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Note

Reversed-phase high-performance liquid chromatographic determination of gadolinium–diethylenetriaminepentaacetic acid complex

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Recent investigations indicate that paramagnetic compounds may be extremely useful in enhancement of contrast in magnetic resonance imaging[1–10]. Of all compounds investigated, gadolinium–diethylenetriaminepentaacetic acid complex (Gd–DTPA) possesses the most suitable characteristiscs of high magnetic moment of the gadolinium atom, high stability (stability constant, 10^{23})[7] and low toxicity[3]. However, one of the major concerns for use in humans is the acute toxicity of the free gadolinium ion which may potentially result from dissociation of the complex *in vivo*[3].

Several investigators have applied ion-pair chromatography in normal- or in reversed-phase mode in analysis of metal complexes[11–16]. Recently, high-performance liquid chromatographic (HPLC) analyses of amine-N-carboxylic acid complexes of Fe(III) and Cu(II) have been reported[11,12,16]. An HPLC method was employed for determination of Gd–DTPA by Weinmann *et al.*[4], but details of the method development have not been reported.

We have developed an HPLC method which can be used in the determination of not only Gd–DTPA, but also free gadolinium ion (Gd^{3+}) and free DTPA ligand. We investigated the effect of various mobile phase variables, such as molarity and pH of the buffer, organic modifier, and ion-pairing agents on the capacity factor of Gd–DTPA, DTPA and Gd^{3+}, which led to improved separation and peak shapes. A UV detector operated at 200 nm was used in detection of Gd–DTPA and DTPA. In order to facilitate the detection of Gd^{3+} and Gd–DTPA, radioactive ^{153}Gd ($t_{\frac{1}{2}}$ = 241.6 d) was used.

In this paper we report our findings related to the retention characteristics of Gd-DTPA, DTPA and Gd^{3+} on a reversed-phase column with on-line UV and radioactivity detectors.

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EXPERIMENTAL

Equipment

The chromatographic system used in this investigation consisted of an Altex Model 110A pump, an Altex 155-40 variable-wavelength detector operated at 200 nm, and an Omniscribe Series D5000 recorder. The Altex injector was fitted with a 20-μl loop. Radioactivity was detected by using an on-line NaI(Tl) well counter coupled to a Nuclear Data Model 60A analyzer operated in multichannel scaling mode. The column used for the chromatographic analysis was an Alltech C_{18} column (10 μm, 25 cm $\times$ 4.6 mm I.D.). The column was operated at ambient temperature and all injections were 20 μl (full loop). A guard column (2 cm $\times$ 2 mm I.D.) filled with C_{18} Analytichem material (40 μm) was used for protection of the analytical column. Another guard column (5 cm $\times$ 4.7 mm I.D.) filled with silica (40 μm) was placed in the flow stream before the injector to saturate the mobile phase with silica.

Mobile phase

Primarily, the mobile phase consisted of 5 m*M* potassium dihydrogen phosphate–acetonitrile (90:10), with varying amount of ion-pairing agent (octylamine, tetrabutylammonium bromide or tetraethylammonium bromide). The pH of the mobile phase was adjusted to a desired value with phosphoric acid (2 *M*) or potassium hydroxide (2 *M*). The mobile phase was filtered through a 0.5-μm filter and degassed ultrasonically under reduced pressure.

Materials

No-carrier-added ^{153}Gd ($t_{\frac{1}{2}}$ = 241.6 d) was cyclotron-produced by irradiation of a gadolinium metal foil which produces mostly the terbium isotopes (^{153}Tb–^{160}Tb). The Tb radioisotopes are separated from the gadolinium target material by ion exchange. The ^{153}Tb ($t_{\frac{1}{2}}$ = 2.34 d) decays to ^{153}Gd, which is purified and separated from the remaining Tb radioisotopes[17].

Gd–DTPA and ^{153}Gd–DTPA (as disodium or dimethylglumine salts) were prepared according to the literature procedures[3]. All samples were prepared in the mobile phase and adjusted to appropriate concentrations. To facilitate the detection of Gd^{3+} and Gd–DTPA, the samples were spiked with radioactive $^{153}Gd^{3+}$ and ^{153}Gd–DTPA, respectively. For quantitation, stock solutions of Gd–DTPA and DTPA in mobile phase (10 mg/100 ml) were prepared. Appropriate dilutions were made to construct standard curves of peak height versus concentration.

The time required for the first unretained peak was used in the calculations of the capacity factors.

