IFOSFAMIDE COMPOSITIONS FOR PARENTERAL ADMINISTRATION AND A PROCESS FOR THEIR PREPARATION

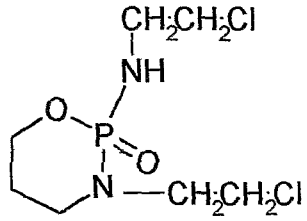
Field of Invention

This invention relates to Ifosfamide compositions for parenteral use. This invention particularly relates to stable, clear, aqueous Ifosfamide compositions having reduced toxicity, for parenteral administration. This invention more particularly relates Ifosfamide compositions comprising 2-hydroxypropyl-β-cyclodextrin (referred to hereinafter as "HPBCD") in addition to Ifosfamide.

Background of the invention

Two main groups of drugs used in the treatment of malignant disease are alkalyting agents and the antimetabolites. Ifosfamide is one of the widely used antineoplastic drug belonging to the alkalyting agents group.

Ifosfamide is chemically 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]-tetrahydro-2H- 1,3,2-oxazaphosphorin-2-oxide and is represented by the formula:



Ifosfamide is a white crystalline hygroscopic powder having a low melting point of 40°C. The powder has a water solubility of about 100mg/ml.

Ifosfamide is used in the treatment of a variety of solid tumours including those of the cervix, endometrium, lung, ovary, testes and thymus as well as in sarcoma and in the treatment of Burkitts lymphoma.

The treatment with Ifosfamide is associated with serious side effects like hemorrhagic cystitis, myelosuppression, cardiac arrythmias, CNS disturbances,

nephrotoxicity, haematological and gastro-intestinal reactions. The LD_{50} in mouse on intravenous administration has been reported to be 338 mg/kg body weight. Combination with the uroprotective agent mesna reduces the incidence of haemorrhagic cystitis. Therefore mesna is normally administered intravenously at a dose of 20% of the Ifosfamide dose at time zero (the time of administration of Ifosfamide), and then at 4 and 8 hours.

Ifosfamide is given intravenously either by injection or by infusion as a diluted solution containing less than 4%w/v of Ifosfamide. Ifosfamide is very susceptible to hydrolytic degradation and accordingly prompt administration of such solutions is generally required. Therefore commercially it is predominately available in dry form and is supplied as sterile packaged dry powder for dissolution in water for injection prior to administration. However, the low melting point and the hygroscopic nature of Ifosfamide make it necessary to fill the powder with great care keeping both temperature and humidity accurately controlled to get a sterile product. Further, prolonged storage of the dry powder also results in sintering and yellowing, which in turn leads to a reduction in dissolution rate thereby increasing time taken for reconstitution.

To overcome difficulties associated with the thermal and hydrolytic susceptibility, lyophilization of the drug has been tried. However, the lyophilization process is quite time consuming requiring specialized equipments. Personnel exposure to the strongly cytotoxic Ifosfamide occurring during reconstitution of lyophilized powder is undesirable.

Hence, attempts are being made to prepare clear liquid Ifosfamide compositions that will be stable over a period of time for parenteral administration.

US 4952575 discloses preparation of ethanolic solution of Ifosfamide containing 96% to 100% ethanol. Even though the degradation of Ifosfamide has

been shown to be minimal, use of solvents in such a high concentration leads to other problems such as volatility, handling during manufacturing, miscibility with blood on administration. As such alcohol is pharmacologically active which may also affect the person on administration of alcoholic solution of Ifosfamide.

There are two patents using polyols for making stable liquid compositions of oxazophosphorine compounds namely

US 4879286 discloses an invention in which Cyclophosphamide is formulated in a ready-to-dilute solution. This invention uses organic polyols namely propylene glycol and polyethylene glycol and their mixtures as a solvent and also 0 to 50% water. The water may be partly replaced by 10 to 30% of ethanol.

WO 0202125 discloses liquid pharmaceutical composition for parenteral administration comprising Ifosfamide, solvent and optionally, conventional pharmaceutical carriers and excipients. According to the invention, the solvent comprises 35-75% lower alcohol and 25-65% polyol. While lower alcohol solvent is usually ethanol, the polyol solvent is propylene glycol, glycerol and / or polyethylene glycol.

In both the patents US 4879286 and WO 0202125, the parenteral administration of larger amounts of polyols and alcohols lead to other problems like pain or irritation on injection, hemolysis, ototoxicity, cardiovascular effects, CNS effects and seizures. It may also lead to hyperosmolarity and lactic acidosis in patients with renal impairment.

WO 9918973 describes a stable, ready-to-use liquor of Ifosfamide using Sodium chloride as a stabilizing agent. The invention also discloses 10-500 mg/ml Ifosfamide composition containing Urea, sodium chloride and sodium dihydrogen phosphate. The compositions of the invention are said to be stable but

there is no mention about the safety and toxicity of the composition. The higher concentration of urea in the formulation may lead to complications like hemolysis, irritation, phlebitis & thrombosis at the site of injection, and elevated blood ammonia & urea concentrations in patients with hepatic and renal function impairment.

