Ascorbic Acid: Useful as a Buffer Agent and Radiolytic Stabilizer for Metalloradiopharmaceuticals

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The goal of this study is to explore the use of ascorbic acid (AA) as a buffer agent and a radiolytic stabilizer for preparation and stabilization of radiolabeled DOTA−biomolecule conjugates. Results from a titration experiment show that 0.1 M AA solution has sufficient buffer capacity at pH 5.0 while 0.5 M AA solution is useful even at pH 6.0. The radiolabeling experiment using TA138, a DOTA-conjugated nonpeptide integrin $\alpha_v\beta_3$ receptor antagonist, clearly demonstrates that AA is a good buffer agent for pH control and an excellent antioxidant for stabilization of metal-labeled diagnostic (^{111}In) and therapeutic (^{90}Y and ^{177}Lu) radiopharmaceuticals if the radiolabeling is performed at pH 5−6. There is no need for the additional stabilizer (e.g., gentisic acid) and buffer agent such as ammonium acetate. The anaerobic AA formulation described in this study is particularly useful for radiolabeling of small biomolecules, which are sensitive to the radiolytic degradation during radiolabeling.

INTRODUCTION

There has been a great current interest in radiolabeled small biomolecules (peptides and nonpeptides) as diagnostic and therapeutic radiopharmaceuticals (*1*−*15*). Radiopharmaceuticals comprising α- or *β*-emitting radionuclides often undergo radiolysis during preparation and storage (*11*). During radiolysis, emissions from the radionuclide attack the metal chelate, targeting biomolecule, and other compounds in proximity, which results in decomposition or destruction of the metal chelate or/and the biomolecule. Since the tumor uptake is largely dependent on the receptor binding of the radiolabeled biomolecule, radiolytic degradation may lead to the decreased therapeutic efficacy and unwanted radiation toxicity to normal organs. Thus, it is important that the radionuclide remains linked to the targeting moiety, and specificity of the targeting biomolecule is preserved.

A radiopharmaceutical composition usually contains a bifunctional chelator-conjugated biomolecule (BFC−BM), a buffer agent for pH control, a weak chelator to prevent metal−colloid formation, and a stabilizer to prevent radiolytic degradation of the radiolabeled BFC−BM conjugate. The pH is critical for the success and reproducibility of the ^{90}Y- or ^{111}In-labeling. When it is used at high concentrations, ammonium acetate can serve as a buffer agent and weak chelator. As a matter of fact, ammonium acetate has been widely used as the buffer agent for the radiolabeling of various DOTA−BM and DTPA−BM conjugates (*16*−*25*). The stabilizer can be added into the reaction mixture before or after radiolabeling. However, the combination of ammonium acetate and stabilizer often results in high osmolarity of the radiopharmaceutical composition. Therefore, there is a need for a new agent, which can serve both as a buffer agent for pH control and as a stabilizer to stabilize the radiolabeled BFC−BM conjugate.

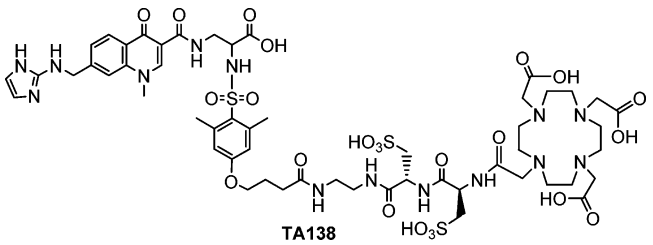

Figure 1. TA138: a DOTA-conjugated vitronectin receptor antagonist.

TA138 (Figure 1) is a DOTA-conjugated nonpeptide integrin $\alpha_v\beta_3$ receptor antagonist that binds with high affinity and specificity to integrin $\alpha_v\beta_3$ receptors overexpressed on endothelial cells of tumor neovasculartur and tumor cells (*26*−*30*). ^{90}Y-TA138 has demonstrated significant therapeutic effects in several preclinical tumor models, including c-neu Oncomouse, HCT116, and HT460 xenografts (*31*). Since ^{90}Y is a pure *β*-emitter, ^{111}In-TA138 was chosen as the imaging surrogate for biodistribution and dosimetry determination. To support the clinical studies, it is necessary to develop a robust formulation for routine preparation of ^{90}Y-TA138 and ^{111}In-TA138.

