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Comparison of 9-fluorenylmethoxycarbonyl and 9-fluoreneacetyl-tagged silica-based derivatization reagents in high-performance liquid chromatography

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Abstract: Two silica reagents based on a 4-hydroxy-3-nitrobenzoyl backbone were synthesized and characterized with 9-fluorenylmethoxycarbonyl (FMOC) and 9-fluoreneacetyl (FA) tags. These reagents were tested by derivatization of primary and secondary amines. Derivatization conditions such as temperature, time and triethylamine catalyst were tested. The FA-tagged silica reagent showed better performance than the FMOC-tagged silica reagent by a comparison of derivatization efficiencies, stabilities of reagents, and blank reagent interferences with derivatization. Finally, cadaverine and an aliphatic amine mixture were analysed using the FA-tagged reagent by pre-column, off-line derivatization and fluorescence detection.

Keywords: *Derivatization; HPLC; 9-fluorenylmethoxycarbonyl chloride; 9-fluoreneacetic acid; amines; silica gel.*

Introduction

HPLC separations of organic compounds have been greatly developed during the last 20 years. Identification and detection of complex samples can be achieved by HPLC in conjunction with a proper detector system. Common HPLC detectors, such as ultraviolet (UV), fluorescence (FL) and electrochemical (EC) detectors, are specifically sensitive to certain compounds with a broad linearity of response. In order to detect compounds without a chromophore or fluorophore with conventional detectors, derivatization methodology has been used, which provides excellent specificity with increased sensitivity [1, 2]. Pre-column, off-line derivatization is the most widely used approach, because of its flexibility in choosing derivatization reaction types [3]. Using a solid phase reagent as an alternative to solution derivatizations has some advantages, in terms of stability and solvent compatibility. A solid phase reagent consists of the solid support, extended spacer and active tags. Based on silica, alumina or organic polymeric materials, detector sensitive tags covalently bonded to the support possess better shelf-life and derivatization solvent stability. The spacer on the solid matrix extends analyte accessibility to the reactive site and sometimes catalyses derivatizations or modifies the support surface properties [4]. Silica gel has some different properties from organic, synthetic polymers with respect to physical, chemical and chromatographic behaviour, such as: (1) good pressure stability in an HPLC solvent for fast flow, on-line, pre-column derivatization; (2) narrow particle size distribution for narrow band broadening in a solid phase derivatization column; (3) high surface area for high loading capacity of derivatization tags; (4) low cost compared with similar organic polymer particles; (5) well-established chemistry for attachment of various spacer and tagging groups; (6) poor pH stability in aqueous solution, due to dissolution of the silica backbone and instability of the bonded ligand [5, 6].

9-Fluorenylmethoxy-chloroformate (FMOC-Cl) has been widely employed in peptide synthesis and solution phase derivatizations of amines and amino acids [7–10]. It has also been successfully introduced into solid phase derivatization for amine samples [11]. In this paper, both FMOC and fluoreneacetyl (FA) tags were immobilized on a silica-based intermediate. They both contained the fluorenyl moiety and had similar structures, but their derivatization efficiencies showed significant differences under identical reaction conditions for the same substrates. This paper is a

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comparison of FMOC and FA tagged solid phase reagents in derivatization efficiency and stability for primary and secondary amines.

Experimental

Reagents

The bare silica was LiChrosorb® Si 100, particle size 10 μm, donated by E. Merck, GmbH (Darmstadt, Germany). The bis-(2-hydroxyethyl)-3-aminopropyltriethoxysilane reagent was obtained from Petrarch Systems, Inc. (Bristol, PA, USA). Thionyl chloride, 4-hydroxy-3-nitrobenzoic acid, 9-fluoreneacetic acid, 9-fluorenylmethoxycarbonyl chloride, *n*-C_3-C_8 alkyl amines and triethylamine (TEA) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without purification. *N*,*N*′-dimethylformamide (DMF), dichloromethane (CH_2Cl_2) and other HPLC solvents were kindly donated by EM Science, Inc. (Gibbstown, NJ, USA).

Apparatus

The HPLC system consisted of an LDC Constametric® III pump (Milton Roy/LDC Division, Riviera Beach, FL, USA), a Model 7010 injection valve (Rheodyne, Inc., Cotati, CA, USA) and an E. Merck LiChrospher® C_{18}, 5 μm, 4 × 250 mm chromatographic column. HPLC detectors were Waters Model 420-AC fluorescence detector (Waters Chromatography, Division of Millipore Corp., Bedford, MA, USA) and an LDC UV III Monitor.

The instruments used to characterize the authentic standards and intermediates were a PE 599 B infrared spectrophotometer (IR), a PE 650-10S fluorescence spectrophotometer, a PE 3B ultraviolet–visible (UV–vis) spectrophotometer (Perkin–Elmer Co., Analytical Instrument Division, Norwalk, CT, USA), a Varian 300 MHz nuclear magnetic resonance (NMR) spectrometer (Varian Associates, Palo Alto, CA, USA) and a Nuclide magnetic sector mass spectrometer (MS) (Nuclide Co., State College, PA, USA).

Synthesis and characterization

(a) *Silica-based, FMOC- and FA-tagged derivatization reagents.* The intermediates used here to immobilize FMOC and FA tags were the same as those described in the literature [Fig. 1(a)] [6]. Steps 1, 2 and 3 in the preparation of both reagents were identical. For the FA-tagged reagent, 9-fluoreneacetyl chloride was prepared from 9-fluoreneacetic acid before the tagging reaction.

