ELSEVIER

Carbohydrate Polymers 39 (1999) 7–16

Carbohydrate Polymers

Amino acids as plasticizers
II. Use of quantitative structure-property relationships to predict the behavior of monoammoniummonocarboxylate plasticizers in starch–glycerol blends

Thomas M. Stein, Sherald H. Gordon*, Richard V. Greene

Biopolymer Research Unit, National Center for Agricultural Utilization Research, US Department of Agriculture, Agricultural Research Service, 1815 N. University St., Peoria, IL 61604, USA

Received 25 July 1998; received in revised form 17 September 1998; accepted 20 September 1998

Abstract

Twenty natural and synthetic amino acids (5 cyclic and 15 acyclic) were blended with a standard starch–glycerol mixture and extruded as ribbons. Glycerol was present in all blends as a co-plasticizer, permitting observation of both increase and decrease in sample flexibility resulting from amino acids. Mechanical testing of the ribbons revealed that amino acids had a dramatic effect on the percent elongation at break (%E) which varied from 13% to 379%. Tensile strength (TS) of the ribbons also varied considerably from 0.96 to 6.29 MPa. In general, samples displaying the greatest elongation had the lowest TS. FT-Raman spectroscopy indicated that the amino acids in these blends existed predominately as zwitterions. Computational models of all test compounds were therefore generated as zwitterions, and the global minimum-energy conformation of each test compound was used as the basis for calculating molecular descriptors. Surprisingly, only two (sum of absolute values of atomic charges and maximum positive charge on the molecule) of the 17 descriptors evaluated were needed to generate predictive quantitative structure–property relationships (QSPR) for both %E and TS data. By calculating these two descriptors from computer models, %E and TS can be predicted for blends with unknown or new monoamine–monocarboxyl compounds.

1. Introduction

Biodegradable plastics offer a solution to the growing disposal problem of petroleum-based materials, which tend to persist in the environment. Fully biodegradable polymers with useful physical properties are available, but they are usually expensive and therefore not competitive with petroleum-based plastic (Lenz, 1995; US Congress, 1993). Starch, by contrast, is economically competitive with petroleum, and was used in several methods for preparing biodegradable plastics, including blending starch with petroleum-derived materials (Imam et al., 1996; Fritz et al., 1995) or grafting a petroleum-derived chain directly onto the polysaccharide backbone (Fanta & Doane, 1986). Although biodegradation of the petroleum-derived portion of the blends or plastic materials remains minimal (Imam et al., 1996), the starch portion is inherently biodegradable. However, a substantial barrier to the development of starch materials (van Soest & Vliegenthart, 1997; US Congress, 1993; Lenz, 1995; Shogren et al., 1992; Yoo et al., 1995; Khalil et al., 1994; Imam et al., 1996; Fritz et al., 1995; Fanta & Doane, 1986) is the brittle nature of blends with high concentrations of starch.

Overcoming the brittleness of starch, while achieving full biodegradability in blends, can be accomplished with the addition of biodegradable plasticizers (van Soest & Vliegenthart, 1997). Common plasticizers for hydrophilic polymers such as starch are glycerol and other low molecular weight polyhydroxy compounds, polyethers, and urea (Khalil et al., 1994; Shogren et al., 1992). Plasticizers lower water activity, thereby limiting microbial growth (Yoo et al., 1995). By adding a non-toxic, yet effective plasticizer to a starch blend, water activity in the blend is lowered, offering potential control of the biodegradation rate. In a previous study (Stein & Greene, 1997), we found the amino acid, proline, to be an exceptionally good plasticizer of starch-based extruded materials. An advantage of proline over common plasticizers such as urea is its low toxicity.

In this study, to identify the structural features which

* Corresponding author.

0144-8617/99/$ - see front matter
PII: S0144-8617(98)00136-2

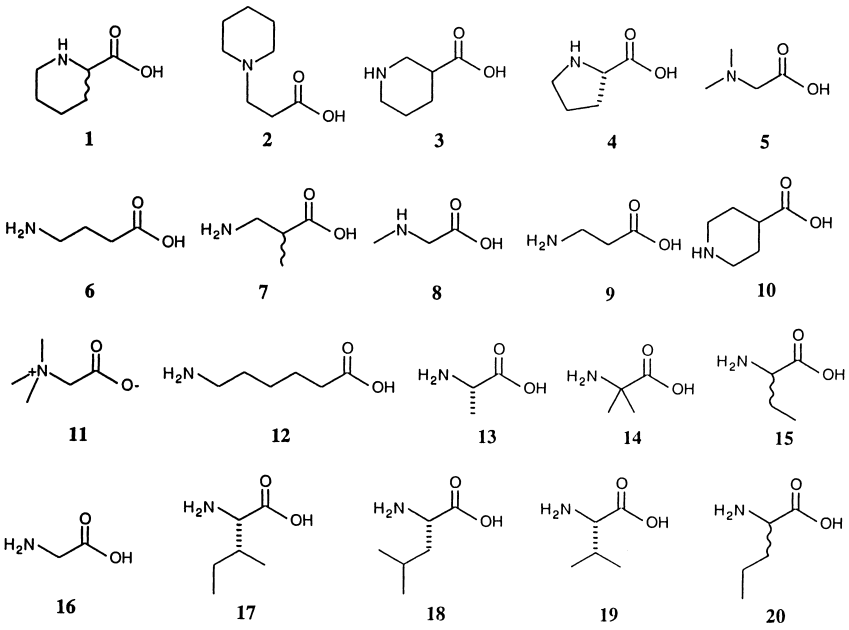

Fig. 1. Amino acids compared in this study and used to generate QSPRs between structure and %E or TS.

make proline an effective starch plasticizer, we tested twenty commercially available amino acids, specifically acyclic and monocyclic monoamino monocarboxylic acids containing 2–8 carbons. Despite these strict structural limitations, many unique geometries are possible. Mechanical testing of blends prepared with these natural and synthetic amino acids, combined with three-dimensional structural models, provided the basis for quantitative structure–property relationships (QSPR) (Murugan et al., 1994; Stanton & Jurs, 1990, Katritzky et al., 1995). This is essentially the same technique as that used to develop quantitative structure–activity relationships (QSAR) which are important in molecular biology, immunology, and pharmacology for designing new molecules with predictable activities. The idea behind QSAR is that structure–activity relationships of a set of compounds can be accounted for by their relative differences in hydrophobic, electronic, stearic, and other molecular properties. The objective is to make and test new compounds with properties predicted from correlations in the explored data set (Hansch & Leo, 1995).

