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Research Article

Preparation and evaluation of iopamidol parenteral formulation

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Parenteral formulation,
Contrast media

Abstract

Parenteral administration offers many advantages over therapy, given by non parenteral routes. Most notably, therapeutics can reliably predict with considerably accuracy, the Pharmacokinetic and Pharmacology of the agents. Despite of these advantages, parenteral administration is not without certain, measurable risks and limitation that the professional must intelligently weigh in terms of risks benefits, and costs. Iopamidol is radio opaque Nonionic contrast media used parenterally for diagnostic purpose. Solubility of iopamidol is very limited in water. Various Marketed formulations suffer the problem of crystallization of Iopamidol; such crystallization is very crucial as it will vary the dose and also effective drug in the solubilized form is minimized.

1. Introduction

Iopamidol is radio opaque Nonionic contrast media used parenterally for diagnostic purpose. Solubility of iopamidol is very limited in water. Various Marketed formulations suffer the problem of crystallization of Iopamidol; such crystallization is very crucial as it will vary the dose and also effective drug in the solubilized form is minimized. After multiple use of Iopamidol injection Vials the iopamidol tends to get crystallize. For this reason many marketed formulations gives indication of "Crystals may form in the solution but are readily redissolved by immersing the container in hot water and gently shaking it". Such redissolution of crystals is a hectic procedure for a Physician. Many times it may take several minutes to get iopamidol redissolved.[1]

Such context of flaws in predecessor approaches in formulation of Iopamidol Injection, scarcity of research work, asked for extensive research work. The overall scenario triggered to aim the development of stable, Noncrystallizable Iopamidol Injection to assist Physician in rapid clinical Intervention. And also to achieve dose uniformity in each dozing, various adjutants/cosolvents were studied to formulate a stable, Noncrystallizable Iopamidol Injection.[2-5]

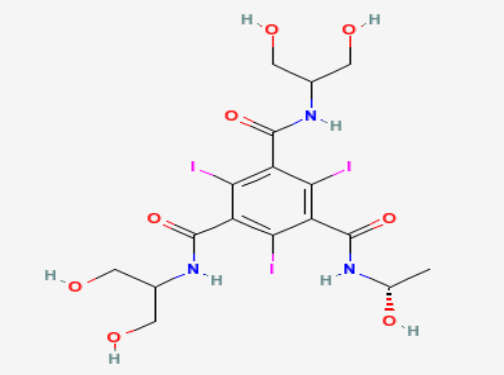


Figure 1: Structure of Iopamidol

2. Experimental work

2.1 formulation of iopamidol injection:

It was desirable to adept the formulation, technique in formulation step to avoid microbial contamination, which is possible to various steps.

Table 1: Formulation of iopamidol injection as a control batch

Sr. No.	Ingredients	Use	Quantity Taken
1	Iopamidol	Active ingredient	1530.85 g
2	Trometamine	Antioxidant	2.5g
3	Calcium disodium EDTA	Chelating agent	1.0g
4	Water for injection	Vehicle	q.s.
5	Buffer(Dil. HCL)	To maintain pH	q.s.

Iopamidol injection 50 ml vial (Batch of 50 vials)

The total volume formulated 2500 ml. The concentration of Iopamidol drug was 612.34mg/ml.

Procedure:

Heat the half quantity of water for injection about 80-90° C, & add accurately weigh iopamidol and stir it for 5-10 min, then add tromethamine and calcium disodium EDTA and stirr gently, slowly they are soluble in water for injection then add little amount of water for injection and stirr, the above ingredient are completely soluble in water for injection. Add remaining amount of water for injection to make up the volumn, adjust the pH with dilute HCl about 6.5-7.5. fill the solution in the vial aseptically and autoclave it at temperature of about 121° C for 45 min.

Table 2: Formulation of iopamidol injection by using cosolvant and adjuvants

S. No.	Ingredient	Use	F1	F2	F3	F4	F5
1	Iopamidol	Active ingredient	306.170g	306.170g	306.170g	306.170g	306.170g
2	Tromethamine	Antioxidant	0.5g	0.5g	0.5g	0.5g	0.5g
3	Calcium Disodium EDTA	Chelating agent	0.2g	0.2g	0.2g	0.2g	0.2g
4	PG (1.0%)	Cosolvant	5.0 ml	-	-	-	-
5	PG (2.0%)	Cosolvant	-	10.0ml	-	-	-
6	PVP	Adjuvant	-	-	0.05 g	-	-
7	Urea	Adjuvant	-	-	-	0.5g	-
8	Mannitol	Adjuvant	-	-	-	-	0.5 g
9	Water For Injection	Vehical	q.s.	q.s.	q.s.	q.s.	q.s.
10	Buffer (Dil.HCl)	To maintain pH	q.s.	q.s.	q.s.	q.s.	q.s

Iopamidol injection 50 ml vial (Batch of 10 vials)

F: Formulation; PG: Propylene Glycol; PVP: Polyvinylpyrrolidon; Dil.HCl: Dilute Hydrochloric Acid

2.2 Packaging

Above formulation was prepared aseptically and packed in 50 ml vial by using type I glass container with rubber closure.

2.3 Stability and Storage:

Table 3: Stability and Storage

Sr. No.	Formulation	Storage Condition (1ST Day)			
		2-8° C	Room Temperature	30-35° C	35-40° C
1	CONTROL SAMPLE	STABLE	STABLE	STABLE	STABLE
2	F1	STABLE	STABLE	STABLE	STABLE
3	F2	STABLE	STABLE	STABLE	STABLE
4	F3	STABLE	STABLE	STABLE	STABLE
5	F4	STABLE	STABLE	STABLE	STABLE
6	F5	STABLE	STABLE	STABLE	STABLE

F: Formulation

Formulation of iopamidol injection was formulated and kept for stability study for 60 days in different temperature along with its control batch sample.

2.4 Evaluations of injection batches

A) Solubility Test

B) pH

C) Assay

Procedure:

Transfer about 300 mg of iopamidol, accurately weighed to a glass stopper. 125 ml conical flask add 40 ml of 1.25M sodium hydro oxide and 1.0 g of zinc powder, connect the flask to a reflux condenser, and reflux the mixture for 30 min. cool the flask to room temperature, rinse the condenser with 20 ml of distilled water, disconnect the flask from the condenser and filter the mixture. Rinse the flask and filter thoroughly adding rinsing to the filtrate. Add 5 ml of glacial acetic acid and titrate with 0.1 N silver nitrate to determining the end point by potentiometrically.[6]

Each ml of 0.1 N silver nitrate is equivalent to 25.90 mg of iopamidol.

