RESEARCH ARTICLE

# Development of Radiolabeling Methods for 5-Fluorouracil (5-FU) with Technetium-99m for Preclinical Nuclear Imaging (PNI)

**Kan Singh Shekhawat[1*], R.S. Lokhande[1], J.K. Bhagat[2]**

[1]School of Life & Basic Science, Jaipur National University, Jaipur, Rajasthan, India

[2]Department of Nuclear Medicine & PET-CT, Bhagwan Mahaveer Cancer Hospital & Research Centre (BMCHRC), Jaipur, Rajasthan, India

[*]**Address for Correspondence:** Mr. Kan Singh Shekhawat, PhD Scholar, Department of Chemistry, Jaipur National University, Bhagwan Mahaveer Cancer Hospital and Research Centre, Jaipur, India

**Received: 26 March 2017/Revised: 23 May 2017/Accepted: 28 June 2017**

**ABSTRACT- Background:** 5-Fluorouracil (5-FU) is a well known anti-metabolite and extensively used in chemotherapy. The search of target specific radiopharmaceutical is always on in the field of radiopharmaceutical and diagnostic nuclear imaging. In this study, we have developed the radiolabling methodology for the 5-FU with Technetium-99m for preclinical nuclear imaging evaluation of $^{99m}$Tc- labeled 5-FU.

**Methods:** We have used radio pharmacy lab and its equipments of Bhagwan Mahaveer Cancer Hospital & Research Centre (BMCHRC), Jaipur, Rajasthan, India. We have used simple basic radiochemical reactions for the radiolabeling of the 5-FU. 99 mTc pertechnetate, an elute from $^{99}$Mo-$^{99m}$Tc generator used for tagging. A series of high grade and analytical chemical were used and a few from outside without further purification e.g. L-Cysteine Monohydrae acid, $SnCl_2.2H_2O$, Normal Saline, Distilled water, pH meter. $SnCl_2.2H_2O$ was used for the reduction reaction. Post radiolableing paper and ITL chromatography techniques used for the standardization and characterization of the labeled radiopharmaceutical.

**Results:** We have observed >95.66% radiolabeling efficiency of 5-Fluorouracil (5-FU) with Technetium-99 m by the paper chromatography (PC) and instant thin layer chromatography (ITLC) radiochemical efficiency labelling techniques.

**Conclusion**: As per our observation and study $^{99m}$Tc-5-FU has a potential to be of becoming a useful tool for the Nuclear Imaging of solid tumor. The labeled drug can be used for the gamma imaging after conducting further clinical trials on animal and human.

**Key-words-** Instant Thin Layer Chromatography (ITLC), 5-Fluorouracil (5-FU), Paper Chromatography (PC), Preclinical Nuclear Imaging (PNI),) Radiolabeling

## INTRODUCTION

5-Fluorouracil is a well know drug for chemotherapy of various types of cancer. In the present study, we have established the radio labeling methods of 5-fluorouracil with $^{99m}$Tc by the different techniques of radiochemistry at radiopharmacy lab of Bhagwan Mahaveer Cancer Hospital & Research Centre (BMCHRC), Jaipur. In the previous reference work and research it is observed that the 99mTc-5-Flurouracil can be potential radiopharmaceutical for the cancer detection & nuclear and molecular imaging[1].

| Access this article online | |
|---|---|
| Quick Response Code | Website: www.ijlssr.com |
| | crossref DOI: 10.21276/ijlssr.2017.3.4.5 |

5-Fluorouracil is a pyrimidine analog that belongs to the antimetabolite family of pharmaceuticals. It is used as an anticancer drug in the chemotherapeutic treatment of various types of cancer as bowel, stomach, pancreatic, breast and esophagus cancer. [2] 5-Fluorouracil is involved in the metabolic activation of RNA to 5-fluoro-2'-deoxyuridine-5-monophosphate, it inhibits the enzymatic activity of thymidylate synthetase that is an important enzyme for DNA synthesis. [3]

In the present study, radiolabeling of 5-fluorourail will be radiolabeled by using $Na^{99m}TcO_4$ to use it as a potential candidate for the diagnosis of cancer. The intention was to deliver high doses of radiation to selected malignant sites in targeted tumor, while minimizing the radiation dose to surrounding healthy cells. [4]

As 5-Fluorouracil has already been in use for chemotherapeutic treatment of cancer, its pharmaceutical characteristics are well known. We, hereby, report a very simple and easy method of its radio labeling that is

performed under physiological conditions.

