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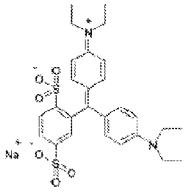
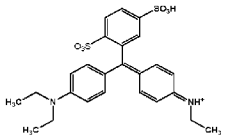
(54) Title: ISOSULFAN BLUE, ITS CRYSTALLINE FORM AND PROCESS FOR PREPARATION THEREOF



Formula I



Formula A

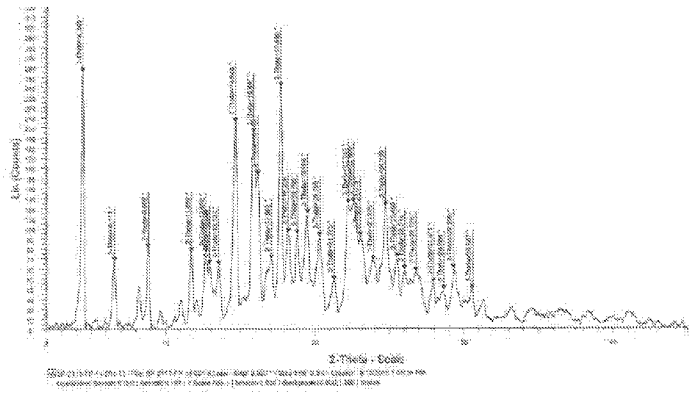


Figure-1: XRPD peaks pattern of Isosulfan blue (I) hydrate

(57) Abstract: The invention relates to an improved process for the preparation N-[4-[[4-(diethyl amino) phenyl] (2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N- ethylethanaminium inner salt sodium salt (Isosulfan blue) of formula I. The invention also relates to highly pure novel crystalline form of Isosulfan blue (I) hydrate and its process for the preparation thereof. The invention also relates to an improved process for the preparation of Isosulfan blue sodium hydrate having not more than 0.2% of desethyl impurity of formula A.

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[Continued on next page]

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ISOSULFAN BLUE, ITS CRYSTALLINE FORM AND PROCESS FOR PREPARATION THEREOF

CROSS REFERENCE

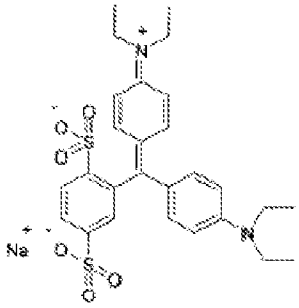
This application claims priority from Indian Patent Application No. 201641023263 filed in Indian Patent Office on July 06, 2016.

FILED OF INVENTION

The invention relates to an improved process for the preparation of Isosulfan blue. More particularly, the invention relates to highly pure crystalline form of Isosulfan blue (I) hydrate and its process for the preparation thereof. The invention also relates to an improved process for the preparation of Isosulfan blue sodium hydrate having not more than 0.2% of desethyl impurity of formula A.

BACKGROUND OF THE INVENTION

The compound N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium inner salt sodium salt (Isosulfan blue) is represented by the formula (**I**)



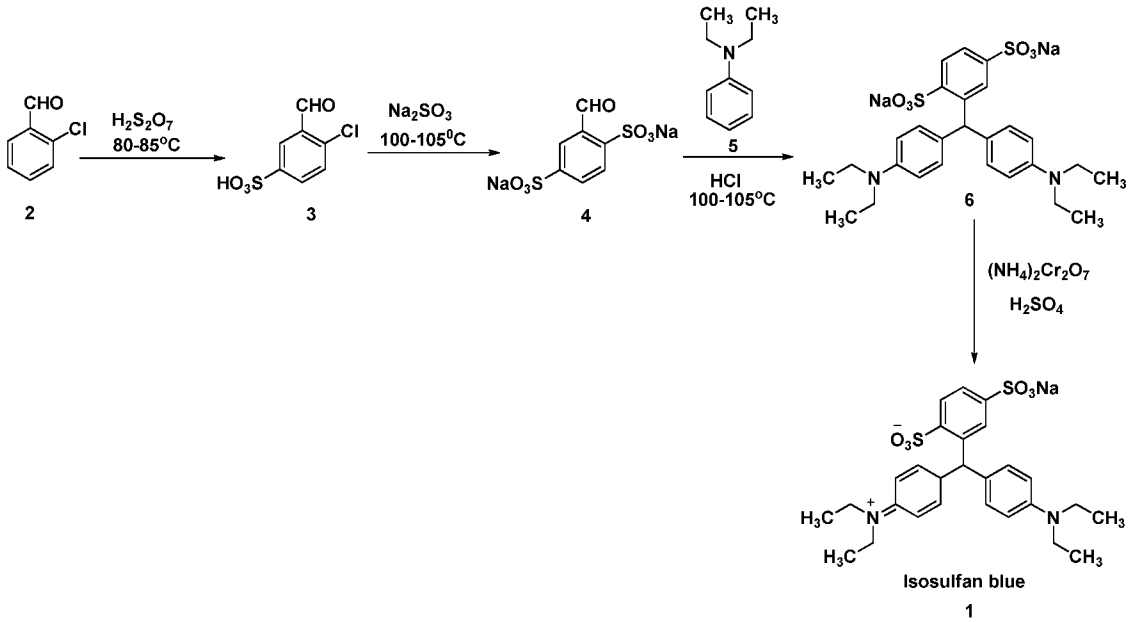
Formula I

Isosulfan blue (I) is a triarylmethane dye used as a contrast agent for delineation of lymphatic vessels and is particularly useful as a cancer diagnostic agent. Isosulfan blue is marketing as lymphazurin blue dye, which is available as 1% (10mg/ml) 5ml solution for injection.

US patents US1531507, US7534911, US8969616 reported various processes for the synthesis of dyes specifically, Isosulfan blue, the contents of which are hereby incorporated as reference in their entirety.

General synthesis of Isosulfan blue was first described in the US patent US1531507. According to this Isosulfan blue is made by converting orthochlorobenzaldehyde to sodium salt of 2-chlorobenzaldehyde-5-sulfonic acid by sulphating using oleum (26% or oleum 65%) and sodium carbonate, which is further treated with sodium sulphite to produce benzaldehyde-2,5-disulfonic acid sodium salt followed by condensation with alkylated aryl amine to produce isoleuco compound. The obtained compound is oxidised to produce Isosulfan blue. The yield and purity of the final compound is not reported in the prior art.

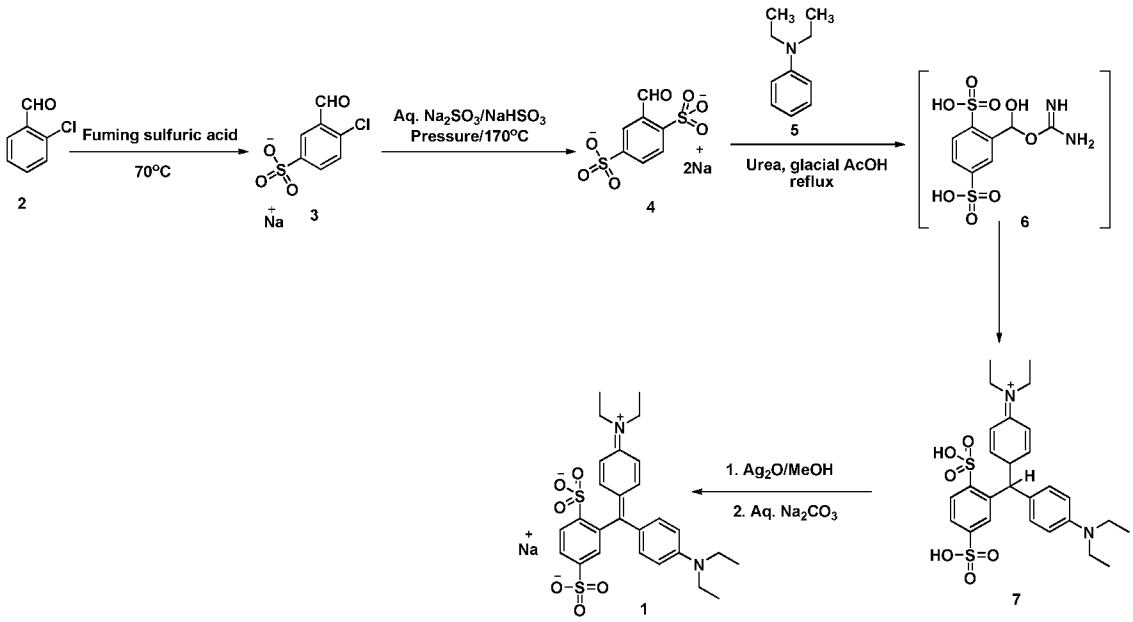
US7534911 patent discloses another route for the synthesis of Isosulfan blue (Schene-1) by sulphonating orthochlorobenzaldehyde with Oleum (23% to 65%) to obtain 2-chlorobenzaldehyde-5-sulfonic acid, which is subsequently treated with sodium sulphite and followed by basification produces sodium salt of benzaldehyde-2,5-disulfonic acid sodium. This is further treated with sodium sulphite to get disodium salt followed by condensation with diethyl aniline to yield sodium 2-(bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonate and oxidizing the obtained compound with ammonium dichromate in presence of sulphuric acid affords Isosulfan blue.



