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A RAPID METHOD FOR THE SYNTHESIS OF 5-IODO-2'- DEOXYURIDINE (IUDR) AND OPTIMISATION OF THE PARAMETERS

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Summary: A modified method for the synthesis of radioiodinated 5-iodo-2'-deoxyuridine based on electrophillic iodination using concentrated HNO_3 as an oxidising agent is described. It is easy, quick, reproducible and yields consistently high (>95%) radiolabelled IUDR. This method avoids refluxing at high temperatures, thereby saving preparation time and improving safety.

Keywords: Iododeoxyyridine, deoxyuridine, radioimmunotherapy, radiotherapy, radiodiagnosis.

INTRODUCTION

5-iodo-2'-deoxyuridine (IUDR) an analogue of thymidine, is an effective inhibitor of growth of neoplasm and the competitive antagonist of the utilisation of thymidine in mice and mammalian system (1,2). IUDR is used in competition with thymidine in the bio-synthesis of DNA (3), and specifically incorporates into the DNA in place of the normal component thymidine (4). Radioiodinated IUDR is a stable tracer for studying DNA metabolism, as the label is not removed from intact DNA in the living cell and reutilisation of IUDR is negligible (5). ^{125}IUDR is a tracer of choice for measuring cell loss from undisturbed solid tumours (6) and ^{125}IUDR is also recommended for radiodiagnosis and radiotherapy of tumours (7,8). By monitoring the ^{125}IUDR loss as a measure of cell loss, it is possible to obtain an index of cell proliferation and therefore the tumours' response to chemo-and radiotherapy (8,9). A recent study indicates that IUDR increases the therapeutic effectiveness of radioimmunotherapy (10).

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The most popular method of synthesising IUDR is based on the work of Prusoff (1). The method consists of refluxing a mixture of 2'-deoxyuridine (UDR), iodine and chloroform or iodide and HNO_3 of different molarities for variable periods of time to achieve up to 95% labelling (8,9,12,13). Other techniques involve refluxing mixtures of UDR and iodine or iodide with NaOH (14), $TlCl_3$ (15), ICl in acetic acid (ethanoic acid) (16) and Chloramine T (17). The latest recoil labelling technique, involves mixing UDR and KIO_3 with radioactive xenon (^{123}Xe) (18). In view of the wide variety of available techniques, the varying yields of radiolabelled IUDR and in-house demand for ^{124}IUDR and ^{123}IUDR for PET and SPECT studies, it was decided to investigate parameters involved and to study their effect on labelling yield. The aim was to optimise conditions so as to reduce the time of preparation, increase the labelling yield and avoid heating the mixtures at high temperature to improve safety.

MATERIALS AND METHODS

2'-Deoxyuridine (UDR) and 5-iodo-2'-deoxyuridine (IUDR) were obtained from Sigma Chemicals Ltd.. AR grade KI, concentrated HNO_3(16M) and ammonia solution (specific gravity 0.88) were obtained from B.D.H. plc.. Na^{125}I 100mCi/ml was supplied by Amersham International plc.. All reagents were used as received. UDR and KI solutions were prepared in deionised and degassed water.

100μl 0.1M UDR, 10μl 0.1M KI and 20μl (1μCi/μl) no carrier added Na^{125}I were mixed thoroughly in a plastic reaction vial; 200μl conc.HNO_3 was added carefully and mixed using a vortex mixer. The plastic reaction vial was placed in thermostatically controlled water bath maintained at 45°C for 3 minutes. The reaction vial was cooled to room temperature and ammonia solution was added dropwise to neutralise excess acid. Radiolabelling of >95% was consistently obtained. The whole operation was performed in the radiochemical fume cupboard.

The identification and separation of ^{125}IUDR was achieved using reverse-phase HPLC. Alphasil 5 OD, C18 RP column, 4.8mm x 25cm (HPLC Technology) equipped with Spectra physics SP 8000 system, SP 4290 integrator, Spectra 100 (UV

and visible) variable wavelength detector (λ=280nm) and Beckmann 170 radioisotope detector provided simultaneous detection of chemically produced and radiolabelled ^{125}IUDR independently. The eluent used consisted of 85:5:10::water:ethanol:0.1M phosphate buffer (pH7). UDR and IUDR were detected at approximately 3.4 and 7.6 minutes respectively. This was confirmed by comparing their elution times with their standard solutions.