2. Experimental section

2.1. Materials

Buffalo Corn Starch was from CPC International (Argo, Illinois, USA). Glycerol was from Fisher Scientific (Pittsburgh, Pennsylvania, USA). The amino acids glycine, L-isoleucine, and DL-pipecolic acid were from Sigma Chemical Company (St. Louis, Missouri, USA). Remaining amino acids, β-alanine, L-alanine, DL-2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoisobutyric acid, DL-3-aminoisobutyric acid, betaine, *N,N*-dimethylglycine, isonipecotic acid, L-leucine, nipecotic acid, DL-norvaline, 1-piperidinepropionic acid, L-proline, sarcosine, and L-valine, were from Aldrich (Milwaukee, Wisconsin, USA).

2.2. Extrusion of test blends

A standard mixture of starch (480 g, $\sim$ 8% moisture) and glycerol (120 g) was prepared by using a Kitchen Aid mixer (3 min on stir setting, 7 min on setting 2) after initially combining these components by hand. Amino acid (10 g) was blended with 50 g of the standard starch-glycerol mixture. Moisture content was determined with an MA30 moisture analyzer (Sartorius AG, Germany) and adjusted to 15% $\pm$ 2%. Thin ribbons of these blends were prepared with a Randcastle 1/2″ single screw extruder fitted with a 2 : 1 compression screw and a 25 $\times$ 1.0 mm thin ribbon die having a land length of 20 mm. The three-zone barrel of the extruder was heated to 100°C, 115°C, 100°C, and the die to 100°C. The screw speed was 30 rpm. Each of the 20 amino acids shown in Fig. 1 was added as potential plasticizers by blending with a standard starch–glycerol mixture. Each blend was extruded and prepared for testing as described later.

2.3. Mechanical testing

Tensile strength (TS) and percent elongation (%E) at break were determined according to ASTM method D638M. Dogbone-shaped test specimens (type M-II with overall dimensions of 25 $\times$ 115 mm and a narrow section length of 33 mm and a width of 6 mm) were cut with a punch press (NAEF model B) from the extruded ribbons

and equilibrated in a humidity- and temperature-controlled room (50% relative humidity, 25°C) for 10 days. Specimen thickness was determined by averaging three measurements along the test length with a Minitest 3001 micrometer (Elektro-Physik, Köln, Germany). TS and %E were measured on triplicate samples (1–3 replicates) with an Instron 4201 universal testing machine (grip distance of 80 mm, crosshead speed of 20 mm/min, data collection rate of 20 points/s).

2.4. Raman spectroscopy

FT-Raman spectra were measured with a Bio-Rad (Digilab Division, Cambridge, MA) FTS 6000 spectrometer and Raman accessory equipped with a liquid nitrogen-cooled Germanium detector. The excitation source was a Nd : YAG laser operating at 1064 nm, and the laser beam with 600 mW of power was focused onto the samples. Laser line rejection was accomplished with a holographic notch filter. Samples were mounted flush against aluminum foil backing (which reflected transmitted laser light back into the sample) and illuminated with 180° scattering optics to maximize the Raman signal-to-noise ratio. Spectra were acquired by in-scan co-addition of 500 scans at a resolution of 4 cm^{-1}.

2.5. Computational methods

HyperChem (HyperCube, Waterloo, Ontario, Canada) and SciQSAR (SciVision, Lexington, MA, USA) were run on a Gateway 200 MHZ personal computer. The force field chosen was MM+, containing force field parameters of MM2 (Allinger, 1977). A constant dielectric of $\epsilon = 1.5$ was used for MM+ calculations.

Low energy conformers were determined by randomly varying dihedral angles of the zwitterionic forms of the amino acids. Using the Conformational Search module of the ChemPlus add-on program for HyperChem v4.5, a usage-directed search method collected energy-minimized structures which were a maximum of 6 kcal above the lowest energy conformation. Pre-optimization tests rejected structures with non-bonded atoms separated by less than 0.5 Å or torsion angles within 15° of a previous conformation. Duplication tests included rejecting conformations of energy within 0.05 kcal/mol, torsions within 5°, or RMS error within 0.25 Å when compared to previous conformations. Protons were ignored in the RMS fit and equivalent atom orders were used when possible. Optimization was determined by an RMS gradient of 0.01 kcal/Å mol, allowing a maximum of 1000 cycles. The random seed number 12345 (default value) was used to control variation of torsional angles. Limits for searches were set at 10 000 iterations and 500 optimizations. Fewer than 100 conformations were kept. The lowest energy conformation was chosen as the basis for QSPR generation.

Structures were then assembled into a SciQSAR database, reminimized with MM+ and a single point calculation was performed with the semi-empirical method PM3 to appropriately distribute charges in the zwitterionic compounds. The minimization algorithm was Polak-Ribiere. MM+ parameters included a 0.1 kcal/Å mol gradient, bond dipoles, and no cutoffs (in vacuo). PM3 parameters included a total charge of 0, spin multiplicity of 1, and spin paring RHF.

