ISSN: 2321-3132

Research Article

International Journal of Chemistry and Pharmaceutical Sciences

www.pharmaresearchlibrary.com/ijcps

Formulation Development and optimization of Lyophilized product of an Anti Ulcer Drug

GY. Srawan kumar, V Deva Prasad, Raveendra, Harish K, Ann Jose, Reny Jose, Durga, Rachel mathai, Kempe gowda.

Hindu College of Pharmacy, Amaravathi road, Guntur, Andhra Pradesh, India.
Strides Arcolab Limited, Strides House, bannerghatta road, bilekhali, Bangalore, 560076, India.

Received: 27 March 2014, Accepted: 10 May2014, Published Online: 27 Ma y 2014

Abstract

The aim of present research work was to formulate an intravenous injection of Omeprazole sodium. Omeprazole drug is very slightly soluble in water. Hence *in-situ* conversion of Omeprazole into Omeprazole sodium was opted. But Omeprazole sodium is not stable in solution form. It is stable only for 1-2 days. Hence lyophilization technology was adopted to increase the stability of Omeprazole sodium injection. The lyophilization was carried out in different batches by varying the total cycle time, freezing and holding time, primary drying and secondary drying time while keeping the quantities of all the active pharmaceutical ingredients constant. Lyophilization was carried out in five different batches with five different lyophilization-cycles of 24.5 hours, 28.5 hours, 30.5 hours, 32.5 hours, and 35 hours respectively. Melt pack was found in Batch 1, Partial melt pack was found in batch 2, cake was sticking to the bottom of vial in batch 3, moisture content was high in batch 4, the optimized good cake was found in batch 5. Lyophilization cycle of 35 hours was optimized. The optimized lyophilized product was subjected to evaluation parameters such as cake appearance, reconstitution time, pH, assay, impurities, particulate matter, water content and DSC. After considering all product characteristics batch-5 was considered as an optimized formulation. All the evaluation parameters complies the limits as per the specification of USP. Accelerated stability studies were also conducted for a period of three months and from the results obtained, it was found that the optimized formulation was found to be stable. Finally, it was concluded that the lyophilization is a suitable technique to enhance the stability of Omeprazole sodium for intravenous injection with a single dose of 40mg/vial.

Keywords: Lyophilization, DSC, HPLC, Freezing, Primary drying, Secondary drying, Infrared.

Contents

***Corresponding author**
V. Deva Prasad
Hindu college of pharmacy,
Amaravathi road, Guntur, A. P, India
E-mail: dev25648@gmail.com
Manuscript ID: IJCPS2049

PAPER-QR CODE

1. Introduction

A peptic ulcer, also known as PUD or peptic ulcer disease, is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. It is defined as mucosal erosions equal to or greater than 0.5 cm. Normally, the lining of the stomach and small intestine is protected against the irritating acids produced in the stomach. If this protective lining is affected it results in the breakdown of lining and hence inflammation (gastritis) or an ulcer. Most ulcers occur in the first layer of the inner lining. A hole that goes all the way through the stomach or duodenum is called a perforation. A perforation is a medical emergency. Gastroesophageal reflux disease (GERD) is a condition in which the stomach contents (food or liquid) leak backwards from the stomach into the esophagus (the tube from the mouth to the stomach). This action can irritate the esophagus, causing heartburn and other symptoms. When refluxed stomach acid touches the lining of the esophagus it may cause a burning sensation in the chest or throat called heartburn or acid indigestion. Erosive Esophagitis is an inflammation and swelling of the esophagus, and is most often caused by acid-containing stomach contents refluxing back up into the esophagus. Proton pump inhibitors are commonly used to treat the above mentioned diseases. Omeprazole for injection is mostly used among the proton pump inhibitors. But the solubility of Omeprazole is very less and it is very unstable in solution form. So the main objective of the research work is to increase the solubility of Omeprazole by *in-situ* conversion of Omeprazole into Omeprazole sodium and increase the stability by lyophilizing the solution form of Omeprazole sodium. Lyophilization is performed for the substances which are Thermo labile and Unstable in the solution form.

