mean = 2084	Mean = 5	Mean = 4		
n = 40		5/4 = 1.2			

[a] Approximate masses of PCD molecular ions after subtraction of the masses of the other "passengers" from the masses enumerated in the Fig. 4 spectrum.

[b] Examples shown here were all from mass numbers rounded to integer values. The "passengers" were solvents, catalysts, and other species, which apparently were retained and carried along with the *quasi*-molecular cations detected in this sample. The arithmetic mean values represent only those of the columns in this table of these assignments. The numbers in bold font refer to a peak containing permutations of molecules each having 6 methacrylate and glutarate substituents, which was the proportion of reagents used in the synthesis.

the same proportions of resulting substituents in the product libraries as provided by the probability statistic calculations, one should minimize the amount of water present. A small amount of water existing in the solvents or introduced during the addition of the reagents could account for the slightly lower-than-predicted numbers of substituents residing on the library components sampled here.

(2) Pyridine or strongly basic amines may not be the solvents or catalysts of choice for the preparation of BCD derivatives that will contain attached carboxylic acid ligand groups (substituents), unless facile means of removing the amines are available. Removal of pyridine and DBU appeared to be difficult, perhaps due to a combination of salt formation and host-guest molecular containment.

(3) Especially with cyclodextrin derivatives, matrix compounds should be selected that have mass values sufficiently different from those of any compound that might be contained in the analyte. The similarity in the average masses of the matrix DHB (154.12 g/mol) and of DBU (154.24 g/mol) caused uncertainty regarding the presence of DBU complexes in the samples analyzed. However, mass peaks corresponding to DHB complexed within the starting (control) BCD were not observed.

(4) Careful calibration with the use of internal standards during each analysis by MALDI-TOF MS would be desirable to ensure precise and accurate structural determinations of mass distributions within libraries. In retrospect, alpha-cyclodextrin and/or BCD could be used as internal standards, because the product library had no peaks above background at their mass values. Measurement uncertainty with regard to the relative abundance of detected ions (a.i.) was estimated to have a standard deviation of about 10, and, with regard to mass values, about 2. Therefore, the masses, rounded herein to integer values, should be considered as illustrative estimates rather than reference mass units.

Table 2. Examples of PCD Masses and Substituents of Enumerations Shown in the Fig. 5 Spectrum

g/mol (Daltons)		BCD Substituents			
PCD Ion[a]	Peak Mass	Methacrylate	Glutarate	Na	Other "Passengers"
1682*	1743*	3*	3*	0	Pyridine - water
1772	1790	6	2	0	water
1658	1835	6	1	1	DBU or DHB
1816	1857	10	0	1	water
1862	1903	9	1	1	water
1901	1926	8	2	0	water
1930	1971	10	1	1	water
1976	1994	9	2	0	water
1976	2017	9	2	1	water
1862	2034	9	1	0	DBU or DHB + water
2043	2085	10	2	1	water
2044	2107	12	1	1	water
1976	2153	9	2	1	DBU or DHB
2112	2153	11	2	1	water
2158	2199	10	3	1	water
2180	2221	12	2	1	water
2044	2221	10	2	1	DBU or DHB
2226	2244	11	3	0	water
2090	2244	9	3	0	DBU or DHB
2226	2267	11	3	1	water
2090	2267	9	3	1	DBU or DHB
2112	2284	11	2	0	DBU or DHB water
2182	2284	7	5	1	Pyridine
2136	2313	8	4	1	DBU or DHB
2272	2313	10	4	1	water
2250	2352	8	5	1	Pyridine
2180	2352	12	2	0	DBU or DHB + water
2226	2398	11	3	0	DBU or DHB + water
2296	2398	7	6	1	Pyridine
2364	2466	8	6	1	Pyridine
2271	2466	12	3	1	DBU or DHB + water
2340	2512	11	4	0	DBU or DHB + water
2410	2512	7	7	1	Pyridine
Mean = 2030 n = 33	Mean = 2125	Mean = 9	Mean = 3 (9/3 = 3)		

[a] Estimated approximate masses of PCD molecular ions after subtraction of the "passenger" masses from the peak mass values enumerated in the Fig. 5 spectrum. The "passengers" were solvents, catalysts, and other species, which apparently were complexed and carried along with the *quasi*-molecular cations detected in this sample. For some of the peaks, there was more than one combination of components that matched provisory integer mass values. One or more of these combinations could have produced these peaks. In addition, a PCD molecular ion could produce more than one peak by carrying different "passengers". The values marked with asterisks (*) match theoretical values that would correspond to PCDs having an intramolecular glutaric diester moiety. The arithmetic mean values represent only those of the columns in this table of these assignments.

Among other potential mechanisms, one provisional mechanism to account for the small amount of dimer formation is that some of the cyclic glutaric anhydride might have reacted with carboxylic acid groups of nascent glutarate substituents of PCD molecules. Upon ring opening, a glutarate-glutarate anhydride group might have formed. Depending on the manner in which this anhydride group reacted with a hydroxyl group of another PCD molecule, a dimer might have formed. If this reaction mechanism is valid, analogous reactions with by-product methacrylic acid molecules could account for the higher ranking of mean methacrylate groups relative to the mean number of glutarate groups in the sampled spectra (Tables 1 and 2). Future use of a methacrylic acid halide or removal of the by-product methacrylic acid molecules before addition of the cyclic glutaric anhydride reagent might obviate the latter anomaly.

Because very small quantities of analyte are required for analysis by MALDI-TOF MS, future studies could compare spectra of library solutes before and after solutions have been exposed to dentin or other substrate surfaces. Such comparisons might indicate the types of library components least bound, and therefore point toward improved proportions of reagents

and resulting substituents in subsequent products.

In conclusion, the use of MALDI-TOF MS was valuable in characterizing this sample of a PCD library. This form of analysis substantiated the value of probability statistics as a basis for the formation of such libraries. The results also indicated how improvements might be made in the synthesis protocol to yield product libraries that more closely approximate the characteristics of the predictions. The use of MALDI-TOF MS provided helpful insights regarding a number of factors relevant to future PCD preparations and developments.

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Certain commercial materials and equipment are identified in this paper to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by NIST, the FDA, the ADA Health Foundation, or the NIDCR, or that the material or equipment identified is necessarily the best available for the purpose.

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