5-Fluorouracil, first synthesized in 1957, is one of the antitumor agents frequently used for treating solid tumors such as breast, colorectal and gastric cancers. 5-FU is poorly tumor selective and therefore its therapeutic use results in high incidences of bone marrow, gastro intestinal tract and central nervous system toxicity. [5]

To tackle these problems, numerous modifications of the 5-FU structure have been performed. Since the chemotherapeutic drug 5-Fluorouracil is used for treatment of various types of cancers, clinical studies can be a good tool for pre therapeutic screening line for the patient. This study was conducted to evaluate the radio metal labeling efficiency by the RCP. We have successfully radiolabeled the drug. The radiolabeled 5-FU can be good tool for nuclear and molecular imaging. We have done radio labeling of 5-FU by using the stannous chloride reduction method with Technetium 99m. There is trans-chelating procedure involved in this process in which radio metal trans-chelates to blood proteins, particularly albumin. [6]

So it was important to study *in vitro* blood protein binding with radiolabeled drug before it is applied to any organism. The percentage of drug binding with blood proteins tells us the efficacy of the drug in the body. The bounded drug remains in the blood stream and the free portion of the drug is extracted and called active part of the drug which may cause pharmacological changes. [7] This parameter was considered more important, when patient might be on other medication. Because certain proteins are already saturated, this may affect the binding of new drugs, thus changing its pharmacological effect.

Partition coefficient is an important parameter to be studied for evaluation of any drug. Hydrophilic and lipophilic characteristics of drugs highly affect the pharmaco dynamics properties of drugs). [8] The lipophilic characteristic of the drug affects their binding to the receptor targets. One drawback of lipophilic drug is that it tends to be toxic because of longer retention and wider distribution in body. While hydrophilic drugs show rapid clearance from body, it is highly recommended to synthesize a drug with high hydrophilic properties. These findings support the earlier findings in which 5-FU exhibited a high intracellular catabolite gradient. Prominent uptake was observed in the liver and the tumor specimens. [9] It shows that uptake of a radiotracer is dependent on various factors, like the nature of the complex, pH, blood flow, plasma concentration, etc., thus, indicating a slow transfer of charge metabolites formed across the cell membrane.

## MATERIALS AND METHODS

### Chemicals

We have procured 5-FU from Aura laboratories a kind of gift. The Acid L-cysteine hydrochloride monohydrate was purchased from Finar Chemical Ltd and Stannous chloride was purchased from Finar Chemical Ltd. We have synthesis the NaOH at our laboratory. The pertechnetate were taken from 99mMo/ 99mTc column generator supplied by Pars Tech, Iran. All other chemicals were also used analytical and reagent grade without further purification. e. g. distilled water, normal saline etc.

### Instruments

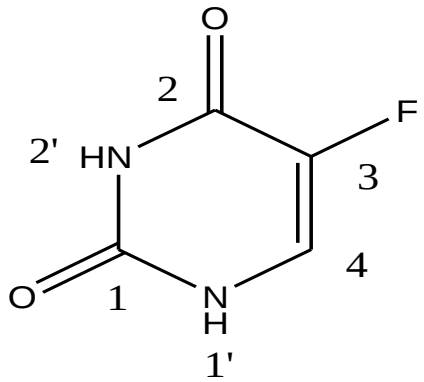
We have used some instruments and equipments from the Nuclear Medicine Department and Radiopharmacy Lab. We have imported Syringe filters from Merck Millipore Ltd, Ireland. The chromatography ITLC and Paper Chromatrography kits from BRIT, Mumbai. The Imaging and counting system, Symbia SPECT System from Siemens, Germany. Other instruments and equipments used from Radio pharmacy lab of nuclear medicine department of Bhagwan Mahaveer Cancer Hospital and Research Centre, Jaipur.

