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CEE 772: INSTRUMENTAL METHODS IN ENVIRONMENTAL ANALYSIS

Lecture #12

Sample Preparation: Basics and Physical Methods

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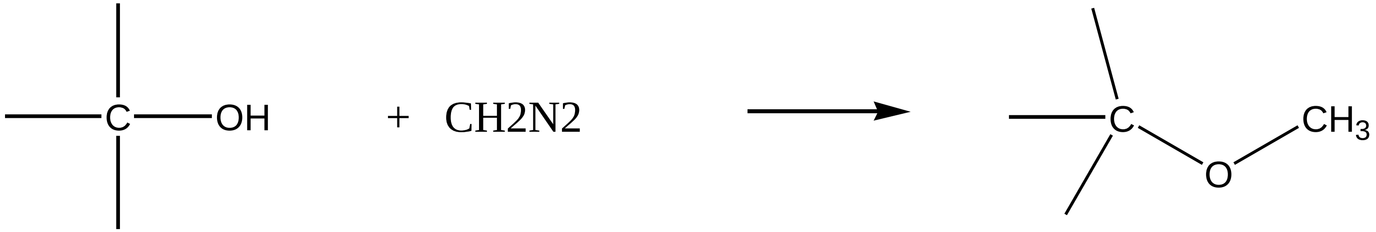
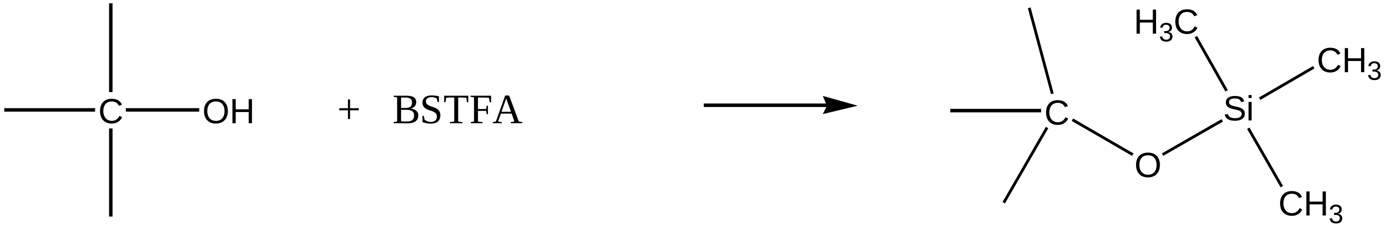
(817-839)

Derivatization of hydroxy groups

- Diazomethane



- Bis(trimethylsilyl)trifluoroacetamide



Functional Group	Silylation	Acylation	Alkylation
Active Hydrogens	BSA, BSTFA, BSTFA/TCMS, Deriva-Sil, Hydrox-Sil, MSTFA, MTBSTFA, TMSI	PFPOH/PFPA	DMF Dialkylacetals, TBH
Carboxylic Acids	BSTFA, Hydrox-Sil Conc., MSTFA, TMSI	PFPOH/PFPA	BF_3/Methanol, BF_3/n-Butanol, DMF Dialkylacetals
Alcohols and Phenols: unhindered and moderately hindered	BSA, BSTFA/TCMS, HMDS, MTBSTFA/t-BDMCS	HFBI, Fluorinated Anhydrides (HFBA, PFPA, TFAA), MBTFA, MCF*	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4, TBH
Alcohols and Phenols: highly hindered	BSTFA/TCMS, Deriva-Sil, Deriva-Sil Conc.	Fluorinated Anhydrides, (HFBA, PFPA, TFAA), HFBI, PFBCI	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4, TBH
Amines: primary and secondary	BSTFA, MTBSTFA/t-BDMCS	Fluorinated Anhydrides, (HFBA, PFPA, TFAA), HFBI, MBTFA, PFBCI, TPC*	DMF Dialkylacetals, TBH
Amides	BSA, BSTFA, BSTFA/TMCS, Deriva-Sil Conc.	HFBI	DMF Dialkylacetals, TBH
Amino Acids	BSTFA, TMSI	HFBI (+ Silyation)	DMF Dialkylacetals, TBH
Catechloamines	TMSI	Fluorinated Anhydrides, (HFBA, PFPA, TFAA), HFBI	
Carbohydrates and Sugars	HMDS, Hydrox-Sil AQ, TMSI	MBTFA	
Inorganic Anions	BSTFA, MTBSTFA		
Nitrosamines		HFBA	
Sulfonamides	BSTFA	Fluorinated Anhydrides, (HFBA, PFPA, TFAA)	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4,

