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Optimization of Solvent Composition for Extraction of Multi-Polarity Molecules

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Abstract: Marine Natural Products (MNP) are currently extracted from a range of organisms that are complex matrices composed of significant organic and inorganic components. In order to optimize the extraction of the MNP, typically large organic molecules composed of polar and nonpolar structural subcomponents, namely a multi-component solvent should be utilized. In this paper an algorithm is outlined that provides an optimal solvent mixture based on dipole moment and molecular volume calculations. In this model the MNP is divided up into subcomponents, with carbon fragments ranging in size from one to six carbons. The subcomponent is then matched by dipole moment and molecular volume to a common solvent (i.e., ethanol, methanol, etc.). This algorithm is experimentally demonstrated by increasing the quantity of bryostatin extracted from the bryozoa *Bugula neritina* and chlorophyll from Common Bermudagrass (*Cynodon dactylon*).

Keywords: Extract, solvent, natural product, marine, bryostatin

INTRODUCTION

Scientists have developed solvent extraction techniques for isolating molecular species from various matrices. Pavikova et al. (1) developed a two-solvent nonpolar extraction to remove hydrocarbons from soil and used IR spectroscopy to categorize the product. The use of a two-phase system in microalgae environments by Leon et al. (2) helped improve solubility and subsequently the extraction efficiency of natural products. Certain

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natural products from terrestrial and marine samples have been isolated by the extraction method outlined by Lewis et al. (3). Lino et al. (4) have developed extraction techniques based on the use of ultrasonication and varying extraction solvents. Horng et al. (5) were able to determine the optimal extraction conditions for geniposide from *gardenia*. Koch et al. (6) created a method to improve and stabilize the extraction of natural products from *Hypericum perforatum* L. so that this extract may be used medicinally. Ni et al. (7) have created a model to determine separation schemes and extractants for natural products. Alvarez et al. (8) developed a percolation technique to extract a desired molecular species, such as corn oil with hexane. Adrian et al. (9) have demonstrated a phase-split phenomenon that led to a new high-pressure liquid-liquid extraction process to isolate natural products. Mohamed (10) used a novel supercritical fluid extraction process to extract natural products. Chandler et al. (11) created a process to purify natural products by using a two-phase, multi-solvent system. Aphios Corporation developed a novel extraction and crystallization procedure related to bryostatin (12). Marcus et al. (13) recently published a detailed text on a number of cutting edge topics in solvent extraction theory and technology. In many of these studies the optimum extraction conditions for a single molecular species from a certain matrix are provided. Our algorithm is designed to be a more comprehensive approach to selecting solvents for a range of molecular structures.

MNPs, such as bryostatin, ET-743, jasplakinolide and dolastatin 10 are extracted from organisms that represent complex matrices potentially composed of lignins, cellulose, chitin, and other organics (aromatic, aliphatic) as well as inorganic (cations, anions, insoluble salts, NH_3, I_2, H_2O, etc.) components (14–17). To optimize the extraction of the MNP, which are large molecules composed of polar and nonpolar subcomponents, a multi-component solvent mixture is estimated using this concept. Drawing on a number of classic physical chemistry experiments (18), a simple analogy can be perceived by considering the two-solvent, two phase system of water and octane, mixed in a 1:1 volume ratio. If sodium chloride salt were added to this system most of the salt would dissociate and dissolve in water, with the ions (Na^+, Cl^-) forming weak electrostatic bonds with the polar water molecules. If hexane were added to this same two-phase system, most of the hydrocarbon would dissolve in octane due to similarities in polarities. Trace amounts of the salt would dissolve in the octane and trace amounts of hexane would dissolve in the water but at a considerably lower concentration than the octane. The solubility of MNP's which have both polar and nonpolar structural fractions will inherently have their solubility lowered when dissolved in a single component solvent.

Specific solvent molecules will align themselves, based on polarity, with the matching subcomponents of the MNP. For example, bryostatin (Fig. 1) has a C_8 component, if considered separately, would be solubilized by a nonpolar solvent such as hexane. Bryostatin has other structural

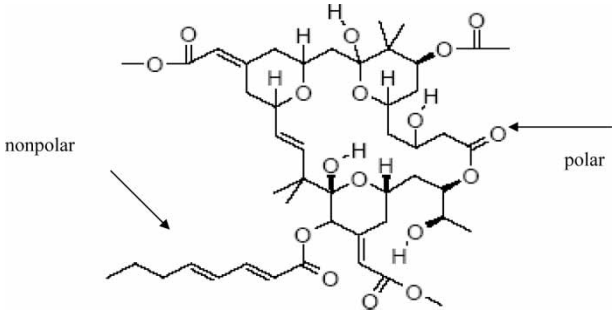


Figure 1. The molecular structure of bryostatin 1 has both polar and nonpolar subcomponents.

features, such as ester bonds and alcohol groups that are fairly polar and, if considered individually, would be soluble in methanol or ethanol. MNPs, such as bryostatin, extracted with a single solvent (e.g. ethanol) or two solvents of similar polarities (i.e., methanol-chloroform), would behave like a micelle with nonpolar components facing inward, away from the fairly polar solvent, impacting on solubility and accelerating aggregation and precipitation.

