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Hydrotropic Solubility Determinations and Evaluation of Dry Powder For Injection of Poorly Water Soluble Diuretic Spironolactone

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Dry powder injection of spironolactone was developed using lyophilization and hydrotropic solubilization method. It is fast acting medication in emergencies like refractory edema associated with heart failure and hepatic cirrhosis. The ultimate aqueous based powder prepared showed 892.85 and 378.57 times increased solubility of spironolactone with sodium salicylate and sodium benzoate as compared to its water solubility. Amongst six hydrotropic agents, the solubility was increased in the order sodium salicylate > sodium benzoate > nicotinamide > sodium ascorbate > urea > sodium acetate. IR graph showed shift of wavenumber of characteristic peaks. Lyophilization technique produced more stable product against different temperature cycles and stability parameters. Degradation was only about 0.45% at room temperature and it was more about 1.3% at higher temperatures. Haemolytic activities of lyophilized formulations observed were 8.54% to 96.85% for sodium salicylate based hydrotropic lyophilized system and 3.50 to 88.17% for sodium salicylate based hydrotropic lyophilized system.

KEYWORDS: Spironolactone, Dry Powder Injection, Lyophilization, Hydrotropic solubilization, Haemolysis, Stability.

INTRODUCTION

Poor water solubility of many drugs causes significant difficulties in getting high bioavailability formulations[1]. Drugs are needed to solubilize completely before they enter the circulatory system or before permeation through muscle or tissue barriers. The solubility of drug in dosage form vehicle and solubility in body fluids are important considerations for intrinsic activity of drug. Aqueous solubilisation of insoluble drugs can be achieved by addition of hydrotropic agents. Hydrotropic solubilisation is considered as one of the safest methods of solubilisation[2-3].

Spironolactone is a crystalline powder. Highly crystalline solutes cannot be solubilized by using surfactant, cosolvents or soluble complexing agents. Crystal interaction energy must be overcame to increase ideal solubility which can be done by altering nature of crystal. The characteristic self association of hydrotropes in aqueous solution can be used to increase this crystal interaction

energy in water. This can then be formulated in terms of dry sterile powder for injection.

The broad range and functionality of hydrotropes has led to various suggested hydrotropic solubilization mechanisms, including complexation[4], self-aggregation[5], changes in the nature or structure of the solvent[6] etc. In most of the hydrotropic solubilization studies it was assumed that the enhancement in solubility of drugs was due to "salting-in" effect or due to change in solvent character[7]. For aromatic hydrotropes such as nicotinamide, sodium salicylate and sodium p-toluenesulfonate, two main mechanisms were proposed. One was stacking complexation, and the other was self-aggregation. Increased solubilisation with temazepam was attributed to an increase in hydrogen bonding between drug and hydrotrope[8]. Experiments were carried out to highlight the solute solvent interactions and related modifications in case of the presence of hydrotropic agents at different temperatures 298.15 to 313.15 °K.[9] The effective hydrotropic agents are those that destabilize water structure and at the same time interact with poorly soluble drug[10].

A desirable solubilizing system should also be acceptable for formulations used in the clinic. The solubilizing properties of hydrotropes can be exploited for this purpose. However, in the development of a hydrotropic solubilizing system suitable for clinical formulations, an important hurdle is that how to exploit the hydrotropic effect without accompanying issue of high doses of the hydrotrope in the formulation[11]. Mixed hydrotropic solubilisation technique was used as a new concept to overcome this hurdle[12].

Spironolactone is mainly used[13] in treatment of refractory oedema associated with heart failure, hepatic cirrhosis. It has adverse effects like drowsiness, GI disturbances including cramp and diarrhea. Its onset of action is gradual from about 24 h to 48 h. For rapid onset of action and minimizing its adverse effects dry powder injection formulation is an ideal formulation for spironolactone.

In the present investigation of the solubilizing attributes of a hydrotropic system, we examined six different candidate agents for their abilities to solubilize spironolactone. Accordingly we processed to aqueous base dry powder formulation[14-19] which is a potentially superior to liquid formulation in their stability and less volume due to increase in solubility with the hydrotropic solubilisation of drug, thereby facilitating mass administration.