Step 1: 5.0 g of bare silica gel, **I**, 8.4 g of bis-(2-hydroxyethyl)-3-aminopropyltriethoxysilane (62% v/v in ethanol), 40 ml of ethanol and 0.2 ml pyridine were refluxed for 8 h under a nitrogen atmosphere. The product, **II**, was washed with 3 × 75 ml of methanol and 3 × 75 ml of CH_2Cl_2 and dried at 40°C for 8 h in a vacuum oven.

Step 2: 5.1 g of 4-hydroxy-3-nitrobenzoic acid (0.03 mol), 9.0 ml of thionyl chloride (0.1 mol), and 0.7 ml of pyridine were mixed with 30 ml of benzene (dried with Na_2SO_4). The reaction was kept at 55–60°C for 4 h. Unreacted 4-hydroxy-3-nitrobenzoic acid was removed by filtration. Excess thionyl chloride and benzene solvent were removed via rotary evaporation.

Step 3: 4.0 g of **II** and 5.7 g of 4-hydroxy-3-nitrobenzoyl chloride were reacted in 120 ml benzene (dried with Na_2SO_4) with 0.5% (v/v) pyridine. The reaction was held at 70–75°C for 2 h. The solid, **III**, was washed with 3 × 75 ml of DMF, 3 × 75 ml of CH_2Cl_2 and dried under vacuum at 40°C overnight.

Step 4: 1.0 g of **III**, 0.6 g of 9-fluorenylmethoxyl chloroformate (2.3 mmol, FMOC-Cl), 1 ml of pyridine and 25 ml of dichloromethane were stirred for 1 h at room temperature. The silica based FMOC reagent was washed with 3 × 30 ml of DMF, 3 × 30 ml of dichloromethane and dried under vacuum at 40°C overnight. FA-tagged silica was made in the same way by using 9-fluoreneacetyl chloride (Fig. 1b).

(b) *Loading determination.*

1. *Hydrolysis method.* A 15 mg mass of FMOC-tagged or FA-tagged silica reagent was hydrolysed in 4 ml of 40% ACN–0.3 N NaOH at room temperature for 10 min. The hydrolytic mixture was acidified with 0.75 ml of 2 N HCl and diluted with 50% ACN–H_2O before HPLC quantitation. 9-Fluorenemethanol and 9-fluoreneacetic acid were obtained in the hydrolysed solution from the FMOC- and FA-tagged reagents, respectively. Calibration curves of 9-fluorenemethanol and 9-fluoreneacetic acid standards were used for quantitation with HPLC–FL.

2. *Elemental analysis method.* The elemental analysis was performed at Galbraith Laboratories, Inc. (Knoxville, TN, USA). The load-