In our previous contribution (*38*), we reported synthesis of complexes ^{90}Y-TA138 and ^{177}Lu-TA138. Through a series of radiolabeling experiments, we developed an anaerobic formulation for routine preparation of ^{90}Y-TA138 and ^{177}Lu-TA138. It was found that ^{90}Y-TA138 and ^{177}Lu-TA138 are very sensitive to radiolytic degradation, and exclusion of oxygen is necessary during the radiolabeling. Using the anaerobic formulation, ^{90}Y-TA138 and ^{177}Lu-TA138 can be prepared in high yield and high specific activity. We also found that Tris (∼0.1 M, pH = 6.0−8.0) and ascorbic acid (AA: ∼0.1 M, pH = 5.0−7.0) can also be used as buffer agents to replace ammonium acetate in the anaerobic formulation. Since AA is a known

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radiolytic stabilizer and has the buffer capacity at pH 4−6, there is no need for gentisic acid and ammonium acetate in the formulation matrix if the radiolabeling is performed at pH 5−6. That led us to explore the possibility of using AA as a buffer agent for pH control and a radiolytic stabilizer for stabilization of the radiopharmaceutical during or/and after radiolabeling. In this report, we present an anaerobic formulation for routine preparation of radiometal-labeled small biomolecule radiopharmaceuticals. This formulation is particularly useful for small biomolecules sensitive to radiolytic degradation during radiolabeling.

EXPERIMENTAL SECTION

Materials. Ammonium acetate, ascorbic acid (sodium salt), and diethylene-triaminepentaacetic acid (DTPA) were purchased from Sigma/Aldrich Chemical Co. and were used as received. $^{90}YCl_3$ and $^{111}InCl_3$ (in 0.05 N HCl) were purchased from PerkinElmer Life Sciences, N. Billerica, MA. High specific activity $^{177}LuCl_3$ was obtained from University of Missouri Research Reactor, Columbia, MO. Synthesis of TA138, 3-sulfon-*N*-[[4,7,10-tris(carboxymethyl)1,4,7,10-tetraaza-cyclododec-1-yl]acetyl]-L-alanyl-*N*-[2-[4-[[[(1S)-1-carboxy-2[[[1,4-dihydro-7-[(1*H*-imidazol-2-ylamino]methyl]-1-methyl-4-oxo-3-quinolinyl]carbonyl]amino]ethyl]amino]sulfonyl]-3,5-dimethylphenoxy]-1-oxobutyl]amino]ethyl]-3-sulfo-L-alaninamide, has been reported in our previous communication (*25*).

Analytical Methods. The radio-HPLC method used a HP-1100 HPLC system with a UV/visible detector (λ = 230 nm), an IN−US radio-detector, and a Zorbax C_{18} column (4.6 mm × 250 mm, 80 Å pore size). The flow rate was 1 mL/min with a gradient mobile phase starting from 92% solvent A (0.025 M ammonium acetate buffer, pH 6.8) and 8% solvent B (acetonitrile) to 90% solvent A and 10% solvent B at 18 min. The mobile phase was isocratic using 40% of solvent A and 60% solvent B from 19 to 25 min. The isocratic condition was used to make sure that more lipophilic radioimpurities were washed out from the column. The TLC method used the C_{18} reverse phase glass plates and a mobile phase containing methanol, acetone, and saline (2:1:1 = v:v:v). By this method, the radiolabeled DOTA−conjugate migrates to solvent front while unchelated radiometal (metal−colloid and metal−acetate complex) remain at the origin. The corrected radiochemical purity (RCP) was calculated by subtracting the percentage of unchelated radiometal obtained by TLC from that obtained by radio-HPLC.