Scheme-1

US8969616 discloses another route of synthesis of synthesis for Isosulfan blue (Scheme-2). In this Isosulfan blue is obtained by the reaction of 4-Chloro-3-

formylbenzenesulfonic acid with sodium sulphite and sodium bisulphite mixture in presence of water at very high temperature (180°C) under high pressure (150PSI) by isolating the crude with huge volumes of methanol further underwent purifications using huge volumes of Dimethylformamide and Dichloromethane solvent to get pure compound and ends in very low yield to get Sodium-2-formylbenzene-1,4-disulfonate.Further reaction of Sodium-2-formylbenzene-1,4-disulfonate with diethyl aniline in presence of urea and acetic acid at reflux temperature for longer hours (20-25hrs) then isolated by cooling the reaction mixture followed by addition of anti-solvent methanol to give crude 2-(Bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonic acid. The isolated crude underwent purifications to give pure 2-(Bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonic acid. The 2-(Bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonic acid gets oxidized in presence of silver oxide in methanol media and further isolation using Diisopropylether to obtain Isosulfan blue.



Scheme-2

Therefore, there is a need in the art for an improved method for the preparation of Isosulfan blue in significantly high yield and purity. In view of this, the present inventors provide a safe and cost-effective process for the preparation of Isosulfan blue in significantly high yield.

OBJECT OF THE INVENTION

One object of the invention is to provide an improved process for the preparation of Isosulfan blue.

Another object of the invention is to provide novel crystalline form of Isosulfan blue hydrate.

Another object of the invention is to provide process for preparing crystalline form of Isosulfan blue hydrate containing 4- 10% of moisture content.

Another object of the invention is to provide purification process to remove desethyl impurity of formula A.

Another object of the invention is to provide Isosulfan blue having desethyl impurity level less than 0.2%.

Another object of the invention is to provide Isosulfan blue with metal content lead level less than 2 ppm, Arsenic level less than 3 ppm and chromium level less than 50 ppm.

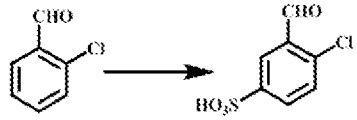
Another object of the invention is to provide process for preparing pure Isosulfan blue, which is having total impurity level less than 3% and desethyl impurity level less than 0.2% and metal content lead level less than 2 ppm, Arsenic level less than 3 ppm and chromium level less than 50 ppm.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides improved process for the preparation of N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium inner salt sodium salt (Isosulfan blue) of formula I, which is schematically represented in Scheme-3.

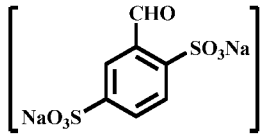
In one aspect, the present invention provides a process for the preparation of Isosulfan blue comprises the following steps:

a) sulphonating 2-chloro benzaldehyde compound of formula II with fuming sulphuric acid to obtain 4-chloro-3-formyl-benzenesulfonic acid compound of formula III;



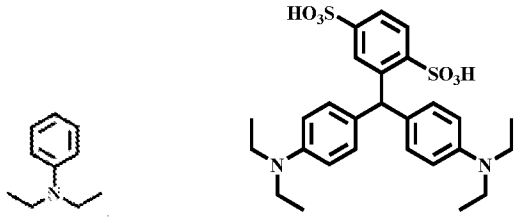
Formula II Formula III

b) treating the 4-Chloro-3-formyl-benzenesulphonic acid compound of formula III obtained in step (a) with alkali metal sulphite to obtain 1-formyl benzene-2,5-disulphonic acid disodium salt of formula IV;



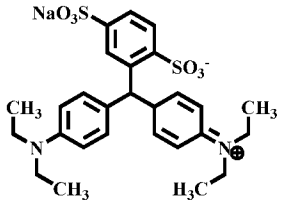
Formula IV

c) condensing 1-formyl benzene-2,5-disulphonic acid disodium salt of formula IV obtained in step (b) with N,N-diethylaniline of formula V in presence of hydrochloric acid to obtain 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid compound of formula VI;



Formula V Formula VI

d) oxidizing 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid of formula VI with an oxidizing agent in presence of sulphuric acid to obtain crude Isosulfan blue of formula VII, and purifying;



Formula VII

e) optionally, recrystallizing the Isosulfan blue of formula VII obtained in step (d) to obtain highly pure Isosulfan blue.

The alkali metal sulphite in step (b) is selected from the group comprising of sodium sulphite, potassium sulphite, Iron sulphite, zinc sulphite and copper sulphite. In one

embodiment the alkali metal sulphite used is sodium sulphite. The reaction in step (b) is carried out in a solvent selected from ethanol, methanol, propanol, isopropanol and water or mixtures thereof.

The process step (c) further comprises purifying the compound of formula VI by treating with a base and converting into free base to get pure compound VI.

The oxidizing agent in step (d) is selected from potassium dichromate, sodium dichromate, zinc dichromate and pyridinium dichromate. In one embodiment the oxidizing agent used is potassium dichromate.

The crude Isosulfan blue of formula VII in step (d) is purified with a solvent selected from the group comprising of methanol, acetone and dichloromethane or mixture thereof.

In one embodiment the crude Isosulfan blue of formula VII in step (d) is purified by column chromatography, wherein the column purification comprises using 100-500 mesh silica gel and selecting a polar aprotic eluent from dichloromethane, acetonitrile, methanol, ethanol and the mixtures thereof. In one embodiment the mess size of Silica gel is selected from100-200, 200-400, 230-400 and 200-425.

The above said process after column purification further comprises the steps of:

(a) slurring the obtained Isosulfan blue in acetone; and
(b) recrystallizing from the mixture of polar protic solvents to obtain highly pure Isosulfan blue;

- wherein the polar protic solvent in step (b) is selected from methanol, ethanol, propanol, isopropyl alcohol and tertiary butyl alcohol or mixture thereof.

In one embodiment the recrystallization in step (e) comprises the steps of:

a) suspending Isosulfan blue in a solvent;
b) stirring the solution for 1-2 hrs at 25-30°C;
c) filtering the solution mass through 0.2-micron filter at 25-30°C;
d) distilling off the filtrate under vacuum below 50 °C;

e) cooling the crude to 25-30°C and adding solvents like methanol, Ethanol, Isopropyl alcohol, n-butanol, acetone, diethyl ether, Isopropyl ether at 25-30 °C;

f) stirring for 2-3 hrs at 25-30 °C;

g) filtering the solid under vacuum at below 30 °C; and

h) drying the solid under vacuum below 50 °C;

wherein,

- the solvent in step (a) is selected from the group comprising of methanol, ethanol, isopropanol, n-butanol, acetone, diethyl ether, Isopropyl ether, dichloromethane, acetonitrile, acetic acid, tetrahydrofuran, toluene, ethylacetate and cyclohexane or mixtures thereof;

- the Isosulfan blue obtained from the process is crystalline Isosulfan blue hydrate characterized by X-ray powder diffraction (XRPD) pattern comprising one or more of the reflections at 4.38, 14.64, 15.84, 16.14, 19.49, 22.25, 22.60 and 24.73± 0.2 degrees 2 theta (2θ°);

- the Isosulfan blue obtained from the process is crystalline Isosulfan blue hydrate further characterized by X-ray powder diffraction (XRPD) pattern comprising of the reflections at 6.51, 8.80, 11.69, 12.66, 12.90, 13.53, 17.05, 17.68, 18.20, 18.79, 20.29, 21.27, 23.01, 23.92, 25.49, 26.01, 26.78, 27.97, 28.66, 29.29 and 30.58 ± 0.2 degrees 2 theta (2θ°).