D) Official test of iopamidol as per USP

Free aromatic amine test:

Transfer 500 mg to a 25 ml volumetric flask, and add 20.0 ml of distilled water heating on a water bath, if necessary to affect solution .to a second 25 ml volumetric flask transfer 18.4 ml of distilled water and 1.6 ml of standard solution prepared by dissolving a suitable quantity of USP iopamidol related compound.

A RS in water and diluting with water to obtain a concentration of 62.5 µg/ml. to a third 25 ml volumetric flask add 20 ml of distilled water to provide a blank. Treat each flask as follows. Place the flask in ice bath, protected form light for 5 min (note – in conducting the following steps, keep the flask in the ice bath and protected from light as much as possible until all the reagent have been added)

Add slowly 1.0 ml of HCL, mix and allow to stand for 5 min. add 1.0 ml of ammonium sulfa mate solution (3 in 25) shake and allow to stand for 5 min (caution-considerable pressure is produce) add 1.0 ml of N-(1-Naphthyl) ethylenediamine dihydrochloride solution (1 in 1000) and mix. remove the flask from ice bath and allow to stand in water bath at about 25 for 10 min. dilute with water to volume mix and without delay (about 5 min from final dilution), concomitantly determine the absorbance of the solution from the substance under test and the standard solution in 1- cm cells at a wavelength of maximum absorbance at about 500 nm with a suitable spectrophotometer, against the prepared blank. The absorbance of the solution from iopamidol is not greater than that of the standard solution (0.02 %).[6]

Free IODINE

Transfer 2.0 g to a stopered, 50.0 ml centrifuge tube add sufficient water to dissolve, heating on a water bath, if necessary to effect solution, and dilute with water to 25.0 ml. add 5.0 ml of toluene and 5.0 ml of 2N sulfuric acid. Shake well and centrifuge the toluene layer shows no red color. [7]

Limit of free iodide:

Transfer about 6 g accurately weight to a suitable container, dissolved in 50 ml of water and add 2.0 ml 0.001 M potassium iodide. Titrate with 0.001 N silver nitrate and determining the end point by potentiometrically using silver indicator electrode and an appropriate reference electrode. Perform a blank determination and make ant necessary correction each ml of 0.001 N silver nitrate is equivalent to 126.9 µg of iodide. Not more than 0.001 % is found.[7]

Thin Layer Chromatography

Prepare a filter and degassed a mixture of water and methanol (3:1). Mobile Phase use various mixture of solution A and solution B as directed under chromatographic system.

System suitability solution

Transfer 10.0 mg of USP iopamidol related compound B RS and 10.0 mg of USP iopamidol RS into 1000.0ml volumetric flask dissolved in and dilute with water volume and mix.

Test solution

Transfer about 1.0 g of iopamidol accurately weighed to a 100.0 ml volumetric flask, add water to volume and mix.

Chromatographic system

The liquid chromatography is equipped with 240 nm detector and 46 mm x 25- cm stainless still that contain 5 µm packing L1, the column temperature maintained at 35 c, and flow rate is about 1.5 ml /min. the chromatograph is programmed to provide variable mixture of solution A and B . the percentage of solution B being 8 % at the time of injection and is held at that % for 6 min, then increase linearly to 35 % at 18 min, after which it is changed to increase linearly to 92 % at 30 min. maintain at that % for 4 min and decrease linearly to 8 % at 36 min where it is held to the end of

run at 40 min. chromatograph the suitability solution and record the peak response as directed under procedure the resolution R between the iopamidol related compound B and iopamidol is not less than 7. [8]

Procedure

Separately inject equal volumn (about 20.0 ml) of the system suitability solution and test solution into a chromatogram and measure the area of peak response calculate the % of each related compound in the portion of iopamidol taken by the formula

$$0.10\ (ri/rs)$$

Where ,

ri = peak response for individual related compound obtained from test solution

rs = peak response for iopamidol related compound B obtained from system suitability solution.

The sum of all related compound does not exceed 0.25 %.

2.5 Animal Study

Toxicity test:

Rabbit test

Take three healthy rabbits of same species having weight not less than 1.5 kg. then inject iopamidol injection of about 5.7 ml to the marginal ear vein of rabbit and keep the rabbit under observation for 5 days.

Mice test

Take 10 healthy mice of same species having weight not less than 15 g then inject about 0.1 ml of iopamidol injection to the mice and keep it for observation for 5 days.

2.6 Evaluation of formulation for stability[9-11]

For a product to be stable, it should be within the limit of monograph of specification, regarding its identity, strength, quality and purity throughout its shelf life.

The purpose of stability testing is to provide evidence on how the quality of drug substance or product varies with time under the influence of variety of the environmental factor like temperature, humidity, light etc. thus enabling recommendation of storage condition and to establish shelf life.

In stress testing of a drug or a product it is exposed to elevated temperature and humidity, over a period of time, called the " accelerated stability condition ' and the data obtained predicts stability, storage condition and shelf life of drug substance or product when kept under specified storage condition.

The prepared formulation was subjected to accelerated stability study at different temperature like 2-8°C, room temperature (27°C), 30-35°c & 35-40°C for a period ranging from 1st day to 60 days.

The formulation was tested for

a) Particulate matter
b) pH
c) % content

2.7 Drug-Excipient Interaction Study

After the selection of emulsifiers all the formulation ingredients were subjected to drug excipient interaction studies to determine any possible interaction with drug molecule. The interaction study was carried by using Fourier Transform Infra red spectroscopy (FTIR).

2.7.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra were obtained using FT-IR spectrometer (Shimadzu).The samples (Iopamidol /Excipient) were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix in 1:5 (sample : KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. Thirty scans were obtained at a resolution of 2 cm^{-1} from 4500 to 400 cm^{-1}.

2.8 Microscopic study of formulation

Microscopic evaluation of marketed formulation (crystals) in the formulation was performed using motic Digital Microscope using Plus 2.0 software in department of Pharmaceutical sciences, R. T. M. Nagpur University under the guidance of Dr. P. R. Itankar to characterize the crystal structure of the crystals of iopamidol formed in the marketed preparation.

3. Results and Discussion

3.1 Test Result

Table 4: Solubility

SR. NO.	Formulation	Solubility
1	Control batch	soluble
2	F1	soluble
3	F2	soluble
4	F3	soluble
5	F4	soluble
6	F5	soluble

For the above table it is clear that iopamidol shows high solubility in polar solvents like water. And in formulation using cosolvant and adjuvants they are soluble in water.

