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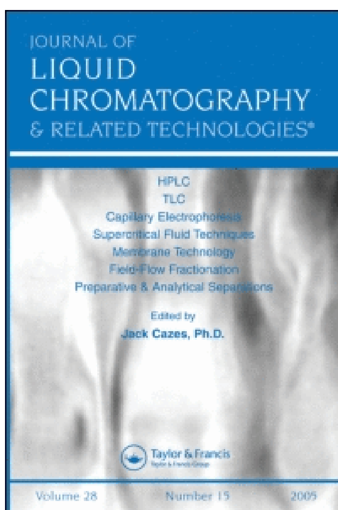

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SOLID PHASE REACTIONS FOR DERIVATIZATION IN HPLC (HPLC-SPR)

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I. INTRODUCTION

As in all other forms of chromatography, high performance liquid chromatography (HPLC) has come to rely quite heavily on derivatization of individual analytes for improved qualitative and quantitative identifications (1-13). Virtually all of this work has involved the use of homogeneous type derivatizations, wherein the sample solution to be injected, in the pre-column approach, or the HPLC effluent, in the post-column approach, are fully mixed with the derivatization reagents in solution. Clearly, homogeneous type derivatizations can be done off-line or on-line, in either the pre- or post-column modes, but in general, pre-column methods have been done off-line, and post-column approaches have been done both on-line and off-line. On-line type derivatizations, in either the pre- or post-column modes, appear to offer some very significant advantages. Major among these is the ability to perform derivatization-separation-detection or separation-derivatization-detection following injection of the sample mixture. The other off-line approach requires an initial derivatization off-line, then injection-separation-detection, in the pre-column mode. Off-line, post-column methods require injection-separation-derivatization off-line, then detection. Ideally, for any derivatization method, be this pre- or post-, on-line or off-line, one would like to be able to avoid any additional sample pre-treatment before injection, and any extra sample/analyte handling after the point of injection. Unfortunate-

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ly, on-line, pre-column derivatizations are very rare in the existing literature, and this has to do with the major problem of using solvents for the derivatization step that will be compatible with the separation needed via the HPLC step. Most recently, various instrument manufacturers have attempted to automate off-line, pre-column derivatizations with automated sample injections-separation-detection of a large number of samples. Automated on-line, post-column derivatizations have also now become available, wherein a homogeneous reagent solution is mixed, after the analytical column, with the HPLC effluent, in a small dead volume, high mixing efficiency chamber before the elevated temperature reaction coil. The final, derivatized eluent solution with unreacted derivatizing reagent are then passed into the detector for the final analyte detection/identification.

There are a number of disadvantages possible via homogeneous type derivatizations, although, by far, these are the most commonly employed approaches for HPLC derivatizations. This approach will generally require the use of additional instrumentation, HPLC pumps, mixing chamber, reaction chamber, heating arrangements, connectors, plumbing lines, etc., depending on whether the pre- or post-column modes are to be utilized. Additional extra dead volume is often encountered, which can/will adversely affect the overall HPLC performance, total apparent plate count, resolution, peak shape, peak heights, and minimum detection limits. In general, this homogeneous approach also increases the overall time for each analysis, along with the corresponding cost per analysis. The addition of extra reagent solutions introduces another possible source of contamination, thus reducing the qualitative validity of the overall analysis. Finally, homogeneous derivatizations often require additional sample handling and manipulations, especially in the off-line form. Despite these overall problems, many, if not most, analytical chemists who use HPLC in one or another form, have used and will continue to use some sort of homogeneous derivatization for their analyses. Derivatizations of any sort are extremely useful, especially wherein the analyte of interest cannot be detected with then available HPLC detectors. The derivatives formed and derivatization reactions employed should be designed to provide a final analyte entering the detector which will provide practical, low levels of detection with high analyte specificity. Thus, in general, any type of derivatization should improve analyte qualitative and quantitative determinations, as well as improving the overall specificity/selectivity of the analysis employed.

Within the past few years, certain investigators have described more novel approaches for performing on-line or off-line, pre- or post-column derivatizations that employ some sort of solid based reactions. We prefer to refer to this field as solid phase reactions or solid phase reactors,

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hence the abbreviation of SPR or HPLC-SPR to indicate that such solid phase approaches are used in combination with HPLC. We have recently reviewed, in part, the area of solid phase reactors in HPLC, although this was somewhat limited to just those papers that had actually used HPLC-SPR methods (14). In the present review, we have attempted to describe virtually all literature reports related to HPLC-SPR, and to present/discuss various solid phase reactions that have been reported for synthetic organic applications, but may not, as yet, have been utilized in HPLC applications/analyses. Solid phase reactions would appear to provide a large number of rather significant advantages with regard to improved HPLC operations. In general, the type and amount of additional instrumentation, hardware, mixing chambers, reaction chambers, etc., will be less in SPR approaches than in homogeneous methods of derivatization. What is required in HPLC-SPR are the solid phase reactor itself, a dummy reactor column, various end fittings, and perhaps a low-cost, low dead volume, on-line switching valve. The method does not introduce any additional extra-column dead volume other than that normally introduced with any conventional HPLC guard column. The time of analysis with SPR derivatizations usually will not increase per analysis. There is a slight increase in the overall cost per analysis, but this is always less than that incurred via homogeneous derivatizations. With regard to contamination, there will be very little, if any, contamination introduced by the SPR, and this can be eliminated or prevented by careful pre-washing and suitable treatment before it is put on-line. SPRs can be used both on-line and off-line, with somewhat different advantages in the off-line mode. There is no excess derivatizing reagent present in the mobile phase with the SPR, and thus the background noise level should be the same with or without the SPR on-line. Detection limits should always be maximally improved via on-line HPLC-SPR approaches. When used in the pre-column mode, Figure 1, then difference chromatography can be employed to improve analyte identification. Such difference chromatography employs two separate injections of the sample mixture, one with a dummy column and the other with the SPR on-line. In the post-column mode, Figure 2, difference chromatography cannot be employed, since retention times cannot be affected via derivatization after the separations, but detector responses between the initial analyte and its derivative can be used for improved analyte/compound identification. With HPLC-SPR, there is no additional sample handling or manipulation as compared with analysis in the absence of derivatization. The only additional requirement is for a separate analysis via the dummy column together with the analysis with the SPR on-line. In general, SPRs are compatible with a wide variety of HPLC solvents, but this is not always the case that all SPRs will be useful and/or usable with both normal phase and reversed phase type solvents. Many

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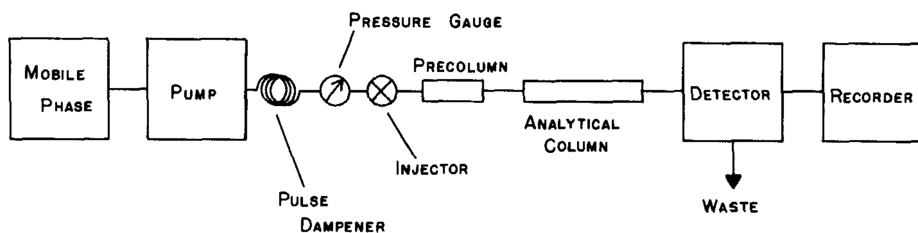

Figure 1. HPLC-SPR with the solid phase reactor in the pre-column mode of operation, before the analytical column.

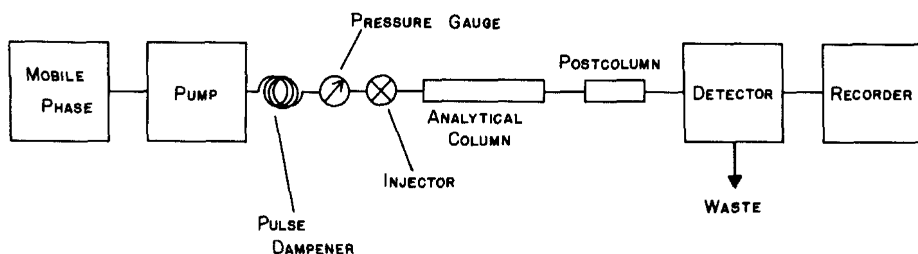

Figure 2. HPLC-SPR with the solid phase reactor in the post-column mode of operation, after the analytical column.

