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Disruption and fractionation of biological materials by matrix solid-phase dispersion

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ABSTRACT

The isolation of drug residues, environmental contaminants or naturally occurring component molecules from biological materials is often a complex undertaking. We report here the development and application of a simple approach to the disruption of biological samples that also allows for the rapid fractionation and isolation of the sample's natural components or incurred residues. This process, called matrix solid-phase dispersion (MSPD), combines the use of mechanical forces generated from the grinding of samples with irregular shaped particles (silica or polymer based solid supports) with the lipid solubilizing capacity of a support-bound polymer (octadecylsilyl or others) to produce a sample/column material from which dispersed sample matrix components can be selectively isolated. The factors governing this process and examples of its various applications are presented.

INTRODUCTION

Methods for the isolation of target molecules from biological matrices, such as tissues, often begin with a process designed to disrupt the general architecture of the sample. By using a mechanical blender or by grinding with abrasives, such as sand, the sample is reduced to fragments of structural components or clusters of cells. A degree of cell lysis occurs and can be enhanced by sonication, extrusion or the addition of chemical agents. To assist in lysis, one may treat the membrane fragments and subcellular structures with surfactants, which tend to completely disrupt and solubilize the component molecules. Such a step is necessary in the isolation of integral membrane proteins or molecular complexes.

In the case of animal cells, relatively mild procedures accomplish cell lysis. However, plants, bacteria and fungi possess cell walls and often require more physically and chemically dynamic procedures to obtain complete cellular disruption. This may involve the weakening of the cell wall by enzymes, extended sonication at maximum intensity, and the use of high concentrations of detergents. Although these processes accomplish cellular disruption their physical and chemical harshness can complicate the procedures for the isolation of the target molecules or lead to their destruction and the generation of sample artifacts. These factors are of concern regardless of the methodology employed.

The fractionation of the lysates involves methodology to isolate a specific type of target compound (*i.e.*, lipid, carbohydrate, protein, peptide, drug, metabolite, pollutant, etc.), often to the exclusion of all others. Such isolation techniques may involve centrifugation, counter-current extractions, pH adjustments, various forms of chromatography or combinations of these and other technologies. While accomplishing the desired task this overall

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approach can be extremely laborious and time and materials intensive. Furthermore, the high degree of sample manipulation often leads to poor recoveries and, thus, an inefficient process.

For the last several years our laboratory has been involved in developing procedures for the isolation of drug residues from tissues using a process called matrix solid-phase dispersion (MSPD) [1]. This process involves the grinding of biological samples with silica solid supports (40 μm particle size) to which lipid solubilizing polymers (octadecylsilyl or others) are chemically bound. We present here a summary of data obtained to date in examining the application of MSPD to the isolation of drug and pollutant residues from a variety of biological matrices. We also present data illustrating its application to the disruption and fractionation of muscle tissue and the bacteria *Mycobacterium paratuberculosis* and *Escherichia coli*. The results of these studies indicate that MSPD is a generic method for the disruption, lysis and fractionation of biological matrices that may possess numerous advantages when compared to classical approaches.

EXPERIMENTAL

Materials

Liquid chromatographic grade solvents from commercial sources were used without further purification. Water for reagents and HPLC mobile phases was triple distilled and passed through a Modulab Polisher I water purification system. Bulk octadecylsilane (C_{18}) derivatized silica (40 μm, 18% carbon load, endcapped from Analytichem, Harbor City, CA, USA) was washed with hexane, dichloromethane (DCM) and methanol and was air dried prior to use. Syringe barrels of 10 ml were thoroughly washed with hot, soapy water, rinsed with distilled water and methanol and air dried for preparation of MSPD columns. Filter paper discs (1.5 cm diameter, Whatman No. 1) were used as column frits to retain the column packing. Standards for the various substances tested were obtained from commercial sources and were >98% purity.

Generic procedures

By adding 0.5 g of sample (milk, fat, liver, kidney, muscle, cultered cell pellet, etc.) to C_{18} derivatized silica or some other appropiate lipophilic polymer-derivatized silica or polymer solid support column packing (2.0 g) in a glass or agate mortar and gently grinding the material for 30 s with a pestle, a nearly homogeneous blend of sample components "dissolved" or dispersed on the solid phase packing material can be obtained. It may be necessary to scrape the sides of the mortar and pestle and repeat this process of blending when dealing with particularly "wet" samples or if homogeneity is not evident. The blend is then transferred with a funnel to a syringe barrel column (10 ml syringe barrel) plugged with a filter paper disc (Whatman No. 1, 1.5 cm). The column head is covered with a second disc and the contents are compressed by a plunger to a volume of 4.5 ml. The column may then be eluted with a single solvent or a series of solvents in order to elute a specific compound, a class of compounds or to perform a total fractionation of the sample matrix. Further purification or the use of co-columns may be required depending on the intended use of the extract and the nature of the compounds being isolated. Elution may be performed by gravity flow, use of a vacuum manifold or centrifugation.

Isolation of drug residues

This generic method has been applied to the isolation of some thirty different compounds representing several major drug classes from a variety of tissue and sample matrices and are summarized in Table I.