Functional group	Derivatization
-OH (hydroxyl group) in primary, secondary and tertiary alcohols; phenols; carbohydrates)	• Silylation • Acylation • Benzoylation • Alkylation • Dansylation • Reaction with Dis-Cl • Reaction with FDNB • Reaction with NBD-Cl • Ion-pair formation
-COOH (carboxylic acids)	• Esterification • Silylation • Ion-pair formation
-C=O (carbonyl group) in aldehydes and ketones	• Oxime formation • Oxime formation and silylation • Ketal/acetal formation • Hydrazone formation • Schiff's base formation • Silylation

From Start GC: http://gc.discussing.info/index_reference.html

Functional group	**Derivatization**
-NH_2 (amino group) in primary amines, amino acids, amino sugars	Acylation Benzoylation Silylation Treatment with CS_2 Thiourea formation Schiff's base formation 2,4-Dinitrophenylation Sulphonamide formation Carbamate formation Treatment with pyridoxal Treatment with NBD-Cl Alkylation Ion-pair formation
-NH-R (amino group) in secondary amines, imino acids, substituted amino sugars	Acylation Benzoylation Silylation 2,4-Dinitrophenylation Sulphonamide formation Treatment with NBD-Cl Ion-pair formation
-NH_2 and -COOH in amino acids	Silylation Esterification + Acylation
-NO_2 (nitro compounds)	Chromatograph without derivatization

Alkylation

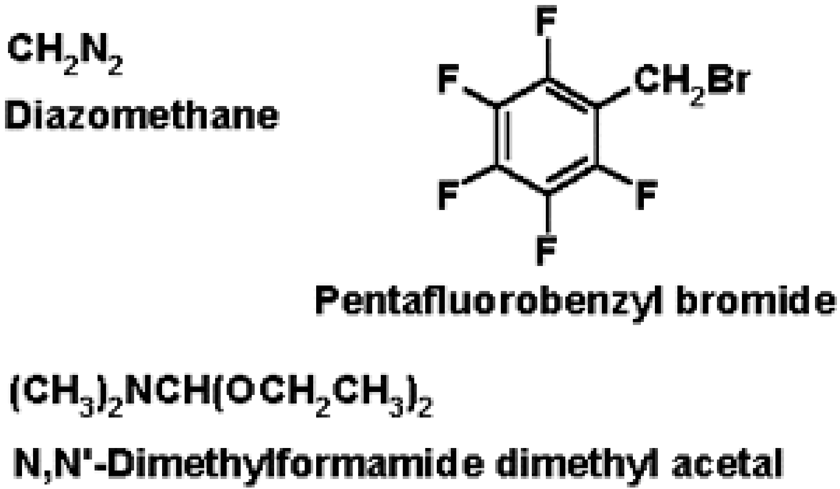


Figure 1. Structures of the most commonly used alkylating reagents

- Alkylation is the replacement of a active hydrogen in R-COOH, R-OH, R-SH, and R-NH2 with an alkyl group or, sometimes aryl group. The gas chromatographic properties of compounds are enhanced because of the decreased polarity of the derivatives as compared with the parent compound. One of the most important areas of chromatography where alkylation has been applied concerns carbohydrates. A number of reagents are available and the structures of the widely used reagents are shown in Figure 1.

- Alkyl halides in the presence of silver oxide will convert any non-hindered carboxylic acid to the corresponding alkyl ester in minutes, and phenolic or thiol groups will also be alkylated rapidly. The alkyl halides most frequently used are the lower molecular weight aliphatic bromides and iodides or benzyl and substituted benzyl bromides.
- Diazoalkane alkylating reagents include diazomethane, diazoethane, diazoisobutane and phenyldiazomethane. The diazomethane is most frequently used. many workers prefer not to make large quantities of these materials because the diazoalkanes are toxic materials.

Figure 2. Alkylation with diazoalkane

- Various N,N'-dimethylformamide dialkyl acetals, $(CH_3)_2 NCH(OR_1)_2$, are commercially available in which $R_1 = CH_3$, C_2H_5, C_3H_7 and C_4H_9. The reagents are easily hydrolyzed to dimethylformamide and the appropriate alcohol so most reactions are performed under scrupulously dry conditions. Other reagents commonly used include quaternary alkylammonium hydroxides such as tetramethyl and tetrabutylammonium hydroxide as a 0.2 M solution in methanol which is used mainly for low-molecular-weight acids.

