Formulation and evaluation of a single vial lyophilized preparation of technetium-99m labeled ethylene dicysteine as a renal scintigraphic agent

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Abstract: ^{99m}Tc labeled N-N-ethylene-L, L-dicysteine (EC) was introduced in form of multiple-step kit as an alternate renal imaging radiopharmaceutical for commonly used ^{99m}Tc-MAG3. We developed a single component lyophilized kit of EC ready to be labeled with ^{99m}Tc. We present the optimization of the components of our formulation, its evaluation in animal models, normal human volunteers and patients of various renal diseases, in comparison with ^{99m}Tc-MAG3. Efficient labeling of EC was achieved with our preparation at pH 7 to 12. The formulation at pH 8 was used further for bio distribution studies in organs of sacrificed Sprague Dawley rats and a live rhesus monkey using scintigraphy. After satisfactory bio-distribution results, the kit was then evaluated in normal human volunteers through renography. But the renogram parameters of ^{99m}Tc-EC (pH 8) were statistically inferior to ^{99m}Tc-MAG3. Surrogate kit at pH 10 was therefore developed and re-evaluated in rats for organ bio distribution. After favorable results the kit was then assessed further in normal volunteers and a group of patients with various renal disorders via scintigraphy. The EC kit developed at pH 10 gave images better than and scintigraphic parameters comparable to ^{99m}Tc-MAG3. It was concluded that single vial kit we formulated easy to prepare than three-vial kit and can be used as an alternate to ^{99m}Tc-MAG3.

Keywords: Ethylene dicysteine (EC), mercapto acetyl triglyrine, renal scintigraphy, animal bio distribution.

INTRODUCTION

Renal radiopharmaceuticals are used to detect functional abnormalities of the kidneys during dynamic studies done under gamma camera. The agents used are either filtered through glomeruli or secreted through peripheral tubules of nephrons. The gold standard for scintigraphic evaluation of renal function to date is radioiodine labeled orthoiodohippurate (OIH), because of its unique renal handling characteristics. However, there are well-known drawbacks associated with the use of radioisotopes of iodine in humans (Britton, 2006). Currently the most popular agent, mercapto acetyl triglyrine (MAG3) labeled with ^{99m}Tc was introduced in 1986 (Fritzberg *et al*., 1986). Like OIH it is removed through the tubules and in the past two decades has exhibited a fairly good performance (Bubeck *et al*., 1990; Taylor *et al*., 1986). It however still falls short in many parameters (Jaffri *et al*, 1988). The quest for better alternates therefore remains active.

A promising ^{99m}Tc labeled compound N-N-ethylene-L, L-dicysteine (EC) was introduced for renal studies and is under evaluation (Verbruggen *et al*., 1992; Lima *et al*., 2010; Senthil *et al*., 2011). The kit is dispensed as three-vial assembly. The vials are used sequentially to finally obtain a labeled compound (Környei, 2007). An attempt was made in our department with collaboration of Isotope Production Division (IPD) of Pakistan Institute of Nuclear Sciences and Technology (PINSTECH) to prepare a practical single vial lyophilized kit for ready to use after reconstruction with pertechnetate ($^{99m}TcO_4^-$) solution. The formulations, quality control procedure, animal studies followed by its evaluation in human is presented here and compared with ^{99m}Tc-MAG3.

MATERIALS AND METHODS

Radiopharmaceuticals

The MAG3 used for the comparative studies was prepared in our laboratory using the methods formulated by Fritzberg *et al*., 1986. EC was synthesized by the method documented by Blondeau *et al*., 1967. Characterization was carried out using melting point determination, IR, NMR and elemental analysis.

Radiochemical Analysis of ^{99m}Tc-L,L-EC Complex

Radiochemical purity of the ^{99m}Tc-EC complex in terms of free $^{99m}TcO_4^-$ and reduced hydrolyzed technetium was assessed by paper chromatography, instant thin layer chromatography (ITLC) and high-performance liquid chromatography (HPLC) (Verbruggen *et al*., 1992; Környei, 2007). By ascending paper chromatography, (Whatman No. 1) 99.9% acetone (system-I) and 50% acetonitrile (system-II), the R_f values ^{99m}Tc-EC complex,

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$^{99m}TcO_2$ and $^{99m}TcO_4^-$ for system-I were 0, 0 and 1, while those for system-II were 1, 0 and 1 respectively. For ITLC 99.9% acetone (system-I) and 0.5 M acetic acid (system-II) were used. The R_f values were similar to paper chromatography. HPLC was done in reverse phase column RP-18 using gradient system 0.0125M phosphate buffer pH 2.5 (solution A) and ethanol solution 99.99% (solution B) as solvent system. Elution was obtained by using gradient 0 min 100% (A), at 10min 91% (A): 9% (B) and at 20 min 91% (A): 9% (B).