MATERIALS AND METHODS

Chemicals

Spironolactone was a gift sample from RPG Lifesciences Ltd. (India). Urea used was of Merck ltd. Mumbai. All other chemicals used were of analytical grade of SD Fine Chemicals, India.

Equilibrium Solubility studies

Solubility of spironolactone was determined by excess solute method. Excess amount of drug was added to screw capped 10 ml glass vials at 25°C ±1°C containing fixed volume of different aqueous solutions such as distilled water, phosphate buffer solutions with different pH from 2.5 to 9.0 and different individual hydrotropic solutions such as sodium benzoate, sodium salicylate, sodium ascorbate, nicotinamide, urea and sodium acetate of known concentrations (0.2M, 0.8M, 1.2M, 1.6M and 2.0M).

The vials were shaken mechanically for 12 h at 25°C ±1°C in an orbital flask shaker. The solutions were allowed to equilibrate for the next 24 h and then transferred into Eppendorf tubes and centrifuged for 30 min at 2000 rpm (Remi Instruments Limited, Mumbai, India). The supernatant of each vial was filtered through Whatman filter paper. Filtrates of saturated solutions of spironolactone were analyzed by spectrophotmetric method using a double beam UV-Visible Spectrophotometer (Shimadzu 160A) after proper dilutions and equilibrium solubility was calculated. Solubility enhancement ratios were calculated using following formula.

Enhancement ratio =Solubility of drug in hydrotropic solution/Solubility of drug in distilled water.

Formulation of dry powder injection

Aqueous solutions were prepared by dissolving together weighed amounts of spironolactone, alongwith solubilizing hydrotrope showing maximum solubility of drug, antioxidant and inert bulking agent with shaking on orbital flask shakers and making up volume aseptically. After complete solubilization and equilibration solutions were filtered through 0.45µm membrane filter aseptically. Then solutions were aseptically filled in sterile glass vials, plugged with sterile butyl rubber caps and sealed with aluminium caps. All the vials were placed in lyophilization chamber and subjected to lyophilization cycle of freezing and drying to give a freeze dried solid plug of solubilized material which after drying again sealed in sterilized glass vials after withdrawing samples for evaluation. (Table 1)

UV spectral studies

In order to check any interaction between drug and the hydrotropic agents, UV spectral studies of spironolactone were performed in selected hydrotropic solutions. Possible spectroscopic changes were subsequently investigated. ƛmax of each solution of spironolactone with hydrotrope prepared for equilibrium solubility study was determined over wavelength range 200-400nm on UV spectrophotometer.

IR spectral studies

Lyophilized product of solubilized mixture of drug with hydrotropes in which maximum solubility was observed were subjected to infrared spectroscopic studies by KBr pellet method.

Stability monitoring

Physical stability

For 45 days color uniformity and clarity of lyophilized plug in sterile vials amber as well as colorless and reconstituted solutions with hydrotropes showing maximum solubility was observed at three different temperature conditions room temperature (25°C) in dark (R.T.D.), Freezing temperature in dark (F.T.D.) at 2 to 4°C and temperature cycling with shaking (T.C.S.) that is at 50°C and 4°C alternatively for 24 hrs and shaking every day for 5 min on a shaker.

Chemical stability

Lyophilized formulations with hydrotropes showing maximum solubility were stored at freezing temperature 4°C, room temperature 25°C and at elevated temperatures 40°C, 50°C and 60°C one in light and one in dark for 45 days. They were reconstituted and assayed for spironolactone content at different day intervals by UV spectrophotometric

method. From % undecomposed drug values different reaction rate plots were ploted and degradation rate constants and shelf lives were calculated of lyophilized formulations.

Reconstitution rate and dilution profiles

Measured amount of lyophilized preparations equivalent to 25 mg of spironolactone were reconstituted with 3ml of water for injection. Completeness and clarity after reconstitution was observed. Series of dilutions were done by diluting reconstituted fluid with different diluents like normal saline and 5% dextrose solution in ratios 1:1, 1:2, 1:5, 1:10, 1:15, 1:20, 1:50 and 1:100. Clarity of solutions was observed for 24 hrs at different time intervals.

Moisture Content

Moisture content of lyophilized plug was determined initially and by keeping a sample for 45 days at 25°C by a titrimetric Karl-Fischer method.