If we consider this a two phase (solvent vs. organism) extraction, the MNP has to be more soluble in the solvent than in the organism. Because it is an intrinsic part of the biological organism, the MNP should be very soluble in its host. While a MNP might be soluble in a solvent (e.g. bryostatin in methanol), the solubility in the solvent may be less than in the organism, leading to an extraction with a very low efficiency or a small partition coefficient. For the extraction efficiency to be optimized, the solubility of the MNP has to be greater in the solvent than in the host. In this paper an algorithm is outlined that leads to an optimal solvent mixture based on dipole moments (D) and molecular volume (V, A^3) values. In the multi-step model a MNP is divided into subcomponents, with carbon fragments ranging in size from one to six carbons. The subcomponents are matched by its D/V value to a common solvent (e.g. ethanol, methanol, etc.).

EXPERIMENTAL

The bryozoa *Bugula neritina* was obtained from Gulf Specimen Marine Lab. (Panacea, Fl). It was chopped (blender) and sonicated (Biologics Homogenizer, VA) and extracted with HPLC grade solvents. Diethyltriaminepentaacetic acid (DTPA), a strong chelating agent, was used to bind, solubilize, and remove cations from the sonicated organism. Bryostatin was quantitiated using the 262 nm absorbance line (19–22). The Matrix Assisted Laser Desorption Ionization-Mass Spec. (MALDI-MS) is located at the University of

Georgia (Athens) mass spectrometer facility. Chlorophyll extractions were carried out using 100 mg of grass, dissolved in a small volume of solvent. The absorbance wavelength of chlorophyll at approximately 660 nm was utilized for quantitation but each sample was scanned from $\lambda 800-300$ nm to ensure the full chlorophyll spectrum was present.

DISCUSSION

This algorithm presents a method to calculate a mixture of common solvents needed to optimize the quantity of natural product extracted from a marine organism. A summary of the algorithm is:

1. Start at a terminal group of the molecule and count carbons until an oxygen atom is reached or six consecutive carbon atoms are tallied.
2. Using the solvents provided in Table 1, a match is made. While this table represents a number of common solvents, others could be added as needed.
3. The process is repeated starting at the next carbon atom. This is done until the molecule is broken into a number of fragments, varying in length from one to six carbon atoms.

An example of the algorithm is demonstrated using decane–3,8-diol (Fig. 2). Starting at the left of the molecule, count three (3) carbon atoms stopping at the oxygen, matching this fragment to 1-propanol and methanol. Next count five carbon atoms stopping at the second oxygen atom, matching it to 1-pentanol and methanol. Finally starting from the right side of the molecule, count three carbon atoms stopping at the oxygen, matching it to 1-propanol and methanol. To extract this molecule from an organism, a solvent composed of three parts methanol, two parts propanol and one part pentanol by volume would be utilized.

Whilst this is a fairly simple structure, the method is intended for larger molecular species (i.e. $> C_{12}$) whose total dipole moment does not represent its real physical property as sensed by much smaller solvent molecules. Ten steps are used to outline the concept presented in this paper. While this algorithm outlines the concept, it does not provide exact details for all molecular variations possible in a large multi-component molecule.

1. In any aliphatic or aromatic structure that contains an oxygen (e.g. ether), nitrogen (e.g. secondary or tertiary amine) or sulfur (e.g. sulfide) atom within the chain or ring structure, the atom (O, N, S) results in stopping the carbon count and matching to the appropriate solvent (Fig. 3a, b). For example the C_2 terminated by an ester (Fig. 3a) results in an ethanol, and the C_3 and C_2 groups terminated by the tertiary amine give propanol and two ethanol components, respectively.

Table 1. The dipole moment, molecular volume, D/V value and the Hildebrand parameter for a number of common solvents