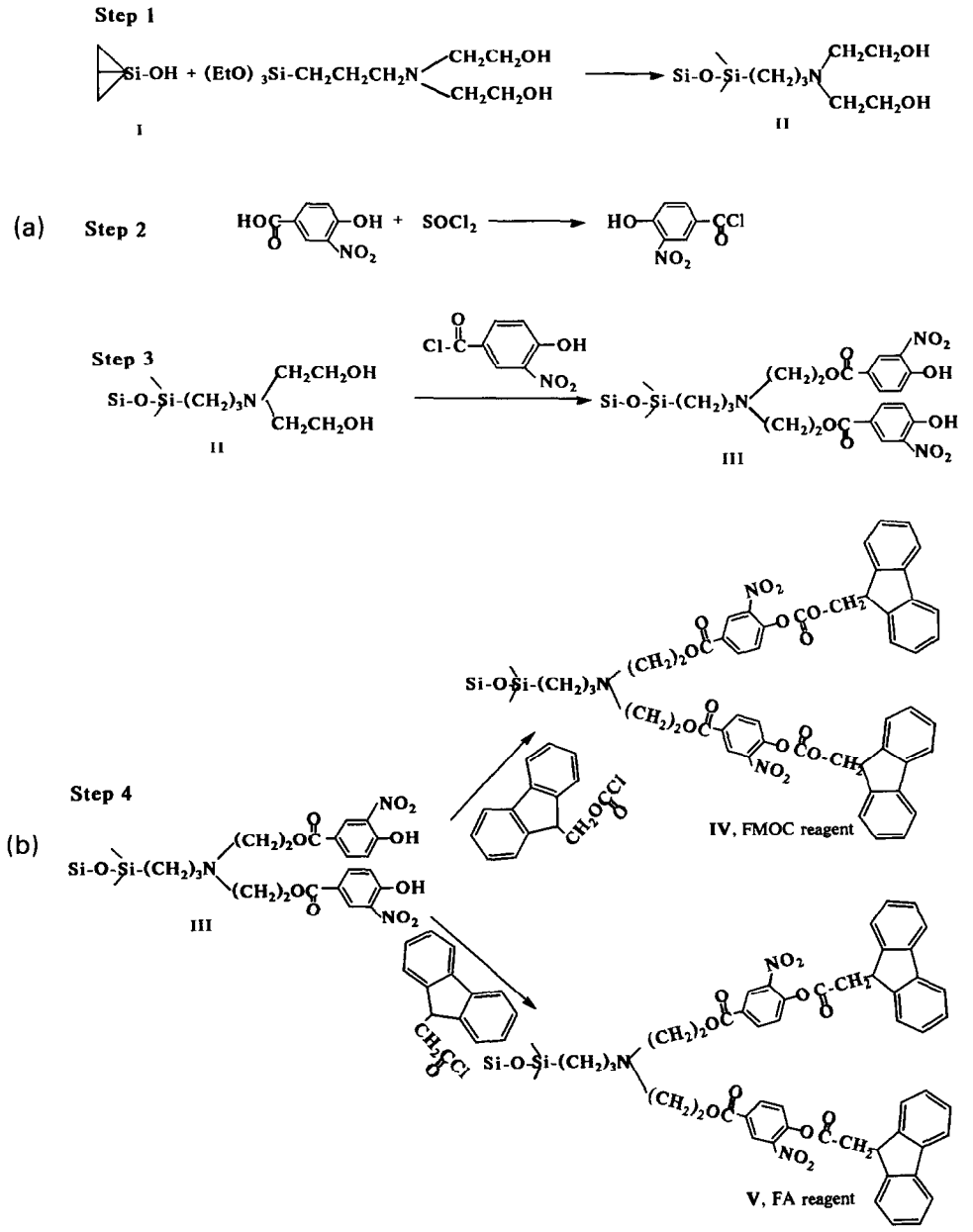


Figure 1
(a) Synthesis of silica intermediate with 4-hydroxy-3-nitrobenzoyl backbone. (b) Synthesis of FMOC- and FA-tagged solid phase reagents.

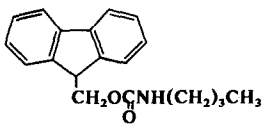
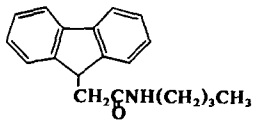
ing capacity was calculated based on carbon content as follows:

$$\frac{X \text{ mg of C}}{100 \text{ mg of reagent}} \times \frac{\text{mmol of C}}{12 \text{ mg}} \times \frac{\text{mmol of ligand}}{n \text{ mmol of C}} \times \frac{1000 \text{ mg}}{g} = \text{mmol ligand}/g,$$

where n is the number of carbon atoms in each ligand formula respectively.

(c) *Synthesis of FA-BA and FMOC-BA standard derivatives.* A 2.0 ml volume of butylamine (20 mmol), 4.8 g 9-fluoreneacetyl chloride (20 mmol) and 10 drops pyridine catalyst were reacted in 25 ml chloroform for 2 h at room temperature. The amide product was extracted with 30 ml ethyl acetate and washed with 30 ml 0.5 N HCl and 30 ml of 0.5 N NaOH, respectively. The organic phase was dried and purified by recrystallization in meth-

Table 1
Physical and spectral properties of FMOC and FA butylamine derivatives

Properties	FMOC-butylamine	FA-butylamine
Structure		
MW	295	279
m.p.	115–116°C	106–108°C
LC–FL response*	5′6″	4′2″
EA†	C 77.94% (77.36%) H 7.14% (7.11%) N 5.00% (5.02%)	C 81.29% (81.72%) H 7.57% (7.53%) N 4.52% (4.74%)
NMR:$CDCl_3$ with 1% TMS		
$-CH_3$	3H (t) 0.9 ppm	$-CH_3$ 3H (t) 0.9 ppm
$-CH_2-$	2H (m) 1.4 ppm	$-CH_2-$ 2H (m) 1.3 ppm
$-CH_2-$	2H (q) 1.5 ppm	$-CH_2-$ 2H (q) 1.4 ppm
$-CH_2N$	2H (q) 3.2 ppm	$-CH_2C{=}O$ 2H (d) 2.6 ppm
$-CH-$	1H (t) 4.2 ppm	$-CH_2N$ 2H (t) 3.2 ppm
$-CH_2-O$	2H (d) 4.4 ppm	$-CH-$ 1H (t) 4.2 ppm
$-NH-$	1H 4.8 ppm broad	$-NH-$ 1H 4.5 ppm broad
Aromatic	8H (m) 7.4 ppm	Aromatic 8H (m) 7.4 ppm
MS	296 (M + 1)	280.1 (M + 1)
Major fragments	235.2, 178, 152.1, 86.1	178, 165, 114.1, 74
IR		
NH stretch	3300 cm^{-1}	NH stretch 3320 cm^{-1}
Aromatic C—H stretch	3050 cm^{-1}	Aromatic C—H stretch 3100 cm^{-1}
Aliphatic C—H stretch	2950 cm^{-1}	Aliphatic C—H stretch 2950 cm^{-1}
C=O stretch	1670 cm^{-1}	C=O stretch 1670 cm^{-1}
N—H bend	1540 cm^{-1}	N—H bend 1570 cm^{-1}
Aliphatic C—H bend	1440 cm^{-1}	Aliphatic C—H bend 1460 cm^{-1}
Amide C—H stretch	1250 cm^{-1}	Amide C—H stretch 1290 cm^{-1}
Ester C—O stretch	1130 cm^{-1}	

*HPLC conditions: 20 μl of 5 ppm of each standard in ACN; mobile phase, 70% ACN–H_2O; column, E. Merck LiChrospher® ODS, 4 × 250 mm; flow rate, 1.5 ml min^{-1}; FL detection at 254/320 nm.
†Elemental analysis, numbers in parentheses are theoretical values.

anol. FMOC-butylamide was prepared using the same procedure. Their structural data are shown in Table 1.

(d) *Off-line derivatization procedure.* The derivatization cartridge was made of a disposable pasteur pipet with one end sealed by Teflon® tape. A 25 μl volume of amine solution was added to 20 mg of the silica derivatization reagent. Derivatization was carried out in a water bath for a specific reaction time. A 1 ml volume ACN was used to wash out the derivative from the solid phase reagent and a 20 μl sample of this solution was injected into the HPLC. A blank test was performed using the same solvent without amine component.