General Procedure for the Synthesis of ^{90}Y-TA138. To a 5 mL vial containing 50 μg of TA138 was added 0.5 mL of the AA buffer (0.1 or 0.5 M; pH = 5.0−7.0). The solution was degassed under vacuum (<1 mmHg) for ∼2 min. Upon addition of 10−15 μL of $^{90}YCl_3$ (∼10 mCi) in 0.05 N HCl, the reaction mixture was heated at 50 °C or 95 °C for 5 or 35 min. After radiolabeling, a sample of the resulting solution was added to a 2 mL HPLC autosampler vial containing a mixture of 0.5 mL of AA and 0.5 mL of 2 mM DTPA solution and was then analyzed by radio-HPLC and TLC. Each sample was run twice, and the RCP data are presented as an average of two independent measurements.

Synthesis of ^{177}Lu-TA138. To a clean sealed 5 mL vial was added 1.0 mL of 0.1 M AA buffer (pH = 5.0) containing 100 μg of TA138. The solution was degassed

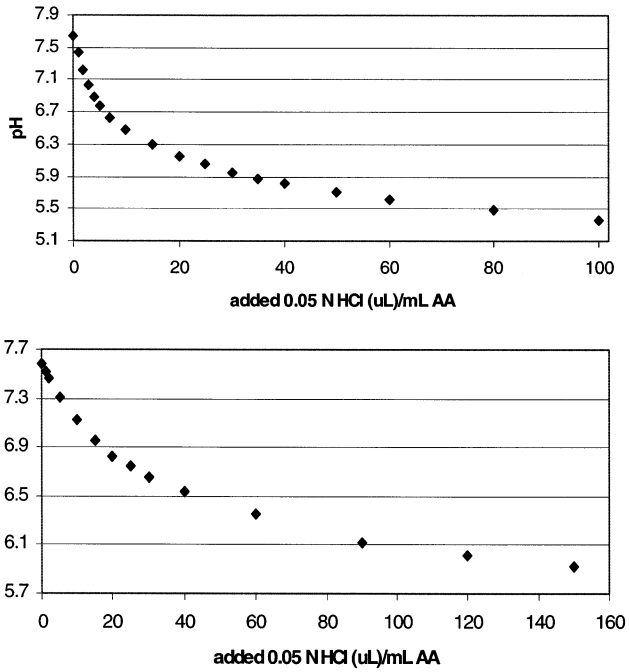

Figure 2. The pH titration curves for 0.1 M (top) and 0.5 M (bottom) AA solutions.

the reaction mixture was heated at 95 °C for 30 min. After being cooled to room temperature, a sample of the resulting solution was diluted 2-fold with 2 mM DTPA solution, analyzed by HPLC and TLC. The mixture was kept at −78 °C for 5 days, and then reanalyzed using the same HPLC and TLC methods.

Synthesis of ^{111}In-TA138. TA138 (40−100 μg) was dissolved in 1.5 mL of AA buffer (0.1 M at pH 5.0 or 0.5 M at pH = 6.0). The solution was immediately degassed under vacuum (<1 mmHg) for ∼2 min. Upon addition of $^{111}InCl_3$ solution (2−2.5 mCi) in 0.05 N HCl, the reaction mixture was heated at 95 °C for 30 min. After being cooled to room temperature, a sample of the resulting solution was analyzed by radio-HPLC and TLC. The resulting mixture was kept at room temperature for 24 h and was then reanalyzed using the same HPLC and TLC methods.

RESULTS

The pH Titration Experiment. In this experiment, sodium ascorbate was used as the starting material to prepare 0.1 M (20 mg/mL) and 0.5 M (100 mg/L) AA solutions. The original pH in both solutions was ∼7.6. Since $^{90}YCl_3$, $^{111}InCl_3$ and $^{177}LuCl_3$ are all dissolved in 0.05 N HCl solution, we used 0.05 N HCl to titrate both AA solutions. Figure 2 shows the titration curves (pH versus added 0.05 M HCl/mL AA) for 0.1 M (top) and 0.5 M (bottom) AA solutions.

