

Formation of a potential tumor therapeutic pharmaceutical ^{186}Re -bleomycin

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Abstract The influence of experimental conditions on the formation of ^{186}Re -bleomycin (BLM) was studied. The results show that the labeling yield is mainly dependent on the pH value in reaction medium and gluconate can effectively protect the re-oxidation of Re reduced by Sn(II). A better method for preparing ^{186}Re -BLM is described with labeling yield up to 95% in the pH range from 3.0 to 4.5.

Keywords ^{186}Re -bleomycin, Tumor therapeutic agent, Sn(II) reducing method, Gluconate protecting agent

1 Introduction

Based on BLM (bleomycin) tumor affinity, several γ -emitting radionuclides have been explored to develop labeled BLM for the diagnosis of tumors.^[1~3] Because $^{99\text{m}}\text{Tc}$ possesses excellent nuclear properties, $^{99\text{m}}\text{Tc}$ -BLM preparation, its biological evaluation and potential application in tumor imaging have been extensively investigated^[4~6], and the results show that $^{99\text{m}}\text{Tc}$ -BLM was a hopeful tumor imaging agent.

In addition to a tumor imaging agent obtained by using γ -emitter bonding to BLM, a new kind of tumor therapeutic agent was suggested to be synthesized by using β -emitting nuclide labeling BLM, which has both chemo and radio therapeutic property. Because ^{186}Re , a β -emitter, has excellent nuclear properties with half life of 90.6 h, maximum β energies of 1.07 MeV (77%) and 0.93 MeV (23%), and moderate energy γ ray very suitable to image, it is a desirable radiotherapeutic nuclide and has been used for treating bone tumor by us. In this paper, a systematical investigation on formation conditions of ^{186}Re -BLM, oxidation state of ^{186}Re in the complex, and the function of protecting agent was described.

2 Experimental

2.1 Materials

All reagents, except ^{186}Re , were avail-

able commercially. ^{186}Re , in the form of $\text{NH}_4\text{Re}(^{186}\text{Re})\text{O}_4$, was prepared by irradiating natural metal Re at the reactor of our institute to a specific activity of $\sim 4.4 \text{ GBq/mgRe}$.

2.2 Preparation of ^{186}Re -BLM

BLM, dissolved in physiological saline, was mixed with the diluted Sn(II) solution, the pH value was adjusted using small portions of HCl or NaOH solutions. After adding $\text{NH}_4\text{Re}(^{186}\text{Re})\text{O}_4$, the mixture was bubbled with nitrogen to prevent oxidation of Sn(II) by atmospheric oxygen, and then was placed in a sealed vial in boiling-water bath for 40 min.

A radiopaper chromatographic method was used to determine the purity of the complex with acetone and physiological saline as developing agents.

The influence of pH value, reductant (Sn(II)), BLM and protecting agent (gluconate) concentrations on the complex yield was investigated by an analysis of variance.

2.3 Oxine extraction

0.1 ml of reaction mixture, which was prepared according to the procedure for ^{186}Re -BLM in the absence of BLM, was added to 2.9 ml of saturated sodium sulphate solution (containing 200 mg of boric acid), followed by the addition of 3.0 ml of 0.4 mol/L oxine in chloroform. After oscillation for 60 min, a 2.0 ml solution was taken from the organic layer and counted, the extraction percentage was calculated from the ratio between counts taken from

the organic layer and the reaction mixture.

3 Results and discussion

3.1 Formation conditions of ^{186}Re -BLM

The influence of pH value on ^{186}Re -BLM yield is given as Fig.1. The yield raises to

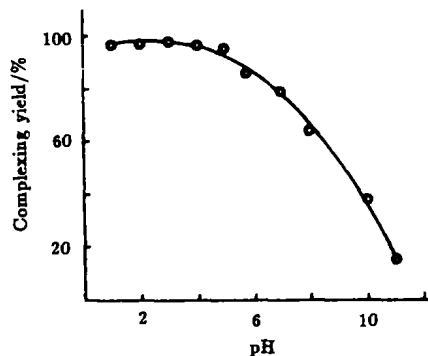


Fig.1 The effect of pH value on ^{186}Re -BLM yield

the maximum as the pH value is ranging from 3.0~4.5, while decreases rapidly with further increasing of pH value. Using Sn(II) as reductant, the complex yield is not less than 95% when ligand amount is above 2.0 mg (Fig.2), pH=3.0~4.5.

