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Research Article

A new method of [^{99m}Tc]-ciprofloxacin preparation and quality control

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Summary

[^{99m}Tc]-ciprofloxacin is a useful radiopharmaceutical for the detection of osteoarticular prosthesis infection. We report a new preparation method based on the use of tartrate anion as an exchange ligand and tartaric acid as a source of tartrate. An improved quality control procedure is also reported that allows measuring the level of [^{99m}Tc]-tartrate present in the radiopharmaceutical preparation. Copyright © 2006 John Wiley & Sons, Ltd.

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Introduction

Ciprofloxacin is a 4-fluoroquinolone antibiotic (Scheme 1) and [^{99m}Tc]-ciprofloxacin is a radiopharmaceutical developed to diagnose infection, being particularly attractive for the detection of osteoarticular prosthesis infection.[1] This radiopharmaceutical has been tested in clinical studies and it has been

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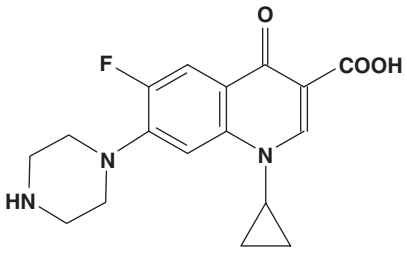

Scheme 1.

claimed that it can distinguish between bacterial infection and sterile inflammation.[2–4] Nevertheless, the preparation method of this labelled compound is a critical point because a significant amount of colloid ($^{99m}TcO_2$) is frequently formed and it can accumulate in inflammation sites yielding false positives.[5] Hence, a wide range of reducing agents has been studied to improve the preparation of this radiopharmaceutical such as tin(II) chloride,[6] formamidine sulphinic acid,[2,3,6] tin(II) tartrate[7] and a polymer with iminodiacetic acid.[8] Among all these reported methods, the synthesis with tin(II) tartrate should be highlighted because it leads to a radiopharmaceutical with small amounts of colloid. However, the reported quality control procedure cannot determine the level of the possible [^{99m}Tc]-tartrate formed in the reaction with tin(II) tartrate. Since tartrate is a common exchange ligand in the preparation of technetium radiopharmaceuticals,[9] we decided to investigate the influence of tartrate anion in [^{99m}Tc]-ciprofloxacin synthesis. It should be borne in mind that although the structure of the [^{99m}Tc]-ciprofloxacin complex is unknown, ciprofloxacin acts as an O-donor ligand in reported metal complexes,[10] so the difference between the stability of [^{99m}Tc]-ciprofloxacin and [^{99m}Tc]-tartrate (complex with exchange ligand) could not be so significant as in other radiopharmaceuticals.

This study reports: (1) an alternative labelling procedure based on tin(II) chloride and L-tartaric acid instead of tin(II) tartrate; (2) an improvement in quality control by means of thin-layer chromatography (TLC) that allows quantification of [^{99m}Tc]-tartrate.

Results and discussion

L-tartaric acid was chosen as a source of tartrate anion because it can supply a small tartrate concentration to the reaction medium, sufficient enough to act as an exchange ligand. The use of tartaric acid and tin(II) chloride instead of tin (II) tartrate allows working at low exchange ligand concentration without dependence on tin(II) concentration. In addition, L-tartaric acid is a compound included in the European Pharmacopeia, which can facilitate its use in clinical trials. Hence, [^{99m}Tc]-ciprofloxacin was prepared by simple

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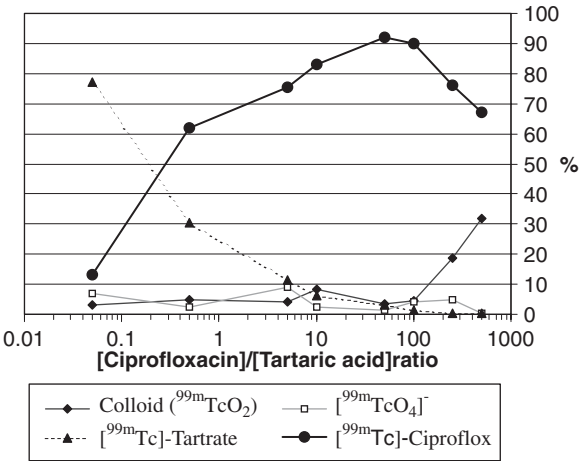

Figure 1. Influence of [ciprofloxacin]/[tartaric acid] ratio in the labelling reaction

reaction at room temperature between ciprofloxacin hydrochloride, L-tartaric acid and tin(II) chloride. Quality control was performed by TLC by means of ITLC-SG plates, developed with acetone in order to determine the pertechnetate content, and ethanol:water:ammonium hydroxide (2:5:1) as the mobile phase to determine the colloid content of the preparations.[7] The amount of [^{99m}Tc]-tartrate formed in the preparation method was monitored using RP-18 plates and saline solution:methanol:acetic acid (55:45:1) as mobile phase. In this system, pertechnetate and [^{99m}Tc]-tartrate move with the solvent front ($R_f = 0.9$) and [^{99m}Tc]-ciprofloxacin remains at the origin ($R_f = 0$). Consequently, the concurrent use of the three TLC systems supplies enough information to determine the percentages of the four species involved in the preparation: [^{99m}Tc]-ciprofloxacin, [^{99m}Tc]-tartrate, [$^{99m}TcO_4$]$^-$ and colloid ($^{99m}TcO_2$). Making use of this methodology, we studied the influence of the ratio [ciprofloxacin]/[tartaric acid] in the labelling reaction and the result is displayed in Figure 1.