WO 03/051297A2 describes a ready-to-use aqueous composition of Ifosfamide comprising 40 – 400 mM (10 – 100mg/ml) of Ifosfamide in pharmaceutically acceptable buffer. The patent suggests the use of buffers preferably from the group of Na_2HPO_4 & NaH_2PO_4 and K_2HPO_4 & KH_2PO_4. There is no report on the toxicity of Ifosfamide compositions disclosed in this patent.

Thus, there remains a need for a stable, concentrated Ifosfamide solution to facilitate handling during administration. In addition, there remains a need for Ifosfamide pharmaceutical compositions that exhibit less toxicity than the currently available compositions.

Our main object of the present invention is thus to develop stable, clear, aqueous Ifosfamide compositions having reduced toxicity for parenteral administration in human beings and other mammals.

Another object of the invention is to develop a process for making such compositions.

Summary of the Invention

Accordingly, the present invention provides stable, clear, aqueous Ifosfamide compositions having reduced toxicity, for parenteral administration comprising

Ifosfamide up to 1100 mg /ml of the composition

and 2-hydroxypropyl-β-cyclodextrin.

The present invention also provides a process for the preparation of Ifosfamide compositions having reduced toxicity, for parenteral administration comprising steps of

i. bringing in intimate contact Ifosfamide, 2-hydroxypropyl-β-cyclodextrin and water;

ii. rendering sterile the composition so obtained at the end of step (i).

In the process of the present invention, required quantities of pharmaceutically acceptable additives may be added while bringing in intimate contact Ifosfamide, 2-hydroxypropyl-β-cyclodextrin and water.

Intimate contact may be brought about by methods such as stirring, mixing, sonicating, heating, homogenizing. Preferably it is carried out under aseptic conditions and under inert gas atmosphere at temperatures below 40°C.

Further the invention provides adding one or more pharmaceutically acceptable buffers, tonicity agents, preservatives, chelating agents, antioxidants, anticrystallising agents.

The present invention also contemplates rendering the composition sterile preferably by passing through sterile 0.2µm sterilizing grade filter and transferring aseptically the sterile composition into sterile containers followed by purging the air in the headspace of the container with inert gas such as nitrogen and sealing the containers.

Detailed description of embodiments of the invention

Ifosfamide content in the composition described in this invention is up to 1100 mg/ml., the preferred range is 1 mg/ml to 200 mg/ml, more preferably 10 mg/ml to 100 mg/ml, most preferably 40mg/ml to 50mg/ml. Other preferred ranges of Ifosfamide content in the composition described in this invention are 200mg/ml to 500 mg/ml and 500 mg/ml to 1000 mg/ml.

In concentrated Ifosfamide compositions, Ifosfamide content is from 200 mg/ml to 1100 mg/ml, preferably, 200 mg/ml to 1000 mg/ml, more preferably 500mg/ml and 1000mg/ml.

2-hydroxypropyl-β-cyclodextrin (HPBCD) is a partially substituted poly(hydroxypropyl)ether of beta cyclodextrin. The hydroxyproplyl groups are randomly substituted onto hydroxyl groups of the cyclodextrin and the amount of substitution is reported as average degree of substitution or number of hydroxypropyl groups per cyclodextrin. Alternatively amount of substitution is reported as molar substitution (MS) or the average number of substitution per anhydroglucose unit in the ring of the cyclodextrin. Molar substitution can have an effect on the binding of guest molecules to HPBCD . At low degree of substitution, binding is very similar to that of the unmodified betacyclodextrin. Increasing molar substitution can lead to weakened binding due to stearic hindrance. HPBCD having molar substitution between 0.05 to about 2 can be useful in this invention. HPBCD having molar substitution between 0.3 to about 1.5 is preferred, HPBCD having molar substitution between 0.5 to about 1.2 is more preferred.

HPBCD may be present in the composition at a molar ratio of Ifosfamide to HPBCD 100 : 0.1 to 1 : 300, preferably 100 : 0.25 to 1 : 100, more preferably 100 : 1 to 1 : 20. Other preferred molar ratio of Ifosfamide to HPBCD is 100 : 3.3 to 1 : 2.5.

While making concentrated Ifosfamide compositions the requirement of HPBCD varies with the concentration of Ifosfamide. Initially it increases with the concentration of Ifosfamide. But at the higher end of the Ifosfamide concentration, HPBCD and water content is limited due to volume constraint. Therefore the ratio of Ifosfamide to HPBCD should be rightly chosen.

The pH of the composition of the present invention may be between 3.0 – 9.0. The preferable pH for the present invention is between 5.0 – 8.0.

The compositions of the present invention may also include pharmaceutically acceptable additives for the purposes of pH stabilization, preservation, isotonicity adjustment, stabilization against oxidation, chelating agents, anticrystallising agents and other suitable additives. Some of the pharmaceutically acceptable additives may be present in the aqueous solution to which the Ifosfamide and HPBCD are added and / or some of them may be added separately as a solution in water before making up the volume in the final composition.

