International Journal of Basic and Applied Sciences, 3 (2) (2014) 78-85
©Science Publishing Corporation
www.sciencepubco.com/index.php/IJBAS
doi: 10.14419/ijbas.v3i2.1766
Research Paper

Isotopic exchange method for preparation of io-dine-125-n-(1-p-nitrobenzaldhydephenyl)-2-(2-iodophenyl)-4-(4-nitrobenzilidene)-imidazolin -5-one and its biological evaluation

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Abstract

N-(1-p-nitrobenzaldhydephenyl)-2-(2-iodophenyl)-4-(4-nitrobenzilidene)-imidazolin-5-one was labeled with radioactive iodine-125.via nucleophilic substitution reaction, the isotopic exchange labeling process was carried out in dry state at 330oC for 20 min. The radiochemical yield was determined by using electrophoresis and the yield is equal to 93%.Ammonium sulphate was used as catalyst to facilitate the exchange reaction in melt state. Maximum heated with iodine-125 at 330oC for 20 min in a reaction mixture with pH between 6 and 8. The results referred to the importance of use NaOH in a molarity of less than 0.02M to prevent the decomposition of the produced tracer due to the change in pH of the reaction mixture. The biodistrubution pattern of the labeled compound was examined in normal as well as in tumor bearing mice and the data show high clearance from the blood as the activity remind in the blood 11.2% at 4 hours post injection. The labeled compound was extracted via liver as the accumulated activity in the liver and intestine were 14.4, 15.4% respectively at 4 hours post injection The biodistrubution of labeled compound in tumor bearing mice accumulation in ascites with high percentage equal to 55.3 % at 24 hours post injection. This demonstrate the ability of this tracer concentrate in tumor site which primate its possible application as therapeutic agent.

Keywords: *Imidazoline Derivatives, Isotopic Exchange Method, Tumor Targeting, Tissues Distribution*.

1. Introduction

The nitroimidazole derivatives such as misonidazole and metronidazole have been shown to enhance the radio sensitivity of hypoxic cells. These compounds are also known to exert a toxic effect selectively on hypoxic cells. The hypo- xic cell toxicity may arise from the formation of reactive reduction products of the nitro compounds. Also, several imidazole derivatives have been developed as diagnostic agents for positron emission tomography (PET) or optical imaging [1-4]. Many imidazolines are biologically active [5, 6]. 2-imidazolines have been investigated as antihyperglycemic, anti-inflammatory, antihypertensive, antihypercholesterolemic, and antidepressant reagents[6, 7].The imidazole nucleus appears in a number of naturally occurring products among which, the most important are the amino acid, histidine , the purines which comprise many of the important bases in nucleic acids and hydration which occurs in beet sap. Also, In recent years, different imidazole and benzoimidazole drugs have been found to be associated with several biological activities such as antiparasitic, antifungal, anti-inflammatory, antibacterial activities [8], [9]. imidazole derivatives are reported to exhibit a wide range of pharmaceutical bactericidal[8] activities. 5-nitroimidazole (azomycine) is active against infections associated with anaerobic conditions. Subsequently, it was shown that changing the substitution pattern from the 5-nitro in nitroimidazole to the2-nitro in misonidazole (M1SO) increases the reduction of the molecule under slightly aerobic conditions. These compounds enter cells by diffusion. In the cytoplasm, the nitro group (NO2) undergoes one-electron enzymatic reduction to the free radical anion. In normoxic cells, this reaction step in reversed by intercellular oxygen, and the oxidized molecule diffuses out of the cell. In

hypoxic tissue, the free radical is further reduced to a reactive species, hydroxylamine and then to an amine free radicals are attached irreversibly to cellular macromolecules and are retained within the cell. Reduction of these molecules occurs in all tissues with viable enzymatic processes, hut retention occurs only in those tissues with low oxygen tension. In addition, radioiodinated imidazoline compounds were used in nuclear medicine[9]. In view of these findings, the author planned to synthesis imidazolinone derivative containing iodine atom in their structure then labeled with iodine (^{125}I).The optimum conditions required to produce high labeling yield with high purity was studied. In-vivo study of the labeled compound in non-tumor and tumor bearing mice was done to elucidate its biological behavior.