Effect of pH and Excipients on the Radiochemical Purity of ^{99m}Tc-EC Complex

To evaluate the effect of pH, freeze dried kits were prepared at pH values ranging from 7 to 12 keeping all other parameters constant. Similarly the optimal amounts of ascorbic acid, tartaric acid and stannous chloride in the final formulation was also assessed by optimization process i.e. the quantity of selected substance was altered and analyzed for the labeling yield of EC with ^{99m}Tc, keeping other parameters constant. Other ingredients were kept same as three vial preparation i.e., EC (2 mg), mannitol (30 mg), disodium ethylene-diamine-tetra-acetic acid (EDTA, 0.35 mg), Na_2HPO_4 (13.3 mg) and KH_2PO_4 (18.2 mg) in each vial (Környei, 2007).

The bulk solution was prepared at room temperature (20-25ºC0 under sterile condition with laminar flow under N_2 gas atmosphere. Ingredients mentioned were dissolved in deoxygenated water. N/20 NaOH was added drop wise to bring the pH close to 10. Then N_2 gas was purged into the prepared stock solution for 15 minutes and $SnCl_2.2H_2O$ (200 µg/100µL/vial) was added. The final pH was again adjusted to 8 or 10. N_2 gas was purged once aging for 5 min and then the stock solution was dispensed into the vials which were freeze dried for 48 hours.

Labeling of EC with ^{99m}Tc

The kit was stored in lead container at room temperature. Two milliliters of freshly eluted $^{99m}TcO_4^-$ solution (740 MBq/mL) from Amertech-II generator was added to the kit with previous elution carried no more than 24 hours prior to use. Having reconstituted the kit it was incubated for 15 minutes at room temperature before the radio pharmaceutical was checked for purity.

Stability of ^{99m}Tc-EC

Six kits of EC were labeled in the morning and incubated for 15 minutes. Paper chromatography was carried out at various intervals over a period 24 hours. Stability of six MAG3 kits was studied simultaneously.

Biodistribution studies in rats

The time course of organ distribution was evaluated in Sprague Dawley rats having average body weight of 300 g. Each radiopharmaceutical i.e., ^{99m}Tc-EC (pH8), ^{99m}Tc-EC (pH 10) and ^{99m}Tc-MAG3 was studied in a group of 5 animals. All animal studies were conducted in accordance with appropriate guide of animal care (National Research Council, 2011). Each animal was injected intravenously through tail vein with approximately 0.2MBq of each radio pharmaceutical in 0.2 mL solution. The rats were sacrificed with lethal dose of ether at 2 or 10 minutes after injections. The organs were excised, weighed and counted in a well counter.

Scintigraphic imaging in monkeys

A 10 year-old rhesus monkey (body weight 12 kg) was selected for both ^{99m}Tc-EC (pH 8 and 10) and ^{99m}Tc-MAG3 scintigraphy. Later was done one week after the initial imaging. The animal was anaesthetized by I/M injection of 100 mg ketamine hydrochloride and sustained by a 10 mg dose after every 25 minutes. An intravenous line was maintained throughout the procedure.

A single headed Siemen® integrated ORBITER™ gamma camera, interfaced with high-resolution parallel-hole collimator and connected with an online dedicated computer, was used for scintigraphy. One-minute images of full syringe before and empty syringe after injection were taken under the camera and input activity was determined by subtracting empty syringe counts from full syringe counts. The monkey was scanned in prone position. A three-phase dynamic study was acquired immediately after injection of radiopharmaceuticals using 64×64 matrix size. The data was acquired in the posterior projection with kidney and urinary bladder in the field of view. Sixty frames of one sec each were acquired in phase-I followed by 24 of five seconds in phase-II and 54 of 30 seconds in phase-III.

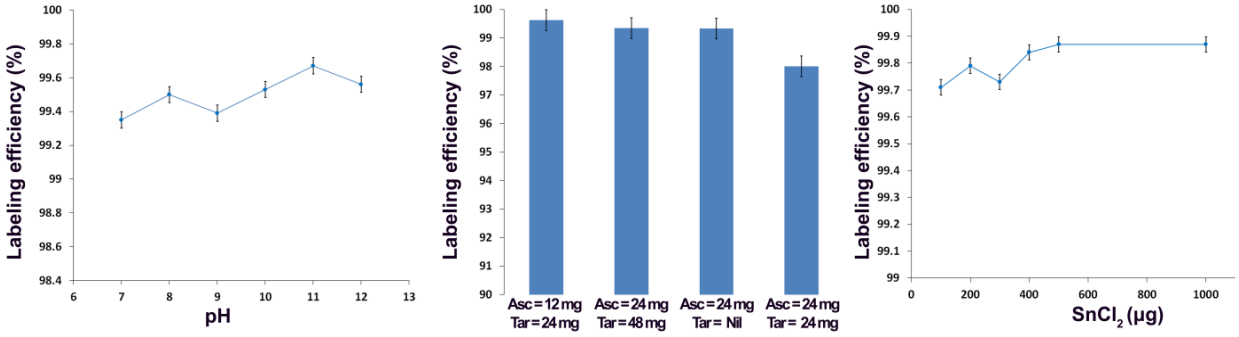

Fig. 1: Effect variation pH and amounts of ingredients on labeling yield of ^{99m}Tc-EC as seen during optimization

Regions of interest (ROI) were drawn over the kidneys with appropriate background subtraction and time to peak activity (T_{MAX}), time to half of peak activity ($T_{1/2}$) and percentage of activity at T_{MAX} to the injected dose (R_{MAX}) were calculated.