Radiolabeling Experiment. This experiment was designed to explore the possibility of using AA as a buffer agent for pH control and as a radiolytic stabilizer for stabilization of ^{90}Y-TA138. We used 100 μg of TA138 for 20 mCi of ^{90}Y to make sure that TA138 was in large excess. We also fixed the ^{90}Y:TA138 ratio (10 mCi/50 μg/mL) to explore other factors influencing the radiolabeling yield of ^{90}Y-TA138. Four factors were considered in the experimental design. These include pH value (5, 6, and 7), heating time (5 and 35 min), AA level (20 and 100 mg/mL), and temperature (50 °C and 95 °C). There were

Table 1. Radiolabeling Results (at ~10 mCi Level)

pH	heating time (min)	temp (°C)	AA level (mg/mL)	average RCP (%) for ^{90}Y-TA138
5.0	35	95	100	96.2 ± 0.8 (n = 2)
5.0	35	95	20	98.5 ± 0.3 (n = 6)
5.0	5	95	100	95.3 ± 1.3 (n = 3)
5.0	5	95	20	98.1 ± 0.4 (n = 6)
6.0	35	95	100	97.8 ± 0.8 (n = 6)
6.0	35	95	20	98.5 ± 0.5 (n = 3)
6.0	5	95	100	97.5 ± 0.7 (n = 2)
6.0	5	95	20	98.7 ± 0.3 (n = 3)
7.0	35	95	100	97.3 ± 0.6 (n = 2)
7.0	35	95	20	98.4 ± 0.3 (n = 3)
7.0	5	95	100	98.0 ± 0.5 (n = 3)
7.0	5	95	20	98.5 ± 0.4 (n = 2)
5.0	5	50	20	17.5 ± 15.3 (n = 3)
5.0	35	50	100	74.4 ± 7.5 (n = 3)
5.0	35	50	20	36.2 ± 9.4 (n = 2)
6.0	5	50	100	38.4 ± 8.6 (n = 3)
7.0	5	50	100	78.5 ± 2.3 (n = 2)
7.0	5	50	20	80.6 ± 3.5 (n = 2)
7.0	35	50	100	91.2 ± 1.5 (n = 3)
7.0	35	50	20	87.1 ± 2.7 (n = 2)

Table 2. RCP Performance of the AA Formulation for ^{90}Y-TA138 (at 100 mCi level)

buffer concn	buffer pH	HPLC RCP (%)	TLC RCP(%)
0.1 M (20 mg/mL)	6.0 (n = 3)	98.4 ± 0.6/T = 0	99.7 ± 0.2/T = 0
0.1 M (20 mg/mL)	6.0 (n = 3)	97.8 ± 0.2/T = 3 d	99.5 ± 0.4/T = 3 d
0.5 M (100 mg/mL)	5.0 (n = 3)	98.9 ± 0.5/T = 0	99.2 ± 0.1/T = 0
0.5 M (100 mg/mL)	5.0 (n = 3)	98.3 ± 0.6/T = 3 d	99.4 ± 0.2/T = 3 d

activity level in each vial was ~10 mCi. The RCP data for the radiolabeled vials are summarized in Table 1.

Solution Stability of ^{90}Y-TA138. In this experiment, we prepared six ^{90}Y-TA138 vials at a 100 mCi level using 0.1 M AA (pH = 5.0: n = 3) or 0.5 M AA (pH = 6.0: n = 3), and studied the solution stability of ^{90}Y-TA138 at −78 °C over 3 days. The TA138 concentration was 100 μg/mL, and the activity concentration was 20 mCi/mL. Radiolabeling was readily accomplished by adding 100 mCi of $^{90}YCl_3$ (in 0.05 N HCl) into the degassed AA solution containing TA138 and heating the reaction mixture at 95 °C for 30 min. After the radiolabeling, a sample of the resulting solution was analyzed by radio-HPLC and TLC. Vials containing ^{90}Y-TA138 were then placed in a lead pig and stored at −78 °C for 3 days. Frozen vials were allowed to thaw for 30−40 min at room temperature. Samples were analyzed by radio-HPLC and TLC. The RCP data at t = 0 and t = 3 days postlabeling are listed in Table 2. As an example, Figure 3 shows the typical radio-HPLC chromatograms of ^{90}Y-TA138 at 0 and 72 h post-labeling.

