Rapid Miniaturized Chromatography Procedures for Iodinated Monoclonal Antibodies: Comparison to Gel Exclusion Chromatography

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Chromatographic quality control testing of radioiodinated monoclonal antibodies (^{131}I MOAB) is necessary to assess radiochemical purity prior to patient injection. Conventional gel exclusion chromatography column scanning (GCS) is time consuming and not practical. We investigated rapid miniaturized chromatographic procedures for evaluating the radiochemical purity of ^{131}I MOAB. Three systems were evaluated using Gelman ITLC-SG and three solvents: acetone, 85% methanol, and 0.9% NaCl. Radiochemical analysis was performed on $Na^{131}I$ of high radiochemical purity and $Na^{131}I$ containing radiochemical impurities, as well as three ^{131}I MOAB preparations. Five separate measurements were obtained for each preparation and solvent, and the results were compared to GCS. The results demonstrated ITLC-SG and 0.9% NaCl was most accurate in assessing radiochemical purity when compared to GCS. With the ITLC-SG and acetone system, and to a lesser degree, the ITLC-SG and 85% methanol system, no separation between ^{131}I iodate/periodate and ^{131}I MOAB was achieved, resulting in some instances in the overestimation of the radiochemical purity of the ^{131}I MOAB.

With the increasing use of radioiodinated monoclonal antibodies (^{131}I MOAB) for immunodetection and immunotherapy (*1–3*), a rapid and accurate chromatography system is necessary to assess the radiochemical purity of ^{131}I MOAB. Gel exclusion chromatography column scanning (GCS) has been used in our laboratory (*4–6*), but proved time consuming and not practical. This study was initiated to develop a rapid and accurate miniaturized chromatography system that would evaluate levels of "free" ^{131}I in ^{131}I MOAB preparations. The results were compared to conventional GCS to assess the reliability of the respective chromatography system.

MATERIALS AND METHODS

Iodination Procedure

Two different MOABs, of IgG1 and IgG2a isotypes, were evaluated, including 443A6*, directed against lung adenocarcinoma cell lines, and T101†, directed against cutaneous T-cell lymphoma. The MOABs were radioiodinated using the chloramine-T method. In a typical reaction, 500–1000 μCi of high specific ^{131}I sodium iodide‡ was added to 100–200 μg MOAB (I/MOAB ratio = 1). After chloramine-T addition (5–10 μg), the reaction was incubated for 10 min at 0°C. The reaction was then terminated by the addition of 10–20 μg metabisulfite.

Rapid Miniaturized Chromatography

The radiochemical purity of the radioiodinated MOAB and the ^{131}I sodium iodide solutions were assessed using silica gel instant thin layer chromatography (ITLC-SG)§. The silica gels were cut into 1 cm × 8 cm strips with the origin and solvent front pencil-marked 1 cm from their respective ends. A cut line was drawn in the center of the strip. To minimize counting difficulties, the section containing the origin was marked "A," and the upper section containing the solvent front was marked "B." A typical chromatography strip is illustrated in figure 1.

Our laboratory initially evaluated three chromatography systems obtained from the literature, including ITLC-SG in acetone, 85% methanol, and 0.9% NaCl (*6–10*). The migration of two ^{131}I sodium iodide solutions, one of which contained high amounts of iodate/periodate as determined by conventional chromatography (*11*), were initially evaluated on the miniaturized chromatography systems described above. The radioiodine solutions were spotted at the origin, placed in a 10-ml serum vial containing about 1 ml of the respective solvent, and developed until the solvent migrated to the solvent front. The elapsed developing time was approximately 2 min. The strips were removed and then scanned for radioactivity with a 1-mm slit collimated NaI(Tl) detector interfaced to a multichannel analyzer‖.

The radiochemical purity of the ^{131}I MOAB preparations was assessed using ITLC-SG in 85% methanol or 0.9% NaCl. The chromatography strips were spotted and developed as described above. The strips were cut at the cut line and counted

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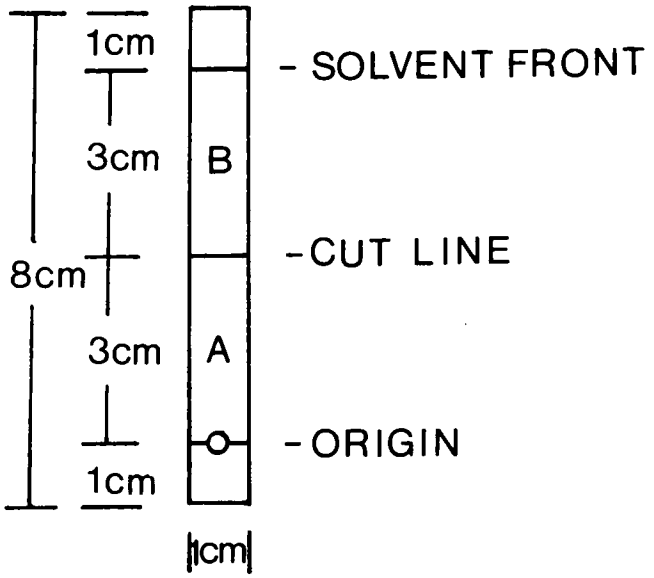