2. Experimental

2.1. Materials

Melting point was determined in an open capillary and is uncorrected. The IR spectrum was recorded on pyeunicam sp-11100 spectrophotometer. Mass spectrum was performed by a shimadzu Gc-MS-QP 100 Ex (shimadzu, Japan) Elemental analysis were carried out the Microanalytical Research Center, Faculty of Science, and Cairo University. All other chemicals were purchased from Merck Co. Radioactive iodine-125 was purchased from Institute of Isotopes Co. Ltd. (IZOTOP) Budapest, Hungary.

2.2. Procedures

N-(1-p-nitrobenzaldhydephenyl)-2-(2-iodophenyl)-4-(4-nitrobenzilidene) imidazolin-5-one was synthesized according to the procedure a solution of 1-(4- aminophenyl)–2(2-iodophenyl)-4-(4-nitrobezylidine)2-imidzolin-5-one (0.01mol) and p-nitrobenzaldehyde (0.01mole) in acetic acid (30ml.) containing freshly fused sodium acetate (0.01mole.) was refluxed for 2 hours. The precipitate was collected, and the solid obtained was crystallized from DMF as yellow crystals, (m.p 330-332^{o}C, yield 55%) as illustrate in figure 1. The structure was confirmed by I.R. Spectrum which revealed bands at 1655 cm−1(C=O), and 1621 (C=N) .1H-NMR spectrum of [46a, DMSO- d6] afforded signals at δ 3.87ppm (s, 3H, OCH3), 7.00 (s, 1H, methylene) and 7.12-8.32 (m, 16H, Ar-H) and 9.88 (s, 1H, CH=N).

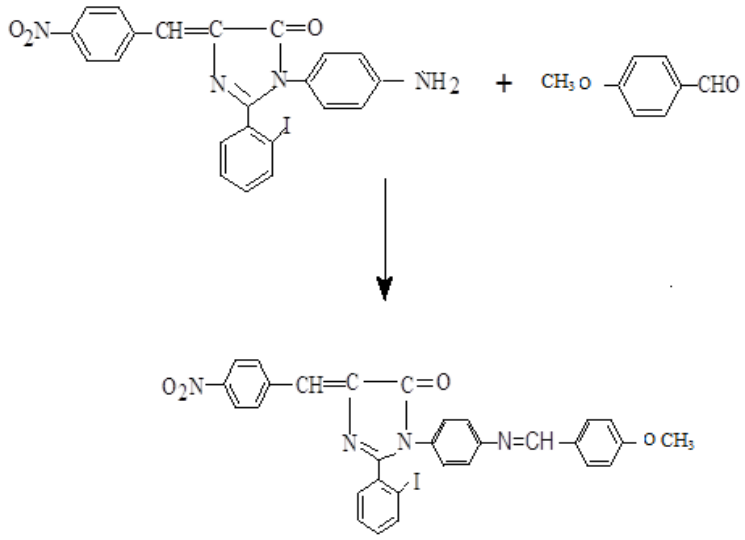

Fig. 1: Synthesis scheme of N-(1-p-nitrobenzaldhydephenyl)-2-(2-iodophenyl)-4-(4-methoxybenzilidene) imidazolin-5-one

2.3. Labeling technique

Labeling in dry state was carried out in a V shaped bottom vial with a screw cap. Radioactive sodium iodide [5µl, 100µCi] was introduced in the reaction vial and evaporated to dryness by a vacuum line, thereafter 5mg of imidazoline was added, and the reaction vial was closed and put in a sand bath at 300^{o}C. After a defined time the reaction was stopped by cooling the vial within an ice bath. The reaction mixture was dissolved in150 ml dimethyl formamide for analysis.