The present work was designed to address the following objectives:

- Preformulation studies on the drug.
- Selection of the excipients for development of injectable dosage form by lyophilization techniques.
- Formulation of the injectable dosage form.
- Performing Lyophilization and study its parameters.
- Evaluation of the optimized formulation
- Perform stability studies on the optimized best formulation.

Table 1: Composition per one vial

Composition	Quantity	Rationale
Omeprazole	40mg	Active
Edetate Disodium	0.4mg	Chelating Agent
Sodium Hydroxide	q.s to solubilize Omeprazole Slurry	For conversion of Omeprazole base to its Sodium salt
Water for Injection	q.s to 4ml	Solvent

Precautions taken during manufacturing

- The vehicle used i.e. sterile water for injection was free from oxygen
- Glass or stainless steel apparatus was used
- Entire manufacturing process was carried out under aseptic conditions and includes washing and sterilization of vials, rubber plugs and disinfection aluminium seals. The vials were filled in class 100 laminar air cabinets

2. Materials and Methods

Method of Preparation

1. Collect Required water for injection and bring down the temperature below 40°C, by nitrogen bubbling
2. Check and record the pH of WFI (Limit 5.0-7.0)
3. Add and dissolve the weighed quantity of Disodium Edetate in 80% of WFI with continuous stirring
4. To the solution of step-3, Omeprazole was added with stirring to get uniform slurry
5. 1N sodium hydroxide was prepared by using WFI separately
6. To the slurry obtained in step-3, sodium hydroxide solution obtained in step-4 was added slowly with stirring till a clear solution was obtained
7. Volume of the solution is made to 100% with Water for injection. pH was checked (limit 10.3 – 12)
8. The solution of the step-7 was filtered through 0.22µm PVDF membrane and filled into USP type 1 flint glass tubular vials (fill volume 4.0 – 4.1ml), half stoppered with slotted grey bromo butyl rubber plugs and loaded into lyophilizer.

Table 2: Composition of Special diluent for reconstitution

S.No.	Ingredients	Qty/ml
1	Citric acid monohydrate	0.5mg
2	Polyethylene Glycol 400	400mg
3	Water for injection	q.s to 1ml

Trail Batches: Trail batches were conducted as per below table

Table 3: Formulation trials of Omeprazole sodium for injection

Constituents	Trail batches				
	I	**II**	**III**	**IV**	**V**
Omeprazole	40mg/vial	40mg/vial	40mg/vial	40mg/vial	40mg/vial
Disodium edetate	0.4mg	0.4mg	0.4mg	0.4mg	0.4mg
Sodium Hydroxide	q.s to solubilize omeprazole	q.s to solubilize omeprazole	q.s to solubilize omeprazole	q.s to solubilize omeprazole	q.s to solubilize omeprazole
Water for injection	q.s to 4ml	q.s to 4ml	q.s to 4ml	q.s to 4ml	q.s to 4ml
Lyocycle	**LYO 1**	**LYO 2**	**LYO 3**	**LYO 4**	**LYO 5**

