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Journal of Chromatography B, 658 (1994) 249–260

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Polymeric 6-aminoquinoline, an activated carbamate reagent for derivatization of amines and amino acids by high-performance liquid chromatography

J.H. Yu[a], G.D. Li[a,1], I.S. Krull[a,*], S. Cohen[b]

[a]*Department of Chemistry, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA*
[b]*Waters Chromatography Division, Millipore Corporation, 34 Maple Street, Milford, MA 01757, USA*

First received 3 November 1993; revised manuscript received 3 May 1994

Abstract

A new polymeric reagent containing the 6-aminoquinoline (6-AQ) tag was developed and applied for the off-line derivatization of amines and amino acids in high-performance liquid chromatography (HPLC). The synthesis and characterization of this polymeric reagent are described. An authentic external standard of a typical amine was synthesized and characterized for the determination of the derivatization efficiency. All amines had a derivatization efficiency higher than 50%; the derivatization of amino acids was performed under optimized phase-transfer catalysis reaction conditions. Derivatized amines and amino acids were separated under conventional reversed-phase conditions and determined by UV and FL detectors. To investigate the practical applications, this polymeric reagent was also used to derivatize protein hydrolysates.

1. Introduction

The analysis of amine-containing compounds by HPLC combined with chemical derivatization has been greatly improved over the past two decades. Compared with liquid-phase derivatizations, the solid-phase derivatization approach has become increasingly attractive due to some unique features, such as good selectivity and ease of operation [1,2]. A large number of solid-phase reagents with different underlying supports, the leash that connects the support to the tag, and the final analytical tags have been described over the last 15 years [3]. Most of these reagents were used for the analysis of amines, amino alcohols, and weak nucleophiles by HPLC. Only a few of them were used for the determination of amino acids and peptides. Recently, a polymeric reagent containing 9-fluoreneacetyl (9-FA) as the fluorescent tag was successfully used for the derivatization of amino acids and peptides by Zhou et al. [4]. A serious drawback of this 9-FA tagged solid-phase reagent is formed by the hydrolysis of the derivatization reagent, giving 9-fluorenylacetic acid, which has a fluorescence spectrum similar to that of the amino acid or peptide derivatives. This hydrolysis product may sometimes seriously interfere with the desired amino acid or peptide derivatives, especially in the on-line approach.

* Corresponding author.
[1] Visiting Scientist from Nanjing Normal University, Nanjing, China.

0378-4347/94/$07.00
SSDI 0378-4347(94)00242-W

To solve this problem, a tag which gives no fluorescent hydrolysis products has to be immobilized on the polymeric support.

Cohen and Michaud have recently developed a new fluorescent derivatization reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, for the analysis of amino acids in solution-phase derivatization via HPLC [5]. This highly reactive amine derivatizing reagent can react with amino acids to form stable derivatives which are readily amenable to analysis by RP-HPLC. Excellent derivative yields were obtained for all primary and secondary amino acids. The hydrolysis product of this reagent is 6-aminoquinoline (6-AQ) (Fig. 1b). 6-AQ does not show fluorescence at the same wavelengths as its amino acid or peptide derivatives [6]. Thus, it does not interfere in the derivatization and detection process. Recently the use of this reagent for the enantiomeric separation of amino acids has also been reported by Pawlowska et al. [7]. Based on this solution-phase derivatization, the feasibility of immobilizing 6-AQ on a polymeric support has been investigated in this paper. 6-AQ was attached to a benzotriazole leash. The structures of this polymeric reagent and its hydrolysis products are shown in Fig. 1. Another hydrolysis product of this polymeric 6-AQ reagent, quinoline-6-diurea (Fig. 1c), is not fluorescent at all due to self-quenching. Thus, this solid-phase reagent should not suffer from serious fluorescent interferences by its hydrolysis products.

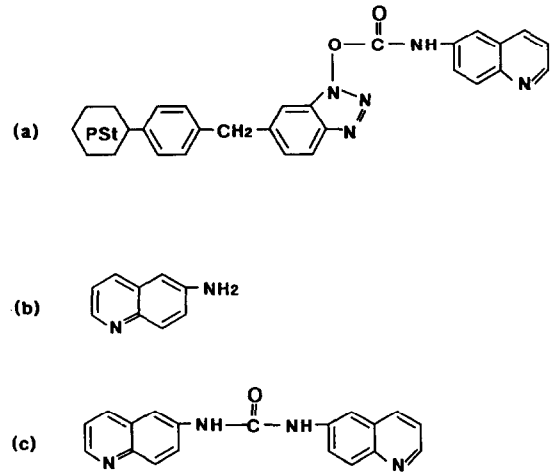


Fig. 1. Structure of polymeric 6-AQ reagent and its hydrolysis products. (a) Polymeric 6-AQ reagent; (b) 6-AQ; (c) quinoline-6-diurea.

A major problem encountered in the solid-phase derivatizations of amino acids was the low derivatization efficiency. Most amino acids are soluble only in aqueous solutions. A buffer with a basic pH has to be used to provide enough nucleophilicity for the amino group. Highly reactive solid-phase reagents were unstable in basic buffer due to the competing hydrolysis process. Moreover, charged amino acids can not penetrate a hydrophobic polymeric support to react with the immobilized reagents. Thus, derivatization efficiencies of amino acids were limited. To solve this problem, Zhou et al. proposed a phase-transfer catalysis (PTC) reaction to perform solid-phase derivatizations of amino acids [4]. The ionized amino acids combine with a hydrophobic cationic surfactant to form neutral ion-paired complexes and are subsequently extracted into the hydrophobic polymer support. The neutral complexes can be derivatized by the immobilized reagents to give the desired derivatives. In this paper, PTC reactions were utilized for the derivatization of amino acids with the 6-AQ tagged polymeric reagent. This polymeric reagent was also applied to protein hydrolysate samples, in order to investigate the practical applications of this derivatization approach.