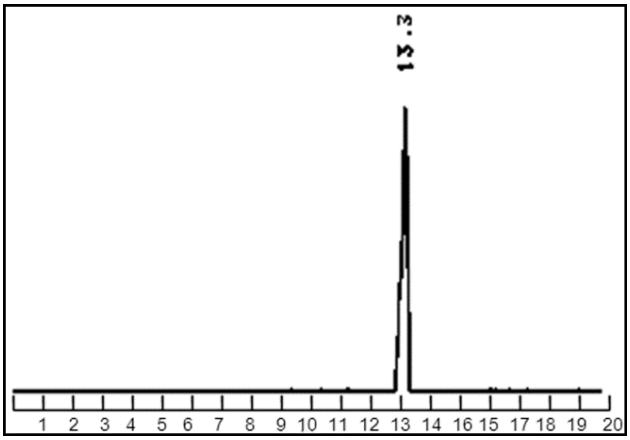

Fig. 2: HPLC of ^{99m}Tc-EC prepared at pH 8

Human Studies

A prior approval was obtained from the ethical committee of PIEAS to conduct the study in human population. Informed consents were acquired from all human subjects before the activity was injected.

Thirteen healthy volunteers with and age range of 23-70 years and weight 45-103 kg were studied first. Dynamic scintigraphy was carried out with ^{99m}Tc-EC prepared at pH 8 in four volunteers (eight kidneys); and at pH 10 in nine volunteers (18 kidneys). Another group of eight patients (14 kidneys) of various renal diseases but with normal serum creatinine levels was also studied with ^{99m}Tc-EC (pH 10) (table 1). Renal scans were repeated in all subjects with ^{99m}Tc-MAG3 one week after the first scan. The subjects were encouraged to drink 1-2 glasses of water before injection to ensure the hydration. Dose ranging from 95.5-148MBq of radiopharmaceuticals was given in anti-cubital vein followed by a flush of 10mL of normal saline. The acquisition of dynamic study was immediately initiated with protocol and positioning same as described in study of rhesus monkey.

The parameters computed during data processing were T_{MAX}, and $T_{1/2}$. Additionally residual activity at 25th minute to the activity at T_{MAX} (A_{25}/A_{MAX}) after appropriate decay correction was calculated manually for comparison of two agents.

The percentage of renal uptake was calculated in each case study with each renal imaging agent as a parameter of renal clearance by the following relationship (Itoh *et al*., 1996).

$$\%RU = \frac{C}{De^{-0.15d}} \qquad (1)$$

Where *RU* is renal uptake, *C* is 1-2 minute renal counts (corrected for background), *D* is total injection dose (corrected for empty syringe counts) and *d* is renal depth. The renal depth was measured by following regression equations for right (d_R) and left (d_L) kidneys.

$$d_R = 13.631(\frac{W}{H})^{0.6996} \qquad (2)$$

$$d_L = 14.0285(\frac{W}{H})^{0.7557} \qquad (3)$$

W and *H* are the weight and height of the patient.

For parameters like T_{MAX}, $T_{1/2}$ and residual activity standard error of mean (sem) was determined. Student's paired t test was applied to quantitative data to compare the means and p value ≤ 0.05 was considered as significant.

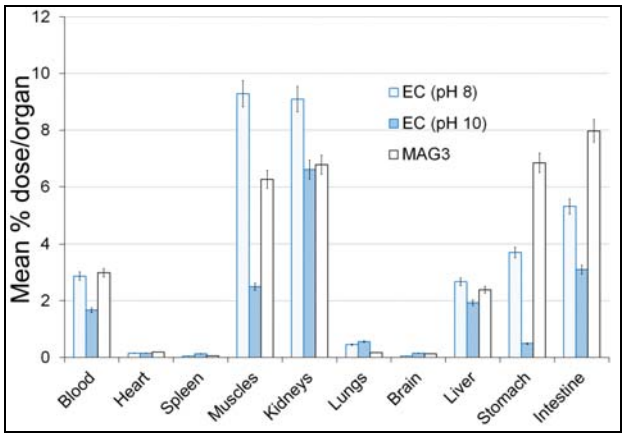

Fig. 3: Biodistribution results showing uptake of ^{99m}Tc-EC prepared at pH 8 and 10, and ^{99m}Tc-MAG3 in various organs of rats (n=5)