### Chemistry of 5-FU:

**Molecular Formula:** $C_4H_3FN_2O_2$

**Molecular weight:** 130.078g/mol

**Molecular Structure of 5-FU**:




(5-Fluorouracil)

**Fig.1:** Molecular Structure of 5-FU

## RESULTS

### Chemical Analysis of 5-FU

**MP**285.0$^0$C (dec), **MS** m/z 129.17($MH^-$). **FTIR** (KBr) NH (Stretching) at 3433.6 & 3417.2 ,C=O (Stretch) at 1723.1, C-N (Stretch) 1666.2, C-H (in plane) 1247.7, C-O 1180.2 in $cm^{-1.}$

**$^{13}$C NMR** (DMSO-$d_6$) δ: 150.370 (C-1), 158.463-158.207 (C-2, d), 141.306-139.050 (C-3, d), 126.709-126.388 (C-4, d). **$^1$H NMR** (DMSO-$d_6$) δ: 7.775-7.761 (H-4, d), 10.752 (NH-1', s), 11.626 (NH-2', s). D2O Exchange: The peak at 10.752 and 11.626 were exchanged.**$^{19}$F NMR** (DMSO-$d_6$) δ: The doublet peak at -172.092 to -172108 was Fluoro peak.

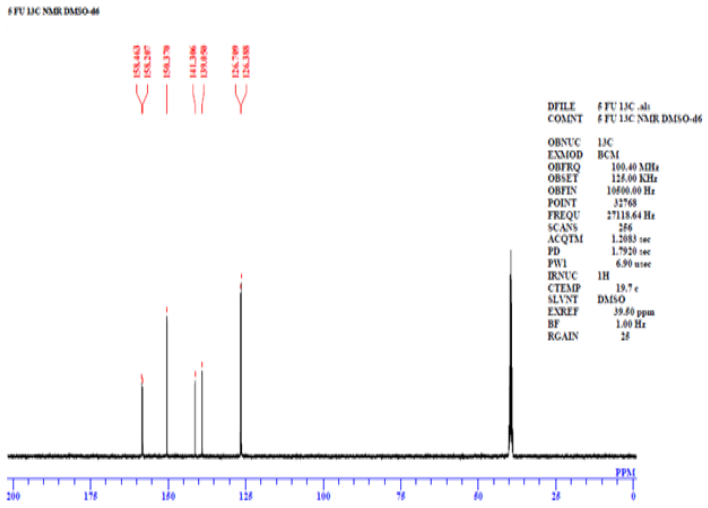


**Fig. 2:** $^{13}C$ NMR of 5-FU

## Radiolableing Procedures

Direct labeling approach involved use of radioisotope, drug of interest and an effective reducing agent. For direct labeling of 5-Flurouracil with Technetium-99 m stannous chloride was used as reducing agent. Fresh pertechnetate ($^{99m}TcO_4^-$) eluted from 99Mo/$^{99m}$Tc Column Generator, Pars Tech, Iran was used for the labeling procedures. The variable concentrations 1 mg to 20 mg of stannous chloride, different pH (2–9) conditions, and variable incubation times (2 minutes, 5 minutes, 10 minutes, 20 minutes and 30 minutes) were tested. $^{99m}$Tc-5-Flurouracil was prepared by disolving 100 mg of 5-Flurouracil in 100 mL distilled water, followed by the addition of a standardized concentration of 2 mg stannous chloride dihydrate and pH was established to 7.2.

The contents were filtered through a 0.22 µm membrane filter (Millipore) into a sterile vial. Aprox 40.0M Bq of pertechnetate was added to the 1 ml of mixture and incubated for15-20 minutes. The resultant radio ligand $^{99m}$Tc-5-Flurouracil was then subjected to various quality control tests.

## Radiopharmaceutical kit preparation steps

We were dissolved 100 mg of 5-fluorouracil in 100 ml of distilled water with continuous stirring and then added 50mg of L-cysteine hydrochloride monohydrate in it. Afterwards, 2mg $SnCl_2.2H_2O$ was added and pH was maintained at 7.2 by using 5 N NaOH, 2 N NaOH, 1 N NaOH and 0.1N NaOH. The product was passed through a 0.22 µm membrane filter. 1 ml/kit of resultant solution was dispensed in sterilized serum vials. 370 MBq in 0.5 ml of $Na^{99m}TcO_4$ eluted from$^{99}$Mo/$^{99m}$Tc Generator added to the kit (1 ml) and incubated at room temperature for 20 min.

