recrystallisation to reduce the Azure levels in this derivative. Again, the final process is reconversion or oxidation to methylene blue using quinones, nitric acid, perchloric acid iodine, hydrochloric acid, sulphuric acid, hydrogen peroxide or UV light, with a preference for 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).

[0017] In summary, the levels of metals and/or organic impurities found within methylene blue samples is not unexpected when such historic synthetic approaches are considered, and may even be acceptable for the commercial product in use for many industrial and cytological purposes where these potential residues do not affect the use. However, with recent requirements for lower levels of these impurities in methylene blue used for pharmaceutical purposes, it has become necessary to address the removal of impurities in an efficient, simple and cost effective purification process.

OBJECT OF THE INVENTION

[0018] It is an aim of this invention to provide a purification method and intermediate which overcomes or ameliorates one or more of the disadvantages or problems described above, or which at least provides a useful alternative or simpler methodology.

[0019] Other preferred objects of the present invention will become apparent from the following description.

SUMMARY

[0020] According to the disclosure, there is provided a process for the purification of a diaminophenothiazinium compound including the steps of:

(i) contacting the diaminophenothiazinium compound with a reducing agent to form a protonated stabilised diaminophenothiazinium complex;

(ii) purifying the protonated stabilised diaminophenothiazinium complex and;

(iii) contacting the purified protonated stabilised diaminophenothiazinium complex with an oxidising agent to convert it back to the diaminophenothiazinium compound,

to thereby purify the diaminophenothiazinium compound.

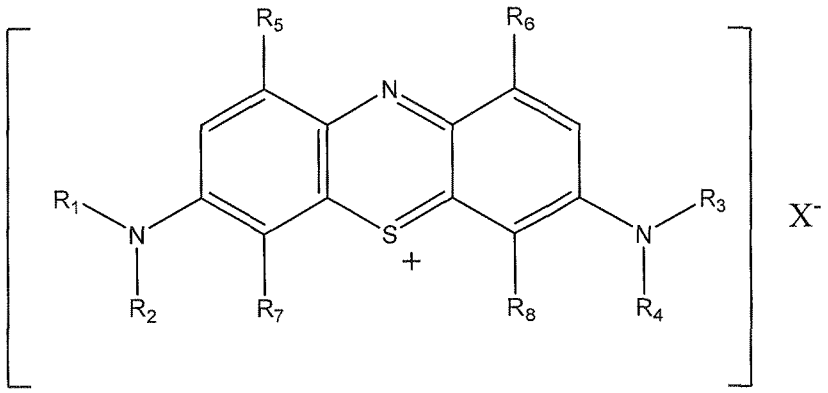
[0020a] In a first aspect of the present disclosure, there is provided a process for the purification of a diaminophenothiazinium compound including the steps of:

(i) contacting the diaminophenothiazinium compound with a metal-free reducing agent to form a protonated stabilised diaminophenothiazinium complex;

(ii) purifying the protonated stabilised diaminophenothiazinium complex; and

(iii) contacting the purified protonated stabilised diaminophenothiazinium complex with an oxidising agent to convert it back to the diaminophenothiazinium compound,

to thereby purify the diaminophenothiazinium compound, wherein the diaminophenothiazinium compound is a compound of formula V:



Formula V

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen, C_1-C_6 alkyl and C_1-C_6 alkenyl wherein each alkyl or alkenyl group may be substituted with hydroxy, halo or alkoxy;

R_5, R_6, R_7 and R_8 are independently selected from hydrogen, nitro, halo, haloalkyl, C_1-C_6 alkyl and C_1-C_6 alkenyl; and

X is an anionic counterion.

[0021] The diaminophenothiazinium compound may be selected from the group consisting of methylene blue, Azure A, Azure B and Azure C.

[0022] In one embodiment, the contact with the reducing agent may form an N-10 protonated stabilised diaminophenothiazinium complex which is subsequently purified and oxidised back to the diaminophenothiazinium compound.