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SPR initiated derivatization reactions will occur in real-time, with no increased analyte/sample hold-up within the SPR prior to the separation-detection steps. However, it is always possible to allow the sample solution to remain in contact with the SPR, at ambient or elevated temperatures, before or after the analytical column, in order to increase the percent conversion to the desired derivative. Most SPR methods will lead to derivatization at or slightly above room temperature, in real-time or hold-up time. SPRs can be held at elevated temperatures *via* the use of a constant temperature water bath or similar column heater device already commercially available *via* many suppliers. In many instances, derivatization reactions are more selective and specific than with the corresponding reactions/reagents in homogeneous solution approaches. Thus, from the analytical point of view, quite often improved analyte identification can result *via* the use of more selective/specific derivatizations using the solid phase approach. Since solid phase derivatizations can be designed to improve detection *via* virtually any HPLC detector, these approaches are not limited to improved overall analysis for a particular detec-

tion method. It is entirely possible that one particular SPR will lead to improved detection _via_ ultraviolet-visible (UV-VIS), fluorescence (FL), or electrochemical (EC) methods. Other SPRs may provide enhanced detectability for more than one particular detection method (UV/FL, UV/EC, etc.). The deciding factors are the nature of the functional group within the analyte being modified, and the nature of the derivatization reaction leading to an altered chemical structure. Some SPR reactions may just change one particular functional group with the analyte, such as oxidizing an aldehyde to a carboxylic acid or reducing an aldehyde to an alcohol. Still other derivatization methods may tag an analyte, by the addition of a specific chromophore/fluorophore/electrophore to the original compound's structure. In general, derivatization _via_ compound structure alteration, as opposed to tagging, will provide for improved qualitative identification/specificity, but not necessarily for improved detection limits. Tagging reactions, with or without SPRs, are designed to improve the detectability of the final product, so that overall detection limits will be reduced/improved.

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Affinity chromatography has long employed enzyme bound/modified solid supports for improved separation of biological materials, but, in general, these have not involved chemical alterations of their structures. However, this use of enzyme bound supports has encouraged various individuals to employ similar solid phase reagents, employing enzymes, for SPR type derivatizations in HPLC. Clearly, this is an area for vast development over the coming years. Solid phase reactions can most easily be broken down into two general fields or areas, those which utilize solid supported reagents and reactions, and those which use polymer bound/attached reagents and reactions. We term these two approaches as solid supported and polymeric SPRs, with the understanding at the start that the nature of the solid support or polymeric backbone can vary from one type of SPR to another. Most solid supported SPRs will be compatible with normal phase type separations and organic mobile phases. They will not, in general, be at all compatible with reversed phase type conditions in HPLC. The polymeric SPRs will or can be compatible with both normal phase and reversed phase type separations, depending entirely on the nature of the reagent/reactions employed. The very nature of the inorganic or organic reagent on the solid support or polymeric backbone can often dictate whether normal or reversed phase HPLC can be used with a particular SPR. This is perhaps one of the most important considerations to be met in all of HPLC-SPR, what is the compatibility of the HPLC mobile phase with the solid supported or polymeric SPR? That is, there may very well be times when the solvents needed for successful SPR derivatizations will just not be those ideal for the desired separations. There may be other times when the solvents necessary for

a given separation are not at all compatible with the SPR needed for that particular derivatization. There will yet be other times when the solvent conditions for the SPR and those for the HPLC separations can be met by the same solvent or solvent mixture. Some amount of solvent screening or evaluation may very well be called for in the development or perfection of any new HPLC-SPR system, and this can require a considerable amount of time and effort. However, this is not necessarily any more time or effort than what is needed for the development and optimization of a new homogeneous derivatization method.

In many instances, the lifetime of SPRscan be vastly greater than that of a derivatization solution that must be prepared fresh for each application. It is entirely possible to store an SPR in an inert atmosphere, or under an appropriate inert solvent, and to have it remain active for several hundred analyses lasting over many months. Thus, an individual SPR can and should be usable over and over, especially wherein the percent loading of the reagent(s) can be intentionally designed to be quite high (mg/g). The loading of the reagent on the solid or polymeric support can be varied, by appropriate preparation designs, the size of the SPR column itself can also be varied, just as is now done with a commonly used guard column before the analytical column. It is also possible to utilize more than one type of SPR, either in series or parallel, on-line, in HPLC, so as to improve analyte identification _via_ a number of related compound/analyte modifications or derivatizations. With the use of appropriate switching valves on-line, it is entirely possible to vary the nature of the analyte modification, just by passing the injected solution to one or another of existing, on-line SPRs, pre- or post-column. This would be the parallel mode of SPR operation. In the case of series operations, a series of appropriate SPRs could be placed one after the other, again either pre- or post-column, so that the initial analyte would be modified, modified again, perhaps modified again, each time selectively, so that perhaps only one particular analyte/compound could possibly undergo the reactions desired to produce a known, finally detectible product.

SPRs are not entirely new HPLC detectors, they employ currently known and available detection methods, but they are designed to improve analyte identification and overall selectivity. Clearly, SPRs can be utilized in an off-line as well as an on-line fashion, as discussed above. In the case of off-line derivatizations _via_ SPRs, although of less significance than for on-line approaches, there are still serious advantages as compared with homogeneous, off-line derivatizations. It is entirely conceivable that SPRs can be developed for off-line approaches, wherein the sample mixture is simply injected onto the SPR, this is heated for a given period of time, cooled to room temperature, eluted with a mobile phase compatible solvent

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or the mobile phase itself, and an aliquot of this final eluate is then injected onto the HPLC. Such SPR columns for off-line derivatizations could be used over and over again, moved from one laboratory to another, and could form a bank of readily accessible and available derivatization systems that could be used at a moment's notice. There could, in fact, be a bank of SPRs present within any given laboratory, from which individual SPRs could be drawn by an analyst, utilized off-line or on-line, and then returned to the SPR bank for future use by others. Such SPR approaches to derivatization could readily be automated, so that samples could automatically be derivatized by passage, off-line, through a particular SPR set in a laboratory rack, and the effluent would then be collected for subsequent work-up, separation, detection, or related analytical steps. Thus, it would appear that the future of SPRs in analytical chemistry, and especially within HPLC applications, appears very bright, and that these somewhat newer methods of derivatization should find much more widespread acceptance and utilization in the coming years.

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II. SOLID SUPPORTED REAGENTS IN HPLC (NON-POLYMERIC)

We define solid supported reagents as those organic or inorganic reagents that are physically or chemically adsorbed onto an inorganic type GC or HPLC solid support, such as silica gel, alumina, clay, Florisil, etc., wherein such supports are not synthetic organic/inorganic polymers. In general, most of the solid supported reagents already described in the synthetic organic literature are adsorbed onto the support, rather than being covalently attached/bonded. It is also possible to use silica based ion exchange materials for the ionic attachment of organic/inorganic reagents, and to then utilize such solid supported, but really ionically attached, reagents for synthetic or HPLC applications. However, as of yet, very few such silica based ion exchange packings have indeed been utilized for any type of HPLC derivatizations. There is some interest at the present time in demonstrating the potential usefulness of this approach for on-line or off-line HPLC reactions. In general, solid supported reagents have some distinct advantages when compared with polymeric type supported materials, especially with regard to the type of mobile phases that are compatible with this type of support. Usually, HPLC applications with silica supported reagents will be limited to normal phase type solvents, hydrocarbons, chlorinated organics, etc. It may prove possible to utilize silica based ion exchange packings for HPLC-SPR, wherein the pH of the mobile phase does not exceed 2-9 in reversed phase approaches. Polymeric based SPRs appear to be more compatible with reversed phase needs, assuming that the chemical reaction to be conducted is also compatible with this type of solvent mixture (MeOH/HOH, EtOH/HOH, ACN/HOH, etc.). In solid

supported reagents, there are at least two important criteria that must be met in HPLC-SPR work: 1) that the solid support be stable and compatible with the HPLC mobile phase; and 2) that the reagents/reactions being employed are also compatible with the mobile phase. If either of these requirements cannot be met with a particular HPLC solvent, then that HPLC-SPR approach is doomed to failure. Alternative SPR methods would have to be investigated for that HPLC mobile phase, or an alternative HPLC approach would have to be found which is compatible with the SPR to be employed.