2. Experimental

2.1. Reagents

Styrene-divinylbenzene copolymer (12% cross-linked, 102 Å templated, 16–20 μm) was obtained from Supelco (Bellefonte, PA, USA). Quinoline-6-carboxylic acid was purchased from ICN Biomedicals (Costa Mesa, CA, USA). Sodium azide was acquired from Eastman Kodak Co. (Rochester, NY, USA). 6-Aminoquinoline, triphosgene, triethylamine (TEA), thionyl chlo-

ride ($SOCl_2$), all amines and cationic surfactant, cetyltrimethyl ammonium bromide (CTAB), were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Amino acids, bovine serum albumin, lyophilized human plasma and sodium tetraborate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ammonium bicarbonate and sodium acetate were obtained from Fisher Chemical Co. (Fair Lawn, NJ, USA). HPLC grade solvents were generously donated by EM Science (Gibbstown, NJ, USA), as their Omnisolv grade.

2.2. Apparatus

The isocratic HPLC system consisted of a Waters Model 590 pump (Waters Chromatography Division, Millipore Corp., Milford, MA, USA), a Rheodyne Model 7125 injection valve with a 20-μl injection loop (Rheodyne, Cotati, CA, USA), a Waters Nova-Pak 4 μm, C_{18} column (150 × 3.9 mm I.D.) or a Supelcosil C_{18} column (150 × 4.6 mm I.D.), a Waters Model 420 fluorescence detector (254 nm/395 nm, ex/em), and a Knauer UV variable-wavelength monitor at 254 nm (Knauer, Berlin, Germany). Data was collected by a Rainin Dynamax data system (Rainin Instrument Co., Woburn, MA, USA) through a Macintosh personal computer (Apple Computer, Cupertino, CA, USA), and a Brown-Boveri SE 120 strip-chart recorder (Brown-Boveri Co., Vienna, Austria).

The gradient HPLC system consisted of two Waters Model 6000A chromatography pumps, a Waters Model 660 solvent programmer, a Waters Nova-Pak 4 μm, C_{18} column (150 × 3.9 mm I.D.) and the same injector, detectors, and data collection system as used for the isocratic HPLC system. Mobile phase A was 140 m*M* sodium acetate with 17 m*M* triethylamine titrated to pH 5.05 with phosphoric acid. Mobile phase B was 60% (v/v) acetonitrile (ACN) in water. Gradient conditions were: initial = 0% B linearly increased to 30 min = 50% B, followed by a wash with 100% B for 5 min and reequilibration for 10 min at 100% A.

2.3. Preparation and characterization of the polymeric 6-AQ reagent

The synthesis of the polymeric benzotriazole intermediate followed a literature procedure [8]. The attachment of the analytical label 6-AQ to the polymeric intermediate involved the synthesis of the acyl azide of 6-AQ [9] (Fig. 2). Quinoline-6-carboxylic acid (1.6 g, 9.2 mmol) was reacted with 2.7 ml $SOCl_2$ (36.8 mmol) in 55 ml of dried benzene at 65–75°C for one hour. After the solvent benzene had been rotovaporated, 21 ml of acetic acid was added and the mixture stirred strongly for several minutes. Sodium azide (600 mg, 9.2 mmol) was slowly added at room temperature and stirred for one hour. Then 53 ml of triethylamine (to neutralize acetic acid) and 100 ml of water were added and stirred for 30 min. The solution was vacuum filtered and the solid was washed with 10 × 25 ml of water. The shiny solid obtained (quinoline-6-acyl azide) was dried under vacuum overnight. A

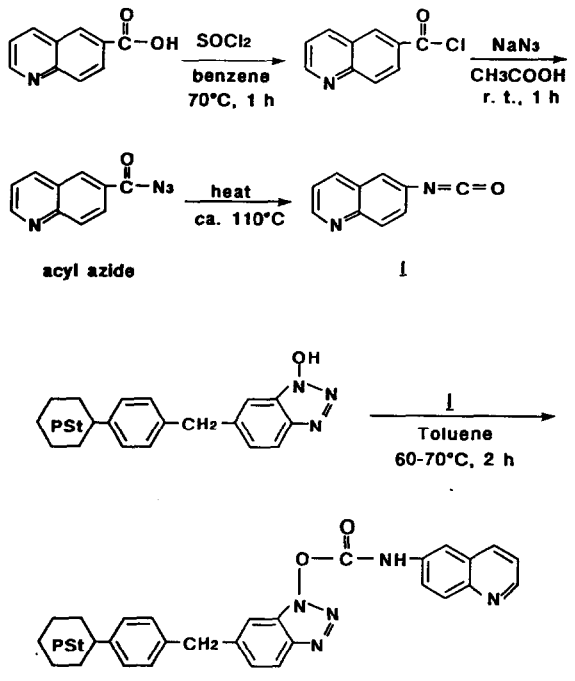


Fig. 2. Immobilization of the analytical tag 6-AQ on the polymeric benzotriazole intermediate.

3.3-g quantity of polymeric benzotriazole intermediate was reacted with 2.0 g of quinoline-6-acyl azide (10 mmol) in 150 ml of CaH_2-dried toluene at ca. 110°C for 30 min. The reaction was continued at 60–65°C for 2 h and at room temperature for 2 h. The polymer was washed over a sintered glass funnel with 600 ml of 40°C dichloromethane (DCM), 50 ml of ACN and dried under vacuum overnight. The weight of the dried polymer was 3.5 g.

The amount of labelling moiety in 1 g of polymeric support was determined by base-promoted hydrolysis of the polymeric reagent. A 6-mg amount of 6-AQ tagged solid-phase reagent was suspended and vigorously stirred in 0.5 ml ACN and 0.5 ml of 0.5 *M* NaOH. The slurry was heated at 75°C for 30 min. Then the solid was filtered and 200 μl of the solution was diluted to 2 ml with 50% (v/v) acetonitrile–water. The released 6-aminoquinoline was determined by HPLC-UV.