## Quality control

The QC method can determine the percentage fraction of radioisotope bound to ligand, hydrolyzed portion, and amount of free pertechnetate. Acetone used as a mobile phase for paper chromatography and saline was used for ITLC. Small aliquots from the reconstituted kit were spotted on the respective strips. The strips, after elution, were cut in fractions of 1 cm and counted for radioactivity in a wipe test counter and RIA gamma counter.

## *In vitro* Quality Control Procedures

Radiochemical purity (RCP) of the $^{99m}$Tc-labeled 5-Fluorouracil studied by using 2 simple chromatographic techniques, e.g. Instant Thin Layer Chromatography (ITLC) and Paper Chromatography (3 mm Whatman paper). For quality control two methods were used viz. Paper chromatography and Instant thin layer chromatography.

## Paper chromatography (PC)

In the technique we have placed approximately 1 ml of acetone into one 10-mL glass vial and 1 ml of 0.9% NaCl in to an identical vial. In the second step, we have loaded a spot of radiopharmaceutical at the bottom of the whatman paper chromatography strip and marked the position of the same with a pencil line.

Developed paper strip in acetone solution for free pertechenatate and in 0.9% NaCl solvent for free hydrolyzed Tc, until solvent front migrated to top. We have cut strips into sections and counted all strips and sections for activity (per unit time) using a RIA gamma counter.

## Instant thin layer chromatography

The radiochemical purity of the labeled complex was determined by instant thin layer chromatography (ITLC) using 100% acetone and 0.9% sodium chloride as solvents. Briefly, 20.0 µL of radio metal complex was dropped onto the ITLC strip at the marked origin point and put into the solvent chamber at room temperature. The percent labeling of $^{99m}$Tc-5-FU was calculated at 15 minutes, 1 hour, 4 hours, and 24 hours by ITLC method. The percentage of free pertechnetate, hydrolyzed pertechnetate, and bound pertechnetate was calculated.

**Putative radio metal structure of $^{99m}$Tc-5-Flurouracil**

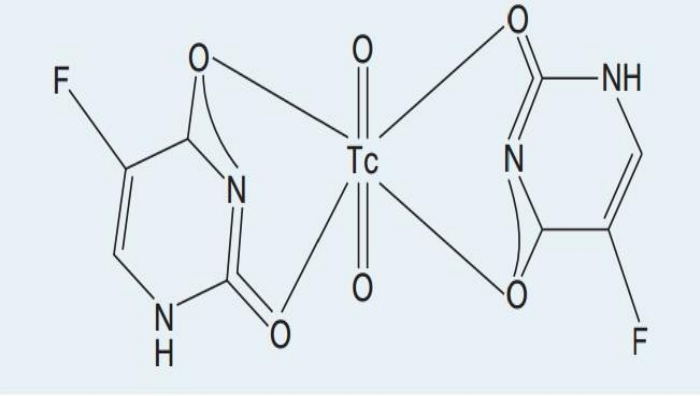



**Fig. 3:** Putative structure of $^{99m}$Tc-5-Flurouracil

**Table 1:** Radiolabeling Efficiency of 5-FU with $^{99m}$Tc

| TIME | PC radiolableing efficiency test (%) | | | | | ITLC radiolableing efficiency test (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **0 hr after Preparation** | 94.2 | 95.6 | 96.1 | 98.4 | 94.0 | 94.3 | 95.9 | 95.4 | 96.7 | 98.5 |
| **4hrs after Preparation** | 91.6 | 93.4 | 92.6 | 95.1 | 90.7 | 92.5 | 89.5 | 90.7 | 93.7 | 93.0 |
| | Avg. 95.66% for 0 hrs and after 4 hrs 92.68% | | | | | Avg. 96.16% for 0 hrs and after 4 hrs 91.88% | | | | |

## *In vitro* Stability at Room Temperature

*In vitro* stability of the 5-fluorouracil radio complex with $^{99m}$Tc was studied at room temperature at time intervals of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hr. The purpose to determine the *in vitro* stability was to detect any dissociation of the complex at room temperature. For this purpose, ITLC-SG and Paper Chromatography (PC) again was used as standard techniques. The percentage dissociation of the complex at a particular time interval was detected by the percentage of free pertechnetate at that time.