The compositions of the present invention may require suitable buffers to adjust or stabilise the pH . Suitable buffering agents for the compositions of the present invention include but are not limited to Phosphate buffer, Citrate buffer, Glycine buffer, Histidine buffer containing any of the commonly used compounds or a mixture of compounds such as Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Histidine hydrochloride, Sodium hydroxide, Phosphoric acid, Sodium citrate, Citric acid, Glycine, Potassium citrate, Hydrochloric acid and Potassium hydroxide. Preferred buffering agent is a mixture of Sodium dihydrogen phosphate and Disodium hydrogen phosphate.

Of the other pharmaceutically acceptable additives, suitable tonicity agent for compositions of the present invention are selected from group of compounds such as glycerin, sodium chloride, maltose, mannitol, dextrose and mixtures thereof.

Similarly, suitable preservatives for compositions of the present invention may include but are not limited to methyl hydroxy benzoic acid, propyl hydroxy benzoic acid, phenol, benzyl alcohol and sodium benzoate.

The composition of the present invention may contain suitable chelating agents such as ethylenediaminetetraacetic acid (EDTA) & its salts and Desferoximine methane sulfonate (Desferal).

The composition of the present invention may also contain suitable antioxidants such as, ascorbic acid, sodium bisulfite, sodium metabisulfite, butylated hydroxy anisole and butylated hydroxy toluene.

Also, the composition of the present may contain substances such as Glycerin as anticrystallising agents.

The aqueous solutions containing Ifosfamide and HPBCD are brought in intimate contact by methods such as stirring, mixing, sonicating, heating, homogenizing. Pharmaceutically acceptable additives such as buffers, tonicity agents, preservatives, chelating agents, antioxidants, anticrystallising agents as required by parenteral dosage form may be present in the aqueous solution to which the Ifosfamide and HPBCD is added. Alternatively, they can be added separately as a solution in water before making up the volume. In the preparation of concentrated Ifosfamide composition, to incorporate larger amounts of Ifosfamide, HPBCD is dissolved in minimum quantity of water and Ifosfamide is solubilized by intimate stirring. Pharmaceutically acceptable additives if required are added as such or as solutions into Ifosfamide - HPBCD solution. Finally the remaining quantity of water is added to makeup to the required volume, followed by mixing to get a homogenous solution and then rendering sterile the composition so obtained. The composition may be rendered non-pyrogenic if required by passing through Tangential Flow Filtration System (TFF) before sterilisation.

The composition is usually rendered sterile by passing through a sterilising grade filter. Preferably, 0.2µm sterilizing grade filters may be used.

The sterile compositions of the present invention may be filled aseptically into sterile containers such as vials, ampoules, plastic containers, purging the air in the headspace of the containers with inert gas such as nitrogen and sealing the filled containers.

Not bound by theory, we believe that the three component system of Ifosfamide, HPBCD and water is a stable aqueous composition. Such systems are being studied at present for different aspects such as solubility, complexation and encapsulation over different temperature and time period. Further the effect of other additives on the stability of the system is also under study.

It is well known that the treatment with Ifosfamide is associated with serious side effect like hemorrhagic cystitis, and to counteract it Mesna is always administered with Ifosfamide injection. However, there are other side effects like myelosuppression, cardiac arrythmias, Central Nervous System (CNS) disturbances, nephrotoxicity, haematological and gastro-intestinal reactions which are not taken care of by the Ifosfamide compositions available as of today.

The toxicity of the compositions of the present invention were evaluated against the conventional dosage form available in the market (Holoxan). The compositions of present invention when studied in swiss albino mice showed lesser toxic effects like convulsions, myelosuppressions, hepatotoxicity. The mortality rate was found to be significantly less in animals treated with the compositions of the present invention compared to conventional marketed product. LD_{50} values of the compositions studied are represented in examples.

Not bound by theory, we believe that the Ifosfamide whole or a portion of it is complexed in HPBCD cavity in the aqueous solution. On systemic administration, the drug is released from the cavity in to the blood stream. The free drug and the drug-HPBCD complex will be in equilibrium and the metabolism of the free Ifosfamide shifts the equilibrium resulting in the release of

free drug. Presence of Ifosfamide in complex form and optimum levels of free drug in the blood may prevent the interaction of Ifosfamide with healthy tissues and organs thereby preventing undesirable side effects.

In the present invention, Ifosfamide and HPBCD may form a synergistic combination to give reduced toxicity both in presence and absence of Mesna as shown by LD_{50} values in mice and hemorrhagic cystitis studies in rats. The Ifosfamide compositions of the present invention having reduced toxicity are of advantage in the treatment of a variety of solid tumours including those of the cervix, endometrium, lung, ovary, testes and thymus as well as in sarcoma and in the treatment of Burkitts lymphoma.

The solubility of Ifosfamide in water is about 100mg/ml. The process of the present invention makes it possible to obtain a composition containing Ifosfamide in a concentration greater than 100mg/ml. These concentrated solutions containing 100mg/ml to 1100mg/ml offer additional advantages like safety by virtue of less handling, thereby less exposure of clinicians to cytotoxic Ifosfamide during administration and increased assurance of sterility.