Table 5: pH of formulatiion

Sr. No.	Formulation	pH (6.5-7.5)
1	Control batch	7.4
2	F1	6.8
3	F2	7.2
4	F3	6.8
5	F4	7.4
6	F5	6.8

pH of all formulation was found in the range of 6.5-7.5. Ideally, the formulation should have possessed the pH in range 6.5-7.5 so as to minimize discomfort and irritation to the body. No modification of pH was attempted for the experimental formulation for the reason of solubility and stability.

Figure 2: pH values of various formulation of Iopamidol Injection

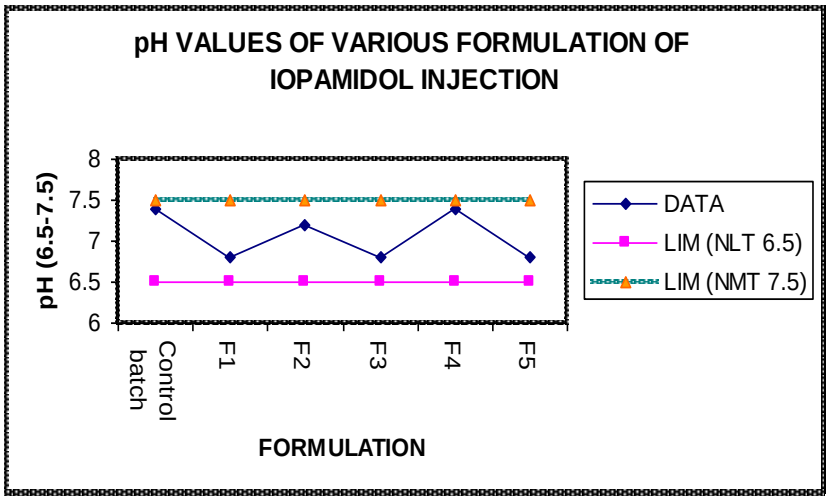


Table 6: ASSAY

Sr. No.	Formulation	Assay (95-105%)
1	Control batch	97.68%
2	F1	96.42%
3	F2	99.37%
4	F3	98.95%
5	F4	98.52%
6	F5	96.16%

From the table it was clear that the % purity of iopamidol injection is within the range.

Figure 3: Assay values of various formulation of iopamidol Injection

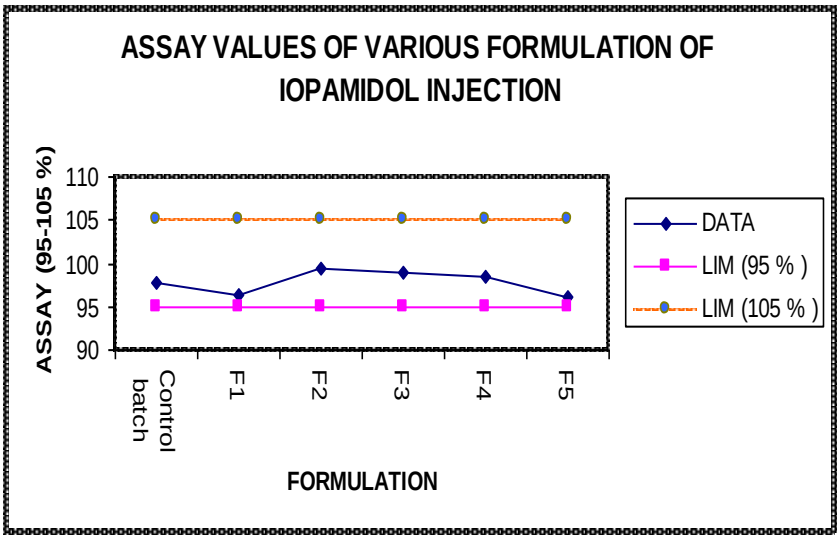


Table 7: Assay of marketed product containing crystals

Sr. No.	Formulation	Assay	
		Clear Solution	Crystal Assay
1	Uniray Product (Iopamidol Injection)	81.04%	126.81%
		79.36%	122.19%

From the above it was clear that in the crystal formation product, the clear solution have less percentage purity and the crystal has more % purity hence it is concluded that the crystal are produce to that of active ingredient i.e. Iopamidol & the pH of the product (clear solution)-7.2 and pH (crystal solution)-7.4, i.e. within limit.

Official Test Result

Table 8: Free aromatic amine test

Sr. No.	Formulation	Test Result
1	Control batch	complies
2	F1	complies
3	F2	complies
4	F3	complies
5	F4	complies
6	F5	complies

The absorbance of the solution from the iopamidol is not grether than that of standard solution. Hence all formulation complies the test.

Table 9: Free iodine test

Sr. No.	Formulation	Test Result
1	Control batch	complies
2	F1	complies
3	F2	complies
4	F3	complies
5	F4	complies
6	F5	complies

As there was no pink or red colour in the toluene layer hence the above formulation complies the test.

Thin Layer Chromatography

Table 10: Rf values of formulation

Sr. No.	Formulation	Rf Value (Less Than 1)
1	Control batch	0.86
2	F1	0.85
3	F2	0.95
4	F3	0.96
5	F4	0.97
6	F5	0.92

As the Retention factor was less than 1 it indicates that the above formulation do not content impurities.

3.2 Animal Study

Table 11: Results of toxicity study of formulation

Sr. No.	Formulation	Toxicity Test	
		Rabbit	Mice
1	Control batch	No toxicity found	No toxicity found
2	F1	No toxicity found	No toxicity found
3	F2	No toxicity found	No toxicity found
4	F3	No toxicity found	No toxicity found
5	F4	No toxicity found	No toxicity found
6	F5	No toxicity found	No toxicity found

As there was no reaction in 10 days of observation so, the above formulation had no toxicological effect in animal. hence passes the test.

