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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF GLUCOSIDES (GLUCOSE CONJUGATES) WITH POST-COLUMN REACTION DETECTION COMBINING IMMOBILIZED ENZYME REACTORS AND LUMINOL CHEMILUMINESCENCE

PHILIP J. KOERNER, Jr.* and TIMOTHY A. NIEMAN*
Department of Chemistry, University of Illinois, 1209 W. California St., Urbana, IL 61801 (U.S.A.)
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SUMMARY

This detection method makes use of sequential immobilized enzyme reactors (IMER) to first hydrolyze β-D-glucosides to β-D-glucose (using β-glucosidase) and then to produce hydrogen peroxide from the β-D-glucose (using glucose oxidase); the hydrogen peroxide is then detected with luminol chemiluminescence. This method has been used for the determination of individual β-D-glucosides (phenyl, *p*-nitrophenyl, and salicin) via flow-injection analysis and has been extended to the determination of a mixture of glucosides following their separation via reversed-phase high-performance liquid chromatography. The use of up to 30% acetonitrile in the buffered mobile phase had very little effect on the efficiency of the β-glucosidase and glucose oxidase enzyme reactors; overall the use of acetonitrile did not affect the linear working range or detection limits for the glucosides. Chromatographic detection limits are approximately 0.1 μM (2 pmol) with a linear working range of more than three decades.

INTRODUCTION

In recent years chemiluminescence (CL) has become an attractive detection method for analytical determinations due to the very low detection limits and wide linear working ranges which can be obtained while using relatively simple instrumentation[1–3]. However, analytical applications of CL based detection have been limited due to the lack of selectivity inherent to the CL reaction. This problem can be overcome by coupling the sensitivity of CL detection with a highly selective chemical or physical step, such as enzymatic reactions[4–7], immunoassay[3,8–10], or liquid chromatography[11–23].

The CL reaction of interest in this study is the luminol (3-aminophthalhydrazide) reaction. This reaction has been used in conjunction with enzyme reactions

* Present address: E.I. DuPont Co., Chemicals and Pigments Dept., Jackson Laboratory/Building 1155(U), Wilmington, DE 19898, U.S.A.

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for the determination of a variety of species. Analytes which can be converted in one or more enzymatic reactions to hydrogen peroxide have been quantitated by luminol CL. The luminol CL reaction has also been applied to the detection of organic species separated by high-performance liquid chromatography (HPLC)[13–17] and for the detection of metal ions separated as their chloro complexes on a strong anion-exchange column[22,23].

There have been two reports in the literature on the combined use of HPLC with enzyme reactions followed by CL detection; however, neither utilized the luminol CL reaction. The first involved the enzymatic hydrolysis of β-D-glucuronides to glucuronic acid which was then detected with the lucigenin CL reaction after potential interferants (other organic reductants) were removed by anion-exchange chromatography[12]. The second involved the reversed-phase separation of a mixture containing acetylcholine and choline followed by enzymatic conversion to hydrogen peroxide and peroxy-oxalate CL detection[21]. We report here the first example of the coupling of HPLC with enzyme reactions and luminol CL detection.

The use of the luminol CL reaction has been investigated in this study for the analytical determination of β-D-glucosides. β-D-Glucosides are conjugates of β-D-glucose and "foreign" molecules (such as herbicides and other potentially toxic organic species). These conjugates are produced primarily in plants (and to a lesser extent in mammals) as a means of metabolizing and eliminating these foreign molecules. The formation of glucosides in plants is analogous to the formation of glucuronides (conjugates of glucuronic acid) in mammals. Glucuronides are formed in the liver and serve to eliminate otherwise insoluble and potentially toxic organic species, thus permitting their elimination from the body.

The enzyme β-glucosidase specifically catalyzes the hydrolysis of β-D-glucosides to produce β-D-glucose and the corresponding organic compound (aglycon). The enzyme glucose oxidase catalyzes the oxidation of β-D-glucose to produce hydrogen peroxide which will then react with an alkaline solution of luminol to generate CL. An example of this scheme is shown for a β-D-glucoside in Fig. 1. In this study the β-glucosidase and glucose oxidase have been immobilized by covalent attachment to controlled pore glass (CPG) and packed into small columns, or immobilized enzyme reactors (IMER). The sample, in a buffered stream, first passed through the β-glucosidase IMER where the glucoside bond is hydrolyzed to produce glucose, and then passes through the glucose oxidase IMER where the glucose reacts to produce hydrogen peroxide. The enzymatically generated hydrogen peroxide is then quantitated by the luminol CL reaction.