Examples :

The invention will now be illustrated by way of Examples. The Examples are by way of illustration only and in no way restrict the scope of the invention.

Ifosfamide used in these Examples was of parenteral grade complying with US Pharmacopoeial specifications. 2-hydroxypropyl-β-cyclodextrin (HPBCD) used was manufactured by Wacker Chemie having molar substitution per anhydroglucose unit by hydroxy propyl groups between 0.5 to 1.2. Equipments used were of conventional nature; the entire processing was done in an area with a controlled environment. Water used in these Examples was of parenteral grade complying with "Water for Injection" specifications. All other additives used in these Examples were of parenteral grade.

Example I: Preparation of Ifosfamide 50mg/ml composition containing 20% HPBCD in water

The following composition was prepared by the procedure given below

i. Ifosfamide 10gm.

i. HPBCD 40gm

ii. Water qs to 200ml.

Weighed quantity of HPBCD was dissolved in 150ml of water. Weighed quantity of Ifosfamide was added and mixed for 3 hours. The volume was made up to 200ml with water and mixed. The resultant solution was filtered through a 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

The composition obtained in this Example was analyzed for Ifosfamide content by High Pressure Liquid Chromatography (HPLC) method and was found to contain 51.73mg/ml of Ifosfamide. The composition had a pH of 6.5.

Example II: Stability of composition of Example I

The composition obtained in Example I was subjected to long term Stability studies at 2-8°C. The stability data at the end of 24 months is shown in Table 1.

Table 1 : Stability data of Composition of Example I

Ifosfamide mg/ml	HPBCD %	Buffer	Initial % Ifosfamide content	24 month at 2-8°C % Ifosfamide content
50	20%	---	103.46	99.12

The above data shows insignificant drop in Ifosfamide content indicating a good stability.

Example III: Toxicity study of composition of Example I

The composition obtained in Example I was subjected to acute toxicity studies in mice

Experimental details are as follows:

Animals used	: Swiss albino mice of either sex.
Weight range of animals	: 20-22 gm.
Number of groups	: 10
Number of animals per group	: 10
Acclimatization	: One week under test conditions under controlled temperature and humidity.
Test Materials	: ***Ifosfamide Injection***
Identity	: Composition of Example I
Description	: Clear colourless solution
Route of administration	: Intravenous
Comparative material	:***Holoxan™ (reconstituted)***
Identity	:Ifosfamide injection U.S.P.
Lot No.	:G 220
Manufacturing Date	:October 2001
Expiry Date	:September 2003
Description	:Dry powder for reconstitution with water for injection
Strength	:40 mg/ml on reconstitution
Manufacturer	: German Remedies Limited.
Route of administration	: Intravenous

Both the drug solutions were suitably diluted with 5% Dextrose Injection and administered intravenously. Ifosfamide in the doses of 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg and 800 mg/kg body weight was administered in 10 different groups of animals, each group consisting of 10 animals.

The animals were kept under observation for 14 days and mortality recorded at the end of 7 days. The LD_{50} dose i.e. the dose that is lethal to 50% of animals is shown in Table 2.

Table 2.

LD_{50} dose of Composition of Example I and Holoxan™

Composition	LD_{50} (mg/kg Body weight)
Example I	648.04
Holoxan™	562.16

The above data clearly indicates that composition of Example I is less toxic compared to the Conventional formulation.

Example IV: Preparation of Ifosfamide composition containing 10% HPBCD in phosphate buffer

The following composition was prepared by the procedure given below

i.	Ifosfamide	-	10g
ii.	HPBCD	-	20g
iii.	Disodium hydrogen phosphate	-	0.1g
iv.	Sodium dihydrogen phosphate	-	0.06g
v.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 160ml of water. Weighed quantity of HPBCD was added and dissolved slowly under stirring in this buffer solution. Weighed quantity of Ifosfamide was gradually added under stirring to the

buffered HPBCD solution and mixed for 3 hours. The volume was made up to 200ml with water and mixed. The resultant solution was filtered through a 0.2μ filter and filled aseptically in sterile glass vials. The air in the headspace of the vials was purged with nitrogen and the glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

The composition obtained in this Example was analysed for Ifosfamide content by High Pressure Liquid Chromatography (HPLC) method and was found to contain 50.23mg/ml of Ifosfamide. The composition had a pH of 7.2.