The Isosulfan blue obtained from the process comprises a purity level of greater than 99%.

The Isosulfan blue obtained from the process contains moisture content less than 10%.

The Isosulfan blue obtained from the process contains desethyl impurity level less than 0.2%.

The Isosulfan blue obtained from the process contains lead less than 2 ppm, arsenic less than 3 ppm and chromium less than 50 ppm.

The Isosulfan blue obtained from the process contains total organic impurities less than 3% and total metal impurities less than 20 ppm.

In one embodiment the present invention provides a process for the preparation of N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium inner salt sodium salt (Isosulfan Blue) comprising the steps of:

(a) sulphonating 2-Chloro benzaldehyde compound with 1.4 equivalents of 20-25% sulphuric acid (Oleum) at 10-15°C to obtain 4-Chloro-3-formyl-benzenesulphonic acid ;

(b) converting 4-Chloro-3-formyl-benzenesulphonic acid to 1-formyl benzene-2,5-disulphonic acid disodium salt by treatment with sodium sulphite in presence of water and sodium hydroxide (NaOH) at temperature 85-110 °C;

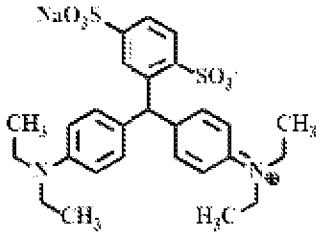
(c) condensing 1-formyl benzene-2,5-disulphonic acid disodium salt with N,N-diethyl aniline in presence of hydrochloric acid to obtain 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid and optionally purifying;

(d) oxidizing 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid using potassium dichromate in presence of sulphuric acid to obtain crude Isosulfan blue;

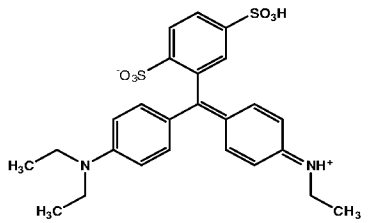
(e) optionally, purifying the crude Isosulfan blue obtained in step (d) by Column chromatography using dichloromethane and methanol to obtain pure Isosulfan blue ; and

(f) optionally, recrystallizing Isosulfan blue obtained in step (d) or step (e) with methanol and Isopropyl alcohol to provide highly pure Isosulfan blue.

In one embodiment, the present invention provides an Isosulfan blue compound of formula-I comprising less than 0.2% of desethyl impurity of the formula A.



(I)



(A)

In one embodiment, the present invention provides an Isosulfan blue compound of formula-I containing metal impurities comprising lead less than 2ppm, Arsenic less than 3 ppm and chromium less than 50 ppm.

In one embodiment, the present invention provides an Isosulfan blue compound of formula-I having moisture content less than 10%.

In one embodiment, the present invention provides an Isosulfan blue compound comprising desethyl impurity less than 0.2% and moisture content 4-10%.

In one embodiment, the present invention provides Crystalline Isosulfan blue hydrate characterized by X-ray powder diffraction (XRPD) pattern comprising one or more of the reflections at 4.38, 14.64, 15.84, 16.14, 19.49, 22.25, 22.60 and 24.73± 0.2 degrees 2 theta (2θ°).

The above said crystalline Isosulfan blue hydrate is further characterized by X-ray powder diffraction (XRPD) pattern comprising of the reflections at 6.51, 8.80, 11.69, 12.66, 12.90, 13.53, 17.05, 17.68, 18.20, 18.79, 20.29, 21.27, 23.01, 23.92, 25.49, 26.01, 26.78, 27.97, 28.66, 29.29 and 30.58 ± 0.2 degrees 2 theta (2θ°).

The above said crystalline Isosulfan blue hydrate is characterized by XRPD peaks pattern as represented in figure-1, XRPD peaks data as presented in table-1 and TGA thermogram as represented in figure-2.

The above said process is further described in details in the description along with examples.

BRIEF DESCRIPTION OF DRAWINGS

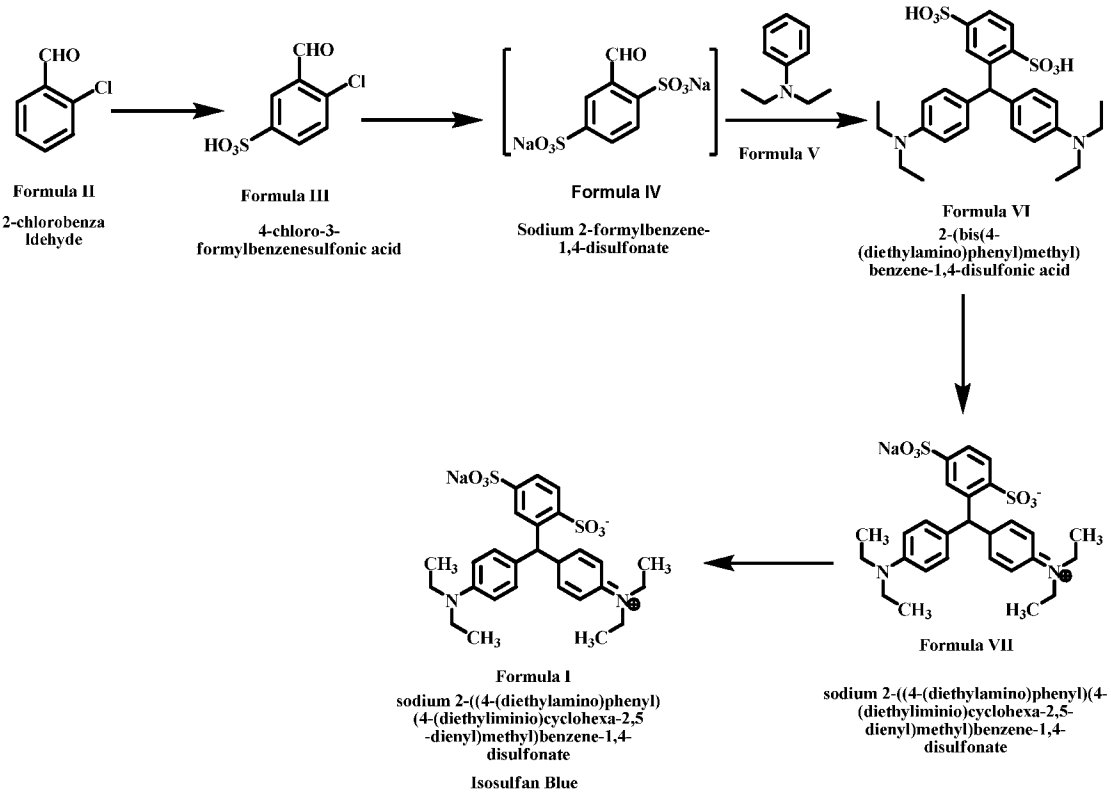
Figure-1: Represents characteristic X-ray powder diffraction (XRPD) pattern of crystalline form of Isosulfan blue (I) hydrate.

Figure-2: Represents characteristic TGA thermogram of crystalline form of Isosulfan blue (I) hydrate.

Figure-3: Represents HPLC chromatogram of desethyl impurity of formula A.

