Synthesis of $^{99m}$Tc(V)-DMSA and radiolabelling of SPIO nanoparticles

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Abstract. $^{99m}$Tc(V)-DMSA has been approved to be a successful tumour-imaging agent in the many types of cancer such as medullary thyroid and bone metastases. $^{99m}$Tc(V)-DMSA was successfully formed from labelling meso-2,3-dimercaptosuccinic acid (DMSA) with $^{99m}$Tc which was done under basic conditions. In this experiment superparamagnetic iron oxide nanoparticles (SPIOs) labelling with $^{99m}$Tc(V)-DMSA is being tested. These nanoparticles have been the most developed for biomedical applications due to their high stability and high biocompatibility. The results of this experiment show that combination between PEGylated SPIOs and $^{99m}$Tc(V)-DMSA has a potential impact on increasing the quality of imaging cancers.

1. Introduction

Technetium-99m is a metastable nuclear isomer of technetium-99 which is utilised as a radioactive tracer for medical diagnostic. A particularly convenient chelator for technetium is meso-dimercaptosuccinic acid (DMSA) due to it was reported high renal cortical concentrations of DMSO in human studies [1]. In addition, technetium forms a stable complex $[^{99m}$TcO(DMSA)$_2$] when $^{99m}$TcO$_4^-$ (pertechnetate) is reduced in alkaline solution by dithionite in the presence of DMSA [2]. Pentavalent $^{99m}$Tc-dimercaptosuccinic acid ($^{99m}$Tc-(V)DMSA) is a tumour-targeting radiopharmaceutical for imaging both benign and malignant tumours [3]. Even though the mechanism of $^{99m}$Tc-(V)DMSA accumulation in the tumour remains unknown, it is approved that the tracer uptake can be used for detect bone metastases and tumour characterisation through classification process [3]. ($^{99m}$Tc-(V)DMSA) was synthesised as a metabolic mimic of phosphate which can be localized in cancer cells by hydrolysis process of the pentavalent DMSA complex within cancer cells to yield the phosphate-like ion $^{99m}$TcO$_4$ [3,4].

Due to present kinetically stable radiopharmaceuticals, functional chelate systems are used to link metallic radionuclides to targeting molecules [5]. Besides, the interaction between radiotracers and nanoparticles can increase the probability of binding upon encountering the target receptor for therapy or imaging [6]. Superparamagnetic iron oxide nanoparticles (SPIOs) as an example of inorganic nanoparticles is the most investigated biomedical applications since they have high stability and high biocompatibility [7]. SPIOs can be utilised for several in vivo applications, such as drug delivery, magnetic resonance imaging (MRI) contrast agents, cell tracking and tissue repair [8,9]. Despite these advantages, there are some fundamental challenges to utilise SPIOs in the clinical test. It includes SPIOs uptake by the reticuloendothelial system (RES), in which SPIOs are rapidly circulated to the liver, bone marrow, spleen, and nonspecific binding of SPIOs to non-targeted areas [10].
2. Material and methods

Before chromatography analyses were started, pertechnetate was prepared by adding 1 mL saline into the Pa vial which contains 1 mL Tc-99m eluate. Besides that, DMSA solution was made by adding 0.0044 g DMSA; 0.0035 g sodium bicarbonate in solid phase; 0.109 g anhydrous dextrose. 0.4 mL sodium bicarbonate 7% was added after replacing the septum, gently agitating until the powder was solved.

2.1. TLC analysis
Before starting the experiment, mobile phase for TLC was made by added n-butanol, glacial acetic acid, and water with ratio: 3:2:3, respectively. Water and acetic acid were added first, then n-butanol. This solution is added to the small beaker to a depth of 0.5 cm. After that, a piece of filter paper was inserted into the beaker to act as a wick and the beaker is sealed with Parafilm. Si-gel strip was marked 1 cm from the end (origin) and 0.5 cm from the top. The sample was spotted on a strip, and a sample of pertechnetate was eluted on a second strip, at the baseline using a 0.5 ml syringe in order to give a small, discrete spots on the baseline. Due to obtain the best resolution, the spots should be as small as possible and allowed the spots to dry at least within 5 minutes. TLC sheets were stood in the mobile phase and were developed until the mobile phase reaches the solvent front mark. It took around 45 minutes as the mobile phase was quite viscous. After that, TLC sheets were taken out and were allowed to dry. Then, those silica gels are covered by sellotape and were marked 0.5 strips along the whole length with a permanent marker. Number each one starting with 1 at the bottom. The strip was cut into 5 mm strips and each one was counted in the dose calibrator.

