

Effect of Polyvinyl Pyrrolidone and Ethyl Cellulose Concentration on Release Profile and Kinetics of Glibenclamide Extended Release Dosage Form System

Amit Kumar, Peeyush Sharma, Anil Bhandari

Abstract—The aim of present work was to optimize the effect of Ethyl Cellulose (EC) and Polyvinyl Pyrrolidone (PVP) concentration in extended release solid dispersion of Glibenclamide using combination of hydrophilic and hydrophobic polymers such as Polyvinyl Pyrrolidone and Ethyl cellulose. The advantage of solid dispersion technique provides a unique approach to particle size reduction and increased rates of dissolution. The compatibility studies of the drug and polymers were studied by TLC and results suggested no interaction between drug and polymers. Solid dispersions of Glibenclamide were prepared by common solvent evaporation method using Polyvinyl Pyrrolidone and Ethyl cellulose. The results indicated that homogeneous or heterogeneous conditions during the preparation methods employed governed the internal structures of the polymer matrices while retaining the drug in an amorphous form. F2 formulation prepared by solid dispersion method, displayed extended drug release followed by Higuchi matrix model indicating diffusion release of GLB from polymer matrices.

Keywords—Ethyl Cellulose, Glibenclamide, Polyvinyl Pyrrolidone, Solid Dispersion.

I. INTRODUCTION

NOW a days as very few drugs are coming out of research and development and already existing drugs are suffering the problem of resistance due to their irrational use and some limitations of conventional dosage forms, as these have to be administered several times a day as to maintain a therapeutically effective plasma level of drug, which is a major drawback in terms of patient compliance. Hence, change in the operation is a suitable and optimized way to make the some drug more effective by slight alteration in the drug delivery system. To overcome such problems greater attention has been focused on extended release drug delivery system [3]. Conventional dosage form as to be administered several times to produce therapeutic efficacy, which yields fluctuations in plasma level. Repetitive dosing of drug causes poor compliance among the patients [12], [19].

Extended release formulations can be utilized to avoid repetitive dosing of drugs in a day. Diabetes is one of the major causes of death and disability in the world [2]. The latest, WHO estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030. The focus of medical community is

on the prevention and treatment of the disease, as is evident from the rising number of research papers every year on the subject. The term solid dispersions was initially used by Sekiguchi and Obi and applied to systems in which the drugs are homogeneously dispersed within a carrier [26]. The methodology to make solid dispersions includes co-fusion, co-dissolution in a proper solvent [1], [7]. Solid dispersions have attracted considerable interest as an efficient means of improving the dissolution rate and hence the bioavailability of a range of poorly water-soluble drugs. Solid dispersions of poorly water-soluble drugs with water-soluble carriers have been reduced the incidence of these problems and enhanced dissolution [28]. Solid dispersion techniques are widely applied to increase the apparent solubility or enhance the oral bioavailability of poorly water-soluble compounds [8], [20]. However, despite many papers, which suggested that the release mechanisms of drugs from a variety of solid dispersions depend on the physical properties of carriers as well as drug substances, preparation methods and so on, basic principles of their dissolution mechanism have not been understood sufficiently [9], [23], [25].

Glibenclamide is an oral hypoglycemic agent, which is a drug for the treatment of patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) Type-2 [6]. Glibenclamide inhibits ATP sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which causes voltage-dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release. Glibenclamide is a weak acid ($pK_a = 5.3$) [27] practically insoluble in water and acidic solutions but highly permeable (class 2) according to the Biopharmaceutical classification System [4], [14], [15].

II. MATERIALS AND METHODS

A. Materials

GLB (Glibenclamide) was a generous gift from Akums Pharma, Haridwar, India. PVP (Polyvinyl Pyrrolidone), EC (Ethyl Cellulose) [22], [24], and all other chemicals and solvents were of analytical grade.