2.4. Synthesis and characterization of 6-AQ butylamine standard

The authentic standard derivative of butylamine was synthesized based on modified literature procedures (Fig. 3) [10,11]. A 2.1-g quantity of triphosgene (6 eq) was dissolved in 13 ml of dry THF. A mixture of 0.5 g of 6-aminoquinoline (3.5 mmol, 1 eq) in 15 ml dry THF and 1.0 ml TEA (2 eq) was added dropwise. The reaction was performed at 40–45°C for 2 h. The extra phosgene was absorbed by a 1 *M* NaOH solution. Then 2.4 ml TEA (5 eq) was added at room temperature and stirred for 10 min. Finally 2.1 ml butylamine (6 eq) was added and stirred at room temperature overnight. The product was vacuum evaporated at 45°C for 1 h to remove the solvent THF. A 20-ml volume of 0.1 *M* NaOH and 60 ml of chloroform were added to the solid and stirred strongly. The solid was dissolved in the organic phase. After removing the 0.1 *M* NaOH solution, the organic phase was washed with 10 ml of pure water and vacuum evaporated to remove chloroform. A very sticky, dark red-brown liquid was obtained. Flash chromatography was run on a silica-gel column to purify the product. The solvent used was ACN–acetone–ethyl acetate (3:1:6, v/v/v). After removing the solvents and drying under a high vacuum overnight, a light yellow solid was obtained.

This compound was characterized by both chromatographic and spectroscopic measurements. In the mobile phase of ACN–H_2O (50:50, v/v) with 0.015 *M* SDS and 0.01 *M* buffer (phosphoric acid and sodium dihydrogen phosphate), the compound showed only a single peak both in FL (254 nm/395 nm, ex/em) and UV (254 nm) detection. Elemental analysis for this compound gave the following results: C% = 68.40, H% = 7.30, and N% = 16.88; while theoretical values gave C% = 68.80, H% = 7.40, and N% = 17.20. The experimental data and theoretical values were consistent with each other. IR was run in the form of a KBr pellet. The spectrum is shown in Fig. 4. Fig. 5 gives the NMR-H spectrum of this compound in dimethyl-d_6 sulfoxide (DMSO). The ionization method for MS analysis (Fig. 6) was chemical ionization with NH_3. The melting point of this compound was not measured due to its sticky state.

2.5. Procedures for off-line derivatizations

Both primary and secondary amines were derivatized with the 6-AQ tagged polymeric reagent. The derivatization reaction is shown in Fig. 7. A 50-μl volume of amine solution and 50 μl of the same concentration of triethylamine solution were added to a disposable-pipet reaction cartridge containing *ca.* 15 mg of the polymeric reagent. Derivatization was performed in a 70–75°C water bath for 10 min. After derivatization, the slurry was washed with 1.0 ml pure ACN. A 20-μl volume of the derivatized solution was injected onto the HPLC system.

Off-line derivatization of amino acids was performed under conditions optimized for the polymeric 9-FA reagent [4]. Around 15 mg of 6-AQ tagged polymeric reagent was reacted with 25 μl of analyte solution, 25 μl of saturated sodium borate solution, 25 μl of 20 m*M* CTAB solution, and 12.5 μl pure ACN at 70°C for 10 min. After derivatization, the solid-phase re-

agent was washed with 1.0 ml of 70% ACN–H_2O and 20 μl of the solution was injected onto the HPLC system.

2.6. Analysis of samples spiked with standard solutions

Three different concentrations of single blind L-phenylalanine spiked samples were prepared in plasma. To each spiked plasma, three different amounts of L-phenylalanine solution were added. Off-line derivatizations were performed with these amino acid spiked solutions. Three injections were made for each spiked sample and a four-point calibration plot was constructed for the determination of absolute levels of L-phenylalanine. Measurement was performed with isocratic chromatography; a solution containing 10 m*M* NaH_2PO_4, 10 m*M* H_3PO_4, 10 m*M* SDS, and H_2O–ACN (40:60, v/v) was used as mobile phase. Analysis of a blank unspiked plasma was performed under the same conditions as for spiked plasma samples.

2.7. Acid hydrolysis of proteins

Aliquots of samples containing ca. 50 μg of bovine serum albumin were vacuum dried in 6×50 mm tubes using a Pico Tag workstation. To the tube were added 200 μl of 6 *M* HCl and one crystal of phenol. The tube was sealed under vacuum and heated at 153°C for 1 h [16]. The samples were reconstituted with borate buffer and derivatized with the polymeric reagent. To compare the derivatization results, the samples were also derivatized with a solution reagent according to the literature [5].

3. Results and discussion

The purpose of this work was to develop a new solid-phase fluorescent reagent to derivatize amines and amino acids. This is the first reagent using immobilization of 6-aminoquinoline carbamate (6-AQC) on a polymer support. The successful synthesis of this polymeric reagent demonstrated its feasibility. A unique feature of this polymeric reagent is that there is no serious interference from the hydrolysis products with the fluorescent (FL) detection. In fact, this was the initial reason to choose 6-AQ as the analytical tag on a polymeric support. Another polymeric reagent with a 9-FA label suffered from interferences of its hydrolysis products in the amino acid and peptide determinations [4].

3.1. Characterization of the standard derivative of butylamine

An authentic 6-AQ tagged butylamine standard (empirical formula: $C_{14}H_{18}ON_3$) was synthesized and purified (Fig. 3). All spectral data for this compound, IR, NMR, MS, were consistent with the expected structure. In the IR spectrum, a peak at 1650 cm^{-1} belonged to a typical stretching vibration of an amide carbonyl group, and a peak at 1560 cm^{-1} in the fingerprint region was due to N–H bending (the amide II band) (Fig. 4). In the NMR-H spectrum, the chemical shifts of protons on the quinoline ring fit very well with the expected structure (Fig. 5). The strong peak with a chemical shift of 3.4 ppm originated from the small amount of water in the sample. The MS spectrum showed only one fragment peak in addition to the molecular-ion peak (Fig. 6). This fragment was consistent with the expected structure. This standard derivative of butylamine was suitable as external standard to determine the derivatization efficiency. With a gradient mobile phase, the minimum detectable amount of this compound was found to be 0.4 ng (20 ppb, 20-μl injection) by normalizing the signal-to-noise ratio to 3:1.