Isolation of pesticides and other environmental pollutants

Polyaromatic hydrocarbons in catfish muscle [19]. Following the procedures given above the MSPD column was eluted with 8.0 ml of acetonitrile (ACN). The extract was evaporated to dryness, reconstituted in 1.0 ml of ACN, filtered and assayed by HPLC with UV and florescence detection. The method extracted 14 different fortified polyaromatic hydrocarbons (PAHs) over a concentration range of 100–2000 ng/g with recoveries ranging from 73–112%.

Pesticides in bovine fat [20] and catfish muscle [21]. Nine chlorinated pesticides were isolated from bovine fat or catfish muscle by elution of the MSPD column with 8.0 ml of ACN through a co-column of Florisil (2.0 g) with a recovery ranging from 62–114% for the concentrations examined.

TABLE I

A LISTING OF COMPOUND CLASSES FOR WHICH MSPD EXTRACTION METHODOLOGY HAS BEEN ESTABLISHED

Compound	Matrix (ref.)	Recovery ($n \geqslant 20$)	MSPD wash and eluting solvent[a]
Aminoglycosides			
Neomycin	Bovine kidney [2]	88.6 ± 4.6	Cyanopropyl; wash; hexane, ethyl acetate, MeOH, water; elute 0.1 *M* HCl
Avermectin			
Ivermectin	Bovine liver [3]	74.9 ± 7.3	Wash; hexane, DCM–ethyl acetate (3:1), ACN; elute MeOH
Benzimidazoles			
Albendazole	Milk [14]	81.1 ± 6.8	Wash; hexane; elute, DCM–ethyl acetate (1:2)
	Bovine muscle [1]	73.9 ± 8.0	Wash; hexane, benzene; elute, ethyl acetate
	Bovine liver [13]	72.4 ± 2.6	Wash; hexane; elute, ACN
	Swine muscle [12]	93.0 ± 6.2	Wash; hexane; elute, ACN
Fenbendazole (FBZ)	Milk [14]	69.7 ± 8.9	
	Bovine muscle [1]	74.0 ± 11.8	
	Bovine liver [13]	62.0 ± 5.3	
	Swine muscle [12]	98.0 ± 5.3	
FBZ-OH	Milk [14]	94.4 ± 5.1	
	Bovine muscle [1]	68.4 ± 10.5	
FBZ-SO_2	Milk [14]	100 ± 4.1	
	Bovine muscle [1]	85.7 ± 15.0	
Mebendazole	Milk [14]	101 ± 4.1	
	Bovine muscle [1]	63 ± 4.2	
	Bovine liver [13]	93.0 ± 5.7	
	Swine muscle [12]	85.2 ± 3.2	
Oxfendazole	Milk [14]	107 ± 2.3	
	Bovine muscle [1]	82.9 ± 9.5	
	Bovine liver [13]	86.8 ± 10.8	
	Swine muscle [12]	92.2 ± 7.8	
Thiabendazole	Milk [14]	88.7 ± 5.8	
	Bovine muscle [1]	63.8 ± 9.6	
	Bovine liver [13]	78.5 ± 1.0	
	Swine muscle [12]	85.5 ± 6.8	
β-Lactams			
Penicillin	Bovine muscle [1]	86.3 ± 6.1	Wash; hexane, benzene, ethyl acetate; elute, methanol
Ampicillin	Bovine muscle [1]	59.8 ± 9.8	
Cephalosporins			
Cephapirin	Bovine muscle [1]	72.4 ± 26.5	Wash; hexane, benzene, ethyl acetate; elute, MeOH
Chloramphenicol	Milk [4]	68.7 ± 8.3	Wash; hexane, benzene; elute, ethyl acetate
Chlorsulon	Milk [5]	99.8 ± 5.3	Diethyl ether
Furazolidone	Swine muscle [7]	89.5 ± 8.1	Wash; hexane; elute, DCM
	Chicken muscle [8]	89	Wash; hexane; elute, DCM
	Milk [6]	81.7 ± 8.0	Wash; hexane; elute, DCM
Nicarbuzin	Chicken liver [9]	87.8 ± 1.9	Wash; hexane; elute, ACN
	Chicken muscle [9]	84.4 ± 7.9	
Sulfonamides (S = sulfa)			
S-diazine	Swine muscle [17]	95.1 ± 15.1	Wash; hexane; elute, DCM
	Milk [15]	81.2 ± 4.8	Wash; hexane; elute, DCM
	Infant formula [16]	99.6 ± 5.3	Wash; hexane; elute, DCM

(Continued on p. 26)