3.3 Stability Results

Table 12: Particulate matter

Sr. No.	Formulation	Particulate Matter	
		1st day	60th day
1	Control batch	Transparent	Transparent
2	F1	Transparent	Transparent
3	F2	Transparent	Transparent
4	F3	Transparent	Transparent
5	F4	Transparent	Transparent
6	F5	Transparent	Transparent

Table 13: Assay

Sr. No.	Formulation	Assay	
		1st Day	60th Day
1	Control batch	97.68%	96.78%
2	F1	96.42%	96.35%
3	F2	99.37%	99.27%
4	F3	98.95%	98.57%
5	F4	98.52%	98.42%
6	F5	96.16%	96.12%

Table 14: pH

Sr. No.	Formulation	pH	
		1st Day	60th Day
1	Control batch	7.4	7.3
2	F1	6.8	6.8
3	F2	7.2	7.2
4	F3	6.8	6.7
5	F4	7.4	7.2
6	F5	6.8	6.7

3.4 Drug excipient interaction study

The interaction study was carried by using Fourier Transform Infra red spectroscopy (FTIR)**.**

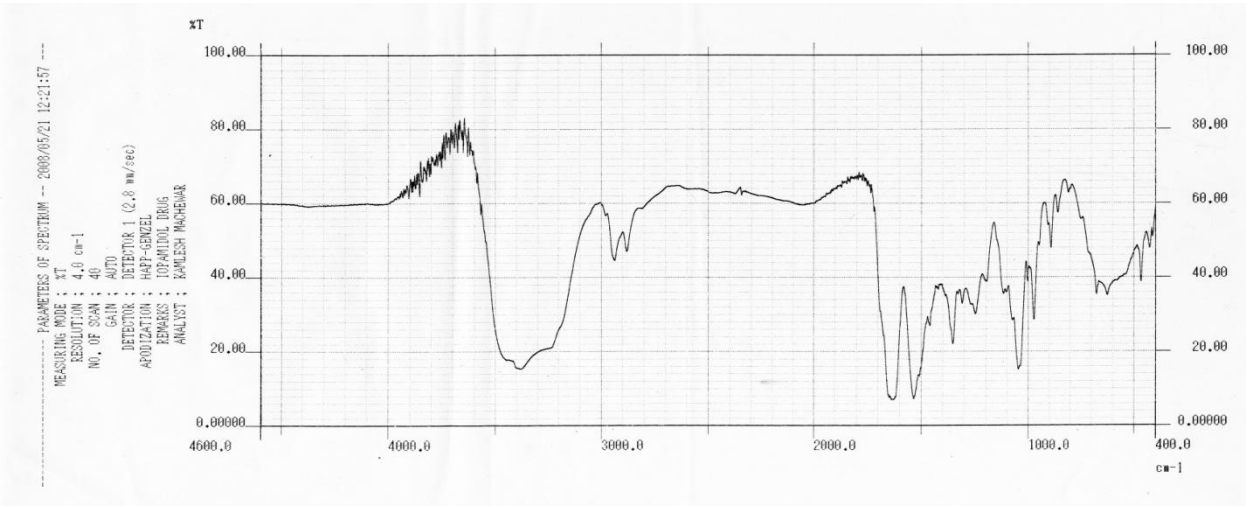
Compatibility study by Fourier transforms infrared spectroscopy (FT-IR)

Shown in figure, FTIR are the absorption Spectrum of A) Iopamidol B) 1:1 Physical mixture of Iopamidol and propylene glycol, C) 1:1 Physical mixture of Iopamidol and urea, D) 1:1 Physical mixture of Iopamidol and PVP, E) 1:1 Physical mixture of Iopamidol and Mannitol, F) 1:1 Physical mixture of Iopamidol and mixture of all excipient.

In order to characterize possible interactions between the drug and the excipient, infrared spectra were recorded. Infrared spectrum of Iopamidol is characterized by the aromatic-0H in plane bend at 1352 cm^{-1}, COOH group stretching at 1535 cm^{-1}, aromatic nitro compounds at 1460 cm^{-1}.

Comparing the spectra of Physical mixtures of various excipient individually and finally with mixture of all excipient with that of Iopamidol gives idea about possible interaction. No any significant shift in absorption bands (which are characteristics of Iopamidol) was observed. Therefore it can be ascertained that no significant interaction in the Iopamidol and excipient exists.

Figure 4A – FTIR spectrum of Iopamidol drug



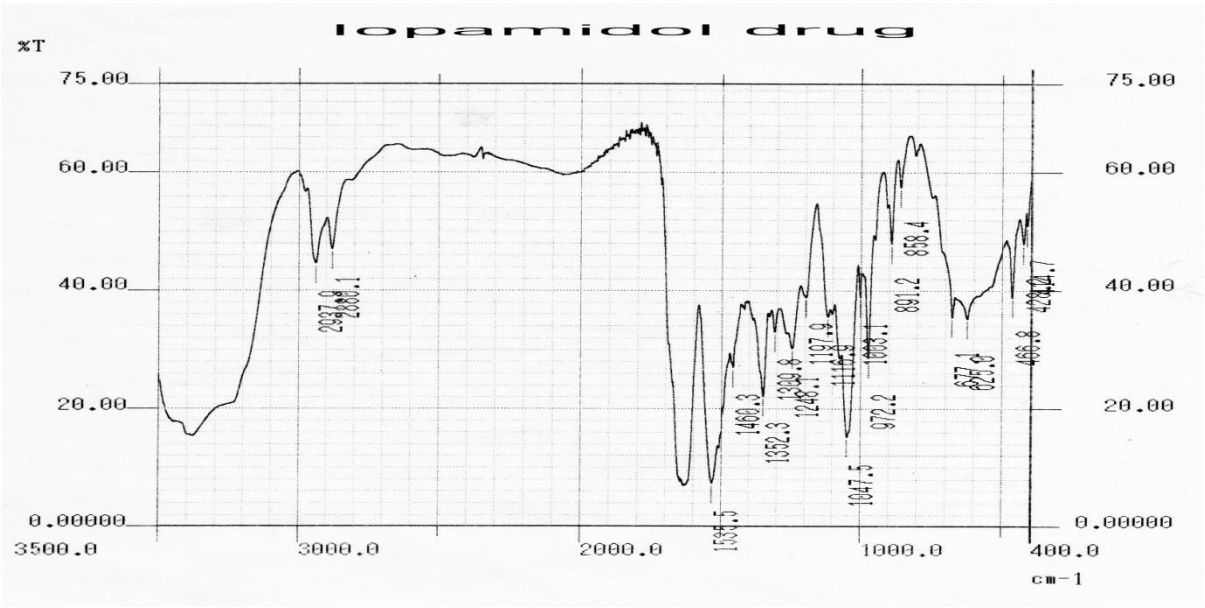
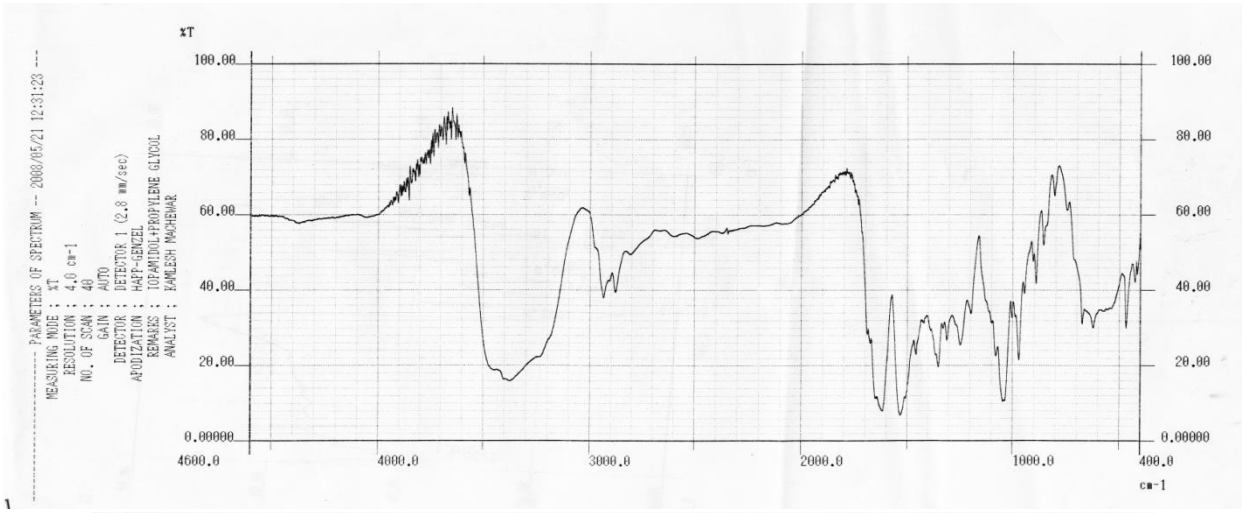


