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Radioiodination of Cefoperazone with ^{125}I and its Biological Distribution in Mice

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Cefoperazone tracer was radioiodinated with ^{125}I by replacing the hydrogen atom with iodonium cation I^+ which is known as an electrophilic substitution reaction. All the reaction parameters were studied to produce maximum Radiochemical yield (RCY) of ^{125}I-Cefoperazone [^{125}I-Cefo] using 3.7 MBq of $Na^{125}I$, 150 µg of cefoperazone as substrate, 100 µg of Chloramine-T as oxidizing agent in water at 60ºC for 20 min, the RCY of ^{125}I-Cefo up to 90.2% The radiochemical yield was determined practically by electrophoresis 0.02M phosphate buffer with pH 7. A theoretical study was performed using Gaussian 09 to calculate the heat of formation of the expected products. The tracer was separated and purified by high-pressure liquid chromatography (HPLC) with phosphate buffer pH 6.8: methanol (3:1 v/v). The biological distribution of the tracer was studied in normal and infected mice, with E.coli bacteria, and sacrificed after different time's intervals. Activity in each organ was counted and expressed as a percentage of the injected activity per organ

Keywords: Cefoperazone/ Radioiodination/ Gaussian 09/ RCY

Introduction

One of the most broad-spectrum antimicrobial agents are third generation Cefalosporins [1], cefoperazone is a member of this family. It gives its bactericidal effect by inhibiting the bacterial cell synthesis [2]. It is mainly used for dealing with bacteria infecting the respiratory and urinary tract, skin, and the female genital tract. Cefoperazone is a broad spectrum antibiotic. It is used for targeting E.coli and Pseudomonas aeruginosa causing urinary tract infections [3]. Ciprofloxacin labeled with ^{99m}Tc was used for imaging infections and inflammation from decades, but the only restriction was the radiochemical stability and purity of the compound. A study was conducted to determine the optimum conditions of labeling of cefoperazone with ^{99m}Tc and the use of the labeled kit in imaging infection [4].

The objective of this work was oriented to label Cefoperazone with ^{125}I and the factors affecting the labeling yield were investigated. The suggested structure of ^{125}I- Cefoperazone which was formed via an electrophilic substitution reaction in the presence of Chloramine-T as an oxidizing agent; where $^{125}I^+$ for H^+ exchange in the ring is in ortho-position as illustrated in Figure (1).

Experimental

Materials and methods

All chemicals used in the present work were of analytical grade. Cefoperazone was obtained from Pharco Company, Egypt and used without any purification. $Na^{125}I$ (185 MBq/5 ml) in diluted NaOH, with specific activity >600 GBq/ml was purchased from Institute of Isotopes, Budapest, Hungary. The statistical analysis was performed using SPSS software, USA. Gaussian 09 is used

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for calculating the heat of formation of the expected product. E.coli obtained from the Microbiology Department of the Institute of Serum and Vaccines of cefoperazone followed by (25-300µg) of chloramine-T. Then 10 µL (3.7 MBq $Na^{125}I$) of NaOH solution was added and the reaction mixture was kept at different temperatures for different time periods. The different parameters that affect the radiochemical yield of ^{125}I-Cefo were studied.

Labeling of Cefoperazone with $Na^{125}I$

The experiment was performed by using (25-300µg)