TABLE I *(continued)*

Compound	Matrix (ref.)	Recovery ($n \geqslant 20$)	MSPD wash and eluting solvent[a]
S-dimethoxine	Swine muscle [17]	95.8 ± 12.4	
	Milk [15]	89.6 ± 8.1	
	Infant formula [16]	103 ± 9.2	
	Catfish muscle [10]	101.1 ± 4.2	Wash; hexane; elute, DCM
S-merazine	Swine muscle [17]	78.1 ± 9.1	
	Milk [15]	82.0 ± 4.5	
	Infant formula [16]	92.7 ± 8.8	
S-methazine	Swine muscle [17]	84.7 ± 8.2	
	Milk [15]	92.7 ± 5.6	
	Infant formula [16]	99.1 ± 8.8	
S-methoxazole	Swine muscle [17]	95.7 ± 14.8	
	Milk [15]	89.4 ± 8.3	
	Infant formula [16]	112 ± 8.2	
S-anilamide	Swine muscle [17]	70.4 ± 12.7	
	Milk [15]	73.1 ± 7.3	
S-thiazole	Swine muscle [17]	80.3 ± 11.1	
	Milk [15]	93.7 ± 2.7	
	Infant formula [16]	75.9 ± 11.1	
Sulfisoxazole	Swine muscle [17]	92.8 ± 11.8	
	Milk [21]	88.6 ± 11.2	
	Infant formula [22]	93.1 ± 9.7	
Tetracyclines			
Chlortetracycline	Milk [18]	77.2 ± 11.3	Wash; hexane; elute, ACN–ethyl acetate (3:1)
Oxytetracycline	Milk [18]	93.3 ± 3.4	
	Fish [11]	80.9 ± 6.6	Wash, hexane; elute, ACN–MeOH (1:1)
Tetracycline	Milk [18]	63.5 ± 19.6	

[a] Washing sequences are the same for compounds of the same class and for the same reference number.

The resulting eluate was assayed directly by GC–electron-capture detection.

Pesticides in oysters [22] and crawfish and lobster [23] hepatopancreas. Similarly, oyster or crustacean hepatopancreas homogenate was eluted with 8.0 ml of ACN—MeOH (9:1, v/v) through a Florisil co-column. The process isolated 14 chlorinated pesticides with recoveries greater than 60% for concentrations ranging from 62–2000 ng/g of tissue. The eluates were assayed, without further manipulation of the sample, by GC with electron-capture detection.

Clorsulfuron in milk [24]. The milk/C_{18} MSPD column was eluted with 9.0 ml of hexane. The hexane was discarded and the column was eluted with 9.0 ml of DCM. The solvent was removed by evaporation with nitrogen, reconstituted in 250 μl of DCM and analysed by GC with nitrogen/phosphorous detection.

Disruption, lysis and fractionation of bovine muscle tissue [25]

Samples were prepared as described above. Several small aliquots were removed and were sprinkled onto scanning electron microscopy (SEM) mounts coated with a thin layer of graphite paint. C_{18} Material alone served as a control. After drying overnight, the excess C_{18}/sample blend was removed by abrupt shaking. The coated mount was then shadowed with gold–palladium and viewed with a Cambridge Stereoscan Model F-150 scanning electron microscope.

The MSPD column was eluted with 8.0 ml each of the following solvents, hexane, DCM, ethyl acetate, ACN, MeOH and water, respectively. The eluates were collected in preweighed conical glass tubes and the solvents were evaporated under a stream of dry nitrogen. The tubes were then reweighed to determine mass balance. The column packing was removed and sonicated in normal sa-

line. Insoluble materials (other than the C_{18} itself) were removed and assayed. Analyses for the determination on the various classes of compounds in each of the different eluates were undertaken. (i) Protein content by the method of Lowry described in ref. 26. (ii) Total cholesterol by the Sigma colorimetric/enzymatic assay, Procedure No. 352. (iii) Triglycerides were assayed by TLC [27]. (iv) Free fatty acid content and bound fatty acid content (triglycerides, phospholipids, etc.) were determined by differential GC–MS analysis, comparing fatty acid content with and without hydrolytic methanolysis of the extract residues. GC–MS analysis of residues with and without derivatization (trimethylsilyl, TMS) afforded identification of several mono- and disaccharides as well as other individual compounds or compound classes [25].

Lysis and fractionation of bacteria [27]

Samples of pelleted *M. paratuberculosis* and *E. coli* (0.5 g) were blended with 3.0 g and 2.0 g of the C_{18} material, respectively. Aliquots were taken for SEM analysis as described above. Similarly, pelleted bacteria were blended with 40 μm underivatized silica particles, containing no octadecylsilane, for comparison.

Columns prepared from these blends were sequentially eluted with 12.0 ml each of hexane, DCM, ACN, MeOH and water into preweighed conical glass tubes. The organic solvents were removed by evaporation with nitrogen and the water extract was lyophilized. The tubes were then reweighed for determination of mass balance. Protein content in the various fractions was determined by the method of Markwell described in ref. 28. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was also performed on all fractions [29,30]. Nucleic acid content was determined by measurement of absorbance at 260 nm and the 260 nm/280 nm ratio [31] and by the presence of florescent bands on a 1.0% agarose minigel after staining with ethidium bromide [32]. Phospholipid content was determined by a direct colorimetric method [33]. Quantitation of *E. coli* lipopolysaccharide content was conducted by the method of Karkhanis described in ref. 34. As described above for muscle tissues, the various eluates were also examined by differential GC–MS analyses to determine the presence of other compounds or compound classes.