(e) *Optimization and per cent derivatization.* A 10 ppm solution of butylamine in ACN was used in the optimization study in the off-line, pre-column mode. Peak heights of the derivatives were measured as a function of derivatization parameters. Derivatization time and temperature were tested. Per cent derivatization was calculated by a comparison of derivative peak height with the peak height of the authentic amide standards.

Results and Discussion

Synthesis of 4-hydroxy-3-nitrobenzoyl chloride

4-Hydroxy-3-nitrobenzoyl chloride contains phenol and carbonyl chloride functional groups (step 2, Fig. 1) [12]. A self-condensation reaction is possible between them to form a phenol benzoyl polyester or an oligomer product, which will decrease the stability of solid phase reagents and introduce interfer-

ences into the derivatization. Infrared and MS spectra results were used to determine the product structure. Comparing the IR spectrum of 4-hydroxy-3-nitrobenzoyl chloride and 4-hydroxy-3-nitrobenzoic acid, the carbonyl absorbance peak at 1685 cm^{-1} (—COOH) shifted to 1750 cm^{-1} (—COCl) in the product, which indicated a carbonyl chloride group in the molecule. The strong absorption at 3250 cm^{-1} showed that free phenol groups existed in the product. Further confirmation of the monomer structure was carried out with mass spectrometry. Major fragments (*m/z*) were: 201 (M^+), 184 (—OH), 166 (—Cl), 149 (—Cl, OH), 120 (—Cl, NO_2), 92 (—COCl, NO_2). All of the main fragment peaks in MS corresponded to the monomeric 4-hydroxy-3-nitrobenzoyl chloride. There was a dimeric peak at *m/e* 366 (M — HCl) in small abundance. Low reaction temperature, short time and smaller amounts of pyridine catalyst in step 2 resulted in decreased condensation polymerization.

Loading determination

Table 2 lists the elemental analysis results and tag loading capacity of the intermediates and tagged solid phase reagents. Loading capacity for the intermediates and tagged reagents were calculated from elemental analysis, based on the hypothesis that each of the reaction products had an ideal expected ligand formula. From the hydrolysis reaction of the FMOC moiety, the 9-fluorenylmethoxy formic acid was unstable and quickly decomposed to 9-fluorenemethanol and carbon dioxide. 9-Fluoreneacetyl (FA) tag was a modification of the FMOC tag, its hydrolysis product was 9-fluoreneacetic acid. With the standard 9-fluoreneacetic acid (FA acid) and 9-fluorenemethanol (FMeOH), loading capacity of the tagged reagents was quantitated from the hydrolysis. They had similar loading capacities to a 3,5-dinitrobenzoyl-tagged silica and a FMOC-tagged polymeric reagent [6, 11]. This demonstrated that the tagging reaction in step 4, Fig. 1(b) was complete.

Derivatization time optimization

For the FMOC-tagged silica reagent, the appropriate derivatization time for a primary amine was 10 min. Diethylamine derivatization efficiency on FMOC-tagged reagent was obtained based on peak area comparison of FMOC-diethylamide with FMOC-butylamide pure standard, assuming both amides have same fluorescence intensity. For the FA-tagged silica reagent, the derivatization efficiency of butylamine did not change very much within 2–30 min. Diethylamine had the best derivatization efficiency under 10 or 15 min (Fig. 2). Overall derivatization efficiencies for both primary and secondary amines on both FMOC- and FA-tagged silica reagents were lower than those on the organic polymeric FMOC reagent, especially for diethylamine.

Derivatization temperature optimization

The FMOC-tagged silica reagent had an optimum temperature for butyl amine derivatization at 60°C. For the butylamine on FA-tagged silica reagent, the derivatization efficiency approached a plateau at around 80°C. Diethylamine had a constant, low derivatization efficiency from 40 to 80°C on both FMOC- and FA-tagged silica reagents (Fig. 3). One of several possible reasons for their low derivatization efficiency was the SiOH effect on the silica surface, which greatly decreased the nucleophlicity of diethylamine.

Table 2
Elemental analysis (EA) and hydrolysis results for loading capacity determination of FA- and FMOC-tagged reagents

					Loading capacity [mmol g^{-1}]	
Substrate	C%	H%	N%	Ligand formula	(EA)*	(hydrolysis)†
Bare silica gel	0.31	0.64	0.1	SiOH		
Intermediate **II**	6.27	1.55	1.42	$SiC_7H_{16}NO_2$	0.75	
Intermediate **III**	19.39	1.66	3.63	$SiC_{21}H_{22}N_3O_{10}$	0.77	
FMOC reagent **IV**	25.50	1.95	2.99	$SiC_{51}H_{42}N_3O_{14}$	0.42	0.32
FA reagent **V**	27.30	2.18	3.53	$SiC_{51}H_{42}N_3O_{12}$	0.45	0.35

* Loading capacity calculated from C% elemental analysis results.
† Obtained by quantitation of 9-fluorenemethanol and 9-fluoreneacetic acid, respectively.