3. Results and Discussion

Lyophilization Cycle

Lyophilization or Freeze drying fills an important need in pharmaceutical manufacturing technology by allowing drying of heat-sensitive drugs and biologicals at low temperature under conditions that allow removal of water by sublimation or a change of phase from solid to vapor without passing through the liquid phase. Lyophilization occurs in three steps: freezing, primary drying and secondary drying. In freezing process water is converted into ice, in primary drying to sublime the ice is subjected to sublimation and in secondary drying process unfrozen water is removed by desorption. During the lyophilization process the material is first frozen and then subjected to drying. To initiate the drying stage, the material in the chamber is subjected to vacuum. Heat is applied carefully to the material, and a condenser is used in the chamber to collect the water. When water is leaving rapidly, its heat of vaporization is taken away from the material and helps to keep it cool and safe. Before carrying out lyophilization for formulation it was subjected for preliminary DSC studies and based on the DSC results obtained the suitable lyophilization cycles were designed. The glass transition temperature obtained from DSC was used to determine the freezing temperature of the formulation filled into the vials. The cycles thus designed were applied and the product obtained after lyophilization was subjected for physical examination. On the basis of the cake obtained the process variables i.e. temperature and duration of the cycle were applied. Duration taken to attain required temperature was termed as ramp temperature and the duration in which the formulation remained in the attained temperature was termed as soak temperature.

Trail 1:

In this cycle, formulation was subjected for 3.5 hours of freezing, 14 hours of primary drying and 7 hours of secondary drying. Here the freezing temperature was fixed purely based on the glass transition temperature of the formulation. The glass transition temperature of the formulation was found to be -15.78°C. Thus the formulation was frozen to a temperature of -35°C which is -15.78°C lesser than the glass transition temperature. This was carried out in order to ensure complete freezing.

Table 4: Lyophilization cycle of 24.5 hours

Process &Temperature	Ramp duration (min)	Soak duration (min)	Pressure (torr)
Freezing (-35°C)	120	90	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	120	0.75
10°C	120	150	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	150	0.1

Trail 2: In this trial formulation was subjected for 5.5 hours of freezing, 16 hours of primary drying and 7 hours of secondary drying. Here the freezing temperature was reduced to -40°C. And the duration of the steps like freezing

and primary drying was increased. These changes were implemented in order to enhance the freezing of the formulation and to ensure proper drying.

Table 5: Lyophilization cycle of 28.5hrs

Process &Temperature	Ramp duration (min)	Soak duration (min)	Pressure (torr)
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	180	0.75
10°C	120	210	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	150	0.1

Trail 3: In this trial formulation was subjected for 5.5 hours of freezing, 18 hours of primary drying and 7 hours of secondary drying. Here the freezing temperature was maintained similar to trial 2. But the duration of the steps in primary drying were increased. These changes were implemented in order to enhance proper drying of the formulation.

Table 6: Lyophilization cycle of 30.5hrs

Process & Temperature	Ramp duration (min)	Soak duration (min)	Pressure (torr)
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	210	0.75
10°C	120	300	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	150	0.1

Trail 4: In this trial formulation was subjected for 5.5 hours of freezing, 19.5 hours of primary drying and 7.5 hours of secondary drying. Here the freezing temperature was maintained similar to trial 2. But the duration of the steps like primary drying and secondary drying were increased. These changes were implemented in order to enhance the proper drying of the formulation.

Table 7: Lyophilization cycle of 32.5 hours

Process & Temperature	Ramp duration (min)	Soak duration (min)	Pressure (torr)
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	240	0.75
10°C	120	360	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	180	0.1

Trail 5: In this trial formulation was subjected for 5.5 hours of freezing, 19.5 hours of primary drying and 10 hours of secondary drying. Here the freezing temperature was maintained similar to trial 2. But the duration of the secondary drying were increased. These changes were implemented in order to enhance the proper drying of the formulation

Table 8: Lyophilization cycle of 35 hours

Process & Temperature	Ramp duration (min)	Soak duration (min)	Pressure (torr)
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	240	0.75