Haemolytic studies

The blood from marginal ear vein of a healthy male rabbit was collected; defibrinated and red blood cells were then washed with normal saline and centrifuged until supernatant was colorless. RBCs were then diluted to original volume (1ml) with normal saline solution. Colorimetric method was used to determine degree of haemolysis in each test solution. Different concentrations of spironolactone were obtained by diluting their formulations with normal saline (equivalent to 8, 10, 15, 20, 30, 40 and 50µg/ml spironolactone). To 10ml of these dilutions one tenth ml of RBCs suspension was added and incubated at 25°C for 45 min. The unhaemolised cells were separated by centrifugation at 3000 rpm for 10 min and absorbance readings of haemolysate were noted at 550nm. Each absorbance reading was compared with a total haemolysis reading obtained by taking RBCs in water (1:10). The degree of haemolysis occurring in each test solution was calculated as a percent of total haemolysis. Also methods were carried out to test the haemolytic activity of different concentrations of hydrotrope solutions used in lyophilized formulations (individual sodium benzoate and sodium salicylate solutions at concentrations 2, 5, 7, 10, 15, 20 and 25 %w/v) at different sodium chloride solution concentrations (0.45% to 1.8%w/v).

RESULTS AND DISCUSSION

Solubility Determinations

The solubility of spironolactone in water was 0.0284mg/ml. The solubility of drug at pH 4.5 was more and found 1.42mg/ml (about 50.71 times). It increased abruptly in hydrotropic solutions of sodium salicylate and sodium benzoate (about 892.85 and 378.57 times) than with the phosphate buffer solutions. Thus the solubility enhancement of water insoluble spironolactone in hydrotropic solutions was not due to pH effect. The solubility of spironolactone in various buffer solutions is shown in Fig. no. 1 and the equilibrium solubility curves in different hydrotropic solutions are shown in Fig. no. 2. The solubilising power of different hydrotropes in terms of solubility enhancement ratio is given in Table 2.

The solubilizing power of sodium salicylate was highest for spironolactone following sodium benzoate.

Spectral studies of solubilisates

In the UV spectral analysis of solubilised system of spironolactone in different hydrotrope solutions (Table 3) it showed different peaks with different maximum wavelength of absorption (λ_{max}) such as for sodium benzoate it was 337.5nm, for sodium salicylate it was 305.6 nm, for sodium ascorbate it was 268.5 nm, for nicotinamide it was 296 nm, for urea it was 239.4 nm and for sodium acetate it was 249 nm. After observing the shifts in the wavelength of absorptions of spironolactone in sodium salicylate solution and λmax of only a sodium salicylate solution it can be explained that there was combination of chromophore carbonyl of α,β- unsaturated ketone and an auxochrome –OH for giving another chromophore.

The overall solubilization due to sodium salicylate and sodium benzoate showed that a bathochromic shift occurred as a result of n-π* transition in carbonyl compounds where polarity of solvent decreases. Also here π electron cloud spreads over at least four carbon atoms. The planner structure of drug allowed their molecular inclusion in the stacked aggregates form after certain hydrotropes concentration. Also the intramolecular hydrogen bonding mediated via 3-hydroxyl group offers the possibility of additional hydrogen bonding between drug and hydrotrope.

The results of IR studies (Fig. 3, 4 and 5) showed that the presence of electron donating groups and conjugation lowered the wavenumber of absorption of typical functional groups of the drug such as a 5-membered lactone carbonyl of drug showed peak at 1775 and 1778 cm^{-1} but in formulation with sodium salicylate hydrotrope it showed peak at 1752.6 cm^{-1} and in formulation with sodium benzoate it showed peak at 1732 and 1749.3 cm^{-1}. The broadband between 3000-2500 cm^{-1} showed presence of O-H str. Absorption in sodium salicylate. In the region 1350-1000 cm^{-1} observed absorption showed the presence of C-O str. of ester and lactone of drug and hydrotrope. The overall lowering in wavenumber and broadening of peak observed was due to intermolecular hydrogen bonding. Thus no indication of complex formation between drug and the hydrotropes was seen. Only a molecular rearrangement through intermolecular and intramolecular hydrogen bonding was seen.