In the synthetic organic literature, there has evolved considerable interest in the use of reagents supported on insoluble inorganic materials for performing various types of reactions (15, 16). There are several possible reasons for the widespread use of these supported reagents, many of which have become commercially available, such as: 1) the increase in the effective surface area whereon the reaction can take place; 2) the presence of various sized pores in the matrix which can hold the two reacting species in the same general proximity, thus lowering the energy of activation for a given reaction; 3) the selectivity of the reaction is often enhanced; and 4) the reactions are often much cleaner with fewer side products. Despite a well developed and recognized literature in synthetic organic chemistry for solid supported reagents, the use of HPLC-SPR with such reagents/supports is not very well developed nor recognized at this particular time. There are some obstacles, as suggested above, that must be overcome before this technique can readily be applied to HPLC applications/problems. Some of these requirements are: 1) the inorganic support and the supported reagent must be stable under the given HPLC operating conditions; 2) the ideal solvent for the derivatization may not be the best mobile phase for separating the derivatized analytes, thus often a compromise is needed; 3) if the reaction is to be carried out on-line, in real time, it must be fast at ambient or slightly above ambient temperatures; 4) the reaction should quantitatively convert all of the starting compound into a single, known product, or at least react a determined, reproducible percentage of the starting compound to the product; 5) by-products of the reaction should either remain adsorbed on the support or not interfere with either the separation or detection of the desired product. Reactions that will form products that are either gases or insoluble precipitates are clearly incompatible with HPLC-SPR requirements for continued/continuous operation.

Despite the above requirements, which are clearly not insurmountable, there are still many, already described, supported reagents that are currently being used almost exclusively for organic synthesis that might be well applied in HPLC-SPR. Solid supported reagents can be either adsorbed onto, intercalated, dispersed in the inorganic support, or ionically/covalently

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attached/bonded. Examples of some inorganic supports that could be used in HPLC-SPR include: silica gel, alumina, Celite, Florisil, graphite, clay, and molecular sieves. The types of chemical reactions that could be used in HPLC-SPR are almost limitless. The supported reagents described and discussed below were only chosen to illustrate those SPRs that can be most directly applicable to HPLC-SPR. However, they do not, by far, represent all of the literature reports on solid supported reagents.

II.A. HPLC-SPR CHEMICAL REDUCTIONS VIA NON-POLYMERIC, SOLID SUPPORTED REAGENTS

By and large, the vast majority of solid supported reagents in synthetic chemistry have to do with either oxidation or reduction type reactions. Remember that we have arbitrarily divided derivatizations in HPLC into two major types or categories: 1) those reactions that will convert one or more functional groups into another type of functional group(s), perhaps best termed functional group conversions; and 2) those reactions that will tag an analyte by adding to the initial compound's structre another compound or part of a derivatizing reagent that provides enhanced UV, FL, and/or EC detectability. Either of these approaches to HPLC derivatization will, in effect, improve analyte identification and/or detection limits. A very large number of organic functional groups are amenable to solid supported reductions, and many papers have already appeared describing a wide variety of suitable organic/inorganic reducing agents on solid supports (17-21).

Quite recently, Krull _et al_. have described the use of sodium borohydride precipitated onto silica gel ($NaBH_3/SiO_2$) as an on-line, pre-column or post-column derivatizing agent in HPLC (17). Difference chromatography was used here to monitor the overall reaction on the SPR for various carbonyl compounds. Standard compounds were injected onto a combination of a dummy (no reagent) pre-column plus analytical column, and then onto a combination of the solid phase reactor (SPR) pre-column plus analytical column. The HPLC-UV chromatogram of the SPR plus analytical column shows a decrease or complete disappearance, depending on the particular analyte, of the starting compound's peak height/area, with a concomitant increase or appearance of the product peak (Figure 3). This $NaBH_4/SiO_2$ type SPR was found to be quite reactive towards most aldehydes, ketones, and some acid chlorides. The SPR was unreactive towards esters, nitro, N-nitroso, alkyl/aryl halides, or amides. The percent reductions of various aldehydes and ketones was monitored as a function of temperature, and this could then be used to differentiate between these two classes of compounds. Products of these reductions, the alcohols, have longer retention times in normal phase HPLC, and these had to be eluted with flow programming. The borohydride reagent was stable with mobile phases

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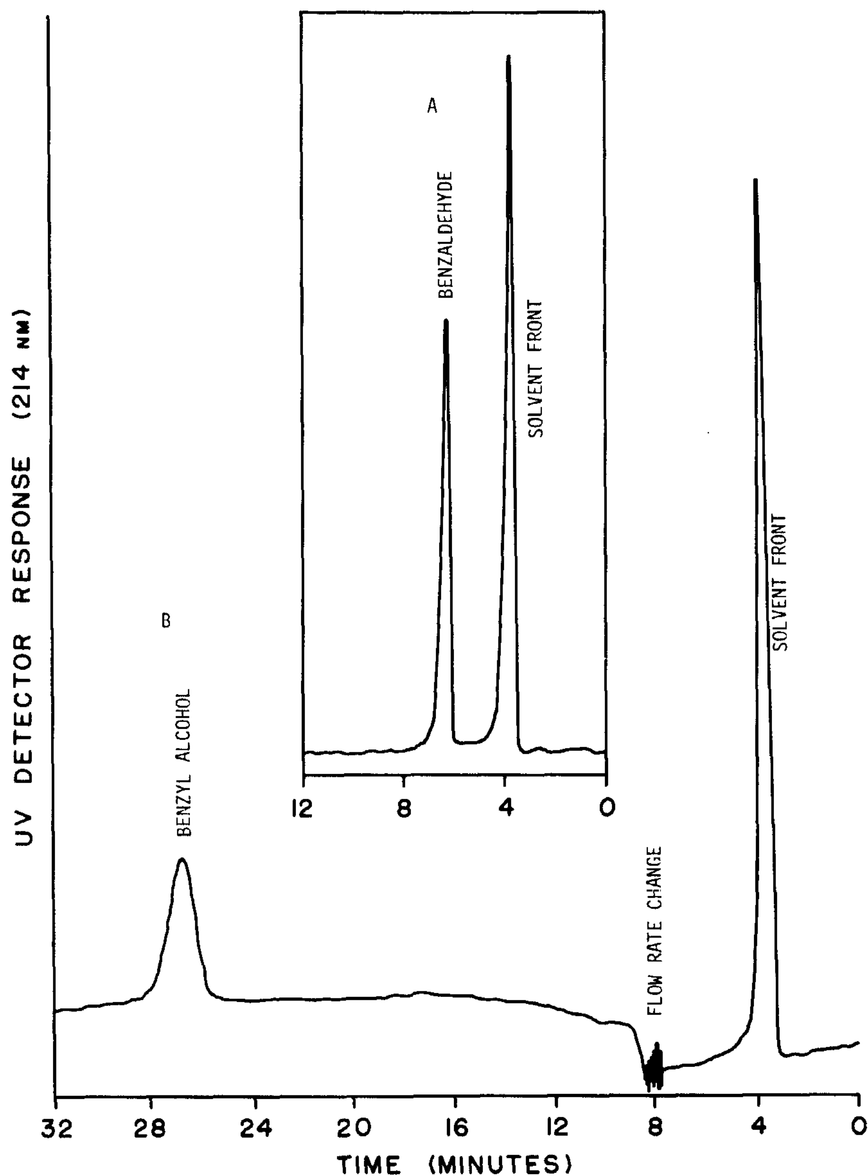

Figure 3. (A) HPLC-UV chromatogram of benzaldehyde using dummy column and silica gel analytical column with 0.3% IPA/hexane at 1 ml/min; (B) HPLC-UV chromatogram of benzaldehyde injected onto reduction column and silica gel analytical column with 0.3% IPA/hexane at 1 ml/min to 8 mins, and then flow rate of 4.3 ml/min to 32 mins. Pre-column mode of HPLC-SPR operation.

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having as much as 15% isopropyl alcohol in hexane, and therefore gradient elution could have been used in order to reduce the overall time of analysis via HPLC-SPR for the alcohol reduction products. The long retention time of the product is not necessarily a disadvantage and may actually prove useful in certain applications. If, for example, an aldehyde or ketone eluted at the same time as another component of interest, then a $NaBH_4/SiO_2$ pre-column would/could eliminate the peak and thus serve to "clean-up", in an on-line manner, the overall chromatogram and improve final selectivity. This same approach could easily be used to monitor impurities in commercial preparations of aldehydes or ketones. Analysis of the aldehyde would only show the impurities initially present, assuming that these have not been derivatized, together with the product alcohol peak coming from the original aldehyde. Krull et al. have used this $NaBH_4/SiO_2$ approach to detect cinnamaldehyde in a commercial food product, cinnamon, as well as in a commercial mouthwash product (17). This same HPLC-SPR approach was used to monitor for vitamins K_1 and K_3, although not in actual samples or food products.