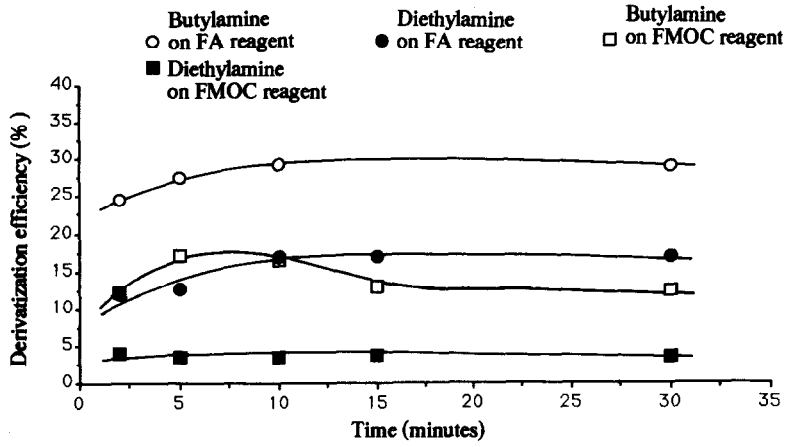


Figure 2
Optimization of derivatization time on FA-tagged reagent and on FMOC-tagged reagent for butylamine and diethylamine. Conditions: 20 μl 100 ppm (μg ml^{-1}) butylamine with 20 mg silica-based reagent; 60°C, elute to 0.5 ml ACN; injection volume, 20 μl; FL detection, 254/313 nm; derivatization efficiency calculated from derivative peak height.

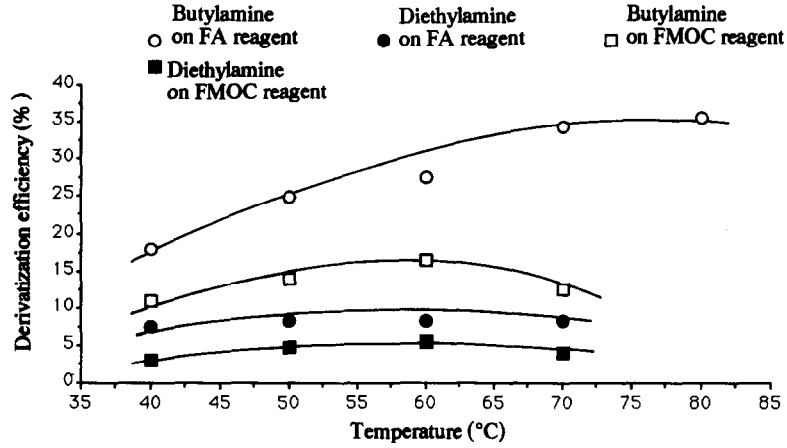


Figure 3
Optimization of derivatization temperature. Conditions as in Fig. 2, derivatization time: 10 min.

Nucleophilic derivatization on FA-tagged silica reagent under triethylamine catalysis

The derivatization efficiency of the FA- and FMOC-tagged silica reagents could be increased by adding a triethylamine (TEA) catalyst. FMOC-tagged silica reagent had a much lower derivatization efficiency than that of the FA-tagged silica reagent even with triethylamine catalysis, which makes the FMOC-tagged silica reagent impractical. Systematic research of triethylamine's effect on FMOC-tagged silica reagent was not performed. In ACN, the optimum molar ratio of TEA to amine was one-to-one for both primary and secondary amines derivatization on FA-tagged silica reagent (Fig. 4). Under these conditions, butylamine and diethylamine derivatization efficiencies were increased to 45 and 15%, as opposed to 23 and 6% without TEA addition. Compared with the FMOC-tagged polymeric reagent (0.32 mmol g^{-1}) [11], the FMOC-tagged silica reagent had a similar tag loading capacity (Table 2), but much lower derivatization efficiency. This is due to the partial silanization of the bare silica gel in step 1, which could not cover all of the active silanol groups on the surface. The $Si—O^-—H^+$ residues on the silica reagent could protonate free amines to decrease their nucleophilic reactivity, especially at low amine concentrations. TEA acted as a base to neutralize $Si—O^-—H^+$ and increase the nucleophilicity of the amine, which resulted in higher derivatization efficiencies.

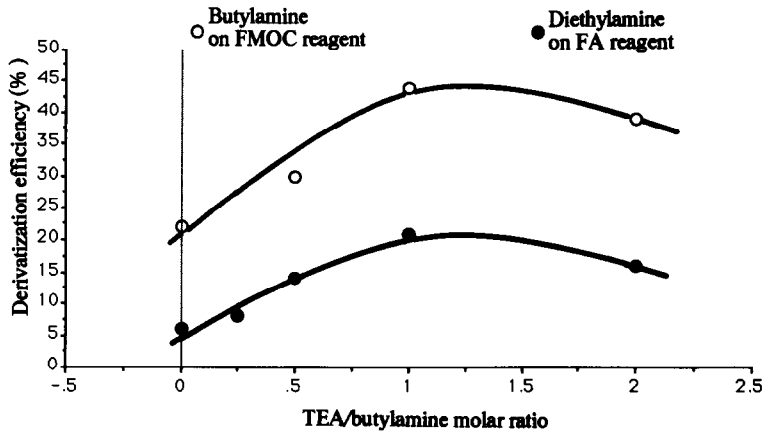


Figure 4
Effect of triethylamine on the nucleophilic derivatization. Conditions: 25 μl sample (100 ppm in ACN) with 20 mg silica-based FA reagent; 5 min at 60°C, elute to 0.5 ml ACN; injection volume, 20 μl; FL detection, 254/313 nm; derivatization efficiency calculated from derivative peak heights.