Species	Molecular volume ($Å^3$)	Dipole moment (D)	D/V	Hildebrandt
Acetone	72.41	3.12	.043	19.7
Acetonitrile	52.93	4.04	.076	
n-amyl acetate	154.13	4.85	.031	17.1
Benzene	98.45	0.0	0	18.7
1-butanol	95.34	1.69	.0177	28.7
Carbon tetrachloride	88.55	0.0	0	18
Chloroform	74.56	1.35	.0181	18.7
Cyclohexane	111.44	0.0	0.0	16.8
Cyclohexene	107.39	.19	.00176	
Diacetone alcohol	133.54	3.69	.0276	20
Diethyl ether	97.19	1.24	.0127	15.4
Dimethyl formamide	85.66	4.09	.0477	24.7
Dimethyl sulfoxide	79.02	5.67	.0717	26.4
Dodecane	234.0	0.0	0	16
Ethanol	58.74	1.74	.0296	26.2
Ethyl acetate	99.23	4.8	.0483	18.2
Ethylene dichloride	79.29	3.14	.0396	20.2
Ethylene glycol	65.92	2.70	.0409	34.9
Freon 11	79.04	0.43	0.0054	
Glycerol	91.02	2.21	.0242	36.2
Heptane	143.06	0.07	0.000489	15.3
Hexane	124.78	0.0	0	14.9
Methanol	40.38	1.87	.0463	29.7
Methyl ethyl ketone	90.66	3.00	.0331	19.3
Methylene chloride	60.77	1.99	.0327	20.2
Morpholine	96.41	1.40	.0145	22.1
1-octanol	168.64	1.89	.0112	
Pentane	106.44	0.07	.00066	14.4
1-pentanol	113.65	1.62	.01425	
1-propanol	77.09	1.84	.0238	24.9
Propylene glycol	84.10	2.55	.0303	30.7
Pyridine	92.02	2.31	.0251	21.7
Tetrahydrofuran	85.35	1.94	.0227	18.5
Toluene	116.57	.27	.00231	18.3
1,1,1-trichloro ethane	93.03	2.07	.0222	15.8
Trichloroethylene	88.08	1.14	.0129	18.7
Water	19.21	2.20	.114	48
m-xylene	134.73	.27	.0020	18.2

2. When the carbon count ends with no oxygen's in the carbon chain, the starting point is reversed. As illustrated in Fig. 4, starting at the left, count three carbon atoms and match it with propanol, count five carbon atoms and match it with pentanol. The final count would result

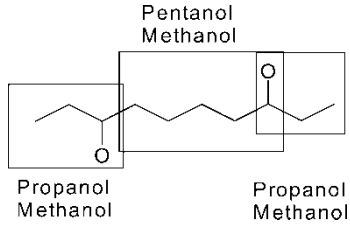


Figure 2. The molecular structure of decane-3,8-diol. Its subcomponents are outlined (boxes) and matched to a common solvent.

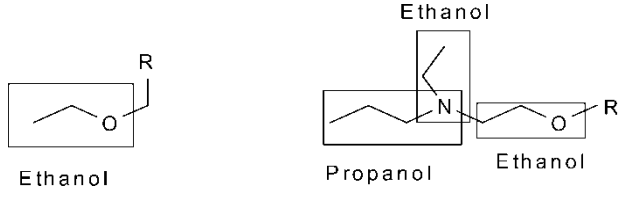


Figure 3. An ester (left) and a tertiary amine (right) demonstrate step 1.

in a four carbon chain and no oxygen. Reversing the starting point and beginning at the right hand carbon, would give a five-carbon atom count and a pentanol match. For optimal extraction of the molecule in Fig. 4, a solvent composed of two parts 1-pentanol and one part 1-propanol by volume would be utilized.

3. For each noncarbon, nonhydrogen nonmetallic atom in a multiatom functional group that links carbon chains together (e.g. esters, anhydrides, and disulfide); an alcohol is added in each direction (Fig. 5).
4. Functional groups or atoms that protrude from the main chain or aromatic ring (e.g. carbonyls, primary amines, chloro, bromo, etc.) are matched by an alcohol and a methanol group (Fig. 6a). Starting from the left, a five carbon subcomponent count is stopped at the carbonyl yielding a pentanol and a methanol; a two carbon count is stopped at the primary amine yielding ethanol and a methanol; a three carbon count is stopped at the chloro group yielding propanol and a methanol, and reversing from the right hand end a two carbon chain is

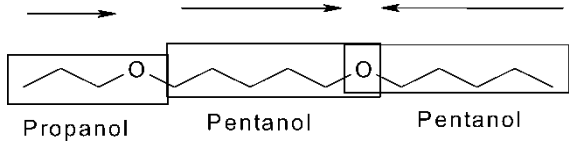


Figure 4. To extract 1-pentyloxy-5-propoxy-pentane (below) from a sonicated organism, 2 parts pentanol and 1 part propanol by volume would be optimal.

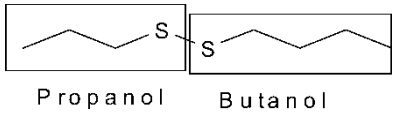


Figure 5. 1-Propyldisulfanyl-butane demonstrates step 4.

counted and stopped at the chloro group yielding ethanol and methanol. The mixture needed to optimally extract this molecule would be two parts ethanol, one part propanol, one part pentanol, and four parts methanol by volume.