In this paper a method for the determination of glucosides using immobilized enzymes and CL detection is reported. This enzyme coupled CL detection method is then extended to the determination of a mixture of glucosides after separation on a reversed-phase column.

EXPERIMENTAL

Reagents

The glucosides used in this study were: salicin (2-(hydroxymethyl)phenyl-β-D-glucoside), phenyl-β-D-glucoside, and *p*-nitrophenyl-β-D-glucoside (all from Sigma). The structures of these glucosides are shown in Fig. 2. The corresponding aglycons

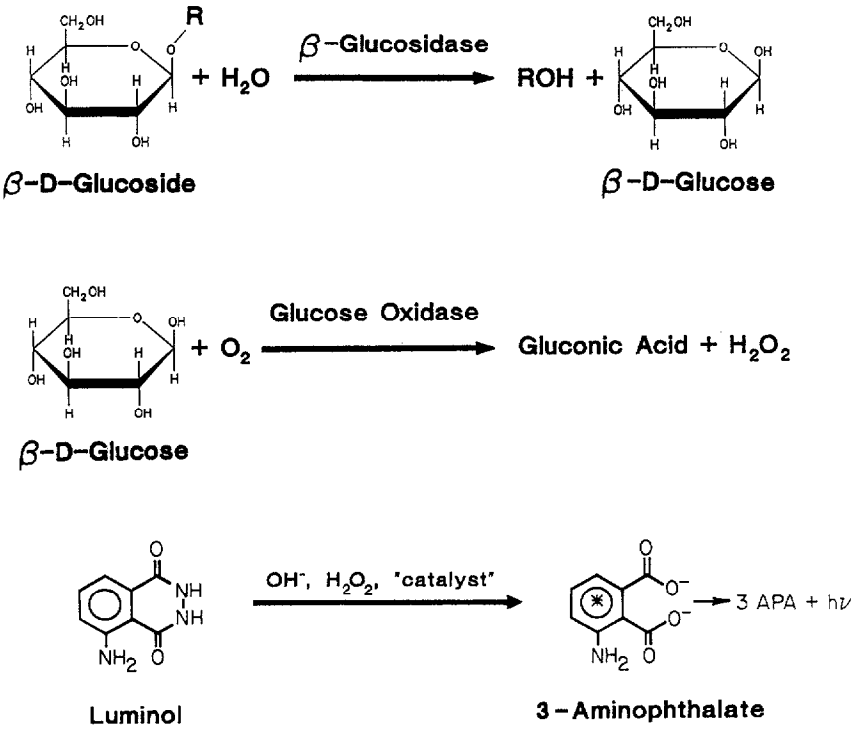

Fig. 1. Reaction scheme for the determination of β-D-glucosides via luminol chemiluminescence.

used to study their effects on CL detection were: phenol (Mallinckrodt), and *p*-nitrophenol (Aldrich).

The enzymes used in this study were β-glucosidase and glucose oxidase. β-Glucosidase (E.C. 3.2.1.21) was obtained from Fluka (from almonds) with an activity of ~6 units/mg with salicin as the substrate at pH 5.0 at 37°C. Glucose oxidase (E.C. 1.1.3.4) was obtained from Amano Enzymes (from *Aspergillus* sp.) with an activity of 109 units/mg with glucose as the substrate at pH 5.1 at 35°C.

The reagents used for enzyme immobilization include 3-aminopropyltriethoxysilane obtained from Petrarch Systems and glutaraldehyde (25% in water) obtained from Eastman Kodak. Enzymes were immobilized onto controlled pore glass (CPG) obtained from Electro Nucleonics with 125–180 μm diameter (80–120 mesh) and 250 Å average pore size, and onto CPG with 37–74 μm diameter (200–400 mesh) and 547 Å average pore size.

Luminol was obtained from Aldrich. Horseradish peroxidase (E.C. 1.11.17) was obtained from Sigma as Type I with an activity of 95 units/mg. Hemin was obtained from Sigma as Type I. All other chemicals were analytical reagent grade. All chemicals were used as purchased without further purification. All solutions were prepared with water purified by a Millipore/Continental Milli-Q water purification system.