B. Methods

1. Preparation of PM and SDs

Physical mixture and solid dispersions of GLB in PVP and EC containing different weight ratio (Table I) were prepared by following methods:

Amit Kumar associates with the Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur-334001, India (Mobile: 00919252059985; e-mail: amit_jana81@rediffmail.com).

Physical Mixture:

Physical mixtures were prepared by mixing the powered drug and polymers in a mortar. Glibenclamide and two different polymers in different drug to polymer ratios were accurately weighed mixed for 15 minutes than sifted through sieve no. #60 and kept in desiccators under vacuum until use [7], [29].

Solvent Evaporation Method:

Solid dispersion consisting of GLB, EC and PVP was prepared as follows. According to the solvent method, different amounts of Glibenclamide and carriers were accurately weighed and separately dissolved in a solvent ethanol and the solutions were mixed with constant stirring. Solvent was removed under vacuum at 40° C using a rotary evaporator. The solid dispersions were obtained were ground in a mortar [20].

it was removed from the chamber. The plates were allowed to dry in the air and placed in the chamber containing iodine vapors.

Solvent system: Methanol: Acetic acid: Cyclohexane: Methylene chloride
 (5:5:45:45 V/V/V/V)

Visualizing agent:

Ultraviolet light chamber
 The RF value was calculated for the drug, carriers and physical mixtures using the following formula: The results are shown in Table II.

$$RF = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

F. Preparation of Standard Calibration Curve

The calibration curve of GLB was prepared in gastric simulated HCl buffer fluid without enzyme (pH 1.2), phosphate buffer (7.4 pH) and borate buffer pH 9.5. The stock solution was prepared by accurately weighing 10mg of GLB and dissolving in 100 ml of respective buffer to get a stock solution of 100µg/ml. From each of the stock solution 1.0ml, 2.0ml, 3.0ml, 4.0ml, 5.0ml, and 6.0ml solution were withdrawn into a 10 ml of volumetric flask and the volume was made with respective buffer to prepare 10-60 µg/ml solution and prepared concentrations were analyzed spectrophotometrically [11].

G. Quantitative Solubility Estimation of GLB

Solubility of GLB was performed by placing an excess amount in 25ml conical flask with 10ml of each of the following different solvents: gastric simulated HCl buffer fluid without enzyme (pH 1.2), phosphate buffer (pH 6.8 & 7.4 pH) and borate buffer (pH 9.5). The samples were subsequently allowed to equilibrate at 37°C±1°C in mechanical shaker for 24 hours. Samples were filtrate and the filtered was analyzed for GLB by UV spectrophotometry [23], [27].

H. Drug content

Physical mixtures and Solid dispersions each equivalent to 5mg of GLB were accurately weighed separately and dissolved in minimum amount of selecting a solvent dimethylsulfoxide (DMSO) in which polymers were insoluble but drug was soluble. The resulted mixtures were diluted with 100ml of gastric simulated HCl buffer fluid without enzyme (pH 1.2) and phosphate buffer (pH 7.4) in 100ml of volumetric flasks separately and after this the solutions were filtered and 2ml of each sample was further diluted with gastric simulated HCl buffer fluid without enzyme (pH 1.2) and phosphate buffer (pH 7.4) up to 10ml and assayed spectrophotometrically for Glibenclamide at 300.0nm using calibration curve based on standard solutions in phosphate buffer (pH 7.4). Results were expressed both as the drug content (mg incorporated drug) and percent incorporation (actual amount of drug in solid in drug in solid dispersion Vs initially added amount). The studies were conducted in triplicate [18].