RESULTS AND DISCUSSION

A summary of results utilizing the MSPD technique for the isolation of drugs from a variety of biological matrices is given in Table I. Similarly, the results of the application of this method to the isolation of chlorinated pesticides and organophosphates from several aquatic and non-aquatic species are given in Table II. Mass balance data for *M. paratuberculosis* is shown in Table III. The identification of various classes of compounds for the bacteria are given in Table IV. Micrographs from the examination of the materials obtained by blending the silica particles with tissue and bacteria are shown in Fig. 1A–E.

The extraction methods for the various drug classes summarized in Table I provided recoveries of greater than 60% for the individual compounds, whether the method was for a given compound or for several compounds, over the range of concentrations examined. All of the procedures developed gave correlation coefficients for linearity of 0.99 or better. The limits of detection obtained for these drugs were at or below the action levels established for the various drugs by the different regulatory agencies at the time of publication. In most cases, no clean-up steps were required post-elution and concentration. The extracts, analyzed by HPLC or GC, were relatively free of contaminating co-extractants. In the LC analyses the drugs were assayed using simple isocratic solvent systems with relatively short run times (6–25 min), even when the analysis was conducted for several compounds simultaneously.

Similarly, the methodlogy developed for the chlorinated pesticides and organophosphates, and summarized in Table II, provided more than adequate limits of detection and excellent recoveries and linearities over the ranges of concentrations tested in the various matrices. The cleanliness of the extracts also greatly enhanced the analyses and reduced the need for any post-elution clean-up. Essentially the samples are assayed directly after elution, reducing the degree of sample manipulation required and the time necessary to obtain results. As with many of the drug methods, the MSPD approach required a minimum of 8.0 ml of solvent and could be performed within 30 min, ready for analysis.

There are several points to be made concerning

TABLE II

THE PERCENT RECOVERIES OF VARIOUS PESTICIDES FROM DIFFERENT MATRICES

Samples were extracted by MSPD and assayed as described in Experimental. Recovery values are the mean for the range of concentrations examined ± S.D.

Pesticides	Matrix					
	Catfish muscle [21]	Bovine fat [20]	Oyster [22]	Crawfish [23]	Milk [24]	Bovine muscle [1]
Lindane	82 ± 5	85 ± 3	78 ± 7	86 ± 7		
Heptachlor	84 ± 9	86 ± 5	73 ± 8	86 ± 10		
Aldrin	94 ± 12	92 ± 13	67 ± 10	80 ± 12		
Heptachlor epoxide	93 ± 12	86 ± 6	82 ± 10	74 ± 9		
p,p'-DDE	91 ± 6	94 ± 6	81 ± 11	94 ± 13		
Dieldrin	91 ± 2	95 ± 3	73 ± 11	90 ± 11		
Endrin	93 ± 7	97 ± 3	74 ± 9	79 ± 2		
p,p'-TDE	97 ± 4	97 ± 5				
p,p'-DDT	97 ± 5	102 ± 5	69 ± 12	96 ± 13		
a-BHC			77 ± 8	83 ± 6		
b-BHC			80 ± 16	81 ± 4		
4,4'-DDD			81 ± 14	90 ± 9		
Endosulfan-SO_4			74 ± 8	93 ± 12		
Methoxychlor			65 ± 8	95 ± 12		
Endrin aldehyde			70 ± 7	54 ± 7		
Clorsulfuron					92 ± 11	
Fenthion						86 ± 8
Coumaphos						77 ± 8
Famfur						82 ± 9
Crufomate						94 ± 6

these results as they relate to the MSPD process. The compounds extracted represent a diversity of molecular structure and polarity characteristics. Nevertheless, an essentially generic methodology afforded high recoveries, with a degree of specificity, of all of these various compounds from several different matrices, even from the same sample. The mechanisms involved in MSPD appear to encompass sample homogenization and cellular disruption, exhaustive extraction, fractionation and purification in a single process. The method involves the dispersal of a sample over a theoretical surface area of 1000 m^2 (500 m^2/g C_{18} solid support) in a thin film (100 Å), utilizing the shear forces of the particles and the blending or grinding action employed to disrupt the sample architecture while the polymer (C_{18} or others) serves to dissolve or disperse the sample components on the basis of hydrophobic–hydrophilic interactions. In this manner the polymer bound to the solid support may literally disrupt and unfold cell membrane or micellar lipids. The disruption process can be envisioned as incorporating the use of shear forces from the particles with tissue solubilization using detergents, two classical approaches to tissue and cellular disruption. However, in MSPD the "detergent" is bound to the particles, eliminating the need to subsequently remove the detergent before the final analysis, and provides a unique column support material for subsequent isolation of the dispersed compounds.

TABLE III

THE PERCENT RECOVERIES (± S.D.) AND MASS BALANCE FOR THE DISRUPTION AND FRACTIONATION OF *M. PARATUBERCULOSIS* [27]

Data are calculated based on the dry weight of the sample (0.5 g wet weight).