RESULTS

Chemicals

The analytical data obtained by paper chromatography and ITLC showed that the radiochemical purity of ^{99m}Tc-EC complex was >99.0% at pH values of 7 to 12 (fig. 1). Only traces of $^{99m}TcO_4^-$ and $^{99m}TcO_2$ were found. HLPC analysis (fig. 2) showed that the percentage yield of ^{99m}Tc-EC complex prepared at pH 8 was 99.05±0.5% and for that prepared at pH 10 was 99.95±0.16% with retention time (t_R) 13.42±0.50 minutes for both complexes. Value of t_R for free $^{99m}TO_4^-$ was 3.00±0.15 minute. The optimal amounts of ascorbic acid and stannous chloride were 24 mg and 200 µg respectively while adequate labeling was possible without tartaric acid in final preparation (table 2). The variations in labeling yields with change in these parameters are also given in fig. 1.

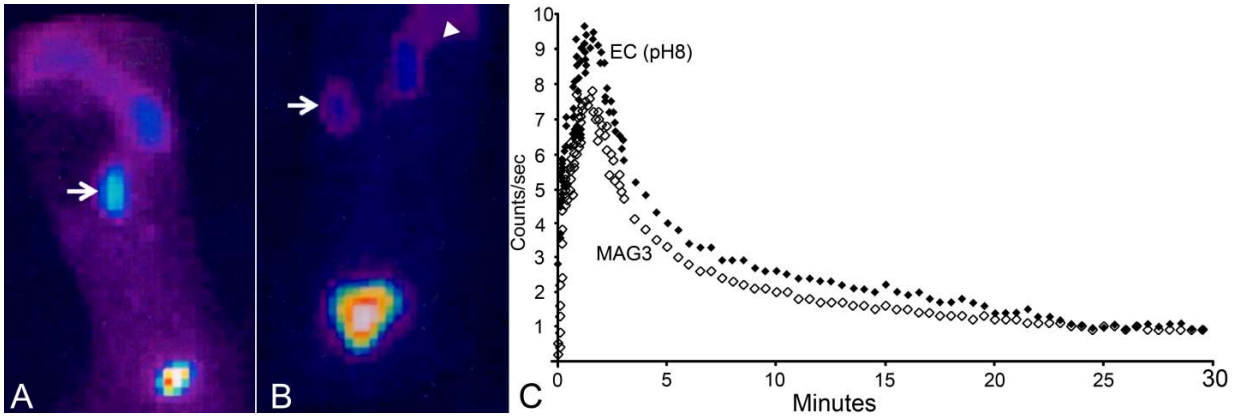

Fig. 4: Posterior views of lower abdomen of rhesus monkey, 10 minutes after injections of ^{99m}Tc-EC (A) and ^{99m}Tc-MAG3 (B) showing kidneys (arrows are left kidneys) and liver (arrowhead). Computer-generated renograms (C) of left kidney of rhesus monkey after IV injections of ^{99m}Tc-EC and ^{99m}Tc-MAG3

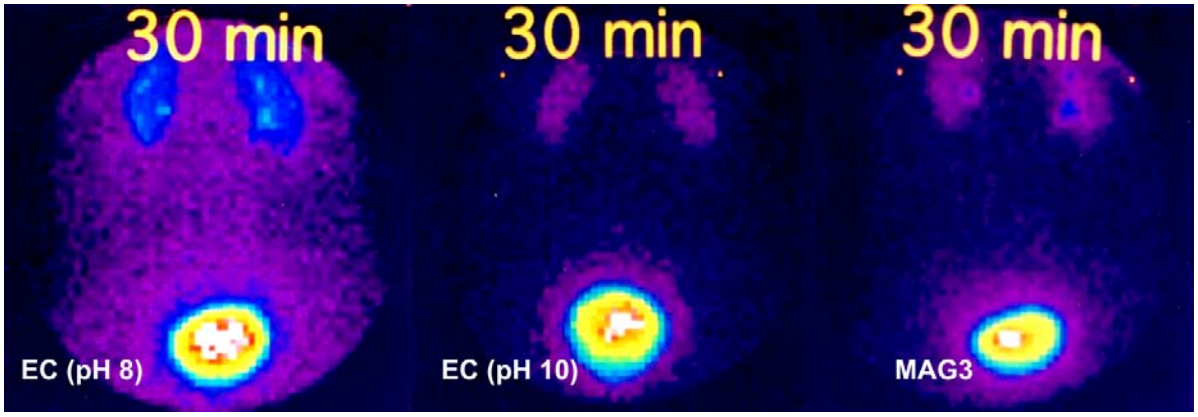

Fig. 5: 30th minute renal images in normal volunteers using the three radiopharmaceuticals studies