TABLE I

COMPOSITION OF FORMULATIONS FOR EC CONCENTRATION OPTIMIZATION

| Formulation | Drug (mg) | EC (mg) | PVP (mg) |
|-------------|-----------|---------|----------|
| F1 | 100 | 150 | 500 |
| F2 | 100 | 100 | 300 |
| F3 | 100 | 50 | 100 |
| F4 | 100 | 150 | 100 |
| F5 | 100 | 50 | 300 |
| F6 | 100 | 150 | 500 |
| F7 | 100 | 100 | 500 |
| F8 | 100 | 100 | 100 |
| F9 | 100 | 50 | 300 |

C. UV Spectrophotometer Study

The standard stock solution was prepared by dissolving 10mg of drug in 10ml of methanolic HCl (pH 1.2) and was appropriately diluted with solvent to get a concentration of 100µg/ml and was kept as the stock solution. The standard solution of GLB (10 ppm or 10 mcg /ml) was scanned in the wavelength region of 200-400 nm and λ_{max} was recorded [16].

D. Drug- Excipient Compatibility Study

Compatibility studies were employed to characterize the possible interactions between the drug and the carriers in the solid state using TLC. For this study, Ethyl cellulose and PVP were selected as excipients. GLB, EC and PVP were taken in ratio similar to that to be taken in formulation. These mixtures were prepared, placed in vials, sealed and kept at 40°C ±2 and 75% ± 5% humidity for one month in a stability chamber. At the end of one month the drug and mixtures were examined for their chemical interaction. Chemical interactions were analyzed by TLC [17], [21].

E. Thin Layer Chromatographic Studied

Preparation of silica plate was carried out with the 2% slurry of silica gel GF₂₅₄. Prepared silica plates were air dried and then activated at 60°C for 1h in oven. 10mg of substance being examined was dissolved in 5ml of (0.2%w/v). 5µl of Glibenclamide solution was applied on plate by capillary tube and plate was developed in TLC chamber pre saturated with solvent system. When solvent traveled 3/4th distance of plate,

1. In-vitro Dissolution Studies

In vitro dissolution study was performed using USP XXXVII Apparatus II in 900 ml of phosphate buffer (pH 7.4) for ten hours at an agitation rate of 100 rpm. The temperature of the medium was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. 10 mg of drug or its equivalent weight of the prepared dispersions was analyzed for dissolution. A 5.0ml sample was withdrawn at specific time points over a 10 hour period and equal volume of fresh dissolution medium was added to maintain a constant volume. The aliquot samples were filtered and the drug concentration was determined by spectrophotometrically [10], [13], [26].

Dissolution test parameters

Name of drug : Glibenclamide
 Temperature : $37 \pm 0.5^{\circ}\text{C}$
 Paddle speed : 100rpm
 Dissolution medium : 900ml
 Volume of sample removed : 5ml

III. RESULT AND DISCUSSION

A. UV Spectrophotometer Study

λ_{max} determination of drug was carried out in 0.01M methanolic HCl (pH 1.2). After scanning the λ_{max} in 0.01M methanolic HCl (pH 1.2) was found to be 300 nm. It was observed that the λ_{max} of drug is similar to the official compendia so, indicate indicating the identity and purity of the drug sample.

B. Drug- Excipient Compatibility Studies

The mixtures prepared for drug excipients compatibility study were kept for 30 days at $40^{\circ}\text{C}/75\%\text{RH}$ for compatibility studies. After a period of 30 days interaction study was analyzed by physical means and chemical means. At the end of one month the drug and mixtures were examined for their physical and chemical interaction. Any interaction between the drug and carrier was ruled out with the help of TLC studies. In TLC, the comparable Rf values of chromatograms and absence of additional spots indicates that there is no interaction between drug and carriers which confirm the compatibility of glibenclamide with excipients (Table II).

TABLE II
 DRUG EXCIPIENTS TLC COMPATIBILITY STUDY

| S. No. | Mixture | Distance travelled by solution (cm) | Distance travelled | | | Rf Value |
|--------|---------------|-------------------------------------|--------------------|------------|-------------|----------|
| | | | by drug (cm) | by EC (cm) | by PVP (cm) | |
| 1 | GLB | 13.7 | 12.6 | - | - | 0.92 |
| 2 | GLB + EC | 14.5 | 13.6 | 6.3 | - | 0.93 |
| 3 | GLB+ PVP | 12.7 | 11.7 | - | 3.6 | 0.92 |
| 4 | GLB + EC +PVP | 14.3 | 13.2 | 5.9 | 4.1 | 0.92 |