Stock solutions (10 m*M*) of all glucosides were prepared fresh daily by weighing accurately and dissolving with water. Serial dilutions of the stock solutions with water yielded the required working solutions which were prepared immediately prior to use.

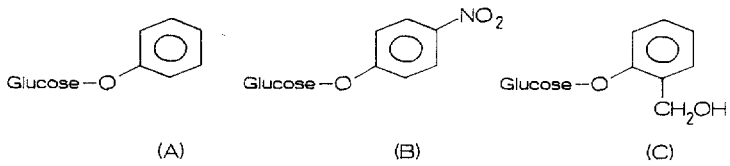

Fig. 2. Structures of glucosides studied: (A) phenyl glucoside, (B) *p*-nitrophenyl glucoside and (C) salicin.

A stock solution (10 m*M*) of glucose was prepared in a similar fashion and could be stored refrigerated for 3–4 days before significant decomposition occurred. Working solutions were prepared from this stock solution prior to use. Working solutions of hydrogen peroxide were prepared fresh daily by serial dilution from 30% (10 *M*) hydrogen peroxide.

The chemiluminescence reagent solution used contained 0.4 m*M* luminol, 8 mg/l horseradish peroxidase (HRP), 2 μM hemin, and 0.1 *M* sodium phosphate dibasic buffered at pH 11.6. This solution was prepared from a stock solution of 2 m*M* luminol in 0.5 *M* sodium phosphate dibasic (pH 11.4) and a stock solution of 2 m*M* hemin by the appropriate dilution in water. The HRP was added as a solid and the solution diluted to volume.

The HPLC mobile phase were prepared fresh daily and filtered through a 0.2-μm Nylon 66 membrane filter (Rainin) and then degassed prior to use.

Enzyme immobilization

Glutaraldehyde-bound CPG was prepared as described previously[24,25]. To 0.5 g of glutaraldehyde-bound CPG was added 10 ml of 0.05 *M* sodium pyrophosphate (pH 7.5) containing either 100 mg of glucose oxidase (10 900 units) or 100 mg of β-glucosidase (*ca.* 600 units). The enzyme immobilization proceeded as described previously[25]. The immobilized glucose oxidase and β-glucosidase were stored in 0.05 *M* sodium pyrophosphate buffer (pH 7.5) at *ca.* 5°C.

For the flow injection analysis studies the immobilized enzymes were slurry packed into plexiglas columns 2.25 in. × 0.125 in. I.D. The column was capped with nylon end fittings containing 20 μm stainless-steel frits and designed to accept 1/4–28 fittings for easy insertion into the flow system.

For the HPLC studies the immobilized enzymes were slurry packed under vacuum into 70 mm × 2.0 mm I.D. stainless-steel HPLC guard columns (Whatman). The columns were equipped with zero-dead-volume end fittings containing 2 μm stainless-steel frits and designed to accept 1/16 in. Swagelok (female) fittings for incorporation into the HPLC flow system.

Instrumentation

The glucoside analyses were carried out with the flow injection system shown in Fig. 3. A Rainin Rabbit peristaltic pump was used to pump each of two channels, one containing buffer and the other containing the CL reagent solution, at a flow-rate of 1.5 ml/min. Samples of the glucoside of interest (77 μl) were injected with a Rheodyne Model 5250 sample injection valve into the buffer stream, which contained 1 m*M* phosphate buffered at pH 6.5 and 0–30% acetonitrile (v/v). The sample was then carried through an IMER containing immobilized β-glucosidase and an IMER