Judging from the existing synthetic organic literature, there are many other reports of supported reducing reagents that could be directly applicable for HPLC-SPR analyses/purposes. Some examples of these reagents would be: 1) $NaBH_4$/alumina; 2) $NaBH_4$/Celite; and 3) potassium or other active metals/reagents intercalated into graphite. However, whichever supported reagents are to be considered for HPLC-SPR utilization, clearly such materials must be pressure stable, so that there is no bed collapse in the pre-column mode during actual operations. All of the other requirements of SPRs in HPLC must also be met, as summarized above.

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II.B. HPLC-SPR CHEMICAL OXIDATIONS VIA NON-POLYMERIC, SOLID SUPPORTED REAGENTS

The synthetic organic literature is replete with references to the use of solid supported oxidizing reagents. Each specific combination of solid support and chemical reagent has different selectivities and reactivities. The most general and widespread use of supported oxidizing reagents has been to convert alcohols to the corresponding aldehyde, ketone, or lactone derivative. Nef type oxidations have also been described, wherein primary and/or secondary nitro compounds are converted to their aldehydes or ketones (15). Oxidative rearrangements are also possible using solid supported oxidizing agents (22).

Regen and Koteel have described the use of activated potassium permanganate ($KMnO_4$) impregnated onto organic supports, such as molecular sieves, silica gel, and clays, for the oxidation of various alcohols (23). Such oxidations were carried out in benzene, although other nonpolar solvents of

more routine HPLC use (e.g., hexane), might also be effective here. In general, high yields of ketones and modest yields of aldehydes were obtained. Thus, supported permanganate should be ideally suited for normal phase HPLC-SPR, because it is virtually insoluble in most organic solvents. In addition, it is a powerful oxidizing agent and should react very quickly with alcohols in organic mobile phases. The oxidation products, viz., aldehydes/ketones, will not be irreversibly bound to the solid support, the reactions are generally very clean, and the reduced reagent, manganese dioxide (MnO_2), should also remain bound to the support and not interfere with either the separation or detection of oxidation products. Such approaches have not, as yet, been described for HPLC-SPR purposes, although work is now underway in the authors' laboratory related to the use of permanganate supported polymeric reagents for organic oxidations.

Santaniello, Ponti, and Manzocchi have reported that chromic acid (H_2CrO_4) adsorbed onto silica instantaneously oxidizes hydroxyl compounds in ether to their corresponding carbonyl derivatives (24). Pyridinium chromate on silica gel can oxidize allylic and benzylic alcohols, as well as saturated primary or secondary alcohols, even when these contain other acid sensitive groups (25). Pyridinium chromate on silica is a stable reagent and showed no deterioration even after one year of storage at room temperature.

One of the most thoroughly investigated supported reagents has been silver carbonate precipitated onto Celite (15). Ag_2CO_3/Celite is a very versatile oxidizing agent capable of oxidizing primary and secondary alcohols, diols, triols, hydroquinones, phenols, and amines. Reaction times vary depending on the ease of oxidation, but these can be as fast as one minute. The most common solvent used for such oxidations has been benzene, although heptane, toluene, and dichloromethane can also be used.

Lalancette et al. have reported that chromic anhydride incorporated into clay is a specific oxidizing agent for the conversion of primary alcohols to the corresponding aldehydes (26). Sensitive structures, such as terpenes and allylic systems can be oxidized with this type of a reagent, but secondary and tertiary alcohols are unreactive. Some other supported oxidizing agents that can oxidize alcohols to their corresponding carbonyl compounds are chromyl chloride chemisorbed onto silica/alumina (27) and manganese dioxide on carbon, but this latter example may not be stable to high back pressures in HPLC (28).

Oxidative rearrangements, as opposed to simple oxidations, are also possible using supported reagents, and these might be ideal for HPLC-SPR purposes. Taylor and Chiang have described such oxidative rearrangements for alkyl aryl ketones by Thallium (III) nitrate (TTN) adsorbed on montmorillo-

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nite K-10 clay (22). The final product in this case was a different alkyl aryl ester. This particular reaction is rapid, selective, and can be carried out in heptane, methylene chloride, carbon tetrachloride, toluene, or dioxane. Oxidations of simple olefins, such as cyclohexene, are very rapid. In non-polar solvents, both Thallium (III) nitrate on the support and Thallium (I) nitrate which is generated during the reaction are tightly bound to the support throughout the reaction.

II.C. HPLC-SPR CHEMICAL DERIVATIZATIONS OF A MISCELLANEOUS NATURE VIA NON-POLYMERIC, SOLID SUPPORTED REAGENTS

Ribhood and Ruthven report that bromine adsorbed on molecular sieves is a selective reagent for the bromination of terminal double bonds (29). This type of an SPR, in the post-column mode, could be used to improve the response of an electron capture detector (ECD) in HPLC-SPR. By choosing a molecular sieve with a pore diameter that is only large enough to admit straight chain hydrocarbons, the brominating agent is then capable of differentiating between a double bond located in a sterically unhindered side chain and one in an accessible position in an alicylic ring. A molecular sieve with a larger pore size would be a more general brominating reagent for HPLC-SPR.

II.D. HPLC-SPR CATALYTIC REACTIONS VIA NON-POLYMERIC, SOLID SUPPORTED REAGENTS

Crown ethers have been shown useful in organic synthesis because of their ability to selectively chelate metal ions from dilute solutions. They have been used to concentrate ionic species/reagents by immobilization on a solid support. Siliça gel immobilized crown ethers could also have an important impact on metal ion chromatography and on-line catalytic reactions. Dibenzo-18-crown-6 is a typical crown ether that has been covalently bonded to silica gel, Figure 4, and has been found to concentrate potassium ions from dilute aqueous solutions of potassium chloride (30). Crown ethers of differing ring size could/would selectively concentrate other metal ions and could conceivably catalyze different reactions.

III. POLYMER SUPPORTED DERIVATIZATION REACTIONS IN HPLC

A very large number of polymeric reagents have been described in recent years, and many of these have been summarized in a recent book (31). There are a very large number of possible polymers that could be used as polymeric reagents, and a large number of reagents that could be attached to any given polymer. Hence, the total possible combinations of polymers and reagents to form polymeric reagents are very large, and many of these could/should be compatible with HPLC-SPR requirements/needs. Although most polymeric reagents

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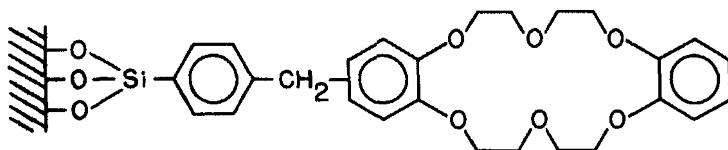

Figure 4. Dibenzo-18-crown-6 covalently bonded to silica gel support (30).

that have already been utilized in HPLC-SPR are organic polymers, there is no reason why inorganic polymers or silica gel based, ionically attached reagents could not be used in HPLC-SPR. These would be sufficiently different from silica gel supported reagents, as described above, to fall into the class of polymer supported/attached reagents, rather than silica or solid supported reagents for HPLC-SPR. Polymer supported reagents, organic polymers, have certain significant advantages as compared with solid supported reagents, not the least of which should be their general compatibility with reversed phase type solvents. Another advantage is that polymeric reagents can often be regenerated by a single synthetic step, and in some instances, this might be accomplished on the HPLC system, merely by passing the appropriate regenerating solution through the spent SPR. Polymeric reagents, ionically or covalently attached, should be usable in a batch process or on-line in HPLC-SPR. Several polymer attached reagents have been described that incorporate an immobilized enzyme on the surface of the polymer beads. This type of polymeric reagent acts as a biological catalyst for certain deconjugation reactions, for example, wherein the catalyst is not consumed, and such SPRs could be used for many separate analyses and/or sample preparations, on-line. The idea of using polymer attached enzymes for on-line catalytic reactions has been used but rarely, although it should have substantial potentials in much of HPLC work. Other polymeric catalysts have already been described in the synthetic organic literature, but very few of these, if any, have ever been utilized in HPLC-SPR type work.