Comparative Example V : Preparation of Ifosfamide 50mg/ml composition in phosphate buffer

To ascertain the toxicity reducing nature of HPBCD by toxicity studies a comparative composition containing Ifosfamide in buffer was prepared by the procedure given below

i.	Ifosfamide	-	10g
ii.	Disodium hydrogen phosphate	-	0.1g
iii.	Sodium dihydrogen phosphate	-	0.06g
iv.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 180ml of water. Weighed quantity of Ifosfamide was gradually added under stirring to the buffer solution and mixed for 3 hours. The volume was made up to 200ml with water and mixed. The resultant solution was filtered through a 0.2μ filter and filled aseptically in sterile 10ml glass vials. The air in the headspace of the vials was purged with nitrogen gas and the glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

The composition obtained in this Example was analysed for Ifosfamide content by High Pressure Liquid Chromatography (HPLC) method and was found to contain 50.1mg/ml of Ifosfamide. The composition had a pH of 6.5

Example VI : Acute toxicity study of Example IV and Example V

Experimental details are as follows:

Animals used	: Swiss albino mice of either sex.
Weight range of animals	: 20-22 gm.
Number of groups	: 25
Number of animals per sub group	: 8
Acclimatization	: One week under test conditions under controlled temperature and humidity.

Test Materials	: ***Ifosfamide Injection***
Identity	: Composition of Example IV
Description	: Clear colourless solution
Route of administration	: Intravenous

Comparative material 1.	: ***Ifosfamide Injection***
Identity	: Composition of Example V
Description	: Clear colourless solution
Route of administration	: Intravenous

Comparative material 2. : ***Holoxan™ (reconstituted)***

(Details of ***Holoxan™*** are shown in Example III

Uroprotective material	: ***Uromitexan™***
Identity	:Mesna Injection
Lot No.	:G 168

Manufacturing Date	:October 2001
Expiry Date	:September 2004
Description	:Clear & colorless solution for Intravenous Injection
Strength	:100 mg/ml
Manufacturer	: German Remedies Limited.
Route of administration	: Intravenous

The compositions obtained in Example IV and Example V were subjected to acute toxicity studies in mice. A conventional formulation, Holoxan™ was reconstituted as directed by the manufacturer and was used as a control. The doses of Ifosfamide selected for the study were 400 mg/kg, 500 mg/kg, 600 mg/kg, 700mg/kg and 800mg/kg body weight. The Ifosfamide solutions of compositions of Example IV, Example V and Holoxan™ were suitably diluted with 5% Dextrose Injection as such and also with Mesna at a dose of 20% of Ifosfamide dose and administered intravenously in 25 different groups of animals, each group consisting of eight animals.

The animals were kept under observation for 14 days and mortality recorded at the end of 7 days. The LD_{50} values of compositions of Example IV, Example V and Holoxan™ are shown in Table no 3.

Table No 3 : The LD_{50} values of compositions of Example IV, V and Holoxan™

Composition	HPBCD concentration	Buffer	Mesna Dose as % of Ifosfamide Dose	LD_{50} (mg/kg Body weight)
Example IV	10%	phosphate	----	661.57
Holoxan™	Nil	nil	-----	562.16
Example V	nil	phosphate	-----	566.00
Example IV	**10%**	**phosphate**	**20**	**669.54**
Holoxan™	**nil**	**nil**	**20**	**580.00**

The above data clearly indicates that composition of Example IV is less toxic both in presence and absence of Mesna compared to the Conventional formulation as well as Ifosfamide in buffered solution.

Example VII: Repeat dose toxicity studies of composition of Example IV

The composition obtained in Example IV along with conventional formulation Holoxan™ were subjected to repeat dose toxicity study in mice to evaluate the effect of Ifosfamide compositions on haematological and biochemical parameters.

Experimental details are as follows:

Animals used	: Swiss albino mice of either sex.
Weight range of animals	: 20-22 gm.
Number of groups	: 7
Number of animals per group	: 10
Acclimatization	: One week under test conditions under controlled temperature and humidity.
Test Materials	: Composition of Example IV
Comparative material .	:***Holoxan™ (reconstituted)***
Uroprotective material	: ***Uromitexan™***

(The details of ***Holoxan™*** and ***Uromitexan™*** are given in Example VI)

The animals were injected Ifosfamide along with Mesna (as 20% of Ifosfamide Dose) at daily doses of 80 mg/kg, 100 mg/kg and 120 mg/kg, intravenously for seven days. The untreated group was used as a control. The animals were observed during the study period of 14 days for mortality, haematological and biochemical changes. The total WBC count was done before and after treatment with composition of Example IV and Holoxan ™. The values are shown in Table no 4

Table no 4 : The total WBC count of animals treated with composition of Example IV and Holoxan ™.

Dose (mg/kg body weight)		Total WBC (cells /microlitre) ± SEM					
		Example IV		Holoxan ™		Control	
Ifosfamide	Mesna	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment
80	16	6220.00 ± 631.73	3170.00 ± 478.21	4870.00 ± 310.94	1840.00 ± 131.83		
100	20	5760.00 ± 435.44	6440.00 ± 1764.2	5980.00 ± 806.77	2220.00 ± 237.02	7920.00 ± 539.93	10980.0 ± 666.65
120	24	5900.00 ± 481.91	4420.00 ± 1102.0	8150.00 ± 904.61	1362.50 ± 301.15		

The animals treated with Example IV and Holoxan ™ showed reduction in total WBC count. However, animals treated with Holoxan ™ showed severe leucopoenia compared to composition of Example IV. This indicates less toxic nature of composition of Example IV.

