2-26-2001

Dansylation of hydroxyl and carboxylic acid functional groups

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Abstract

Fluorescent labeling of primary and secondary amines using dansyl chloride has been widely used in the past. Its application provides an extremely sensitive means to detect amine functional groups to amounts of less than 1 mg of material. This work describes a method for the dansylation of hydroxyl (–OH) and carboxylic acid (–COOH) functional groups. This technique is demonstrated with ethanol, gamma hydroxy butyric acid (GHB), benzoic acid, and p-chloroaniline. Sensitivity of detection for all compounds are microgram or microliter. For the compounds ethanol and GHB which are liquids at room temperature, as little as 1 ml quantity can be detected. Benzoic acid and p-chloroaniline which are solids at room temperature can be detected at levels of 1 mg. Fast thin layer chromatography was accomplished using acetone as the resolving solvent, which resulted in good differentiation of analytes for R_f measurement. The dansylation reaction performed similarly at pH 11, 10 and 9.6 and uses 2 molar $Na_2 CO_3$.

Keywords:

Fluorescence; Ethanol; Dansylation

1. Introduction

The compound dansyl chloride (DNS-Cl; 5-dimethylaminonaphthalene-1-sulfonylchloride) has been used extensively in analytical chemistry to fluorescent label substances having primary and secondary amines on their molecular structure [1,2]. This process of dansylation creates a fluorescent derivative which can be detected with ultraviolet light with great sensitivity. Amounts of material of less than 1 mg can be observed when illuminated with ultraviolet light. The technique of dansylation is versatile, and has been applied to many types of analytical applications in biology and toxicology [2].

Other methods exist for the derivatization of either a hydroxyl group (–OH) or carboxylic acid (–COOH) group. For electrochemical detection, phenolic hydroxyl groups can be converted to quinoneimines and alcoholic may be reacted to form urethanes [3,4]. For ultraviolet-visible (UV–VIS) detection the derivatives of carboxylic acids may be syntheized using alkyl halides (panacyl bromide, *p*-bromophenacy bromide), aromatic amines (*p*-methoxyaniline, *p*-chloroaniline, 1-naphthylamine), hydrazines

(nitrophenylhydrazines), or hydroxylamines [5,6]. For UV–VIS detection of hydroxyl groups, labeling with acyl halides, acid anhydrides, isocyanates, and phenyldimethylsilyl chloride are effective [7]. The fluorescent detection of hydroxyl groups can be achieved by forming benzofuran and naphthalene fluorophores [8] and for carboxylic acids, formation with fluorophores such as coumarin, phenanthrene, pyrene, quinoxaline, and benzofurazan are accomplished [9]. Chemiluminescence detection of phenolic hydroxyl groups can be accomplished by reacting with 10-methyl-9-acridinium or Lissamine Rhodamine B sulfonyl chloride, whereas carboxylic acids are reacted with compounds having the fluorophores of quinoxalines and coumarins [10].

Fluorescent detection of compounds by dansylation of chemical groups such as alcoholic hydroxyls and carboxylic acids has applications in studies concerning toxicology. An alcohol such as ethanol (CH_3 CH_2 OH), which is an abused substance, can be fluorescent labeled by dansyl chloride utilizing the method presented here. The compound gamma hydroxy butyric acid ($HOCH_2$ CH_2 CH_2 COOH) can also be dansylated by this method, it being an abused drug with both a hydroxyl and a carboxylic acid functional group. Other compounds having medicinal applications can be labeled with DNS–Cl by the method shown.

2. Materials and methods

2.1. Materials

Reagents were obtained from the Sigma Chemical Company, St. Louis, MO 63178, USA. The gamma hydroxy butyric acid was provided by the Chemistry Department, College of Arts and Sciences, University of Nebraska, Omaha, NE 68182 USA. A hand held ultraviolet light source was used for sample illumination.

2.2. Dansylation of compounds

2.2.1. Part 1

Solid or liquid samples should be solubilized in a minimal amount of water. Then an amount of 50–100 ml of the sample is added to an equal volume of 2.0 molar Na_2CO_3 (pH 11.0) in small eppendorf tube or reaction glass tube. Add 30 ml of dansyl chloride (5 mg / ml in acetone) to the mixture. Store the reaction solution in dark, and occasionally mix 30 min for hydroxyl group dansylation or 60 min for carboxylic acid group dansylation.

2.2.2. Part 2

Extract the reaction solution with about 300 ml of diethyl ether. Remove the organic layer (top) from the aqueous portion and it can be dried with a small amount (1 / 5 of total volume) of $MgSO_4$. Do not store over $MgSO_4$, but samples can be saved in dark for

several hours. The samples can be examined by ultraviolet light source, injected into high performance liquid chromatography (HPLC) instrument, or placed onto thin layer chromatography (TLC) plates for analysis. Samples were spotted onto silica TLC plates and resolved by using acetone as resolving solvent.