Silylation

- Silylation is the most widely used derivatization technique. Nearly all functional groups which present a problem in gas chromatographic separation (hydroxyl, carboxylic acid, amine, thiol, phosphate) can be derivatized by silylation reagents. It involves the replacement of an acidic hydrogen on the compound with an alkylsilyl group, for example, $-SiMe_3$. The derivatives are generally less polar, more volatile and more thermally stable.
- The introduction of a silyl group(s) can also serve to enhance mass spectrometric properties of derivatives, by producing either more favorable diagnostic fragmentation patterns of use in structure investigations, or characteristic ions of use in trace analyses employing selected ion monitoring and related techniques.
- The most common reagents for silylation are the trimethylsilyl (TMS) reagents. There are a number of TMS donor reagents available and they show the wide applicability and ease of use of the TMS reagents. In addition the reaction for TMS derivatives occurs cleanly without artifact or byproduct formation. The structures of the most widely used trimethylsilylating reagents are shown in Figure 1.

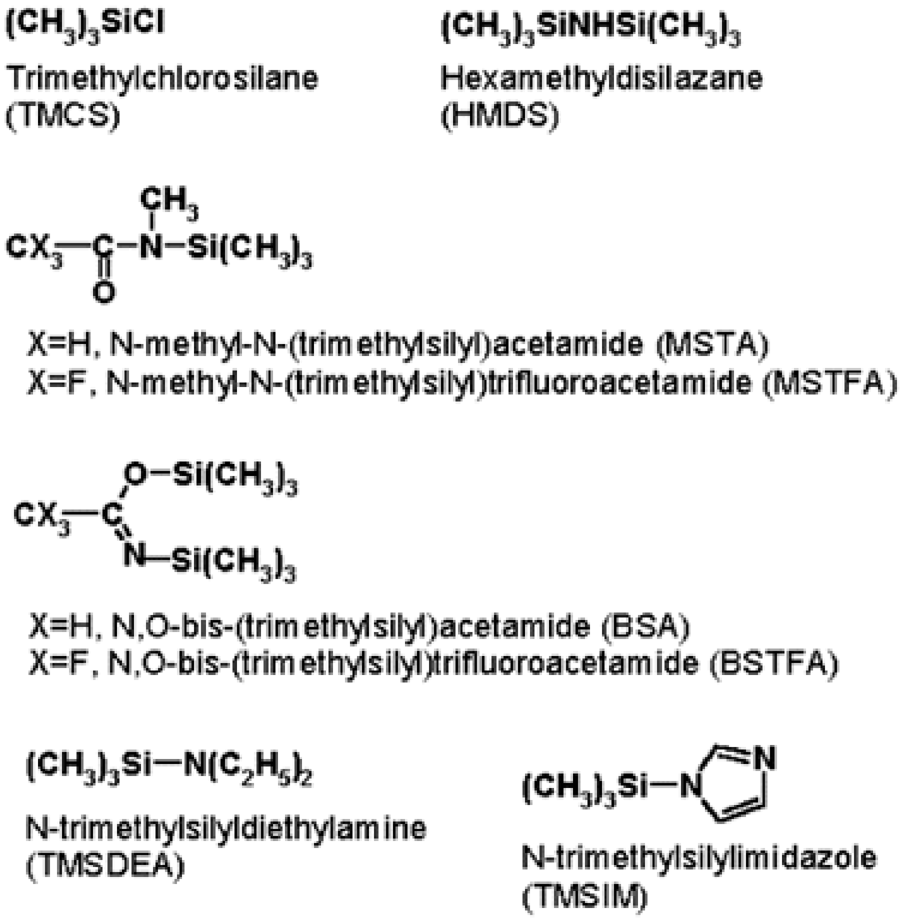


Figure 1. Structures of the most commonly used trimethylsilylating reagents

- Silylation reactions generally proceed very rapidly (within 5 min) with pyridine being the most frequently used solvent. GC columns used for analysis of silyl derivatives are conditioned by HMDS before use to block any acidic sites and avoid possible reactions with silyl derivatives. Many varied and improved silylation have been developed. Examples are the substituted acetamides such as BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide), BSA (N,O-bis(trimethylsilyl)acetamide) and N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).
- A examples of silylation is shown below:

  For N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)