To study the effect of composition of Example IV and Holoxan ™ on Liver function, the Serum Glutamate Oxaloacetate Transaminase (SGOT) levels of treated and untreated control group were analysed. The SGOT levels of treated and untreated control groups are shown in Table no 5.

Table 5 : The average SGOT levels of animals treated with composition of Example IV, Holoxan™ and untreated control.

Dose of Ifosfamide (mg/kg body weight)	Dose of Mesna (mg/kg body weight)	SGOT level (U/L)		
		Example IV	Holoxan™	Control
80	16	**129.15**	**158.50**	**136.4**
100	20	**147.8**	**299.65**	
120	24	**164.25**	**309.15**	

As per the above data the elevation of SGOT levels in animals treated with Example IV was slightly higher than that of control group, whereas in

animals treated with Holoxan™ increase in SGOT values was highly significant. This indicates reduced hepatotoxic nature of composition of Example IV.

Example VIII : Hemorrhagic cystitis studies of composition IV

The composition obtained in Example IV along with conventional formulation Holoxan™ were subjected to Hemorrhagic cystitis studies in rats to evaluate their bladder toxicity.

Experimental details are as follows:

Animals used	: Wistar rats of either sex.
Weight range of animals	: 100-150 gm.
Number of groups	: 9
Number of animals per group	: 2
Acclimatization	: One week under test conditions under controlled temperature and humidity.

Test Materials	: **Composition of Example IV**
Comparative material .	:***Holoxan™ (reconstituted)***
Uroprotective material	: ***Uromitexan™***

(The details of *Holoxan™* and *Uromitexan™* are given in Example VI)

Study design

Animals were divided into 9 groups and each group comprised two animals. Ifosfamide alone and with Mesna was administered via the intravenous route at doses of 400mg/kg and 500mg/kg bodyweight. The group treated with Dextrose Injection was used as a control.

The animals were sacrificed 24 hours after injection. The urinary bladders of all the animals were collected and were fixed in 10% formalin for 48 hours.

Histopathological slides of the organ were prepared and subjected to microscopic examination.

Observations

Table 6 depicts the evaluation results on hemorrhagic cystitis of two formulations of Ifosfamide

Table 6 : Scoring of hemorrhagic cystitis

Sl no	Ifosfamide Dose	Mesna Dose	Score	
			Example IV	Holoxan™
1	400	-	1+	2+
2	400	-	N	3+
3	500	-	1+	3+
4	500	-	1+	1+
5	400	80	N	1+
6	400	80	N	1+
7	500	100	N	1+
8	500	100	N	2+

N : Normal

1 + : Mild hemorrhagic cystitis

2 + : Moderate hemorrhagic cystitis with or without epithelial atypia

3 + : Severe hemorragic cystitis with or without epithelial atypia

Results:

Holoxan : **moderate to severe** hemorrhagic cystitis

Holoxan with Mesna : **mild to moderate** hemorrhagic cystitis

Example IV : **mild to moderate** hemorrhagic cystitis

Example IV with Mesna : **No** hemorrhagic cystitis

Conclusion:

The above findings conclusively proved that the composition of Example IV has less bladder toxicity than the conventional formulation Holoxan™

Example IX: Stability study of composition of Example IV

The composition obtained in Example IV was subjected to stability studies at 2°C -8°C. The samples at the end of 6 and 12 months were analysed by HPLC method. The data is shown in Table no 7

Table 7: Stability data of composition of Example IV.

Storage condition	Description	% Ifosfamide content
Initial	Clear, colourless liquid	50.2 mg/ml
2°C-8°C – 6 Months	Clear, colourless liquid	50.9 mg/ml
2°C-8°C – 12 Months	Clear, colourless liquid	49.1 mg/ml

The above data shows insignificant drop in Ifosfamide content at 2°C - 8°C indicating good stability over a period of 12 months

The other compositions of the present invention comprising different concentrations of Ifosfamide and HPBCD are shown in the Table no 8

Table: 8 : Other compositions of the present invention

Ingredients	Example						
	X	XI	XII	XIII	XIV	XV	XVI
Ifosfamide	10.0g	10.0g	10.0g	20.0g	100g	100g	200g
HPBCD	40.0g	80.0g	20.0g	40.0g	40.0g	80.0g	10.0g
Disodium hydrogen phosphate	0.1g	0.1g	-	0.1g	-	-	-
Sodium dihydrogen phosphate	0.06g	0.06g	-	0.06g	-	-	-
Water to make up the volume	qs to 200 ml	qs to 200 ml	qs to 200 ml	qs to 200 ml	qs to 200 ml	qs to 200 ml	qs to 200 ml
Ifosfamide concentration	50mg/ml	50mg/ml	50mg/ml	100mg/ml	500mg/ml	500mg/ml	1000mg/ml

The compositions shown in table no 8 were prepared by the procedure given below

Example X :

Ifosfamide (10g) and HPBCD (40g), Disodium hydrogen phosphate (0.1 g) and Sodium hydrogenphosphate (0.06g) were taken in a graduated flask. Water for injection was slowly added into it with intermittent mixing to get 200ml clear homogenous solution. The resultant solution was passed through sterile 0.2µm filter and filled aseptically in sterile 10ml glass vials. The air in the headspace was purged with nitrogen gas and the glass vials were closed under aseptic conditions with sterile teflon ™ coated rubber bungs and sealed using flip-off seals.