^{90}Y-Labeling Efficiency of TA138 Using the AA Formulation. We studied the ^{90}Y-labeling efficiency of TA138 by determining the minimal amount of TA138 required to achieve 95% RCP for ^{90}Y-TA138. We prepared ^{90}Y-TA138 using 10, 20, 50, and 100 μg of TA138 for 20 mCi of $^{90}YCl_3$ in 1.0 mL of 0.5 M AA buffer (pH = 6.0). The heating temperature was 95 °C, and the heating time was 30 min. Figure 4 shows the effect of TA138 concentration on the RCP for ^{90}Y-TA138. At pH 6.0, the minimal amount of TA138 required to achieve 95% RCP for ^{90}Y-TA138 is about 20 μg for 20 mCi of $^{90}YCl_3$ corresponding to a TA138:^{90}Y ratio of ~30:1. In all cases, the formation of [^{90}Y]colloid was minimal.

AA Formulation for ^{177}Lu-TA138. We also used the AA formulation to prepare ^{177}Lu-TA138. Radiolabeling was easily accomplished by adding $^{177}LuCl_3$ solution (~20

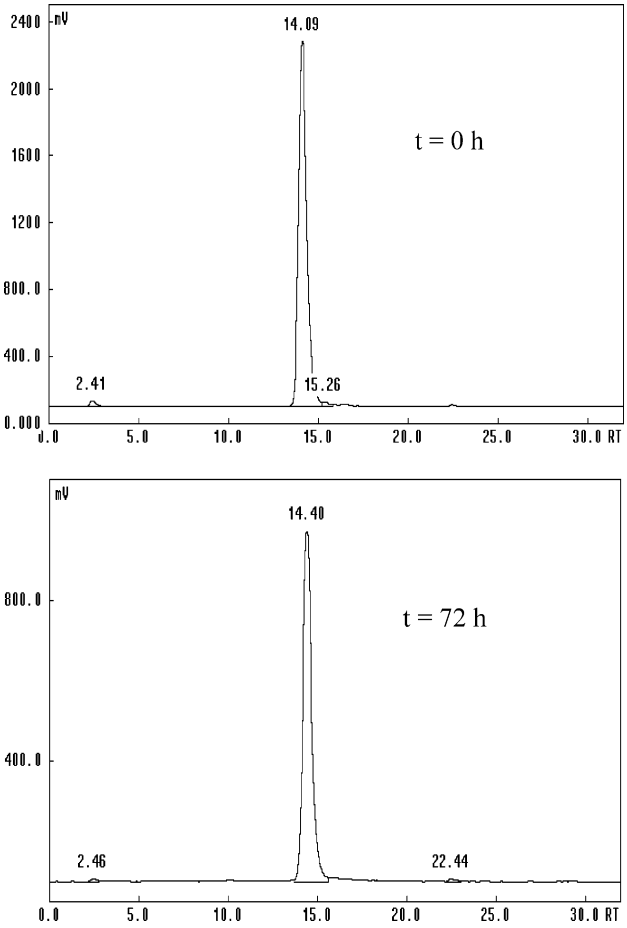

Figure 3. Radio-HPLC chromatograms of ^{90}Y-TA138 at −78 °C.