2.4. Determination of radiochemical purity of imidazoline 5-one.

The radiochemical purity was determined using the electrophoresis technique (EC 3000P-series 90 programmable (E-C apparatus corporation) power and chamber supply units using cellulose acetate strips. Cathode and anode poles and application points were indicated on cellulose acetate strips and these strips were moistened by buffer solution [0.05 M phosphate of pH 7]. They were putted in electrophoresis chamber after the samples set on the strips [5 µl]. Standing time and applied voltage for one and half-hour at 300 volts Developed strips were dried and cut into 1cm segments. They were counted by a well-type NaI (4%Tl) scintillation counter. The radio-histogram of the labeled compound was presented in figure 2.

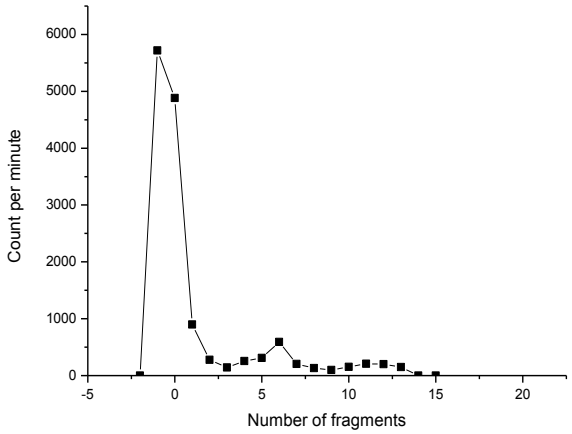

Fig. 2: Paper Electrophoresis Pattern of N-(1-p-nitrobenzaldhydephenyl)-2-(2-iodophenyl)-4-(4-nitrobenzilidene) imidazolin-5-one

2.5. Tumor implementation

2.2×106 cells (200 µl) of Erlich culture were implemented in the left leg of female Swiss Albino mice. The tumor volume was read every 3 days, until it reached 100mm2 as an example of hypoxic tumor. For non-hypoxic tumor, the same cell count was injected intraprotenial and the mice were kept for 10 days on normal diet in a metabolic cage until the growth was appeared.

2.6. Bio distribution studies

This experiment was done by diluting the neutral solution of the labeled imidazoline-one with 1 ml saline for injection ,and filtered through 0.22µm Millipore filter into a sterile sealed vial.100µl (250-300KBq) was injection, in the tail vein of the healthy and tumor bearing Albino mice, weighing approximately 25g each (3groups each of 3 mice). The mice were maintained on normal diet in a metabolic cage. The mice were sacrificed at 2h and 4h post injection and in addition at 24 h in the case of tumor bearing mice. Samples of fresh blood, bone and muscle were collected in pre-weighing vials and counted .The different organs were removed, counted, and compared to the standard solution of the labeled imidazoline -5-one. The average percent values of the administrated dose/organ were calculated. Blood, bone and muscles were assumed to be 7, 10 and 40% of the total body weight, respectively

3. Results and discussion

N-(1-p-nitrobenzaldhydephenyl)-2-(2-iodophenyl)-4-(4-nitrobenzilidene) imidazolin-5-one compound can be labeled with radioactive iodine through nucleophilic substitution reaction. This exchange reaction can be done in melt state . In melt state, the imidazolin-5-one compound must be stable at its melting point and has a high dielectric constant to dissolve the radioiodine. In our study imidazolin-5-one was labeled with radioactive iodine in melt state, and the factors that affect the labeling reaction were studied. The results of this study can be presented as follow.