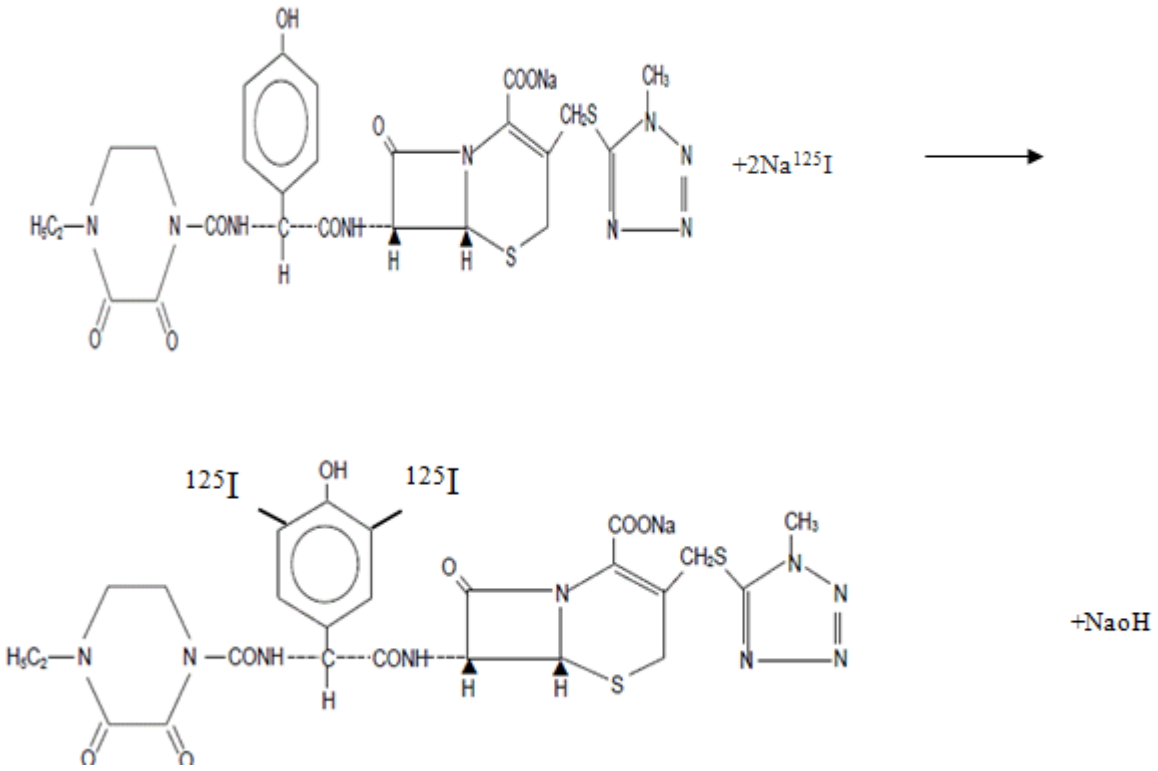

Fig (1): The expected reaction of Cefoperazone

Radiochemical yield and purity

The radiochemical yield was determined by Electrophoresis while the radiochemical purity was determined by HPLC.

Electrophoresis analysis

Electrophoresis was performed using cellulose acetate strips. These strips were moistened with 0.02M phosphate buffer pH 7 and then were placed in the chamber. Samples of 5 µL were applied. Standing time and applied voltage were continued for 60 min. Developed strips were removed, dried and cut into 1cm segments. They were counted using a well-type NaI (Tl) detector connected with a single-channel γ-counter. The analysis of samples from the reaction mixture resulted in two peaks as shown in Figure (2), one corresponding to the free iodide which move towards the anode about 12 cm distance while the second peak [^{125}ICefo] remains at the point of spotting , depending on their charge and ionic mobility. It gives RCY equal to 90.2%.

HPLC analysis

The RCY and the purity of Cefoperazone were determined by injection of 10 µL of drug sample into the column (Rp-18. 250.4 mm^2, 4-6 µm, Lichrosorb) built in HPLC Merck model 2010 Shimadzu model, which consists of pumps LC-9A, Rheohydron injector (Syringe Loading Sample Injector -7125) and UV spectrophotometer detector(SPD-6A) adjusted to the 254 nm

wavelength using phosphate buffer pH 6.8 : methanol (3:1 v/v) as mobile phase with a flow rate of 1ml/min [5] as shown in Figure (3).