2.6. QSPR molecular descriptors

The SciQSAR program calculated 14 descriptors of the MM+/PM3 treated HyperChem structures: *Weiner Index*, ovality, *dipole*, polarizability, *volume*, molecular weight, *sum of absolute values of atomic charges* (ABSQ), *sum of absolute charges on nitrogen and oxygen atoms, lowest unoccupied molecular orbital*, highest occupied *molecular orbital*, maximum partial *positive charge on the molecule* (MaxQp), *maximum positive charge on an H atom in the molecule*, maximum partial *negative charge on the molecule*, and specific *polarizability*. In addition to the 14 calculated descriptors, three user-defined descriptors were imported before the development of the QSPR. Two of these user-defined descriptors, surface area and hydration energy, were calculated with the QSAR module in ChemPlus. Values calculated by ChemPlus were imported into the SciQSAR program using an Excel Visual Basic macro. Published octanol partition coefficient (log P) values (Hansch et al., 1995) were also included as the third imported descriptor.

2.7. QSPR generation

The QSPR parameters (dependent variables), %E and TS, were fitted to the descriptors (independent variables) by multiple linear regression in separate databases. QSPR were generated by first removing descriptors that showed little correlation ($R^2 < 0.04$) with the QSPR parameter. Next, high cross correlations ($R^2 > 0.85$) between descriptors were resolved by retaining the descriptor with the higher product of QSPR parameter and correlation equation coefficient. Improvements in statistical parameters justified removal of subsequent descriptors. The remaining descriptors were then removed on a trial-and-error basis to determine their influence on the fit, error and *F*-test. Descriptors that were earlier removed were then examined for their effect on the statistics of the newly generated relationships. None had a substantial positive effect, either with the individual descriptors or the combination, that would justify inclusion in the equation.

2.8. QSPR validation

To assess the validity of each of the QSPR, six of the test compounds were chosen at random (L-proline, *N,N*-dimethylglycine, 4-aminobutyric acid, DL-2-aminobutyric acid, L-isoleucine, and DL-norvaline), removed from the database, and new QSPR were derived. QSPR parameters

Table 1

	%E exp	Relative %E	TS exp	Relative TS	Plasticizer effectiveness
1 DL-pipecolic acid	379	10.1	1.14	0.18	Good
2 1-piperidinepropionic acid	290	7.7	0.96	0.15	
3 Nipecotic acid	222	5.9	1.46	0.23	
4 L-proline	149	4.0	1.53	0.24	
5 *N,N*-dimethylglycine	83	2.2	2.50	0.40	Moderate
6 4-aminobutyric acid	61	1.6	2.01	0.32	
7 DL-3-aminoisobutyric acid	61	1.6	2.36	0.38	
8 Sarcosine	59	1.6	2.53	0.40	
9 β-alanine	52	1.4	3.10	0.49	
10 Isonipecotic acid	48	1.3	1.85	0.29	Non-effective
11 Betaine	42	1.1	1.79	0.28	
12 6-aminocaproic acid	38	1.0	1.90	0.30	
0 Starch–glycerol control	37	1.0	6.29	1.00	
13 L-alanine	27	0.71	5.19	0.83	
14 2-aminoisobutyric acid	25	0.66	3.91	0.62	
15 DL-2-aminobutyric acid	21	0.55	3.57	0.57	Poor
16 Glycine	19	0.50	4.90	0.78	
17 L-isoleucine	17	0.44	4.29	0.68	
18 L-leucine	16	0.43	3.78	0.60	
19 L-valine	16	0.43	3.66	0.58	
20 DL-norvaline	13	0.43	3.47	0.55	

for this set of six removed compounds (the validation set) were then recalculated based on the QSPR of the reduced set and compared with parameters from the full set. As a second test of the QSPR validity, %E and TS values were randomly reassigned among the compounds, effectively destroying the correlations. Statistical significance of the QSPR were computed by analysis of variance (ANOVA) using the *F*-test for multiple regression, where *R* is the multiple correlation coefficient, *n* is the number of compounds, and *m* is the number of descriptors.

$$F = \left(\frac{R^2}{1 - R^2}\right)\left(\frac{n - m - 1}{m}\right).$$

3. Results

3.1. General appearance of blends

Of the 20 blends tested, those with greater elongation than the control starch–glycerol mixture were generally translucent, whereas those with lesser elongation were opaque. β-Alanine was an exception in that it had a substantial amount of dusty bloom that appeared as blotches covering the majority of the sample surface. Although the melting point of β-alanine (205°C) is substantially above the extrusion temperature (130°C), the combined conditions of heat, moisture and shear forces within the extruder likely lowered the melting point of β-alanine, allowing it to migrate to the surface where it solidified.

3.2. Mechanical test results

Mechanical testing was conducted with extruded ribbons equilibrated at 50% relative humidity. The data of Table 1 show that the test compounds had highly variable effects on %E at break and TS of the starch–glycerol control (%E = 37%, TS = 6.29 Mpa). When %E was used as a measure of plasticization, the test compounds could be placed into four groups: good ($>$ 100 %E), moderate (50–100 %E), non-effective (24–50 %E), and poor ($<$ 24 %E). The non-effective range was defined by one standard deviation from the %E of the control.

Pipecolic acid had the greatest effect on %E, increasing the elongation of the starch–glycerol sample 10-fold. Piperidinepropionic acid was nearly as effective, increasing elongation nearly eight-fold. Nipecotic acid and L-proline were also good plasticizers, increasing elongation six-fold and four-fold, respectively. *N,N*-dimethylglycine was a moderate plasticizer, more than doubling the elongation of the standard blend. 4-Aminobutyric acid, DL-3-aminoisobutyric acid and sarcosine had nearly identical effects on %E (1.6-fold). β-alanine increased elongation 1.4-fold over the standard mixture, but still qualified as a moderate plasticizer. Remarkably, isonipecotic acid, despite its structural similarity to nipecotic acid, was a non-plasticizer, increasing elongation only 1.3-fold. Betaine, 6-aminocaproic acid, L-alanine and 2-aminoisobutyric acid also had little effect on elongation. DL-2-Aminobutyric acid, glycine, L-isoleucine, L-leucine, L-valine and DL-norvaline decreased the %E of the standard mixture in the range of one-half to one-third, all classifying as poor plasticizers.