Solvent	% Recovery *M. paratuberculosis*
Hexane	14.7 ± 4.7
DCM	40.6 ± 3.3
ACN	16.6 ± 6.1
MeOH	17.2 ± 2.8
Water	11.2 ± 0.4
Remaining on column	6.8 ± 0.5
Total accounted for	100 ± 4.7

TABLE IV

THE MAJOR COMPOUNDS OR CLASS OF COMPOUNDS IDENTIFIED IN THE VARIOUS ELUATES OBTAINED FROM THE DISRUPTION AND FRACTIONATION OF THE BACTERIA *M. PARATUBERCULOSIS* AND *E. COLI* BY MSPD [27]

Solvent	Major compounds identified	
	M. paratuberculosis	*E. coli*
Hexane	Neutral lipids	Neutral lipids
DCM	Phospholipids	Neutral lipids
		Short chain fatty acids
		Indoles
		Quinolines
ACN	Short chain fatty acids	Pyrimidine
	Sterols	Indoles
		Aromatic acids
		Sterols
MeOH	Phospholipids	Phospholipids
	Amino acids	Amino acids
	Inositols	Purines
	Mono-, disaccharides	Pyrimidines
	Citric acid	Mono-, disaccharides
Water	Nuleotides/nucleotides	Nucleotides/nucleotides
		Lipopolysaccharides
	Protein: Water > DCM > MeOH > ACN	Protein; Water ≫ MeOH > ACN

By transferring the material to a column and performing a solvent elution one obtains a distribution of the compounds as well as other sample components that is dependent on (i) interactions with the bound polymer phase and solid support, (ii) interactions with the dispersed sample matrix components, (iii) molecular size and (iv) interactions with the eluting solvent(s). Combinations of these factors for individual components are also certain to be involved as well. Although having many of these properties in common with classical solid phase extraction (SPE) the MSPD process is distinctly different, possessing elution and retention properties that appear to be a mix of partition, adsorption and paired ion/paired component chromatography that is somewhat unique. These properties are effected by the following variables. (i) The solid support and the bound phase utilized. (ii) The nature of the sample matrix. (iii) The ratio of sample to solid support. (iv) The solvent elution sequence performed. (v) The use of matrix modifiers. One may influence the disruption, distribution and subsequent elution profile of an MSPD column by blending the sample in the presence of acids, bases, salts, chelators, preservatives or other modifiers. (vi) The use of various solid support combinations or tandem column configurations. We have observed that for many of the drugs and matrices examined, little or no further clean-up or chemical manipulation of the sample is necessary following elution. However, several classes of compounds co-elute with sample matrix components that interfere with detection or that foul the instrumentation after several injections. In some cases a simple back-extraction or re-solubilization process has eliminated such interferences. For several drugs a more efficient process has been the use of tandem columns. For example, the MSPD isolation of nine pesticides from bovine fat or catfish muscle and the fourteen pesticides for oysters and crawfish hepatopancreas is assisted by including in the bottom of the same column 2 g of Florisil, which has little retention for such compounds but readily removes lipids and other materials that adversely effect subsequent GC–electron-capture detection. Similarly, Schenck *et al.* have utilized alumina SPE columns post elution of nicarbazin from chicken liver [9] and muscle [9] and ivermectin from beef liver [3]. We have also observed that the incorporation of up to 1 g of C_{18} in the bottom of an MSPD column prior to addition of the matrix blend can often provide extra fractionation and clean-up of eluates.

These factors come into play whether one is isolating drugs, pollutants or the naturally occurring components of the sample matrix itself. As shown in Table III one is able to account for the entire sample through isolation of eluted components or removal of uneluted components from the column solid support. As with the drugs and pollutants, there is a discernable specificity of elution of sample structural components from the MSPD column. Based on the analyses conducted on the various tissue fractions [25], the elution sequence employed efficiently fractionated triglycerides (hexane fraction) from steroids (DCM fraction), fractionated proteins into several eluates (MeOH > water > ACN > ethyl acetate) and provided a degree of separation of the various carbohydrate and other components of muscle tissue. Connective tissues were neither disrupted by the process nor were they eluted from the MSPD column. Sonication of the