This graph shows that when the ratio [ciprofloxacin]/[tartaric acid] is very low, [^{99m}Tc]-tartrate is a major by-product of the labelling reaction. This result agrees with the idea that tartrate concentration in the reaction medium should be low because the thermodynamic stability of [^{99m}Tc]-ciprofloxacin probably is not much higher than [^{99m}Tc]-tartrate. On the other hand, when the ratio [ciprofloxacin]/[tartaric acid] is very high, the amount of colloid becomes considerable. This result is consistent with the role of tartrate as an exchange ligand, because when the concentration of tartrate is very low, the reduced technetium is not stabilized and forms colloid. It should be emphasized that Figure 1 shows clearly a maximum in the representation of [^{99m}Tc]-ciprofloxacin percentage versus [ciprofloxacin]/[tartaric acid] ratio in

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the range 50–100. Therefore, this is the optimal range for the [ciprofloxacin]/[tartaric acid] ratio to obtain the most favourable labelling yield of [^{99m}Tc]-ciprofloxacin using the preparation method reported in this work.

Experimental

Materials and methods

Tin(II) chloride dihydrate and L-tartaric acid were purchased from Merck and ciprofloxacin hydrochloride from Bayer. TLC was performed on 200 mm PALL ITLC-SG plates and Merck TLC-RP-18 plates. The plates were analysed in a BIOSCAN System 200 Imaging Scanner. Pertechnetate was obtained from the commercial ^{99}Mo/^{99m}Tc generators DRYTEC (Amersham).

Labelling procedure

A solution of tin(II) chloride dihydrate (0.1 mg in 0.1 ml of hydrochloric acid 0.01 N) was added to a solution of ciprofloxacin hydrochloride (2 mg) in 1 ml of saline. Next, 0.1 ml of an aqueous solution of L-tartaric acid was added to the resulting solution and it was vigorously stirred. In order to study the influence of tartaric acid concentration, the labelling procedure was analysed using tartaric acid solutions with the following concentrations: 1 M, 100 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.2 mM and 0.1 mM. These values correspond to the molar ratios [ciprofloxacin]/[tartaric acid] of 0.05, 0.5, 5, 10, 50, 100, 250 and 500, respectively. Finally, freshly eluted pertechnetate (0.2–1.1 GBq) was added, the resulting solution was vigorously stirred and kept at room temperature for 15 min. Data were obtained by means of three or five experiments (N) as it is shown in Table 1.

Table 1. Labelling results

R	Colloid ($^{99m}TcO_2$)	$^{99m}TcO_4^-$	[^{99m}Tc]-tartrate	[^{99m}Tc]-Ciproflox.	*N*
0.05	3 ± 2	7 ± 3	77 ± 5	13 ± 4	3
0.5	5 ± 1	3 ± 2	31 ± 6	62 ± 6	3
5	4 ± 1	9 ± 5	12 ± 6	76 ± 3	3
10	8 ± 4	2 ± 1	6 ± 3	83 ± 5	5
50	3 ± 1	1 ± 1	3 ± 2	92 ± 2	5
100	5 ± 1	4 ± 2	1 ± 1	90 ± 2	5
250	19 ± 5	5 ± 2	1 ± 1	76 ± 4	3
500	32 ± 3	1 ± 1	1 ± 1	67 ± 4	3

Data are in percentage ± SD.
R = [ciprofloxacin]/[L-tartaric acid] ratio, *N* = number of experiments.

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TLC procedure

(a) Pertechnetate analysis: A 10 μl sample of the preparation was spotted on a silica-gel strip. It was developed using acetone as mobile phase and free pertechnetate migrates to the solvent front ($R_f = 1$).[7]
(b) Technetium colloid analysis: A silica-gel strip was prepared as before and developed using *ethanol:water:ammonim solution* (25%) {2:5:1}. The colloid is found at the origin of the strip ($R_f = 0$).[7]
(c) Technetium tartrate analysis: A 10 µL sample of the preparation was spotted on a RP-C18 plate. It was developed using *saline solution:methanol:acetic acid* (55:45:1). [^{99m}Tc]-tartrate and free pertechnetate migrate with the solvent front ($R_f = 0.8 - 0.9$) and [^{99m}Tc]-ciprofloxacin is found at the origin ($R_f = 0$). To estimate the [^{99m}Tc]-tartrate content, the value of free pertechnetate found in (a) was subtracted from the percentage obtained with this procedure.

Conclusions

The observed relationship between [ciprofloxacin]/[tartaric acid] and [^{99m}Tc]-ciprofloxacin labelling is consistent with the exchange ligand role for tartrate anion. Furthermore, tartaric acid and tin(II) chloride can be a useful alternative to tin(II) tartrate because the labelling reaction can be performed at low tartrate concentration. The highest radiochemical purity in [^{99m}Tc]-ciprofloxacin labelling was achieved when a [ciprofloxacin]/[tartaric acid] molar ratio in the range 50–100 was used. Finally, the study has shown that it is necessary to control the [^{99m}Tc]-tartrate formed in the reaction and TLC-RP-C18 is a convenient method to achieve this.

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