DETAILED DESCRIPTION OF THE INVENTION

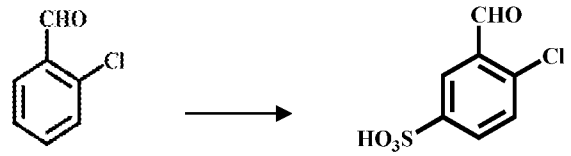
The present invention relates to an improved process for the preparation of Isosulfan blue of formula I in significantly high yield and substantially pure form. The present invention is schematically represented in Scheme-3 as follows:



Scheme-3

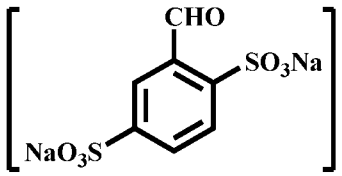
In one aspect the present invention provides a process for preparation of N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium inner salt sodium salt (Isosulfan Blue) of formula I comprising the steps of:

(a) sulphonation of Orthochloro benzaldehyde compound of formula II using fuming sulphuirc acid (Oleum) to obtain 4-chloro-3-formylbenzenesulfonic acid compound of formula III;



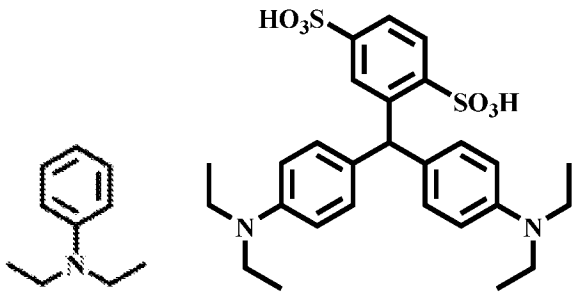
Formula II **Formula III**

(b) conversion of 4-Chloro-3-formyl-benzenesulphonic acid compound of formula III to 1-formyl benzene-2,5-disulphonic acid disodium salt compound of formula IV by treatment with alkali metal sulphite;



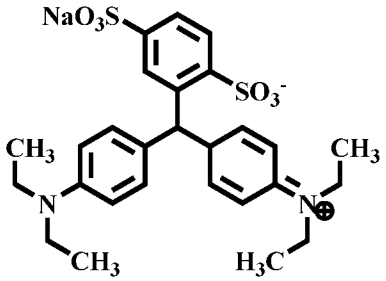
Formula IV

(c) condensation of 1-formyl benzene-2,5-disulphonic acid disodium salt compound of formula IV with N,N-diethyl aniline of formula V in presence of hydrochloric acid to obtain a 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid compound of formula VI; optionally purifying the crude intermediate from a mixture of solvents;



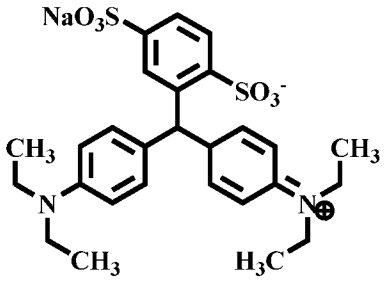
Formula V **Formula VI**

(d) oxidizing 4-[Bis[4-(diethylamino) phenyl] methyl] benzebe-2,5-disulphonic acid of formula VI with a suitable oxidizing agent in presence of inorganic acid to obtain Isosulfan blue of formula VII; optionally purifying crude compound by column chromatography;



Formula VII

(e) optionally purifying the crude Isosulfan blue of formula VII by recrystallizing from suitable solvents to provide highly pure Isosulfan blue of formula I.



Formula I

Wherein, in step a) 20-25% of fuming sulphuric acidis used as a sulphonating agent and the sulphonating reaction was carried out at1.4 equivalents of 20-25% fuming sulphuric acid.

In step b) the suitable reagent alkali metal sulphite is selected from the group comprising sodium sulphite, potassium sulphite, Iron sulphite, zinc sulphite, copper sulphite, or the like; preferably in one embodiment the alkali metal sulphite used is sodium sulphite. The solvent is selected from alcohols comprising ethanol, methanol, propanol, isopropanol and water or mixtures thereof.

In step c) the reaction was carried out with 0.7 to 1.0 V of N,N-diethyl aniline in presence of hydrochloric acid.

In step d) the suitable oxidizing reagent is either potassium dichromate or sodium dichromate or zinc dichromate or pyridinium dichromate; the suitable acid used is sulphuric acid; suitable solvents used for the purification of Isosulfan blue crude is selected from the group methanol, acetone, dichloromethane, or the mixture thereof.

In step e) Isosulfan blue obtained in the above process is further purified from the solvents selected from group comprising alcohols like methanol, ethanol, isopropyl alcohol; water; acetonitrile; tetrahydrofuran; acetone; ethyl acetate; dichloromethane or mixtures thereof.

A preferred embodiment of the present invention provides a process for the preparation of Isosulfan blue of formula I, comprising the steps of:

(a) sulphonating Orthochloro benzaldehyde compound of formula II with 1.4 equivalents of 20-25% Oleum at 10-15 °C to obtain 4-Chloro-3-formyl-benzenesulphonic acid of formula III;

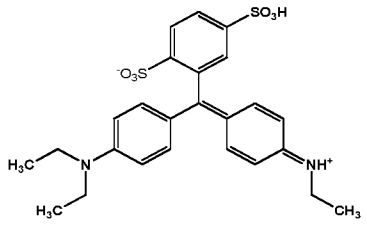
(b) conversion of 4-Chloro-3-formyl-benzenesulphonic acid of formula III to 1-formyl benzene-2,5-disulphonic acid disodium salt of formula IV by treatment with sodium sulphite and sodium hydroxide at temperature ranging 85-110 °C;

(c) condensation of 1-formyl-benzene-2,5-disulphonic acid disodium salt of formula IV with N,N-diethyl aniline of formula V in presence of hydrochloric acid to obtain a 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid of formula VI; optionally the obtained product is further purified by treatment with base followed by converting into free base to get pure compound VI;

(d) oxidation of 4-[Bis[4-(diethylamino) phenyl]methyl]benzene-2,5-disulphonic acid of formula VI using potassium dichromate in presence of sulphuric acid and solvent to obtain Isosulfan blue of formula VII; optionally the obtained crude is further purified by Column chromatography using dichloromethane and methanol to obtain pure Isosulfan blue;

(e) The final step involves optional purification of compound of formula VII by recrystallizing from methanol and Isopropyl alcohol to provide highly pure Isosulfan blue of formula I.

In one aspect the present invention provides a purification process to produce of Isosulfan blue devoid of the below impurity identified at RRT 0.9 by HPLC analysis.



Desethyl impurity (RRT: 0.9 in HPLC)
Formula A

The HPLC chromatogram of desethyl impurity of formula A is represented in figure-3.

In another aspect Isosulfan blue obtained in the present invention is having desmethyl impurity of formula A level less than 0.2% and preferably below 0.1%.

Another aspect of the invention pertains to method of purification of Isosulfan blue to control desethyl impurity at RRT 0.9 (HPLC) by column chromatography.

The purification is carried out using 100-500 (like 100-200 or 200-400 or 230-400 or 200-425) mesh silica gel and an eluent is selected from the solvent from polar aprotic solvent such as dichloromethane, acetonitrile, methanol, ethanol and the mixtures thereof.

Optionally Isosulfan blue obtained in the above column purification is further purified to remove other process related impurities as described herein.

Isosulfan blue thus obtained in the above process is further purified by slurring in acetone followed by recrystallization from the mixture of polar protic solvents.

In one aspect polar protic solvents used for the purification of Isosulfan blue after column is selected from methanol, ethanol, propanol, isopropyl alcohol and tertiary butyl alcohol.

In particular, the present invention provides a process for the purification of Isosulfan blue comprising the steps of;

(a) suspending crude Isosulfan blue in suitable solvents like methanol, ethanol, isopropanol, n-butanol, acetone, diethyl ether, Isopropyl ether, dichloromethane, acetonitrile, acetic acid and tetrahydrofuran, toluene, cyclohexane solvents or mixtures thereof;

(b) stirring the solution for 1-2 hrs at 25-30°C;

(c) filtering the solution mass through 0.2-micron filter at 25-30°C;

(d) distilling off the filtrate under vacuum below 50 °C;

(e) cooling the crude to 25-30 °C and adding solvents like methanol, ethanol, isopropyl alcohol, n-butanol, acetone, diethyl ether, isopropyl ether at 25-30 °C;

(f) stirring for 2-3 hrs at 25-30 °C;

(g) filtering the solid under vacuum at below 30 °C; and

(h) drying the solid under vacuum below 50 °C.

Isosulfan blue obtained by the above purification is having moisture content less than 10 %.

Isosulfan blue obtained by the above purification is having moisture content less than 8%.

Isosulfan blue obtained by the above purification is having moisture content less than 6%.

Isosulfan blue obtained by the above purification is having moisture content less than 4%.

In particular, the preparation or purification process of the present invention provides Isosulfan blue of formula I, which comprises metal contents i.e lead level less than 2 ppm, arsenic level less than 3 ppm and chromium level less than 50 ppm.

The process of the present invention is the only one which gives Isosulfan blue sodium comprising impurity level less than 3% organic impurities, preferably less than 2% impurities and most preferably less than 1% of impurities and total metal impurities less than 20 ppm.