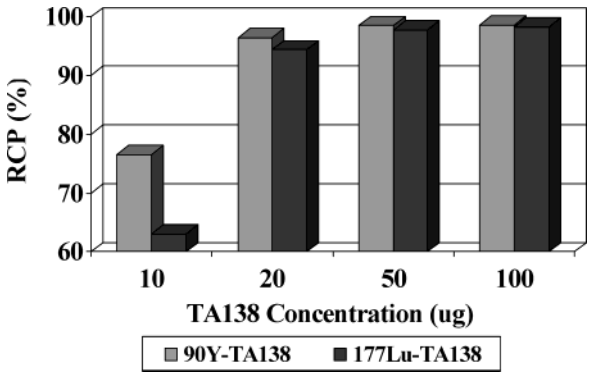

Figure 4. The effect of TA138 concentration on RCP of ^{90}Y-TA138 and ^{177}Lu-TA138.

reaction mixture at 95 °C for 30 min. The RCP for ^{177}Lu-TA138 was >95%. ^{177}Lu-TA138 remains stable at −78 °C for at least 5 days. Under optimized conditions (0.1−0.5 M, pH = 5.0−6.0), the minimal amount of TA138 required to achieve 95% RCP for ^{177}Lu-TA138 is ~20 μg for 20 mCi of $^{177}LuCl_3$ (Figure 4) even though the specific activity of ^{177}Lu is much higher than that of ^{90}Y.

AA Formulation for ^{111}In-TA138. We also carried out a radiolabeling study using the AA formulation for preparation of ^{111}In-TA138. Radiolabeling was accomplished by simply adding $^{111}InCl_3$ (~2.0 mCi) into 1.5 mL of the degassed AA solution (0.5 M at pH = 6.0 or 0.1 M at pH = 5.0) containing TA138 (67 μg/mL) and heating the mixture at 95−100 °C for 30 min. The RCP data for

Table 3. RCP Performance of the AA Formulation for RP748 (at ~ 2.5 MCi Level)

^{111}In source	buffer pH	RCP (%) HPLC	TLC RCP(%)
NEN ($n = 4$)	6.0	97.5 ± 0.4	99.6 ± 0.3
Indiclor ($n = 3$)	6.0	97.2 ± 0.6	99.5 ± 0.3
NEN ($n = 3$)	5.0	98.5 ± 0.2	99.6 ± 0.2
Indiclor ($n = 3$)	5.0	97.2 ± 0.5	99.7 ± 0.2

2.5 mCi of $^{111}InCl_3$. Apparently, the ^{111}In-labeling efficiency was not as high as that for ^{90}Y and ^{177}Lu, probably due to the presence of trace metal contaminants from the $^{111}InCl_3$ source (*11*).

DISCUSSION

A radiolytic stabilizer is a radical scavenging antioxidant, which reacts readily with hydroxyl and superoxide radicals (*32*). In general, the ideal stabilizer possesses the following characteristics: low or no toxicity, no interference with the receptor binding of the radiolabeled compound, and the ability to stabilize the radiopharmaceutical composition for a reasonable period of time during preparation, release, storage, and transportation. AA is a known antioxidant and has been used for stabilization of both diagnostic and therapeutic radiopharmaceuticals (*23*, *24*, *33*−*37*). It has a pK_a of 4.2 with high buffer capacity at pH 3.5−5.5. At higher concentrations (>50 mg/mL or 0.25 M), it may also have sufficient buffer capacity at the pH range 5.5−6.0. Since AA contains two hydroxyl groups, one of which is deprotonable at pH > 4.2, it can also be used as a weak chelator to prevent the formation of radiometal colloid. If AA is used at pH 4.0−6.0, there is no need for extra buffer agent in the formulation matrix. In doing so, it will eliminate the use of ammonium acetate and reduce the osmolarity of the radiopharmaceutical composition.

To demonstrate the utility of AA as a buffer agent, we performed a titration experiment, in which 0.1 M (20 mg/mL) and 0.5 M (100 mg/L) AA solutions were titrated with the 0.05 N HCl. Figure 2 shows the titration curves for 0.1 and 0.5 M AA solutions. For an ideal buffer agent, the pH change should not exceed 0.2 units after addition of 20 μL of 0.05 N HCl solution and during radiolabeling. If one assumes that the activity concentration for $^{90}YCl_3$ and $^{177}LuCl_3$ is about 1.0 mCi/μL, the volume of the $^{90}YCl_3$ or $^{177}LuCl_3$ stock solution for a 20 mCi activity in 1.0 mL of the AA buffer solution is about 20 μL. Obviously, 0.1 M AA solution has sufficient buffer capacity at pH 5.0 while 0.5 M AA solution has the buffer capacity even at pH 6.0.