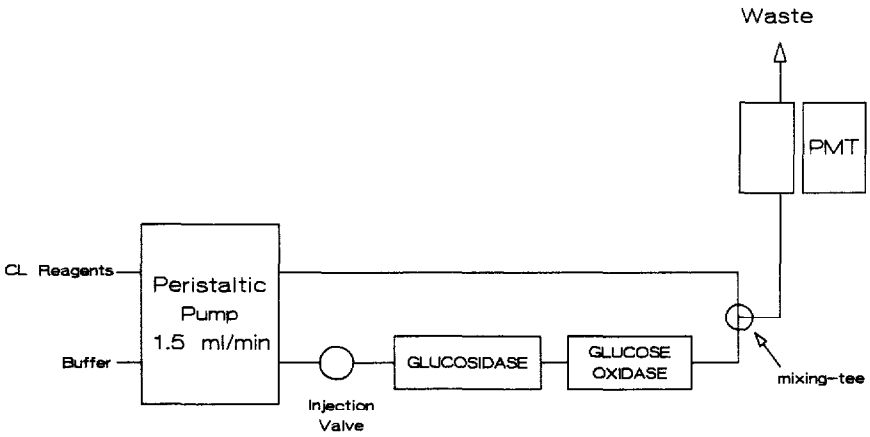

Fig. 3. Flow system used for flow injection determinations.

containing immobilized glucose oxidase. The sample in the buffer stream then combined at a mixing-tee with the CL reagent solution and passed through a short segment (80 mm × 1.0 mm I.D.) of microporous PTFE tubing (Anspec) to remove any air bubbles present and on to the flow cell.

For the analysis of mixtures of glucosides the HPLC flow system shown in Fig. 4 was used. Isocratic HPLC separations were carried out using a modular HPLC system consisting of an Altex Model 110A HPLC pump, a silica pre-column, an Altex Model 210 injection valve equipped with a 20-μl sample loop, a Partisil ODS-2 guard column (70 mm × 2.0 mm I.D.), a high-pressure in-line filter (Scientific Systems) with replaceable 0.5-μm frits, and an HPLC column. Chromatographic separations were obtained using a DuPont Zorbax ODS column (5 μm, 250 mm × 4.6 mm I.D.). In this system the buffer, 1 mM phosphate (pH 6.5) with 0–30% acetonitrile (v/v), was delivered at a flow-rate of 1.0 ml/min. After passing through the HPLC system the column effluent passed through the β-glucosidase and glucose IMERs, then was combined at a mixing-tee with the CL reagent solution which was delivered with

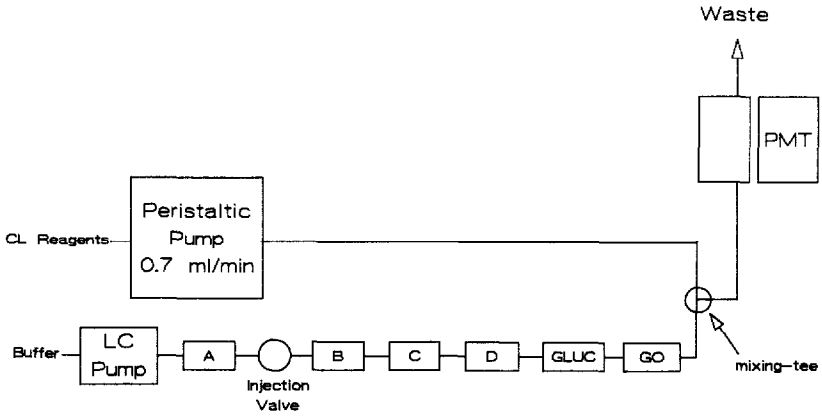

Fig. 4. HPLC flow system. A = Silica pre-column; B = guard column; C = column prefilter; D = analytical column; GLUC = glucosidase IMER; GO = glucose oxidase IMER.

a peristaltic pump at a flow-rate of 0.7 ml/min. The combined solution then passed through the flow cell.

In both the flow injection and HPLC systems dscribed above, the flow cell (with a volume of 50 μl) was placed directly adjacent to the photomultiplier tube (Hamamatsu 1P28 biased at -960 V) where the CL emission was detected. The signal was then amplified by a Pacific Precision Instruments Model 126 photometer and output to a strip chart recorder.

Optimization of separation conditions, and determination of the retention times of the various glucosides, was initially carried out with the HPLC system shown in Fig. 4 using an Altex Model 153 UV–VIS detector to monitor the column effluent.

Determination of IMER activities

The efficiency of the glucose oxidase IMERs were determined by measuring the quantity of hydrogen peroxide produced from the oxidation of β-D-glucose in a single pass through the enzyme reactor. A 77-μl sample of 10 μM D-glucose was carried through the IMER in a buffered flowing stream and the hydrogen peroxide produced quantitated by luminol CL. The signal obtained was corrected for the fact that a solution of D-glucose at equilibrium contains only 64% of the β-anomer, and then compared with the signal obtained for a similar injection of 10 μM hydrogen peroxide.