Example XI :

Ifosfamide (10g) and HPBCD (80g), Disodium hydrogen phosphate (0.1 g in 10ml of water) and Sodium hydrogen phosphate (0.06g in 10ml of water) were taken in a graduated flask. Water for injection was slowly added into it with intermittent mixing to get 200ml clear homogenous solution. The resultant solution was passed through sterile 0.2µm filter and filled aseptically in sterile 10ml glass vials. The air in the headspace was purged with nitrogen gas and the glass vials were closed under aseptic conditions with sterile teflon ™ coated rubber bungs and sealed using flip-off seals.

Example XII:

HPBCD (20g) was dissolved in 40 ml of water. To the concentrated solution of HPBCD, Ifosfamide(10g) was added gradually and the mixture was stirred at a moderate speed for 1 hour. The clear solution was then diluted to 200ml with water. The resultant solution was filtered through 0.2µm filter and filled aseptically in sterile 10ml glass vials. The air in the headspace of the vials was purged with nitrogen gas and the glass vials were closed under aseptic

conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

Example XIII:

The composition was prepared by following the procedure of Example IV using the components in the amounts mentioned in table no 8.

Example XIV:

HPBCD(40g) was dissolved in 80 ml of water. To the concentrated solution of HPBCD, Ifosfamide(100g) was added gradually and dissolved by stirring. The volume was made up to 200ml with water and mixed. The resultant solution was filtered through 0.2µm filter and filled aseptically in sterile 10 ml glass vials. The air in the headspace of the vials was purged with nitrogen gas and the glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals. The composition of this Example was analysed for Ifosfamide content by HPLC and was found to contain 500.3mg/ml of Ifosfamide.

Example XV:

The composition was prepared by following the procedure of Example XIV using the components in the amounts mentioned in table no 8. The composition of this Example was analysed for Ifosfamide content by HPLC and was found to contain 500.28mg/ml of Ifosfamide.

Example XVI:

Ifosfamide (200g) and HPBCD (10g) were taken in a in a graduated flask. Water for injection was slowly added into it with intermittent mixing to get

200ml clear homogenous solution. The resultant solution was passed through sterile 0.2µm filter and filled aseptically in sterile 10ml glass vials. The air in the headspace was purged with nitrogen gas and the glass vials were closed under aseptic conditions with sterile teflon™ coated rubber bungs and sealed using flip-off seals. The composition of this Example was analysed for Ifosfamide content by HPLC and was found to contain 1025.5mg/ml of Ifosfamide.

CLAIMS

1. Stable, clear, aqueous Ifosfamide compositions having reduced toxicity, for parenteral administration comprising
   Ifosfamide up to 1100 mg /ml of the composition
   and 2-hydroxypropyl-β-cyclodextrin.

2. Ifosfamide compositions as claimed in claim 1, wherein the molar substitution by hydroxy propyl groups in 2-hydroxypropyl-β-cyclodextrin is 0.05 – 2.

3. Ifosfamide compositions as claimed in any claim 1 or 2, wherein the molar substitution by hydroxy propyl groups in 2-hydroxypropyl-β-cyclodextrin is 0.3 – 1.5

4. Ifosfamide compositions as claimed in any claims 1 - 3, wherein the molar substitution by hydroxy propyl groups in 2-hydroxypropyl-β-cyclodextrin is 0.5 – 1.2

5. Ifosfamide compositions as claimed in any claims 1-4, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:0.1 - 1:300

6. Ifosfamide compositions as claimed in any claims 1-5, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:0.25 - 1: 100

7. Ifosfamide compositions as claimed in any claims 1-6, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:1 - 1: 20

8. Ifosfamide compositions as claimed in any claims 1-7, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:3.3 - 1:2.5

9. Ifosfamide compositions as claimed in claim 1, further comprising pharmaceutically acceptable additives which are selected from a group of substances such as buffers, tonicity agents, preservatives, chelating agents, antioxidants, anticrystallising agents.

10. Ifosfamide compositions as claimed in any claims 1-9, wherein the Ifosfamide content of the composition is 1 - 200 mg/ml.

11. Ifosfamide compositions as claimed in any claims 1-10, wherein the Ifosfamide content of the composition is 10 - 100 mg/ml

12. Ifosfamide compositions as claimed in any claims 1-11, wherein the Ifosfamide content of the composition is 40 - 50 mg/ml.

13. Ifosfamide compositions as claimed in any claims 1-9, wherein the Ifosfamide content of the composition is 200- 500 mg/ml.

14. Ifosfamide compositions as claimed in any claims 1-9, wherein the Ifosfamide content of the composition is 500-1000 mg/ml.