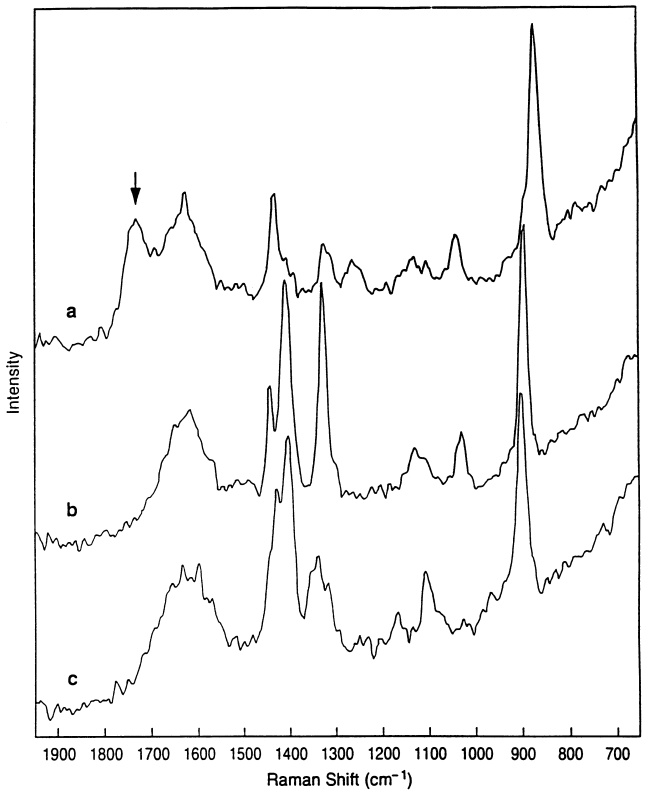

Fig. 2. FT-Raman spectra of 1 M glycine solutions at: (a) pH 1; (b) pH 7; and (c) pH 12. Deprotonation of the carboxyl group is detected by the disappearance of the peak at 1740 cm^{-1} (designated by arrow) in traces b and c.

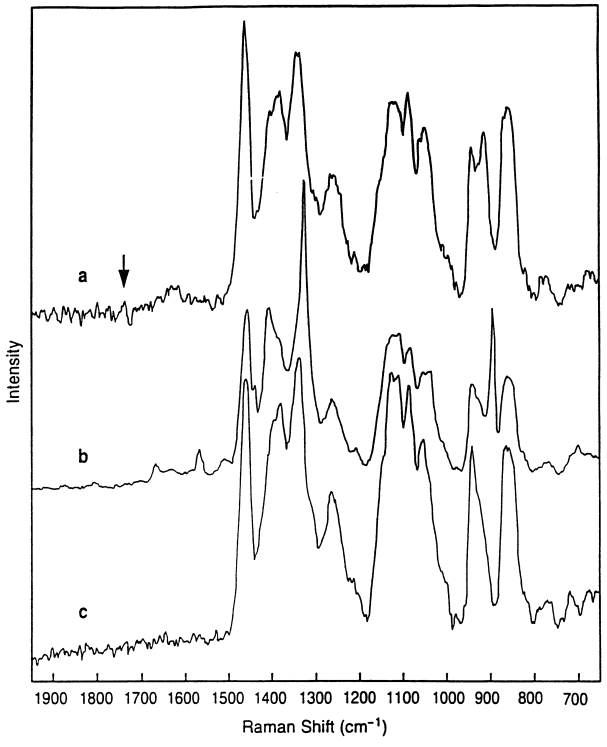

Fig. 3. FT-Raman spectra of extruded blends: (a) starch–glycerol blend containing proline, (b) starch–glycerol blend containing glycine and (c) starch–glycerol control. Arrow marks 1740 cm^{-1}.

All test compounds lowered the TS of the standard mixture (6.29 MPa), with alanine and glycine having the least effect and thereby producing the strongest samples. The remaining compounds followed a trend in which samples displaying greater elongation generally possessed lower TS.

3.3. Raman spectroscopy

To establish spectral changes that occurred after ionization of functional groups in amino acids, aqueous glycine solutions (1 M) were prepared, varying from pH 1 to pH 12, and FT-Raman spectra of the solutions were collected (Fig. 2). Disappearance of the carbonyl stretching band at 1740 cm^{-1} and appearance of carboxylate bands (1330 and 1410 cm^{-1}) correlated with the deprotonation of the carboxyl group between pH 2 and pH 3. FT-Raman spectra were then collected on samples of two of the solid starch-based blends (Xue, 1997). Absence of the 1740 cm^{-1} band in the solid glycine-containing and proline-containing blends (Fig. 3) confirmed the absence of the protonated carboxyl group and indicated the deprotonated monoammonium monocarboxylate was the dominant form of the test molecules.

Direct observation of the ammonium form of the compounds (absorption bands centered at 1450 and 1050 cm^{-1} would be expected) was not possible because of large interfering bands from starch (Fig. 3). As compounds were added in their overall electrically neutral (isoelectric) forms, evidence of a carboxylate implies the presence of a proton acceptor. An amine group, capable of proton exchange, was present in each test compound. Betaine is an exception in that it contains a quaternary amine and a carboxylate with no exchangeable proton. The amines of the remaining test compounds are more basic than the hydroxyl groups of either starch or glycerol and, therefore, represent the most reasonable proton acceptors in the blends, implying that the amino acids in the blends existed predominately as zwitterions. Further support comes from ab initio calculations showing that when glycine is hydrated with at least two water molecules, the zwitterion form predominates. This level of hydration would be possible in a 60 g sample which, at 15% moisture, would contain 500 mmol water and 120 mmol glycerol relative to 60–130 mmol amino acid (Jensen & Gordon, 1995).