## Partition coefficient

200 µL phosphate buffer of pH 6.6, 7.0 and 7.6 taken in separate vials and the same quantity of octanol was added to $^{99m}TcO^{4-}$ had an Rf of 0.8–0.9, while the $^{99m}$Tc-ligand that $^{99m}$Tc labeled 5-fluorouracil shows maximum binding complex. The reduced or hydrolyzed $^{99m}TcO_2$ appeared at Rf= 0.00 – 0.01. The reduced or hydrolyzed fraction can be separated from $^{99m}$Tc-ligand complex by using saline, in which case the $^{99m}$Tc-ligand complex and free $^{99m}TcO_4$ appeared at Rf = 0.9–1.0, and reduced or hydrolyzed fraction ($^{99m}TcO_2$) was detected at Rf = 0.00–0.01. The overall labeling yield of $^{99m}$Tc-ligand complex, as calculated by these methods was more than 96.3±2.1%.

## *In vitro* stability of radiolabeled drug

*In vitro* stability of the radio labeled 5-fluorouracil was evaluated as a function of time by determining the amount of free pertechnetate and colloid formed with constant intervals of time up to 4 hrs.

It is observed that the labeling efficacy was > 93.5±0.6 % after 2 hr with minor fractions of free and colloid formation as per previous data. The high labeling efficacy shows the validity of labeling technique with the radio metal. These data indicate that the radio metal complex should be quite suitable for its further evaluation as a potential scintigraphic agent.

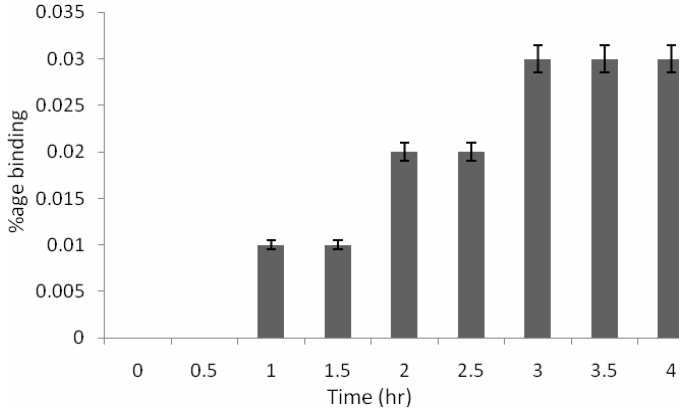



**Fig. 4:** Graphical presentation of increase in colloid formation (%) with time from earlier available data

## Partition coefficient

$^{99m}$Tc labeled 5-fluorouracil evaluated for its hydrophilic and lipophilic characteristics. Results performed in hydrophilic conditions (phosphate buffers of pH 6.6, 7.0, 7.6), it shows that the radiolabeled compound is hydrophilic in nature. Data are shown in the Table 2 for the Binding of radio metal-drug complex (%) in phosphate buffers and octanol.

**Table 2:** Binding of radio metal-drug complex (%) in phosphate buffers and octanol

| Sample | | Radiolabeling in hydrophilic layer (%±1.0) | Radiolabeling in lipophilic layer (%±0.2) |
|---|---|---|---|
| **Hydrophilic solvent** | **Lipophilic solvent** | | |
| Phosphate buffer pH= 6.6 | Octanol | 99.7 | 0.3 |
| Phosphate buffer pH= 7.0 | Octanol | 99.1 | 0.9 |
| Phosphate buffer pH= 7.6 | Octanol | 99.6 | 0.4 |

This shows that the radiolabeled drug is more hydrophilic in nature compare to lipophilic. This ability of the radiolabeled radio pharmaceutical reduces its radio metal toxicity which occurs after binding with blood protein and lipid.

## DISCUSSION

The presented study was aimed to develop such a radio metal or radiopharmaceutical which is able to detect the process of carcinogenesis at an early stage. We have utilized drug delivery efficacy of nano systems (albumin in indirect labeling), sensitivity of radionuclide $^{99m}$Tc and target specific ability of 5-Fluorouracil. The combined utilization or in the other words fusion of these promising areas resulted in the successful development of a unique radiopharmaceutical which will allow early detection of process of carcinogenesis.