C. Preparation of Calibration Curve

After scanning the wave length in gastric simulated HCl buffer without enzyme pH 1.2, phosphate buffer 7.4 pH and borate buffer pH 9.5, it was found 300nm in all solutions therefore 300 was selected as λ_{max} . After selection of λ_{max} of drug, linearity was observed with an $r^2 = 0.9937$, $r^2 = 0.9957$ and $r^2 = 0.9914$ for gastric simulated HCl buffer

without enzyme pH 1.2, phosphate buffer (7.4 pH) and borate buffer pH 9.5 respectively, when analyzed at 300 nm. (Figs. 1-3)

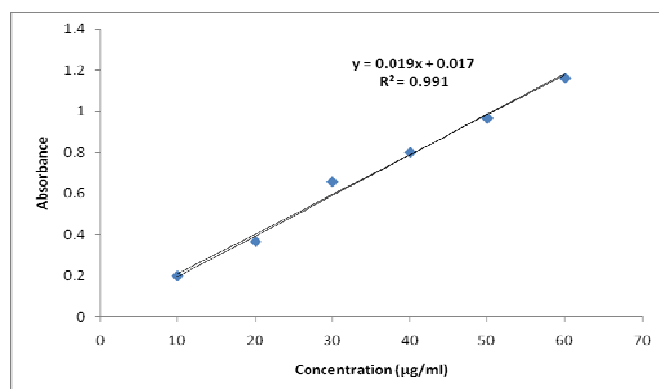


Fig. 1 Calibration Curve of GLB in HCl Buffer (pH 1.2) at λ_{max} 300nm

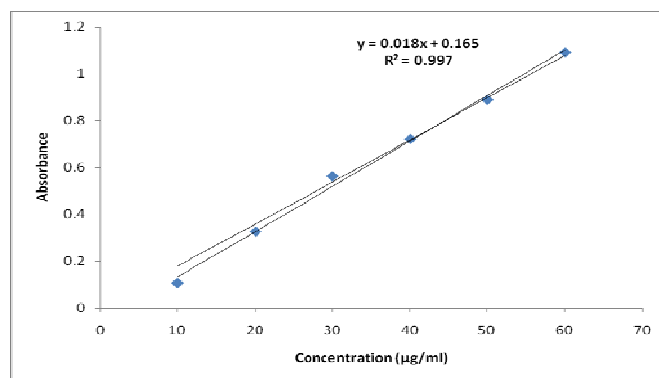


Fig. 2 Calibration Curve of GLB in Phosphate Buffer (pH 7.4) at λ_{ma} 300.0 nm

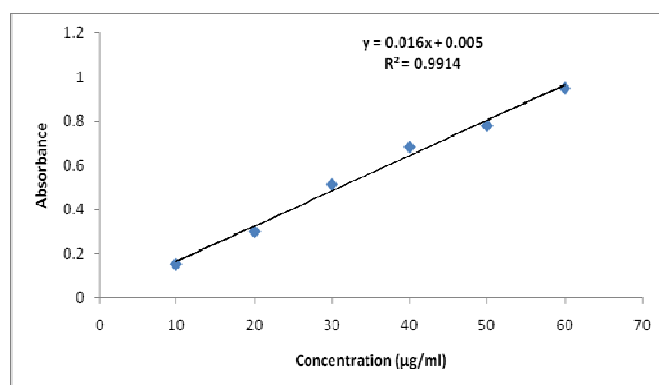


Fig. 3 Calibration Curve of GLB in Borate Buffer (pH 9.5) at λ_{m} 300.0nm

D. Quantitative Estimation of Glibenclamide

The quantitative solubility estimation of glibenclamide was performed in different solvent and it was observed that the solubility of drug in gastric HCl buffer pH 1.2 was found minimum and in borate buffer pH 9.5 was maximum (Table III).