[0023] There is also provided a process for the purification of methylene blue including the steps of:

(i) contacting the methylene blue with a reducing agent to form a stabilised protonated leucomethylene blue complex;

(ii) purifying the stabilised protonated leucomethylene blue complex; and

(iii) contacting the purified stabilised protonated leucomethylene blue complex with an oxidising agent to form methylene blue,

to thereby purify the methylene blue.

[0023a] In a second aspect of the present disclosure, there is provided a process for the purification of methylene blue including the steps of:

(i) contacting the methylene blue with a metal-free reducing agent to form a stabilised protonated leucomethylene blue complex;

(ii) purifying the stabilised protonated leucomethylene blue complex; and

(iii) contacting the purified stabilised protonated leucomethylene blue complex with an oxidising agent to form methylene blue,

to thereby purify the methylene blue.

[0024] The reducing agent of the present disclosure is a metal-free reducing agent.

[0025] In one embodiment, the reducing agent is selected from the group consisting of ascorbic acid, sodium dithionite, hydrogen, formic acid, oxalic acid, dithiothreitol, sodium

amalgam, sodium borohydride, hydrazine, phosphites, hypophosphites and phosphorous acid.

[0026] Suitably, the reducing agent of the present disclosure is ascorbic acid. Preferably it is L-ascorbic acid.

[0027] Preferably, the diaminophenothiazinium compound or methylene blue is contacted with the reducing agent at an acidic pH. More preferably, the diaminophenothiazinium compound or methylene blue is contacted with the reducing agent at below pH 3. Even more preferably, the diaminophenothiazinium compound or methylene blue is contacted with the reducing agent at below pH 1.

[0028] Preferably, the reducing agent is in a solution made acidic with an acid chosen from a monoprotic or diprotic mineral acid.

[0029] Suitably, the acid is selected from the group consisting of hydrochloric, sulphuric, nitric, phosphoric, boric, hydrofluoric, hydrobromic and perchloric acids.

[0030] In one embodiment, the mineral acid is hydrochloric or sulphuric acid.

[0031] Suitably, the mineral acid is hydrochloric acid.

[0032] Preferably, the contacting with the reducing agent of the present disclosure occurs in an organic solvent. Suitably, the organic solvent is a C1 to C8 alcohol or C1 to C4 alcohol. Preferably, the alcohol is methanol or ethanol.

[0033] The purification of the protonated stabilised diaminophenothiazinium complex (which in one embodiment may be an N-10 protonated stabilised diaminophenothiazinium complex) or stabilised protonated leucomethylene blue complex is preferably by recrystallisation.

[0034] In one embodiment, the recrystallisation is from a solvent selected from the group consisting of water, an alcohol and an ether.

[0035] If at least one of the recrystallisation solvents is an alcohol then it may be selected from the group consisting of C_1 to C_{10} alcohols inclusive of methanol, ethanol, *n*-propanol and *iso*-propanol.

[0036] If at least one of the recrystallisation solvents is an ether it may be tetrahydrofuran or diethyl ether.

[0037] Suitably, the oxidising agent of the present disclosure is a metal-free oxidising agent.

[0038] Preferred oxidising agents of the present disclosure may be selected from the group consisting of oxygen, ozone, chlorine, fluorine, bromine, iodine, hydrogen peroxide, nitric acid and nitrate compounds, phosphoric acid, peroxydisulphuric acid, peroxymonosulphuric acid, sulphuric and sulphurous acids, chlorite, chlorate, perchlorate and other analogous halogen-containing compounds, hypochlorite and other hypohalite compounds, permanganates, nitrous oxide and quinones.

[0039] In one embodiment the oxidising agent of the present disclosure is selected from the group consisting of oxygen and a quinone.

[0040] Preferably, the oxidising agent of the present disclosure is 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) or, more preferably, *p*-benzoquinone.

[0041] The stabilised protonated leucomethylene blue complex may be a salt or other ion complex.

[0042] In one embodiment, the stabilised protonated leucomethylene blue complex may be a leucomethylene blue:ascorbate complex.

[0043] In one embodiment of the disclosure there is provided a protonated stabilised diaminophenothiazinium:ascorbate complex.

[0044] In one embodiment, the protonated stabilised diaminophenothiazinium complex is a stabilised protonated leucomethylene blue:ascorbate complex.