Figure 6b shows the difference in matching the solvent to an oxygen atom that is in a hydrocarbon and subsequently produces a smaller dipole moment and an oxygen atom protruding (e.g. carbonyl) from the chain with a higher dipole moment. The addition of the relatively polar methanol is attributed to the increase in a dipole moment typically noted for molecules that have an electronegative element protruding from the structure. For example, comparing the calculated dipole moment on pentan-3-one (6.64D) to diethyl ether (3.97D) illustrates the impact that the position of the oxygen atom in the structure has on the polarity of the molecule.

5. In addition to the previous rules, a charged species (e.g. $-NH_3^+$; $R\text{-}COO^-$) draws an additional part of water. Figure 7a shows a carboxylate (-1 charge) that would count as 1 part water, 1 part methanol, and 1 part propanol, whereas the uncharged carboxylic acid (Fig. 7b) would

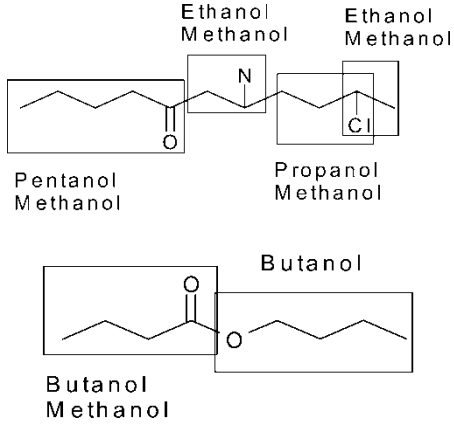


Figure 6. (top) A C_{11} chain (7-amino-10-chloro-undecan-5-one) with a carbonyl, amine, and a chloro group is used to demonstrate step 4. (bottom) An ester (butyric acid butyl ester) illustrates the difference in matching solvent molecules to an oxygen in the hydrocarbon chain (butanol) and the oxygen protruding from the chain (butanol, methanol).

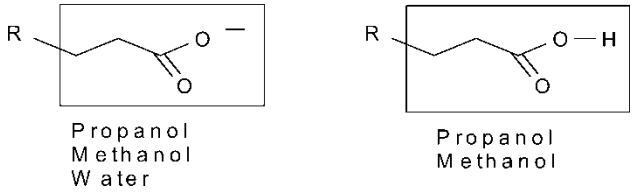


Figure 7. A carboxylate (left) and a carboxylic acid (right) are used to demonstrate step 5.

be 1 part propanol and 1 part methanol. The pH of a matrix can be measured and the pK_a of the functional group estimated to help select more accurately the optimal solvent composition. While a charged functional group such as a carboxylate will bind cations, a DTPA wash (below) will minimize their presence.

6. Six-member aromatic carbon rings are considered as two cyclohexenes (benzene is avoided due to health concerns). If the ring is substituted with a hydrocarbon, the aromatic ring is treated as oxygen at the end of the hydrocarbon chain. For example, as illustrated in Fig. 8a, the ethyl group is counted as ethanol and the benzene structure is counted as 2 cyclohexenes. If an aromatic structure is monosubstituted by a polar species (e.g. $-$Cl, $-$F, $-$Br, $=$O, NO_2^-, etc.), it is counted as 2 parts cyclohexenes and 1 part methanol. If an aromatic structure is disubstituted by polar elements or groups, it is treated as a series of small carbon fragments (i.e. no cyclohexenes). The 4-chloro-3ethyl-phenol structure illustrated in figure 8b would result in a solvent mixture that is two parts propanol, 2 parts methanol and 1 part ethanol by volume. Cyclic structures (Fig. 8c) substituted with polar elements such as O, N, or S, are treated as a series of small carbon fragments. For example, to extract 2-propyl [1,4] dioxane, a solution composed of 1 part butanol, 1 part ethanol and 1 part methanol by volume would be used. Similarly, other non aromatic ring structures are treated as small carbon fragments.
7. For long aliphatic chains, hexanes and the appropriate alcohol are used to match the polarity. The 1-decanol structure in Fig. 9 shows how the first six carbons are counted as 1 part hexane and the remaining four carbons and the alcohol result in 1 part butanol, 1 part methanol by volume. For branched hydrocarbons, side chains composed of carbons backbones, are counted as part of the chain. As shown in Figure 9b, the two-methyl groups are counted in selecting pentanol as one of the solvents.
8. Solvent mixtures may be predicted with this algorithm that do not form a single phase (e.g. hexane and water). For example, heptanoate ($C_7H_{13}O_2^-$) would result in a solvent composed of two parts water, one part methanol and one part hexane (Fig. 10). In these cases, we propose a calculation based on the dipole moment per unit volume (D/V) that matches the solvent polarity to the MNP subcomponent's