Figure 4B – FTIR spectrum of iopamidol+ propylene glycol



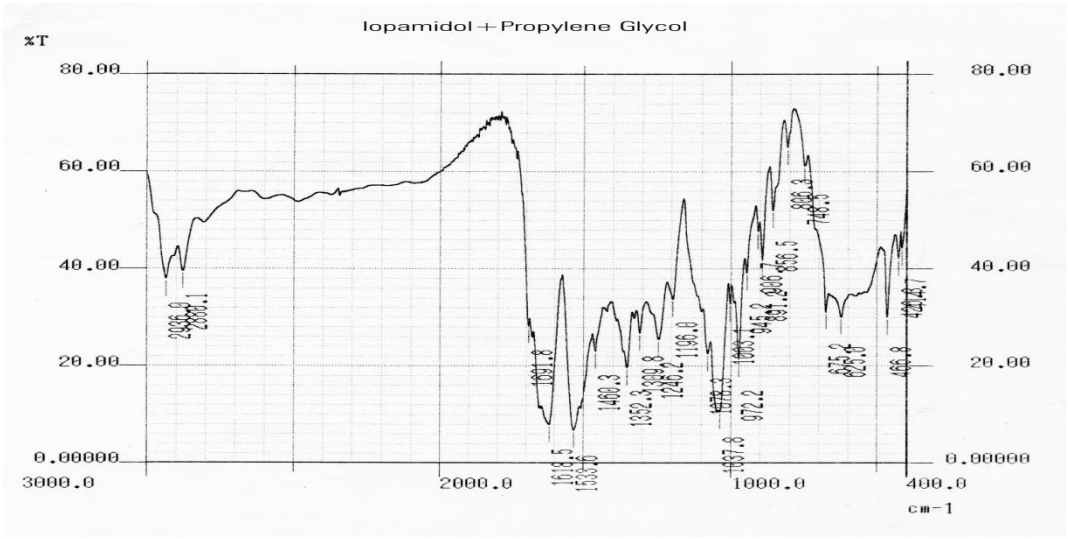
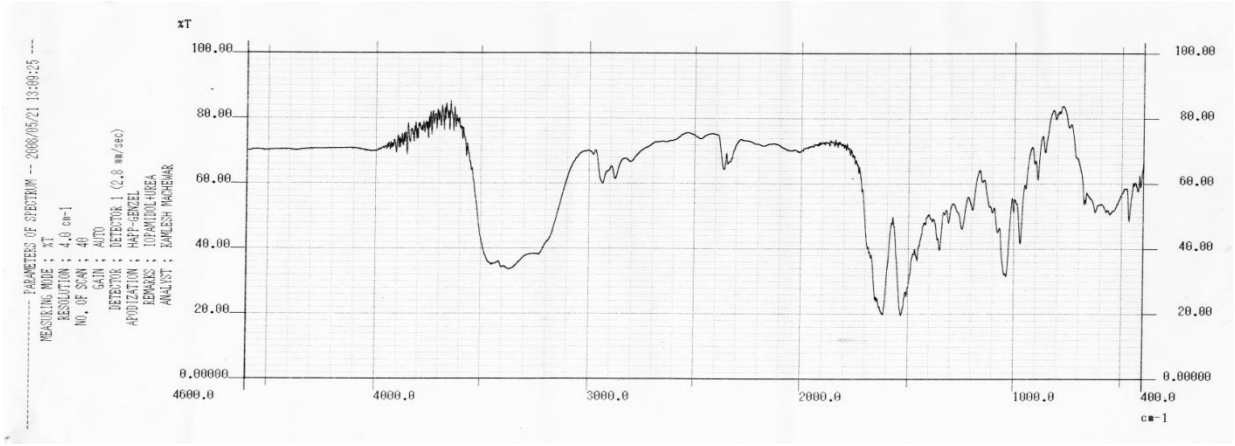