3.4. Molecular modeling and descriptor calculation

Computational models of all test compounds were thus generated as zwitterions. Low energy global minimum conformations were determined by randomly varying the dihedral angle containing the carboxyl carbon and extending to the γ-carbon or, if no γ-carbon was present, the first branching carbon on the secondary or tertiary amine. The resulting conformers were reminimized and the lowest energy conformer was accepted as the global minimum-energy conformation. Cyclic compounds and those without

Table 2
Abbreviations: Surface area (SurfA), hydration energy (HydrE), Partition coefficient (LogP), Wiener Index (WienI), specific polarizability (Sp. Pol), volume-dependent polarizability (Polar), maximum partial positive charge (MaxQp), maximum partial positive charge on H atoms (MaxHp), sum or absolute values of atomic charges (ABSQ), sum of absolute charges on N and O atoms (ABSQon), maximum partial negative charges (MaxQn), highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

	SurfA	HydrE	Log P	Volume	WienI	Ovality	MW	Sp. Pol	Dipole	Polar	MaxQp	MaxHp	ABSQ	ABSQon	MaxQn	HOMO	LUMO
1 DL-pipecolic acid	300	− 1.16	− 2.31	125.5	89	1.377	129.2	0.0915	13.6	11.48	0.682	0.122	3.87	1.77	− 0.641	− 8.26	− 0.714
2 1-piperidinc-propionic acid	357	0.76	− 2.45	158.7	175	1.471	157.2	0.0955	20.9	15.15	0.734	0.142	4.99	1.90	− 0.667	− 7.14	− 1.125
3 Nipecotic acid	298	− 1.55	− 2.89	125.4	89	1.370	129.2	0.0916	19.5	11.48	0.663	0.125	3.95	1.82	− 0.637	− 7.24	− 1.542
4 L-proline	277	− 1.50	− 2.50	108.9	63	1.345	115.1	0.0886	13.7	9.65	0.682	0.118	3.63	1.77	− 0.639	− 8.25	− 0.772
5 *N*,*N*-dimethyl-glycine	265	− 0.09	− 2.19	101.8	49	1.351	103.1	0.0844	13.6	8.59	0.774	0.149	4.10	1.87	− 0.640	− 8.27	− 0.586
6 4-aminobutyric acid	280	− 5.30	− 3.17	102.2	53	1.381	103.1	0.0840	24.3	8.59	0.753	0.115	3.40	1.95	− 0.683	− 6.67	− 2.416
7 DL-3-amino-isobutyric acid	273	− 4.53	No data	102.3	47	1.359	103.1	0.0840	14.4	8.59	0.756	0.133	3.23	1.91	− 0.649	− 7.87	− 1.349
8 Sarcosine	249	− 1.59	− 2.78	86.0	33	1.317	89.1	0.0785	13.8	6.75	0.707	0.110	3.32	1.79	− 0.637	− 8.30	− 1.011
9 β-alanine	251	− 5.51	− 3.05	85.3	33	1.317	89.1	0.0492	19.3	6.75	0.731	0.135	3.09	1.88	− 0.658	− 7.33	− 1.892
10 Isonipecotic acid	301	− 1.60	− 3.05	125.5	89	1.380	129.2	0.0915	21.3	11.48	0.677	0.128	4.01	1.85	− 0.654	− 7.00	− 1.746
11 Betaine	284	0.62	No data	118.8	67	1.374	117.1	0.0877	13.9	10.42	0.782	0.162	4.71	1.89	− 0.631	− 8.12	− 0.489
12 6-aminocaproic acid control	339	− 4.53	− 2.95	136.6	115	1.487	131.2	0.0897	36.2	12.26	0.752	0.121	3.87	1.99	− 0.700	− 5.78	− 3.170
13 L-alanine	243	− 4.73	− 2.96	85.2	30	1.302	89.1	0.0792	13.0	6.75	0.772	0.120	2.98	1.85	− 0.634	− 8.37	− 0.959
14 2-amino-isobutyric acid	264	− 4.00	No data	102.4	43	1.350	103.1	0.0839	13.3	8.59	0.753	0.122	3.18	1.86	− 0.581	− 8.25	− 0.816
15 DL-2-amino-butyric acid	272	− 3.71	− 2.53	102.3	47	1.348	103.1	0.0839	13.5	8.59	0.763	0.125	3.28	1.87	− 0.581	− 8.25	− 0.888
16 Glycine	219	− 5.57	− 3.21	68.6	19	1.264	75.1	0.0717	13.2	4.92	0.799	0.110	2.82	1.88	− 0.633	− 8.38	− 1.121
17 L-isoleucine	315	− 3.05	− 1.72	135.8	93	1.431	131.2	0.0903	12.6	12.26	0.765	0.114	3.55	1.85	− 0.637	− 8.37	− 0.853
18 L-leucine	320	− 2.67	− 1.52	135.9	97	1.447	131.2	0.0902	12.9	12.26	0.768	0.117	3.61	1.85	− 0.637	− 8.36	− 0.830
19 L-valine	290	− 3.57	− 2.26	118.9	66	1.393	117.1	0.0877	12.6	10.42	0.767	0.114	3.38	1.85	− 0.637	− 8.37	− 0.860
20 DL-norvaline	301	− 3.87	− 2.11	119.2	71	1.416	117.1	0.0874	12.7	10.42	0.774	0.123	3.34	1.85	− 0.623	− 8.34	− 0.994

