LABELING WITH ^{131}I OF CHLOROQUINE-ANALOGUES FOR THE DETECTION OF OCULAR MELANOMA

Cees N.M. Bakker, Frans M. Kaspersen
Institute for Nuclear Physics Research (IKO)
P.O. Box 4395, Amsterdam, The Netherlands
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SUMMARY

Electrophilic iodination of chloroquine with ^{131}I by the chloramine-T method results in labeled 3-iodochloroquine (maximum yield 30%) and a number of other labeled quinolines. This method also gives 3-chlorochloroquine in mass amounts. Higher yields (up to 60%) of labeled 3-iodochloroquine are obtained by isotopic exchange with ^{131}I-iodide as its phosphate-salt.

Key words: Radiopharmaceuticals, ^{131}I-chloroquine-analogues, Electrophilic Iodination, Melanoma, Isotopic Exchange.

INTRODUCTION

Quinoline derivatives have an affinity for melanine and it is suggested that they can be used for the detection of melanoma [1]. Described are ^{131}I-iodoquine [2] (fig. 1(1)), ^{131}I-dimethylamino-propylamino-7-iodoquinoline [3] (fig. 1(2)) and the electrophilically ^{131}I iodinated chloroquine [4] (fig. 1(3)). We are interested in the applicability of these labeled chloroquine-analogues for the detection of ocular melanoma [5]. Therefore the need was felt for a rapid method of labeling with high and reproducible yields, especially when ^{123}I should be used as label. In the literature three methods are given for the labeling of quinolines with radioactive iodine:

1) Isotopic exchange with iodo-quinolines in solution [6] or in a melt [7], according to the method of Elias [24]. We were able to show [8] that for dimethylamino-propylamino-7-iodoquinoline (fig. 1(2)) higher yields were obtained when the exchange was carried out with the phosphate-salt of the quinoline instead of the free base.
2) Replacement of an amino-group by iodine by a Sandmeyer-reaction [9]
3) Electrophilic iodination by the chloramine-T method as described

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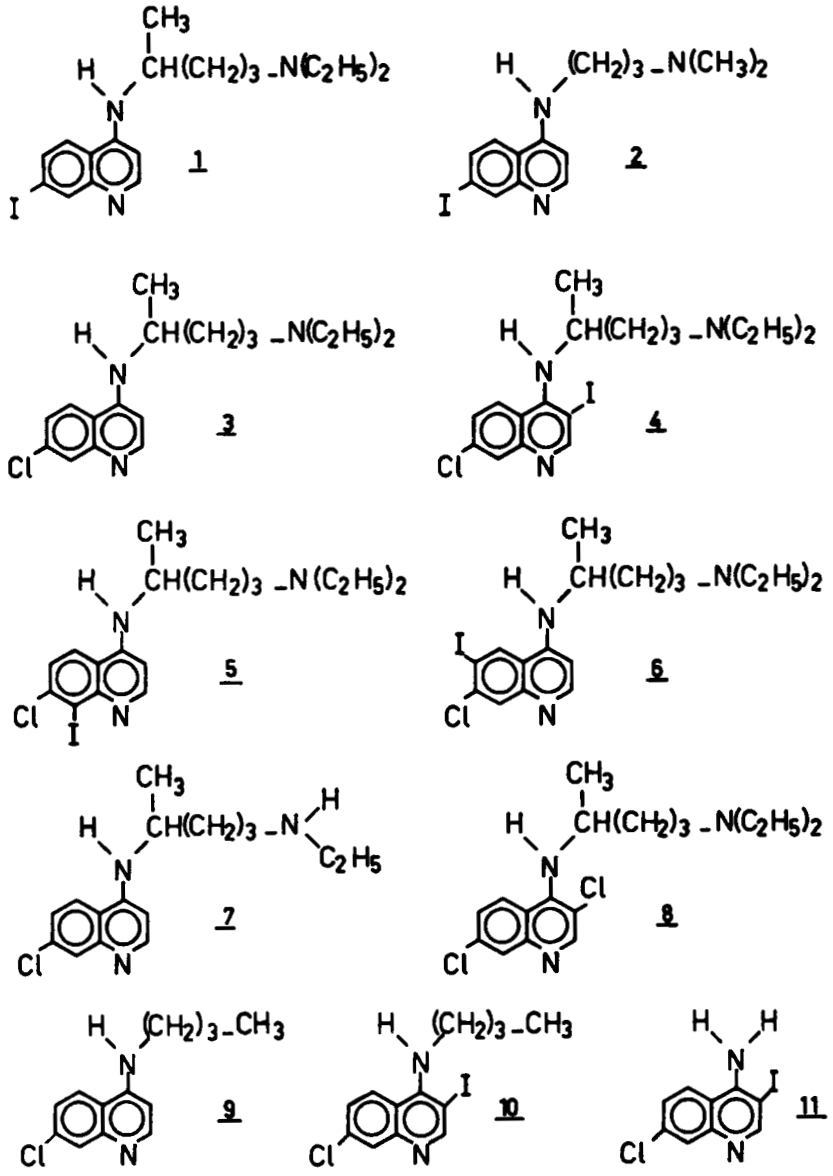