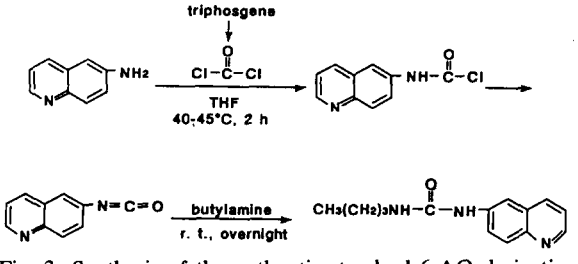


Fig. 3. Synthesis of the authentic standard 6-AQ derivative of butylamine.

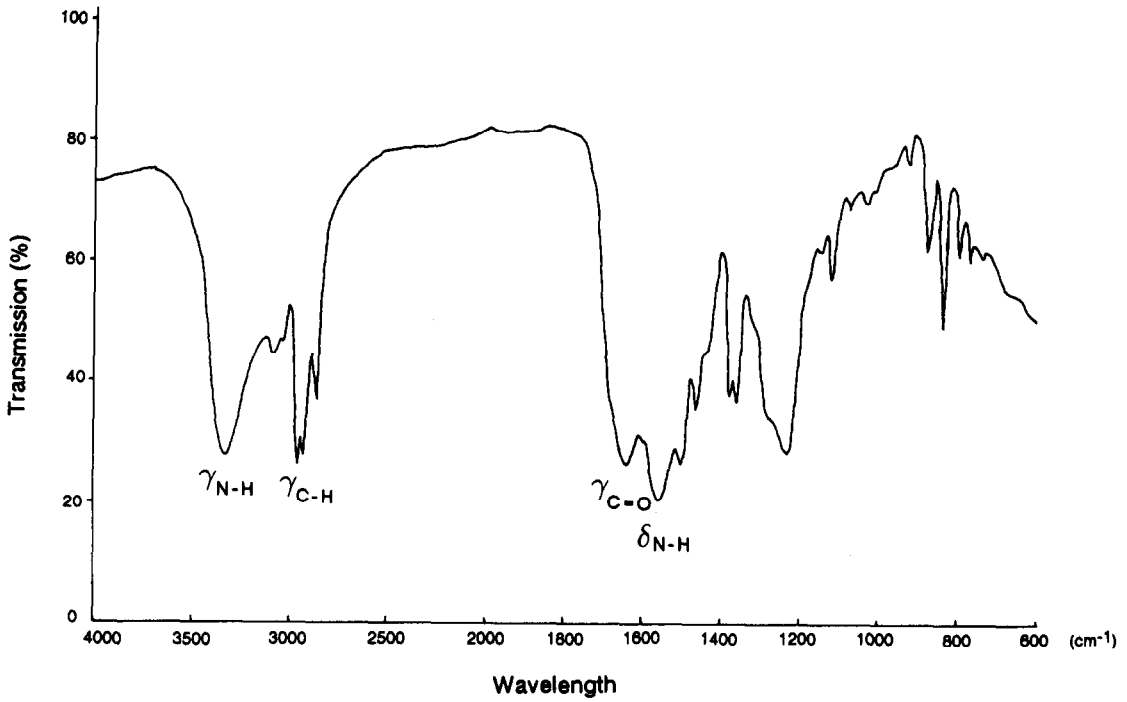


Fig. 4. IR spectrum of the standard derivative of butylamine.

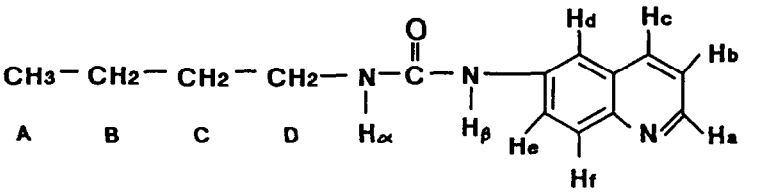
3.2. Characterization of polymeric 6-AQ reagent

Characterization of the polymeric reagent was performed by determination of the loading capacity [2]. The loading of the 6-AQ tag on the solid-phase reagent was found to be 0.18 ± 0.01 mmol/g ($n = 3$). This loading capacity was not very high, but enough for the derivatization reactions.

This solid-phase reagent can be stored for a relatively long time without loosing its reactivity. The stability of the polymeric 6-AQ reagent was tested by comparing the reactivity of a freshly prepared reagent to that of a one-month stored reagent. The reagent was stored in a capped glass bottle at room temperature without any special protection. The same concentrations of amino acids were derivatized with the fresh and the stored polymeric reagents under the same conditions. Almost the same derivatization yields were obtained using the same external standard derivative; this showed the good storage stability of the 6-AQ tagged polymeric reagent for at least one month under laboratory conditions.

3.3. Reactivity of different amines with the polymeric 6-AQ reagent

The off-line derivatization of amines with the 6-AQ tagged polymeric reagent was performed under conditions optimized for some other solid-phase reagents [1,2,13] (Fig. 7). The separation characteristics, such as column, mobile phase, and detection conditions were selected according to the literature [5,12,14]. The calibration curve for the standard derivative of butylamine was used to determine the derivatization efficiencies for different amines. Derivatization efficiencies of propylamine, butylamine, hexylamine, heptylamine, and diethylamine were 67.5 (65.2), 65.0 (66.0), 62.4 (64.4), 60.0 (67.0), and 57.6%, respectively (off-line derivatizations were performed twice for each amine except diethylamine). It seemed that the hydrophobicity of the amines did not have a great influence on the