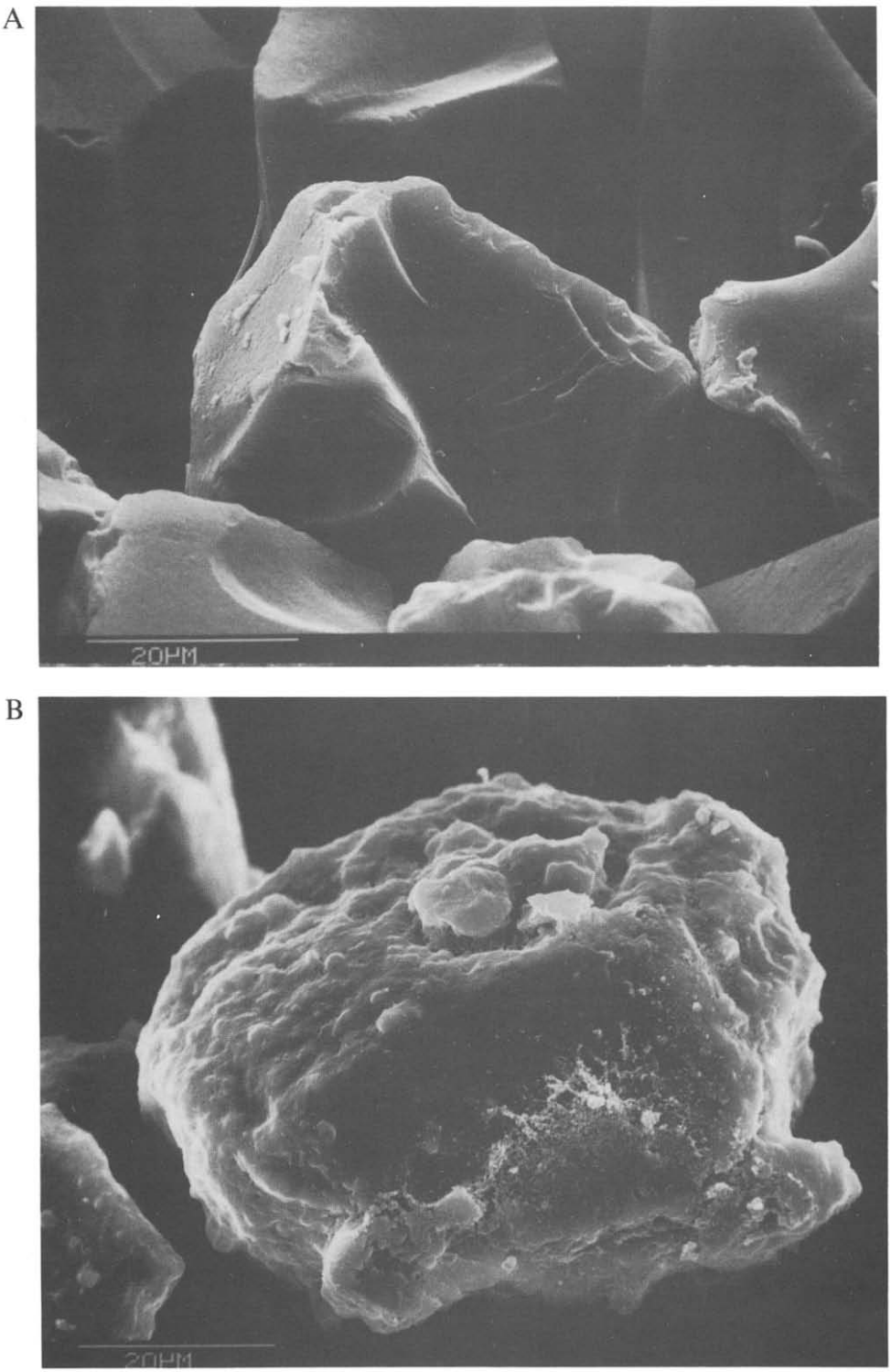

Fig. 1.

