Preparation of Neohydrin Labelled with Mercury-197 or Mercury-203 by Exchange Reaction

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Abstract.

A new method for the preparation of neohydrin labelled with $^{197}$Hg or $^{203}$Hg based on isotopic exchange is described. The parameters of this reaction are studied. A yield of 72-76% ± 5% and radiochemical purity of 99% is attained.

Neohydrin labelled with $^{197}$Hg or $^{203}$Hg has found in the last four years widespread application in brain and renal scanning. It also localizes in other tumors in all areas of the body. (1, 2, 3) Mercurial compounds are protein bound and their clearance in blood has been found to be between 3-6 hours. It was found recently that this radiopharmaceutical is concentrated by tissues that has undergone carcinomatous change and its wide application in radioscanning will help in the delineation of the carcinoma area.

Most methods for the preparation of labelled neohydrin have been basically carried by synthesis (4, 5) such as by refluxing allyl urea in methanol with high specific activity mercuric acetate for 2-6 hours and then treating with hot 10% NaCl and allowing to cool where crystals of neohydrin separate. A yield of 50-70% was claimed. Mani (6) improved these techniques especially when labelling with short lived $^{197}$Hg.

The work of Reyoutof (7) on the mechanism of isotopic exchange between aromatic organic mercury compounds and mercury halides attracted our attention for the preparation of labelled neohydrin by isotopic exchange. Reyoutof used organic solvents such as dimethyl sulphoxide, aqueous dioxan or pyridine. However, our exchange studies using neohydrin an aliphatic organic mercury compound (3-chloromercury-2methoxy propyl urea) in dioxan or methanol, gave unreliable results. On the other hand, we found that the isotopic exchange with a high yield is possible in an aqueous medium using short reaction time and simple manipulation.

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PREPARATION OF NEOHYDRIN WITH $^{197}$Hg OR $^{203}$Hg

The different factors on which this exchange reaction in aqueous medium depend are studied and a method of production of labelled neohydrin is described, a radiochemical purity of 99% and a yield of 72-76%±5% being attained. The rate of the isotopic exchange was followed radiochromatographically and the fraction exchanged was calculated as follows:

$$F = \frac{^{\text{Neohydrin}}_{\text{Neohydrin}} + ^{\text{HgCl}_2}}{^{\text{Neohydrin}}_{\text{Neohydrin}} + ^{\text{HgCl}_2}}$$

Where:

- $F$ = Fraction exchanged
- $^{\text{Neohydrin}}_{\text{Neohydrin}}$ = Activity of Neohydrin on the chromatogram
- $^{\text{HgCl}_2}$ = Activity of $\text{HgCl}_2$ on the chromatogram
- $^{\text{Neohydrin}}_{\text{Neohydrin}}$ and $^{\text{HgCl}_2}$ = Molar concentrations of Neohydrin and $\text{HgCl}_2$

**EXPERIMENTAL**

Inactive neohydrin was supplied by kind permission of Lakeside Laboratories, U.S.A. The radioactive mercuric halide was prepared by irradiating a reagent grade mercuric oxide in the U.A.R. 2 M.W. research reactor.

The irradiated $\text{HgO}$ is dissolved in 3N $\text{HCl}$ to which a drop of nitric acid is added, followed by evaporation to dryness, and redissolution in double distilled water. The radioactive purity of the mercuric halide was checked on a multichannel analyzer after decay of the $^{197}$Hg activity: only the two peaks of $^{203}$Hg, mainly the 0.078 MeV and the 0.28 MeV appeared.

The exchange reaction was carried out in a double jacketed vessel, of 50 ml capacity, fitted with a condenser and heated by circulating thermostatically controlled water. The contents of the vessel were agitated by a magnetic stirrer. The whole equipment shown in Fig. 1. is fixed in a ventilated, shielded production box.

An aqueous solution of 10 ml of 0.01N $\text{HgCl}_2$, containing 1 mc of $^{208}$Hg and 0.01 N of inactive Neohydrin, adjusted to pH 3 by the addition of 1N HCl solution, were introduced in the reaction vessel.