Figure –4 C: FTIR spectrum of iopamidol +urea



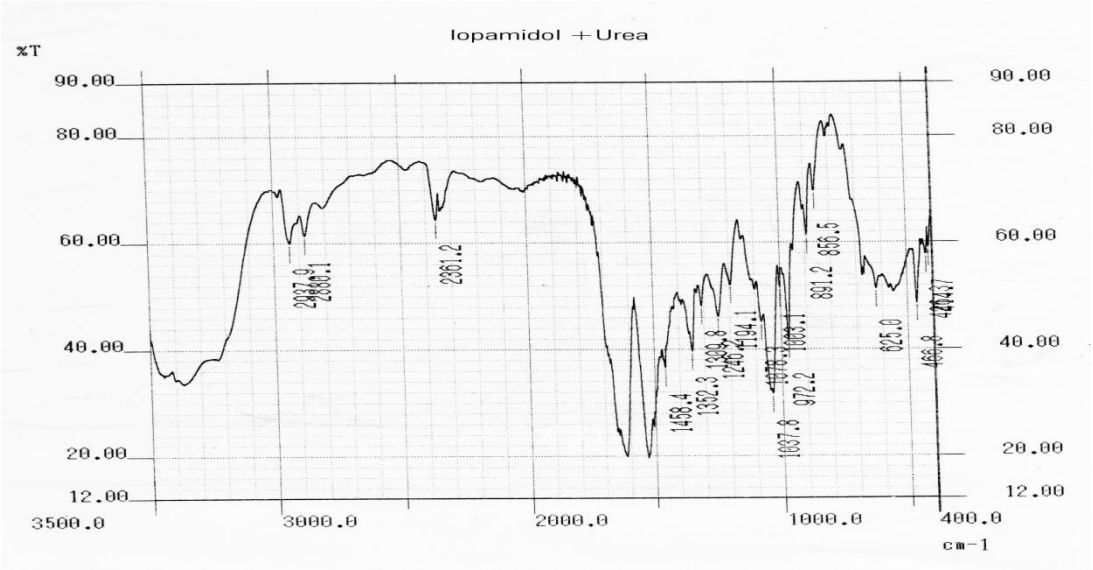


Figure – 4D FTIR spectrums of Iopamidol + PVP

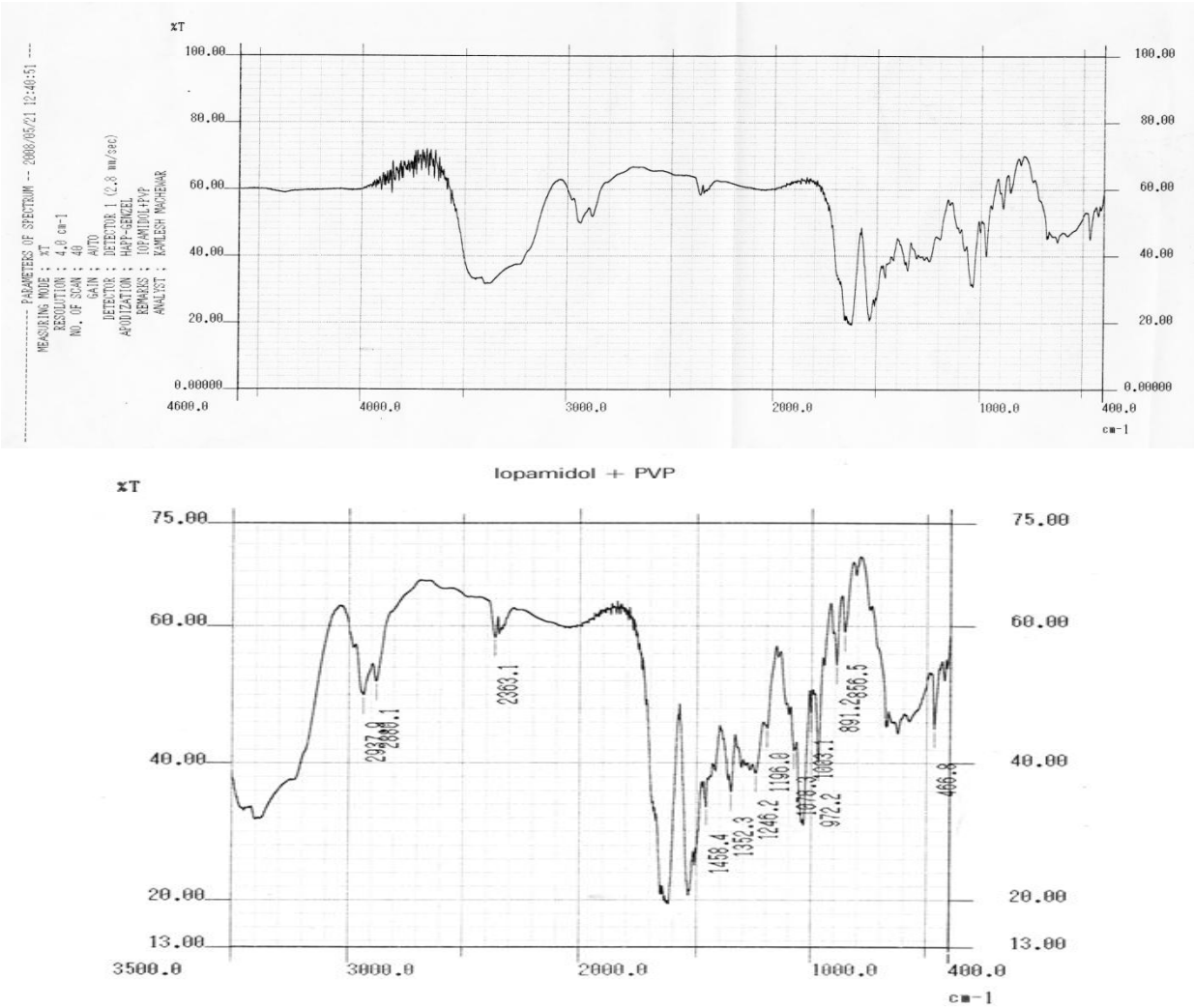
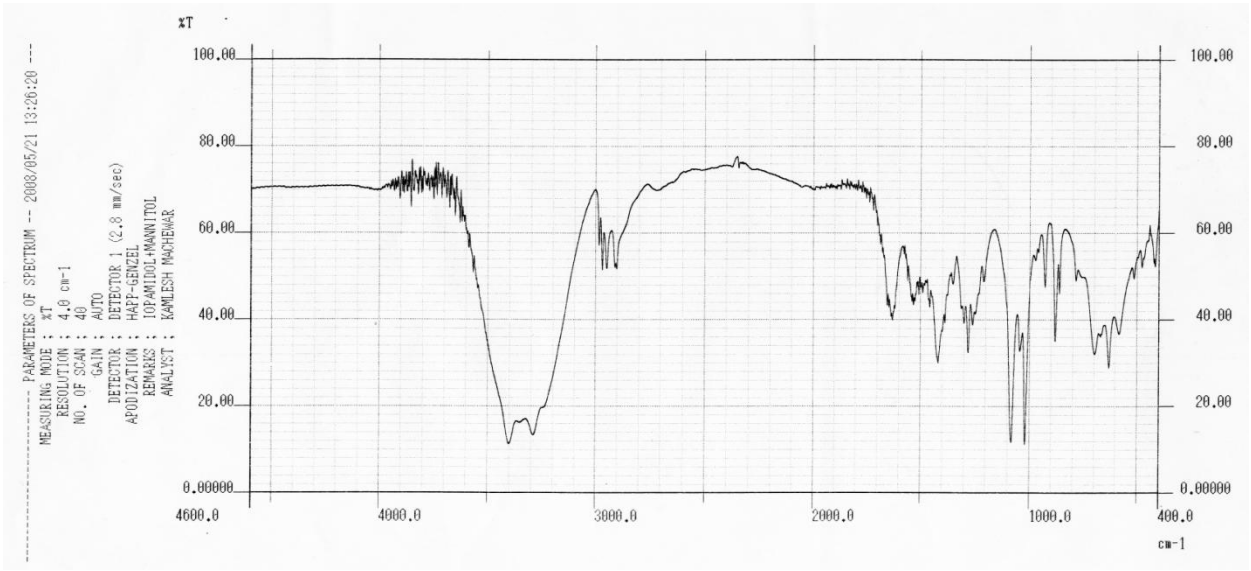