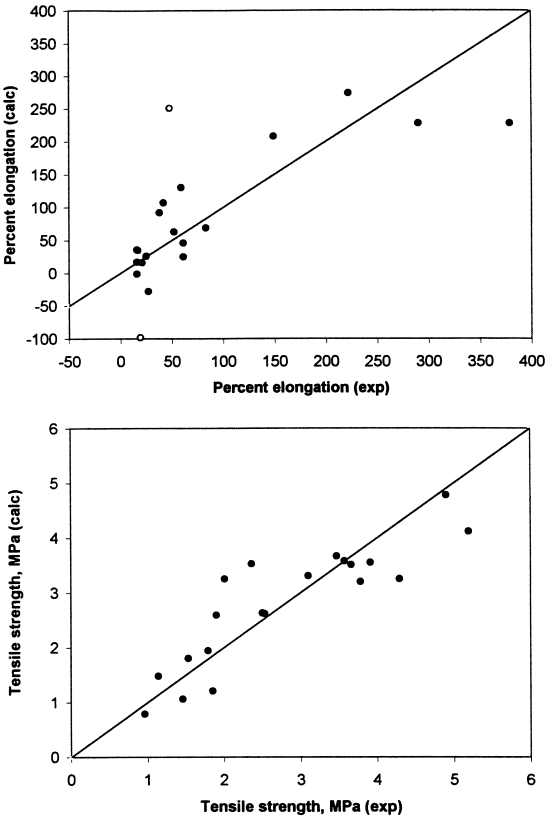

Fig. 4. Plots of calculated versus measured %E (top) and TS (bottom). Calculated values were based on QSPR. Isonipecotic acid and glycine (open symbols, top) were significant outliers and were not included in the %E QSPR. The lines have a slope of one.

branching at either the β-carbon or at the amine were energy-minimized and used in QSPR calculations with a less than exhaustive conformational search as their limited flexibility was expected to produce little change in the molecular descriptors. Numerical values of the descriptors for each amino acid are provided in Table 2.

The global minimum-energy conformation of each test compound was used as the basis for calculating descriptors (Table 2) upon which QSPR were built. Descriptors were calculated with SciQSAR as well as the ChemPlus module of HyperChem.

Table 3

SPR parameter	Coefficients[a] n	a	b	c	RMSD	R^2	F
%E	20	837	71.17	−1360	66.89	0.54	9.96
%E[b]	18	1220	90.29	−1970	52.98	0.73	20.51
%E[c]	12	1430	88.81	−2240	61.16	0.73	12.19
%E[d]	18	−343	8.52	537	100.62	0.03	0.27
TS	20	−4.12	−1.36	15.92	0.59	0.76	27.24
TS[b]	18	−5.27	−1.37	17.49	0.60	0.73	20.32
TS[c]	14	−3.26	−1.41	15.11	0.55	0.82	25.92
TS[d]	20	−2.89	0.02	7.59	1.20	0.05	0.42

[a] Coefficients in the equation, y = a + b ABSQ + c MaxQp where y = QSPR parameter (%E or TS).
[b] Outliers (Isonipecotic acid and glycine) removed from data set.
[c] Reduced data set.
[d] QSPR parameter was randomly reassigned.

3.5. QSPR generation

In the process of creating separate QSPR for %E and TS, appropriate descriptors were selected to minimize cross-correlations and thereby minimize the number of descriptors. Models with three or more descriptors showed little improvement in goodness-of-fit over two-descriptor models, and single-descriptor models were decidedly worse. Hence, the 17 molecular descriptors available were filtered to two descriptors.

Generated in this manner, both %E and TS QSPR were best modeled using the sum of absolute values of atomic charges (ABSQ) and the maximum partial positive charge on the molecule (MaxQp) as descriptors:

$$\%E = 1220 + 90.29\text{ABSQ} - 1970\mathit{MaxQp}$$

$$(n = 18;\ \text{RMSD} = 52.98;\ R^2 = 0.73;\ F = 20.51 > F_{0.05(1),2,15} = 3.68)$$

$$TS = -4.12 - 1.36\text{ABSQ} + 15.92\text{MaxQp}$$

$$(n = 20;\ \text{RMSD} = 0.59;\ R^2 = 0.76;\ F = 27.24 > F_{0.05(1),2,17} = 3.57)$$

Comparison of measured and calculated %E and TS values is shown graphically in Fig. 4.

Isonipecotic acid and glycine were significant outliers in the %E data set (Fig. 4). Removal of these compounds resulted in a dramatic increase in correlation coefficient (0.54–0.73) for the %E QSPR. Their removal from the TS data set produced a modest change in correlation coefficient (0.76–0.73). The TS QSPR was therefore based upon the full set of twenty compounds.

3.6. QSPR validation

Both randomization of the QSPR parameter and random structure removal were performed to confirm the suitability of the QSPR equations. Resulting QSPR are summarized in Table 3. When the QSPR parameter (%E or TS) was randomized, correlation between the QSPR parameter and the QSPR model was abolished (Table 3). Random removal of six of the compounds from each of the test sets generated new QSPR equations. Statistics for %E remained unchanged (0.73) as a result of the reduced set, whereas the correlation coefficient in the TS equation improved (0.76–0.82). QSPR of the reduced sets adequately predicted %E and TS of the removed compounds (validation sets, Table 4). The generally accepted validation criterion of five compounds for every descriptor in the QSPR was

Table 4

	%E exp (SD)	%E calc	%E calc (validation set)	TS exp (SD)	TS calc	TS calc (validation set)
1 DL-pipecolic acid	379 (42)	228		1.14 (0.05)	1.48	
2 1-piperidineproponic acid	290 (22)	228		0.96 (0.06)	0.79	
3 Nipecotic acid	222 (13)	274		1.46 (0.17)	1.06	
4 L-proline	149 (15)	208	166	1.53 (0.11)	1.80	1.90
5 *N,N*-dimethylglycine	83 (3)	69	74	2.50 (0.04)	2.63	2.63
6 4-aminobutyric acid	61 (5)	46	52	2.01 (0.07)	3.25	3.31
7 DL-3-aminoisobutyric acid	61 (19)	25		2.36 (0.15)	3.53	
8 Sarcosine	59 (8)	130		2.53 (0.23)	2.62	
9 β-alanine	52 (8)	63		3.10 (0.03)	3.31	
10 Isonipecotic acid	48 (10)	251		1.85 (0.19)	1.21	
11 Betaine	42 (8)	107		1.79 (0.26)	1.94	
12 6-aminocaproic acid	38 (12)	92		1.90 (0.17)	2.59	
0 Starch–glycerol control	37 (13)			6.29 (0.59)		
13 L-alanine	27 (2)	−28		5.19 (0.32)	4.12	
14 2-aminoisobutyric acid	25 (4)	26		3.91 (0.63)	3.55	
15 DL-2-aminobutyric acid	21 (2)	16	30	3.57 (0.48)	3.58	3.64
16 Glycine	19 (3)	−98		4.90 (0.70)	4.78	
17 L-isoleucine	17 (4)	35	46	4.29 (0.46)	3.25	3.29
18 L-leucine	16 (5)	36		3.78 (0.83)	3.20	
19 L-valine	16 (4)	17		3.66 (0.33)	3.51	
20 DL-norvaline	13 (3)	−1	19	3.47 (0.61)	3.67	3.74