Physical and Chemical stability

The Chemical stability data of formulations AMSS1, AMSS2, AMSB1 and AMSB2 after 45 days is given in Table 4 and physical stability data is given in Table 5. The observations showed that at room temperature and freezing temperature in dark formulations were almost stable against coloration and precipitate formation in amber as well as colorless vials.

From UV spectrophotometric data of spironolactone formulations, undecomposed drug at different temperatures and in light and dark was calculated. The initial drug content was taken as 100%. There was no appreciable loss of spironolactone observed. Degradation was only about 0.45% at room temperature and it was more about 1.3% at higher temperatures. Spironolactone followed a first order reaction kinetics. The degradation rate constants estimated were 1.29, 0.54, 1.00 and 0.28 ($Days^{-1}$ x 10^{-3}) for four formulations AMSS2 in dark, AMSS2 in light, AMSB2 in dark and AMSB2 in light

respectively. The shelf lives calculated were 363 days, 398 days, 380 days and 409 days for four formulations AMSS2 in dark, AMSS2 in light, AMSB2 in dark and AMSB2 in light respectively. Thus the formulations are stable at accelerated conditions.

The Moisture content of the lyophilized formulation was tested as the moisture is most degradative factor. It was observed that the moisture content of formulations AMSS1, AMSS2, AMSB1 and AMSB2 was only a 0.47%, 0.46%, 0.34% and 0.34% respectively. This will not degrade a drug extensively.

The stability enhancement achieved with this powder formulation is likely attributable to the effect of lyophilisation in stabilizing the solubilised components of the spironolactone with hydrotrope and other excepients. The hydrotrope: spironolactone ratios described here (576:1 and 518:1, of sodium salicylate and sodium benzoate respectively) are well above those typically required for formulation of dosage forms. The excess hydrotrope here is used as a solubilising agent as well as bulking agent, to simplify powder handling operations. Further stability improvements may be achievable by modifying the formulation with respect to parameters such as hydrotrope: spironolactone ratio by mixed hydrotropy, lyophilization and milling conditions.

Reconstitution rate and dilution profiles

An appropriate quantity of lyophilized formulations equivalent to 25 mg of spironolactone was reconstituted with 3ml of water for injection. Solid dissolved completely leaving no residual matter. For AMSS1 and AMSS2 reconstitution time was 20 seconds and 19 seconds and for AMSB1 and AMSB2 it was 17 seconds and 16 seconds respectively. For all the formulations reconstitution was rapid and solutions were clear transparent except AMSS2 which was slightly translucent which may be due to excessive drying.

There was no precipitation in solutions reconstituted by normal saline and 5% dextrose solution in 1:1, 1:2 1nd 1:5 dilutions over 24 hrs (Table 6). This can be considered as a stable against dilution as it is necessary for any diluted preparation to remain in solution at least for 2 hrs at room temperature. There was not a much difference in effects of normal saline solution and in 5% dextrose solution. Also after dilution in 1:10 ratio precipitation occurred in reconstituted AMSS1, AMSS2 and in AMSB1 and AMSB2 after 24 hrs. As the dilution ratio was increased the precipitation in case of AMSS reconstituted preparations disappeared at much higher dilution. This may be due to a redissolution of the precipitate but in case of AMSB reconstituted preparations asdilution increases precipitation occurs earlier which may be due to a supersaturation of solution.

Haemolytic activity

Haemolytic activities of lyophilized formulations observed were 8.54% to 96.85% for AMSS2 and 3.50 to 88.17% for AMSB2 (Table 7). The haemolytic property of sodium salicylate was more than in case of sodium benzoate. As the vehicle is diluted 20 times after injection in total blood volume the risk of precipitation into the blood is decreased.

CONCLUSION

The new dry powder formulation of spironolactone described here is more potent than conventional oral tablet formulations and may ultimately provide a safe and effective alternative parenteral dosage form to currently available spironolactone tablets as well as a means to avoid the logistical burden and high cost of packaging and space. The introduction of room temperature stable, powder forms of injections can be expected to result in significant reductions in transportation, cold storage and wastage costs.