Figure – 4E: FTIR spectrum of Iopamidol + Mannitol



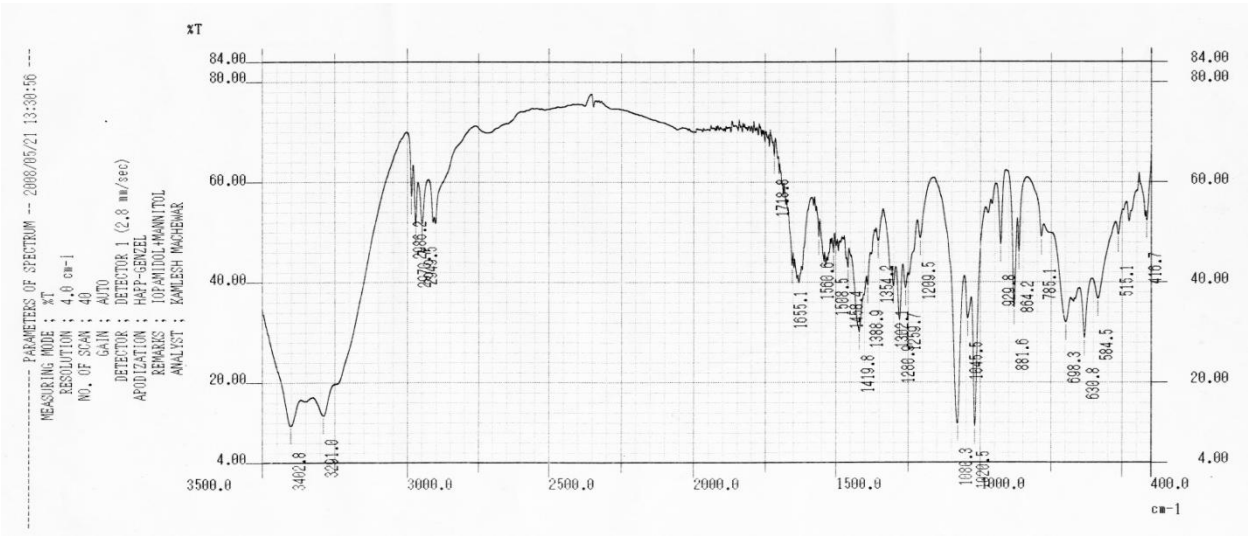
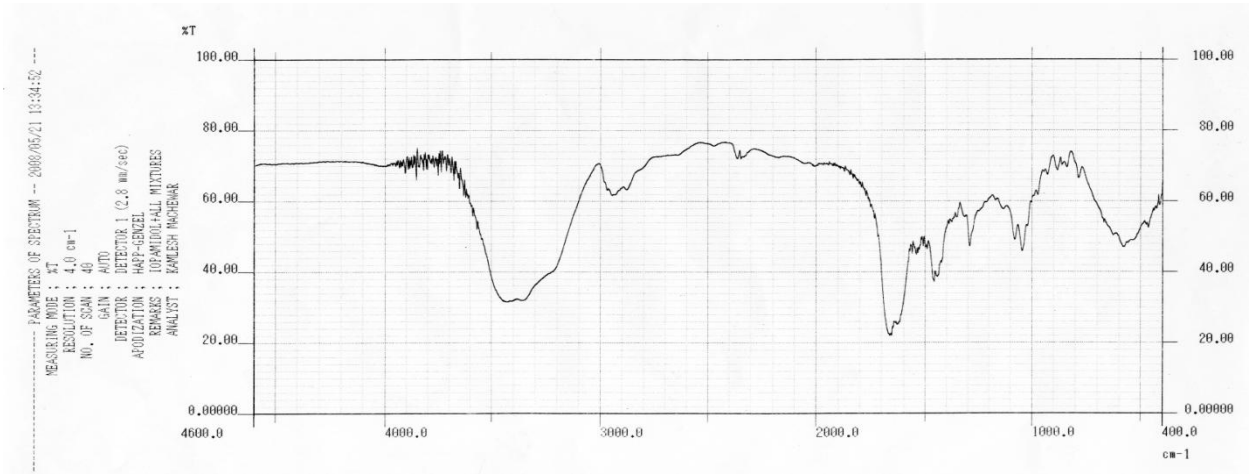
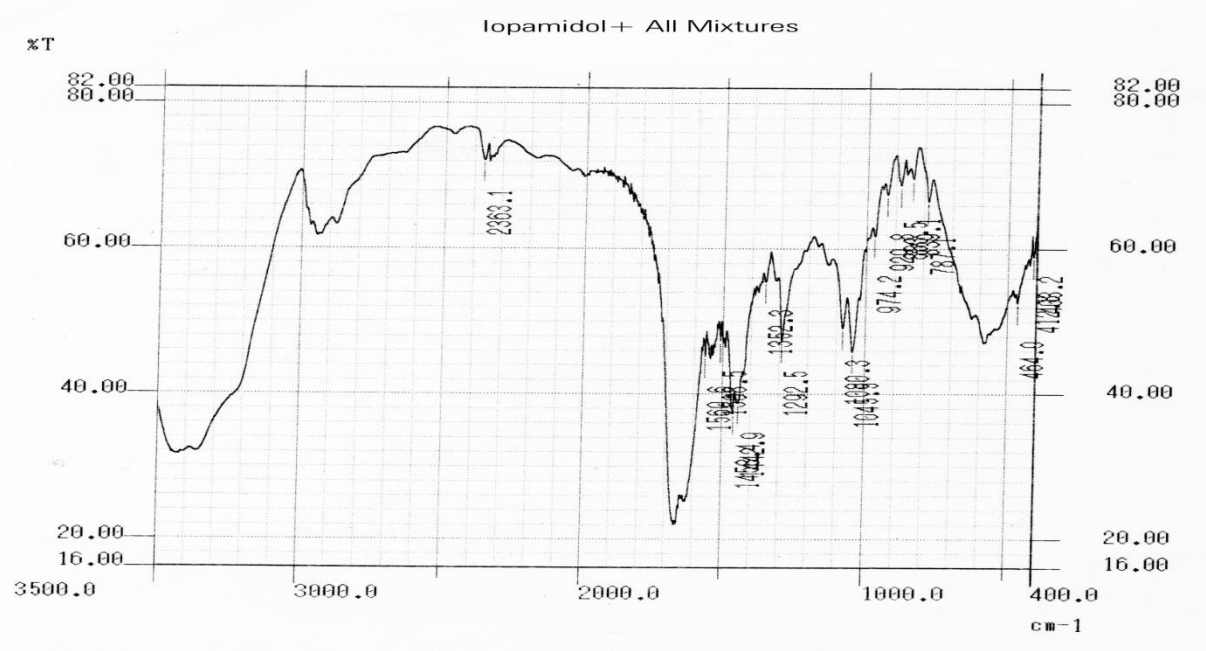