In a similar fashion the efficiency of the β-glucosidase IMERs were determined. A 77-μl sample of 10 μM *p*-nitrophenyl-β-D-glucoside was carried through the β-glucosidase and glucose oxidase IMERs and the hydrogen peroxide produced quantitated by luminol CL. The signal obtained was corrected for the efficiency of the glucose oxidase column and then compared with the signal obtained for a similar injection of 10 μM hydrogen peroxide. The efficiency of the β-glucosidase IMERs were also determined by measuring the quantity of glucoside which was hydrolyzed in a single pass through the enzyme reactor. A 77-μl sample of 1.0 mM *p*-nitrophenyl-β-D-glucoside was carried through the IMER in a buffered flowing stream, the hydrolyzed glucoside was collected in a volumetric flask, and the amount of *p*-nitrophenol in this solution was quantitated by measuring the absorbance of the solution at 400 nm.

RESULTS AND DISCUSSION

Flow injection detection of β-D-glucosides

The flow injection system used in this study is essentially the same as that used for the determination of glucose and other sugars using immobilized enzymes and luminol CL detection[25]. Of these sugars only glucose has a corresponding oxidase enzyme which will produce hydrogen peroxide as a product of an enzymatic reaction. Therefore, in order to quantitate the other sugars by CL they must first be converted to glucose via other enzymatic reactions. Similarly, β-D-glucosides cannot react directly with an enzyme to produce hydrogen peroxide but must first be hydrolyzed by β-glucosidase to produce β-D-glucose which can then be quantitated by CL after reaction with glucose oxidase to produce hydrogen peroxide. Since the hydrolysis of β-D-glucosides produces only β-D-glucose, there is no need for the presence of the enzyme mutarotase, which catalyzes the interconversion of the α and β anomers of D-glucose.

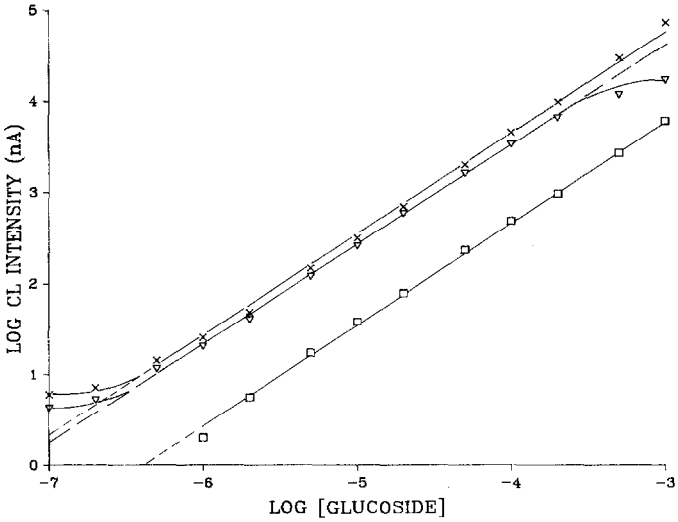

Fig. 5. Flow injection working curves for β-D-glucosides. ▽ = Salicin, □ = phenyl glucoside and × = *p*-nitrophenyl glucoside.

Using the flow injection system shown in Fig. 3 with an aqueous carrier stream that contained 1 m*M* phosphate (pH 6.5), working curves for the three glucosides were obtained and are shown in Fig. 5. The log–log working curves for the three "model" glucosides (salicin, phenyl glucoside, and *p*-nitrophenyl glucoside) are linear and cover over three orders of magnitude. Detection limits for these glucosides are on the order of ~0.5 μ*M* (~40 pmol). These glucoside detection limits are comparable to those obtained for the flow injection analysis of sugars using immobilized enzymes and luminol CL detection[25].