10°C	120	360	0.75
Secondary Drying			
20°C	90	120	0.3
35°C	150	240	0.1

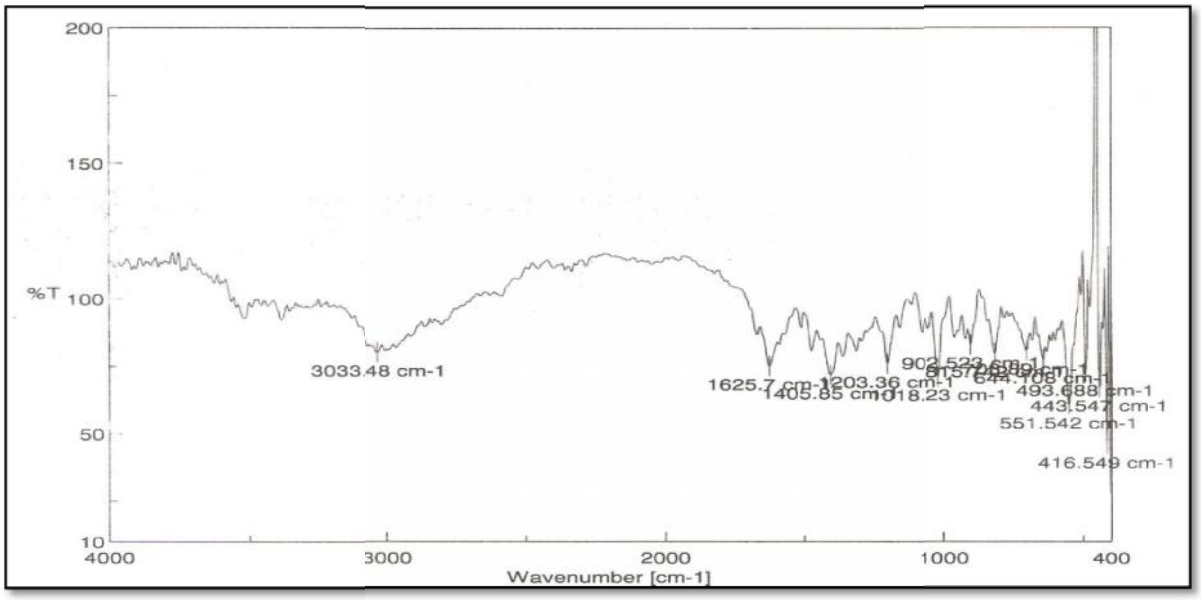

Figure 1: IR Spectra of Disodium Edetate

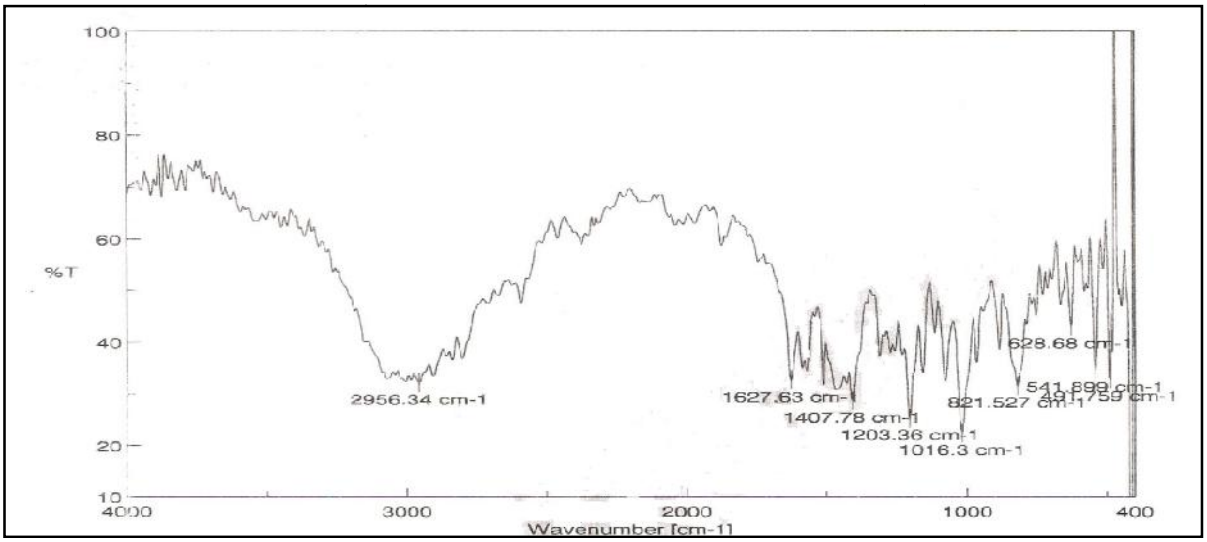

Figure 2: IR of optimized batch V

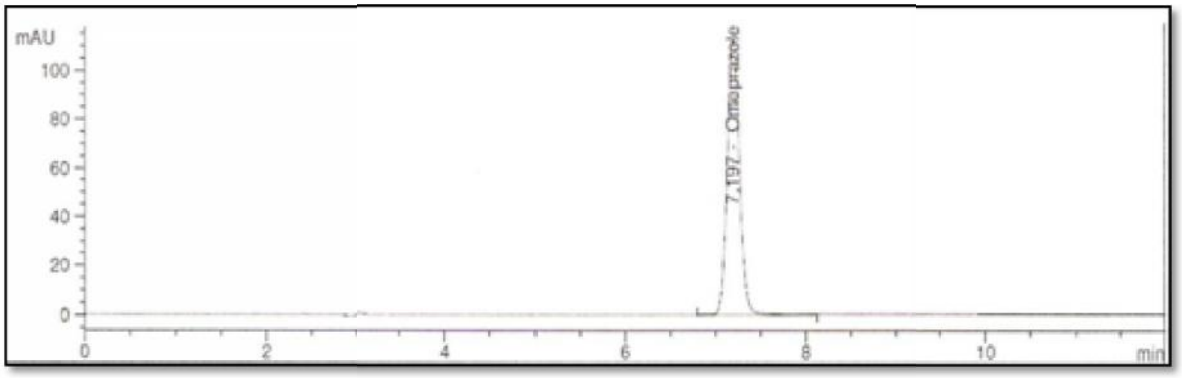

Figure 3: HPLC for Pure drug

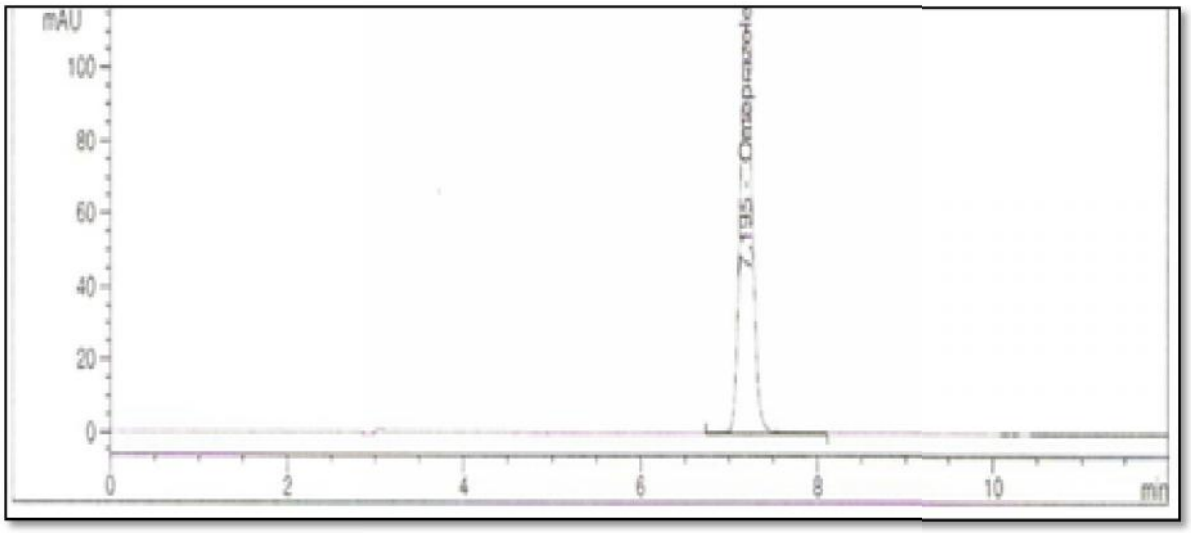

Figure 4: HPLC graph for optimized batch V

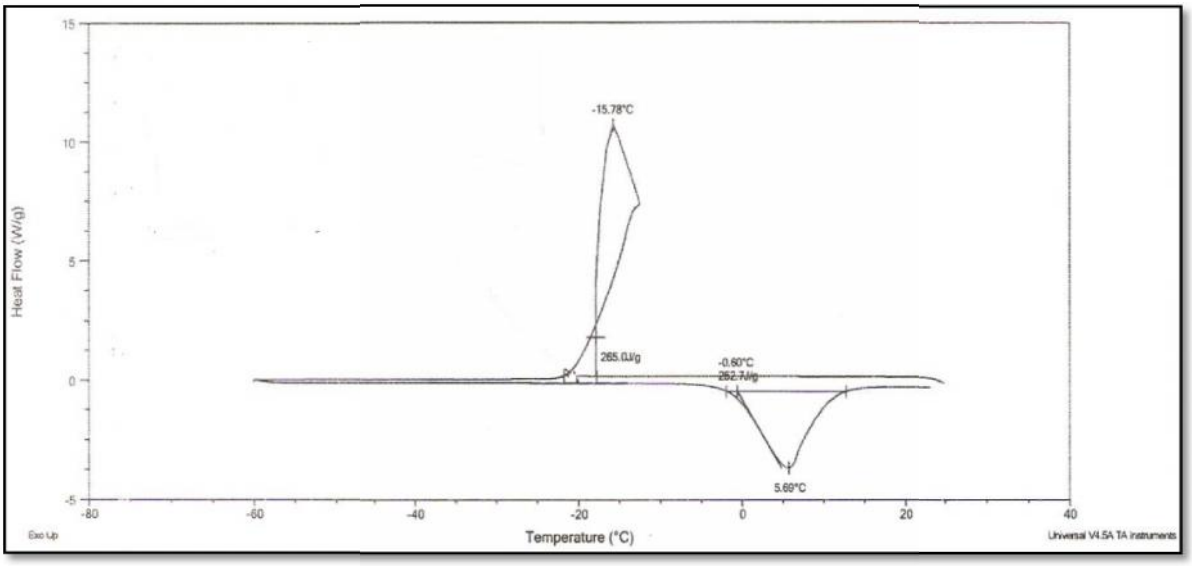

Figure 5: DSC Post-lyophilization

4. Conclusion

The present research work was designed to develop a lyophilized injectable dosage form of an Anti-Ulcer drug Omeprazole Sodium. The drug is unstable if dispensed as liquid dosage form. Hence the present project was envisaged to overcome the drawbacks associated with Omeprazole sodium and to formulate a stable and therapeutically effective formulation by lyophilization technique which provides extended shelf life. Based on the physicochemical properties of the drug , disodium edetate (chelating agent) and Sodium Hydroxide (Solubilizing agent), lyophilization technique was adopted to improve the cake characteristics of the lyophilized form of Omeprazole sodium Five different lyo cycle protocols were investigated sequentially to optimize the product characteristics. The batch-V of total duration of 35 hours was considered as the best formulation because it exhibited a good cake formation and the assay, pH, particulate matter and also percentage water content was found to be within the USP limits. Stability studies were conducted for the optimized formulation as per ICH guidelines for a period of three months which revealed that the formulation is stable. From the above results, it was concluded that the lyophilization technique proves to be an advantage for development of stable injectable dosage form of Omeprazole sodium, hence our objective to develop a stable and therapeutically effective lyophilized injection of Omeprazole sodium was achieved.

5. References

1. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd ed. Bombay: Verghese Publishing House, **1991**: 639-40.