Isosulfan blue obtained by the purification process of the present invention is having purity of greater than 98%, preferably greater than 99% and desethyl impurity less than 0.2% and preferably less than 0.1%.

The present inventors also found that the crystalline form of Isosulfan blue exists as further hydrated forms by exposing Isosulfan blue sodium at ambient temperature

having a relative humidity 50-75% with increase in the moisture content from 10 to 15%. Ambient temperature means a temperature from 15 to 30°C, preferably from 25 to 30°C.

In one embodiment of the invention Isosulfan blue obtained in the above process is crystalline form of Isosulfan blue hydrate.

In one aspect of the invention, a crystalline polymorphic form of Isosulfan blue hydrate is reported, whose X-ray powder diffraction (XRPD) pattern is represented by figure 1 and XPRD values are tabulated in Table - 1.

The crystalline polymorphic form of Isosulfan blue hydrate may produce an X-ray diffraction pattern comprising one or more of the following reflections at 4.38, 14.64, 15.84, 16.14, 19.49, 22.25, 22.60, 24.73 $\pm$ 0.2 degrees 2 theta (2θ°) or that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections at 6.51, 8.80, 11.69, 12.66, 12.90, 13.53, 17.05, 18.20, 18.79, 20.29, 21.27, 23.01, 23.92, 25.49, 26.01, 26.78, 27.97, 28.66, 29.29 and 30.58 $\pm$ 0.2 degrees 2 theta (2θ°) or that produces an X-ray powder diffraction pattern comprising the 2 theta values as tabulated in Table -1.

The crystalline polymorphic form of Isosulfan blue hydrate may also be identified by the TGA thermogram as depicted in figure 2.

Isosulfan blue obtained by the process of the present invention is analysed by TGA and XRPD. The XRPD data of the crystalline form of Isosulfan blue hydrate obtained is tabulated in below table-1:

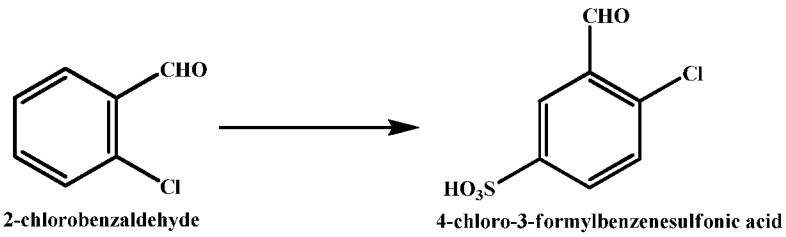
Table 1: X-Ray powder diffraction (XRPD) data of crystalline polymorphic form of Isosulfan blue hydrate

S.No.	2 Theta°	d value Angstrom	Relative intensity %
1.	4.38	20.12	100
2.	6.51	13.56	25.9
3.	8.80	10.03	31.2
4.	11.69	7.55	29.8
5.	12.66	6.98	29.4
6.	12.90	6.85	24.8
7.	13.53	6.53	24.4
8.	14.64	6.04	80.2

9.	15.84	5.58	76
10.	16.14	5.48	59.7
11.	17.05	5.19	26.9
12.	17.68	5.00	94
13.	18.20	4.86	37.4
14.	18.79	4.71	36.8
15.	19.49	4.54	44.7
16.	20.29	4.37	35.7
17.	21.27	4.17	18.7
18.	22.25	3.99	48.7
19.	22.6	3.93	49
20.	23.01	3.86	35.9
21.	23.92	3.71	26.5
22.	24.73	3.59	47.6
23.	25.49	3.49	27.7
24.	26.01	3.42	22.7
25.	26.78	3.32	21.9
26.	27.97	3.18	17.7
27.	28.66	3.11	14.9
28.	29.29	3.04	22.9
29.	30.58	2.92	14.9

The following examples further illustrate the present invention, but should not be construed in any way as to limit its scope.

EXAMPLE 1: PREPARATION OF 4-CHLORO-3-FORMYL-BENZENESULFONIC ACID OF FORMULA III

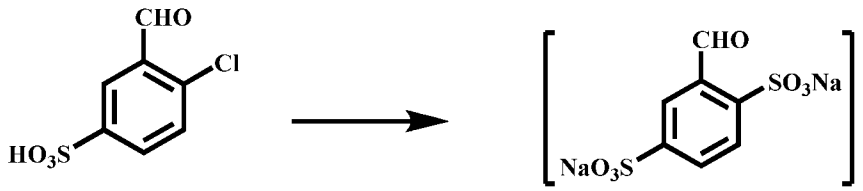


2-chlorobenzaldehyde — 4-chloro-3-formylbenzenesulfonic acid

Formula II — Formula III

100 g of 2-chlorobenzaldehyde was charged to 4 volumes of 20-25% of fuming sulphuric acid over a period of 1-2 hrs at 10-15 °C. stirred the reaction mass temperature for 30-40 minutes at 10-15 and gradually raised the temperature to 25-30 °C and then to 65-70 °C. The obtained reaction mass was further stirred for 5-6 hrs at 65-70 °C and quenched in cold water. To this 300 g of sodium chloride was added and stirred for 3-4 hrs at 25-30°C. Filtered the precipitated solid and dried under vacuum at below 75°C. Yield: 50% Purity: NLT 95%

EXAMPLE 2: PREPARATION OF SODIUM 2-FORMYLBENZENE-1,4-DISULFONATE



Formula III

4-chloro-3-formylbenzenesulfonic acid

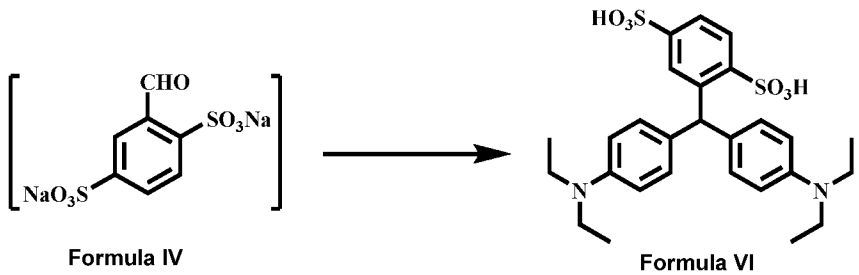
Formula IV

Sodium 2-formylbenzene-1,4-disulfonate

10 g of 4-Chloro-3-formyl-benzenesulfonic acid was dissolved in 25 ml of water and adjusted pH of the reaction mass to 9-10.5with aqueous Sodium hydroxide solution. To this 2.3 g of Sodium sulphite and 4 ml of water were added and heated to 100-105 °C. Stirred the reaction mass for 12-14 hrs at 100-105 °C. Water was distilled off and the residue was stripped off with 20 ml of Isopropyl alcohol. The reaction mixture was stirred in 300 ml of Isopropyl alcohol at 20-30 min, then cooled to 10-15°C, stirred for 1 hr, filtered, washed with of Isopropyl alcohol and dried under vacuum at temperature below 50°C to get Sodium 2-formylbenzene-1,4-disulfonate.

Purity: NLT 85 %

EXAMPLE 3: PREPARATION OF 2-(BIS(4-(DIETHYLAMINO)PHENYL)METHYL)BENZENE-1,4-DISULFONIC ACID



Formula IV

Sodium 2-formylbenzene-1,4-disulfonate

Formula VI

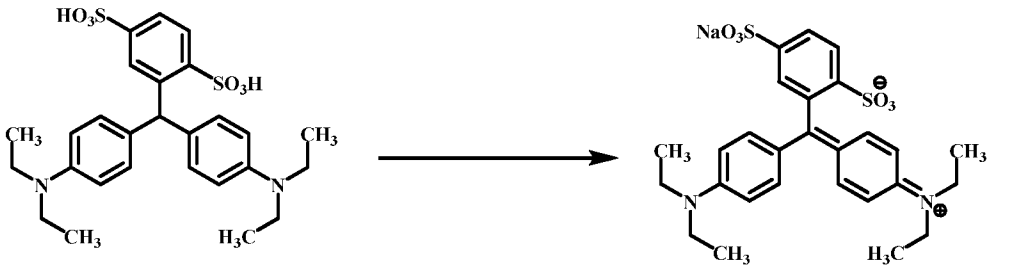
2-(bis(4-(diethylamino)phenyl)methyl) benzene-1,4-disulfonic acid

90 ml of N, N-diethyl aniline was added to 100 g of Sodium2-formylbenzene-1,4-disulfonate at 25-30°C. To this 33- 66 ml of Concentrated Hydrochloric acid was added over a period of 20-30 min. The reaction mixture was heated to 100-105°C and maintained for 12-14 hrs. After reaction completion, cooled to 25-30°C then to 0-5°C. Charged 50 ml of Isopropyl alcohol, stirred for 20-30 min, filtered and washed with

chilled (0-5°C) Isopropyl alcohol. Slurried above solid in Isopropyl alcohol and added 400 ml of Ethanol and cooled to 0-5°C. The pH was adjusted between 11.5-12.0 using aqueous Sodium hydroxide solution, stirred for 1 hr, filtered and washed with 200 ml of chilled (0-5°C) Ethanol. Distilled off the filtrate and charged 100 ml of water and cooled to 0-5°C. The pH was adjusted between 1-2 using conc. Hydrochloric acid and maintained for 4-5 hrs, filtered, washed with 200 ml chilled water and dried under vacuum at temperature below 60°C to get 2-(Bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonic acid of formula VI.