Figure 1

(1) iodoquine
(2) 4-(3-dimethylamino-propylamino)-7-iodoquinoline (8)
(3) chloroquine
(4) 3-iodochloroquine
(5) 8-iodochloroquine
(6) 6-iodochloroquine
(7) deethylchloroquine
(8) 3-chlorochloroquine
(9) 4-butylamino-7-chloroquinoline
(10) 4-butylamino-7-chloro-3-iodoquinoline
(11) 4-amino-7-chloro-3-iodoquinoline

by Safi et al. [4] and Moretti et al. [10] for the labeling of chloroquine (3).

We have studied the latter method and we have compared it with the isotopic exchange method.

EXPERIMENTAL

Chloroquine (3) was a gift from Specia, France.

3-iodochloroquine (4):

7-chloro-4-hydroxyquinoline was iodinated with ICl in acetic acid at 80 °C and the resulting 7-chloro-4-hydroxy-3-iodoquinoline was converted with $POCl_3$ into 4,7-dichloro-3-iodoquinoline (m.p. 110 °C, litt. [11]: 111°-112° C). Reaction with 2 equivalents 4-diethylamino-1-methylbutylamine in refluxing ethanol during 30 hours and purification of the resulting reaction-mixture over SiO_2 with ethanol/triethylamine (9:1) gave 3-iodochloroquine (4) as a brown oil. Yield 30%. This reaction was carried out in refluxing ethanol instead of the amine itself, because otherwise, as is described by Surrey and Cutler [11] the iodine is split off and chloroquine(3) is formed. In refluxing ethanol this side-reaction could be suppressed and the 3-iodochloroquine could be isolated (yield 30%). The product was not very stable but by conversion into the phosphate salt a rather stable product was obtained.

Free quinoline:

IR (liq.cap): 1575, 1610 cm^{-1}

PMR ($CDCl_3$): aromatic protons: 7.35 double doublet (J = 2 and 9 Hz) H_6; 7.89 doublet (J = 9 Hz) H_5; 7.93 doublet (J = 2Hz) H_8; 8.83 singlet H_2.

Mass spectrum (field desorption) M^+ = 445 and 447 (3:1).

The quinoline was converted into the phosphate-salt by adding 0.1 ml 85% H_3PO_4 to a solution of 100 mg 3-iodo-chloroquine in 5 ml ethanol. The resulting precipitate was recrystalized from water/ethanol (m.p. 140°-150° C under decomposition).

8-iodochloroquine (5):

This quinoline was prepared from 3-chloro-2-iodoaniline and methoxymethylenomalonic ester using the same method described by Counsell [6] for 4-(3-dimethylaminopropylamino)7-iodoquinoline

The product was isolated as a light brown oil.

IR (liq.cap. :1540, 1590, 1600 cm^{-1}.

PMR ($CDCl_3$): aromatic protons: 6.58 doublet (J=6 Hz) H_3; 7.52 doublet (J=9 Hz) and 7.95 doublet (J=9 Hz) H_5 and H_6; 8.72 (J = 6

Hz) H_2.
Mass spectrum (field desorption): M^+ = 445 and 447 (3:1).

Iodoquine (1):

This quinoline was prepared by coupling of 4-chloro-7-iodo-quinoline [6] with 4-diethylamino-1-methylbutamine at 160 °C.
The product was obtained as slightly yellow crystals m.p. 110°-115° C (litt. [12]): 113°-116° C).