The lower CL intensities observed for equal concentrations of phenyl glucoside relative to those observed for salicin and *p*-nitrophenyl glucoside may have resulted from the inhibition of either enzyme by the aglycon produced after hydrolysis of the glucoside by β-glucosidase. To check this possibility, solutions containing equimolar amounts of β-D-glucose and the aglycons of interest (phenol and *p*-nitrophenol) were prepared. The CL signal obtained after a sample of each solution was carried through the glucose oxidase IMER was compared with the signal obtained for a solution containing only an equimolar amount of β-D-glucose. The CL signals were all of equal intensity. Repeating the same series of injections through the β-glucosidase and glucose oxidase IMERs yielded similar results. Therefore, the lower intensity CL signal observed for phenyl glucoside does not appear to be the result of any inhibition of the enzymes by the aglycon produced upon hydrolysis. The difference in signal intensity observed for a given glucoside concentration is probably caused by the difference in reaction rates and efficiency of the enzyme β-glucosidase for each glucoside. The rate of reaction, as well as the efficiency, of β-glucosidase has been shown to vary with the identity of the substrate[26,27].

Mobile phase considerations

To convert the flow injection system to an HPLC system required insertion of a chromatographic column between the injector and the first enzyme column. It was therefore necessary that the mobile phase requirements of the separation be compatible with those of the enzyme reactions and CL detection.

It was expected that in order to obtain a separation of a mixture of these glucosides via reversed-phase HPLC, a significant amount of an organic solvent as modifier would be required in the buffered mobile phase. Therefore, it was necessary to first investigate the effects such an organic solvent would have on the enzyme reactions and the luminol CL reaction. Acetonitrile and methanol are commonly used as organic modifiers in reversed-phase HPLC; however, methanol is known to cause the denaturing of enzymes, thereby making them inactive. In addition, Van Zoonen *et al.*[28] have recently shown that a glucose oxidase IMER retained 25% of its original efficiency when a buffered carrier stream containing 80% (v/v) acetonitrile was used. Therefore, the use of acetonitrile in the buffered carrier stream, and its effect on the enzyme reactions and the luminol CL reaction, was investigated.

Increasing the acetonitrile from 0–30% (v/v) in the 1 m*M* phosphate carrier stream (buffered at pH 6.5) led to an increase in the observed CL signals for hydrogen peroxide, glucose, and *p*-nitrophenyl glucoside. This is demonstrted in Fig. 6, which shows a plot of CL intensity *versus* percentage acetonitrile for 10 μM hydrogen peroxide, 10 μM glucose, and 10 μM *p*-nitrophenyl glucoside. Using a 1-m*M* phosphate carrier stream (pH 6.5) containing 0–30% acetonitrile, the efficiencies of the β-glucosidase and glucose oxidase IMERs used in the flow injection studies were determined by CL. The efficiency of the glucose oxidase IMER varies between 80 and 100% while the efficiency of the β-glucosidase IMER varies between 85 and 100%. There is no apparent correlation between the efficiencies of the IMERs and the

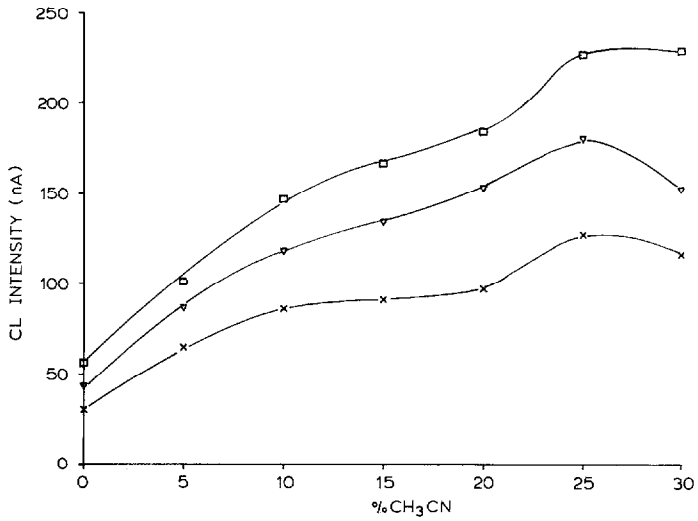

Fig. 6. Plot of CL intensity *vs.* percentage acetonitrile in 1 m*M* phosphate (pH 6.5) carrier stream. □ = 10 μM Hydrogen peroxide, × = 10 μM glucose and ▽ = 10 μM *p*-nitrophenyl glucoside. The solid lines are drawn merely to connect the data points and not to indicate a particular model for the data relationship.

percentage acetonitrile in the carrier stream. The efficiency of the β-glucosidase IMER was also determined by the absorbance method using *p*-nitrophenyl glucoside as the substrate. For a carrier stream containing 0% acetonitrile, the efficiency determined by absorbance was in agreement with the value obtained by CL.