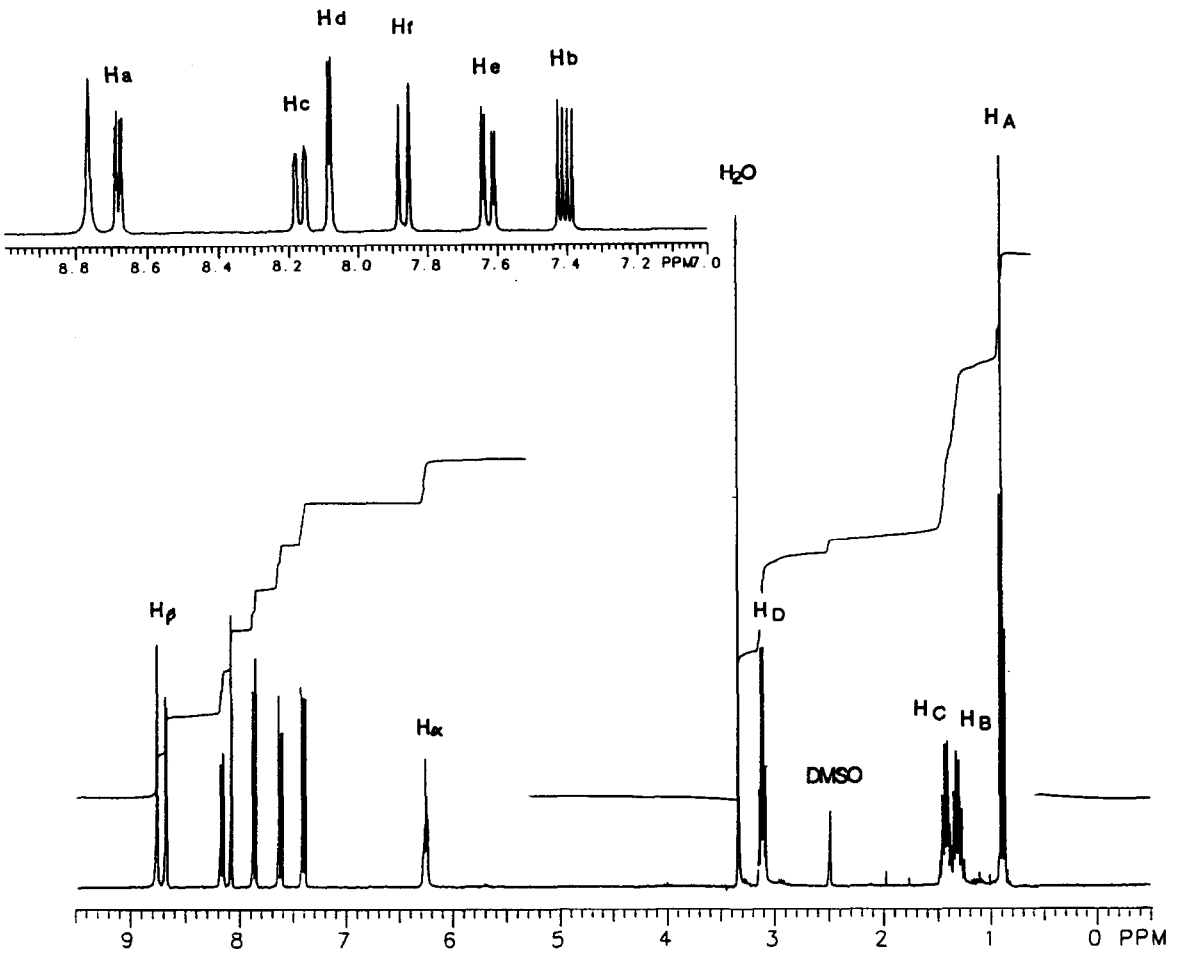
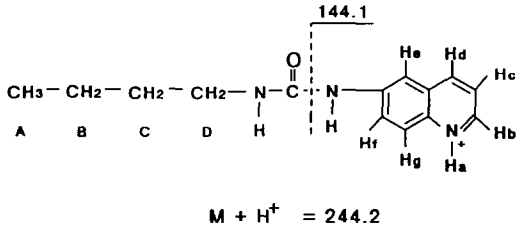


Fig. 5. NMR-H spectrum of the standard derivative of butylamine.

derivatization. Solid-phase derivatization was slightly dependent on the size of the amines. Larger amines showed a slightly lower derivatization efficiency. Also, both primary and secondary amines showed good reactivity with the polymeric reagent. However, morpholine could not be derivatized with the 6-AQ tagged solid-phase reagent. Fig. 8 shows a chromatogram of the off-line derivatization of an amine mixture with the 6-AQ tagged polymeric reagent obtained with FL detection. Compared with UV detection (Fig. 9), the interference peaks from the hydrolysis products, 6-AQ (A) and quinoline-6-diurea (B), did not appear in the



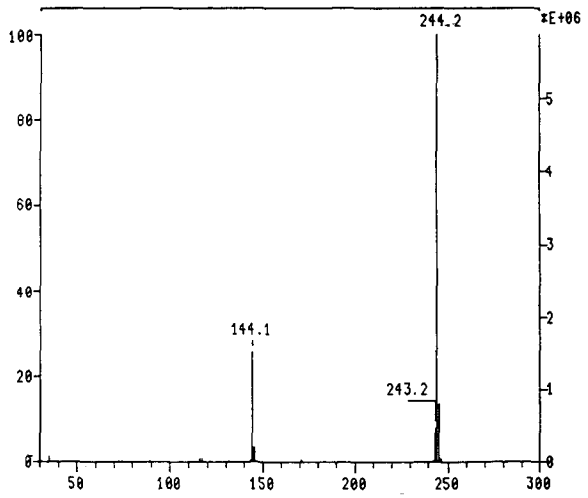


Fig. 6. MS spectrum of the standard derivative of butylamine.