C

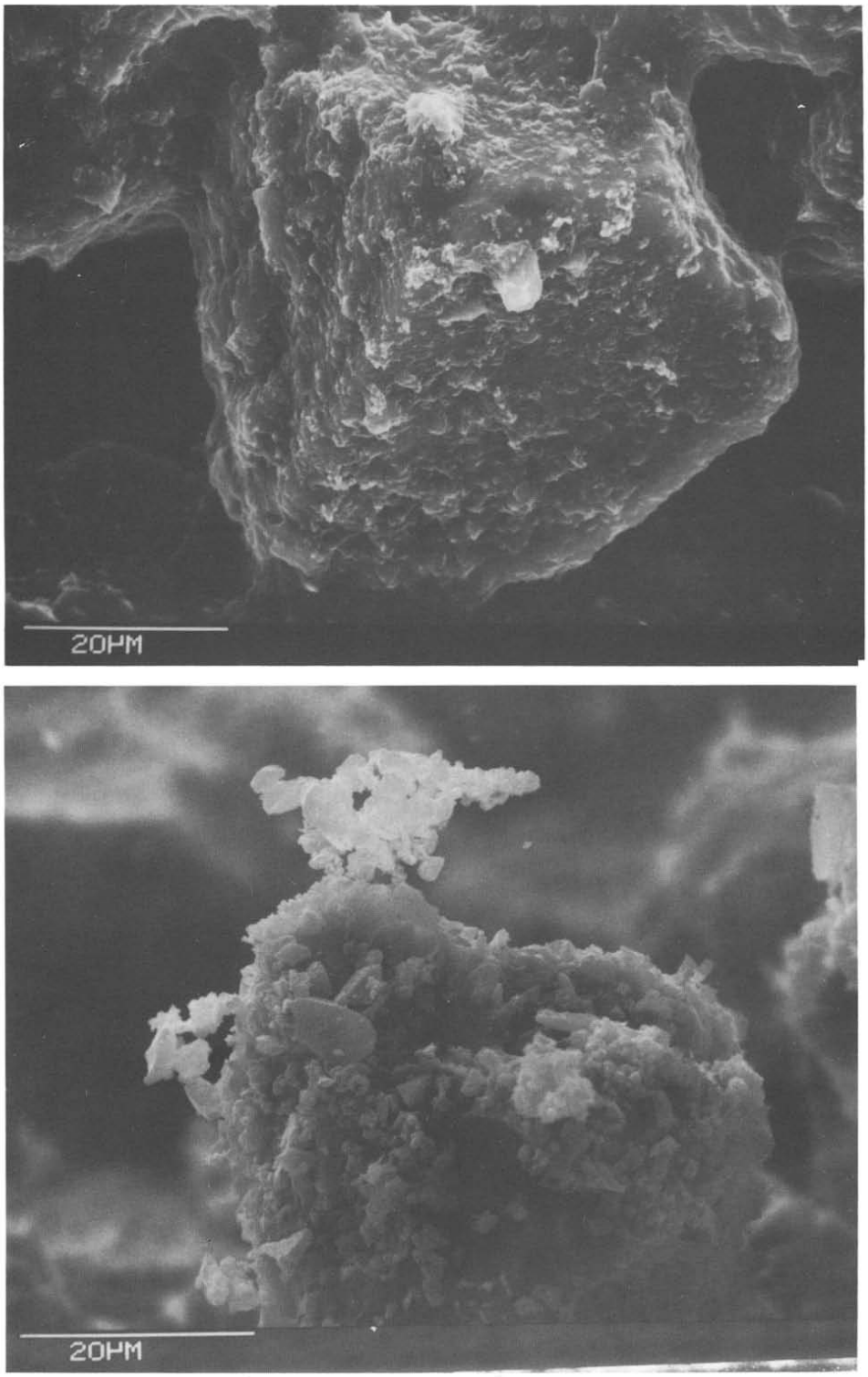

Fig. 1.

(Continued on p. 32)

E

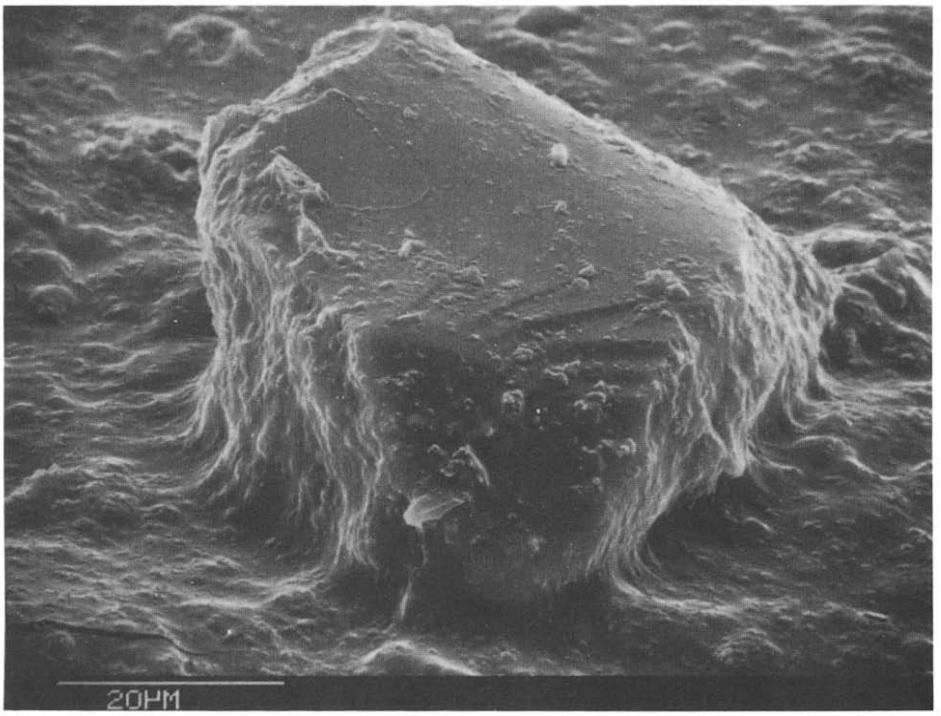

Fig. 1. Scanning electron micrographs of (A) C_{18}-derivatized silica particles used in the studies presented; (B) C_{18}-particles after blending with bovine liver tissue, as described in Experimental; (C) C_{18}-material after blending with pelleted *Mycobacterium paratuberculosis*; (D) underivatized silica particles after blending with *M. paratuberculosis* (Note the degree of silica debris created and the clumping of cells and debris in comparison to B and C); and (E) C_{18}-particle blended with *M. paratuberculosis* showing where two particles had apparently been in contact and broken away. The layer of dispersed material is evident.

column packing allowed isolation of these compounds as an insoluble agglomerate on the surface of the saline sonicate supernatant. Some proteins and materials extractable with a saturated solution of EDTA remained on the column as well but were isolatable by these procedures. The solvent sequence employed no doubt contributed to denaturation of some of the proteins and of larger nucleoside (DNA, RNA) components. Although not examined in the case of muscle tissues we have observed for bacteria that DNA, RNA and other higher-molecular-mass components can be eluted from the less polar components of an MSPD column by beginning the elution sequence with aqueous buffers and reducing the solvent strength as one progresses, *i.e.*, reversing the order of solvent strength elutions performed here. However, the degree of fractionation specificity is greatly diminished [27]. This difficulty may be resolved by including a more polar polymer phase solid support in the blend.

Thus, in the MSPD process for the isolation of drugs or pollutants, one is simultaneously performing a sample matrix fractionation and distinct matrix components will be found to be associated with different drug or pollutant classes. Indeed, the unique elution characteristics of MSPD colums may be directly related to the association of target molecules with the co-eluting tissue components that distribute in the eluting solvents and their interaction with the solid support and the remaining tissue components.