RESULTS AND DISCUSSION

Fig. 1 represents a typical chromatogram of a mixture of Gd–DTPA and DTPA. Gd^{3+} eluted at the solvent front in all chromatographic conditions investigated. The retention time of each analyte was established individually. It was also confirmed with the use of radioactive Gd–DTPA that there was no dissociation of the complex into free Gd^{3+} and DTPA under the chromatographic conditions used. Initially, 5 m*M* phosphate buffer (pH 4.5) was used as the mobile phase without any

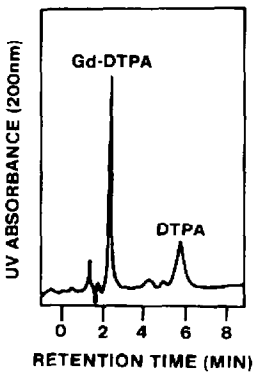

Fig. 1. Simultaneous HPLC analysis of Gd–DTPA and DTPA. Column: Alltech C_{18} (10 μm, 25 cm × 4.6 mm I.D.); mobile phase: potassium dihydrogen phosphate–acetonitrile (85:15) containing 2.5 m*M* octylamine; pH 7.0; flow-rate: 2 ml/min.

added organic modifier. All three compounds were completely unretained and eluted with the solvent front. Upon changing the pH of the buffer to 2–3, there was a slight retention of Gd–DTPA and DTPA. This is expected as ion-suppression at lower pH causes the unionized Gd–DTPA and GTPA to become more hydrophobic and therefore have greater interaction with the lipophilic column bed. Also, Gd–DTPA was retained longer than DTPA, perhaps because the Gd–DTPA has fewer ionizable carboxylic groups than the uncomplexed DTPA.

Upon addition of an ion-pairing agent, *n*-octylamine or tetrabutylammonium bromide, both Gd–DTPA and DTPA were retained. However, Gd^{3+} was not retained under these conditions and eluted at the solvent front. An organic solvent was added to the mobile phase to obtain reasonable capacity factors. Acetonitrile was chosen as the organic modifier because of its lower UV cut-off wavelength.

As generally occurs in reversed-phase chromatography, retention of Gd–DTPA and DTPA on the column decreased with increased percentage of acetonitrile in the mobile phase[18,19]. Similarly, capacity factors of Gd–DTPA and DTPA decreased upon increase in molarity of the phosphate buffer. This is expected since the increased ionic strength reduces the formation of ion pairs[18,19].

Ion pairing occurring only with ion-pairing agents such as *n*-octylamine and tetraalkylammonium bromide. No retention was observed with sulfonic acid ion-pairing agents, indicating that the ion pairing occurs at the carboxylic moieties and not at the amine moieties in Gd–DTPA and DTPA. In the Gd–DTPA complex, metal–ligand bonds are formed and broken constantly, resulting in at least one, if not two, carboxylic moiety which is not complexed with the central Gd atom and therefore available for ion-pair formation[20]. The order of elution of Gd–DTPA and DTPA is reversed as compared to that in the ion-suppression mode in the absence of an ion-pairing agent. This can be explained by consideration of the ion pairing at carboxylic moieties. DTPA ligand, having more carboxylic groups available for ion pairing than Gd–DTPA, is retained longer due to greater ion pairing.

The retention of Gd–DTPA (Fig. 2) increased rapidly upon increase in the concentration of the ion-pairing agent, while maintaining constant pH, and then reached a plateau. This is in accordance with the theoretical considerations of the

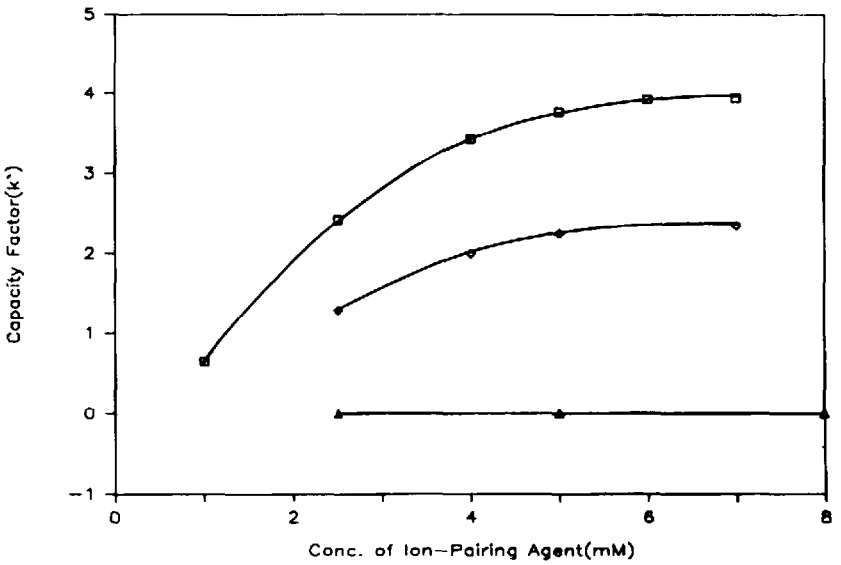

Fig. 2. Effect of concentration of ion-pairing agent in the mobile phase on capacity factor of Gd–DTPA. Mobile phase: 5 m*M* potassium dihydrogen phosphate–acetonitrile (90:10) with a variable amount of ion-pairing agent, pH 7.0. Flow-rate: 2 ml/min. □, Octylamine; ◇, tetrabutylammonium bromide; △, tetraethylammonium bromide.