15. Ifosfamide compositions as claimed in any claims 1-14, wherein the pH of the composition is between 3.0 – 9.0.

16. Ifosfamide compositions as claimed in any claims 1-15, wherein the pH of the composition is between 5.0 -8.0.

17. Ifosfamide compositions as claimed in claim 9, wherein the buffering agent is selected from the group of buffering agents such as Sodium dihydrogen phosphate, Disodium hydrogen phosphate. Dipotassium hydrogen phosphate, Potassium dihydrogen phosphate, Phosphoric acid,

Histidine hydrochloride, Sodium Hydroxide, Hydrochloric acid and mixtures thereof.

18. Ifosfamide compositions as claimed in claim17, wherein the buffering agent is a mixture of Sodium dihydrogen phosphate and Disodium hydrogen phosphate.

19. A process for the preparation of Ifosfamide compositions as claimed in any claims 1-18 comprising steps of

i. bringing in intimate contact Ifosfamide, 2-hydroxypropyl-β-cyclodextrin and water;

ii. rendering sterile the composition so obtained at the end of step (i).

20. The process for the preparation of Ifosfamide compositions as claimed in claim 19 further comprises addition of required quantities of pharmaceutically acceptable additives while bringing in intimate contact Ifosfamide, 2-hydroxypropyl-β-cyclodextrin and water.

21. The process for the preparation of Ifosfamide compositions as claimed in any claims 19 - 20, wherein the composition is rendered sterile by passing through sterile 0.2μm sterilizing grade filter.

22. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-21, comprising further steps of transferring aseptically the sterile composition into sterile containers, purging the air in the headspace of the containers with inert gas and sealing the containers.

23. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-22, wherein the molar substitution by hydroxy propyl groups in 2-hydroxypropyl-β-cyclodextrin is 0.05 – 2

24. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-23, wherein the molar substitution by hydroxy propyl groups in 2-hydroxypropyl-β-cyclodextrin is 0.3 – 1.5

25. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-24, wherein the molar substitution by hydroxy propyl groups in 2-hydroxypropyl-β-cyclodextrin is 0.5 – 1.2

26. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-25, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:0.1 - 1:300

27. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-26, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:0.25 - 1: 100

28. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-27, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:1 - 1: 20

29. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-28, wherein the molar ratio of Ifosfamide to 2-hydroxypropyl-β-cyclodextrin is 100:3.3 - 1:2.5

30. The process for the preparation of Ifosfamide compositions as claimed in claim 20, wherein the said pharmaceutically acceptable additives are selected from a group of substances such as buffers, tonicity agents, preservatives, chelating agents, antioxidants, anticrystallising agents.

31. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-30, wherein the Ifosfamide content of the composition is 1 - 200 mg/ml.

32. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-31 , wherein the Ifosfamide content of the composition is 10 - 100 mg/ml

33. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-32, wherein the Ifosfamide content of the composition is 40 - 50 mg/ml.

34. The process for the preparation of Ifosfamide compositions as claimed in any claims 19 -30, wherein the Ifosfamide content of the composition is 200- 500 mg/ml.

35. The process for the preparation of Ifosfamide compositions as claimed in any claims 19 - 30, wherein the Ifosfamide content of the composition is 500-1000 mg/ml.

36. The process for the preparation of Ifosfamide compositions as claimed in any claims 19-35, wherein the pH of the composition is between 3.0 - 9.0.

37. The process for the preparation of Ifosfamide compositions as claimed in claims 19-36, wherein the pH of the composition is between 5.0 - 8.0.

38. The process for the preparation of Ifosfamide compositions as claimed in claim 30, wherein the buffering agent is selected from the group of buffering agents such as Sodium dihydrogen phosphate, Disodium hydrogen phosphate. Dipotassium hydrogen phosphate, Potassium

dihydrogen phosphate, Histidine hydrochloride, Sodium Hydroxide and mixtures thereof.

39. The process for the preparation of Ifosfamide compositions as claimed in claim 38, wherein the buffering agent is a mixture of Sodium dihydrogen phosphate and Disodium hydrogen phosphate.

40. A stable, clear, aqueous Ifosfamide composition having reduced toxicity, for parenteral administration as claimed in claim 1, wherein each millilitre of the composition comprising

Ifosfamide: 50 mg,
2-hydroxypropyl-β-cyclodextrin : 100mg,
sodium dihydrogen phosphate: 0.3mg and
disodium hydrogen phosphate 0.5mg.

41. A stable, clear, aqueous Ifosfamide composition having reduced toxicity, for parenteral administration as claimed in claim 1, wherein each millilitre of the composition comprising

Ifosfamide: 500 mg
and 2-hydroxypropyl-β-cyclodextrin: 400mg.

42. A stable, clear, aqueous Ifosfamide composition having reduced toxicity, for parenteral administration as claimed in claim 1, wherein each millilitre of the composition comprising

Ifosfamide: 1000 mg
and 2-hydroxypropyl-β-cyclodextrin : 50mg

43. Ifosfamide compositions and a process for their preparation substantially as herein described in the text and examples.