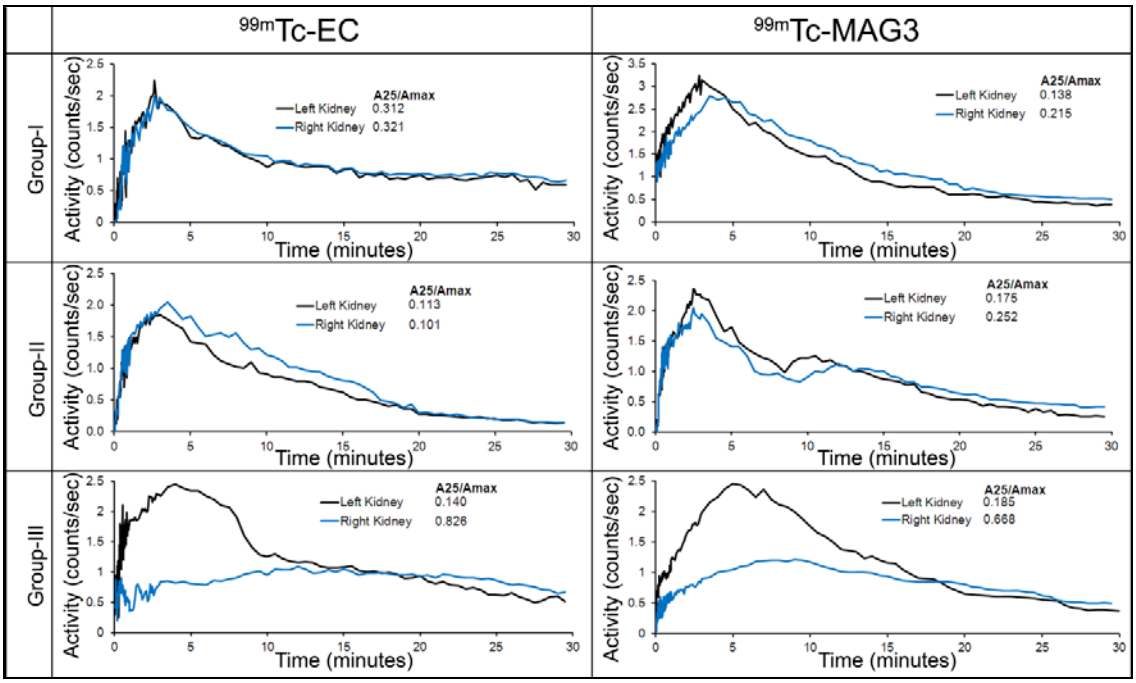

Fig. 6: Renograms of both kidneys of normal volunteers in groups I and II; and patient № 5 in group III using ^{99m}Tc-EC and their corresponding renograms using ^{99m}Tc-MAG3

