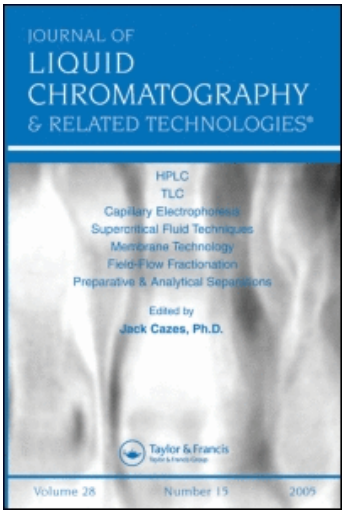

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HPTLC Separation of Homologous and Isomeric Poly(ethyleneamine)s

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HPTLC SEPARATION OF HOMOLOGOUS AND ISOMERIC POLY(ETHYLENEAMINE)S

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ABSTRACT

A simple, reliable HPTLC method has been developed for analysis of poly(ethyleneamine)s (PEAs). Ethylenediamine and all dimeric, trimeric, and tetrameric PEAs are separated without prior derivatization. Treatment with fluorescamine and visualization under ultraviolet light detects each PEA in quantities as low as 100 ng.

INTRODUCTION

Poly(ethyleneamine)s (PEAs) are versatile chemical intermediates with industrial applications in lubricants, corrosion inhibitors, sizing and wet strength agents, epoxy curing agents, fungicides, and surfactants. As a result, PEAs have been the subject of extensive analytical research. While individual PEAs have been detected by titration (1-3), electrochemical (4,5), and optical (6-8) techniques, the most

accurate qualitative and quantitative results have been obtained by chromatography. Gas chromatography (9-14), liquid chromatography (15), electrophoresis (16), paper chromatography (17-20), and conventional thin layer chromatography (TLC) (21-27) have been successfully used for separation of a few selected PEAs. Great difficulty, however, has been encountered in analysis of complex mixtures of either free or derivatized PEAs with a single technique.

Although individual compounds such as ethylenediamine have been examined by conventional TLC, mixtures of homologous PEAs have not been completely separated, and mixtures of isomeric PEAs such as triethylenetetramine (TETA) and tetraethylenepentamine (TEPA) have not been resolved (21-27). In addition to incomplete resolution, the qualitative methods developed for conventional, linear TLC of PEAs are typically limited by severe component tailing, lengthy development times, use of noxious or expensive solvents, and low sensitivity. In this report, we describe a simple, rapid high performance thin layer chromatographic (HPTLC) method for complete separation of the homologous monomeric through tetrameric PEAs, and of all the isomeric TETAs and TEPAs. In addition, the sensitivity of this new method is good. Visualization with fluorescamine permits routine detection of individual components at the 100 ng level.

EXPERIMENTAL

Chemicals

All solvents for sample preparation were obtained from Burdick and Jackson (Muskegon, MI, USA) in chromatographic grade. Reagent grade ethylenediamine (EDA), diethylenetriamine (DETA), piperazine (PIP), N-aminoethylpiperazine (AEP), TETA and TEPA were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Certified ACS grade absolute methanol was supplied by VWR

Scientific (Philadelphia, PA, USA); 28% aqueous ACS certified ammonia was purchased from Fisher Scientific (Springfield, NJ, USA). Fluorescamine was obtained as a 0.2 weight percent solution in acetone from Supelco (Bellefonte, PA, USA); taurine and ACS certified potassium dihydrogen phosphate and sodium hydroxide pellets were obtained from Fisher Scientific. All of the above chemicals were used as received without further purification.

Chromatography Supplies

TLC separations were performed on silica gel HETLC-GHL 10 x 10 cm Uniplates 150 microns thick with inorganic binder and no fluorescent indicator supplied by Analtech (Newark, DE, USA). Standard solutions of PEAs were applied to the plates with disposable 0.5, 1.0, and 2.0 μl microcapillaries from Drummond Scientific (Broomall, PA, USA). Visualization agents were applied with the Camag Unispray Device supplied by Applied Analytical Industries (Wilmington, NC, USA). Visualization was accomplished with the UVS-12 Mineralight obtained from Ultra-Violet Products (San Gabriel, CA, USA).

Methods

Standard solutions (1% V/V) of fluid PEAs were prepared in acetonitrile and were diluted with the same solvent to 0.5 volume %, 0.1 volume %, 0.05 volume %, and 0.01 volume % for analysis. For the solid PIP, a 1% W/V standard solution was prepared, with dilutions to 0.5%, 0.1%, 0.05%, and 0.01%. More concentrated solutions of DETA (6 wt%) in water, methanol, acetonitrile, and chloroform were prepared for evaluation of the influence of solvent on sample spot size.

Prior to use, Analtech HETLC-GHL Uniplates were activated by heating at 70°-80°C for 30 min with subsequent cooling to room temperature for 10-30 min (28).

Solutions of PEAs were applied to the activated plates with the Camag Nanomat. Volumes of 0.5 to 2.0 µl were applied incrementally with the Nanomat in the automatic repetition mode. Spots of decreasing concentration were applied at 30° intervals in a circle 10 mm from the center of the plate. The amount of each polyamine deposited ranged from 100 to 20,000 ng; typical quantities were 100 ng, 200 ng, 500 ng, and integral multiples of these amounts. Two amounts (10,000 ng and 1,000 ng) were duplicated to check for reproducibility, and two tracks (150° and 330°) were blank to provide reference baselines in scanning. After spotting, plates were air dried for one hr at room temperature prior to development.

Circular chromatography was performed with the Camag U-Chamber at 25° ± 1°C. Chromatograms were developed with 750-800 µl of eluant to a distance of approximately 3 cm from the center of the plate. After development, the plates were air dried at room temperature for 24 hrs.