Storage stability of derivatization reagents

The derivatization reproducibility was tested with butylamine under identical conditions for freshly prepared and stored reagent. The FA-tagged silica reagent had a constant derivatization efficiency after being stored for 6 months at room temperature. There were some unknown peaks in the blank test of the stored FA reagent compared to that of the fresh reagent (Fig. 5). The FMOC-tagged silica reagent was not as stable as the FA reagent under the same storage conditions. Stored FMOC reagent had a much lower derivatization efficiency than the fresh one after 6 months storage (Fig. 6). The storage instability was observed from different batches of the FMOC-tagged silica. These results indicated that the stability of the FMOC-tagged silica reagent was worse than that of the FA-tagged silica reagent. The FMOC-tagged reagent could be used up to 3 weeks after synthesis without much decrease in derivatization efficiency. The structural difference between —O—CO—O— in the FMOC tag and —O—CO— in the FA tag was the reason for their different stability behaviour.

Although there are two ways for nucleophiles to attack the carbonyl position (—O—CO—O—) in the FMOC-tagged reagent, only one of them produces a fluorescent amide derivative. This reaction decreased the FMOC reagent's effectiveness in derivatizations. The reagent's solvolytic degradation by a nucleophilic reaction with H_2O may be the reason for poor derivatization efficiency and instability of the reagent in storage. Derivatization and detection behaviours of the FA-tagged reagent were improved over the FMOC reagent with regard to derivatization efficiency and storage stability. For the FMOC-tagged silica reagent, its derivatization efficiency was around half that of the FA-tagged silica reagent. Due to the advantages of the FA-tagged silica reagent, the following application of solid phase derivatization was performed with it.

Derivatization of primary amines

A 25 μl volume of an aliphatic amine mixture, with 100 ppm each of *n*-propyl, *n*-butyl, *n*-amyl, *n*-hexyl, *n*-heptyl and *n*-octylamine, was derivatized with FA-tagged silica reagent (Fig. 7). The derivative peaks were identified by comparison with derivatization chromatograms from each individual amine component. Chromatographic resolution and detectability of the amines were greatly improved with pre-column derivatization, but this derivatization was not as good as on a polymeric reagent or in solution derivatization. Detection limit of butylamine was 1 ppm (15 ng injected) by off-line, pre-column derivatization on this FA reagent. Compared with 34 ppb (0.42 ng) butylamine derivatization by polymeric FMOC reagent, the silica reagent was not an ideal reagent for determining a low concentration amine sample [13]. Interference of blank peaks is a major reason for poor derivatization sensitivity. The formation of blank peaks from the solid phase reagent was caused by: (a) decomposition of solid phase reagent during storage; and (b) hydrolysis of solid phase reagent during blank test. The catalysis effect of Si—OH on the silica re-

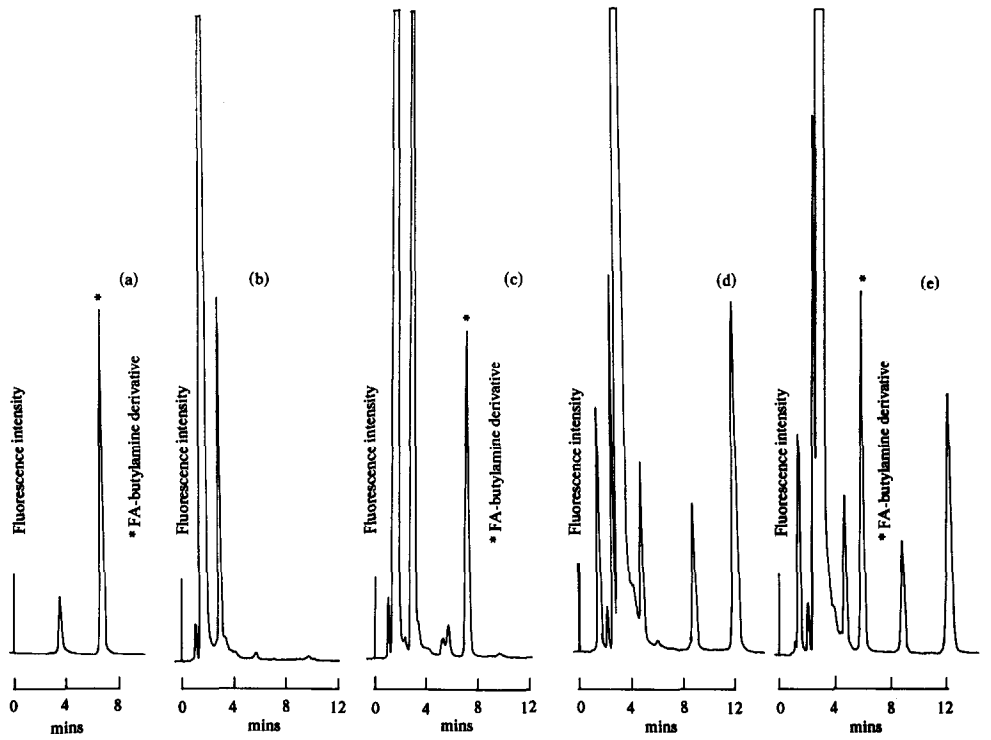


Figure 5
Shelf-life stability of FA-tagged reagent: (a) standard FA-butylamide; (b) blank test of fresh reagent; (c) derivatization of 100 ppm butylamine with fresh reagent; (d) blank test of stored reagent (6 months); (e) derivatization of 100 ppm butylamine with stored reagent (6 months). Conditions: 15 μl sample with 10 mg silica based FA reagent; 10 min at 60°C, elute to 1 ml ACN; injection volume, 20 μl; column, LiChrospher® 5 μm C_{18}, 4 × 250 mm; mobile phase, ACN–H_2O (60:40, v/v) at 1.5 ml min^{-1}; FL detection, 254/313 nm, × 8.

agent's surface increased the intensity of blank peaks. Any factors which could accelerate the decomposition of a reagent will increase the interference of blank peaks, such as: (1) a high pH value of the blank solution; (2) the water content of the aqueous blank solution; (3) high temperature and long time during blank derivatization; and (4) the presence of catalyst of nucleophilic substitution reaction, e.g. triethylamine. By a more complete coverage of free silanol groups with end-capping silanization, the minimum detectable amount of an amine may be decreased. Unlike the polymeric reagents, silica-based reagents were incompatible with on-line derivatization for reversed-phase HPLC. Continuous breakdown of tags was due to the poor water stability of the silica-based reagents in aqueous mobile phases. Differences in chemistry and surface properties between the silica support and polymeric support were the major reasons for the variance in derivatization performance.