Polymeric reagents can also be used to modify a particular functional group within an analyte's structure, and thereby convert it to a suitable derivative for improved identification, as above for solid supported reagents. Again, a very large number of polymeric supported/attached reagents have been described, many of which should be immediately applicable to HPLC-SPR.

Another type of polymeric reagent consists of a ligand or molecule that is covalently or ionically attached to the polymeric backbone, which can be transferred to a suitable substrate, thereby forming a new derivative that

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incorporates all or most of the initial analyte and the tagging molecule. Several of these potential tagging polymeric reagents have also been described in the synthetic organic literature, although very few of these have ever been used in HPLC-SPR applications.

Polymers provide some very useful features, as opposed to solid supports, such as being able to be custom designed so as to provide a micro-environment for specific/selective reactions of the pendant groups. Thus, special electronic and steric conditions can be created in close proximity of the reacting species, leading to enhanced rates of reaction and improved reagent specificity. The chemical and steric structure of the polymer can affect its overall polarity, and this may further influence the overall reactivity of the attached organic/ inorganic reagents. The specific steric requirements of the channels and pores of a cross-linked polymer may impart size and structure selectivity on certain reactions of reagents or pendant groups attached to the polymer or diffused into its pores/channels. This area of polymeric reagents in HPLC has recently been reviewed (14).

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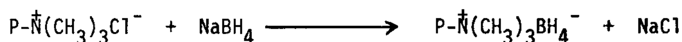
III.A. POLYMER SUPPORTED REDUCTIONS FOR DERIVATIZATION IN HPLC-SPR

The synthetic organic literature is replete with descriptions of various polymeric reducing reagents, selective for the conversion of aldehydes, ketones, and other carbonyl compounds into the expected alcohol product (31). Borohydride, cyanoborohydride, and related reducing agents have all been used as polymeric reagents, and polymeric borohydride resins are even used commercially for removing traces of aldehydes from process streams of various alcohols being produced commercially (32, 33). Since borohydride is a stronger anion than either chloride or borate, when a solution of sodium borohydride is stirred in the presence of a suitable anion exchange resin, this resin is converted to the borohydride form, as below.



$$\text{P-}\overset{+}{\text{N}}(CH_3)_3Cl^- + NaBH_4 \longrightarrow \text{P-}\overset{+}{\text{N}}(CH_3)_3BH_4^- + NaCl$$

Krull et al. have recently described the utilization of this type of a solid phase reactor for performing on-line, pre-column derivatizations/reductions of aldehydes under reversed phase HPLC separation conditions (34). Various classes of carbonyl derivatives were evaluated as potential substrates for these reactions, using either MeOH/HOH or ACN/HOH as the mobile phases, including: aldehydes, ketones, amides, acid chlorides, aryl halides, and N-nitroso derivatives. Only the aldehydes could be reduced, under real time, ambient temperatures, using reversed phase type solvents. Identification of an

aldehyde was based on disappearance of the starting substrate on the SPR plus analytical column, along with the formation of the expected, known reduction product (alcohol). Retention times and difference chromatography were obtained using an analysis on the dummy column plus analytical column together with the same analysis of the aldehyde on the SPR plus analytical column. No work has been done, thus far, with this polymeric borohydride SPR in the post-column mode, nor has it been evaluated as a possible SPR for normal phase HPLC. Analysis of the borohydride loading on any given polymeric borohydride SPR was accomplished via elemental boron analysis (ICP) and in-house titrations for borohydride by established literature methods (34). A number of applications of this selective method of analyte identification have been described, including the analysis for cinnamaldehyde in both cinnamon spice and a commercial mouthwash sample. Because of the heavy loading of borohydride possible on the anion exchange resins utilized in this study, the final SPRs had very long lifetimes and chemical reactivities, extending over several months and several hundred individual anlayses. Figure 5 illustrates the utilization of this polymeric borohydride SPR in the analysis for cinnamaldehyde from a hexane extract of cinnamon (34). This figure indicates the initially present peak for cinnamaldehyde in this extract on the dummy plus analytical column, followed by the complete disappearance (reduction) of this same peak and the appearance of the expected peak for cinnamyl alcohol on the SPR plus analytical column.

Sodium cyanoborohydride ($Na^+CNBH_3^-$) is known to be a much milder and more selective reducing agent than sodium borohydride. An anion exchange resin incorporating sodium cyanoborohydride has also been described in the literature, and this is also more selective than the polymeric borohydride resin indicated above. There is a commercially available polymeric borane complex, sold by Aldrich Chemical Co., that is also somewhat selective for aldehydes and ketones (35). This particular polymeric reducing agent was prepared by treating poly(2-vinylpyridine) with a borane-methyl sulfide complex in tetrahydrofuran (THF). The final polymeric reagent is capable of reducing both aldehydes and ketones into the expected alcohols at room temperature, but only in the presence of boron trifluoride etherate. Some of the above polymeric reducing agent might find direct applications in HPLC-SPR work.

III.B. POLYMER SUPPORTED OXIDATIONS FOR DERIVATIZATION IN HPLC-SPR

A number of polymeric oxidizing reagents have been described in the existing literature, and these have shown significant advantages when compared with homogeneous oxidation type reactions (31, 36). Cainelli et al. have described the preparation and utilization of a macroreticular anion exchange resin incorporating $HCrO_4^-$ as the oxidizing agent. This was shown effective for

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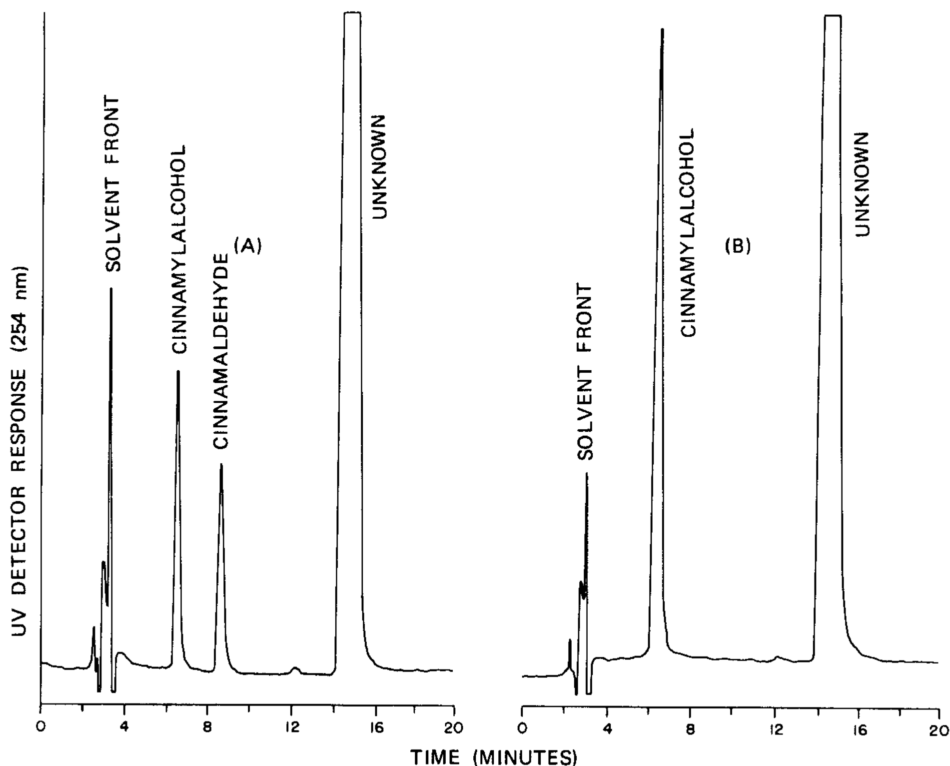

Figure 5. HPLC-UV chromatograms of methanolic extract of commercial sample of cinnamon for determination of cinnamaldehyde. HPLC conditions used a C-18 reversed phase column with mobile phase of 50% ACN/HOH at 1.0 ml/min flow rate, UV detection at 254nm. (A) polymeric dummy column in-line with analytical column; (B) polymeric borohydride reducing column in-line before analytical column.

the complete oxidation of primary and secondary alcohols to the expected aldehydes and ketones. The nature of the solvent used is crucial for effective oxidations, and the most effective ones were: hydrocarbons, chlorinated hydrocarbons, benzene, and ethers. However, acetonitrile with water in various ratios was not as effective as the organic solvents. The rate of the reaction depends both on the structure of the alcohol and on the substrate:resin ratio, this rate being faster for the allylic and benzylic alcohols. Higher ratios of resin:substrate, as expected, also leads to greater overall conversions for such

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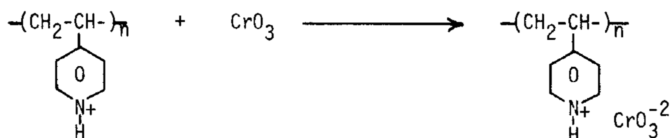
reactions. Regeneration of the spent resin can be readily accomplished _via_ simple treatment with 2N chromic acid.