Deethylchloroquine (7):

This product was isolated from a reaction of chloroquine (3) in acetic acid with 1 equivalent ICl at 80 °C. The product was purified by repeated chromatography over SiO_2 with ethanol/triethyl-amine (9:1) and isolated as a yellow oil.
m.p. as oxalate: 217°-218° C under decomposition (litt. [13]): 217°-218° C under decomposition).
PMR($CDCl_3$): aromatic protons: 6.32 doublet (J=6 Hz) H_3; 7.25 double doublet (J=2 and 9 Hz) H_6; 7.75 doublet (J=9 Hz) H_5; 7.88 doublet (J=2 Hz) H_8; 8.44 doublet (J=6 Hz) H_2.

3-chlorochloroquine (8):

This quinoline could be isolated from a reaction mixture of chloramine-T and chloroquine-phosphate in water (15 minutes reaction at roomtemperature) by chromatography over SiO_2 with a mixture of benzene/triethylamine (1:1) saturated with water.
IR (liq. cap.): 1585 and 1610 cm^{-1}.
PMR($CDCl_3$): aromatic protons: 7.12 doublet doublet (J=3 and 9 Hz) H_6; 7.65 doublet (J=9 Hz) H_5; 7.68 doublet (J=3 Hz) H_8; 8.27 singulet H_2
UV(0.01 N HCl): maxima at 228, 259, 342, 353 nm (litt. [16]): 227, 259, 342 and 352 nm).

4-butylamino-7-chloroquinoline (9):

This compound was prepared from 4,7-dichloroquinoline [15] by reaction with butylamine as described by Craig and Pearson [14].
m.p. 130°-132° C (litt. [14]: 130°-131° C).
Reaction of this compound with 1 equivalent ICl in acetic acid at 80 °C during one hour gave a mixture of 4-butylamino-7-chloro-3-iodoquinoline (10) and 4-amino-7-chloro-3-iodoquinoline (11).
The quinolines were isolated by chromatography over SiO_2 with as eluent $CHCl_3$: ethylacetate (1:1).

4-butylamino-7-chloro-3-iodoquinoline (10):

Yield: 70%.
m.p. 75°-78° C.
IR(KBr): 1500, 1575, 1610 cm^{-1}.
PMR($CDCl_3$): aromatic-protons: 7.28 double doublet (J=2 and 9 Hz) H_6; 7.90 doublet (J=2 Hz) H_8; 7.95 doublet (J=9 Hz) H_5; 8.76 singlet H_2.
Mass spectrum (field desorption): M^+ = 360 and 362 (3:1).

4-amino-7-chloro-3-iodoquinoline (11):

Yield: about 4%
m.p. 220 °C under decomposition
IR(KBr): 1570, 1610, 1650 cm^{-1}.
PMR(d_6-DMSO): 6.90 singlet NH_2; 7.45 double doublet (J=2 and 9 Hz) H_6; 7.80 doublet (J=2 Hz) H_8; 8.42 doublet (J=9 Hz) H_5; 8.69 singlet H_2.
Analysis: Cl: 12.1% I: 41.9% calculated Cl: 11.64% I: 41.67%

Exchange reaction:

To a solution of $Na^{131}I$ (Philips Duphar, specific activity more than 5000 Ci/g iodine, free of reducing agents) in water 2 mg 3-iodochloroquine-phosphate was added and the resulting solution was evaporated to dryness under reduced pressure. A colourless oil was obtained. Exchange was carried out in vacuum. After addition of 100 µg sodium-sulphite the free iodide was removed by chromatography over DEAE-Sephadex with 0.9% NaCl in water.

Electrophilic iodinations:

The reaction was carried out in a mixture of aqueous chloroquine-phosphate solution (0.2 ml, adjusted to the right pH by adding NaOH or H_3PO_4), $Na^{131}I$-solution (0.05 ml) and chloramine-T (0.05 ml, 5 mg/ml). The reaction was stopped after 15 minutes by the addition of 0.1 ml $NaHSO_3$ (5 mg/ml).

Analysis:

The analysis of the ^{131}I-products was performed on thin layer plates of SiO_2 on plastic foil. As eluent the following systems were used:
- the organic phase of a mixture of benzene, triethylamine, butanol-1, H_2O (5:5:2:1½)
- the organic phase of a mixture of benzene, triethylamine, H_2O (5:5:1½)
- methanol, triethylamine (40:1).