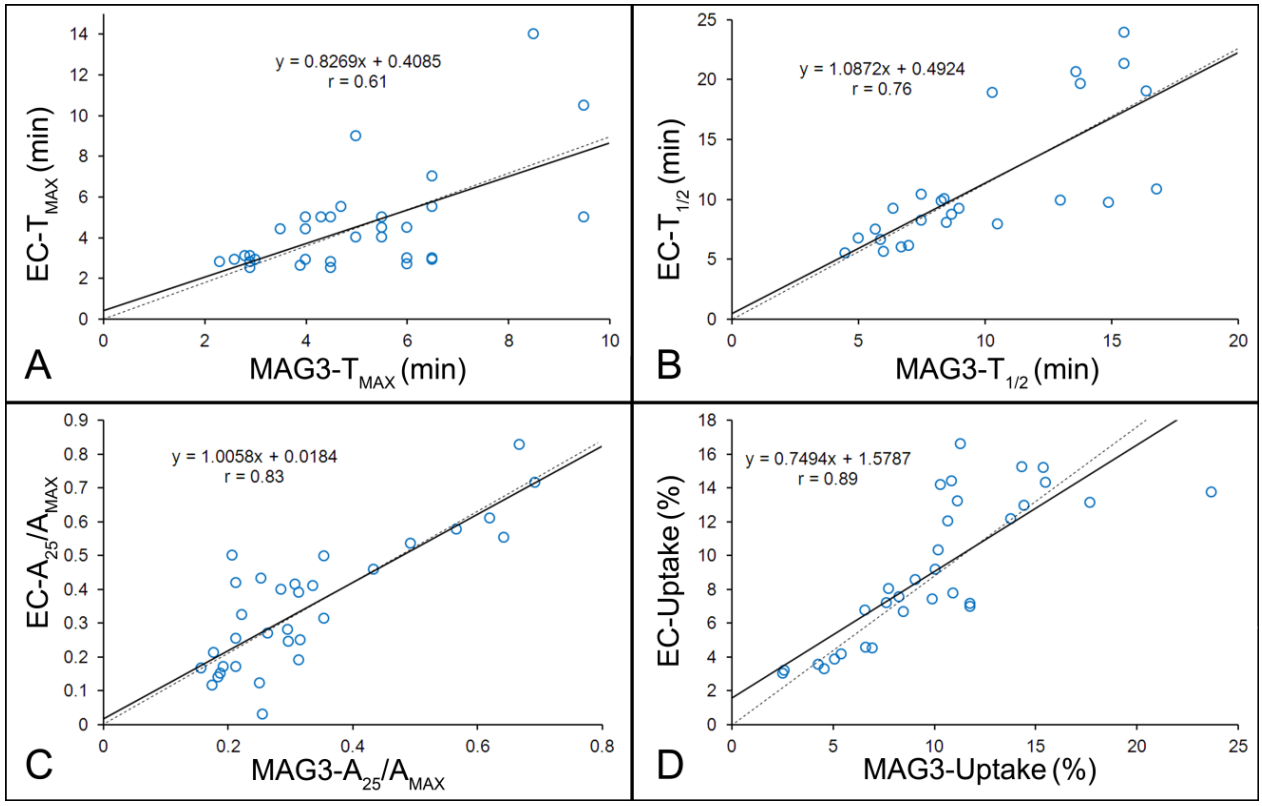

Fig. 7: Correlations of renogram parameters of ^{99m}Tc-EC (pH 10) and ^{99m}Tc-MAG3 in group II and III showing trend-lines (solid lines) and lines of identity (broken lines); (A) T_{MAX}, n=32; (B) $T_{1/2}$, n=25; (C) A_{25}/A_{MAX}, n=32; (D) % uptake, n=32

Table 1: Demographic profile of renal patients of group-III (n=8)

Pt. No.	Age (Yrs)	Sex	Height (cm)	Weight (kg)	Diagnosis	Sr. Creat. (mg/ml)
1	37	F	149	64	Rt. Ureteric calculus	0.9
2	32	F	163	71	Lt. Hydronephrosis	0.8
3	48	M	174	91	Rt. Hydronephrosis	0.6
4	41	F	149	59	Lt. Ureteric calculus	0.7
5	33	M	170	56	Rt. Ureteric calculus	1.0
6	32	F	148	51	Lt. PUJ obstruction and non-functioning Rt. kidney	0.9
7	24	M	177	87	Lt. PUJ obstruction and non-functioning Rt. kidney	1.0
8	59	M	176	70	Bilateral Ureteric calculus	1.1
Mean±SD	38.3±11.0		163.3±12.8	68.6±14.3		0.88±0.33

^{99m}Tc-EC complex was found to be stable even after 24 hours. The percentage yield of the labeled at 15 minutes was 99.67±0.53% which after 8 hours was still 99.59±0.60%. After 4 hours the labeling yield was found to be 98.40±0.55%. ^{99m}Tc-MAG3 complex was however less stable which after reconstruction was dropped down from 96.60±0.32% to 94.60±0.40% after 24 hours.

Biologicals

Biological studies in rats of ^{99m}Tc-EC complex prepared at pH 8 showed that the uptake of activity in blood and liver was almost similar to the uptake of ^{99m}Tc-MAG3 in these organs (fig. 3). The uptake of EC (pH 8) in stomach and GIT was lower while in kidneys and a muscle was higher than MAG3. ^{99m}Tc-EC at pH 10 however exhibited low accumulation in all the organs compared to ^{99m}Tc MAG3 except kidneys which showed almost equal uptake.

After satisfactory results of biodistribution in rats, ^{99m}Tc-EC (pH 8) was studied for renal images in a Rhesus monkey. Good quality images were obtained identical to ^{99m}Tc-MAG3 images acquired week later. The uptake in the liver was significantly less than ^{99m}Tc-MAG3. The

Table 2: Components of our single-vial EC kit compared with three-vial kit.

Single-vial EC kit		Three-vial EC kit [9]	
N,N,-Ethylene-L,L-dicysteine	2.0 mg	Vial A:	
Ascorbic acid	*24 mg*	N,N,-Ethylene-L,L-dicysteine	2.0 mg
Sodium EDTA	0.35 mg	Ascorbic acid	0.5 mg
Na_2HPO_4	13.3 mg	Sodium EDTA	0.35 mg
KH_2PO_4	18.2 mg	Na_2HPO_4	13.3 mg
Mannitol	30 mg	Mannitol	30 mg
$SnCl_2$	*0.2 mg*	Vial B:	
Tartaric acid	*Nil*	$SnCl_2$	0.2 mg
		Tartaric acid	48 mg
		Ascorbic acid	12 mg
		Vial C:	
		$SnCl_2$	0.2 mg
(Amounts of ingredients in italics were optimized in this study)		KH_2PO_4	18.2 mg
		Ascorbic acid	12 mg

renograms of the two agents are shown in fig. 4 and their parameters are given in table 3. No adverse effects were seen in monkey.