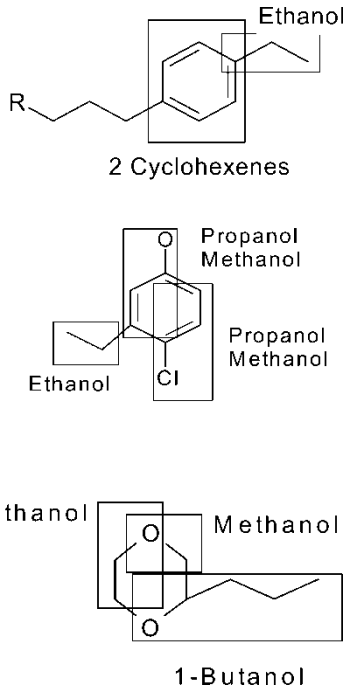


Figure 8. (top) A disubstituted aromatic structure is used to demonstrate step 6. The trisubstituted aromatic structure, 4-chloro-3-ethylphenol (middle) shows that aromatic structures substituted by polar groups are treated as small carbon chains. The 2-propyl [1,4] dioxane structure (bottom) illustrates how a non-aromatic ring structure is counted.

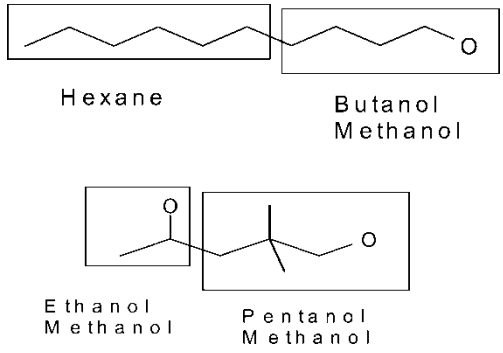


Figure 9. 1-decanol (top) and branched hydrocarbon, 2,2-dimethyl-pentane-1,4-diol (bottom) illustrate step 7.

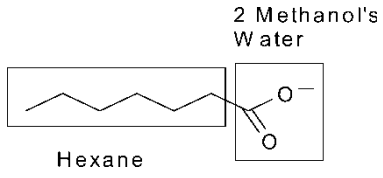


Figure 10. Heptanoate demonstrates step 8.

polarity. Figure 11 is a graph of a solvent's dipole moment (D) by molecular volume (V) and is calculated from data in Table 1. From a visual perspective, using a H_2O/hexane mixture as an example, the graph shows them to be at opposite ends of the range and would result in an inefficient solvent extraction system for a MNP. However using solvents with closer D/V values that results in a two component, single phase solvent could be achieved by using a butanol/methanol mixture. Applying this technique to the extraction of heptanoate, the three-component system (water, methanol, and hexane) could be reduced to a two-component single phase system. Applying the general equation,

$$(P_{v1} * D_1/V_1) + (P_2 * D_2/V_2) + (P_3 * D_3/V_3) + \ldots = (D_v)/n \quad (1)$$

Where: P_{v1} is the parts by volume of solvent component 1 (unitless), D_1 is the dipole moment of solvent 1, V_1 is the molecular volume of solvent component 1, D_v is the sum of the dipole moments per molecular volume, and n is the total parts of solvent (unitless). Using the data in Fig. 11 and Table 1 and applying this approach to heptanoate (Fig. 10) gives:

$$1(.114) + 2(.0463) + 1(.000) = .2066/4 = .0516 \quad (2)$$

For heptanoate, water and 1-propanol are selected because they are miscible but still have distinct polar and nonpolar characteristics. Equation (3) optimizes the solvent mixture by volume percent. Water and 1-propanol, have dipole moment per unit volume (D/V) values of .114 and .0238 respectively. Therefore:

$$(.114)x + (.0238)y = .0516 \quad (3)$$

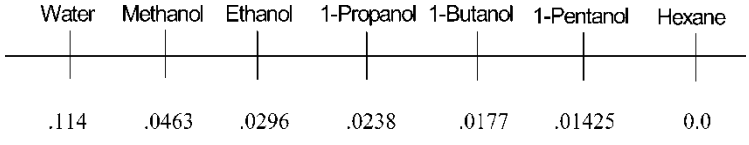


Figure 11. Graph of molecular dipole moment per unit molecular volume (D/V).

Setting $y = 1$, then $x = 0.244$. Hence, 1 part propanol to 0.2430 parts water would give a miscible solution which should successfully extract heptanoate from an organism.