RESULTS AND DISCUSSION

Preliminary work indicated that all solutions should be prepared fresh to avoid variation in the labelling yield. The reactant volumes were kept constant to avoid possible errors arising from different reaction volumes. Since low concentration of Na^{125}I (1uCi/ul) were available for the reaction, KI was added as a carrier. In dilute solutions, other anions may compete for the reaction with the active species of iodine formed during oxidation affecting labelling adversely (19). The reactant volumes were kept constant to avoid possible errors arising from different reaction volumes.

On using different concentrations of UDR from 0.5mM to 1.0M, the yield of ^{125}IUDR increased from 0% to 97.38% Figure 1. It was not possible to obtain significant and reproducible yields below 0.1M concentration as reported by Keough (11). On varying the concentration of KI (added carrier) from 0.01M to 1.0M the labelling yield increased to 95% at 0.1M and then remained constant up to

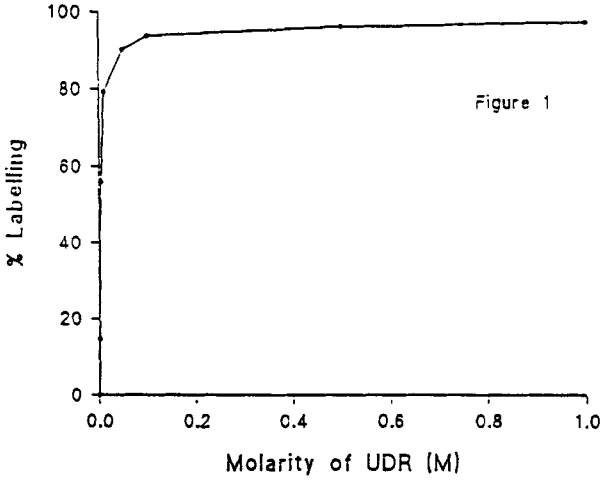


Figure 1

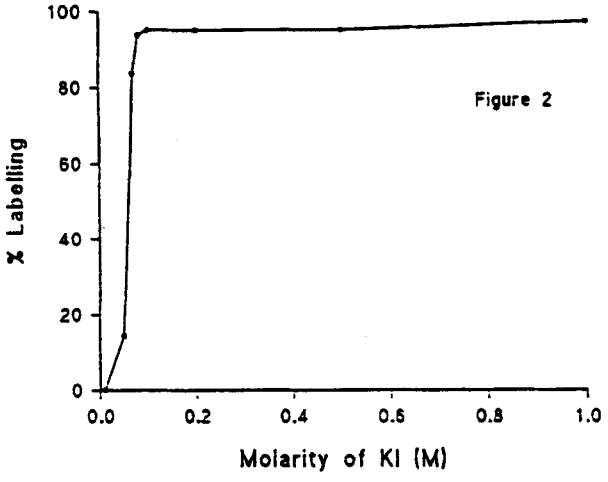


Figure 2

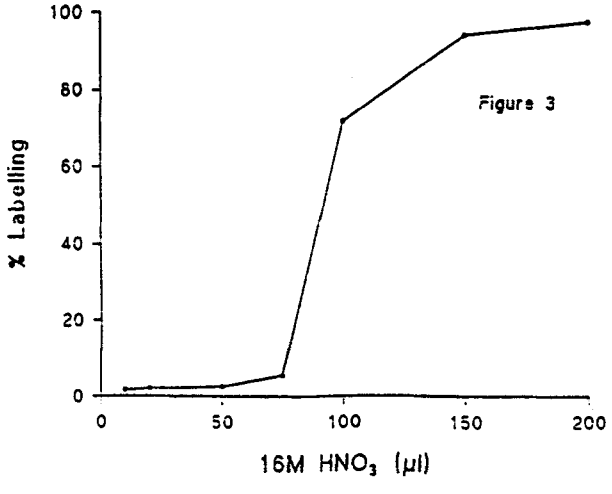


Figure 3

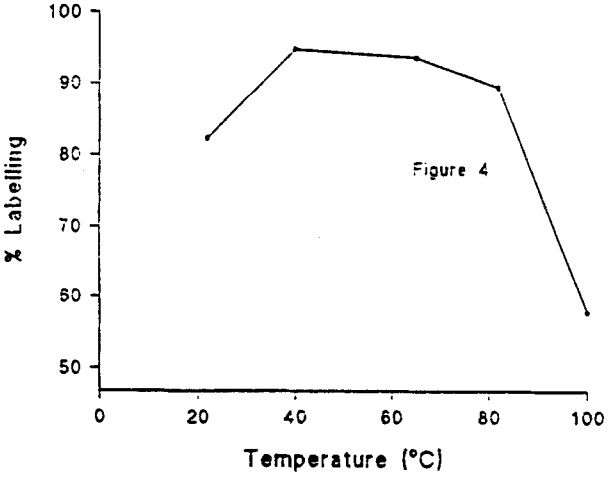
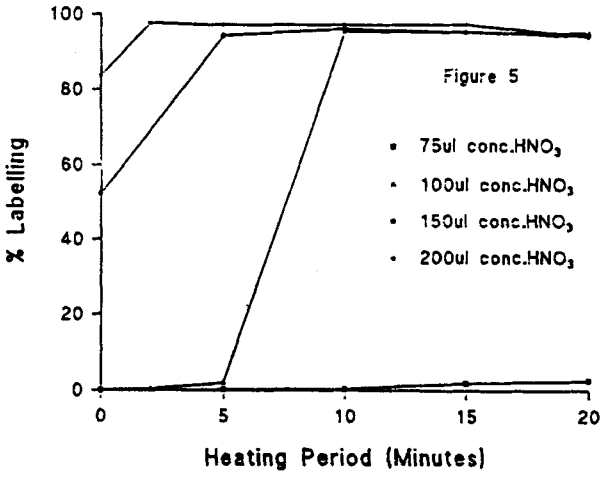


Figure 4



1.0M, Figure 2. Presumably, the higher activity of 10mCi used by other workers (11,12) provided enough iodide to overcome any dilution effect. No ^{125}IUDR was produced until the volume of conc. HNO_3 used exceeded 75ul, then the yield increased sharply to 72% for 100ul and 97% for 200ul as shown in Figure 3. On increasing the reaction temperature from 22°C to 100°C, for the constant period of 3 minutes, it was found that the labelling yield increased from 80% at room temperature (22°C) to 95% at 45°C and then remained constant up to 75°C. Raising the temperature further resulted in reduction of labelling efficiency, Figure 4. The labelling efficiency can be increased by increasing the heating period, provided the sample contained at least 100μl conc.HNO_3 as shown in Figure 5.