In group-I of four human volunteers, ^{99m}Tc-EC (pH 8) showed higher background activity than corresponding ^{99m}Tc-MAG3 (fig. 5). The downslopes of renograms in this group were also sluggish for ^{99m}Tc-EC (fig. 6). table 4 shows that most renogram parameters of ^{99m}Tc-EC (pH 8) were statistically different from ^{99m}Tc-MAG3 (p-values<0.05).

Table 3: Renograms parameters of renal study in rhesus monkey

Parameter	^{99m}Tc-EC (pH 8)		^{99m}Tc-MAG3	
	Rt	Lt	Rt	Lt
T_{MAX} (min)	1.25	1.25	1.33	1.30
$T_{1/2}$ (min)	4.05	4.13	3.90	3.93
R_{MAX} (% of counts at T_{MAX} to ID)	8.87	8.73	6.67	7.60

Further human population was therefore studied with ^{99m}Tc-EC (pH 10). In nine normal volunteers (group-II), the background of ^{99m}Tc-EC was similar to ^{99m}Tc-MAG3 while there was no significant difference in renogram parameters. The performance of the two agents was also similar in eight renal patients studied (group-III). The correlations of various renogram parameters in combined human subjects of group-II and group-III is given in fig. 7.

DISCUSSION

In the current study we made an attempt to formulate a single vial EC kit ready to be labeled with radioactive technetium and used as renal imaging agent. The kit we formulated was easy to prepare and unlike ^{99m}Tc-MAG3 did not require a boiling step. The amounts of legend (i.e. EC, 2mg), mannitol (30mg), EDTA (0.35mg), Na_2HPO_4 (13.3mg) and KH_2PO_4 (18.2mg) in our single vial kit were kept same as reported in three-vial formulation (Környei, 2007). Optimal levels of pH, ascorbic acid, tartaric acid and stannous chloride were however determined for this single vial kit. Although labeling efficiency at all pH levels was >99%, however it was lowest at pH 7 (99.35%) and highest at pH 11 (99.67%). Kit at pH 8 with labeling efficiency of 99.5% was selected for further studies due to its closeness to blood pH and slightly better labeling than that at pH 7.

Technetium-99m (VII) obtained from its generator is in the form of pertechnetate ($^{99m}TcO_4^-$). Its reduction to lower oxidation states is a prerequisite for formation of ^{99m}Tc complex with chelating agents in high yield and purity. The choice of a suitable reducing agent for single-step labeling and at mild pH conditions is a major research effort (Spies *et al*., 2007). The three kit of EC labeling with ^{99m}Tc contains stannous chloride, ascorbic acid and tartaric acid as reducing agents. We optimized the amounts of these agents by assessing the labeling yields with variations in their quantities in the aliquot (fig. 1). Preparation with 24 mg ascorbic acid without tartaric acid gave labeling yield of 9.33±0.11%. Optimal amount of $SnCl_2$ was found to be 200µg. The final ingredients in our single-vial preparation are summed up in table 2 in comparison with three-vial kit.

After reconstitution the kit remained stable throughout the day. The results of bio distribution ^{99m}Tc-EC (pH 8) in rats (fig. 3) showed that it was comparable to ^{99m}Tc-MAG3 with even higher uptake in the kidneys. It was therefore evaluated further in higher animals. Parameters of dynamic renal study done in monkey, using ^{99m}Tc-EC (pH 8) were in agreement with the parameters of ^{99m}Tc-MAG3. It however fell short of ^{99m}Tc-MAG3 in renogram parameters in first four normal volunteers (group-I). ^{99m}Tc-EC was therefore reformulated at pH 10 which exhibited even better bio distribution in rats. Further human subjects (group II and group III) were studied with

Table 4: Comparison of parameters of renal studies in groups of human population studies with ^{99m}Tc-EC (pH 8), ^{99m}Tc-EC (pH 10) and ^{99m}Tc-MAG3

	T_{MAX} (min)	$T_{1/2}$ (min)	A_{25}/A_{MAX}	1-2 minute uptake (%)
Group-I (n = 4)				
^{99m}Tc-EC (pH 8)	5.71±1.37	9.30±1.27	0.574±0.089	5.60±0.77
^{99m}Tc-MAG3	4.91±1.36	6.58±1.05	0.168±0.013	8.91±0.73
p value	0.047	0.034	0.002	7.5E-05
n	8	8	8	8
Group-II (n=9)				
^{99m}Tc-EC (pH 10)	3.97±0.39	7.86±0.42	0.292±0.034	9.27±0.98
^{99m}Tc-MAG3	4.26±0.31	7.81±0.72	0.290±0.031	10.61±1.09
p value	0.58	0.92	0.93	0.13
n	18	16	18	18
Group-III (n=8)				
^{99m}Tc-EC (pH 10)	4.99±0.70	17.03±1.47	0.419±0.059	8.8±1.2
^{99m}Tc-MAG3	5.91±0.56	13.48±0.71	0.375±0.047	9.2±1.2
p value	0.09	0.06	0.57	0.20
n	14	14	14	14

this formulation. None of EC formulations showed any untoward effect in animal trials.