Much of the modern extraction and solubility work of organic compounds is built on the Hildebrandt model (23, 24). This theory is based on a cohesive energy density (c), which is calculated from the heat of vaporization of a liquid (ΔH), the Gas constant (8.314 J/molK), temperature (K), and the molar volume (V_m).

$$c = (\Delta H - RT)/Vm \tag{4}$$

This parameter can be interpreted as the energy needed to separate the molecules, which can be related to the solubility of two molecules. For example, in Table 1, pentane and hexane are miscible, which is reflected by the Hidebrandt parameters (∂) being similar.

$$\partial = (c)^{1/2} \tag{5}$$

This parameter (∂) works quite well for comparing small molecules that can be vaporized. Most natural products would undergo a thermal decomposition or rearrangement upon heating and can not be vaporized. Figure 12 illustrates that the Hildebrandt parameters for a number of common solvents and the D/V parameter proposed here are poorly correlated (linear $r^2 = 0.51$). A number of exponential and polynomial fits were also attempted with no r^2 value above 0.59 being achieved.

9. Organisms are typically blended and sonicated before extraction. A matrix is washed with a solution of 0.002 M Diethyltriaminepentacetic acid (DTPA) an octadentate aminocarboxylate. The aqueous phase supernatant is discarded before the solvent extraction takes place. The hexadentate aminocarboxylate EDTA has also been used. Most

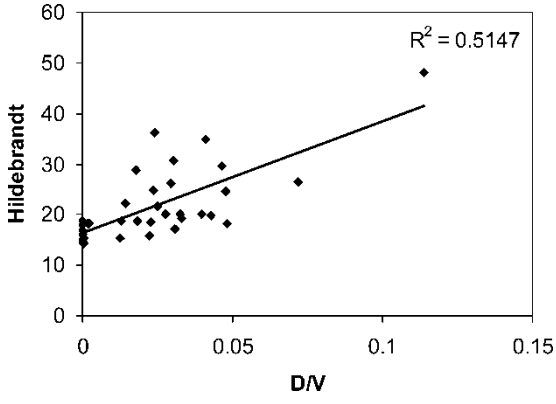
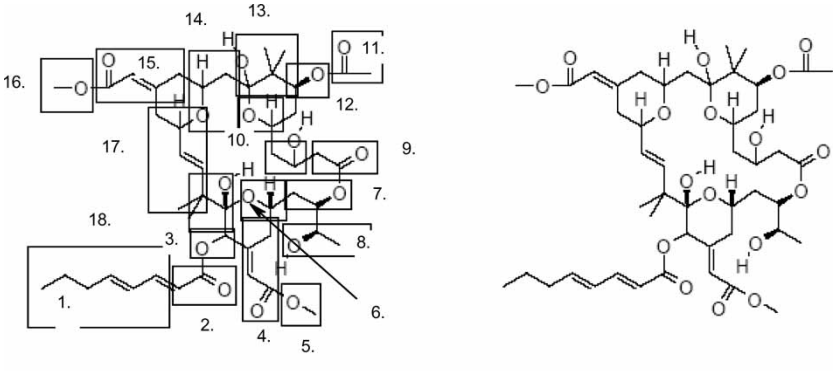


Figure 12. The Hildebrandt parameter (y-axis) for a series of small, volatile molecules plotted verses the calculated D/V value.

MNP's have very low water solubility; we assume the washing stage will not extract an appreciable amount of the nonpolar MNP. A number of salts commonly found in the marine environment (e.g. $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$) are well known flocculating agents for suspended organics. By washing with DTPA or EDTA, which binds and removes the cations from the matrix and dissolves them in the aqueous phase, the flocculation of any natural product is reversed.

10. If the molecule strongly binds a cation, correlate the number of waters added to the extraction solvent to the cation charge. Specifically a 1+ cation (e.g. Na^+) is allowed 1 part water, a 2+ cation (e.g. Ca^{2+}) is allowed 1.5 parts water, and a 3+ cation (e.g. Fe^{3+}) is allowed 2 parts water by volume. Although an approximation, the ability to attract waters into inner sphere coordination sites and form outer sphere complexes increases with charge density, justifying the increase in the number of waters allocated. Many macrolid structures as well as phorphyinms bind cations (i.e. Fe(III), Mg(II), etc.) strongly and can be removed by a competitive binding reaction with a multidentate aminocarboxylate.

The calculated dipole moment for a MNP provides an average of the molecule's subcomponents but does not provide insight into its true solubility in a multicomponent solvent. A single solvent molecule such as ethanol has a molecular volume less than 3% of bryostatin. In terms of the different dipole moments and weak electrostatic attractions needed to dissolve a large molecular species, ethanol can only optimally align itself with a fraction of the MNP. Figure 13 demonstrates how bryostatin ($C_{47}H_{68}O_{17}$) is divided into 24 subcomponents, (2 parts hexane, 3 parts butanol, 1 part



1. Hexane 2. Ethanol, Methanol 3. Methanol, 4. Butanol, Methanol
5. Methanol, 6. Methanol 7. Ethanol 8. Ethanol, 9. Ethanol, Methanol
10. Ethanol, 11. Ethanol, Methanol, 12. Methanol, 13. Butanol, 14. Ethanol
15. Butanol, Methanol, 16. Methanol, 17. Hexane 18. Propanol, Methanol

Figure 13. Bryostatin 1 is partitioned in carbon fragments that are between one and six carbon atoms long. Each fragment is matched to a solvent with a similar D/V value.

propanol, 7 parts ethanol, 11 parts methanol) using the method described, which would be the optimum mixture. Using data in Table 1 and Equation (1), it is possible to calculate a simpler, but less efficient solvent mixture composed of propanol and methanol or two other compatible solvents.