2.2. ITLC analysis
2-Butanone and saline were added to two difference beakers to a depth of 0.5 cm and covered with parafilm. The ITLC-SG strips were marked 1 cm from the bottom (origin) and 0.5 cm from the top. They were spotted in a similar way to the TLC method. The strips for SPIO radiolabelling analysis were allowed for 5 minutes drying. The ITLC strips were stood in the mobile phase and left to develop to the mark. After drying, the strips were covered with sellotape, cut and counted in a similar fashion to the TLC. Radiolabelling of SPIO Nanoparticles is with 99mTc(V) DMSA. Two aliquots of 99mTc(V) DMSA (50microL each tube) were added to two tubes containing 50 microL coated and uncoated SPIO. They were incubated with shaking for 15 minutes at room temperature. To test the radiolabelling, TLC and ITLC were used.

2.3. Chromatography method
Two ITLC strips were spotted (2microL each) and the same technique using butanon and saline methods described above. The results were compared to the 99mTc(V)-DMSA to calculate the radio labelling yield for the SPIO samples.

2.4. Size exclusion method
Two filters were washed by adding 0.5mL H2O and centrifuging tube+filter (3 minutes at 5000 rpm). For each SPIO sample >0.5ml was added using a pipette into a clean size exclusion filter. The filter was inserted into the collection tube. The filter and tube were centrifuged in 3min at 5000 rpm. After centrifugation, a clear solution in the collection tube was presented. H2O was added to the retentate and was repeated until the retentate was washed with 1.5ml of water. After this process, the radioactivity in the filtrate and the retentate was measured to work out the radiolabelling yield.

3. Results

In this experiment, we calculate the accumulation of 99mTc(V)-DMSA and test the radiolabelling of the uncoated SPIOs and coated SPIOs.
Figure 1. The radioactive counts of the TLC results of 99mTc(V)-DMSA and pertechnetate.

Table 1. Rf values of Tc (v) DMSA and Pertechnetate.

| Rf Value of Tc(v) DMSA | Rf Value of Tc Pertechnetate |
|------------------------|-----------------------------|
| 0.6                    | 0.8                         |

In figure 1, it can be seen that Tc(V)-DMSA had less migration compared to pertechnetate (TcO4⁻). This is showed that the less polar substance which was the Tc(V)-DMSA as a product had formed. The control which was pertechnetate had higher polarization than the product. In the other hand, table 2 shows that the Rf value of the product is smaller than the Rf value of pertechnetate.

Figure 2. The radioactivity counts from ITLC of 99mTc(V)DMSA and pertechnetate with saline as a mobile phase.

Figure 2 shows the migration of both Tc(V)-DMSA and pertechnetate with saline as the mobile phase. It shows that both have similar polarity because the highest reading for both is 7.5 cm. However, Pertechnetate has extremely higher count number rather than Tc(V)-DMSA.

Figure 3. The radioactivity counts from ITLC of 99mTc(V) DMSA and pertechnetate with butanone as a mobile phase.
Figure 3 presents a high radioactivity count of the Tc(V)-DMSA near the baseline compared to the pertechnetate which is the control which had migrated to the solvent front. This approves that the reaction had taken place due to the drastic difference in migration.

![Figure 4: The radioactivity of ITLC with saline as the mobile phase for uncoated (a) and coated (b).](image)

Figure 4. The radioactivity of ITLC with saline as the mobile phase for uncoated (a) and coated (b).

![Figure 5: The radioactivity of ITLC with butanone as the mobile phase for uncoated (a) and coated (b).](image)

Figure 5. The radioactivity of ITLC with butanone as the mobile phase for uncoated (a) and coated (b).

Figure 4 shows both coated SPIOs and uncoated SPIOs migrated. Although there was a high amount of coated SPIOs labeled by Tc(V)-DMSA in the baseline. However, figure 5 shows both uncoated SPIOs and PEGylated SPIOs did not migrate due to butanone was the mobile phase.

| Table 2. Radioactivity yield of coated and uncoated SPIOs. |
|------------------------------------------------------------|
| Uncoated (Butanone) | Coated (Butanone) | Uncoated (Saline) | Coated (Saline) |
|----------------------|-------------------|-------------------|-----------------|
| Radioactivity Yield  | 56.35             | 60.47             | 76.90           | 83.85           |
Table 3. The radioactivity yield of the coated and uncoated nanoparticles from the size exclusion.

| Radioactivity Yield (%) | Uncoated SPIOs | Coated SPIOs |
|-------------------------|---------------|--------------|
|                         | 24            | 26.9         |

From the chromatography result (table 2) the PEGylated SPIOs had slightly higher radioactivity yield value than uncoated SPIOs both in butanone or saline as the mobile phase. It was also supported with the size exclusion result when coated nanoparticles had higher radioactivity yield value than uncoated nanoparticles (26.9% and 24%, respectively).