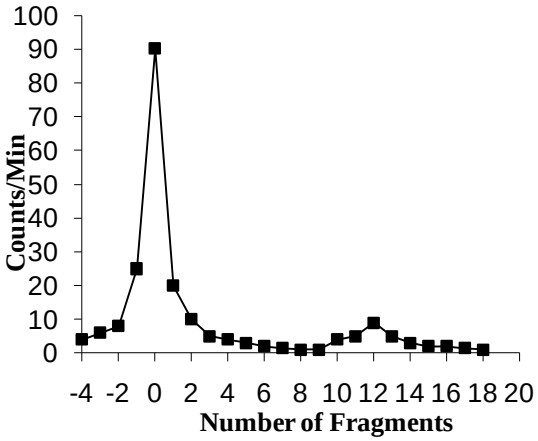

Figure (2) Electrophoresis of ^{125}I Cefoperazone

Biodistribution study

The biodistribution studies were carried out in a group of three male Albino mice. Each animal was injected into the tail vein with 0.2 ml solution containing 3.7 MBq of ^{125}I- Cefo. The mice were put in metabolic cages for the recommended time then sacrificed after (30 min, 1 h, and 3 h) post injection. The organs as well as other body parts and fluids were dissected. Activity in each organ was counted and expressed as a percentage of the injected activity per organ. The weight of blood, bone, and muscles assumed to be 7, 10 and 40% of the total body weight, respectively [6,7].

Results and Discussion

Effect of substrate amount

The radiochemical yield of ^{125}I-Cefo as a function of Cefoperazone amount was studied as shown in Figure (4). The results indicate that the maximum yield of ^{125}I-cefoperazone (90.2% at 150µg) had a significant percentage labeling yield, Other yields at different amounts of cefoperazone were significantly lower than the maximum yield at 150µg according to one-way ANOVA test with subsequent least significant difference (L.S.D) test ($P \leq 0.05$).A further increase in the amount of the substrate does not affect the radiochemical yield. This may be attributed to the fact that the yield reaches the saturation value (90.2%) because the entire generated iodonium ions in the reaction are captured at that concentration of Cefoperazone [8].

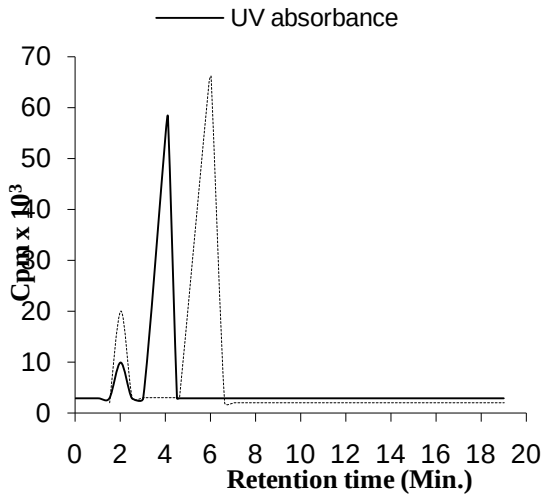

Figure (3) HPLC of Cefoperazone and ^{125}I Cefoperazone

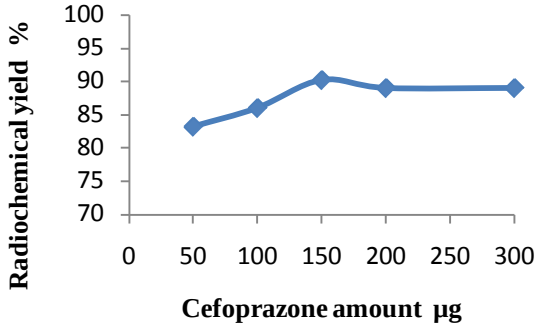

Figure (4): Variation of the radiochemical yield of ^{125}I-Cefo as a function of Cefo amount [100µg CAT and 10 µL of 3.7 MBq $Na^{125}I$, x µg Cefo] at 60°C for 20 min.