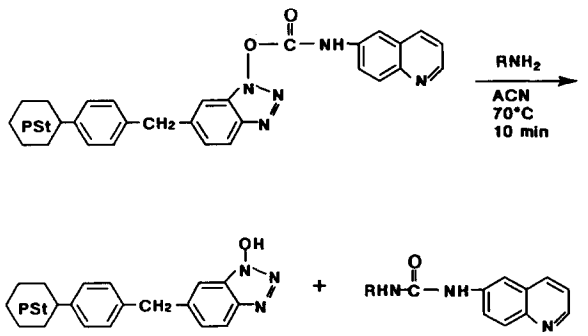


Fig. 7. Derivatization reaction of amines with polymeric 6-AQ reagent.

chromatogram obtained with FL detection. Peak B was assumed to be quinoline-6-diurea; but no characterization experiments were performed.

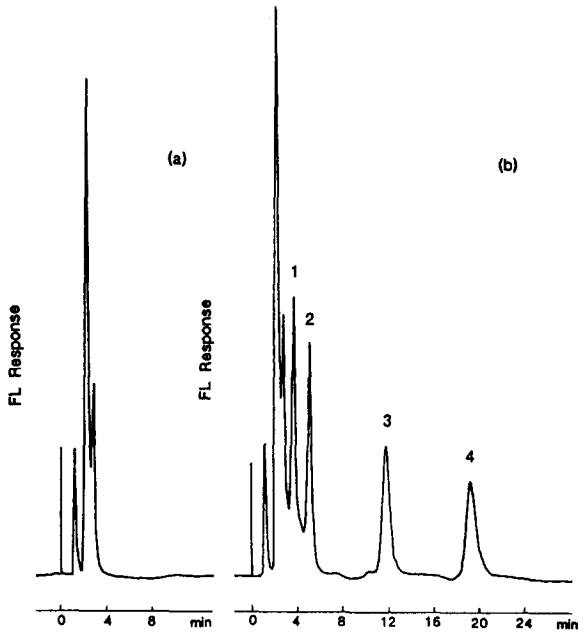


Fig. 8. FL detection of solid-phase derivatization of amine mixture. (a) Blank; (b) 100 ppm amine mixture. Peaks: 1 = propylamine; 2 = butylamine; 3 = hexylamine; 4 = heptylamine. Conditions: column, Supelcosil LC-C18-DB 150 × 4.6 mm I.D.; mobile phase, 50% ACN–H_2O with 0.01 *M* SDS and 0.01 *M* buffer (phosphoric acid and sodium dihydrogen phosphate) at flow-rate 1.5 ml/min; detection, FL. Off-line derivatization conditions: 50 μl of 100 ppm amine mixture and 50 μl of 100 ppm triethylamine were derivatized at 70–75°C for 10 min. After derivatization, the solid-phase reagent was washed with 1.0 ml pure ACN. The blank was obtained by using pure ACN to replace the 100 ppm amine mixture.

3.4. Off-line derivatization of amino acids

In the solid-phase derivatization of amino acids, derivatization efficiency is affected by the actual concentration of the paired amino acid complexes in the polymeric reagent, which depends on the partition coefficient of the paired amino acid complexes. This partition coefficient depends on the hydrophobicity of the amino acids. More hydrophobic amino acids have a stronger ion-pair formation ability, and thus can be more efficiently extracted by the hydrophobic polymeric reagent. The derivatization efficiency would be higher. This has been proven by the

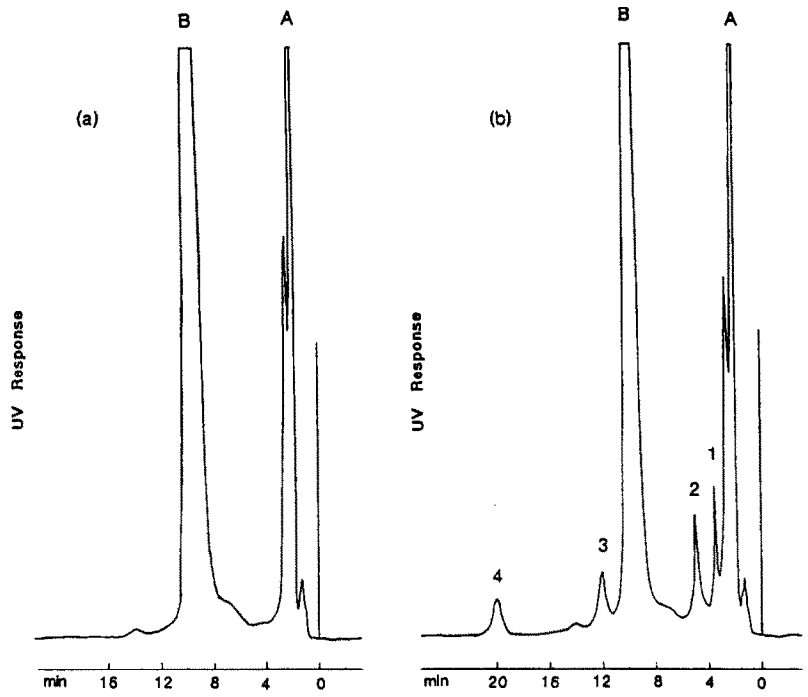


Fig. 9. UV detection of solid-phase derivatization of amine mixture. (a) Blank; (b) 100 ppm amine mixture. Peaks: 1 = propylamine; 2 = butylamine; 3 = hexylamine; 4 = heptylamine. A = 6-AQ; B = quinoline-6-diurea. Separation and off-line derivatization conditions are the same as in Fig. 8.