[0045] In one embodiment, the protonated stabilised diaminophenothiazinium complex is an N-10 protonated stabilised diaminophenothiazinium complex.

[0046] Another embodiment of the disclosure resides in the protonated stabilised diaminophenothiazinium:ascorbate complex of the previous embodiment when produced by the process of contacting the diaminophenothiazinium compound with ascorbic acid to form the protonated stabilised diaminophenothiazinium:ascorbate complex.

[0047] In one embodiment, the protonated stabilised diaminophenothiazinium:ascorbate complex is an N-10 protonated stabilised diaminophenothiazinium:ascorbate complex.

[0048] In one embodiment, the protonated stabilised diaminophenothiazinium:ascorbate complex is the stabilised protonated leucomethylene blue:ascorbate complex as disclosed above when produced by the process of contacting methylene blue with ascorbic acid to form the stabilised protonated leucomethylene blue:ascorbate complex.

[0049] The various conditions for the process of the before said embodiment may be as described for the first aspect.

[0050] According to the disclosure, there is provided a use of a protonated stabilised diaminophenothiazinium complex in the purification of a diaminophenothiazinium compound.

[0051] In one embodiment, the protonated stabilised diaminophenothiazinium complex is an N-10 protonated stabilised diaminophenothiazinium complex.

[0052] In one embodiment, the protonated stabilised diaminophenothiazinium complex is a stabilised protonated leucomethylene blue complex used in the purification of methylene blue.

[0053] In one embodiment, the stabilised protonated leucomethylene blue complex is a stabilised protonated leucomethylene blue salt.

[0054] In one embodiment, the stabilised protonated leucomethylene blue complex is the stabilised protonated leucomethylene blue:ascorbate complex as disclosed above.

[0055] The various features and embodiments of the presentdisclosure, referred to in individual sections above apply, as appropriate, to other sections, *mutatis mutandis*. Consequently features specified in one section may be combined with features specified in other sections as appropriate.

[0056] Further features and advantages of the present disclosure will become apparent from the following detailed description.

DETAILED DESCRIPTION

[0057] The present invention is predicated, at least in part, on the surprising finding that methylene blue can be simply and effectively purified by reducing a post-synthesis or commercially available sample to form a reduced stabilised protonated leucomethylene blue complex, which may be a salt or other ion complex, which can then be purified by, for example, recrystallisation before being allowed to oxidise back to methylene blue. Crystallisation of methylene blue itself, as a purification method to remove both Azure type impurities and metal contaminants, has proven to be ineffective on its own in achieving even close to pharmaceutical grade material and so it is particularly surprising that recrystallisation of the reduced leucomethylene blue form, even with the close structural similarities to the Azures still remaining, is an effective means for separation of that leucomethylene blue form from both the Azures and metal contaminants. A simple oxidation step then provides the methylene blue but in a highly purified form.

CLAIMS

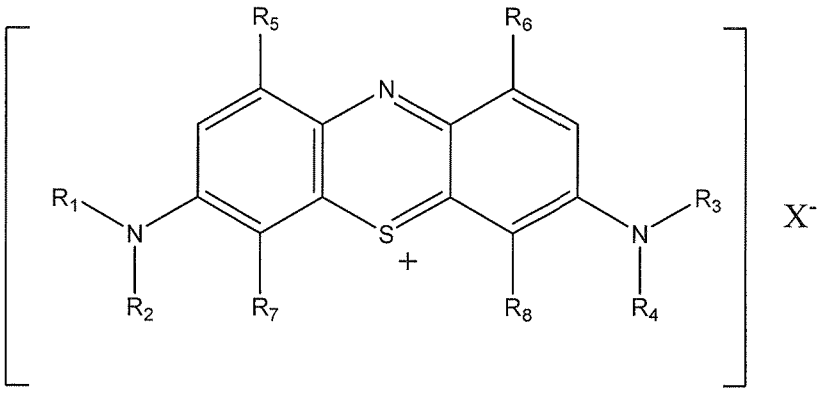
1. A process for the purification of a diaminophenothiazinium compound including the steps of:

(i) contacting the diaminophenothiazinium compound with a metal-free reducing agent to form a protonated stabilised diaminophenothiazinium complex;

(ii) purifying the protonated stabilised diaminophenothiazinium complex; and

(iii) contacting the purified protonated stabilised diaminophenothiazinium complex with an oxidising agent to convert it back to the diaminophenothiazinium compound,

to thereby purify the diaminophenothiazinium compound, wherein the diaminophenothiazinium compound is a compound of formula V:



Formula V

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen, C_1-C_6 alkyl and C_1-C_6 alkenyl wherein each alkyl or alkenyl group may be substituted with hydroxy, halo or alkoxy;

R_5, R_6, R_7 and R_8 are independently selected from hydrogen, nitro, halo, haloalkyl, C_1-C_6 alkyl and C_1-C_6 alkenyl; and

X is an anionic counterion.

2. The process of claim 1 wherein the protonated stabilised diaminophenothiazinium complex is an N-10 protonated stabilised diaminophenothiazinium complex which is subsequently purified and oxidised to give the diaminophenothiazinium compound.

3. The process of claim 1 or claim 2 wherein the diaminophenothiazinium compound is selected from the group consisting of methylene blue, methylene green, Azure A, Azure B and Azure C.

4. A process for the purification of methylene blue including the steps of:

(i) contacting the methylene blue with a metal-free reducing agent to form a stabilised protonated leucomethylene blue complex;

(ii) purifying the stabilised protonated leucomethylene blue complex; and

(iii) contacting the purified stabilised protonated leucomethylene blue complex with an oxidising agent to form methylene blue,

to thereby purify the methylene blue.

5. The process of any one of the preceding claims wherein the reducing agent is selected from the group consisting of ascorbic acid, formic acid, oxalic acid, dithiothreitol, hydrazine, phosphites, hypophosphites and phosphorous acid.

6. The process of claim 5 wherein the reducing agent is ascorbic acid.

7. The process of any one of the preceding claims wherein the diaminophenothiazinium compound or methylene blue is contacted with the reducing agent at an acidic pH, optionally at below pH 3 or below pH 1.

8. The process of any one of the preceding claims wherein the reducing agent is in a solution made acidic with an acid selected from a monoprotic or diprotic mineral acid.

9. The process of claim 8 wherein the acid is selected from the group consisting of hydrochloric, sulphuric, nitric, phosphoric, boric, hydrofluoric, hydrobromic and perchloric acids.

10. The process of any one of the preceding claims wherein the contacting with the reducing agent occurs in an organic solvent.

11. The process of claim 10 wherein the organic solvent is a C1 to C8 alcohol.

12. The process of any one of the preceding claims wherein purification of the protonated stabilised diaminophenothiazinium complex or stabilised protonated leucomethylene blue complex is by a process selected from the group consisting of chromatography, ion-exchange, filtration, washing and recrystallisation.

13. The process of claim 12 wherein the purification is by recrystallization, optionally from a solvent selected from the group consisting of water, an alcohol and an ether.

14. The process of any one of the preceding claims wherein the oxidising agent is a metal-free oxidising agent.

15. The process of claim 14 wherein the oxidising agent is selected from the group consisting of oxygen, ozone, chlorine, fluorine, bromine, iodine, hydrogen peroxide, nitric acid and nitrate compounds, phosphoric acid, peroxydisulphuric acid, peroxymonosulphuric acid, sulphuric and sulphurous acids, chlorite, chlorate, perchlorate and other analogous halogen-containing compounds, hypochlorite and other hypohalite compounds, nitrous oxide and quinones.

16. The process of claim 4 wherein the stabilised protonated leucomethylene blue complex is a salt or other ion complex.

17. The process of claim 16 wherein the stabilised protonated leucomethylene blue complex is a leucomethylene blue:ascorbate complex.

18. The process of any one of the preceding claims further including the step, prior to step (i), of recrystallising the diaminophenothiazinium compound or methylene blue from an acidic solution.

19. The process of any one of the preceding claims further including the step, subsequent to step (iii), of recrystallising the purified diaminophenothiazinium compound or methylene blue from an acidic solution.