2. Tse FLS, Willing PG. Sterile Dosage Forms: J Parenteral drug Assoc **1980,** 34: 409.
3. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd ed. Bombay: Verghese Publishing House, **1991**: 681.
4. Motola S, Agharkar S, Editors. Preformulation research in pharmaceutical dosage forms-Parenteral medications. 3rd ed. New York: Marcel Dekker Inc, **1984**.
5. Rey L, May JC. Freeze drying/Lyophilization of pharmaceutical and biological products. 2nd ed. New York: Marcel Dekker Inc, **2004**.
6. http://www.wisegeek.com/what-is-lyophilization.htm (Date of Access: 05/08/11)
7. Akers MJ. Parenteral preparations. In; Gennaro AR. Remington Editor : The Science and Practice of Pharmacy. 21st ed. vol-1, Pennsylvania: MackPublishing Company, **1995**: 828-31.
8. http://www.freezedrying.com/freeze_drying_principles.html (Date of Access: 05/08/11)
9. http://www.qualbio.com/lyophilization.htm (Date of Access: 07/09/11)
10. http://www.inventors.about.com/library/inventors/blfrdrfood.htm (Date of Access: 05/01/12).
11. Guide to inspections of lyophilization of parenterals, office of regulatory affairs U.S food and drug administration. Available at: http://www.fda/ora/inspect_ref/igs/lyophi.html. (Date of Access: 02/02/12).
12. Motola S, Agharkar S, Editors. Preformulation research in pharmaceutical dosage forms-Parenteral medications. 3rded. New York: Marcel Dekker Inc, **1984**.
13. Cartenson JT. Pharmaceutical Preformulation. Technomic publishing co. Inc, **1998**: 165.
14. Shanghai tofflon of science & technology co, Ltd. Available at: http://www.tofflon.com (Date of Access: 05/01/12)
15. Snowman JW. Lyophilization: Freeze Drying a Downstream Process. Thanaset S, editor, Lyophilization: Dept.of Biochemistry, Science KKU;1-6.
16. Pharmaceutical freeze-drying a comprehensive course reviewing freeze drying technology. Mumbai: Biopharma Technology Limited, **2006**.
17. Principles of Lyophilization. Available at:
18. A guide to freeze drying for the laboratory, LABCONCO, An industry service publication. p. 1-11. (www.labconco.com). (Date of Access: 05/02/12).
19. Jenning TA. Effect of formulation on lyophilization part: 1. IVD Technology, **1997**: 38-41.
20. Nail SL, Gatlin LA. Freeze Drying: Principle and Practice. Liberman H, Lachman L, Avis KE, Eds. Pharmaceutical Dosage forms: Parenteral medications Vol-2. 2nd ed. New York: Marcel Dekker Inc; 1993. p. 163.
21. Jennings TA. Role of the Phase diagram of water in primary drying. Available at: http://www.phasetechnologies (Date of Access: 05/02/12).
22. Bindschaedler C. Lyophilization process validation. Rey L, May JC, editors. Freeze drying/Lyophilization of pharmaceutical and biological products- Vol-96. New York: Marcel Dekker Inc, **1999**.
23. http://www.SSpharma.com/ppi (Date of Access: 05/02/12)
24. Wallen AJ, Van Ocker SH, Sinacola JR, Phillips BR. The effect of loading process on product collapse during large-scale lyophilization. J Pharm sci, **2009**, 98(3): 997-1004.
25. Rambatla S, Ramot R, Bhugra C, Pikal MJ. Heat and mass transfer scale-up issues during freeze drying:II. Control and characterization of the degree of super cooling. AAPS Pharm Sci Tech, **2004**, 5(4): 58.
26. Tsinontides SC, Rajniak P, Hualee WA, Placele J, Reynolds SD. Freeze drying, principles and practice for successful scale-up, manufacturing. Int J Pharm, **2004**, 280: 1-6.
27. Betageri GV, Makarla KR. Enhancement of dissolution of glyburide by solid dispersion and Lyophilization techniques. Int J Pharm.,**1995**, 126: 155-60.
28. Randolph, James A, Searles, John F, Carpenter, Theodore W. The ice nucleation temperature determines the primary drying rate of lyophilization for sample frozen on a temperature-controlled shelf. J Pharm Sci **2001**; 90(7): 860-71.
29. Xiang J, Hey JM, Liedtke V, Wang DQ. Investigation of freeze-drying sublimation rates using a freeze-drying microbalance technique. Int J Pharm, **2004**, 279: 95-05.
30. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles:Formulation, process and storage considerations. Int J Pharm, **2005**, 309:178-88.
31. Bhatnagar BS, Pikal MJ,Bogner RH. Study of the individual contributions of ice formation and freeze-concentration on isothermal stability of lactate dehydrogenase during freezing. J Pharm Sci., **2008**, 97(2): 798-812.
32. Dodav MG, Kumbaradzi EF, Goracinova K, Simonoska M,Calis S, Jolevska ST, Hincal AA. The effects of lyophilization on the stability of liposomes containing 5- FU. Int J Pharm. **2005**, 292: 79-86.
33. Kuu WY, Hardwick LM, Ankers MJ. Correlation of laboratory and production freezedrying cycles. Int J Pharm, **2005**, 302: 56- 67.
34. Zimmermann E, Muller E, Madar K. Influence of different parameters on reconstution of lyophilized SLN. Int J Pharm, **2000**, 196: 211-213.