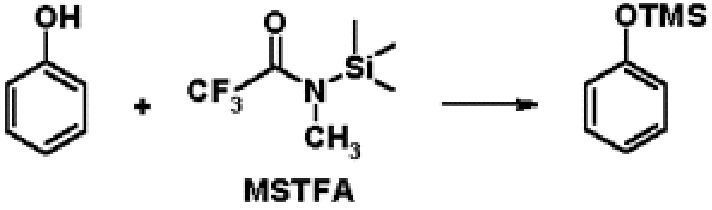
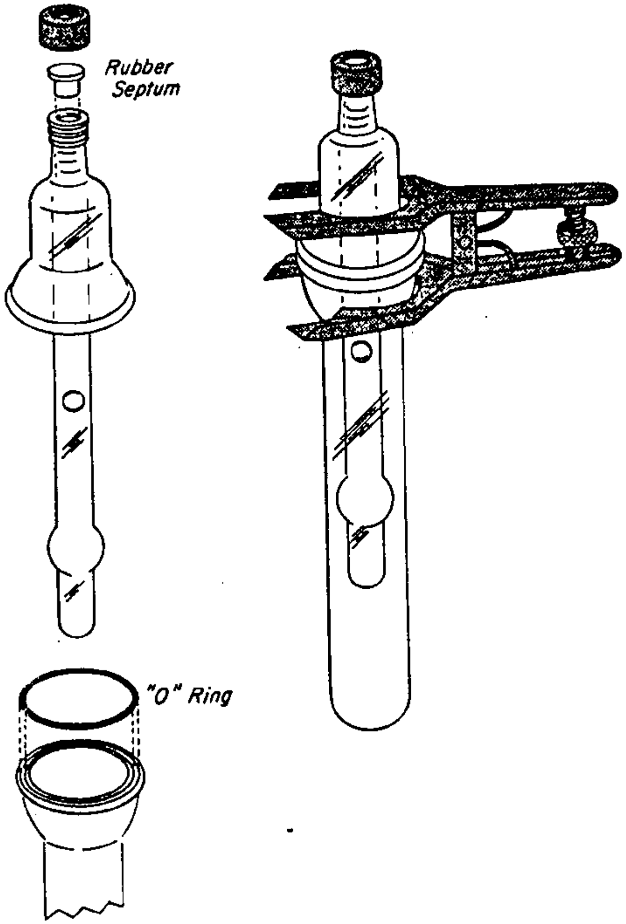
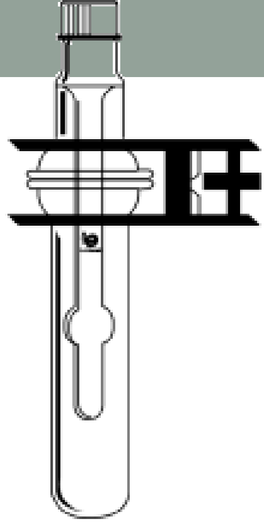


Figure 2. Trimethylsilylation of phenol

Diazomethane generation I

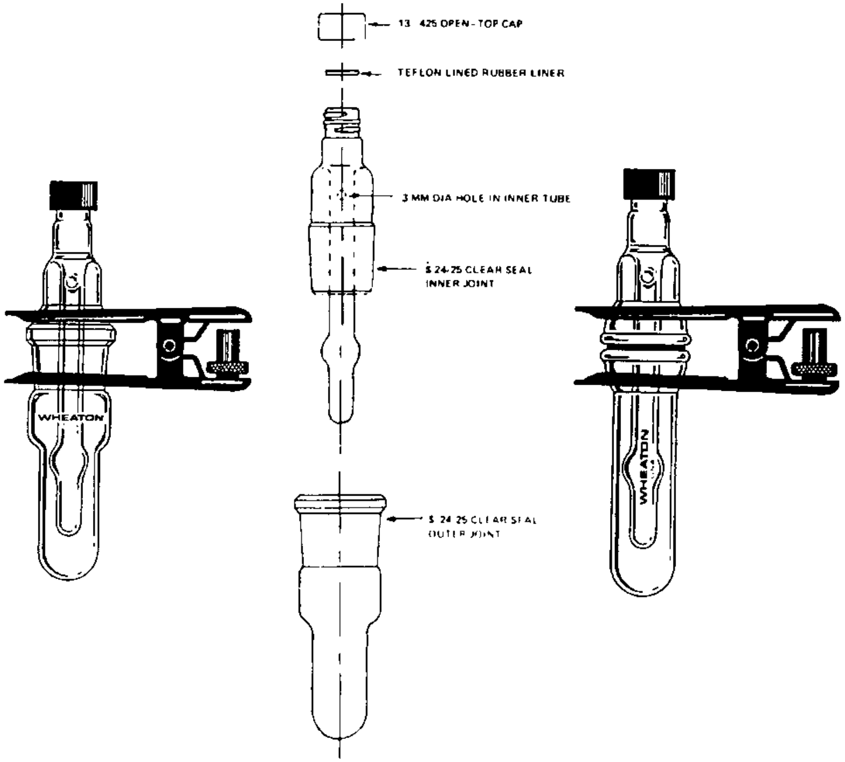
- (N-Methyl-N'Nitro-N-Nitrosoguanidine) MNNG produces diazomethane when treated with an aqueous alkalai. The co-distillation effected with this apparatus produces small quantities required for analytical purposes.
- One millimole (147 mg) of nitrosoguanidine is transferred to the inner tube with 0.5mL of water to dissipate heat generated by the reaction.