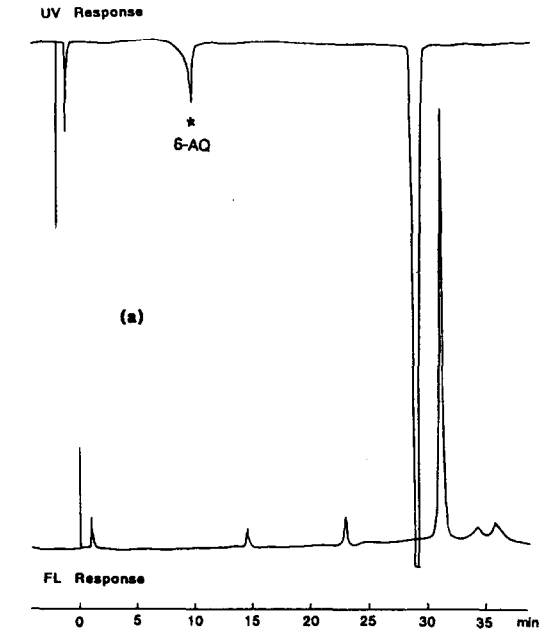
actual derivatization efficiencies of L-methionine, L-isoleucine, and L-phenylalanine, which were $18.7 \pm 2.1\%$, $41.0 \pm 5.2\%$ and $74.6 \pm 1.8\%$ (average number ± standard deviation, $n = 3$ derivatizations), respectively. All these derivatization efficiencies were obtained based on the standard derivative of butylamine, since amine and amino acid derivatives have the same chromophore tag and nearly the same response at UV 254 nm [5]. Figs. 10 a and b show the gradient separation of an amino acid derivative mixture from a solid-phase derivatization with the polymeric 6-AQ reagent. The peaks in the blank test and solid-phase derivatization originated from impurities adsorbed on the polymeric reagent in the immobilization reaction (Fig. 2). Compared with FL detection, UV detection showed more interferences and was less sensitive.

The detection limits in the off-line derivatization of amino acids with the 6-AQ tagged solid-phase reagent were at the ppm level. The minimum derivatizable amount of L-phenylalanine was found to be 20.0 ng (1 ppm), by normalizing the signal-to-noise ratio to 3:1. The detection limits were not very low due to the dilution effect in off-line derivatization approaches.

3.5. Single blind spiked detection

To validate the off-line derivatization of amino acids with the polymeric 6-AQ reagent, three single blind spiked plasma samples were analyzed by the standard additions method (see Experimental). A four-point calibration curve was used to determine the absolute levels of spiked L-phenylalanine ($y = 15.875x + 636.43$; $r^2 = 0.996$). The results are shown in Table 1. The detected amino acid concentrations were in agreement with the spiked levels. The relative error and standard deviation were larger in the low spiked concentration range, since the low

concentration level was close to the detection limit, which would naturally introduce a larger error.



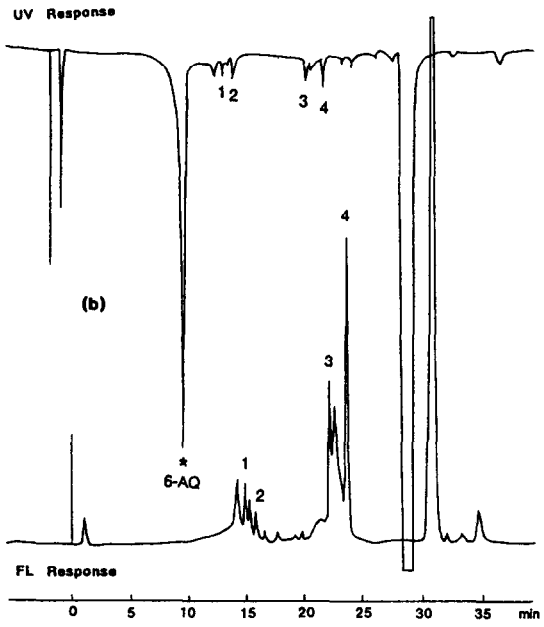


Table 1
Determination of L-phenylalanine spiked plasma

Sample	No. 1	No. 2	No. 3
Spiked (ppm)	16.0	39.0	50.0
Found (ppm)	17.60	40.14	51.07
S.D. ($n = 3$)	1.5	2.4	2.0
%R.S.D.	8.52	5.98	3.92

3.6. Derivatization of protein hydrolysates

The blank test of the polymeric 6-AQ reagent by off-line derivatization was the same as in Fig. 10a. Fig. 11a shows a chromatogram of derivatized bovine serum albumin hydrolysate. Nineteen derivative peaks were obtained with FL detection. To compare the results, the chromatogram of a solution derivatization of the hydrolysates is also shown in Fig. 11b. The ratio of peak areas in the chromatograms were quite different because of the low derivatization efficiencies of the hydrophilic amino acids. However, these qualitative results demonstrated that the 6-AQ tagged solid-phase reagent had derivatized the protein hydrolysate.

Generally, this polymeric 6-AQ reagent showed no serious interferences from its hydrolysis products. High sensitivity can be obtained by FL detection. Compared with the analogous solution-phase derivatization reagent, it has some special advantages, such as long lifetime, good stability, and better selectivity.

Fig. 10. Chromatograms of solid-phase derivatization of amino acid mixture. (a) Blank; (b) 200 ppm amino acid mixture. Peaks: 1 = L-alanine; 2 = L-methionine; 3 = L-isoleucine; 4 = L-phenylalanine. Column: Waters 4 μm Nova-Pak C_{18}, 150 × 3.9 mm I.D. Detection: FL, UV at 254 nm, 0.08 AUFS. Gradient separation in 30 min at flow-rate 1.5 ml/min. Mobile phase A: 140 m*M* sodium acetate with 17 m*M* TEA titrated to pH 5.05 with phosphoric acid. Mobile phase B: 60% ACN–H_2O. Gradient conditions: initial = 0% B linearly increased to 30 min = 50% B. Off-line derivatization conditions: 25 μl of 200 ppm amino acid mixture, 25 μl of saturated sodium borate solution, 25 μl of 20 m*M* CTAB solution and 12.5 μl pure ACN were derivatized at 70°C for 10 min. After derivatization, the solid-phase reagent was washed with 1.0 ml of 70% ACN–H_2O. The blank was obtained by using pure H_2O to replace 200 ppm amino acid mixture.