ion-pair reversed-phase chromatography of weak acids[18,19]. Of interest is that *n*-octylamine and tetrabutylammonium bromide caused retention of Gd–DTPA and DTPA; however, as much as 10 m*M* concentration of tetraethylammonium bromide had no retaining effect. This is perhaps related to lower lipophilicity of tetraethylammonium bromide compared to tetrabutylammonium bromide. The mobile phase containing tetrabutylammonium bromide had UV absorption at 200 nm due to tetrabutylammonium bromide itself and consequently could not be used in conjunction with the UV detector at this wavelength. In this situation, retention time of Gd–DTPA was assessed with the radioactivity detector only. All retention times were corrected for the time-lapse between the two detectors. For routine analysis, the mobile phase containing *n*-octylamine was employed.

Capacity factors of the analytes are greatly affected in ion-pair chromatography by a change in pH of the mobile phase[18,19]. This mode of mobile phase control is often a powerful tool in ion-pair chromatography for regulating the separation selectivity of the analytes of interest. Although weak acids are generally retained longer upon increase in pH of the mobile phase due to the increased ion pairing, retention times of Gd–DTPA and DTPA decreased rapidly upon increasing pH. Fig. 3 shows the effect of pH on capacity factor in the presence of octylamine. Similar behavior was observed when tetrabutylammonium bromide was used as the ion-pairing agent. This seemingly anomalous behaviour has been well documented in literature[21,22].

Relative separation (selectivity, α) between Gd–DTPA and DTPA can be optimized by a combination of pH of the mobile phase and the concentration of the ion-pairing agent. Although the retention times of Gd–DTPA and DTPA decreased with increase in pH, increasing the amount of the ion-pairing agent increased the

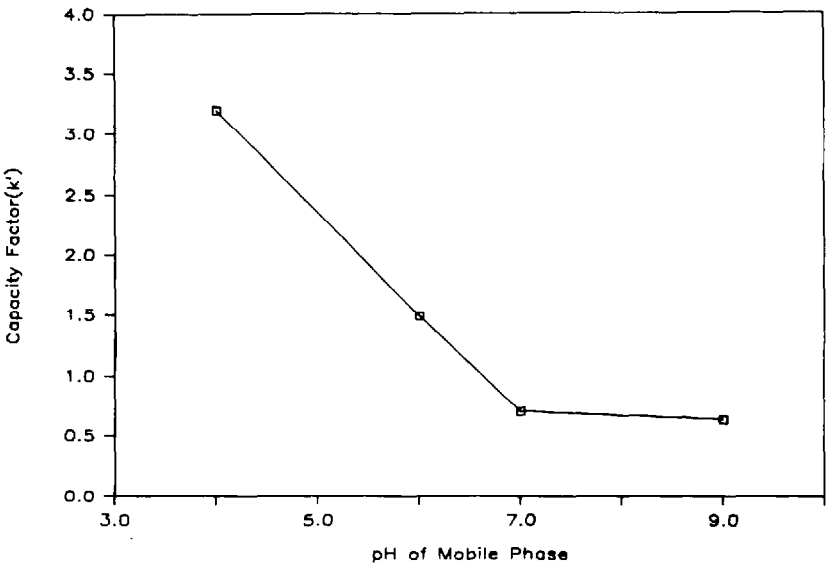

Fig. 3. Effect of pH of the mobile phase on capacity factor of Gd–DTPA in presence of octylamine. Mobile phase: potassium dihydrogen phosphate–acetonitrile (85:15) containing 2.5 m*M* octylamine; pH 7.0; flow-rate: 2 ml/min.

selectivity since DTPA by the virtue of having greater number of free carboxylic moieties is more affected than Gd–DTPA. There is an additional advantage in performing the analysis at higher pH ($\geq$ 7). The peaks were noticeably sharper due to greater and complete ion pairing at higher pH as compared to incomplete ion pairing at lower pH, leading to split or broadened peaks[23]. This is particularly true of DTPA. At lower pH, not only was the retention of DTPA too high, multiple peaks were observed due to different degrees of ionization of the five carboxylic moieties and consequently incomplete ion-pairing.