3.1. Effect of Substrates Content

The dependence of ^{125}I- imidazolin-5-one yield on the amounts of imidazolin-5-one was studied. The results of this study are summarized in figure. (3), The data represented in the figure clearly show that both reaction yields and velocity increased with increasing the amount of imidazolin-5-one.The optimum amount of imidazolin-5-one sufficient to give radiochemical yield more than 93±2.2% was 5 mg when the reaction mixture was heated up to 330°C for 10 min. By increasing the time of heating up to 60 min. the radiochemical yield increased slowly by 5 %. This can be explained as 5 mg when melted, it gives volume enough to react with active iodine. Also figure 3. Show that

imidazolin-5-one compound more reactive for radioiodination which may be due to the presence of NO2 group which is more electrondonner than CHO group.

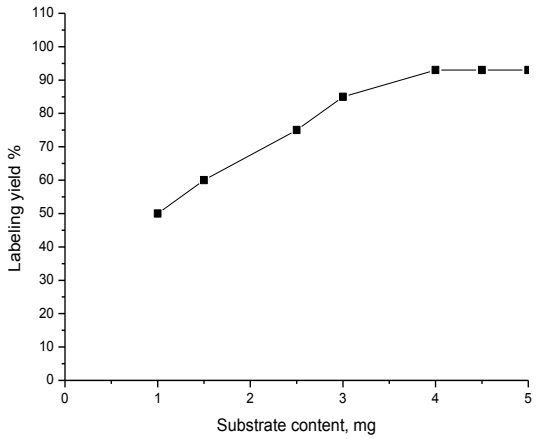

Fig. 3: Effect of imidazoline-5-one amount on the

Percent labeling yield of ^{125}I-imidazoline-5-one; reaction condition: X mg imidazoline, 5µl Na^{125}l, pH between 6 and 8 and reaction temperature 330^{o}C.

3.2. Effect of pH

The hydrogen ion concentration of the reaction mixture has a dramatic effect on the labeling of imidazolin-5-one with iodine-125 .Figure 4. Represent the data obtained from experiments. The data shows that the nucleophilic substitution reaction for imidazolin-5-one proceeded well around pH 6-8 giving radiochemical yield that exceeds 93±2.2%.

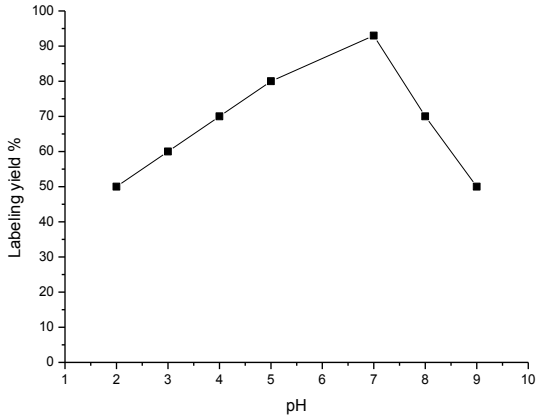

Fig. 4: Variation of the^{125}I-imidazoline-5-one yield as a function of pH; reaction condition: 4 mg imidazoline,5µl Na^{125}l, at different pH values and reaction temperature 330^{o}C.

3.3. Effect of reaction temperature

The ^{125}I imidazolin-5-one was prepared by an exchange reaction between radioiodine and the inactive one in the iodocompound in which iodine is bound to an aromatic carbon atom. The reaction proceeds by nucleophilic substitution of iodine atom via an intermediate where both radioactive and non-radioactive iodine atoms are symmetrically bound to the same carbon. The velocity of the reaction depends on the rupture of the C-l bond which is dependent on the temperature.