exceeded, even for the reduced data sets (12 or 14 compounds per 2 descriptors). *F*-test values (20.51 for %E and 27.24 for TS) comfortably exceeded their critical values (3.68 for %E and 3.57 for TS), proving that the QSPR were statistically significant. In fact, a multiple ANOVA rejected the null hypotheses (no dependence of %E or TS on ABSQ and MaxQp) with 95% confidence.

4. Discussion

Many of the amino acids in this study functioned as plasticizers; that is, their addition to the standard starch-glycerol mixture increased the flexibility of the extruded blends. While glycerol is a common plasticizer for polysaccharides, its chief role in this study was to produce a starch-based ribbon that was flexible enough to detect both increases and decreases in flexibility resulting from incorporation of the amino acids. Test compounds that decreased the flexibility of the standard mixture were not classified as antiplasticizers, because TS was not increased as is typical of antiplasticization.

When subsets of the tested amino acids are compared, some generalizations regarding the effect of molecular structure on plasticizer effectiveness can be made. For instance, branching at nitrogen appears to be optimally effective when the amine is tertiary, rather than primary, secondary or quaternary. This is apparent when *N,N*-dimethylglycine (83%), glycine (19%), sarcosine (59%), and betaine (42%) are compared (parenthetic numbers represent %E). Branching at the α-carbon has a strong influence on plasticizer behavior. Alanine (27%) is a better plasticizer than glycine (19%). It is also better than other tested molecules with more branching at the α-carbon (proline is an expected exception as discussed in the next paragraph). The series of glycine (19%), β-alanine (52%), 4-aminobutyric acid (61%), and aminocaproic acid (38%) provides insight pertaining to the optimal distance between amine and carboxyl groups in acyclic amino acids. Although 5-aminopentanoic acid was not tested, the optimal separation between functional groups would presumably be three or four methylene units.

Amino acids which contain ring structures unequivocally function as the best plasticizers and a six-membered ring is favored over a five-membered ring as demonstrated by comparing pipecolic acid (379%) with proline (149%). The effect of nitrogen position within the six-membered ring is shown by pipecolic acid (379%), nipecotic acid (222%) and isonipecotic acid (48%). %E drops as the amine is further removed from the carboxyl group. This drop is so pronounced in isonipecotic acid (48%) that this compound appears as an outlier on the %E plot. The anomalous behavior of isonipecotic acid is likely related to the linear arrangement of its ionic groups, permitting a particularly favorable orientation in the solid state. This is supported by the fact that isonipecotic acid has the highest melting point in the series: DL-pipecolic acid (282°C), nipecotic acid (261°C) and isonipecotic acid ($>$ 300°C).

Unlike the charged amino acids in this study, common industrial plasticizers are neutral, relatively hydrophobic molecules used in conventional petroleum-based plastics, especially polyvinylchloride (Sears & Darby, 1982; Wilson, 1995). The free volume theory of plasticization (Wilson, 1995; Barton, 1991) suggests that the small molecules are

more efficient plasticizers and their polarity should resemble the polymer for adequate miscibility (Sears & Darby, 1982). Solubility parameters have proved useful in estimating the miscibility of polymers and plasticizers (Barton, 1991; Brandrup & Immergut, 1966; Tomka, 1990). Additive schemes were devised to predict solubility parameters of small molecules based on their chemical structure but unfortunately these schemes are not appropriate for charged or extensively hydrogen-bonded molecules such as amino acids (Brandrup & Immergut, 1966).

QSPRs offer excellent predictability (Hansch & Leo, 1995; Katritzky et al., 1995) and are not limited to neutral molecules. Predictions of chemical and physical properties based on models of charged compounds can be readily incorporated into such relationships. In the present study, QSPRs were created for each of the two QSPR parameters: %E and TS. Surprisingly, both contained only the descriptors ABSQ and MaxQp. Relationships based solely on these two descriptors were predictive over a wide range of %E (13%–379%) and TS (0.96–6.29 MPa). Equally surprising was the poor correlation between plasticizer behavior and log P, despite the wide applicability of log P values in modeling the effects of hydrophobicity on physical and physiological behavior (Katritzky et al., 1995). Partial charge characteristics (Stanton & Jurs, 1990) were thus more important than hydrophobicity in determining plasticizer behavior.

Generally, as plasticizer concentration increases, %E increases and TS decreases. This inverse correlation between %E and TS can be seen in the fact that both QSPR equations contain the same two descriptors, ABSQ and MaxQp, but with coefficients of opposite sign. The absence of a corresponding maximum of partial negative charges descriptor (MaxQn) was a result of all compounds in the set having similar environments about their carboxyl groups. By contrast, many of the skeletal changes involved alteration in the amines. As indicated by the relative magnitudes of the coefficients of ABSQ and MaxQp, distribution of the positive charge was the main factor in determining strength and flexibility of each blend. Aliphatic groups on ammonium cations tend to disperse the positive charge, making the cation larger and more easily hydrated.