PurityNLT 96 %; Yield 25%

EXAMPLE 4: PREPARATION OF ISOSULFAN BLUE USING POTASSIUM DICHROMATE AS OXIDIZING AGENT



Formula VI
2-(Bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonic acid

Formula VII
N-[4-[[4-(Diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanamimium inner salt sodium salt

To 100 g of 2-(Bis(4-(diethylamino)phenyl)methyl)benzene-1,4-disulfonic acid, 50 ml of methanol, 100 ml of water and 15 g of Sodium hydroxide were added at 25-30°C. Cooled to 0-5°C, 25 g of Potassium dichromate was added over a period of 20-30 min, stirred for 15-20 min, 200 ml of 25% aqueous sulphuric acid solution was added over a period of 30-40 min. The reaction mixture temperature was raised to 25-30°C, stirred for 10-12 hrs and charged 500 ml of water, cooled the reaction mixture to 0-5°C and adjusted pH of the reaction mass between 8.5-9.0 using 600 ml of aqueous sodium hydroxide solution, 25 g of potassium dichromate was added over a period of 20-30 min, stirred for 15-20 min, 200 ml of aqueous 25% sulphuric acid solution was added over a period of 20-30 min. The reaction mixture temperature was raised to 25-30°C and stirred for 4-5 hrs. After reaction completion, cooled to 0-5°C, pH was adjusted between 9.5-10.0 using 300 ml of 20 % aqueous Sodium hydroxide solution. The reaction mixture temperature was raised to 25-30°C and stirred for 60-90 min, filtered

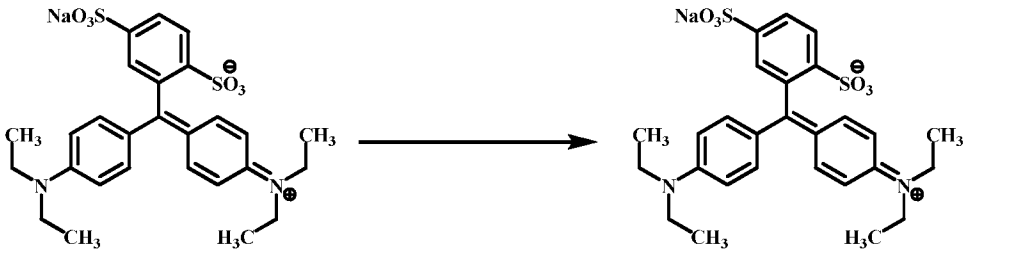
through hyflo and washed with methanol. Filtrate was distilled off under vacuum below 70°C, then charged 1000 ml of ethanol. Stirred the reaction mass for 20-30 min, filtered and washed with chilled (0-5°C) ethanol followed by chilled (0-5°C) methanol.

Purification of Isosulfan blue to remove desethyl impurity:

Filtrate was distilled off to half of its total volume at below 65°C, cooled to 25-30°C, the obtained crude (purity ~ 65%) was impregnated with 500 g of (230-400 mesh) Silica gel and dried at NMT 55°C. Column was packed with (230-400 mesh) Silica gel and loaded the impregnated crude, eluted the column using dichloromethane and methanol gradient mixture as eluent. Compound was eluted in8-12% of methanol in dichloromethane, collected the fractions distilled off the solvent from pure fractions under vacuum at 40°C. The obtained crude was slurred in acetone for 1 hr, filtered and washed with acetone. The obtained compound was dried under vacuum below 50 to get Isosulfan blue sodium.

Purity NLT 98 %; yield 30 %.

EXAMPLE 5: PURIFICATION OF ISOSULFAN BLUE (I)



Formula VII

N-[4-[[4-(Diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanamimium inner salt sodium salt

Formula I

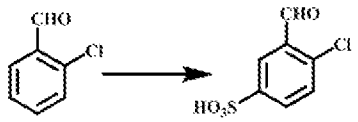
N-[4-[[4-(Diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanamimium inner salt sodium salt

To a 30 g of Isosulfan blue sodium, 300ml of methanol was added and stirred for 1 hr at 25-30°C. Filtered the reaction mixture through 0.2µ micron filter and distilled off the solvent under vacuum below 50°C. Cooled the crude mass to 25-30°C and stirred for 2-3 hrs in Isopropyl alcohol. Filtered and dried the solid below 50°C to get Isosulfan blue sodium. Purity NLT 99.9 %; yield 90 %.

We Claim:

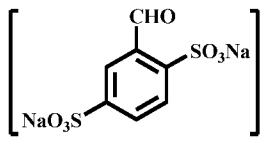
1. A process for preparation of N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium inner salt sodium salt (Isosulfan Blue) comprising the steps of:

a) sulphonating 2-chloro benzaldehyde compound of formula II with fuming sulphuric acid to obtain 4-chloro-3-formyl-benzenesulfonic acid compound of formula III;



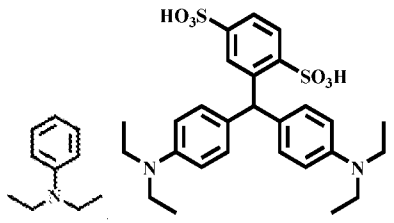
Formula II Formula III

b) treating the 4-Chloro-3-formyl-benzenesulphonic acid compound of formula III obtained in step (a) with alkali metal sulphite to obtain 1-formyl benzene-2,5-disulphonic acid disodium salt of formula IV;



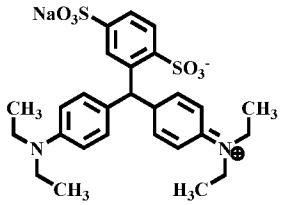
Formula IV

c) condensing 1-formyl benzene-2,5-disulphonic acid disodium salt of formula IV obtained in step (b) with N,N-diethylaniline of formula V in presence of hydrochloric acid to obtain 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid compound of formula VI;



Formula V Formula VI

d) oxidizing 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid of formula VI with an oxidizing agent in presence of sulphuric acid to obtain crude Isosulfan blue of formula VII, and purifying;



Formula VII

e) optionally, recrystallizing the Isosulfan blue of formula VII obtained in step (d) to obtain highly pure Isosulfan blue.

2. The process as claimed in claim 1, wherein the alkali metal sulphite in step (b) is selected from the group comprising of sodium sulphite, potassium sulphite, Iron sulphite, zinc sulphite and copper sulphite.

3. The process as claimed in claim 1, wherein the step (b) is carried out in a solvent selected from ethanol, methanol, propanol, isopropanol and water or mixtures thereof.

4. The process as claimed in claim 1, wherein the oxidizing agent in step (d) is selected from potassium dichromate, sodium dichromate, zinc dichromate and pyridinium dichromate.

5. The process as claimed in claim 1, wherein the crude Isosulfan blue of formula VII in step (d) is purified with a solvent selected from the group comprising of methanol, acetone and dichloromethane or mixture thereof.

6. The process as claimed in claim 1, wherein the crude Isosulfan blue of formula VII in step (d) is purified by Column chromatography.

7. The process as claimed in claim 6, wherein purification by column chromatography comprises using 100-500 mesh silica gel and selecting a polar aprotic eluent from dichloromethane, acetonitrile, methanol, ethanol and the mixtures thereof.

8. The process as claimed in claim 7, wherein the mess size of Silica gel is selected from 100-200, 200-400, 230-400 and 200-425.