This modified method has been shown to be an easy and highly efficient method giving consistently high yields (>95%) of radiolabelled IUDR. This simple method avoids the hazards of refluxing at high temperatures, saves preparation time and simultaneously improves safety.

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REFERENCES

1 Prusoff W.H. Biochim.Biophys.Acta.,**32**:295-296(1959)

2 Prusoff W.H., Jaffee J.J., Gunther H. and Welch A.D..Proc.Amer.Assoc.Cancer Res., **3**:1,54(1959)

3 Prusoff W.H. Fed.Proc.,**18**:305(1959)

4 Fox B.W. and Prusoff W.H. Cancer Research, **25** :234-240(1965)

5 Commerford S.L. Nature(London),**206**:949(1965)

6 Dethlefsen L.A. Cell Tissue Kinet., **4** :123-138 (1971)

7 Bagshawe K.D. Cancer Treatment Reviews, **14** :397-399(1987)

8 Robins A.B. and Taylor D. Int.J.Nucl. Med. and Biol., **8** :53-63 (1981)

9 Hofer K.G.,Choppin D.A. and Hofer M.G. Cancer, **38** :279-287 (1976)

10 Santos O., Pant K.D., Blank E.W. and Ceriani R.L. J.Nucl.Med., **33** :8,1530-1541,(1992)

11 Keough W.G. and Hofer K.G. J.Lab.Compds and Radiopharm., **14** :1,83-90(1977)

12 Hughes W.L., Commerford L., Giltin D. et al. Fed.Proc.Chem.in Med., **23** :640-648(1964)

13 Massaglia A., Rosa U. and Sosi S. J.Chromatog., **17**:316-321 (1965)

14 Johnson T.B. and Johns C.O. J.Biol.Chem., **1**:305 (1905-1906)

15 Commerford S.L. Biochemistry,**10**:1993-1999 (1971)

16 Brownstone A.D. Nature,**199**:1285 (1963)

17 Bakker C.N.S. and Kaspersen F.M. Int.J.Appl.Radiat. and Isotop.,**32**:176-178 (1981)

18 El-Garhy M. and Stocklin G. Radiochem.Radioanal.Letters,**18**:5,281-290 (1974)

19 Baldwin R.M. Appl.Radiat.Isotop.,**37**,8,817-821(1986)