Figure 14 a,b,c provides experimental data for the extraction of bryostatin 6 or 9 (+ Na^+, −4 H) from *Bugula neritina* harvested from the Gulf of Mexico. The intensity (y-axis) of the peak increases from water (Fig. 14a), methanol (Fig. 14b), and ethanol/DTPA treatment (Fig. 14c). Past work in

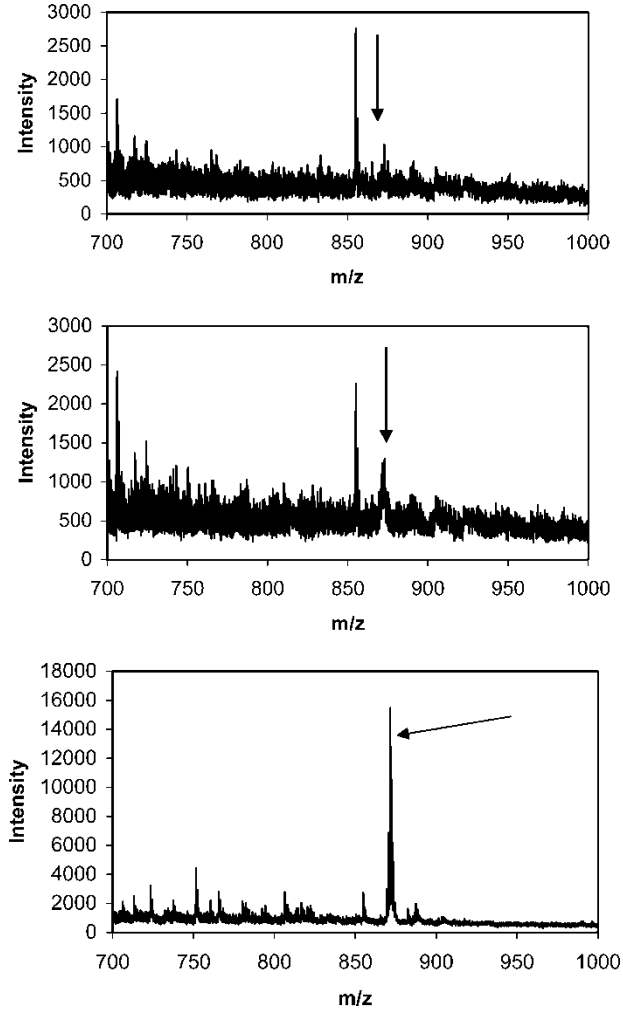


Figure 14. Mass spec analysis of water (top), methanol (middle) and ethanol with DTPA (bottom) of Bugula neritina. The molecular species being extracted is a bryostatin structure.

this lab has shown that *Bugula* has a high mineral content so DTPA is critical in this extraction (20). As the solvent composition comes closer to the calculated value, the yield of material extracted increases. UV/Vis absorbance data, showing bryostatin spectral features in the λ230–235 nm and λ260–265 nm range, was used to support the mass spec. data in terms of the quantitative extraction into different solvents. Table 2 provides UV/Vis quantitative data confirming that the calculated solvent mix is the most efficient. The calculated two solvent mixture was the second most efficient extractant.

Thermodynamic and kinetic effects also need to be taken into account, e.g. in addition to the temperature impacting on the partition coefficient between the sonicated organism and the optimal solvent mixture, the temperature will also be critical if the entire extraction process is endothermic. Increasing the temperature will also accelerate the kinetics of the system and these effects must be assessed on an individual basis with the stability of the MNP and the entire system taken into consideration. Although common, economical HPLC grade solvents were the solvents of choice, additional solvents such as different alcohols (e.g. 2-propoanol, diols) and concentrated solutions (e.g. glacial acetic acid, ammonium hydroxide) could also be considered for specific extractions. Although the focus here is on MNPs, the logic of matching solvent species to

Table 2. Fourteen samples (2.0 grams each) of Bugula neritina were extracted with different solvents at two temperatures. Scatter indicates the spectral features followed the $I_{scatter}\ \alpha\ v^4$ profile indicative of particles scattering light, due to aggregation. The mixture (#7) was the optimized solvent. A UV/Vis spectrometer (b = 1 cm) was used to quantize the bryostatin (λ265 nm). Mass spec was used to confirm the presence of the bryostatins.