9. The process for purification as claimed in claim 7, wherein the process further comprises the steps of:
   (a) slurring the obtained Isosulfan blue in acetone; and
   (b) recrystallizing from the mixture of polar protic solvents to obtain highly pure Isosulfan blue.

10. The process as claimed in claim 9, wherein the polar protic solvent in step (b) is selected from methanol, ethanol, propanol, isopropyl alcohol and tertiary butyl alcohol or mixture thereof.

11. The process as claimed in claim 1, wherein the recrystallization in step (e) comprises the steps of:
   a) suspending Isosulfan blue in a solvent;
   b) stirring the solution for 1-2 hrs at 25-30°C;
   c) filtering the solution mass through 0.2-micron filter at 25-30°C;
   d) distilling off the filtrate under vacuum below 50 °C;
   e) cooling the crude to 25-30°C and adding solvents like methanol, Ethanol, Isopropyl alcohol, n-butanol, acetone, diethyl ether, Isopropyl ether at 25-30 °C;
   f) stirring for 2-3 hrs at 25-30 °C;
   g) filtering the solid under vacuum at below 30 °C;
   h) drying the solid under vacuum below 50 °C.

12. The process as claimed in claim 11, wherein the solvent in step (a) is selected from the group comprising of methanol, ethanol, isopropanol, n-butanol, acetone, diethyl ether, Isopropyl ether, dichloromethane, acetonitrile, acetic acid, tetrahydrofuran, toluene, ethylacetate and cyclohexane or mixtures thereof.

13. The process as claimed in claim 11, wherein the Isosulfan blue obtained from the process is crystalline Isosulfan blue hydrate characterized by X-ray powder diffraction (XRPD) pattern comprising one or more of the reflections at 4.38, 14.64, 15.84, 16.14, 19.49, 22.25, 22.60 and 24.73± 0.2 degrees 2 theta (2θ°).

14. The process as claimed in claim 11, wherein the Isosulfan blue obtained from the process is crystalline Isosulfan blue hydrate further characterized by X-ray powder diffraction (XRPD) pattern comprising of the reflections at 6.51, 8.80, 11.69, 12.66, 12.90, 13.53, 17.05, 17.68, 18.20, 18.79, 20.29, 21.27, 23.01, 23.92, 25.49, 26.01, 26.78, 27.97, 28.66, 29.29 and 30.58 ± 0.2 degrees 2 theta (2θ°).

15. The process as claimed in claim 1, wherein the step (c) further comprises purifying the compound of formula VI by treating with a base and converting into free base to get pure compound VI.

16. The process as claimed in claim 1, wherein the Isosulfan blue obtained from the process comprises a purity level of greater than 99%.

17. The process as claimed in claim 1, wherein the Isosulfan blue obtained from the process contains moisture content less than 10%.

18. The process as claimed in claim 1, wherein the Isosulfan blue obtained from the process contains desethyl impurity level less than 0.2%.

19. The process as claimed in claim 1, wherein the Isosulfan blue obtained from the process contains lead less than 2 ppm, Arsenic less than 3 ppm and chromium less than 50 ppm.

20. The process as claimed in claim 1, wherein the Isosulfan blue obtained from the process contains total organic impurities less than 3% and total metal impurities less than 20 ppm.

21. A process for the preparation of N-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium inner salt sodium salt (Isosulfan Blue) comprising the steps of:

(a) sulphonating 2-Chloro benzaldehyde compound with 1.4 equivalents of 20-25% sulphuric acid (Oleum) at 10-15°C to obtain 4-Chloro-3-formyl-benzenesulphonic acid ;

(b) converting 4-Chloro-3-formyl-benzenesulphonic acid to 1-formyl benzene-2,5-disulphonic acid disodium salt by treatment with sodium sulphite in presence of water and sodium hydroxide (NaOH) at temperature 85-110 °C;

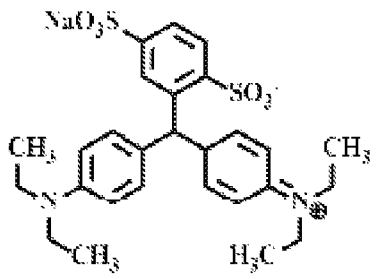
(c) condensing 1-formyl benzene-2,5-disulphonic acid disodium salt with N,N-diethyl aniline in presence of hydrochloric acid to obtain 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid and optionally purifying;

(d) oxidizing 4-[Bis[4-(diethylamino)phenyl]methyl]benzebe-2,5-disulphonic acid using potassium dichromate in presence of sulphuric acid to obtain crude Isosulfan blue;

(e) optionally, purifying the crude Isosulfan blue obtained in step (d) by Column chromatography using dichloromethane and methanol to obtain pure Isosulfan blue ; and

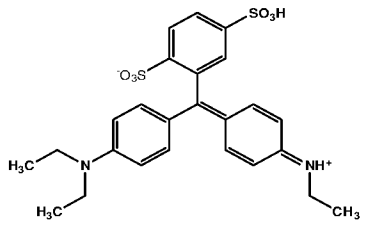
(f) optionally, recrystallizing Isosulfan blue obtained in step (d) or step (e) with methanol and Isopropyl alcohol to provide highly pure Isosulfan blue.

22. An Isosulfan blue compound of formula-I



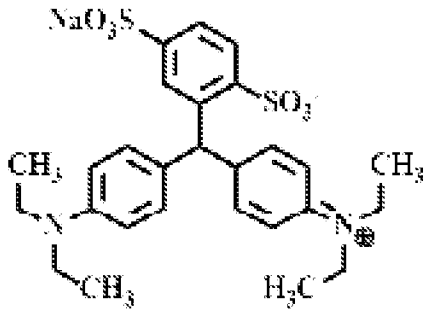
(I)

comprising less than 0.2% of desethyl impurity of the formula A.



(A)

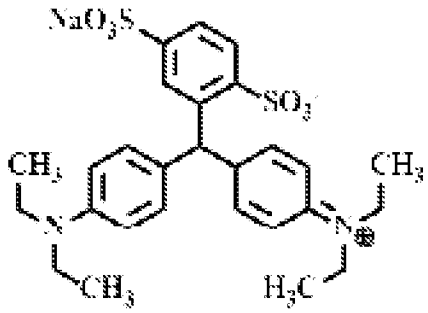
23. An Isosulfan blue compound of formula-I



(I)

containing metal impurities comprising lead less than 2ppm, Arsenic less than 3 ppm and chromium less than 50 ppm.

24. An Isosulfan blue compound of formula-I having moisture content less than 10%.



(I)

25. An Isosulfan blue compound comprising desethyl impurity less than 0.2% and moisture content 4-10%.

26. A Crystalline Isosulfan blue hydrate characterized by X-ray powder diffraction (XRPD) pattern comprising one or more of the reflections at 4.38, 14.64, 15.84, 16.14, 19.49, 22.25, 22.60 and 24.73± 0.2 degrees 2 theta (2θ°).

27. The crystalline Isosulfan blue hydrate as claimed in claim 26, further characterized by X-ray powder diffraction (XRPD) pattern comprising of the reflections at 6.51, 8.80, 11.69, 12.66, 12.90, 13.53, 17.05, 17.68, 18.20, 18.79, 20.29, 21.27, 23.01, 23.92, 25.49, 26.01, 26.78, 27.97, 28.66, 29.29 and 30.58 ± 0.2 degrees 2 theta (2θ°).