FIG. 1. Example of an ITLC-SG miniaturized chromatography strip used for evaluating ^{131}I-labeled monoclonal antibodies.

for radioactivity using a NaI(Tl) well detector system. The labeling efficiency (percentage) was calculated as follows:

$$\text{Efficiency (\%)} = \left[\frac{\text{(net counts A)}}{\text{(net counts A) + (net counts B)}}\right] \times 100$$

For each ^{131}I MOAB preparation and chromatography system, five separate measurements were obtained. The data were statistically summarized by calculating means and standard deviations for each preparation (Table 1).

Gel Exclusion Chromatography Column Scanning

A small volume (20 μl) of the radioiodinated MOAB was applied to a glass column (2 cm × 20 cm) filled with a gel slurry of Biogel P-10, 50–100 mesh**. The column was eluted with 0.1% human serum albumin (HSA) phosphate buffered saline (pH 7.2). After removing the void volume, the column was sealed and scanned for radioactivity as described. Labeling efficiency was measured by calculating the areas under the specific activity curves generated by the scan.

TABLE 1. Chromatographic Evaluation of Radioiodinated Monoclonal Antibodies

^{131}I MOAB	Labeling Efficiency (%)		
	Gel chromatography	ITLC-SG* 85% methanol	ITLC-SG* normal saline
^{131}I 443A6	54.9	52.7 ± 1.8	53.0 ± 1.7
^{131}I T101	74.0	71.5 ± 0.7	73.2 ± 1.1
^{131}I T101	82.1	87.2 ± 1.4	81.7 ± 1.5

*$\bar{x}$ ± Standard deviation (N = 5).

RESULTS

The activity distribution curves of impure ^{131}I sodium iodide preparations using ITLC-SG with acetone, 85% methanol, and 0.9% NaCl are shown in figure 2. For ITLC-SG with acetone, a major amount of activity remained at the origin, whereas significantly less activity remained at the origin using ITLC-SG with 85% methanol. For ITLC-SG with 0.9% NaCl, greater than 99% of the activity migrated with the solvent front. The free iodide in the pure ^{131}I sodium iodide preparations migrated with the solvent front in all systems evaluated. A typical chromatography scan for pure Na^{131}I is shown in figure 3B.

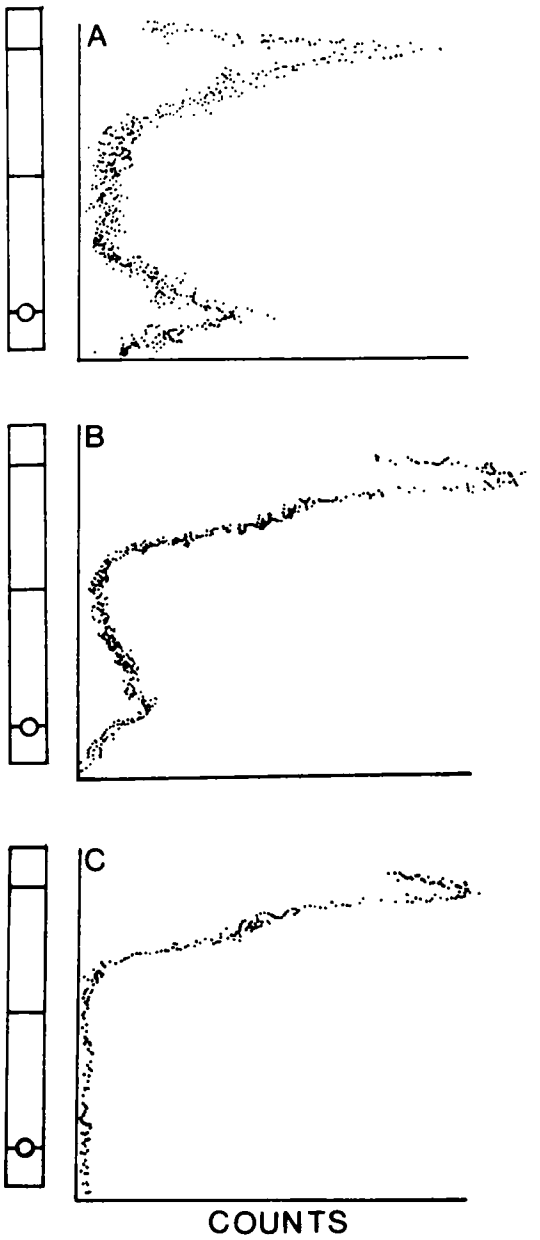

FIG. 2. Activity distribution of impure Na^{131}I preparations on Gelman ITLC-SG with the following solvents: (A) acetone, (B) 85% methanol, (C) 0.9% NaCl. The origin is at the bottom of each plot.