Five lambdas (5 $\lambda$) of the above solution were taken at suitable interval times (30 minutes) and applied to a chromatographic paper Whatman No 1 (25 x 30cm), air dried and eluted by a 1:1 mixture of Methanol and 1 N-Ammonia, in a chromatographic jar using the ascending technique. This eluting solvent gave good resolution with definite peaks of constant $R_f$ values at ambient temperature (28°C±1°C). After 20 cm elution the chromatogram was air dried and cut into equal strips of 1 cm width; their activities were measured on a gamma scintillation counter (Ecko type).

The following $R_f$ values were obtained:

- $R_f \text{ HgCl}_2 = 0.00 - 0.04$
- $R_f \text{ neohydrin} = 0.72 - 0.75$
Fig. 1. Schematic diagrams of the apparatus for the preparation of labelled Neohydrin-\(^{203}\)Hg.

A—Decanning equipment  B—Dissolution vessel  C—Reaction vessel
D—Purification column  E—PH adjustment vessel
F—Filtration unit  a and v = For air and vacuum.

c—Chemicals  H—heater  M—magnetic stirrer.

Fig. 2. Radiochromatographic studies (A, B, C).
Figure 2 shows the following facts:

a) Before any exchange, the peak is that of $^{203}\text{Hg}$, $R_f = 0.045$.
b) After three hours reaction time, two peaks are obtained with $R_f = 0.04$ for $^{203}\text{Hg}$ and $R_f = 0.72$ for labelled neohydrin.
c) After purification of solution (B), a single peak, that of pure labelled Neohydrin appears, with an $R_f$ value of 0.74.

The final solution was purified from unreacted $\text{HgCl}_2$ by adjusting its pH to 7-8 by adding a NaOH by the addition of IN NaOH solution and passing it down an alumina column conditioned to the OH$^-$ form using dilute NaOH, then washing with distilled water until free from alkali (6). Chromatographic grade alumina (100 - 200 mesh) was used in the column (10 cm x 1 cm$^2$).
RESULTS AND DISCUSSION.

A study of the stability of the aqueous reaction medium showed that the most suitable pH is 3-4. At higher pH values 5-6, a lower yield is obtained as is shown in Figure 3. By further increasing the pH to the alkali side, HgCl₂ precipitates forming insoluble mercury oxychloride. On the other hand, at a lower pH, ~ 2, neohydrin starts to precipitate and the solution becomes turbid. At still a lower pH, ~ 1.2, complete precipitation of neohydrin occurs, due to salting out effect as is seen in the following sequence:

<table>
<thead>
<tr>
<th>pH</th>
<th>1.2</th>
<th>2</th>
<th>3-4</th>
<th>5-6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation of neohydrin</td>
<td>Start of Clear homogeous solution</td>
<td>Turbid solution</td>
<td>Precipitation of HgCl₂ due to colloidal Hg oxychloride</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different studies were carried at fixed given experimental condition: pH, (3-4), temperature (70°C) activity, 1 cm and reaction time (3 hours), so as to investigate the effect of neohydrin and HgCl₂ concentrations on the reaction rate and the exchange yield.

From Figure 4 we could notice the following:

a) At a constant mercuric chloride concentration, (0.01 N), the yield increases with increase of neohydrin concentration. Increasing the neohydrin concentration above a certain limit is not recommended due to its high toxicity as a mercurial pharmaceutical.

b) At a constant neohydrin concentration, (0.01 N) the yield decreases with increase of HgCl₂ concentration.

In order to have neohydrin with a high specific activity we should use highly active mercuric chloride, either by long time irradiation in the reactor for getting ²⁰³Hg or by using enriched ¹⁹⁸Hg for ¹⁹⁷Hg.

Fig. 5. Effect of temperature on rate of exchange.
The exchange reaction is favored by increase of temperature. Figure 5. At room temperature a yield of only 5% is obtained after four hours reaction time, whereas at 70°C or 85°C a quick exchange occurs in a relatively short time, (15-30 minutes).

Nevertheless, it is very probable that at these high temperatures, destruction of the neohydrin or hydrolysis of the mercuric chloride to a colloidal oxychloride takes place. The optimum temperature for this exchange reaction is therefore 50°C.

The addition of neutral chlorides did not increase the exchange rate.

The rate of this exchange reaction was found to be first order with respect to neohydrin concentration and the rate constant at 50°C was found to be \( K_1 = 0.022 \) per minute. Samples of labelled neohydrin with \(^{203}\)Hg have been tested pharmacologically and satisfactorily used in our hospitals.

REFERENCES