These study shows that solubility of glibenclamide was increases as pH increases. The maximum solubility was observed at borate buffer pH 9.5 (Fig. 4).

TABLE III
QUANTITATIVE ESTIMATION OF GLIBENCLAMIDE IN DIFFERENT SOLVENT

| S. No. | Medium | Result ($\mu\text{g/ml}$) |
|--------|---------------------------|-----------------------------|
| 1 | Gastric HCl (pH 1.2) | 1.63 \pm 0.0005 |
| 2 | Phosphate buffer (6.8 pH) | 6.94 \pm 0.001 |
| 3 | Phosphate buffer (7.4 pH) | 8.72 \pm 0.001 |
| 4 | Borate buffer (pH 9.5) | 9.75 \pm 0.002 |

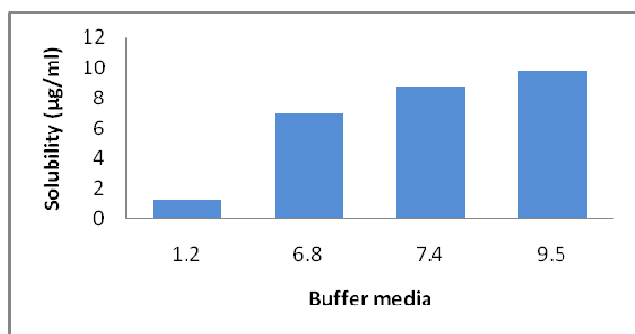


Fig. 4 Quantitative Solubility Profile of Drug at Different pH

E. Optimization of Ethyl Cellulose and Polyvinyl Pyrrolidone Concentration:

Ethyl cellulose and Polyvinyl Pyrrolidone were optimized by evaluating drug content and *in vitro* release kinetics study. The all batches were subjected for evaluation of the drug content (Table IV) and *in vitro* dissolution studies in phosphate buffer pH 7.4 [5]. The drug content of all the formulation was found to be within limit all formulation had the drug content from 91.66 \pm 0.40 to 100.00 \pm 0.7001% (Fig. 5) and it was found that all the batches performance release of drug very from 5 to 10 hours. However, among all nine batches, the batch no. F2, F7 and F8 are showing good retard release of drug. F2 and F7 the drug above 80% -90% within 8 hrs. and one of them batch no. F2 retard released of drug 99 % within 10 hours, which is one of the best release performance among all nine batches, whereas F4 and F6 formulation retard the release about 66% within 10 hrs that may be suitable for controlled release (Figs. 6 and 7).

TABLE IV
DRUG CONTENT PROFILE OF SOLID DISPERSION PREPARED BY SOLVENT EVAPORATION METHOD

| S. No. | Formulations | Loading efficiency (mg) Theoretical drug content (wt. of solid dispersion) | Actual drug* content | Percent incorporation |
|--------|--------------|---|----------------------|-----------------------|
| 1 | F1 | 10 (75) | 9.61 | 96.11 \pm 0.4041 |
| 2 | F2 | 10 (50) | 9.69 | 96.94 \pm 0.4359 |
| 3 | F3 | 10 (25) | 9.22 | 92.22 \pm 0.3047 |
| 4 | F4 | 10 (35) | 9.44 | 94.44 \pm 0.4041 |
| 5 | F5 | 10 (65) | 10.00 | 100.00 \pm 0.7001 |
| 6 | F6 | 10 (55) | 9.75 | 97.50 \pm 0.7000 |
| 7 | F7 | 10 (70) | 9.94 | 99.44 \pm 0.7002 |
| 8 | F8 | 10 (30) | 9.81 | 98.15 \pm 0.4041 |
| 9 | F9 | 10 (45) | 9.16 | 91.66 \pm 0.4099 |