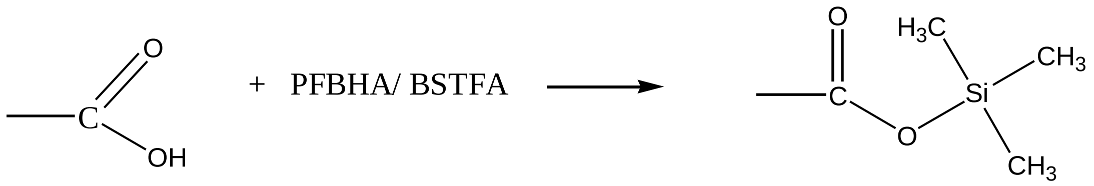
Diazomethane generation II

- Approximately 3mL of ether is measured into the outer tube and the generator is assembled using a Viton® O-ring and a pinch clamp.
- The lower part of the assembled apparatus is placed in an ice bath and 0.6 mL of 5 N sodium hydroxide is injected very slowly. A drop by drop addition is suggested, to avoid excessive foaming and back pressure. The injection is made through the silicone septum using a syringe having a 22 gauge needle.
- The generated diazomethane collects in the ether and can be stored for short periods of time

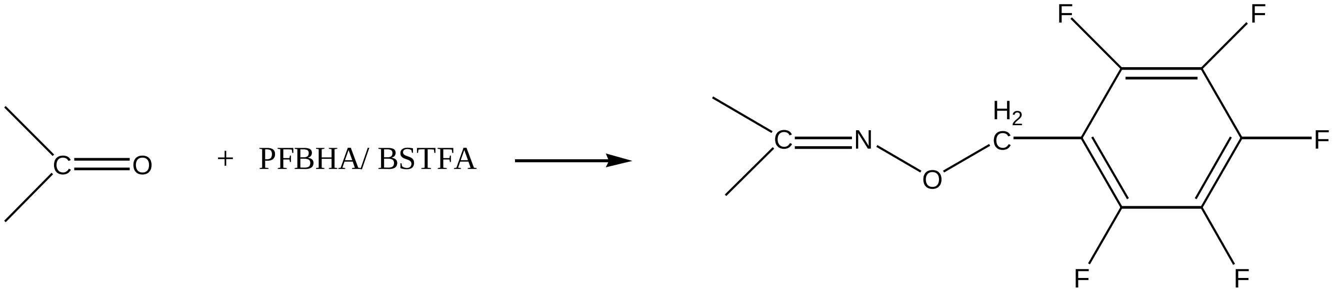


PFBHA/ BSTFA Derivatizations

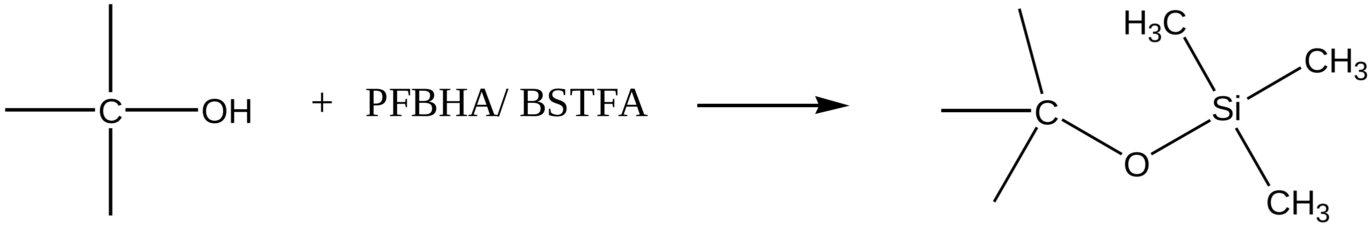
- Carboxylic groups



- Keto-groups:



- Hydroxy-groups



- To next lecture