A linear relation between the peak height and concentration was observed for both Gd–DTPA and DTPA within the range of concentration investigated (100 ng–2 μg). The limit of detection was established at 100 ng (20 μl injection) with a signal-to-noise ratio of four.

CONCLUSION

A reversed-phase ion-pair chromatographic method has been developed for determination of Gd–DTPA, which can be extended to analyze free Gd^{3+} (with radioactivity detector only) and free DTPA which may result from dissociation of Gd–DTPA. It is shown that the regulation of mobile phase variables, such as pH, ion-pairing agent, organic modifier, and ionic strength leads to improved peak shapes and separation selectivity for Gd–DTPA and DTPA. This method may be applicable in quality control of samples containing Gd–DTPA and possible impurities of Gd^{3+} ion and DTPA ligand. Biological samples may be analyzed with the use of radioactive Gd–DTPA in conjunction with a radioactive detector.

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REFERENCES

1 G. Strich, K. Gerber and R. A. Slutsky, *Magn. Reson. Imaging*, 3 (1985) 37.
2 V. M. Runge, J. A. Clanton, A. C. Price, C. J. Wehr, W. A. Herzer, C. L. Partain and A. E. James, *Magn. Reson. Imaging*, 3 (1985) 43.
3 H.-J. Weinmann, R. C. Brasch, W.-R. Press and G. E. Wesby, *Am. J. Roentgenol.*, 142 (1984) 619.
4 H.-J. Weinmann, M. Laniado and W. Mutzel, *Physiol. Chem. Phys. Med. NMR*, 16 (1984) 167.
5 D. H. Carr, J. Brown, G. M. Bydder, H.-J. Weinmann, U. Speck, D. J. Thomas and I. R. Young, *Lancet*, i (1984) 484.
6 D. H. Carr, J. Brown, G. M. Bydder, R. E. Steiner, H.-J. Weinmann, U. Speck, A. S. Hall and I. R. Young, *Am. J. Roentgenol.*, 143 (1984) 215.
7 E. J. Golstein, K. R. Burnett, J. R. Hansell, J. Casaia, J. Dizon, B. Farrar, D. Grelblum and G. L. Wolf, *Physiol. Chem. Phys. Med. NMR*, 16 (1984) 97.
8 M. Laniado, H.-J. Weinmann, W. Schorner, R. Felix and U. Speck, *Physiol. Chem. Phys. Med. NMR*, 16 (1984) 157.
9 D. H. Carr, *Physiol. Chem. Phys. Med. NMR*, 16 (1984) 137.
10 V. M. Runge, J. A. Clanton, C. M. Lukehart, C. L. Partain and A. E. James, Jr., *Am. J. Roentgenol.*, 141 (1983) 1209.
11 A. Yamaguchi, A. Toda, K. Ohzeki and T. Jambera, *Bull. Chem. Soc. Jpn*, 56 (1983) 2949.
12 K. Ohzeki, A. Yamaguchi, A. Toda and T. Kambara, *Bunseki Kagaku*, 32 (1983) E133.
13 S. Igarashi, M. Nakano and T. Yotsuyanagi, *Bunseki Kagaku*, 32 (1983) 67.
14 S. H. Wong, P. Hosain, S. J. Zeichner, L. A. Spitznagle and F. Hosain, *Int. J. Appl. Radiot. Isot.*, 32 (1981) 185.
15 A. R. Timerbaev, O. M. Petrukhin and Y. A. Zolotov, *J. Anal. Chem., USSR (Engl. Transl.)*, 36 (1981) 811.
16 G. A. Perfetti and C. R. Warner, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 1092.
17 T. E. Boothe, R. D. Finn, M. M. Vora, A. M. Emran, P. J. Kothari and S. Wukovnig, in R. R. Muccino (Editor), *Synthesis and Applications of Isotopically Labeled Compounds 1985, Proceedings of the Second International Symposium, Kansas City, MO, U.S.A., 3–6 September 1985*, Elsevier, Amsterdam, 1985, p. 453.
18 L. R. Snyder and J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, p. 453.
19 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
20 E. Brucher, Cs. E. Kukri and R. Kiraly, *Inorg. Chim. Acta*, 95 (1984) 135.
21 R. C. Kong, B. Sachok and S. N. Deming, *J. Chromatogr.*, 199 (1980) 307.
22 R. H. A. Sorsel and A. Hulshoff, *Adv. Chromatogr. (N.Y.)*, 21 (1983) 115.
23 R. F. Adams, in M. T. W. Hearn (Editor), *Ion-Pair Chromatography: Theory and Biological and Pharmaceutical Applications*, Marcel Dekker, New York, 1985, Ch. 4, p. 160.