T_{MAX} extracted from renogram represents the uptake of the radio Pharmaceutical by the renal parenchyma with a slight component of flow through the nephron. While $T_{1/2}$ depends upon the swift flow of the tracer through the tubules of nephron, collecting duct, outflow though renal pelvis and ureter. High A_{25}/A_{MAX} values suggest either higher retention of the tracer in renal parenchyma or low uptake. 1-2 minutes uptake represents the clearance of the tracer.

Higher T_{MAX}, $T_{1/2}$ and A_{25}/A_{MAX} values of ^{99m}Tc-EC (pH 8) in four normal volunteers shown in table 4 indicated significant retention of the tracer in renal parenchyma compared to ^{99m}Tc-MAG3. Low 1-2 minute uptake also suggested lesser rate clearance of the former agent. ^{99m}Tc-EC at pH 10 was therefore used for further studies. All the parameters of ^{99m}Tc-EC prepared at pH 10 however had no statistical difference in normal volunteers as well as patients ($p>0.05$). They also correlated well with each other (fig. 7).

Verbruggen *et al*. in 1992 compared bio distribution of ^{99m}Tc-EC and ^{99m}Tc-MAG3 in mice and reported renal accumulation of 4.87±2.48% and 6.67±2.28% of injected dose respectively. In our study 9.09±1.85, 6.61±2.92 and 6.78±2.69 of ^{99m}Tc-EC (pH 8), ^{99m}Tc-EC (pH 10) and ^{99m}Tc-MAG3 respectively was taken up by the kidneys. The higher accumulation of ^{99m}Tc-EC (pH 8) was perhaps because of retention of activity in renal parenchyma. This was evident from lower blood levels of ^{99m}Tc-EC (pH 8) i.e. 2.87±0.93 of ^{99m}Tc-EC (pH 8) vs 1.67±1.80 of ^{99m}Tc-EC (pH 10).

During studies in rhesus monkey, activity of ^{99m}Tc-EC (pH 8) in the liver was significantly lower than ^{99m}Tc-MAG3 indicating kidneys as the only major route of clearance from the blood. Renogram parameters of ^{99m}Tc-EC were also comparable with ^{99m}Tc-MAG3. The percentage of the injected dose of ^{99m}Tc-EC taken up by the kidneys at T_{MAX} (table 3) was higher than ^{99m}Tc-MAG3 and in agreement with the R_{MAX} value of 8.20% reported by Verbruggen *et al*., 1992.

In the first four human volunteer (group-I, table 4), ^{99m}Tc-EC (pH 8) performed inefficiently compared to ^{99m}Tc-MAG3 ($p<0.05$). Its lesser 1-2 minute uptake was due to its lower renal clearance than ^{99m}Tc-MAG3. This was also seen as higher background of the tracer (fig. 7). The fig also showed higher visibility of kidneys at 30 minutes in case of ^{99m}Tc-EC due to retention in renal parenchyma. This was supported by higher $T_{1/2}$ and A_{25}/A_{MAX} values of ^{99m}Tc-EC (pH 8). ^{99m}Tc-EC (pH 10) in group-II however showed all reno gram parameters similar to ^{99m}Tc-MAG3 ($p>0.05$). The 30-minute images of this tracer were also comparable with ^{99m}Tc-MAG3. Comparisons of other parameters like renal clearance in normal human volunteers have also been successful (Van Nerom *et al*., 1993).

We also evaluated ^{99m}Tc-EC (pH 10) in patients of various renal diseases and compared it ^{99m}Tc-MAG3. All the renogram parameters of ^{99m}Tc-EC were not statistically different from ^{99m}Tc-MAG3 (p-value>0.05). In a group of 25 renal transplant patients studied with two vial of ^{99m}Tc-EC at pH 12 Stoffel *et al* showed similar results (Stoffel *et al*., 1994). Parallel performance of both the radio Pharmaceuticals has also been documented in many other studies (Gupta *et al*., 1995; Kibar *et al*., 1997; Prvulovich *et al*., 1997; Ozker *et al*., 1994).

CONCLUSIONS

The single vial kit that we formulated at pH 10 gave good labeling yields, does not require boiling step like ^{99m}Tc-MAG3 and exhibited animal bio distribution better than ^{99m}Tc-MAG3. It can be used clinically for renal functional studies in human population as an alternate to ^{99m}Tc-MAG3 due to hepatic excretion of the later radio Pharmaceutical. Performance level of kits formulated at lower pH levels was not enough to be used clinically.

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