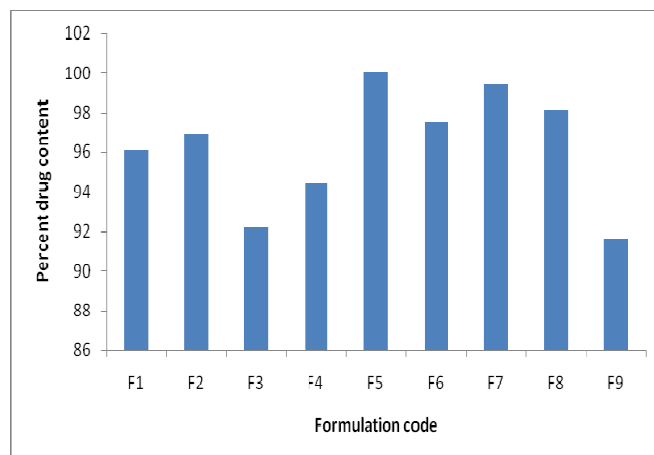


Fig. 5 Comparative Drug Content Profile of Formulation F1-F9 in Phosphate Buffer pH 7.4

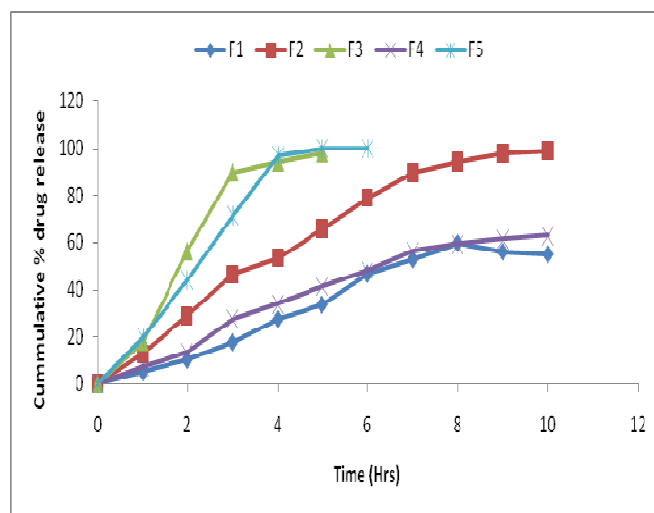


Fig. 6 Comparative % Drug Release of Formulations F1-F5 in Phosphate Buffer pH 7.4

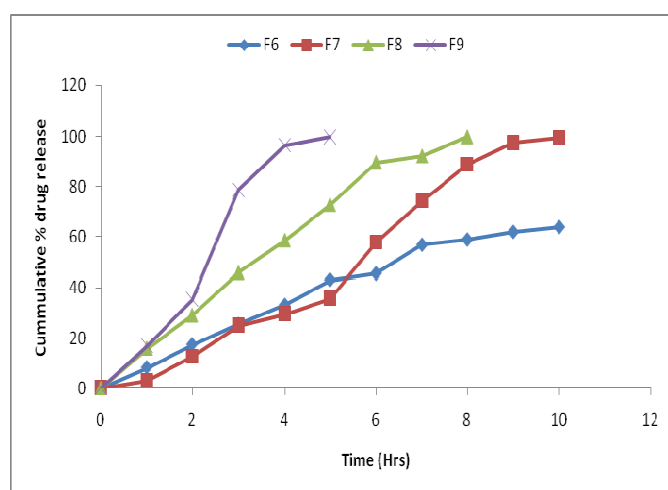


Fig. 7 Comparative % Drug Release of Formulations F6-F9 in Phosphate Buffer pH 7.4

IV. CONCLUSION

The results revealed that the preparation conditions governed the internal structure and dissolution mechanism by solid dispersions. EC as a hydrophobic carrier polymer was capable of sustaining the drug release rate at all weight ratio employed, but was insufficient alone to give complete drug release. When hydrophilic PVP was combined along with EC in 1:3 ratios, the drug release profiles were modified towards more gradual and complete release of GLB from combined polymer matrix. F2 was successfully incorporated to formulate directly to prepare extended drug release profile. The studies provided better forecasting and understanding of particulate systems to be incorporated to develop delivery systems.