Before the analysis 1 µg KI was added to the samples to prevent

losses of free iodide. After development of the chromatogram over about 15 cm, it was wrapped in adhesive tape and cut in segments of 0.5 cm. These were counted in a NaI(Tl) well-type crystal on the 364 keV photopeak of ^{131}I.

Results and discussion

When chloroquine (3) was labeled with the chloramine-T method (as described by Safi et al. [4]) the following results were obtained.

1. The yield of labeled products was rather low. When concentrated solutions of chloroquine were used (0.5 M) the yields were in the order of 50%, while more diluted solutions (10 mM) gave yields of about 2%. It seems that chloroquine is not a very reactive substrate for the electrophilic iodination. For comparison phenol, even at a concentration of 10 mM, is labeled for more than 90%.
2) A quite complex composition of labeled products is found upon analysis. In figure 2a and b chromatograms with different solvents of such a chloramine-T labeling are shown.
3) The ratio of the products formed, depended on the pH of the reaction-mixture and the amount of iodine carrier present during the labeling. Some results are given in table I. In reactions with the pH > 4 the product with R_F 0.4-0.5 was the main product. As is discussed later this product was identified as 3-iodochloroquine (4). Analysis of samples of commercial ^{131}I-chloroquine gave similar product-distributions as is shown in figure 2c and 2d also with 3-iodochloroquine (4) (fig. 1) as the main product.
4) Thin-layer chromatography revealed that besides labeled products also a new mass peak was formed, with a R_F-value about equal to that of the main labeled product. This product was isolated and on basis of its PMR- en UV-spectra identified as 3-chloro-chloroquine (Fig. 1(8)).

Table I Yield of 3-iodochloroquine (4) by electrophilic substitution of chloroquine (3)

pH	carrier-free	1 µg KI
3.6	2.0 %	7.2%
4.8	7.7 %	26.4 %
5.6	13.0 %	32.2 %
6.8	11.3 %	33.1 %