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Table 1 Composition of aqueous injection formulation of spironolactone

Formulation	Lactose	Spirono lactone	Sodium salicylate	Sodium Benzoate	Sodium Bisulfite	Water for injection (q.s.)
AMSB1	100 mg	25mg	-	0.5187gm	0.1%	3ml
AMSB2	1.66gm	0.4166gm	-	8.6466gm	0.05gm	50ml
AMSS1	100mg	25mg	0.576gm	-	0.1%	3ml
AMSS2	1.66gm	0.4166gm	9.606gm	-	0.05gm	50ml

Table 2 solubility enhancement ratios of spironolactone in different hydrotrope solutions

S. No.	1	2	3	4	5	6
Hydrotrope solution (2M)	Sodium salicylate	Sodium benzoate	Nicotinamide	Sodium ascorbate	Urea	Sodium acetate
Solubility enhancement ratio	892.85	378.57	190.35	142.85	118.92	64.28

Table 3 UV spectral analysis wavelength of maximum absorption of spironolactone in hydrotrope solution

System	Peak λ_{max} (nm)
Spironolactone/ Sodium benzoate	337.5
Spironolactone/Sodium salicylate	305.6
Spironolactone/Sodium ascorbate	268.5
Spironolactone/Nicotinamide	296.0
Spironolactone/Urea	239.4
Spironolactone/Sodium acetate	249

Table 4 Chemical stability data of spironolactone in formulations at different temperatures

Formulation in glass vial	Log % Undecomposed spironolactone at different temperatures				
	4°C	25°C	40°C	50°C	60°C
Colourless AMSS1	98.1	95.8	91.6	89.6	87.4
Colourless AMSS2	98.2	95.8	91.8	89.3	87.6
Colourless AMSB1	98.5	96.5	93.1	89.2	86.9
Colourless AMSB2	98.6	96.2	93.1	89.2	87.5
Amber AMSS1	98.6	96.3	92.8	89.0	87.9
Amber AMSS2	98.4	96.4	92.4	88.9	88.3
Amber AMSB1	99.0	97.5	92.8	89.5	87.3
Amber AMSB2	98.9	97.6	92.9	89.7	87.2

Table 5: Color uniformity and Physical stability of Spironolactone Formulations

Formulation	Color of formulation and physical stability					
	R.T.D.		F.T.D.		T.C.S.	
	Initial	After45 days	Initial	After45 days	Initial	After45 days
Colourless AMSS1	Slightly Yellow No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Slightly Yellow No ppt	Slightly Brown Yellow ppt
Colourless AMSS2	Slightly Yellow No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Slightly Yellow No ppt	Slightly Brown Yellow ppt
Colourless AMSB1	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless ppt
Colourless AMSB2	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless ppt
Amber AMSS1	Slightly Yellow No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Slightly Yellow No ppt	Slightly Brown Yellow ppt
Amber AMSS2	Slightly Yellow No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Slightly Yellow No ppt	Slightly Brown Yellow ppt
Amber AMSB1	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless ppt
Amber AMSB2	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless No ppt	Colorless ppt

Table 6 Clarity of reconstituted solutions of spironolactone formulations after dilution with normal saline and 5%w/v dextrose solution after 24 hrs

Formulation	Dilution Solution	Dilution							
		1:1	1:2	1:5	1:10	1:15	1:20	1:50	1:100
AMSS1	Normal Saline solution	-	-	-	+	+	+	+	+
AMSS2		-	-	-	+	+	+	+	+
AMSB1		-	-	-	+	+	+	+	+
AMSB2		-	-	-	+	+	+	+	+
AMSS1	5% w/v Dextrose solution	-	-	-	+	+	+	+	+
AMSS2		-	-	-	+	+	+	+	+
AMSB1		-	-	-	+	+	+	+	+
AMSB2		-	-	-	+	+	+	+	+

Table 7 Haemolytic activity of spironolactone formulations at different concentrations of spironolactone

Spironolactone Concentration (µg/ml)	% Haemolysis			
	AMSS1	AMSS2	AMSB1	AMSB2
8	8.16	8.54	3.2	3.50
10	14.23	14.57	5.59	6.25
15	25.65	25.71	10.36	10.20
20	48.00	47.58	28.89	28.54
30	65.48	65.44	45.16	46.08
40	81.29	81.32	60.85	61.74
50	96.40	96.85	89.31	88.17