REFERENCES

- [1] A.A. Elbary, H.F. Salem and M.E. Maher, *In vitro* and *in vivo* Evaluation of Glibenclamide using Surface Solid Dispersion (SSD) Approach, *British Journal of Pharmacology and Toxicology*, 2011, Vol. 2, Issue 1, pp. 51-62.
- [2] Ammer H. and Khalil R. (1997) 'Preparation and Evaluation of Sustained release solid dispersion of drugs with Eudragit polymer', *Drug Dev. Ind. Pharm.*, Vol. 23, pp. 1043-1054.
- [3] Boddeda B., Kumari P.V. K. and Chowdary K.P.R., Formulation and Evaluation of Glipizide Sustained Release Tablets, *International Journal of Biomedical Research*, 2012, Vol. 3, Issue 1, pp. 44-48.
- [4] British pharmacopoeia, The Stationary office- MHRA, Vol 1, 2009, pg no. 5176.
- [5] Costa P. and Jose M. S. L., Modeling and Comparison of Dissolution Profiles, *European Journal of Pharmaceutical Sciences*, 2001, Vol. 13, pp. 123-133.
- [6] Coppack S. W., Lant A. F., Meintosh C.S. And Rodgers A.V., Pharmacokinetic and Pharmacodynamic Studies of Glibenclamide in Non-Insulin Dependent Diabetes Mellitus, *British Journal of Pharmacology*, 1990, Vol. 29, pp.673-684.
- [7] Chauhan Bhaskar, Shimpi Shyam And Paradkar Anant, Preparation and Evaluation of Glibenclamide-Polyglycolized Glycerides Solid Dispersions with Silicon Dioxide by Spray Drying Technique, *European Journal of Pharmaceutical Sciences*, 2005, vol. 26, pp. 219-230.
- [8] Drugs K Patidar, M D Kshirsagar, V Saini, P B Joshi and M Soni, Solid Dispersion Technology: A Boon for Poor Water Soluble Drugs, *Indian journal of Novel Drug Delivery*, 2011, Vol. 3, Issue 2, pp.83-90.
- [9] Dixit AK, Singh RP and Singh Stuti, Solid Dispersion - A Strategy for Improving the Solubility of Poorly Soluble Drugs, *International Journal of Research in Pharmaceutical and Biomedical Sciences* Vol. 3 (2) Apr – Jun 2012.
- [10] Dash S., Murthy P.N., Nath L. and Chowdhury P., Kinetic Modeling on Drug Release from Controlled Drug Delivery Systems, 2010, *Acta Polonica Pharmaceutica Drug Research*, Vol. 67, Issue 3, pp. 217-223.
- [11] Hindustan A.A., Meghana M., Navya K., Mallika B., Aaslesha A., Srilatha V. and Chitta S.K., Designing and Evaluation of Glibenclamide Azadirachta Indica Mucilage Based Controlled Release Matrix Tablets, *Der Pharmacia Lettre*, 2010, Vol. 2, Issue 1, pp. 117-121.
- [12] Kushal M., Monali M., Durgavati M., Mittal P., Umesh S. and Pragna S., Oral Controlled Released Drug Delivery System: An Overview, *International Research Journal of Pharmacy*, 2013, Vol. 4, Issue 3, pp. 70-78.
- [13] Massik M.A., Darwish I.A., Hassan E.E. and El-Khordagui L.K., Development of a Dissolution Media for Glibenclamide, *International Journal of Pharmaceutics*, 1996, vol140, pp. 69-76.
- [14] Mehta A, Agarwal S, Narang. R. K, Preparation and Evaluation of Solid Dispersion of Nimesulide for Enhancing the Solubility, *Int. J. of Pharm. Sci.*, May- August-2012, Vol. 4, Issue-2, pp. 1871-1876.
- [15] Mutalik S. and Udupa N., Formulation Development, In Vitro and In Vivo Evaluation of Membrane Controlled Transdermal Systems of Glibenclamide, *Journal of Pharmaceutical Sciences*, 2005, Vol. 8, Issue 1, pp. 26-38.