The efficiency of the IMERs was found to be reversibly affected by the presence of acetonitrile in the carrier stream. The efficiency of the IMERs could be restored by flushing the IMERs with 10 m*M* phosphate buffer (pH 6.5) for 20 min after it had been exposed to a carrier stream containing acetonitrile.

The higher CL intensities observed in the presence of acetonitrile were somewhat puzzling. Profiles of the CL intensity *versus* time for 0 and 20% acetonitrile were obtained via stopped-flow analysis and were found not to differ significantly in shape. Both profiles show that peak CL intensity is reached after a short induction period, remains at the peak level for several seconds, and then decreases exponentially back to the background over a period of 2 min. In the system containing 20% acetonitrile the peak CL intensity is *ca.* 3.5 times greater than in the system containing no acetonitrile. The background CL emission (*i.e.*, in the absence of hydrogen peroxide) is also increased in the presence of acetonitrile. The presence of acetonitrile may be affecting the quantum efficiencies of the CL process; however, further experiments are required before the effect of acetonitrile on the luminol CL reaction can be more fully understood.

The presence of 0–30% acetonitrile in the buffered carrier stream does not significantly affect the working curves for the model glucosides. The working curves remain linear over greater than three orders of magnitude and the slope changes only slightly, increasing from 1.1 to 1.2 as the percentage acetonitrile increases. The detection limits also remain approximately equal to those obtained in the absence of acetonitrile.

Since the mobile phase pH is a parameter that might be adjusted in optimizing a chromatographic separation, the effect of mobile phase pH on the detection of glucosides was investigated. The work was centered around pH 6.5 because the optimum pH for the use of immobilized glucose oxidase has been reported to be 6.5 (ref. 25). The data obtained are tabulated in Table I. Although pH 6.5 gave the best results, any pH between 6 and 7 would give satisfactory results. For a separation to be carried out at a pH much lower than 6, a pH change between the analytical column and the IMERs would be required since enzymes typically suffer from a loss of activity at very acidic pHs. The buffering capacity of the mobile phase carrying the analyte through the analytical column and the IMERs was kept low (1 m*M*) so that when this

TABLE I

EFFECT OF pH ON CL SIGNALS FOR MODEL GLUCOSIDES

	CL intensities (nA)		
	pH 6.0	*pH 6.5*	*pH 7.0*
50 μ*M* Salicin	16	32	31
200 μ*M* Phenyl glucoside	5	12	11
50 μ*M* *p*-Nitrophenyl glucoside	211	230	204

stream mixed with the CL reagent solution (buffered at pH 11.6 with 100 m*M* phosphate) the resulting solution would remain close to pH 11.6, which is optimal for the luminol CL reaction.

Chromatographic separations

Separations of the glucosides were obtained with a Zorbax ODS (5 μm) column using a mobile phase containing 25% acetonitrile in 1 m*M* phosphate buffered at pH 6.5.

The HPLC system was configured for use with the IMERs and CL detection as diagrammed in Fig. 4. To minimize resolution degradation within the enzyme reactors and associated tubing several changes were made in going from the flow injection system to the HPLC system. The enzymes were immobilized on smaller size CPG (37–74 μm) and packed into stainless-steel guard columns, and smaller tubing (0.012 in. I.D.) was used to connect the IMERs to the CL flow cell. With these changes to the flow system the resolution of the glucoside separation obtained with CL detection was 1.2 while with UV detection the resolution was 1.9. The difference in resolution is attributable to dispersion of the analyte bands in the IMERs and to some dilution of the analyte bands caused by the post-column addition of the CL reagent solution.