An alternate, non-acidic polymeric reagent, poly(vinylpyridinium) dichromate (PVPDC), can be easily prepared by treatment of a poly(vinylpyridine) resin with a slight excess of chromium trioxide (CrO_3) in water at room temperature. After suitable washing, the final polymer can be utilized directly. Frechet _et al_. have recently described this polymeric reagent, pyridinium dichromate, as an effective oxidizing reagent for alcohols, as below (37, 38). PVDPC is quite stable to prolonged storage. Best oxidation results



are obtained by using the wet reagent in a nonpolar solvent, such as cyclohexane at elevated temperatures, as expected. It is yet possible that this type of a polymeric oxidizing reagent will be compatible with reversed phase HPLC. Recycling of this reagent can be done by using a simple washing reactivation procedure, in which the spent reagent is washed with acid to remove the spent chromium salts. After regeneration of the poly(vinylpyridinium) resin, addition of CrO_3 and water reactivates the PVPDC.

Krull _et al_. have recently developed a polymeric permanganate oxidizing resin, which has been used in HPLC-SPR for the selective oxidation of both alcohols and aldehydes (39). This material can be used on-line, pre-column fashion, at room temperature and above, for the successful oxidation of various alcohols and aldehydes. Derivatizations can be accomplished using reversed phase, ACN/HOH, separation conditions, as indicated in Figure 6. This is a typical HPLC-SPR application of the polymeric permanganate oxidizing column, here for the oxidation of p-nitrobenzyl alcohol to p-nitrobenzaldehyde, using 50% HOH/ACN as the mobile phase. The maximum percent oxidation for this particular alcohol, at about 46°C, in real-time, on-line with the HPLC, has been about 60%. Other alcohols can be oxidized in amounts/efficiencies ranging from 10% to 50%, very much dependent on the particular structures. Aldehydes, in most cases thus far studied, are oxidized to much greater extents, usually about 100% at elevated temperatures, again on-line, in real-time _via_ HPLC-SPR. Normal phase applications of this SPR have yet to be determined and/or evaluated fully. Clearly, there are a very large number of possible applications for both alcohols and aldehydes _via_ this particular HPLC-SPR approach, and it is possible that other classes of compounds, such as catechols and catecholamines, might be suitable substrates for these derivatization methods (39).

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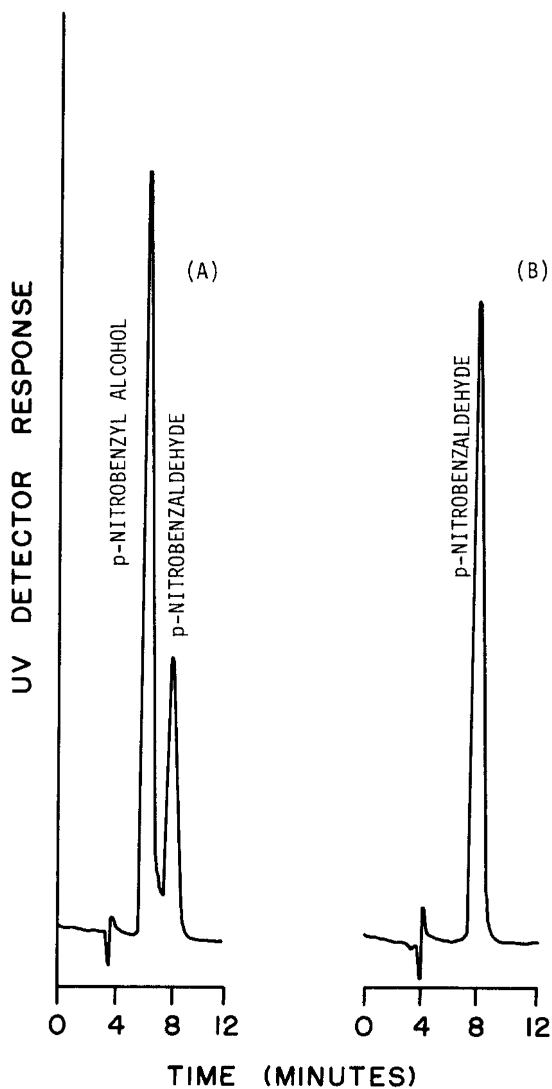

Figure 6. HPLC-UV chromatograms for the polymeric permanganate oxidation of p-nitrobenzyl alcohol to p-nitrobenzaldehyde using C-18 RP-HPLC with 50% HOH/ACN at 0.8 ml/min flow rate, SPR at 46^{0}C on-line, pre-column mode.

III.C. POLYMER SUPPORTED TAGGING REACTIONS FOR DERIVATIZATION IN HPLC-SPR

Despite the fact that several polymeric reagents have already been described for tagging specific classes of analytes, none of these have, as yet, been described for HPLC-SPR derivatizations (31, 40-42). Gelbard and Colonna and Iversen and Johansson have described the preparation and utilization of certain polymeric resins containing phenoxide, p-nitrophenoxide, and naphthoxide anions. These will undergo SN_2 type derivatization reactions with a wide variety of suitable substrates, such as alkyl halides, allyl halides, benzylic halides, and related compounds, at room temperature or above in ethanol or benzene as the solvent. The products in all cases have been aromatic ethers, which has suggested the possible use of these polymeric reagents for HPLC-SPR derivatizations under either reversed phase or normal phase conditions. Clearly, by suitable modification of the aromatic phenoxide or naphthoxide moiety attached to the anion exchange resin, in this case Amberlyst A-26 or Amberlite IRA 900, the final aromatic ethers could improve detection limits for suitable substrates via UV, FL, or EC detection. These remain areas for future development in HPLC-SPR derivatizations, with either normal phase or reversed phase conditions. Derivatizations via polymeric tagging reactions in on-line HPLC-SPR have the advantage of converting, in one fast, clean reaction, the non-UV absorbing substrate into a suitable tagged derivative with pre-tailored UV absorbing characteristics. This is, in many ways, the ideal approach to take for improved specificity and selectivity in HPLC analyses.

Another approach to on-line derivatizations in HPLC has been described by Werkhoven-Goewie et al. (42). However, this particular method has not yet involved a polymeric reagent, although in the future such a modified approach is quite possible. In the work reported here, calcein, a strongly fluorescent compound, is complexed with various divalent metal species, such as palladium, copper, and nickel. These complexes are non-fluorescent, but once the calcein is released, it can then be detected at trace levels via FL methods in HPLC. If the calcein-Pd complex is reacted, off-line or on-line, with thiols, thioethers, thioketones, and disulphides, these will quantitatively release the calcein prior to the FL detector. Thus, this derivatization approach does not actually tag the analyte with a fluorophore, but rather it releases a fluorophore from another complex in the presence of the analyte of interest. Detection of the released fluorophore is then an indirect method of analyzing for the initial, sulphur containing analyte of interest. Clearly, such methods are eminently adaptable to polymeric reactions in HPLC-SPR.

III.D. POLYMER SUPPORTED ENZYME CATALYZED DERIVATIZATIONS IN HPLC-SPR

By treating reactive, insoluble polymeric carriers with enzymes, it is possible to prepare covalently bonded, insoluble, and immobilized

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enzymes that retain their biological activity (43-46). Immobilization by covalent attachment of the enzyme to the solid support or polymer is the most important method of immobilization. For this purpose, insoluble polymers that swell only slightly in water are required, together with reactive groups that will covalently bond to the enzyme under mild reaction conditions. If this polymeric binding does not occur at the biologically active center of the enzyme, then the catalytic activity of the final bound enzyme should be retained, perhaps to a slightly reduced extent. Some enzyme bound polymers have utilized cellulose or Sephadex as the backbone using a cyanogen bromide coupling method. Poly(aminostyrene) has also been converted into an enzyme carrier by diazotization of the amine followed by reaction with the enzyme, or treatment of the aniline portion of the polymer with thiophosgene to form the thiocyanate and reaction of this with the enzyme (47, 48).