- [16] Malamataris S. and Kaplani P., (2000) 'Preparation and characterization of a new insoluble polymorphic form of glibenclamide' *Int. J. Pharm.*, Vol. 195, pp. 239-246.
- [17] Martin A., Bustamante P. and Chun A. H. (1999) 'Physical pharmacy', Fourth edition, B.I. Waverly Pvt. Ltd., New Delhi, pp.257-263.
- [18] Narasimharao R., Anusha R. M., Swetha R.N., Divyasagar P. and Keerthana K., Design and Evaluation of Metformin Hydrochloride Extended Release Tablets by Direct Compression, 2011, Vol. 2, Issue 3, pp. 1118-1225.
- [19] Narasimharao R, Anusha Reddy M, Swetha Reddy N, Divyasagar P and Keerthana K, Design and Evaluation of Metformin Hydrochloride Extended Release Tablets by Direct Compression, *International Journal of Research in Pharmaceutical and Biomedical Sciences* Vol. 2 (3) Jul – Sep 2011.
- [20] Ohara T., Kitamura S., Kitagava T. and Terada K., (2005) 'Dissolution mechanism of poorly water soluble drug from extended release solid dispersion system with ethylcellulose and hydroxypropylmethylcellulose' *Int. J. Pharm.*, Vol. 202, pp. 95-102. (doi :10.1016/j.ijpharm.2003.11.025).
- [21] Patel Manvi, Pandya Narendra and V. H. Bhaskar, Preparation, Characterization and In Vitro Evaluation of Repaglinide Binary Solid Dispersions with Hydrophilic Polymers, *Int. J. Drug Dev. & Res.*, April-June 2011, 3 (2): 111-121.
- [22] Rawo R. C., Sheskey P.J. and Owen S. C. (2006) 'Handbook of pharmaceutical excipients', Fifth edition, The Pharmaceutical Press, Great Britain, pp. 217-220, 346-349.
- [23] R. Kavitha and Sathali A. A. H., Enhancement of Solubility of Repaglinide By Solid Dispersion Technique, *Int. Journal of Chem. Sci.*, 2012, Vol. 10, Issue 1, pp. 77-85.
- [24] Swarbrick J. and Boylen J. C. (2000) 'Encyclopedia of pharmaceutical technology', Second edition, Vol. 1, Marcel Dekkar, New York, pp.532-542.
- [25] Shinde S.S., Patil S.S., Mevekari F.I. and Satpute A.S., An Approach For Solubility Enhancement: Solid Dispersion, 2010, Vol. 1, pp. 299-308.
- [26] Shivanand Pandey, Development, Optimization and *In-Vitro* Evaluation of Glibenclamide Solid Dispersion Using Different Polymer, *Scholars Research Library Der Pharmacia Lettre*, 2009, 1 (2) 255-261.
- [27] Shams Tahiatul, Sayeed Muhammad Shahdaat Bin , Kadir Mohammad Fahim, Khan Rajibul Islam, Jalil Reza-ul and Islam Md. Saiful, Thermal, infrared characterization and in vitro evaluation of Repaglinide solid dispersion, *Der Pharmacia Lettre*, 2011, Vol. 3, Issue- 6, pp. 142-150.
- [28] Tiwari R, Tiwari G, Srivastava B and Rai A K. Solid dispersions: An overview to modify bioavailability of poorly water soluble drugs. *International Journal of PharmTech Research*, vol 1, Issue 4, 2009, pp 1338-1349.
- [29] Zajc N, Obreza A, Bele M, Srcic S., Physical properties and dissolution behaviour of nifedipine/mannitol solid dispersions prepared by hot melt method, *Int J Pharm.* 2005 Mar 3;291(1-2):51-8.