While the enzymes immobilized on the larger size CPG were useful in the flow injection determination of glucosides, the use of the smaller size CPG was necessary to maintain the resolution of the glucoside separation. Due to the excessive back pressure generated, the use of the smaller CPG as a support for immobilized enzymes is not amenable for use in a flow injection system unless a HPLC pump is used to deliver the buffered carrier stream through the IMERs. A peristaltic pump cannot maintain a constant flow-rate against the higher back pressures created by the use of the 37–74 μm CPG. The efficiencies of the β-glucosidase and glucose oxidase IMERs used in the HPLC studies were determined using the chromatographic mobile phase and found to be 72% for glucose oxidase (using the CL method) and 62% for β-glucosidase (using *p*-nitrophenyl-β-D-glucoside as the substrate and the absorbance method).

Another factor which could affect the resolution of the separation, as well as the peak CL intensities observed, is the flow-rate of the CL reagents added to the mobile phase downstream of the IMERs. A post-column reagent flow-rate which is too fast could lead to significant dilution of the analyte bands and result in broader and less intense peaks. The flow-rate used to deliver the CL reagents which gave the best results was 0.7 ml/min. Faster flow-rates led to a slight decrease in resolution and less intense peaks, while slower flow-rates gave somewhat lower CL intensities.

Using the conditions which were found to give the best results, a chromatogram demonstrating the separation and CL detection of a mixture containing salicin, phenyl glucoside, and *p*-nitrophenyl glucoside was obtained and is shown in Fig. 7. The chromatographic detection limit for *p*-nitrophenyl glucoside was determined to be *ca.* 0.1 μM (*ca.* 2 pmol).

A somewhat better detection limit, in terms of number of moles injected, was observed under the chromatographic conditions compared with the flow injection conditions. This is probably due to the difference in the overall solution flow-rate through the flow cell. Since the efficiencies of the β-glucosidase and glucose oxidase IMERs are approximately equal in the flow injection and HPLC systems, the amount of hydrogen peroxide being produced from a given glucoside concentration is about

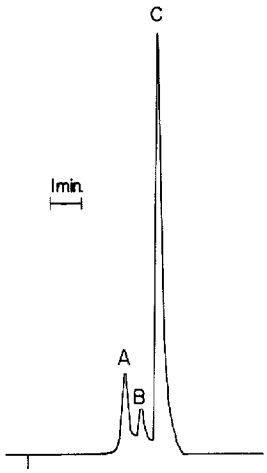

Fig. 7. Isocratic separation of glucoside mixture on Zorbax ODS column (5 μm) with a mobile phase containing 25% acetonitrile in 1 mM phosphate (pH 6.5). Flow-rate = 1.0 ml/min. A = 50 μM Salicin, B = 200 μM phenyl glucoside and C = 50 μM p-nitrophenyl glucoside.

the same. Thus, a faster total flow-rate for the solution passing through the flow cell will lead to the observation of somewhat lower peak CL intensities.

CONCLUSIONS

Determinations of glucosides typically found as metabolic products of herbicides and pesticides in plants have been reported using a variety of methods[29–31]. Typically the determination of glucosides involves hydrolysis, followed by a chromatographic separation, and detection of the aglycon by UV absorbance or photoconductivity[30,31]. The aglycon 5-hydroxychlorsulfuron, formed by hydrolysis of the glucoside of chlorsulfuron, has been detected at 1.5 μg/ml (*ca.* 30 pmol) using absorbance detection at 254 nm and at 0.10 μg/ml (*ca.* 2 pmol) using photoconductivity detection[31].

The detection method for glucosides described here using enzymatic reactions and CL detection is simple, fast, yields wide working ranges, and detection limits which are comparable to those reported using other methods. This method offers several advantages over the alternate methods described above. First, it is easier and faster since it utilizes HPLC followed by on-line enzymatic hydrolysis of the glucosides. The use of on-line enzymatic reactions avoids the long reaction times (up to 6 h) and elevated temperatures (37°C) required for the complete enzymatic hydrolysis of glucosides in solution. Second, this method can be extended to the determination of any β-D-glucosides. The photoconductivity detector, while sensitive to the detection of molecules containing sulfur, nitrogen, phosphorous, and halogen atoms, is not suitable for the detection of simpler glucosides. Absorbance methods are limited to the detection of glucosides containing a native chromophore. The detection limits achieved for the glucosides in this investigation are better than those achieved with UV absorbance detection and are comparable to those reported using photoconductivity

detection. Finally, although not done here, the method could be easily adapted to detection of α-glucosides by using an IMER containing α-glucosidase instead of, or in addition to, the β-glucosidase column used here.

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