Visualization of PEAs was effected by spraying fluorescamine solution on the plates with the Camag Unispray device. Application was carried out from a distance of 30-40 cm according to D. Waldi's design for uniform spraying (29). With air drying at room temperature, all the PEAs except PIP formed fluorescent derivatives in 3-5 min. PIP, the only PEA with only secondary amine functionality, required application of a second reagent for conversion to a fluorescent derivative. This reagent consists of 0.1 M taurine in 0.1 M sodium phosphate (pH 7.5) (30). After spraying with the buffered taurine solution and fluorescamine, heating at 60°C for 5 min initiated fluorescent development of PIP (30). PEA components and the solvent front were located with a hand-held shortwave UV light. For calculation of hR_f values, distances were measured to the center of the lowest visible concentration for a given component, since this produced the most clearly defined zone.

RESULTS AND DISCUSSION

Solvent Selection for Spotting HPTLC Plates

Minimum spot size in sample application requires a solvent that is volatile and as nonpolar as possible. Based on PEA solubilities and the eluotropic series for silica gel (31), chloroform, water, acetonitrile, and methanol were selected for evaluation. To determine a representative spot size, 1 μl of a solution of DETA (6 wt%) in each solvent was applied to an HETLC plate. The following order of sizes (solvent; average diameter of five spots in mm) was found: acetonitrile (3.0); chloroform (3.1); methanol (3.5); water (4.2). Since acetonitrile provided the smallest spots, it was used for sample preparation.

Selection of Eluant

The mobile phase is a modification of a multicomponent solvent system that was initially developed for separation of simple aliphatic amines (26). In the original work, relatively nonpolar mixtures of chloroform, methanol, and 17% aqueous ammonia were used. Attempted separations of PEAs with these eluants were characterized by low component mobilities (hR_f < 50), and the inability to resolve TETA and TEPA isomers.

To obtain higher component mobilities, more polar eluant systems containing a higher concentration of ammonia were investigated in this study. Specifically, PEA mixtures were separated with varying proportions of methanol and 28% aqueous ammonia. Results are summarized in Table I. At high concentrations of ammonia, PEAs are poorly adsorbed by the silica gel. Consequently, they migrate at or near the solvent front (hR_f close to 100), and resolution is lost. At moderate concentrations of ammonia, PEAs are adsorbed on the silica gel,

TABLE I

PEA Migration In Methanol:Aqueous Ammonia Eluants

Polyamine	[a] hR_f Values in Methanol:28% Aqueous Ammonia [b] 0:100	10:90	25:75	50:50	75:25
Ethylenediamine	71	70	66	60	38
Piperazine	100	65	65	57	26
Diethylenetriamine	68	68	61	52	36
N-(2-aminoethyl)piperazine	82	78	74	63	28
Tris(2-aminoethyl)amine	41	34	24	11	21
Triethylenetetramine	71	67	61	46	34
Bis[N,N'-(2-aminoethyl)]piperazine	100	100	72	63	45
N-[1-(piperazino)ethyl]-ethylenediamine	100	100	84	72	56
N'-(2-aminoethyl)-tris-(2-aminoethyl)amine	52	44	31	16	24
Tetraethylenepentamine	70	66	56	39	30
N"-(2-aminoethyl)-bis[N,N'-(2-aminoethyl)]-piperazine	100	100	81	67	42
N-(2-aminoethyl)-N'-[1-(piperazino)ethyl]-ethylenediamine	100	100	100	79	48

[a] $hR_f = \frac{\text{distance of compound from origin} \times 100}{\text{distance of solvent front from origin}}$

[b] 28% aqueous ammonia used; ratios refer to quantities (V/V) of methanol and aqueous ammonia, respectively.

and resolution improves. However, at low concentrations of ammonia (less than 75:25 V/V methanol:28% ammonia), PEAs are very strongly adsorbed and remain near the origin. An acceptable balance of component mobility and resolution was found by development with a 75:25 V/V mixture of methanol:28% aqueous ammonia.

Visualization

Owing to the high sensitivity of fluorescence measurements (32), PEAs were visualized by treatment with fluorescamine (30,33). Visualization of piperazine, a PEA with only secondary amine functionality, required application of 0.1 M taurine in 0.1 M sodium phosphate buffer (pH 7.5) (30) prior to treatment with fluorescamine. Under short wavelength ultraviolet light, PEAs are revealed as yellow-green spots (emission maximum centered at 475 nm) against a deep purple background. Serial dilutions of each PEA showed that all components are detected with high sensitivity ($\leq$ 100 ng).

Separation of PEAs

Separation of PEAs was evaluated by calculation of hR_f values for each component and isomer. Resolution of EDA, and dimeric, trimeric, and tetrameric PEAs is summarized in Table I. In the 100 to 10,000 ng range, visualized zones for individual components and isomers were symmetrical and compact in the low to middle hR_f region. With these loadings, all PEAs, including TETA and TEPA isomers, were reproducibly (at least five replications) separated by a ΔhR_f of at least $\pm$ 2, the criterion for satisfactory separation of compounds by paper or thin-layer chromatography (34).

The small sample size (0.5 - 1.0 µl) required by this method, in conjunction with use of circular TLC, permits rapid

(15-20 min) analysis of a large number of samples (up to 24 per plate). Visualization is readily performed, and is complete in at most 5 min. Key to the success of this method is the use of HPTLC plates. Owing to their uniform particle size, dense silica packing, and homogeneous plate surface, HPTLC plates reduce band broadening, which leads to better resolution and higher detection sensitivity.

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