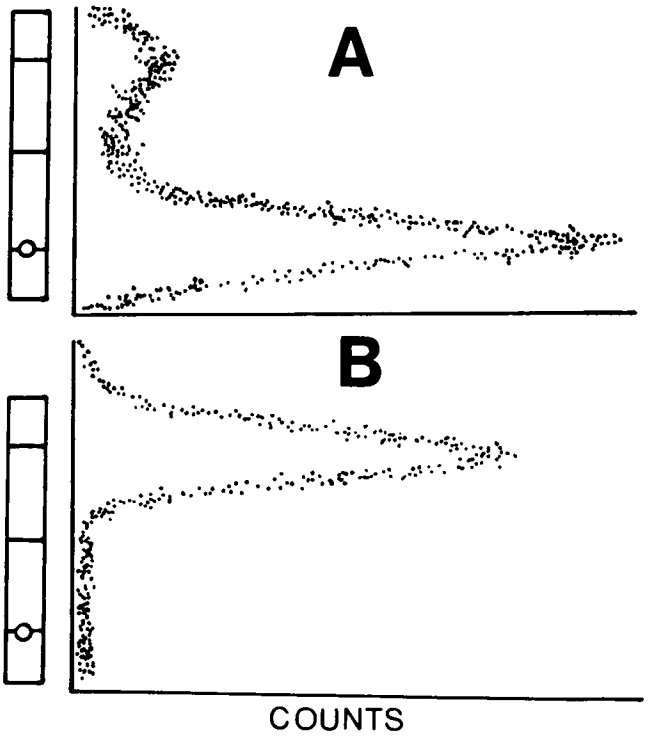

FIG. 3. Activity distribution of ^{131}I monoclonal antibodies (A) and $Na^{131}I$ (B) Gelman ITLC-SG with 0.9% NaCl. The origin is at the bottom of each plot.

For ITLC-SG and acetone, an incomplete migration of radioiodine was observed (Fig. 2A). This could result in an underestimation of the "free" iodine in radioiodinated MOAB, and for this reason, the chromatography system was not further evaluated.

In general, good correlation in labeling efficiencies, with little variability, was observed between the miniaturized chromatography systems and GCS. In one ^{131}I MOAB preparation, however, an overestimation of radiochemical purity was measured using the ITLC-SG 85% and methanol system (87.2% ± 1.4%), when compared to GCS (82.1%).

DISCUSSION

Miniaturized chromatography systems have proven to be reliable, rapid, and accurate in assessing the radiochemical purity of radiopharmaceuticals (*6–10*). Specific miniaturized systems evaluated in this research project have all been utilized in the past to evaluate radiochemical purity (*6–8*). For ITLC-SG and acetone, incomplete resolution of impure $Na^{131}I$ was observed, and this could result in a radiochemical purity overestimation because ^{131}I MOAB does not migrate in the respective chromatography systems evaluated. In fact, we are currently using ITLC-SG with acetone to evaluate the purity of $Na^{131}I$ solutions prior to radioiodination of monoclonal antibodies (*12,13*).

The other two chromatography systems, ITLC-SG 85% with methanol and ITLC-SG with 0.9% NaCl appeared to be accurate in assessing the radiochemical purity of ^{131}I MOAB when compared to GCS. For the ITLC-SG and 85% methanol system, however, incomplete separation of some radioiodine solutions could again result in the overestimation of the radiochemical purity of ^{131}I MOAB (Fig. 2B). The ITLC-SG and 0.9% NaCl appeared to be the most accurate in assessing the radiochemical purity of ^{131}I MOAB preparations. In addition, maximal separation between radioiodinated MOAB and all forms of iodine (iodate, periodate, iodide) was observed, as shown in figure 3.

TABLE 2. Procedure for Determining Percentage Labeling Efficiencies of Radioiodinated Monoclonal Antibodies

1. Place approximately 1 ml of 0.9% NaCl in 10 ml serum vial.
2. Spot about 1 μl ^{131}I MOAB at the origin of the strip.
3. Develop until solvent migrates to solvent front.
4. Cut strip at cut line into sections A and B.
5. Count sections for activity using a gamma counter.

$$\text{Labeling efficiency (\%)} = \left[\frac{\text{(net counts A)}}{\text{(net counts A) + (net counts B)}}\right] \times 100$$

The miniaturized chromatography system described, ITLC-SG and 0.9% NaCl, has been incorporated into a routine quality control procedure to assess the radiochemical purity of ^{131}I MOAB. The quality control procedure, as described in Table 2, is currently used in our laboratory to assess the radiochemical purity of ^{131}I MOAB. The procedure is easy to perform and results in rapid and accurate assessment of ^{131}I MOAB labeling efficiencies.

NOTES

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‡NEN Dupont Products, North Billerica, MA.
§Gelman Instrument Co., Ann Arbor, MI.
¶Nuclear Data Inc., Schaumburg, IL.
**Bio-Rad Laboratories, Richmond, CA.

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