Sample	Solvent	Temp (°C)	Bryo conc.
1	Water	35	Scatter
2	MeOH	35	1.43×10^{-5}
3	Ethanol	35	2.47×10^{-5}
4	Propanol	35	1.39×10^{-5}
5	Butanol	35	1.52×10^{-5}
6	Pentanol	35	2.22×10^{-5}
7	Mixture	35	2.86×10^{-5}
1	Water	23	Scatter
2	MeOH	23	1.95×10^{-5}
3	Ethanol	23	2.47×10^{-5}
4	Propanol	23	1.26×10^{-5}
5	Butanol	23	1.52×10^{-5}
6	Pentanol	23	2.21×10^{-5}
7	Mixture	23	2.56×10^{-5}

macromolecular subcomponents using a D/V ratio could be extended to other applications such as the extraction of herbicides and pesticides, selective extraction of proteins and other macromolecules, and the predictable folding of proteins.

This extraction algorithm is also demonstrated with chlorophyll extracted from Common Bermudagrass (*Cynodon dactylon*). The experimental conditions were normalized (same mass of plant extracted, same extraction times, etc.). The two solvents at extreme ends of the polarity range that were used (water and hexane) had no absorbance, but the hexane did show strong spectroscopic evidence indicating aggregation. The maximum absorbance and subsequently the maximum amount of chlorophyll extracted was derived from the optimal solvent composition predicted using this algorithm (see Fig. 15). Because there is a blend of chlorophyll a and b, which have different extinction coefficients at the selected wavelength of analysis, absolute concentrations are not calculated.

CONCLUSION

The method presented here is loosely based on the "like dissolves like" theory, but rather than considering the whole molecule it treats the molecule as a sum of fragments. The method allows for the calculation of an approximate solvent composition based on the distribution of dipole moments over a molecular volume. The exact atom selected as the starting point for identifying

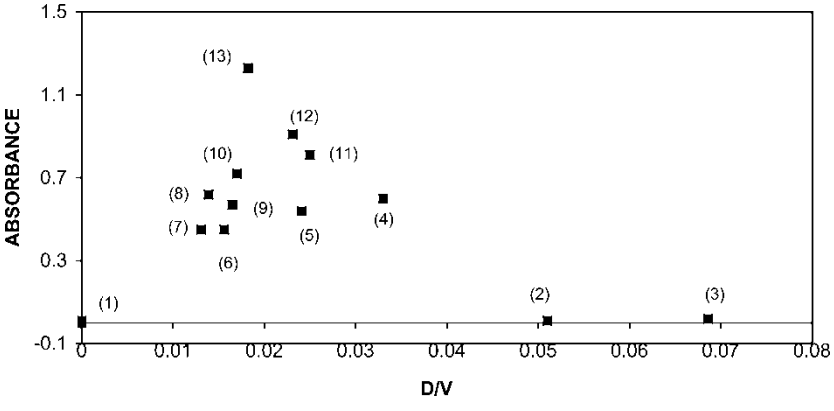


Figure 15. Chlorophyll extracted from grass showing that maximum absorbance (proportional to concentration), was achieved with the optimal solvent system calculated by the described method. (1) hexane, benzene (two overlapping points). (2) 50/50 water/methanol. (3) water. (4) methanol. (5) ethanol. (6) 1-pentanol. (7) 1-butanol. (8) 1-propoanol. (9) 1/1 methanol/hexane. (10) 1/1/1 methanol, butanol, hexane. (11) 1/1: methanol/propanol. (12) 1/1 methanol/pentanol. (13) water: methanol: propanol: pentanol: hexane ideal solvent mix.

subcomponents can affect the calculated solvent composition slightly, but the $(D_v)/n$ value (Eq. (1)), are typically very close regardless of the starting point. Previous work in this lab utlizing solvent extraction systems involved analysis of humic substances for natural products (19). This progressed to scanning an ecosystem in the Gulf of Mexico for a precursor to bryostatin (20). While focusing on marine natural products in this study, the logic can also be applied to a wide variety of extraction problems such as plant natural products and the extraction of herbicides or pesticides from tissue or soil samples. In addition, the concept of matching solvent molecules up to specific regions of much larger molecules may also be applied to not only the extraction of macromolecules such as proteins but also the composition of the solvent can be used to predict their shape in the liquid phase.

Currently an algorithm that breaks the molecule down into fragments and calculates the D/V ratio for each fragment is being developed. The D/V ratio for each fragment is than matched to the solvent with the closest D/V ratio. The model presented here can be used to estimate a multicomponent solvent quickly by hand using only the data in Table 1. The newer algorithm will be computer based. Predictable protein folding is an important but elusive goal in science. Adapting the D/V model developed here in which the solvent composition can be matched to certain protein subcomponents and bend or fold it in a predictable fashion should be achievable with this approach.

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