3. Results and discussion

Detection of analytes via fluorescent emission is an extremely sensitive technique with many applications. Detection sensitivity for the liquid compounds ethanol and GHB, is at 1 ml. For benzoic acid and *p*-chloroaniline, which are solids at room temperature, detection was as little as 1 mg. The procedure is simple and reproducible. The dansylation of primary and secondary amines has been widely used, is a well known reaction, and proceeds efficiently [1,2]. When monitoring the time of the dansylation reaction, followed by separation using TLC, it was found that *p*-chloroaniline (a positive control) is dansylated in 15 min or less.

The acidity of the hydrogens on the amine group plays an important factor in the dansylation reaction. Carboxylic acids have acidity strengths greater than alcohols [11]. The acidity of alcohols can play an important role in chemical reactions, and its known that primary alcohols are more acidic than secondary alcohols [12]. In the case of ethanol, the p*Ka* is about 16 [13].

Although the acidity of ethanol is low, it is found that by using a very high buffer strength with $Na_2\ CO_3$ the reaction with dansyl chloride will take place. The reaction of ethanol ($CH_3\ CH_2\ OH$) with dansyl chloride should proceed 30 min or more. The compound GHB ($HOCH_2\ CH_2\ CH_2\ COOH$), also referred to as 4-hydroxybutanoic acid, which has a primary alcohol group in addition to the carboxylic acid group was dansylated in 30 min by this procedure. GHB (gamma hydroxy butyric acid) is a controlled substance which induces an effect of disorientation on the user. Relative time periods for the dansylation of representative compounds in this work is shown in the table below (Table 1).

Upon reaction in aqueous solution the dansyl chloride that does not bind to an analyte becomes hydrolyzed and can be separated out by TLC. Alcohols and amines react with sulfonyl chlorides more rapidly than does water [14]. Many derivatization reagents for carboxylic acids are of the fluorescent type (i.e.coumarin, phenanthrene, pyrene, quinoxaline, etc.) and many of these require drastic reaction conditions. The reaction conditions for DNS labeling using this protocol is not drastic however. Complex washing steps with organic solvents is not required when using this protocol. Derivatization of –COOH groups using this method is direct and essentially a single-step protocol. Complexing agents such as crown ethers are not needed. Catalysts such as potassium carbonate and potassium hydroxide are not required. Also, activation of the carboxylic acid group prior to labeling is not required when using this method to label with DNS.

Table 1

Time of reaction (min)	Compound
15	*p*-chloroaniline
30	Ethanol
30	Gamma hydroxy butyric acid
60	Benzoic acid

Organic acids in general have no strong absorption, no significant fluorescence, and low reactivity. Sensitive detection of carboxylic acids are achieved with this protocol which uses sodium carbonate buffer. Elevated temperature for driving the derivatization reaction is not required for this method, so heat labile compounds are suitable for labeling by this protocol. Also, this protocol does not require caustic acids or bases to drive the labeling reaction, so the danger of degradation by strong acid or base is removed. The reaction of DNS–Cl with analytes, while in Na_2CO_3 aqueous buffer, was not allowed to proceed longer than 1.5 h.

Derivatives obtained after reacting with DNS–Cl in aqueous buffer were extracted with diethyl ether and placed on silica TLC plates for separation. Prior to TLC separation, the diethyl ether extracts were placed over anhydrous $MgSO_4$ to remove residual H_2O. The ether extracts were placed in air tight containers, kept in the dark, and were found to be stable for greater than 5 h. They were not allowed to dry out to a residue. Ether extracts should not be stored over the $MgSO_4$.

Similar results are obtained when the Na_2CO_3 buffer is used at pH 9.6 and pH 10.0. The ability to apply this technique at various pH buffer conditions expands the versatility of this technique to matrix systems requiring lower pH levels. Detection of 1mg quantity of analyte is readily obtained. At the levels of pH 9.6, 10.0 and 11.0 the

important anion species is CO^2_3 . This is the desired condition, as CO^2_3 is a stronger base than HCO_3 [15], by approximately 10^4 [16]. At the levels of pH 9.6 to 11.0 the predominant species will be the CO^2_3 [15,16].

After sample preparation, the dansylated compounds were then separated from non-bound DNS by TLC using acetone as the resolving solvent. Shown in Fig. 1 are results for labeled analytes p-chloroaniline, GHB, benzoic acid, and ethanol. Use of acetone as resolving solvent permits analyte differentiation by R values. Approximately 1 ml of analytes ethanol and GHB are fluorescing on the TLC. For the cases of benzoic acid and p-chloroaniline, 1 mg of analyte. Using acetone as resolving solvent allows the analytical TLC to be completed in several minutes. Fig. 2 shows the Lewis structures of the compounds studied here, suggesting the versatility of application of dansylation for fluorescent detection of organic compounds.