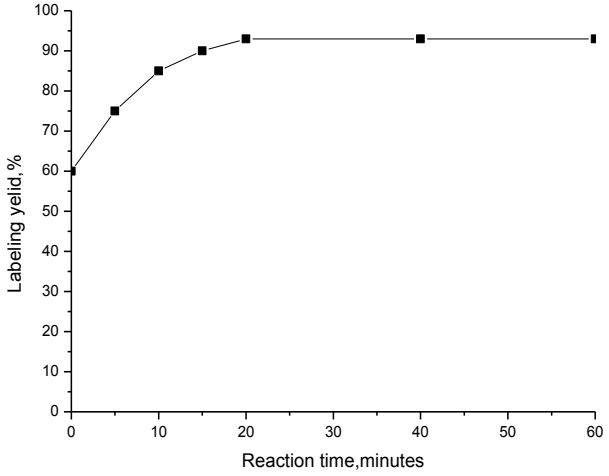

Fig. 5: Variation of the ^{125}I-imidazoline-5-one yield as a function of reaction time; reaction condition: 4 mg imidazoline,5µl Na^{125}I, at pH 7.The reaction mixture was heated at 330^{o}C for different reaction time.

There is a significant effect of temperature on the rate of this exchange reaction as cleared from figure 5. When the reaction was done at 330°C, the yield of ^{125}I- imidazolin-5-one was ~93% after 10 min reaction time. When the reaction temperature was decreased to 25°C, the yield of ^{125}I- imidazolin-5-one was very poor and not exceeds 20% even when the reaction time was extended to 50 min as cleared from figure 5. These findings can be explained, as in any chemical reaction the reactants must be in good contact with each other, so in melt state the reactants must be completely melted.

3.4. Effect of added amounts of ammonium sulphate catalysis

Depending on the fact that catalysis enhance the reaction toward product formation and can be reduce time of the reaction and also decrease the kinetic energy required to attain maximum yield, The use of ammonium sulphate as a catalysis arise from its role in the formation of H_2SO_4 as the result of its in-situe decomposition, that control the acidity of the reaction medium. Figure.6. represent the data obtain from the use of ammonium sulphate as a catalyst. In the labeling of imidazoline 5-one with iodine–125.The data clearly show that, the use of 5 mg of ammonium sulphate was sufficient to give yield more than 93±2.2% of ^{125}I- imidazoline 5-one. By increasing the quantity of ammonium Sulphate, the yield decreased drastically to less than 50% for both labeled compounds. This data can be explained as follow, in low concentration of ammonium sulphate (5mg), its dissociation is complete and become sufficient to attain the desired acidic pH required to proceed the reaction well and also the formation of volatile iodine is minimized. While in the case of large concentration of ammonium sulphate, the dissociation is reduced and also the formation of volatile iodine increased leading to poor radiochemical yield. The observed decrease in the radiochemical yield with the increase of ammonium sulphate was found in good agreement with the result obtained earlier on the labeling of 15 p-iodophenyl pentadecanoic acid and on the labeling of other iodoaromatics.

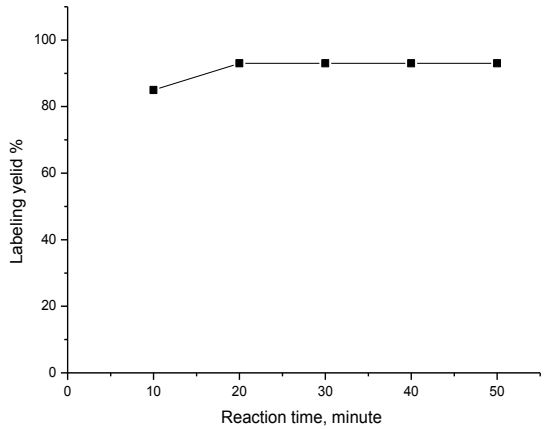

Fig. 6: Variation of the ^{125}I-imidazoline-5-one yield with time as a function of the amount of ammonium sulphate in dry state; reaction condition: 4 mg Imidazoline, 5µl Na^{125}I, 5 mg amm.sulphate, at pH 7.The reaction mixture was heated at 330^{o}C for different reaction time.