With the increasing interest in biodegradable plastics based on starch, a range of plasticizer options for starch can allow us to tailor blends for various applications. Plasticization by water was observed in biopolymers such as polysaccharides (Roos, 1993; Roos & Karel, 1991; Slade et al., 1993; Kalichevsky et al., 1993), elastin (Lillie & Gosline, 1990), caseinates (Le Meste et al., 1990; Kalichevsky et al., 1993) and PHBV (Harrison et al., 1992), synthetic polymers such as nylon (Ellis, 1988) and PVOH (Hodge et al., 1996). Plasticization by amino acids could be a similar general phenomenon for hydrophilic polymers, and hence become particularly critical for the emerging field of biodegradable polymers.

5. Conclusions

Amino acids, both natural and synthetic, demonstrated a wide range of plasticizing behavior in extruded starch-based blends. Cyclic amino acids similar to proline, with nitrogen in the rings, were the best plasticizers. Branching at nitrogen in tertiary amines and branching at the α-carbon were also effective. To predict the plasticizing efficiency of the amino acids, mechanical test data were combined with three-dimensional computational models in the development of QSPR. The QSPR were successfully established with only two molecular descriptors and were predictive for compounds within the set we tested. Consequently, they can be used to estimate the plasticizing behavior of related monoamino monocarboxyl compounds and to design new plasticizers. Development of new synthetic plasticizers requires a substantial investment in time and materials. QSPR thus allow preliminary screening and judicious selection of synthetic targets.

Acknowledgements

Ms. McShell Hairston assisted in acquiring FT-Raman spectra. Ms. Jan Lawton prepared the glycine solutions for FT-Raman studies. Ms. Paulette Smith assisted in blend preparation. Mr. Pat Ward and Ms. Robin Haken provided helpful discussions on the mathematics of linear relationships.

References

Allinger, N. L. (1977). *J. Am. Chem. Soc.*, *99*, 8127–8134.

Barton, A. F. M. (1991). *CRC Handbook of Solubility Parameters and Other Cohesion Parameters*. Boca Raton: CRC Press.

Brandrup, J., & Immergut, E. H. (1966). *Polymer Handbook*. New York: Wiley.

Ellis, T. S. (1988). *J. Appl. Polymer Sci.*, *36*, 451–466.

Fanta, G. F., & Doane, W. M. (1986). In: O. B. Wurzburg (Ed.), *Modified starches: properties and uses* (pp. 149–178). Boca Raton: CRC Press.

Fritz, H. G., Aichholzer, W., Seidenstücker, T., & Widmann, B. (1995). *Starch/Stärke*, *47*, 475–491.

Hansch, C & Leo A. (1995). *Exploring QSAR: fundamentals and applications in chemistry and biology*. Washington, DC: American Chemical Society.

Hansch, C., Leo, A., & Hoekman, D. (1995). *Exploring QSAR: hydrophobic, electronic and steric constants*. Washington, DC: American Chemical Society.

Harrison, S. T. L., Chase, H. A., Amor, S. R., Bonthrone, K. M., & Sanders, J. K. M. (1992). *Int. J. Biological Macromolecules*, *14*, 50–56.

Hodge, R. M., Bastow, T. J., Edward, G. H., Simon, G. P., & Hill, A. J. (1996). *Macromolecules*, *29*, 8137–8243.

Imam, S. H., Gordon, S. H., Greene, R. V., & Niño, K. A. (1996). In: J. C. Salamone, (Ed.), *Polymeric Materials Encylclopedia* (vol. 10) (pp. 7893–7901). Boca Raton: CRC Press.

Jensen, J. H., & Gordon, M. S. (1995). *J. Am. Chem. Soc.*, *117*, 8159–8170.

Kalichevsky, M. T., Blanshard, J., & Tokarczuk, P. F. (1993). *Int. J. Food Sci. Technol.*, *28*, 139–151.

Katritzky, A. R., Lobanov, V. S., & Karelson, M. (1995). *Chem. Soc. Rev.*, *24*, 279–287.

Khalil, M. I., Farag, S., Mostafa, K. M., & Habeish, A. (1994). *Starch/Stärke*, *46*, 312–316.

Le Meste, M., Viguier, L., Lorient, D., & Simatos, D. (1990). *J. Food Sci.*, *55*, 724–727.

Lenz, R. W. (1995). *JTEC Monograph*. Loyola College, Maryland.

Lillie, M. A., & Gosline, J. M. (1990). *Biopolymers*, *29*, 1147–1160.

Murugan, R., Grendze, M. P., Toomey, J., Joseph, E., Katritzky, A. R., Karelson, M., Lobanov, V., & Rachwal, P. (1994). *CHEMTECH*, *24*, 17–23.

Roos, Y. (1993). *Carbohydrate Res.*, *238*, 39–48.

Roos, Y., & Karel, M. (1991). *Biotechnol. Prog.*, *7*, 49–53.

Sears, J. K., & Darby, J. R. (1982). *The technology of plasticizers*. New York: Wiley.

Shogren, R. L., Swanson, C. L., & Thompson, A. R. (1992). *Starch/Stärke*, *44*, 335–338.

Slade, l., Levine, H., Ievolella, J., & Wang, M. (1993). *J. Sci. Food Agri.*, *63*, 133–176.

van Soest, J. J. G., & Vliegenthart, J. F. G. (1997). *Trends Biotechnol.*, *15*, 208–213.

Stanton, D. T., & Jurs, P. C. (1990). *Analytical Chemistry*, *62*, 2323–2329.

Stein, T. M., & Greene, R. V. (1997). *Starch/Stärke*, *49*, 245–249.

Tomka, I. (1990). Patent Application 90/05161, WPO, Switzerland.

US Congress. *Office of Technology Assessment (1993)*. Washington, DC: US Government Printing Office.

Wilson, A. S. (1995). *Plasticizers; principles and practice*. Cambridge: Cambridge University Press.

Xue, G. I (1997). *Prog. Polym. Sci.*, *22*, 313–406.

Yoo, S.-H., Kweon, M.-R., Kim, M.-J., Auh, J.-H., Jung, D.-S., Kim, J.-R., Yook, C., Kim, J.-W., & Park, K. H. (1995). *J. Food Sci.*, *60*, 516–519.