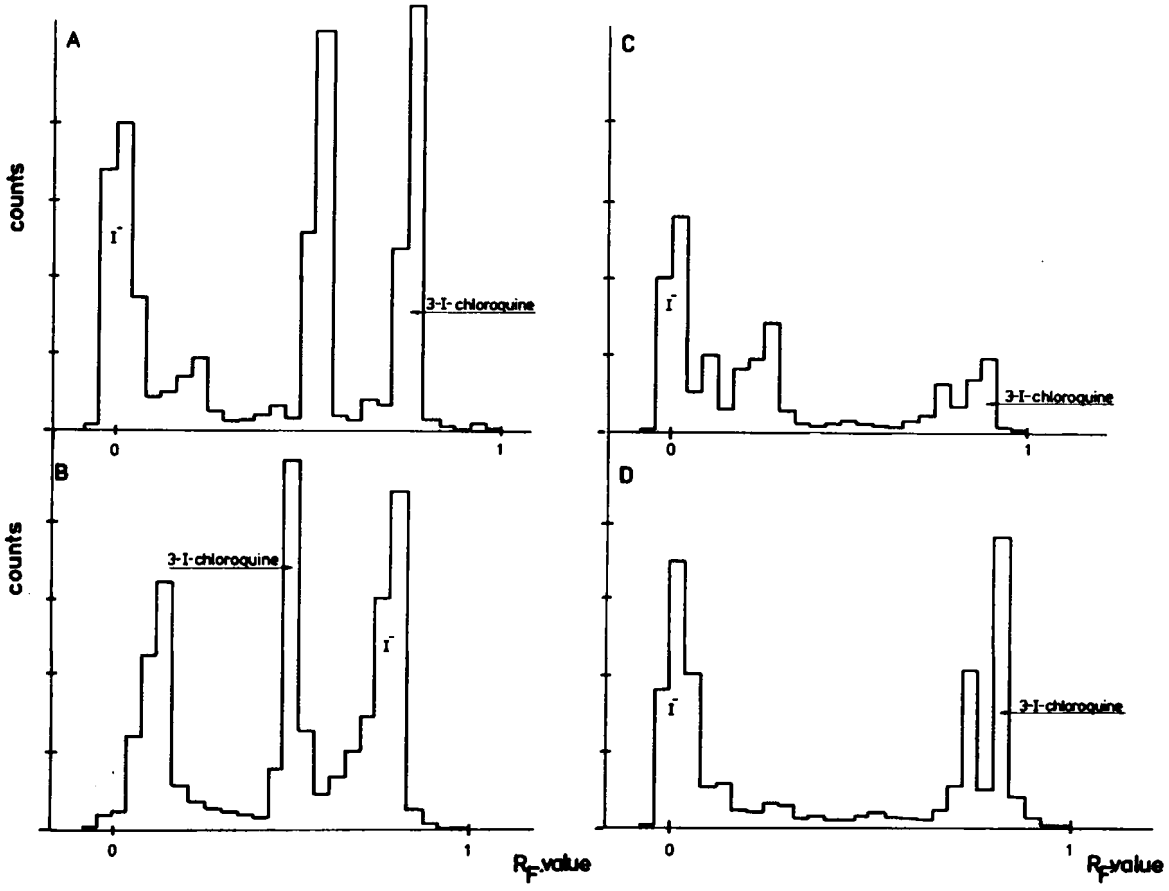

Figure 2

Thin-layer-radio-chromatograms on SiO_2 of reaction-products of the reaction of chloramine-T and A and B: reaction carried out at pH = 4.8 in the presence of 1 µg KI. C and D: different commercial preparations. A, C and D: eluent benzene/triethylamine/butanol-1/H_2O (5:5:2½:1½); B: eluent methanol/triethylamine (40:1).

Other reagents for the generation of a positive iodine-species were also tried like HNO_2 [4], $K_2S_2O_8$ and H_2O_2/lactoperoxidase. In all cases the yields were low (10-20%) and again more than one labeled product was formed.

The low yields for this iodination-reaction are probably a consequence of unwanted reactions of the alkylamino-side chain with the I^+-species. This was shown in a reaction of chloroquine with ICl or I_2 in acetic acid. In this experiment a complex mixture of products was formed. The main product (yield about 10%) was isolated by repeated chromatography over SiO_2 and it was identified by its PMR-spectrum as deethylchloroquine (fig. 1(7)). This product must be formed by an oxidative cleavage of the amine-side-chain; such-like reactions are known for amines with Br_2 and N-Br-succinimide [18,19]. The electrophilic iodination of chloroquine is further complicated by the fact that the oxidative agents used, such as chloramine-T and HNO_2 [20] can react with chloroquines.

As mentioned earlier the reaction of chloroquine (3) with I_2 or ICl resulted in a complex mixture of products including large amounts of tarry products. We have found the same results for the reaction of N-I-succinimide and chloroquine (3). So it was not possible to synthesize in this way iodinated chloroquine-derivatives for the identification of the labeled quinolines in the reaction of chloroquine (3) with $Na^{131}I$/chloramine-T. To get an indication of the structure of these products a reaction was carried out of 4-butylamino-7-chloroquinoline (9) with ICl in acetic acid. It was expected that no disturbing reactions with the amino-side chain would occur with this quinoline. As product of this reaction 4-butylamino-7-chloro-3-iodoquinoline (10) could be isolated in good yields. As a by-product 4-amino-7-chloro-3-iodoquinoline (11) was found indicating that also dealkylation of aromatic amines is possible under these circumstances.

The formation of the 3-iodoquinolines suggests that iodination of chloroquine (3) would result in 3-iodochloroquine (4). This product would also be expected on basis of the π-electron-densities and HOMO-coefficients of 4-alkylamino-7-chloroquinolines [21]. These calculations showed that also 8-iodo-chloroquine (5) could be expected as (by)product, especially when chloroquine is iodinated in its protonated form. The formation of 6-iodochloroquine (6) [21] would be less probable.

For comparison 3-iodochloroquine (4) and 8-iodochloroquine (5) were synthesized (see experimental part). The 6-iodochloroquine (6) was not synthesized because of lack of the starting aniline. Also

iodoquine (1) was synthesized because Safi [4] mentions this quinoline as the product of the electrophilic iodination of chloroquine.