Effect of chloramine-T(CAT) amount

Sodium iodide is the simplest and versatile source of iodinating reagent for organic molecules [9]. Elemental iodine was formed by the oxidation of sodium iodide with oxidizing agents. Chloramine-T is the most common oxidizing agent used to produce H_2OI^+ and HOI from sodium iodide [10]. The influence of CAT amount on the radioiodination of Cefoperazone was studied as shown in Figure (5). One-way ANOVA with (L.S.D) test shows that the maximum percentage of labeling yield for ^{125}I-cefo (90.2% at 100µg of CAT) is significantly higher than yields at other amounts of CAT ($P < 0.05$) , 25µg and 50 µg of CAT was not sufficient to oxidize radioiodine ions in the reaction mixture [11]. The decrease in the yield has occurred when increasing CAT concentrations above 100µg due to the formation

of undesirable oxidative side reactions such as chlorination and polymerization [12].

Effect of pH

The effect of the hydrogen ion concentration of the reaction mixture on the radiochemical yield of ^{125}I-Cefo in presence of CAT as an oxidizing agent was studied. Different buffers system at pH ranging from 2 to 11were used.

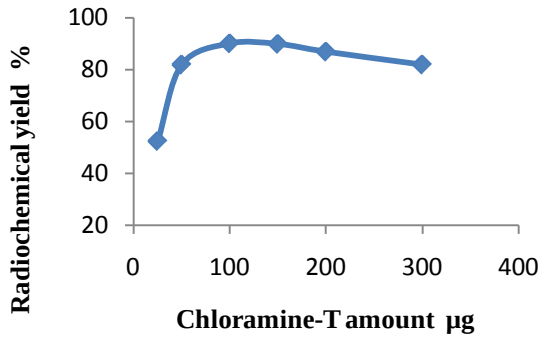

Figure (5): Variation of radiochemical yield^{125}I-Cefo as a function of Chloramine-T amount [150µg Cefo., (x µg) CAT and 3.7MBqNa^{125}I] at 60°C within 20 min

The results of this study were presented in Figure (6), the maximum yield for ^{125}I-cefo (90.2% at pH 7) is significantly higher than other yields according to one-way ANOVA test with (L.S.D) test ($P \leq 0.05$).

When the pH was shifted toward alkaline pH, the radiochemical yield of ^{125}I-Cefo decreased. This could be explained in terms of increasing the pH value leads to a decrease in HOI which is responsible for electrophilic substitution reaction [13].

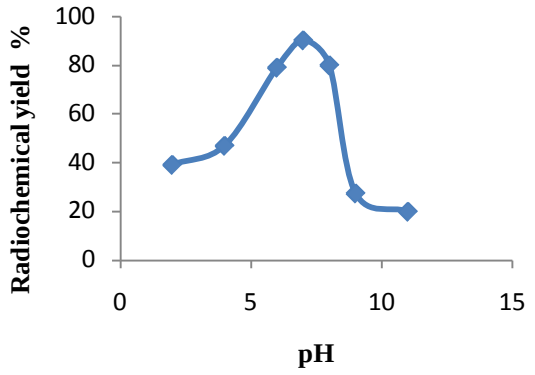

Figure (6): Variation of the radiochemical yield of ^{125}I-Cefo as a function of pH [150 µl Cefo, 100 µl CAT and Na^{125}I in 100µL of a buffer with different pH] at 60°C within 20 min.

Effect of the reaction temperature

The effect of the reaction temperature (25- 100ºC) on the radioiodination of Cefoperazone with ^{125}I was determined using the optimum concentration of both Cefo and CAT (150µg Cefo and 100µg CAT).The data indicate that the reaction temperature was found to be a significant factor affecting the radiochemical yield. As shown in Figure (7), the maximum yield for ^{125}I-cefo (90.2% at 60 ºC) is significantly higher than other yields at different reaction temperatures (room, 40 ºC and 100 ºC,) according to one-way ANOVA test with (L.S.D) test ($P \leq 0.05$).This may be attributed to the thermal decomposition of the labeled compound or to the distortion of the oxidizing agent [14].