3.5. Effect of base

Because $Na^{125}I$ soluble in sodium hydroxide solution, the effect of the molarity of NaOH on the percent yield of ^{125}-I imidazoline 5-one was studied. The test was carried out by adding 0.01 to 0.5 M NaOH(10µl) to the $Na^{125}I$ in the reaction vial and the solution was evaporate under a vacuum, then the substrate was added. The reaction proceeds as usual; the data obtained was summarized in figure 7. Which show that NaOH solution had a marked deleterious effect on the labeling of imidazoline 5-one with iodine-125 even at molarity less than 0.02. By increasing the molarity of added NaOH up to 0.5 the yield of the labeled compounds decreased markedly to ~ 70 %. This assures the necessity to keep sodium Hydroxide molarity as low as possible.

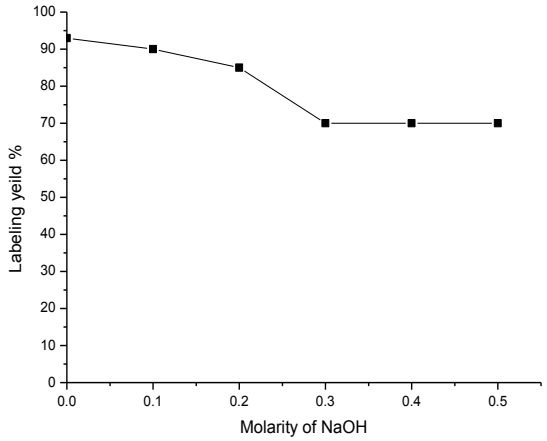

Fig. 7: Variation of the125I-imidazoline-5-one yield at different NaOH Molarities ; reaction condition: 4 mg imidazoline,5µl Na^{125}l, x µl 1M NaOH, at pH between 6-8 and reaction temperature at 330°C.

3.6. Biological study

The biological distribution pattern as well as the ability of the compound imidazoline 5-one to localized in cancer site and carry the radiotherapeutic nuclide iodine–125 to cancer site are examined in mice as described in recent literature [13]. The mice were injected intravenous with 0.1 ml of the tracers. The bio-distribution of ^{125} I- imidazoline 5-one tracer in normal mice was presented in table 1. Its clearance from the blood is high as the percentage reaches to ~11.2±1.8 % at 4 hours post injection. The high liver uptake of the tracer was attributed to their high lipophelicity. This finding was supported by determination of the octanal /saline partition coefficient as its equal to 78. Because the excretion route of the tracer was done via liver, the intestine content increased by time from 9.1±1.2 to 15.4±2.2% at 2 and 4 h. post injection receptively. Also, some ratios of this compound were excreted through the kidney, as activity detected in urine was 16.7±2.4. Otherwise, the uptakes of other organs (bone, muscle, lung, heart, and spleen) are with in normal values. The in-vivo decomposition of this tracer can be seen as the thyroid uptake increased by time from 1.4±0.2% to 3.4±0.9 % at 4 h post injection.

Table 1: Bio-distribution of ^{125}I- imidazoline5-one in normal mice Vial content: 4mg of imidazoline-5-one, 5µl Na125 I, 200µl DMF, at pH between 6 and 8 and reaction temperature=330°C

Organ or body fluid	Time after injection	
	2h	4h
Blood	16.4±2.2	11.2±1.8
Bone	1.4±0.2	0.9± 0.4
Muscle	0.2±0.9	0.6 ± 0.2
Liver	2.7±15.4	14.4± 2.6
Lung	0.1±0.4	1.1± 0.5
Heart	0.2± 0.6	1.1±0.4
Intestine	9.1 ± 1.2	15.4±2.2
Spleen	0.4±0.02	1.1±0.4
Stomach	13.4±1.9	9.1±1.5
Kidney	4.1±1.2	2.4±1.2
Collected-urine	10.4±1.2	16.7±2.4
Thyroid	1.4±0.2	3.4±0.9

Table 2: Bio-distribution of ^{125}I- imidazoline5-one tumor-bearing mice Vial content: 4mg of imidazoline-5-one,5µl Na^{125} I,200µl DMF at pH between 6 and 8 and reaction temperature=330^{o}C.