The radiolabeling results showed >95.66 labeling efficacy of 5-fluorouracil with $^{99m}$Tc. The in vitro stability of the radiolabeled drug at room temperature for 4 hr of post-labeling was detected 91.88%. This radiolabeling approach does not require any additional ligand or any other assistance by foreign molecule. It involves the use of radioisotope, drug of interest and an effective reducing agent.

In the present study, stannous chloride was used as reducing agent for the direct labeling of 5-Fluorouracil with $^{99m}$Tc. The chemical and agent used in the trace amount, which is at the pecogram level. We tested various concentrations of both stannous chloride and drug, in order to achieve easy labeling with high efficiency.

In nuclear medicine, the process of labeling of cells and molecules with Technetium$^{99m}$ almost always requires the use of a reducing agent, since the elute obtained from the generator as the pertechnetate ion was not easily connect to other chemical species.

Standardization of radiolabeling method is done by the changing the concentration of reducing agent and other chemicals. The radiolableing efficiency in confirmed by the paper chromatography and the instant thin layer chromatography (ITLC) techniques. So, the present study, we concluded that target specific anticancer drug 5-Fluorouracil and its radiolabeled form more efficiently by reducing method of radio-labeling using stannous chloride.

## CONCLUSIONS

We were observed good radiolableing efficiency of radiolabeling of 5-fluorouracil with $^{99m}$Tc, >95.66% by PC and >96.16% by ITLC after preparation of radiopharmaceutical at 0 hrs. The in vitro stability of the radiolabeled drug at room temperature at 4 hrs of post-labeling was detected >92.68% by PC and >91.88% by ITLC. This newly developed radiopharmaceutical has promising avenues for early detection of deadly disease of cancer. Further, investigations are needed for clinical validation via animal experimental as well as clinical studies.

## REFERENCES

[1] Heidelberger C. On the rational development of a new drug: The example of the fluorinated pyrimidines. Cancer Treatment Rep. J., 1981; 65: 03-09.

[2] Jain RK. Delivery of molecular and cellular medicine to solid tumors. Microcirculation, 1997; 1: 01-23.

[3] Cvek B. Proteasome inhibitors. Prog Mol. Biol. Transl Sci., 2012; 109:161-226.

[4] Kumar P, Singh B, Sharma S, Mittal BR. Evaluation of $^{99m}$ Tc-Labeled Doxorubicin as a Potential Scintigraphic Probe for Tumor Imaging. Cancer Bio. Radio Pharm., 2012; 27(3): 221-26.

[5] Gerber DE. Targeted Therapies. American Family Physician, 2008; 77(3): 311-19.

[6] Huang WT, Yao HH, Lo JM. Preparation and biolocalization of technetium-99m complexes with $N_2O_2$ schiff-base compounds. Ann. Nucl. Med. Sci., 2004; 17: 13-19.

[7] Eisenberg D, McLachlan AD. Solvation energy in protein folding and binding. Nature, 1986; 319: 199-203.

[8] Hull WE, Port RE, Herrmann R, Britsch B, Kunz W. Metabolites of 5-Fluorouracil in plasma and urine, as monitored by 19F Nuclear Magnetic Resonance spectroscopy, for patients receiving chemotherapy with or without methotrexate pretreatment. Cancer Res.,1988; 48: 1680-88

[9] Scaglione F, Demartini G, Arcidiacono MM, Dugnani S, Fraschini F. Influence of protein binding on the pharmacodynamics of ceftazidime or ceftriaxone against gram-positive and gram-negative bacteria in an in vitro infection model. J. Chemother., 1998; (10): 29-34.



**How to cite this article:**

Shekhawat KS, Lokhande RS, Bhagat JK: Development of Radiolabeling Methods for 5-Fluorouracil (5-FU) with Technetium-99m for Preclinical Nuclear Imaging (PNI). Int. J. Life Sci. Scienti. Res., 2017; 3(4): 1132-1136. DOI:10.21276/ijlssr.2017.3.4.5

**Source of Financial Support:** Nil, **Conflict of interest:** Nil