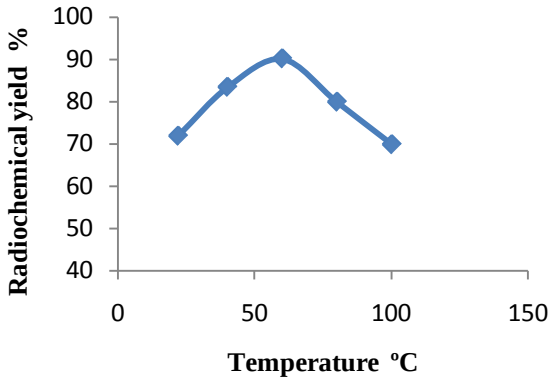

Figure (7) : Effect of the reaction temperature on the radiochemical yield using 150 µg cefo and 100 µg CAT in 60°C within 20 min.

Effect of reaction time

The radiochemical yield of ^{125}I-Cefo was determined at different time intervals using 150 µg cefo and 100 µg CAT at 60ºC as shown in Figure (8) which clearly shows that the maximum yield for ^{125}I-Cefo (90.2% at 20 minutes reaction time) is significantly higher than other yields at different reaction times (5 – 45 minutes) according to one-way ANOVA test with (L.S.D) test ($P \leq 0.05$).

Stability study

The stability of ^{125}I-Cefo was studied in order to determine the suitable time for imaging to avoid the formation of undesired radioactive products that result from the radiolysis of the labeled compound. These undesired radioactive products may be toxic or accumulated in undesired organ. Table (1) shows the stability of ^{125}I-Cefo, which was stable up to 24 h.

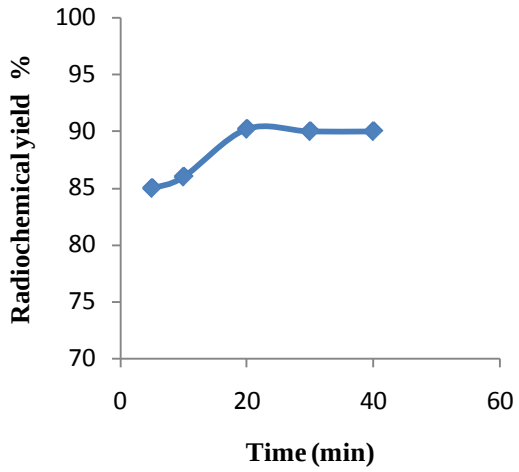

Figure (8) : Effect of the reaction time on the radiochemical yield using 150 µg cefo and 100 µg CAT in 60 ºC at X min.

Table (1) The in-vitro stability of [^{125}I]Cefo at room temperature at different time intervals ranging from 0.5-24h

Time h	Radiochemical yield %
0.5	90.2 ± 0.05
1	90.1 ± 0.2
2	89.5 ± 0.03
3	89.5 ± 0.14
4	88.7 ± 0.21
8	88.3 ± 0.33
12	87.3 ± 0.2
16	87.2 ± 0.43
24	87 ± 0.32

Biodistribution study

Biodistribution in normal mice was studied to elucidate the biological pathway of the tracer. Table (2) shows the data collected from the injection of 200 µl (3.5 MBq) of ^{125}I-Cefo intravenously (i.v.) in the tail vein of normal mice which were sacrificed after 0.5, 1 and 3 h post injection. Data shows that the blood uptake was 11% at 0.5 h and decreased to 1.7% at 3 h due to the binding of cefoperazone with plasma protein [13].The tracer was excreted through hepatobiliary and urinary route, about 15-36% of the dose was recovered in the urine[15] .The urine activity reached to 32% after 3h post-injection. The thyroid uptake was mostly ranged from 0.06% to 0.1% with- in 3 h indicating the stability of [^{125}I]Cefo in-vivo. A single clinical isolation of E.coli from bacteriological samples was used to induce focal gastrointestinal infection. Individual colonies containing 10^5-10^6 organisms were emulsified in saline using microbiological lope in sterile saline to obtain a turbid suspension. The infected mice were left for 48h then sacrificed also after 0.5, 1and 3h.