Organ or body fluid	Time after injection		
	2h	4 min.	24h
Blood	6.4±2.1	3.2±1.8	1.3±0.1
Bone	2.4±0.2	2.6±0.2	1.9±0.1
Muscle	1.6±0.1	0.6±0.2	0.3±0.1
Liver	9.4±2.5	9.2±1.2	0.0±0.0
Lung	0.4±0.1	0.5±1.1	0.3±0.1
Heart	0.2±0.2	0.3±1.1	0.0±0.0
Intestine	9.1±1.2	7.1±2.2	3.3±0.9
Spleen	0.3±0.5	0.2±0.3	0.9±2.1
Stomach	4.4±0.9	1.1±0.5	0.8±0.1
Kidney	0.3±1.2	0.1±0.2	0.7±0.1
Collected-urine	3.4±1.2	5.7±2.4	6.3±2.1
Thyroid	1.0±0.2	0.4±0.9	0.2±0.1
Ascites fluid	44.3±0.1	50.3±0.1	55.3±0.6.

The bio-distribution of ^{125} I-imidazoline 5-one in tumor bearing mice was examined and the data was presented in table 2. The uptake of the tracer in bone, muscle, lungs, heart, and spleen are similar to that of normal mice. Also, the increased uptake of liver, kidneys is due excretion pathway of these tracers. Total ascites weight represent 50.3±0.1% of the mice body weight catch high activity reaches to 44.3±0.1% at 2 h. post injection and increased markedly up to 55.3±0.6 % after 24h post injection. The localization of this tracer with this high percentage in the tumors site for this long period indicates the specificity of this tracer to the tumorized cell. This specificity can be attributed to the following factors; the high metabolic activity of the tumor cells, the high veciency correlated to the tumor and the presence of high concentration of hydrolytic enzymes.

All the obtained data demonstrate that, the tracer distributed rapidly throughout the body after intravenous injection (2 hours) and cleared rapidly through the hepatobiliary system (4 hours). The liver was the organ with highest radioactivity initially and activity was quickly excreted into the intestinal tract. The presence of small activity in the urinary bladder suggests the excretion of the tracers through the kidneys. Also, the biodistribution of the tracer in tumor bearing mice demonstrate the ability of use this tracer as imaging or therapeutic agent for cancer; but many biological studies are required to establish this findings as: examination in-vitro hypoxic tissue and quantitatively determination of how many tissue uptake from this tracer.

4. Conclusion

The optimum amount of imidazoline 5-one that is sufficient to give radiochemical yield more than 93±2.2 % was 4 mg when the reaction mixture was heated up to 330°C for 20 min in dry state. The substitution reaction for imidazoline compound was proceeded well when pH of the reaction medium is in the range of 6-8 ,At pH value lower than 6, the radiochemical yield decreases drastically reaches -35% with the formation of precipitate. The reaction temperature plays an important role in this substitution reaction. As the reaction takes place below the melting point (330°C), the reaction doesn't complete and the yield decreased markedly. Depending on the fact that catalysis enhances the reaction toward product formation ammonium sulphate was added to the reaction mixture. In spite of this the result obtained referred to the role of ammonium sulphate was clear during this substitution reaction, as 5 mg of ammonium sulfate was found sufficient to increase the radiochemical yield. It is also clear from data obtained that NaOH molarity must be kept low as possible as radiochemical yield decreased markedly to 60% when the molarity of NaOH was 0.5 M .The biological distributions of I^{125}-imidazoline-5-one showed marked localization in cancer site with percentage more than 55.3±0.6% at 24h-post injection. Also, the data show that the tracer cleared fast from non-target organs, as the percentage of the radioactivity detected in the blood was 1.3±0.1 % at 24-h. post injection

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