As seen in Table III the application of MSPD to bacteria also afforded a high degree of sample recovery and specificity of elution (Table IV). It should be noted that mycobacteria, such as *M. paratuberculosis*, often require rather severe procedures for complete cellular disruption, such as treatment with antibiotics and high concentrations of detergents or extended sonication at maximum intensity [27]. Indeed, mycobacteria are among the most difficult bacteria to disrupt due to the thick-

ness of their cell walls. *E. coli*, on the other hand, can be readily disrupted by treatment with lysozyme and low concentrations of detergents or short bursts of sonication. However, the results seem to indicate that the MSPD process can perform facile lysis and fractionation of both of these bacterial classes in a chemically and physically mild manner while providing the ability to perform sample fractionation in a single step.

The protein elutions/distributions for *E. coli* (water ≫ MeOH > ACN) and *M. paratuberculosis* (water = DCM > MeOH > ACN) were somewhat different. This may be due to differences in hydrophobicity of certain mycobacterial *versus E. coli* proteins and differences in the interactions between the individual proteins and the remaining constituents on the column. The distribution of proteins suggests that those eluting in the more non-polar solvents (DCM, ACN) may be structurally more lipophilic and possibly associated with cell membranes in their native environment. Further research is being conducted to answer this possibility.

The data also indicate that the MSPD process separated the neutral lipids (hexane fraction) from the phospholipids (DCM and MeOH fractions) in the bacteria. Likewise, fractionation of sterols, indoles, amino acids, purines, pyrimidines, inositols and other mono- and disaccharides was observed for each of the bacteria. However, their individual solvent distributions varied between the two types of bacteria. Since *E. coli* also has a high lipopolysaccharide content each fraction was examined for this compound and was found to reside only in the water fraction. Similarly, nucleic acids and nucleotides were found only in the water fraction but did not account for the total nucleotide content for the mass of cells extracted. We have observed that by beginning the elution sequence with water that a much higher recovery of genomic and plasmid DNA can be obtained from these bacteria and that digestion of the DNA with various restriction endonucleases can be accomplished [27,35].

Taken together these data lend strong support to the proposal that the MSPD process provides a new and generic technique for the homogenization, lysis and/or fractionation of a variety of biological matrices. This conclusion is further borne out by the data obtained from SEM analysis of the various materials. Fig. 1A shows the nature of the solid support/C_{18} particles themselves. The material used is irregular in shape and contains serrated and sharp edges. These characteristics, as in the use of sand, alumina or other abrasives, assist in the initial disruption of the matrix achitecture. Fig. 1B shows the same material after blending with bovine liver. Examination of these and other tissues gave no indication for the existence of intact subcellular structures or individual cells, supporting the idea that complete cellular lysis and dispersion is occurring. Similarly, Fig. 1C shows the result of blending the C_{18} with mycobacteria. Again, no intact cells were noted. This should be contrasted to the results seen with mycobacteria after grinding with identical silica particles that differed only in the fact that they were not derivatized with C_{18} (Fig. 1D). In this case fractured silica particles and clusters of intact mycobacteria were observed. Thus, the use of silica particles alone and the application of shearing forces by grinding were inadequate to obtain lysis of the mycobacteria.

That the cellular components are dispersed is somewhat evident from the various figures wherein C_{18} derivatized particles were used. For example, as shown in Fig. 1E, we often observed particles that had apparently been in contact with others and had broken away. These micrographs show what appears to be a thin layer or film of dispersed material over the surface of the particles. Measurement of the thickness of this layer gave a value of approximately 100 Å.

This approach has several possible advantages over classical methods for the disruption, lysis and fractionation of biological matrices. The process of blending is easy to perform and does not require expensive equipment or special devices. For the types of samples examined the process provides a homogeneous blend of the biological and incurred components dispersed over a large surface area in a thin film. It is applicable to easy or difficult to disrupt tissues as well as bacteria without modification of the process or extra exertion. It is chemically and physically mild, exposing the sample to no excessive heating, mechanical forces or strong chemical or detergent-based reagent. The material obtained can be packed into a column from which compounds may be isolated by various elution schemes. There is sufficient flexibility in the method to allow use of the

listed variables to make the approach applicable and efficient for a variety of needs. It uses, relatively speaking, very small volumes of extracting solvents, greatly reducing solvent need and disposal costs. The method, as applied here, is capable of providing a rapid isolation of the target molecule(s) with a high degree of specificity. The inclusion of co-columns and the use of other column technologies (SPE, immunoaffinity, etc.) could further enhance this specificity while retaining its overall efficiency. The process is also amenable to automation, using an automated mortar and pestle to prepare the samples and eluting the columns using robotics or batch processing instrument configurations. The overall process is also quite rapid and can greatly reduce the time required to isolate and process samples for various needs.

Given these possibilities there remains a wide range of analytical problems to which MSPD may be applicable. However, it is recognized that it will not be applicable in all cases. Nevertheless, if it is truly generic, it will provide an additional method to the analyst for assisting in resolving difficult analytical problems when dealing with biological matrices.

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