The data presented in Table (3) summarize the change in the biochemical parameters of the mice following the bacterial infection. All serological inflammatory parameters showed a significant rise in inflamed versus the normal mice.

It was found that the blood uptake was 18% at 0.5h and 5% after 3h. It clearly shows the high level of the tracer in kidney and urine, through which elimination up to 45% post injection occurs as shown in Table (4). This may be expected from the partial damage of liver with E.coli,[15] which eventually affect the hepatobiliary excretion pathway.

According to this high ratio in urine, this tracer (^{125}I cefo) can be used in imaging of urinary tract infections or disorder. We used the Student's unpaired test, statistical differences were assumed to be reproducible when $p<0.05$, the difference of the uptake of the tracer by the organs between the normal and the infected mice was found to be significant.

Theoretical part

Gaussian 09 [16] is used for optimization and calculate the energy of combination of each structure by using (b3lyp) method of calculation which is one of the density functional theory DFT [17] with basis set (3-21g). Figure (9-A) shows the cefoperazone after optimization without labeling by radioactive iodine 125 , Figure (9-B) shows the structure after labeling by radioactive iodine 125 in one ortho position as shown in Figure(9-B), Figure(9-C) is the structure after labeling by iodine in the other ortho position.

Figure(9- C) shows radioactive iodine in the two ortho positions. Such radioactive iodine inters cefoperazone according to the reaction which is shown in eq. 1, the heats of formations are calculated for each case (Figure 9-B, 9-C and 9-D) and the difference between reactant and products

are calculated for each case at 0 k and 298 k and this is shown in Table(5). According to these calculations, it is clear that the case of the competition of tow iodine atoms in the two ortho-positions is the more stable case because of it has the lowest value.

Table (2): Biodistribution of [^{125}I] Cefoperazone in normal mice (x ± S.D., N=3)

Organs & body fluid	Detected dose per organ percent at different time intervals post injection (min)		
	30 min	**60 min**	**180 min**
Blood	11 ± 0.2	5.8 ± 0.31	1.7 ± 0.2
Bone	2 ± 0.01	1 ± 0.3	0.12 ± 0.01
Muscle	5 ± 0.1	3.5 ± 0.02	1 ± 0.01
Heart	0.3 ± 0.02	0.4 ± 0.01	0.04 ± 0.01
Lung	0.3 ± 0.03	0.5 ± 0.04	0.13 ± 0.01
Stomach	7.7 ± 0.1	9.8 ± 0.2	11 ± 0.02
Intestine	40 ± 0.6	44 ± 0.2	50 ± 0.8
Liver	10 ± 0.3	8 ± 0.1	3.4 ± 0.12
Kidney	3.5 ± 0.04	1.6 ± 0.03	0.6 ± 0.01
Urine	20 ± 0.2	25 ± 0.9	32 ± 1.3
Spleen	0.2 ± 0.01	0.4 ± 0.01	0.07 ± 0.01
Thyroid	0.06 ± 0.02	0.09 ± 0.01	0.18 ± 0.07

Table (3) Results of inflammatory markers, liver functions, hematological parameters in normal and infected male albino mice