Derivatization of a polyamine

Cadaverine is an important polyamine present in physiological fluids, such as plasma and urine. Cadaverine in human urine was derivatized with the FA-tagged silica reagent. A 50 μl volume of a urine sample was derivatized using the same procedure as described (Experimental). The cadaverine peak from the urine sample was identified by retention time comparison with the derivative peak from the standard. Urine spiked with cadaverine, when derivatized, showed an increased derivative peak height in the chromatogram (Fig. 8). This qualitative result showed the existence of cadaverine in the urine sample.

Conclusions

Two silica-based derivatization reagents containing FA and FMOC tags were evaluated. The FA-tagged reagent showed better stability and derivatization efficiency for amines. The carbonyl group (O—CO—O) decomposition in the FMOC-tagged silica resulted in instability of the FMOC reagent. The FA-tagged solid phase reagent was used for derivatization of a primary amine mixture. Cadaverine in urine

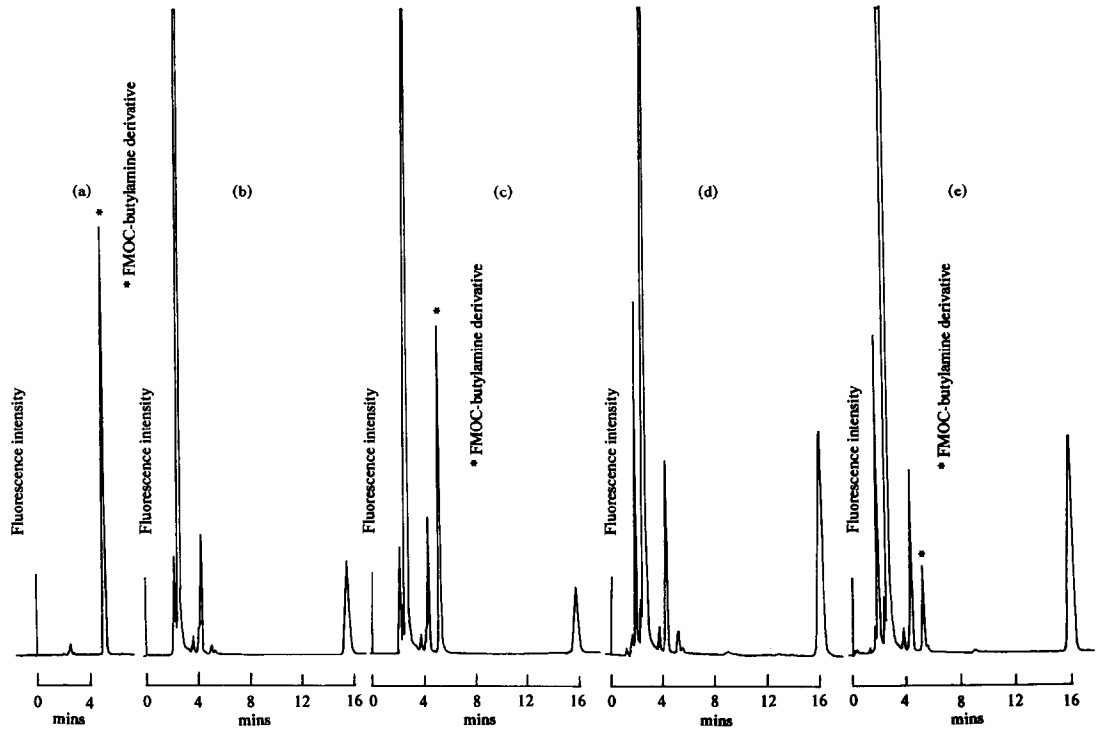


Figure 6
Shelf-life stability of FMOC-tagged reagent: (a) standard FMOC-butylamide; (b) blank test of fresh reagent; (c) derivatization of 100 ppm butylamine with fresh reagent; (d) blank test of stored reagent (6 months); (e) derivatization of 100 ppm butylamine with stored reagent (6 months). Conditions: 15 μl sample with 10 mg silica-based FMOC reagent; 10 min at 60°C, elute to 1 ml ACN; injection volume, 20 μl; column, LiChrospher® 5 μm C_{18}, 4 × 250 mm; mobile phase, ACN–H_2O (70:30, v/v) at 1.5 ml min^{-1}; FL detection, 254/313 nm, × 8.