Figure – 4F: FTIR spectrum of Iopamidol + all mixture





3.5 Microscopic study of formulation.

Microscopic evaluation of marketed formulation (crystals) in the formulation was performed using motic Digital Microscope using Plus 2.0 software in Department of Pharmaceutical sciences, R. T. M. Nagpur University under the guidance of Dr. P. R. Itankar to characterize the crystal structure of the crystals of iopamidol formed in the marketed preparation.

Orthorhombic and Rhombic crystals were found in the formulation which are dispersed and are of size 4-5 mm in size. Moreover crystal settles at the bottom and forms a stuff cake which is not dispersed easily.

On the contrary, batches prepared using various adjutants and cosolvents viz. F1, F2, F3, F4, F5 were clear and has total absence of crystals.

Figure 5: Shown in the figure are the Photographic of marketed formulation (UNIRAY) having crystals settled at bottom.

Figure 6: Shown in the figure are the motic images of crystals of iopamidol in marketed preparation.

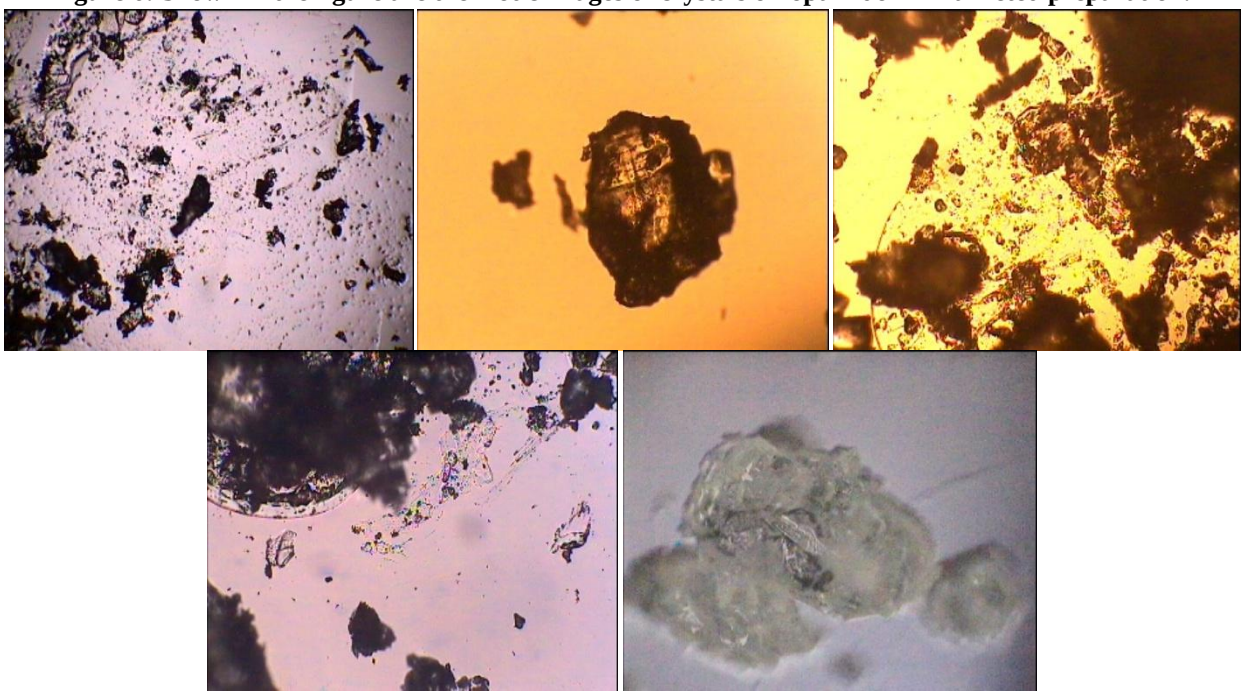
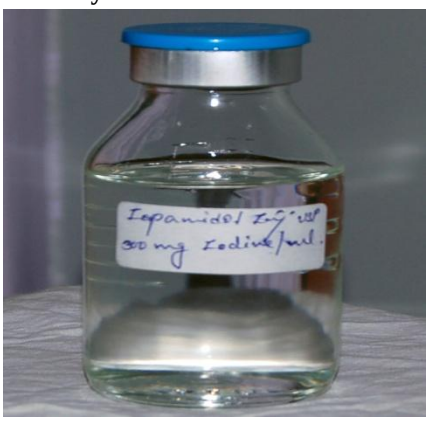


Figure 7: Shown in the figure are the Photographic of optimized formulation (F2) having clear solution without any crystals settled at bottom.



4. Summary & conclusion

The Parenteral formulation of Iopamidol using cosolvents and adjuants provide a better alternative for increasing stability of formulation.

Iopamidol is a radio opaque nonionic contrast media, having limited solubility in water.

In the present work the parenteral formulation of Iopamidol was formulated by using various cosolvents and solubility enhancers. Use of cosolvents and solubility enhancers provide a unique possibility for increasing solubility of Iopamidol and thereby decreaseing its recrystalization in the formulation

Cosolvents and solubility enhancers exhibit some characteristic feature when used in formulation. It was observed during study that various cosolvents and solubility enhancers increases the solubility of Iopamidol. Parenteral formulation batches composed of active pharmaceutical ingredient (Iopamidol), antioxidant (Tromethamine), chelating agent(Calcium disodium EDTA), cosolvents, adjutants were prepared and evaluated. In the present study all excipients which are extensively used in the pharmaceutical industry and widely accepted were selected.[12-14]

Formulation batches F2 and F3 were selected as optimum batches which have shown the least crystallization of Iopamidol.

Stability studies of formulation at different temperatures (2-8 ^{0}C, Room temperature, 30-35^{0}C and 35-40^{0}C for

two months showed no significant effect on physical properties and assay.

No subtle variation in infrared absorption patterns FTIR study, it can be concluded that no significant drug – excipients interaction exists.

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