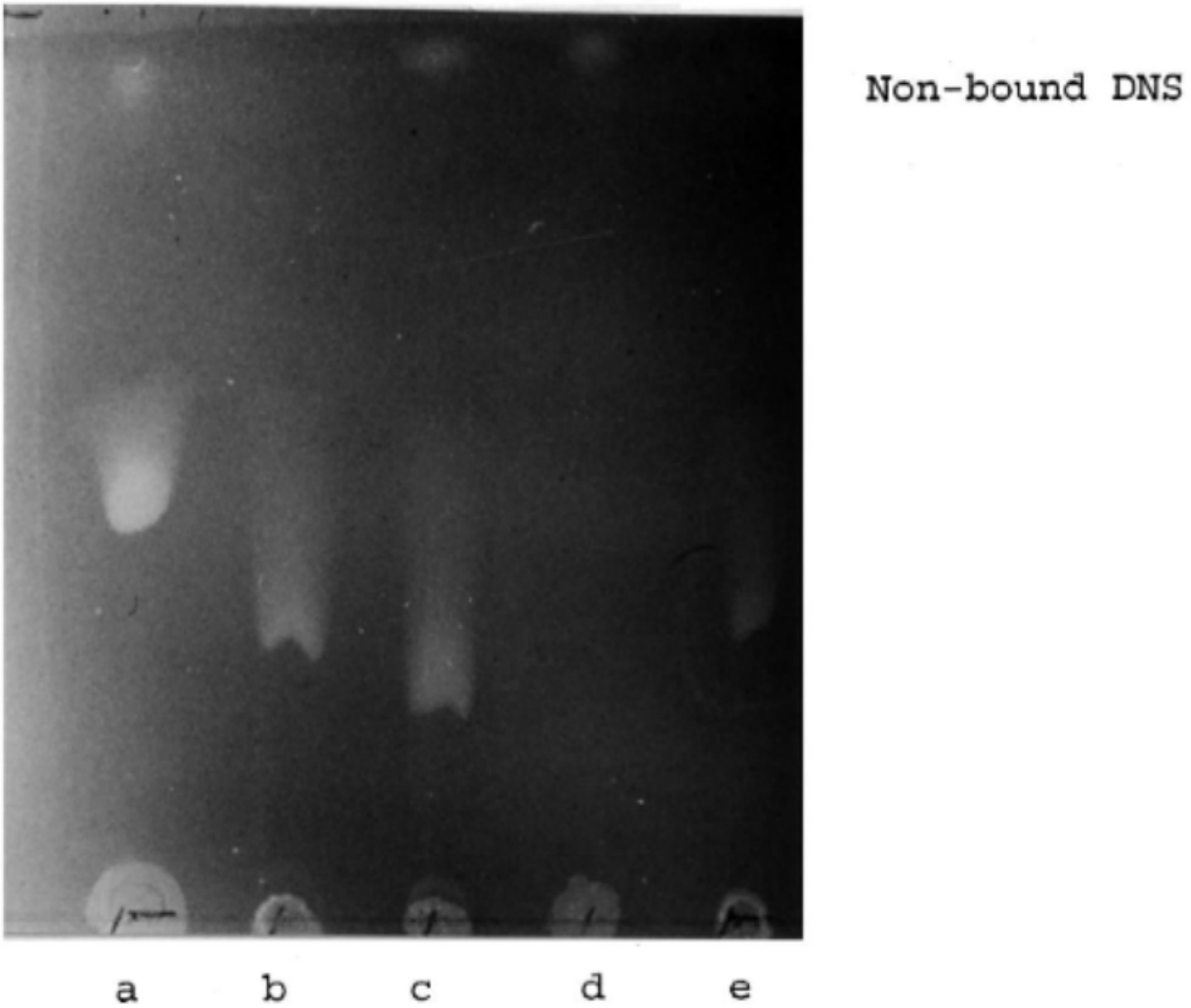

Fig. 1. All dansylated compounds were resolved by TLC using acetone as resolving solvent. Shown here: (a) dansylated benzoic acid; (b) dansylated gamma hydroxy butyric acid; (c) dansylated ethanol; (d) negative control (diethyl ether extract of buffer solution containing DNS–Cl, only); and (e) dansylated p-chloroaniline as positive control. One microgram of each analyte is detected under ultraviolet light. Non-bound DNS is material not reacted with the analytes.

