DETERMINATION OF RADIOCHEMICAL PURITY OF SOME RADIO-CHEMICALS AND PHARMACEUTICALS BY PAPER CHROMATOGRAPHY, THIN-LAYER CHROMATOGRAPHY AND HIGH-VOLTAGE ELECTROPHORESIS

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INTRODUCTION

Much attention has been paid during the last few years to the radiochemical purity of radiochemicals, now generally defined as the portion of the radioisotope concerned that is in the stated chemical form. Chromatography and electromigration techniques have been utilized mostly for these determinations, and the subject has been dealt with by several authors[1-7]. In our laboratory the methods mentioned are used for control of the routine production of radioisotopes and imported materials. This paper gives a review of methods and some practical details which may be of value to other laboratories.

Prior to radiochemical analysis the samples under investigation are analyzed with regard to radioisotopic purity or the proportion of total radioactivity which is in the form of the main or stated radioisotope. This is usually done by gamma spectroscopy or beta absorption measurements. The chemical purity of radiochemicals and pharmaceuticals is examined by methods such as emission spectrography, infrared spectrometry, U.V.-spectrometry, melting point determinations, etc. Chemical impurities may also, of course, be identified by partition paper chromatography followed by specific colour reactions on the paper strips.

The various procedures for radiochemical purity determinations described in this paper have been tested experimentally, and found to give reasonably good reproducibilities, whereas the accuracy has been difficult to establish for some of the methods.

EQUIPMENT

Paper chromatography (PC) is mainly carried out by the ascending technique on Whatman paper No. 1 and 3. Application of the samples to paper strips and thin layer plates is done by means of lambda pipettes connected to a micro syringe. Shandon equipment is used for thin-layer chromatography (TLC), ordinary electrophoresis and high-voltage electrophoresis (HVE).

Scanning of paper strips and plates for radioactivity is usually undertaken with a Packard Radiochromatogram Scanner Model 2701. The quantitative distribution of activity along the strips is determined by registering with a scaler the counts under each peak.

EXPERIMENTAL

The sample should have a reasonable counting rate in a suitable volume (say 0.005 ml), and to obtain this it is frequently necessary to dilute the sample with an appropriate solvent. In the case of so-called carrier-free radiochemicals, for instance sodium iodide—[131]I and sodium phosphate—[32]P, it is advisable to add a carrier. If a certain radioactive impurity is sought or suspected in the sample under investigation, a carrier for the impurity should also be included. In some instances the solution in which the radiochemical is supplied commercially, may cause serious interference in the chromatographic procedure. In such cases the solvent has to be removed completely before starting the chromatogram.

Iodide-^{131}I ions

This compound is conveniently analyzed by PC, over a period of 2–3 h, using a mixture of methanol and water in the ratio 70:30[8].

If rapid results are required, the sample may be analyzed by HVE using 0.1 N NaOH as electrolyte[9]. The spot is placed in the middle of a paper sheet (Whatman No. 1 paper, dimensions about 56 × 2.5 cm) and constant current is applied for 5 minutes (10 mA/cm width of paper). Under these conditions I⁻ migrates approximately 130 mm against the anode, whereas the IO_3^- moves around 40 mm. The presence of periodate is indicated by a tail between the point of application and the iodate spot. A recently published TLC method[10] provides a good and rapid separation of iodide and iodate ions.

Phosphate-^{32}P ions

The chemical composition of phosphate-^{32}P products has been thoroughly investigated previously[11,12]. It was found that the product may contain up to 2 % radiochemical impurities, comprising pyro-, tri-, tetra- and other long-chain polyphosphates. Routine analysis of this product is performed by ascending PC using the following solvent: isopropanol–trichloroacetic acid (20 % w/v)–5 M ammonia solution in water (75:25:1)[13]. The R_F value for orthophosphate in this system is around 0.8. Small amounts of impurities in phosphate-^{32}P can be determined with TLC, using a layer of cellulose and starch[14].

Sulphate-^{35}S ions

This radioisotope is produced by pile irradiation of potassium chloride and contains [36]Cl, [38]Cl, [42]K and ^{32}P from secondary nuclear reactions. After chemical separation the product still contains minor amounts of [32]P. By passing it through a column of aluminium shavings the content of ^{32}P in the product is brought down to 0.1 % or less. The radiochemical purity is easily determined by PC, using Whatman No. 1 paper. A suitable solvent mixture is isopropyl alcohol and 1.5 N ammonia in the ratio 50:50[15]. The R_F value for sulphate under these conditions is approximately 0.7.