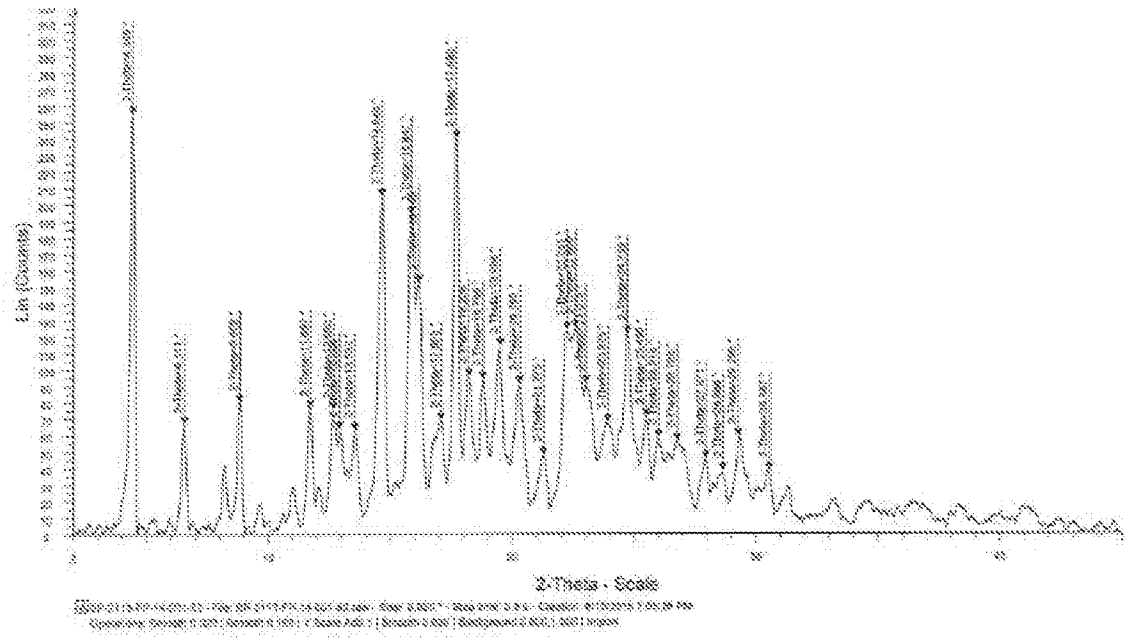


Figure-1: XRPD peaks pattern of Isosulfan blue (I) hydrate

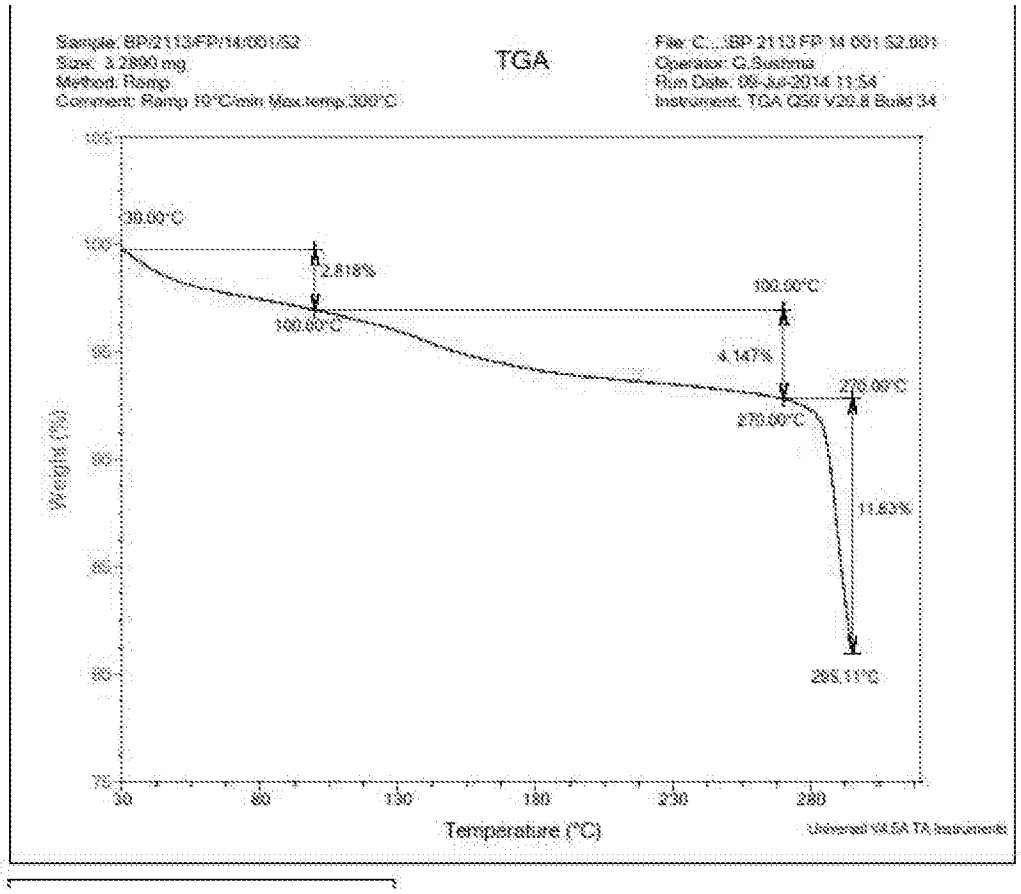


Figure-2: TGA thermogram of crystalline form of Isosulfan blue (I) hydrate

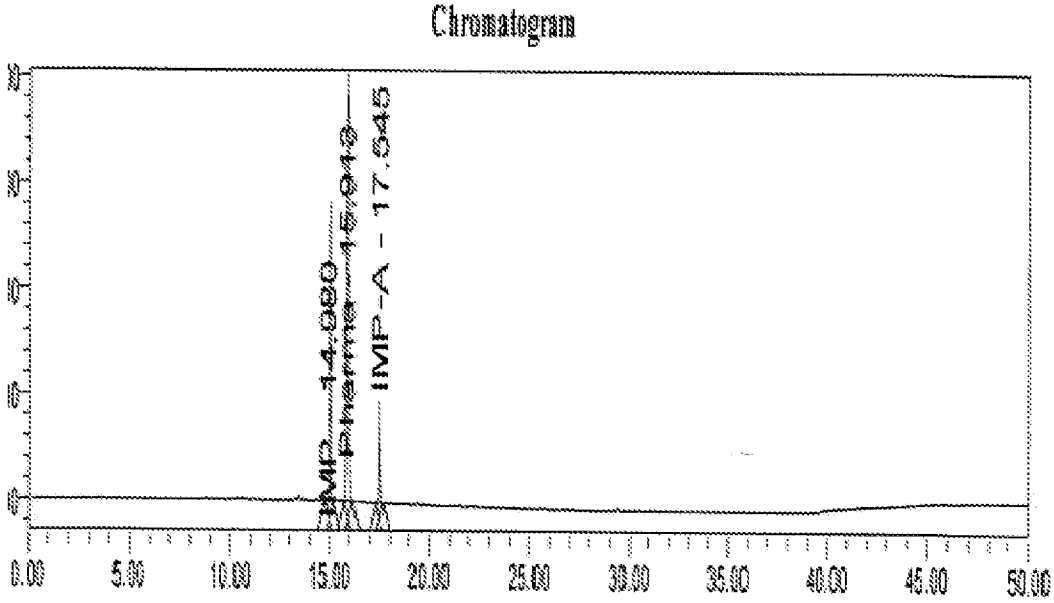


Figure 3: HPLC chromatogram of desethyl impurity of formula A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2016/050297

A. CLASSIFICATION OF SUBJECT MATTER
C07C303/00 Version=2017.01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patseer, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US20060224003 A1 KULKARNI BALKRISHNA K [IN], SANKAR CHINNAKULANDAI [IN], KHANDEKAR GIRISH M [IN], MAURYA HAWALDAR T [IN], ARJUN ASHOK V [IN], SOPE SANDEEP S [IN], SUMIT KAR [IN] 5 OCTOBER 2006 (05-10-2006) Claims 1-9.	1-8, 21
Y	IN 03879/MUM/2014 DISHMAN PHARMACEUTICALS AND CHEMICALS LTD (IN) 10 JUNE 2016 (10-06-2016) Claims 9-15 ,Example -5.	1-27
Y	IN 263/CHE/2014 OPTIMUS DRUGS(P) LIMITED [IN] 01 JULY 2016 (01/07/2016) Claims 1-8	1-27
Y	US20130310600 A1 APICORE LLC [US] 21 NOVEMBER 2013 (21/11/2013) Claim 23	1-27

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27-02-2017	27-02-2017

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2016/050297

Citation	Pub.Date	Family	Pub.Date
US 2006224003 A1	05-10-2006	US 7534911 B2	09-05-2009
		IN 2005MU00420 A	15-06-2007
IN 03879MUM2014 A	10-06-2016	IN 03879MUM2014 A	10-06-2016
IN 0263CHE2014 A	01-07-2016	IN 0263CHE2014 A	01-07-2016
US 20130310600 A1	21-11-2013	US 2016221936 A1	04-08-2016
		WO 2008140564 A1	20-11-2008
		IN 4223KOLNP2009 A	09-05-2014