4. Conclusion

Dansylation is an effective means to derivatize compounds with for fluorescent detection and provides high levels of sensitivity. Many types of molecules for biological or toxicology studies can be dansylated. The procedure presented here is an effective method for using dansyl chloride to fluorescent label compounds that have hydroxyl groups and carboxylic acid functional groups. This can be accomplished at different levels of pH from 9.6 to 11.0. The reaction time period for the dansylation of hydroxyl groups is determined to be 30 min and for carboxylic acid functional groups it is 60 min. This expands the scope of compounds which can be fluorescent labeled using dansyl chloride. Fluorescent labeling by dansyl chloride has found applications in liquid chromatography, high performance liquid chromatography, thin layer chromatography,

and mass spectrometry. This technique will greatly expand the application of dansyl chloride in analytical chemistry.

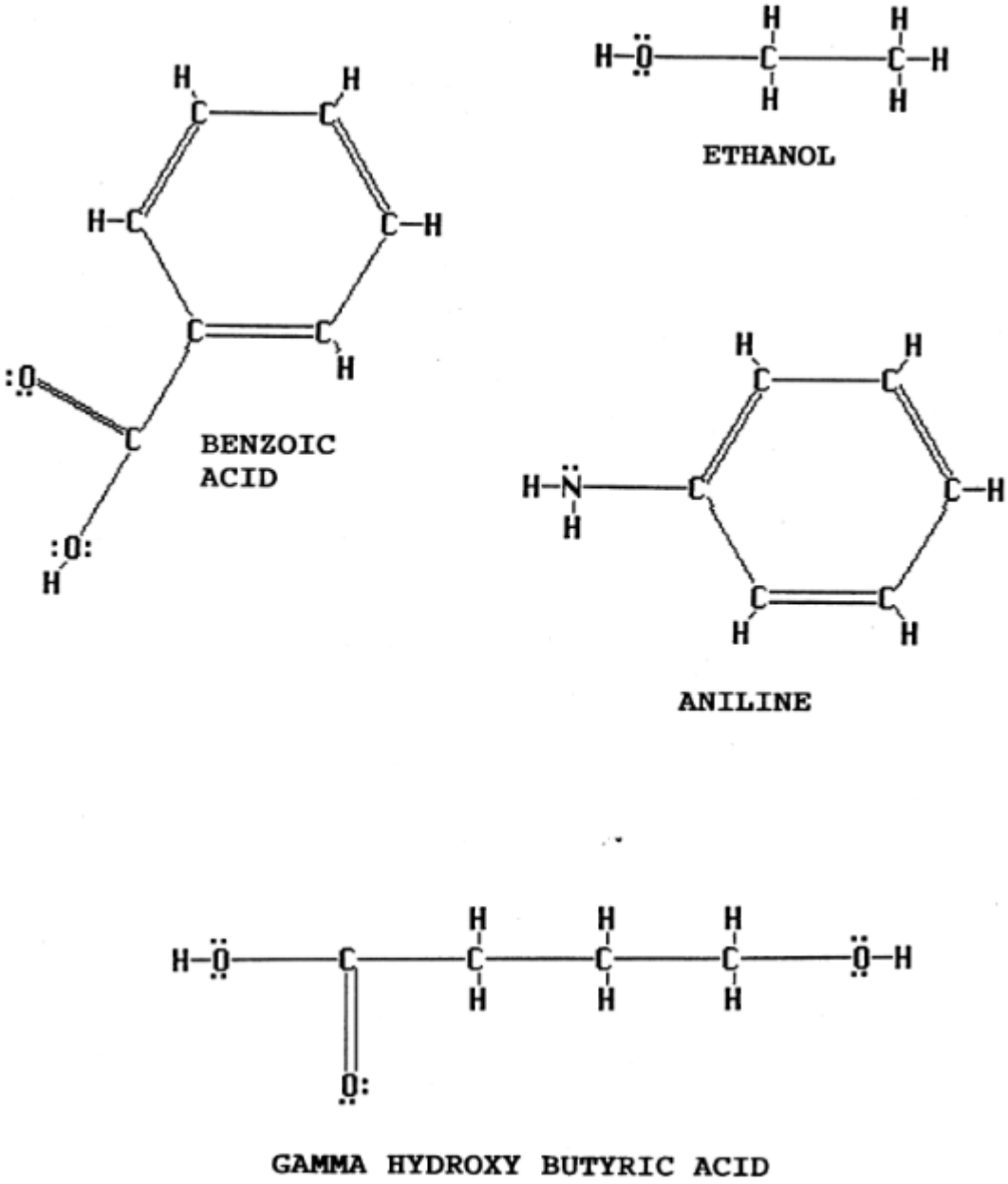

Fig. 2. The Lewis structures for dansylated compounds.

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