The radiolabeling experiment was designed to explore the optimal conditions for routine preparation of ^{90}Y-TA138. Since ^{90}Y-TA138 is sensitive to radiolytic degradation, exclusion of oxygen is required during radiolabeling. Results from the radiolabeling experiments show that heating temperature is the most dominant factor. Heating the reaction mixture at 95 °C for 5−35 min is required to achieve high yield radiolabeling (RCP > 95%). It is interesting to note that the pH shows little effect on the RCP at pH = 5.0−7.0 if the heating temperature is 95 °C. At pH < 6.0, lower AA level seems to give a slightly better RCP for ^{90}Y-TA138 while it does not have a significant effect on the RCP of ^{90}Y-TA138 at pH = 7.0. Longer heating time gives slightly better RCP at 95 °C, particularly at pH = 5.0.

It should be noted that the radiolabeling experiment was performed at ~10 mCi level of ^{90}Y. There is often a relatively large variability (5−15%) between vials heated reported for the ^{90}Y-labeling of DOTA-conjugated antibodies (*16*−*20*). Thus, the RCP difference of ~2.0% between different vials may not be significant within the experimental error. Results from the radiolabeling experiment suggest that ^{90}Y-TA138 can be prepared in high yield (RCP > 95%) under the following conditions: 100 μg TA138 for 20 mCi of ^{90}Y in 1 mL of degassed AA buffer solution (0.1−0.5 M at pH 5.0; 0.5 M at pH 6.0), and heating the reaction mixture at 95 °C for 30−35 min.

To further validate the anaerobic AA formulation, we prepared six ^{90}Y-TA138 vials at 100 mCi level using 0.1 M AA (pH = 5.0: $n = 3$) or 0.5 M AA (pH = 6.0: $n = 3$). We studied the solution stability of ^{90}Y-TA138 at −78 °C over 72 h. The radio-HPLC and TLC data clearly demonstrated that ^{90}Y-TA138 can be prepared in high yield (RCP > 95%) using the anaerobic AA formulation (0.1−0.5 M, pH = 5−6), and it remains stable at −78 °C for at least 72 h. Very high specific activity can be achieved for ^{90}Y-TA138. At pH 6.0, the minimal amount of TA138 required to achieve 95% RCP for ^{90}Y-TA138 is ~20 μg for 20 mCi of $^{90}YCl_3$ (Figure 4) corresponding to a TA138: ^{90}Y ratio of ~30:1. The anaerobic AA formulation is also good for routine preparation of ^{177}Lu-TA138 and ^{111}In-TA138. Very high ^{177}Lu-labeling efficiency has been achieved even though the specific activity of ^{177}Lu is much higher than that of $^{90}YCl_3$. The source of $^{111}InCl_3$ has no significant effect on the RCP of ^{111}In-TA138 (Table 3).

CONCLUSION

This study clearly shows that ascorbic acid is a good buffer agent for pH control and an excellent antioxidant for stabilization of metal-labeled diagnostic (^{111}In) and therapeutic (^{90}Y and ^{177}Lu) radiopharmaceuticals. If the radiolabeling is performed at pH 5−6, there is no need for an additional stabilizer or buffer agent in the formulation matrix. The anaerobic AA formulation described in this study is simple and is particularly useful for radiolabeling of small biomolecules sensitive to the radiolytic degradation during radiolabeling.

Acknowledgment is made to Dr. Thomas D. Harris for the synthesis of TA138 (3-sulfon-*N*-[[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]acetyl]-L-alanyl-*N*-[2-[4-[[[(1*S*)-1-carboxy-2-[[[1,4-dihydro-7-[(1*H*-imidazol-2-ylamino]methyl]-1-methyl-4-oxo-3-quinolinyl]carbonyl]amino]ethyl]amino]sulfonyl]-3,5-dimethylphenoxy]-1-oxobutyl]amino]ethyl]-3-sulfo-L-alaninamide).

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