Some work has already been described with regard to the on-line or off-line use of polymer supported/attached enzymes in HPLC-SPR, and the work of most recent importance is that of Bowers and Johnson, although others have also utilized similar approaches (49-52). In general, enzyme catalyzed reactions in HPLC-SPR will release an analyte of interest from its biological conjugate, on-line, and often provide a more simple and convenient sample clean-up procedure for direct analysis of the released analytes. However, it is very difficult to imagine an enzyme supported reagent tagging an analyte for improved overall detection. Other chemical reactions, enzyme initiated, are another possibility, other than deconjugation. Bowers and Johnson have described the use of an immobilized enzyme, beta-glucuronidase, as an on-line, pre-column modification reagent in the HPLC-SPR analysis of certain steroids and steroidal-glucuronide conjugates. In the trace analysis for the free steroid, it is often required to cleave either the glucuronide or sulfate conjugates initially, prior to extraction, derivatization, and/or pre-concentration steps in the sample preparation. The reaction catalyzed here is the enzymatic hydrolysis of the glucuronide conjugates of the analytes of interest, here estriol and estradiol. These steroids, once released, can then be separated and detected using standard reversed phase HPLC conditions and procedures. The beta-glucuronidase was immobilized on controlled pore glass or polyacrylamide. The release of the steroids from their conjugates does not chemically alter/change the structures of the steroid molecules, but only their initial attachment to the glucuronide moiety is altered. Difference HPLC would provide information regarding the relative ratio of bound vs free steroids present in the initial sample matrix, using SPR to provide chromatographic differences. Thus, analysis of the sample without any initial enzymatic cleavage of conjugates would indicate how much free steroids are initially present in the sample. Analysis of the same sample

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with enzymatic hydrolysis of the conjugates, followed by analysis for the newly formed/released steroids, would then provide the amount of initially conjugated steroids present in the same sample.

In a typical operation of the enzyme bound SPR, a sample is introduced onto the SPR in one mobile phase that has a low methanol content, and there is then complete conversion of the glucuronide conjugates before the sample travels 1% of the reactor length/bed. The excess enzyme present in the reactor assures a long operational lifetime for the reactor. With the initial mobile phase of 0.05M phosphate buffer, pH 6.8, the cleaved steroids are immediately transferred to the top of the C_{18} analytical column. Once several column volumes of this first mobile phase have passed through the SPR, the reactor bypass valve is turned, removing the SPR from the analytical system. Now a gradient elution mobile phase pattern is started, and this eventually reaches 100% of a strong solvent consisting of 42.5/57.5 phosphate buffer/methanol. As each deconjugated steroid is eluted from the analytical column and reaches the UV detector, it is detected using a wavelength of 280 nm. The immobilized enzyme cannot tolerate a mobile phase consisting of more than 15% methanol, for above this level, it becomes permanently denatured and useless for further reactions. Difference chromatograms for the analysis of estriol and estradiol from their conjugates in biological matrices have been presented by Bowers and Johnson in the literature (49, 50). This is an excellent example of a solid phase reactor system used in the pre-column mode, together with conventional reversed phase HPLC-UV detection. Immobilized enzyme technology has developed to the point where a very large number of commercially available enzymes could be used in this type of SPR for improved HPLC qualitative analyses.

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III.E. POLYMER SUPPORTED TRANSESTERIFICATION OR DISULFIDE INTERCHANGE TYPE REACTIONS FOR DERIVATIZATION IN HPLC-SPR

In principle, polymeric transesterification reactions should be quite similar to polymeric disulfide interchange type reactions, both of which have already been described in synthetic organic chemistry (31). In principle, a transesterification reaction can tag a carboxylic or sulfonic acid substrate with an alcohol initially bound to the polymeric ester, wherein the ester was bound to the polymer _via_ the carboxylic (sulfonic) acid portion. If the ester is bound to the polymer _via_ the alcohol end, then a transesterification reaction with another alcohol would release the carboxylic acid portion in the form of a free ester with the substrate alcohol. Another possible scenario might be wherein an ester bound to the polymer _via_ the carboxylic acid portion is reacted with an alcohol, this alcohol substrate undergoes transesterification, displaces the initial alcohol bound to the polymer, and this released

alcohol is then detected after the HPLC column. In one type of transesterification reaction, the initial substrate is tagged, while in the other type of reaction, one portion of the initial ester is displaced and the final compound to be detected is released from its initial attachment to the polymeric SPR. This same type of a scenario is also possible with disulfide interchange type reactions, which involve the replacement of one thiol group in the initial disulfide by another thiol, or the tagging of an initial thiol by another thiol initially on a polymer bound disulfide, leading to a new disulfide which is then detected. All of these various sequences are indeed theoretically possible, but very few of them have ever been utilized in HPLC-SPR type derivatizations. This is despite the fact that a very large number of polymeric transesterification type reactions have already been described in the synthetic organic literature, especially by Patchornik et al. (53-55). In view of the relatively mild conditions often needed for transesterification type reactions, we would expect that polymer supported reactions of this nature in HPLC-SPR applications will shortly be described (56).

In the area of disulfide interchange reactions, some work has been described with regard to HPLC-SPR interfacing, most notably that of Studebaker et al. (57, 58). This approach has proven useful for the detection of thiols, disulfides, and proteolytic enzymes in HPLC eluates. In each case, the analytes of interest release a chromophoric reagent from the polymeric SPR after the analytical column (post-column mode). This once released chromophoric reagent is then detected with a conventional UV-VIS detector in HPLC, at 412 nm for thiols and disulfides or 520 nm for certain enzymes.

These SPRs contain Sepharose polymers with covalently bound thiol groups. Such thiol-Sepharose groups can then form mixed disulfides with a strong chromophore such as m-dinitrophenylcysteine (DNP-cysteine). Clearly, a fluorophore or electrophore could just as readily be incorporated within the mixed disulfide attached to the Sepharose backbone. Wherein this mixed disulfide SPR is placed after the analytical column, individually eluting thiols, the analytes in this case, will undergo, as a function of pH, a rapid and efficient disulfide interchange reaction with the polymer bound disulfide. The eluting thiol thereby forms a new mixed disulfide with the DNP-cysteine moiety, which is now released from the polymeric backbone. The newly formed disulfides, all of which will now have the same chromophoric moiety, then elute from the SPR and enter the UV-VIS detector. This is the basic method for the analysis and identification of thiols by disulfide interchange, but it may also be possible to utilize this same approach for alcohols/phenols.

For the application of this SPR approach to disulfide analysis, one modification of the above described system must be made, on-line. A separate

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column of Sepharose-thiol is placed on-line before the Sepharose-disulfide SPR, after the HPLC separation column and before the detector. In operation, as a disulfide elutes from the HPLC column, it first enters the polymeric thiol SPR. Here, the eluting disulfide undergoes a disulfide interchange reaction with the polymer bound thiol. This forms a polymer bound disulfide and a newly released thiolate anion from the initially eluting disulfide. From this point forward, the reactions already described for the direct analysis of a thiol apply, as above. That is, the newly formed thiolate anion now enters the second SPR, containing the DNP-cysteine bound chromophore that will undergo another disulfide interchange reaction with the formed thiolate anion. A new disulfide is then formed, containing the thiolate anion initially released from the analyte disulfide together with the DNP-cysteine initially polymer bound to the disulfide SPR. This new UV active disulfide then elutes from the second SPR and is detected, as above.