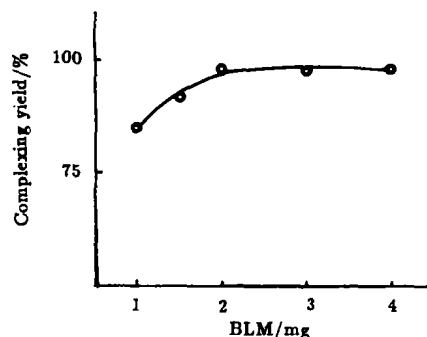
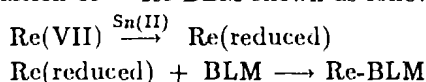


Fig.2 The influence of ligand amount on ^{186}Re -BLM yield

After analysis of the components in uncomplexed Re mixture, it is found that the percentage of reduced Re is not more than one, while that of unreduced Re(VII) increases sharply with the increase in pH value as shown in Fig.3.

The above results suggest that the reduction of Re(VII) may be a controlling step in the formation of ^{186}Re -BLM shown as following



3.2 Function of protecting agent in ^{186}Re -BLM formation

Re and Tc belong to Group VII B of the periodic table and have similar structural and chelation chemistry. In general, rhenium is more difficult to be reduced than technetium and the complexes are more kinetically inert towards ligand substitution reaction than their technetium counterparts, furthermore, the reduced Re in lower oxidation state is re-oxidated more easily than technetium.

Using Sn(II) as reductant, the results of Re(VII) reduction are listed in Table 1, the percentage of reduced rhenium increases with the addition of Sn(II), but it is still below 40% as Sn(II) amount is up to 500 μg , which contrasts

sharply with that of Tc(VII)^[7], while the percentage of reduced Re is not less than 95%, in the presence of gluconate as a protecting agent, in the similar reaction conditions for the complex formation.(see Fig.4) A reasonable explanation is the avoidance of the re-oxidation of reduced rhenium by trace oxygen in atmosphere in the presence of gluconate.

Table 1 Influence of Sn(II) amount on the reduction of Re(VII)*

SnCl ₂ ·2H ₂ O/ μg	100	200	300	400	500
Reduced Re/%	20.1	31.4	32.3	36.2	38.7

*The data are mean values of three experiments

3.3 The oxidation state of ^{186}Re in ^{186}Re -BLM

In 1974, Majec and his collaborators found that Tc(V) could be separated from other species according to the fact that only Tc(V) and oxine could form a complex which was soluble in chloroform. Furthermore, Re(V) has a similar property as that of Tc(V).

In our experiments, using oxine extraction method, we measured the distribution of the oxidation state of rhenium under similar preparative conditions for ^{186}Re -BLM complex, and the results are given in Table 2. Using Sn(II) as reducing agent and gluconate as protecting

agent, the percentages are 34, 62 and 4 for Re(V), lower oxidation states and Re(VII), respectively.

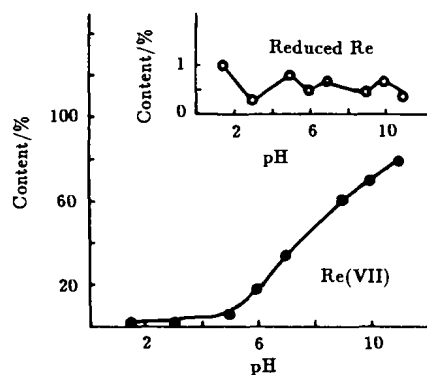


Fig.3 Relationship between Re composition and pH value

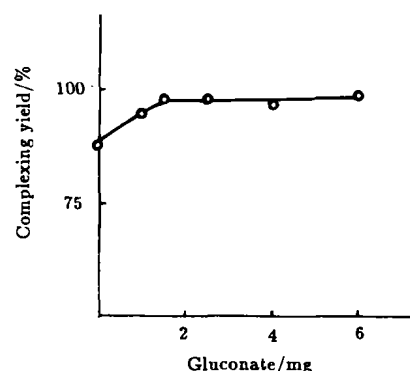


Fig.4 The effect of protecting agent on complex yield

Table 2 Distribution of Re oxidation state percentage using Sn(II) as reductant*

Reducing condition	Re(V)	Re(IV,III)	Re(VII)
Gluconate: 2 mg, Sn(II): 300 μ g, pH: 3.5	34	62	4

*The data are mean values of three experiments

4 Conclusion

In the medium of pH ranging from 3.0~4.5, ^{186}Re -BLM was prepared with labeling yield up to 95%, using Sn(II) as reductant agent, and it is effective that gluconate is used as protecting agent for avoiding the re-oxidation of reduced rhenium due to trace oxygen in the atmosphere.

Using oxine extraction method, the distribution of the oxidation states of rhenium in ^{186}Re -BLM was measured, and the composition of the complex may be 34% for ^{186}Re (V)-BLM

and 62% for ^{186}Re -BLM with lower oxidation states of ^{186}Re , respectively.

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