4. Discussion

In this experiment, we used a mixture of n-butanol, glacial acetic acid, and water as a mobile phase with ratio 3:2:3, respectively. In order to mix those solutions, water and glacial acetic acid were added first before adding n-butanol. If the glacial acetic was mixed first with n-butanone, it will form neutralization reaction which produces salt and water. When the TLC was being done, filter paper to act is a wick was used due to saturating the air and ensures proper separation of the product and standard. Additionally, for the development of chromatograms of TLC or ITLC methods, a small or medium beaker might be used which were closed with a glass plate to maintain a saturated air within the vessel. If the beakers were not closed, solvent evaporation might affect the separation of the product.

In the TLC and ITLC methods pertechnetate was used as a control. This compound is an oxyanion and water-soluble source with extremely polar. However, from the figure 1, we can see that Tc(V)-DMSA was less soluble than pertechnetate in TLC test, in order to pertechnetate is more polar. However, there are two peaks on the Tc(V)-DMSA which are a sign of impurity of the compound. A high reading is occurred due to spillage or bigger dot of the sample. The sample size has a considerable effect on the separation of a certain system. Therefore the dot diameter on the paper should be kept as small as possible. In addition to that, from this test we got 0.6 as the Rf value for product and 0.8 as the Rf value for pertechnetate. It shows there was Tc(V)-DMSA formed and the reaction had occurred. In general, the optimum resolution for medical imaging using radioisotope is achieved by choosing the mobile phase to give a Rf approximately between 0.3 and 0.6⁴.

In order to prove and characterize the reaction that occurred, ITLC tests for Tc(V)–DMSA and pertechnetate were used two types of mobile phase solvents which have different polarity. Saline was utilised as a polar solvent and butanone as a protic polar solvent. Figure 2 shows that both pertechnetate and Tc(V)-DMSA were migrated with the solvent front. However, when the pertechnetate had migrated to the solvent front with butanone as the mobile phase, Tc(V)-DMSA had remained in the baseline. It is showed in figure 3. Polar solvent can be further divided into protic and aprotic. N-butanol is one type of polar protic solvents. This type of solvent solves anions (negatively charged solution) strongly via hydrogen bond [11]. Due to a strong interaction between pertechnetate (oxyanion with extremely strong negative charge) and n-butanol, TcO₄⁻ was migrated to the solvent front. However, saline as a neutral solvent helped to approve that the reaction had occurred. Saline or salt water contains water and dissolved salts (mainly NaCl). Thus, it is possible to migrated pertechnetate and Tc(V)-DMSA because both are soluble in the water. The extremely high count value of the pertechnetate again is due to inappropriate lab technique which can cause dispersion when spotting the sample. However, the low count value of the Tc(V)-DMSA due to we did not include sellotape that covered the strips when we did the radioactivity measurements.

Figure 4 shows that the PEGylated SPIOs have a higher radioactivity count reading. Coated nanoparticle is more stable due to polyethylene glycol being highly hydrophilic. Thus, it should be easier to ⁹⁹mTc(V)-DMSA to interact with the PEGylated SPIOs through the carboxyl group. However, in figure 4 for coated nanoparticle shows that there is dispersion occurred due to a big spot of the sample on the paper. On the other hand, in figure 5, we can see that both coated nanoparticle and uncoated nanoparticle stay still on the baseline with butanone as the mobile phase. It again happened due to butanone characteristic which will only have a strong interaction with the anion which has a negative
charge. In addition, there are two high peaks in the figure 5 which were caused by the sample size that was too big.

The radiolabelling yields from ITLC results were measured by dividing the area of the radioactivity peak obtained for coated/uncoated nanoparticles by the total area of the radioactivity peaks obtained for both coated/uncoated nanoparticles and Tc(V)-DMSA. However, radioactivity yield for size exclusion was calculated by dividing radioactivity in retentate by total radioactivity both in retentate and filtrate. Filtrate consists free 99mTc(V)-DMSA. The radioactivity yield from both ITLC and size exclusion method show the PeGylated SPIOs had a higher yield value rather than uncoated SPIOs (table 2 and table 3) which potentially linked with the explanation above.

5. Conclusion

In conclusion, the experiment was performed a successful experiment which is approved by TLC and ITLC results with TcO$_4^-$ as the standard and $^{99m}$Tc(V)-DMSA as the product. The charge difference between pertechnetate and $^{99m}$Tc(V)-DMSA effects the viability of both to be solved in a different solvent such as butanone. However, due to those compounds relatively polar, they can migrate to the solvent front with saline as the mobile phase. On the other hand, due to PEGylated SPIOs were more stable than uncoated SPIOs, they can interact with more $^{99m}$Tc(V)-DMSA through their carboxyl groups.

The study limitation is this study only used TLC as a technique to compare the distribution of $^{99m}$Tc(V)-DMSA and pertechnetate. Furthermore, alternative conditions, such as co-precipitation in a constrained environment, may have benefitted the synthesis of smaller nanoparticles, which leads to higher radioactivity yields.

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