Chlormerodrin-[203]Hg

The lack of commercial supplies of 3-chloromercuri-2-methoxy-propyl urea as reference material complicates the establishment of a convenient analytical procedure for control of the corresponding labelled compound. By using locally made reference

material, however, we found that TLC gives satisfactory reproducibility. Silica Gel G was used in 0.2 mm layers with n-butanol–acetic acid–water (60:15:25) as solvent. Under these conditions the labelled chlormerodrin has a R_F value of approximately 0.65. A second small peak appeared at around R_F 0.4 in all preparations, and it was found that it did not consist of ^{203}Hg ions. Running its reference material stained with iodine vapour gives a corresponding unidentified spot. Probably this effect is due to the same phenomenon as previously mentioned in the literature[16]. The spot usually amounts to 1–3 % of the total activity. Reference runs of mercuric chloride stained with 1 % diphenyl carbazide in alcohol followed by exposure to ammonia revealed that Hg^{2+} ions move nearly to the solvent front.

A PC method, using n-butanol–methanol–water–ammonia (5:7:1:3)[17] as solvent has also been used. On Whatman No. 1 paper the following R_F values were found: Chlormerodrin-^{203}Hg: 0.28–0.33, 3-chloromercuri-2-methoxy-propyl urea as reference: 0.28, mercuric ions: 0, with slight tail formation. The small separate peak appearing on the TLC plates mentioned above does not emerge on the paper. Otherwise the analytical results from the two methods coincide.

Cyanocobalamin-^{58}Co (Vitamin B_{12})

The radiochemical purity of this product has been examined by PC using a mixture of $sec.$-butanol–acetic acid–water–5 % KCN solution (100:1:50:0.25) or $sec.$-butanol–aqueous NH_3–water–5 % KCN solution (100:1:50:0.25)[18]. The R_F values for cyanocobalamin are 0.25 and 0.30 for the two solvents respectively.

A more rapid TLC method for separation of mixed water soluble vitamins is published by GÄNSHIRT AND MALZACHER[19]. The conditions are: Silica Gel G layer and a solvent consisting of glacial acetic acid–acetone–methanol–benzene (5:5:20:70). The R_F value for cyanocobalamin is 0 in this system. In addition if a Silica Gel G layer is used with water as developing solvent[20] the cyanocobalamin has a R_F value of 0.22. The latter system has been found particularly useful for our purposes and is now used for the control of cyanocobalamin-^{58}Co preparations in general.

Gold-198 colloid

Several methods for PC analysis of these solutions mentioned in the literature have been tried, but most of them seem to suffer from either tail formation or inadequate reproducibility. The best solvent was found to be a mixture of acetone, water and HCl (70:20:10)[21].

Recently we introduced paper electrophoresis (PE) in this connection[22] and later HVE and thin-layer electrophoresis (TLE). In these experiments 0.075 M sodium thiosulphate is used as electrolyte. The experimental conditions are: For HVE the spot is placed in the middle of a paper sheet (Whatman No. 1, 56 × 2.5 cm), and constant current is applied for 10 minutes at 6000 volts. Auric ions move around 160 mm against the anode, while colloidal gold is kept primarily at the zero point. Negligible tail formation is observed after this procedure. For TLE, the plates (20 × 5 cm) are prepared from Silica Gel G, layer thickness 2 mm. After air-drying for some minutes the plates are activated in an oven for one hour at 100°. The sample is applied in the form of a streak in the middle of the plate. Using constant current (2.5 mA/cm width of plate), the auric ions move approximately 60 mm against the anode. Colloidal gold moves a few millimeters toward the anode.

Comparing the analytical results from these three techniques, it has been found that PC nearly always gives the highest percentage of auric ions. The following is typical: PC—1.2 % auric ions, HVE—0.8 % auric ions and TLE—0.6 % auric ions.

Human serum albumin-[131]*I*

[131]I-labelled human serum albumin for blood volume measurements, etc. requires a reliable knowledge of the percentage of free iodide in the product. The conventional PC method (methanol–water, 75:25) does not always give reproducible results. We therefore found it necessary to verify the results by other methods. The PE method described in the British Pharmacopoeia[23] has been adopted for our HVE equipment, and has given excellent results. The experimental conditions are: Carrier (0.5 mg normal serum albumin, iodide and iodate) plus the sample under investigation is placed on a sheet of Whatman No. 1 paper (56 × 3 cm) and submitted to electrophoresis for 10 min using constant current (about 3.4 mA/cm width of paper). Under these circumstances the albumin moves around 10 mm towards the anode, the iodate around 70 mm and the iodide around 150 mm towards the anode.