One final application or modification of the above polymer bound disulfide interchange reactions has been described by Studebaker _et al_., and this allows for the analysis of hydrolytic enzymes _via_ HPLC-SPR. If we have at the start a hydrolyzable substrate, UV active, bound to a solvent insoluble support, then this could be cleaved by an appropriate enzyme in solution. The overall sensitivity of this particular method would depend on how many chromophoric substrate fragments are cleaved by an enzyme molecule during its lifetime within the SPR. In the application described, Azocoll, a strong vis-absorbing dye molecule is attached to a polymer _via_ an amide linkage (-CO-NHRAz, Az = Azocoll). Since this is basically a peptide bond, a suitable peptidase enzyme could hydrolyze this bond, thus releasing a protonated amine-dye molecule (NH_3^+RAz). The amine-dye molecule then enters the optical detector and its response is recorded at a particular wavelength. The formation and detection of the amine-dye molecule thus indicates, in an indirect manner, an initial presence of the correct enzyme in the initial sample solution. Since the enzyme itself has not been consumed in this reaction, it elutes along with the amine-dye molecule, and it can therefore be analyzed by an alternative method, if desired (57, 58). It should be possible to utilize an analogous approach for the HPLC-UV-VIS analysis of other suitable enzymes, assuming that they can also release a detector-active moiety from the SPR.

This entire area of transesterification or disulfide interchange type reactions has been rarely utilized in HPLC-SPR, despite the very large number of possible reactions that have already been utilized on a polymeric support. One would hope that with the wide availability of polymeric and silica bound ion exchange packing materials available today, some enterprising analytical/

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organic chemists will creatively utilize such materials for solid phase reactions and derivatizations in improved HPLC qualitative and quantitative analyses.

IV. ACKNOWLEDGEMENTS

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This is contribution number 154 from the Institute of Chemical Analysis at Northeastern University.

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V. REFERENCES

1. Lawrence, J.F. and Frei, R.W., Chemical Derivatization in Liquid Chromatography, Elsevier Scientific Publ. Co., Amsterdam, Holland, 1976.

2. Lawrence, J.F., Organic Trace Analysis, Academic Press, Inc., New York, 1981, Chapter 7.

3. Frei, R.W. and Lawrence, J.F., eds., Chemical Derivatization in Analytical Chemistry. Volume 1: Chromatography, Plenum Press, Inc., New York, 1981. Chapters 1, 3, and 4.

4. Knapp, D.R., Handbook of Analytical Derivatization Reagents, J. Wiley & Sons, Inc., New York, 1979.

5. Blau, K. and King, G., eds., Handbook of Derivatives for Chromatography, Heyden & Son, Ltd., London, 1977.

6. Snyder, L.R. and Kirkland, J.J., Introduction to Modern Liquid Chromatography, Second Edition, J. Wiley & Sons, Inc., New York, 1979. Chapter 17.

7. Stewart, J.T., Trends in Analytical Chemistry (TRAC), 1, 170 (1982).

8. Clark, C.R. and Wells, M.M., J. Chrom. Sci., 16, 332 (1978).

9. Lawrence, J.F., J. Chrom. Sci., 17, 147 (1979).

10. Ahuja, S., J. Chrom. Sci., 17, 168 (1979).

11. Gfeller, J.C., Huen, J.M., and Thevenin, J.P., Chromatographia, 12, 368 (1979).

12. Schwedt, G., Ang. Chem. Int. Ed., English, 18, 180 (1979).

13. Ross, M.S.F., J. Chromatogr., 141, 107 (1977).

14. Krull, I.S. and Lankmayr, E.P., American Laboratory, 18 (May, 1982).

15. McKillop, A. and Young, D.W., Synthesis, 401, 481 (1979).

16. Posner, G.H., Ang. Chem. Int. Ed., English, 17, 487 (1978).

17. Krull, I.S., Colgan, S., Xie, K-H., Neue, U., King, R., and Bidlingmeyer, B., J. Liquid Chrom., in press (1983).

18. Santaniello, E., Ponti, F., and Manzocchi, A., Synthesis, 891 (1978).

19. Santaniello, E., Farachi, C., and Manzocchi, A., Synthesis, 912 (1979).

20. Schwartz, D.P. and Reynolds, R., Microchem. J., 20, 50 (1975).

21. Fung, N.Y.M., de Mayo, P., Schauble, J.H., and Weedon, A.C., J. Org. Chem., 43, 3977 (1978).

22. Taylor, E.C., Chiang, C-S., McKillop, A., and White, J.F., J. Amer. Chem. Soc., 98, 6750 (1976).

23. Regen, S.L. and Koteel, C., J. Amer. Chem. Soc., 99, 3837 (1977).

24. Santaniello, E., Ponti, F., and Manzocchi, A., Synthesis, 534 (1978).

25. Singh, R.P., Subbarao, H.N., and Dev, S., Tetrahedron, 35, 1789 (1979).

26. Lalancette, J.M., Rollin, G., and Dumas, P., Can. J. Chem., 50, 3058 (1972).

27. Filippo, S.S., Jr. and Chern, C-I., J. Org. Chem., 42, 2182 (1977).

28. McKillop, A. and Young, D.W., Synthesis, 481 (1979).

29. Risbood, P.A. and Ruthven, D.M., J. Amer. Chem. Soc., 100, 4919 (1978).

30. Leyden, D.E., J. Org. Chem., 46, 2406 (1981).

31. Mathur, N.K., Narang, C.K., and Williams, R.E., Polymers as Aids in Organic Chemistry, Academic Press, New York, 1980.

32. Demko, P.R., Wagner, S.E., Mikulski, R.A., and Clements, J.G., in Ions in Polymers, ed. by Eisenberg, A., Advances in Chemistry Series No. 187, American Chemical Society, Washington, D.C., 1980. Chapter 13.

33. Ventron Corporation Technical Bulletin No. TB478. Resin-borohydride developmental polymeric reducing agent. Ventron Division, Thiokol Corp., Danvers, Mass.

34. Krull, I.S., Xie, K-H., Colgan, S., Neue, U., Izod, T., King, R., and Bidlingmeyer, B., J. Liquid Chrom., in press (1983).

35. Menger, F.M., Shinozaki, H., and Lee, H-C., J. Org. Chem., 45, 2724 (1980).

36. Cainelli, G., Cardillo, G., Orena, M., and Sandri, S., J. Amer. Chem. Soc., 98, 6737 (1976).

37. Frechet, J.M.J., Darling, P., and Farrall, M.J., J. Org. Chem., 46, 1728 (1981).

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38. Frechet, J.M.J., Warnock, J., and Farrall, M.J., J. Org. Chem., 43, 2618 (1978).

39. Krull, I.S., Xie, K-H., Colgan, S., Santasania, C., Neue, U., King, R., Bidlingmeyer, B., and Newhart, A., J. Liquid Chrom., in preparation (1983).

40. Gelbard, G. and Colonna, S., Synthesis, 113 (1977).

41. Iversen, T. and Johansson, R., Synthesis, 823 (1979).

42. Werkhoven-Goewie, C.E., Niessen, W.M.A., Brinkman, U.A.Th., and Frei, R.W., J. Chromatogr., 203, 165 (1981).

43. Manecke, G., Chimia, 28, 9 (1974).

44. Zaborsky, O., Immobilized Enzymes, CRC Press, Inc., Chemical Rubber Company, Inc., Boca Raton, Florida, 1973.

45. Weetall, H.H., Immobilized Enzymes, Antigens, Antibodies, and Peptides, Volume 1, Marcel Dekker, New York, 1975.

46. Mosbach, K. FEBS Lett., 62 (Suppl. 1), E80 (1976).

47. Hartley, F.R. and Vezey, P.N., Adv. Organometal. Chem., 15, 189 (1977).

48. Grubbs, R.H., Chemtech, 7, 512 (1977).

49. Bowers, L.D. and Johnson, P.R., Anal. Biochem., 116, 111 (1981).

50. Johnson, P.R. and Bowers, L.D., Anal. Chem., 54, 2247 (1982).

51. Ogren, L., Cisky, I., Risinger, L., Nilsson, L.O., and Johansson, G., Anal. Chim. Acta, 117, 71 (1980).

52. Okuyama, S., Kokubun, N., Higashidate, S., Uemura, D., and Hirata, Y., Chem. Lett., 12, 1443 (1979).

53. Kraus, M.A. and Patchornik, A., J. Polymer Sci.: Macromol. Revs., 15, 55 (1980).

54. Kraus, M. and Patchornik, A., Chemtech, 118 (February, 1979).

55. Kraus, M.A. and Patchornik, A., J. Polymer Sci., 47, 11 (1974).

56. Krull, I.S., LaCourse, W., Neue, U., Bidlingmeyer, B., and Newhart, A., unpublished results (1983).

57. Studebaker, J.F., J. Chromatogr., 185, 497 (1979).

58. Studebaker, J.F., Slocum, S.A., and Lewis, E.L., Anal. Chem., 50, 1500 (1978).

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