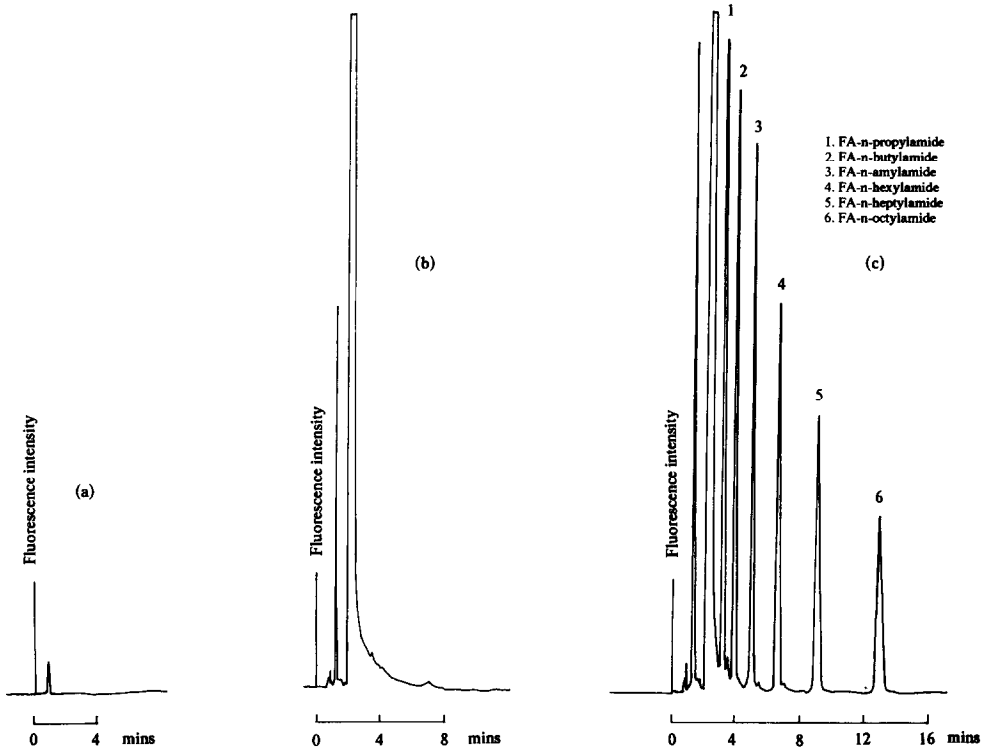


Figure 7
Derivatization of (1) *n*-propylamine, (2) *n*-butylamine, (3) *n*-amylamine, (4) *n*-hexylamine, (5) *n*-heptylamine and (6) *n*-octylamine mixture with FA-tagged silica reagent: (a) amine mixture without derivatization; (b) blank test of silica reagent; (c) amine mixture with derivatization. Conditions: 25 μl (100 ppm each) with 10 mg silica-based FA reagent; 10 min at 60°C, elute to 1 ml ACN; injection volume, 20 μl; column, LiChrospher® 5 μm C_{18}, 4 × 250 mm, ACN–H_2O (70:30, v/v) at 1.5 ml min^{-1}; FL detection, 254/313 nm, × 8.

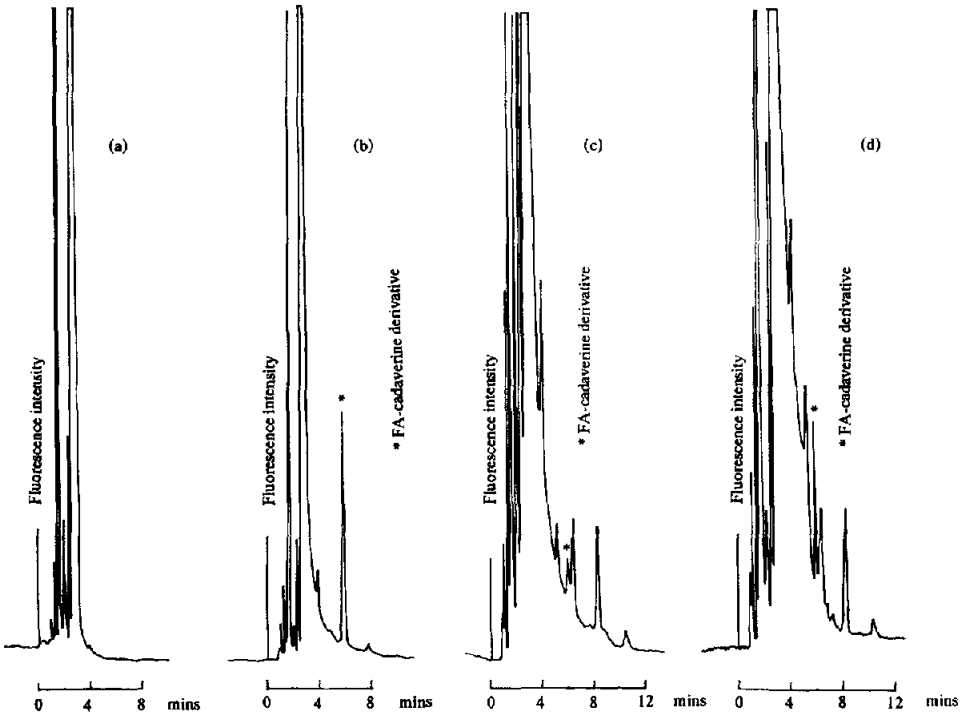


Figure 8
Derivatization of cadaverine in urine: (a) FA reagent blank with ACN, FL × 16; (b) 50 ppm standard cadaverine in ACN, FL × 16; (c) urine sample, FL × 64; (d) spiked urine with 10 ppm cadaverine, FL × 64. Conditions: derivatization, 50 μl sample with 10 mg silica-based FA reagent; 10 min at 60°C, elute to 1.0 ml ACN; injection volume, 20 μl; column, LiChrospher® 5 μm C_{18}, 4 × 250 mm; ACN–H_2O (70:30, v/v) at 1.5 ml min^{-1}; FL detection, 254/313 nm.

was analysed by off-line, pre-column derivatization on the FA-tagged reagent.

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