Groups Parameters	Normal mice	Infected	t-value	p-value	Significantly
C3, mg dL^{-1}	78.8±4.4	152.4±1.8	-34.6	<0.0001	*
C4, mg dL^{-1}	8.12±0.2	24.8 ±1.3	-28.7	<0.0001	*
CRP, mg dL^{-1}	2.8 ±0.2	6.1 ±0.5	-12.7	<0.0001	*
ESR(1st hour)	4.4 ±0.5	12.4 ±0.5	-25.3	<0.0001	*
ASAT,unitsL^{-1}	27.4±2.4	150 ±1.1	103.8	<0.0001	*
ALAT,units L^{-1}	27 ±2	92.6 ±2.5	-45.8	<0.0001	*
GGT,units L^{-1}	9 ±0.7	39.4 ±0.8	-63.9	<0.0001	*
WBCs	6.6 ±0.7	15 ±1.6	-10.7	<0.0001	*
Lymphocyte%	71.2±2.1	31.2 ±1.1	37.7	<0.0001	
Neutrophil%	23.2±1.9	60.8 ±0.8	-40.7	<0.0001	*

*C3: Complement component 3
*C4: Complement component 4
*ESR: Erythrocyte sedimentation rate
*ASAT: Aspartate aminotransferase
*ALAT : Alanine transaminase
*GGT : Gamma Glutamyl transpeptidase
*WBC : White blood cell

Table (4): Biodistribution of [^{125}I] Cefo in infected mice (x ± S.D.,N=3)

Organs & body fluid	Detected dose per organ percent at different time intervals post injection (min)		
	30 min	**60 min**	**180 min**
Blood	18 ± 0.4	12 ± 0.8	5 ± 0.2
Bone	4 ± 0.4	3 ± 0.3	1 ± 0.03
Muscle	15 ± 1	8 ± 0.8	3 ± 0.01
Heart	0.98 ± 0.02	0.8 ± 0.01	0.1 ± 0.01
Lung	0.9 ± 0.03	0.5 ± 0.04	0.2 ± 0.01
Stomach	8.4 ± 0.1	16.4 ± 0.2	21 ± 0.02
Intestine	15 ± 0.9	19 ± 1.1	21 ± 0.8
Liver	8.6 ± 0.3	6.5 ± 0.3	1.6 ± 0.02
Kidney	5.5 ± 0.04	3.6 ± 0.04	1 ± 0.01
Urine	22 ± 1.2	28.5 ± 0.9	45 ± 1.3
Spleen	1.5 ± 0.01	1.2 ± 0.01	1 ± 0.01
Thyroid	0.06 ±0.02	0.09 ± 0.01	0.18 ± 0.07

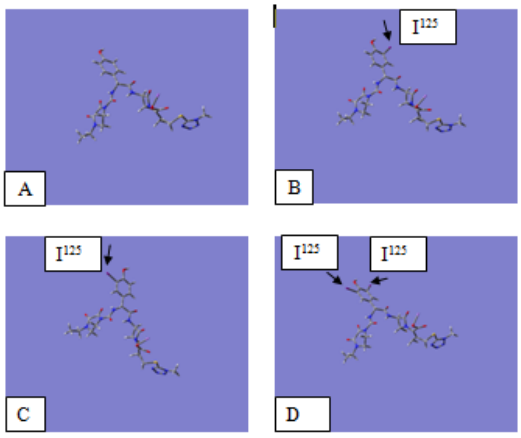

Figure (9A,B,C,D) : Structure of optimized cefoperazone before and after labeling with iodine as expected in different positions

Table (5): heats of formation of different structures of cefat 0 k and 298

	DfH°0	**DfH°298**
Case A	-37.983	-35.591
Case B	-29.915	-27.492
Case C	-65.530	-60.268

Conclusion

It can be concluded that Cefoperazone was successfully radioiodinated with iodine 125 via an electrophilic substitution reaction using CAT as an oxidizing agent. Maximum RCYs of [^{125}I] Cefoperazone was up to 90.2%. Tracer shows a good biodistribution in different organs, especially urinary tract, which recommends the utility of radioiodinated cefoperazone for imaging of inflammation in the urinary system. ^{123}I-labeling of cefoperazone may, therefore, be of interest.

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