A technique based upon precipitation of the albumin by trichloro-acetic acid is also used for checking purposes, and this method probably gives even more precise results. 0.1 ml 0.1 % KI solution in water is added to 0.4 ml of the solution of labelled albumin in a centrifuge tube. 0.1 ml of the mixture is calibrated in a scintillation counter. 0.4 ml of 10 % trichloroacetic acid in water is added to the tube, followed by shaking. The tube is then immersed for a moment in boiling water and afterwards in ice. After approximately three minutes' centrifugation at 3000 r.p.m. or filtration using blue band filter, 0.1 ml of the supernatant containing possible free ions is counted and the percentage of free ions calculated.

Typical results for a sample analyzed by the three methods are as follows: PC—3.3 % iodide, HVE—2.0 % iodide, precipitation techniques—2.9 % free ions. In the PC and HVE analysis the free ions have been identified as iodide only.

Insulin-[131]*I*

The radiochemical purity of insulin—[131]I for immunoassay work is principally determined by PC in this laboratory. The procedure in use has been described previously[24]. Due to the high purity requirements for this product and also the uncertainty concerning the nature of the impurities appearing we found it desirable to verify the analytical results, mainly by using various solvent systems. At present the best solvents appear to be veronal buffer (pH 8.6)[25] and a mixture of *n*-butanol, acetic acid and water (30:10:40)[26]. The first solvent keeps the insulin at the point of application, while the second gives an R_F value of approximately 0.4 for insulin. This work is still in hand.

Iodohippurate-[131]*I*

Radiochemical analysis of iodohippurate—[131]I is described in detail by MAGNUSSON[27], and the PC method (*n*-butyl alcohol–acetic acid–water) was found convenient for our purpose. However, when commercial sodium iodohippurate is applied on a reference strip for identification, the spot appears at an entirely different place, R_F around 0.3 when stained with bromophenol blue as compared with R_F around 0.9 for the ^{131}I-labelled sodium iodohippurate. Some twenty arbitrarily chosen solvents

were tried in order to test the discrepancy, out of which six solvents gave acceptable separation of iodohippurate—[131]I from iodide. The R_F values for commercial sodium iodohippurate and ^{131}I-labelled sodium iodohippurate differed, however, for all the six solvents, the labelled material running faster on the strips than commercial iodohippurate. Products from different suppliers were analyzed during these tests, all showing the same behaviour. The identity of the iodohippurate used was verified by infrared spectrometry.

Thin-layer chromatography with silica gel layer and acetic acid–water (3:97) as solvent was found to give corresponding R_F values for labelled and unlabelled sodium iodohippurate, R_F around 0.75, while iodide ions move with the solvent front. Spraying techniques for iodohippurate do not give a distinct band in this case. Better results are obtained by colouring the material before application on the glass plates. The following procedure may be useful: 10 % sodium iodohippurate (w/v) in water is mixed in the ratio 1:1 with a solution of 40 mg bromophenol blue in 100 ml ethyl alcohol, to which has been added 0.5 ml 3 % (w/v) sodium hydroxide solution. Around 5 μl is applied on the plate. A reference spot of bromophenol blue on the same plate leaves a yellow tail behind and has a R_F value of about 0.9.

Rose Bengal-[131]I

Several methods for examination of the radiochemical purity of this labelled compound have been published[28–31]. It seems to be generally accepted that Rose Bengal—[131]I preparations contain an unknown colourless component, which is separated from the main component when subjected to paper chromatography. This is in agreement with our experience, as all examinations so far performed have revealed a colourless peak, which is not "free" iodide. Using TLC (Silica Gel G layer) in the system water–acetic acid (97:3) the colourless component moves with the solvent front, and the main component is kept at the point of application, whereas in the system ethyl acetate–acetic acid (90:10) the two components travel in the opposite manner. As to the chemical composition of the unknown fraction it has been indicated that some less iodinated tetrachloro-(P)-fluoresceins—[131]I may be found in these preparations[30]. It is also stated that their clinical behaviour is similar to that of the main component.

The TLC method published by MITTA et al.[31] has been found to give reproducible and reliable results. The thin-layer plates are prepared with silica gel G according to STAHL[32], and butyl acetate–acetic acid (90:10) is used as solvent. R_F values are 0.9 for Rose Bengal and o for iodide. The colourless labelled component was found as a tail in our experiment. The tail formed a maximum at about R_F 0.6.

SUMMARY

Procedures for determination of the radiochemical purity of some important radiochemicals are described. The methods have been verified and found to give reliable results with good reproducibility. Some practical details of experience gained during the study are given.

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