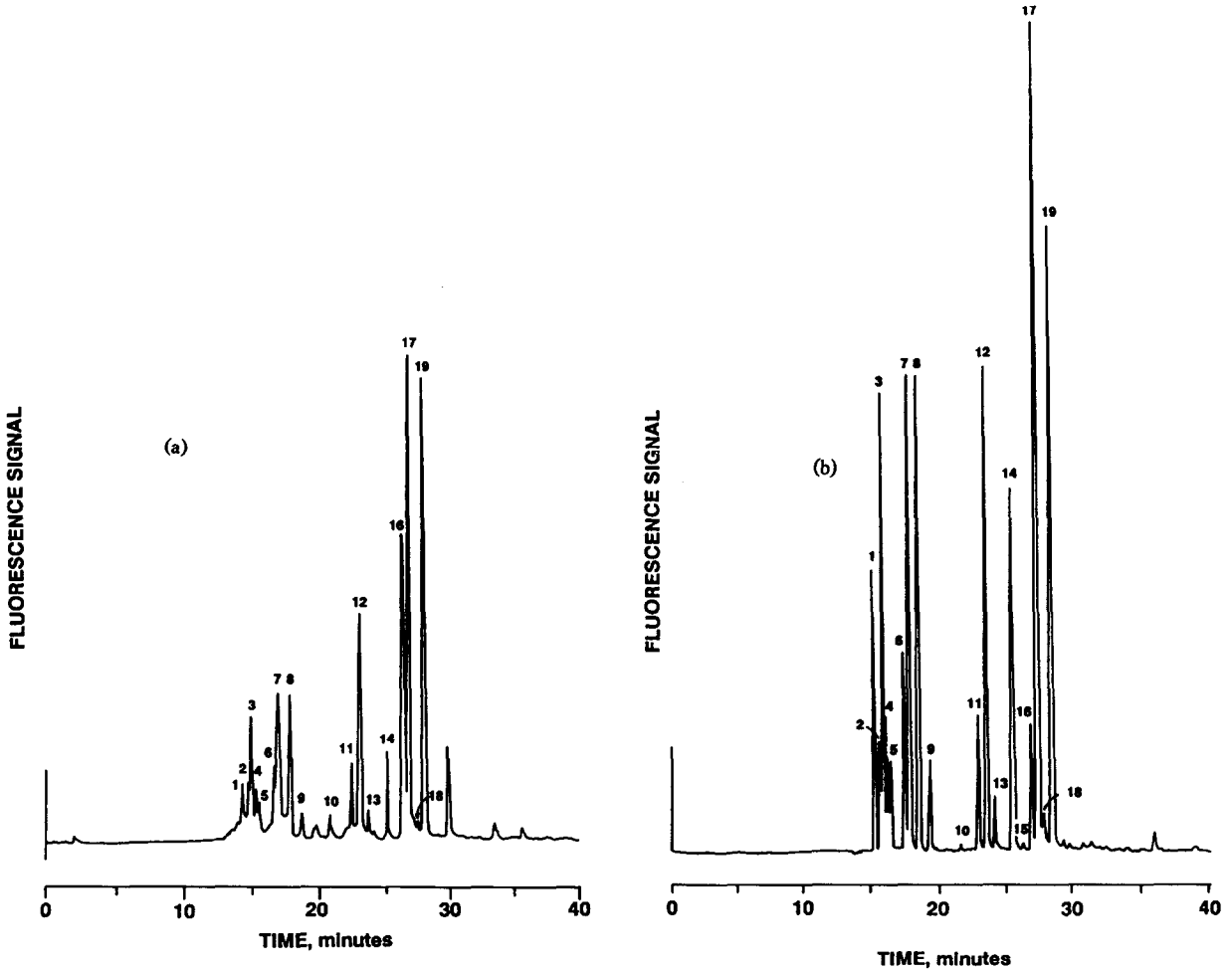


Fig. 11. Solid-phase derivatization of bovine serum albumin hydrolysates. (a) Derivatization with the polymeric reagent; (b) derivatization with solution reagent. Detection: FL 254/395 nm, ex/em. Separation conditions are the same as in Fig. 10.

Thus, it is a very promising derivatization approach for the analysis of amines and amino acids. A major problem with this 6-AQ tagged solid-phase reagent is the strong interferences from impurities in the immobilization reaction. Theoretically, these impurities are physically adsorbed on the polymeric support and can be removed by washing the solid-phase reagent. However, the loading capacity of the reagent will be greatly decreased after washing the reagent with organic solvents. By using a mild washing method, such as supercritical fluid extraction, these impurities may be removed. Another possibility is to change the detection method. Electrochemical detection (ED) is a very sensitive and selective method, which has been commonly used for the analysis of biological samples by HPLC [15]. This is a possible approach to solve the problem of interferences from the impurities on the 6-AQ tagged polymeric reagent.

4. Conclusions

In the present paper we described the syn-

thesis and evaluation of a polymeric 6-aminoquinoline, activated carbamate reagent. This reagent was used for the off-line derivatization of amines and amino acids. The interferences caused by the hydrolysis products of the reagent can be removed by selective FL detection. An authentic external standard derivative of butylamine was synthesized and fully characterized. This derivative was used to determine the derivatization efficiencies of different amines and amino acids. Good derivatization efficiencies can be obtained for amines and typical amino acids under optimized conditions. Low detection limits were obtained. Single blind, amino acid spiked plasma analysis gave satisfactory results. A protein hydrolysate was derivatized with this 6-AQ tagged polymeric reagent, which demonstrated the practical applications of this method. However, the impurities in the immobilization reaction strongly interfered with the detection of some derivatives. This has, thus far, limited the overall utility of this polymeric reagent for amino acid derivatizations.

Acknowledgements

This work was supported by Supelco (Bellefonte, PA, USA). We gratefully acknowledge Waters Chromatography Division, Millipore Corp. (Milford, MA, USA) and EM Science, (Gibbstown, NJ, USA) for their donations of HPLC columns/samples and solvents, respectively. Special thanks are given to Dr. F.X. Zhou for his experimental help. Mr. M.E. Szulc and Mr. R. Mhatre are thanked for helpful discussions. Guodong Li wishes to express his appreciation to the Chinese Academy of Sciences for financial support while a Visiting Scientist at Northeastern University (1993–94). Additional financial support was provided by the Analytical Research Department of Pfizer (Groton, CT, USA).

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