Comparison of these three quinolines (3-iodochloroquine (4), 8-iodochloroquine (5) and iodoquine (1)) with the products formed in the reaction with $Na^{131}I$ and chloramine-T in different chromatographic systems gave the following results:

1) The main activity peak had the same R_F-value as 3-iodo-chloroquine (4)
2) The maximum yield of 8-iodochloroquine(5), if formed at all, is 5%.
3) Iodoquine is certainly not a reaction-product of the electrophilic iodination of chloroquine.
4) The other labeled quinolines could not be identified.

In conclusion it can be said that the electrophilic iodination of chloroquine (3) is not an ideal method of labeling quinolines because low yields are obtained and several products are formed, even in mass-amounts.

As mentioned earlier we are interested in the melanine -affinity of labeled quinolines. It is impossible to draw conclusions about this affinity for one particular compound from experiments with electrophilically labeled chloroquine. Therefore we have labeled 3-iodo-chloroquine (4), the main product of the formed mixture by another method.

High yields were obtained in the labeling of dimethylamino-propylamino-7-iodoquinoline (2) by an exchange reaction in a melt, if the reaction was performed with the phosphate salt of this quinoline [8]. We have found that also other 7-iodo-quinolines are labeled with high yields (80-95%) if their phosphate salts are used [23]. The results for the exchange reaction of 3-iodochloroquine (4)-phosphate are given in fig. 3. The maximum yield of this reaction is 60%. The 3-iodochloroquine slowly decomposes on heating into chloroquine (3) and free iodide. The iodide can be removed by chromatography over DEAE-Sephadex with a 0.9% NaCl-solution. This results in a ^{131}I-3-iodochloroquine preparation with a purity of about 95%. For animal-experiments we have labeled this quinoline by reaction at 120 °C during 10 minutes with a yield of (62 ± 4)%. The total time required for labeling and purification is about 30 minutes. The results of the animal experiments are described elsewhere [5].

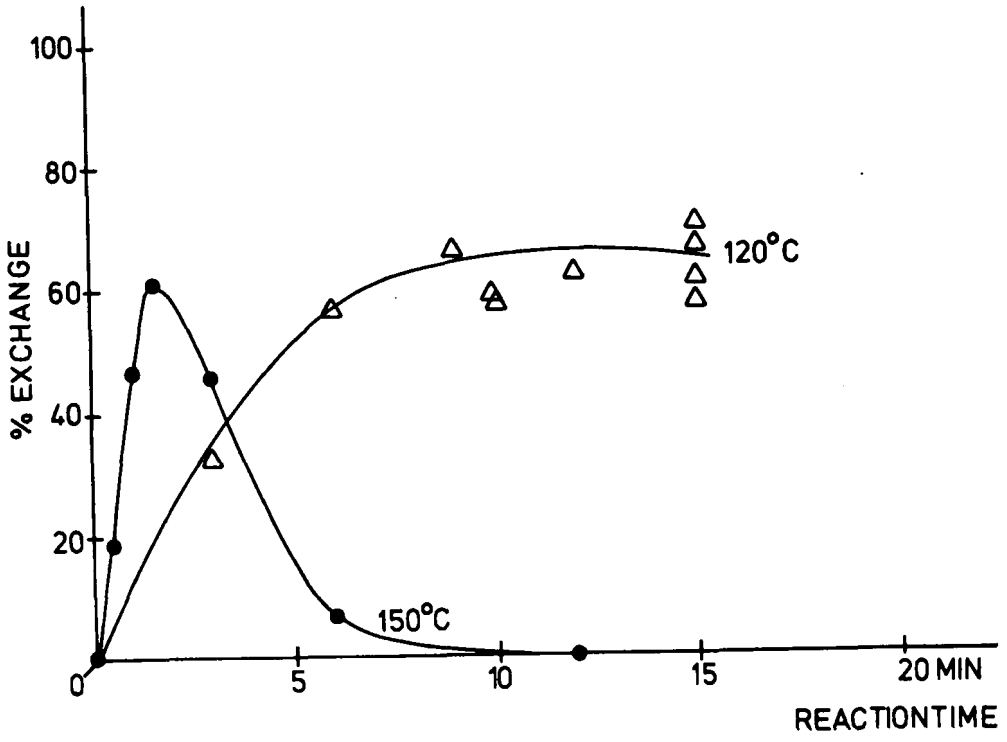

Figure 3 Exchange labeling of 3-iodochloroquine-phosphate with $Na^{131}I$

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