35. Zillies JC et al. Formulation development of freeze dried oligonucleotide loaded gelatin microparticles. Eur J Pharm Biopharm, **2008**, 70:514-21.
36. Anhorn MG, Mahler HC, Langer K. Freeze drying of human serum albumin (HSA) nanoparticles with different excipients. Int J Pharm, **2008**, 363: 162-9.
37. Zhang L, Liu L, Qian Y, Chen Y. The effects of cryoprotectants on the freeze drying of ibuprofen loaded solid lipid microparticles. Eur J Pharm Biopharm, **2008**, 69:750-9.
38. Hansen RK, Broadhurst RW, Skelton PC, Arkin IT. Hydrogen deuterium exchange of hydrophobic peptides in model membranes by electrospray ionization mass spectrometry. J Am Soc Mass Spectrum **2002**, 13: 1376-87.
39. Lim SB, Rubinstein I, Onyuksel H. Freeze drying of peptide drugs self associated with long circulating biocompatible and biodegradable sterically stabilized phospholipids nanomicelles. Int J Pharm, **2008**, 356: 345-50.
40. Franks F. Freeze–drying of bioproducts: putting principles into practice. Eur J Pharm Biopharm, **1998**, 45: 221-29.
41. Fakes MG, Dali MV, Haby TA, Marris KR, Varia SA, Serajuddin AT. Moisture sorption behavior of selected bulking agents used in lyophilized products. J Pharm Sci Tchnol, **2000**, 54(2):144-9.
42. Meyera JD, Nayar R, Manninga MC. Impact of bulking agents on the stability of a lyophilized monoclonal antibody. Eur J Pharm Sci, **2009**, 38: 29-38
43. http://pubchem.ncbi.nimnih.gov (Date of Access: 05/02/12)
44. http://medsafe.govt.nz/proof/datasheet (Date of Access: 05/02/12)
45. Raymond C Rowe,Paul J Seshkey and Sain. Hand book of pharmaceutical excipients, 7th ed., **2012**; pp. 489-513: pp. 255.
46. Raymond C Rowe,Paul J Seshkey and Sain. Hand book of pharmaceutical excipients, 7th ed., **2012**; pp. 489-513: pp. 185.
47. Raymond C Rowe,Paul J Seshkey and Sain. Hand book of pharmaceutical excipients, 7th ed., **2012**; pp. 489-513: pp. 545.