EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 8
581-586
Year: 2017

FORMULATION & EVALUATION OF ETHYL CELLULOSE MICROSPHERES CONTAINING GLIPIZIDE BY EMULSIFICATION METHOD

***Maya Sharma, P.K. Choudhury, Kajal L. Jain, Suresh Kumar Dev and Rajnish Shrivastava**

Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur, Rajasthan-313001, India.

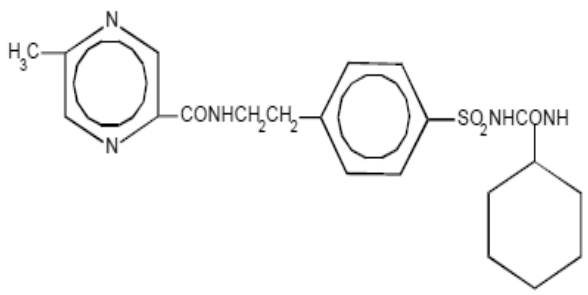
***Corresponding Author: Maya Sharma**
Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur, Rajasthan-313001, India.

Article Received on 08/06/2017 **Article Revised on 28/06/2017** **Article Accepted on 18/07/2017**

ABSTRACT
The aim of the study was to formulate and evaluate microspheres sustained release preparations of glipizide using ethyl cellulose as the entrapment material with high entrapment effectiveness and extended release. Microspheres were prepared by the double emulsion solvent diffusion method. Microspheres prepared by acetone as solvent and light liquid paraffin was chosen as the primary oil phase. Span-80 was used as the surfactant for stabilizing the liquid paraffin as oil phase. The prepared microspheres were characterized by percentage yield, shape, drug entrapment, optical microscopy and scanning electron microscopy (SEM). The in vitro release studies were performed in a series of buffer solutions with variable pH (1.2-7.4). The drug loaded microspheres showed 55–85% of entrapment and the release were extended for up to 10 hrs. SEM studies exposed that the microspheres were spherical and porous in nature. Data obtained from *in vitro* release studies were fitted to various kinetic models and high correlation was obtained with the Zero order kinetic. The drug release was found to be diffusion controlled.

KEYWORDS: Microspheres, Sustained release, Glipizide, Floating microspheres.

INTRODUCTION
Glipizide is an anti-diabetic drug used to treat type II diabetes mellitus. The drug has a short half life of about 2-3 hrs. It is available as single dosage formulation for conventional and sustained release tablet for oral use. Glipizide was model drug because its frequent administration short half life. A model dosage form is one, which attains the desired therapeutic concentration of drug in plasma and maintains constant for entire duration of treatment. This is achievable through administration of a conventional dosage form in a particular dose and at particular rate.[1]



A high potency, long acting (high plasma protein binding and hepatic recycling) second-generation sulfonylurea, factors again resulting from the Ar- moiety present in this compound. Absorption rate reduced when taken with meals; take on an empty stomach. Shortest half-life (2-4 hrs) as the parent drug but extended released formulation ("XL") also is available providing 24-hrs action. It is extensively metabolized by the liver (90%); 10% excreted unchanged by the kidney.[2]

The main function of the stomach is to for the time being store food, start its digestion and to release the resulting chyme slowly through the pylorus in to the duodenum, because of small surface area of the stomach, absorption in to the systemic circulation is restricted. The jejunum and ileum is the most important site for absorption of nutrients and drugs. The concept of Floating Drug Delivery System was described in the literature as early as 1986, when **Davis** discovered a method for overcoming the difficulty experienced by some persons of gagging or choking while swallowing medicinal pills. The author suggested that difficulty could be overcome by providing pills having a density of less than 1.0 g/ml so that pill will float on water surface.[3,4]

Experimental procedure
Glipizide IP was gifed by Kreative organics (p) ltd Hyderabad, Ethyl cellulose was obtained from Loba Cheme, Mumbai, India.

Method

Ethyl cellulose was dissolved in acetone. Span 80 was added to liquid paraffin and drug was mixed and stirred at 800 rpm to get a uniform dispersion. Polymer solution was added drop wise and stirring was continued until all the solvent evaporated. The product was filtered; air dried and stored in amber colored bottles and kept in desiccator until used (Table 1).

Table-1 Formulation Process

Glipizide Ethyl Cellulose emulsification miorospheres						
Code	**Drug**	**Ethyl Cellulose**	**Span 80 (1%) v/v**	**Acetone**	**Liquid Paraffin**	**D:P**
GEC1	100 mg	+	+	20 ml	100 ml	1:0.5
GEC2	+	+	+	+	+	1:1
GEC3	+	+	+	+	+	1:1.2
GEC4	+	+	+	+	+	1:1.4
GEC5	+	+	+	+	+	1:1.6
GEC6	+	+	+	+	+	1:1.8
GEC7	+	+	+	+	+	1:2

Evaluation of microspheres

Size distribution and size analysis

Microspheres were separated into different size fractions by sieving for 10 mins using a mechanical shaker containing standard sieves as per Indian Pharmacopoeia specifications. The particle size distribution was determined and means particle size of gel beads was calculated by the formula.[5] (Figure 1).

$$\text{Mean particle size} = \frac{\sum (\text{Mean particle size of the fraction} \times \text{weight fraction})}{\Sigma (\text{Weight fraction})} \quad \text{Eqn.1}$$

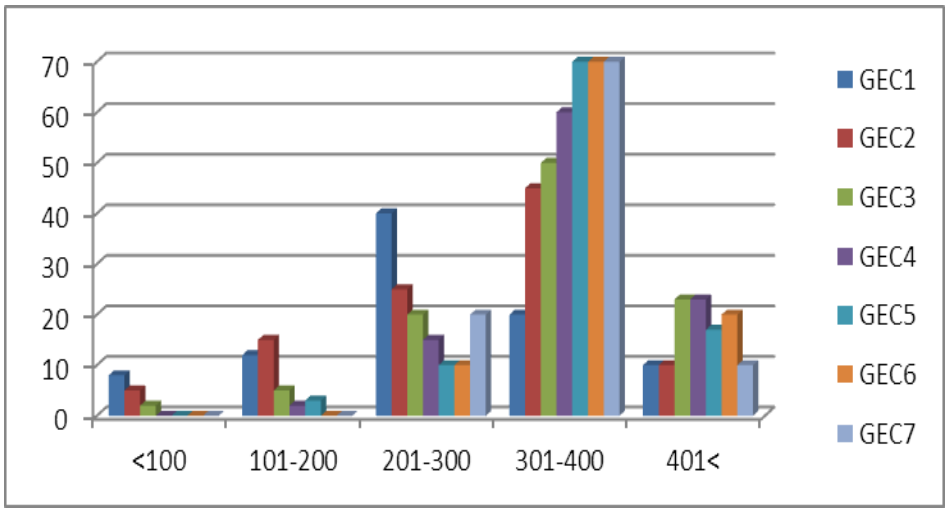


Figure 1- Size distribution of ethyl cellulose microspheres

Study of morphology of ethyl cellulose microspheres

The mean diameter of 50 dried microspheres and morphological examination of dried beads were performed using optical microscopy. Glipizide–ethyl cellulose microspheres were prepared by emulsification solvent evaporation method. Round shaped and small size distinct microspheres were produced. Effect of various factors like stirring rate, concentration of polymer etc. were described and calculated. Larger microspheres showed maximum drug loading and smaller microspheres showed fast drug release.[6] (Table 2).

Scanning electron microscopy (SEM)

The samples for the SEM analysis were performed by sprinkling the microspheres on one side of the double adhesive stub.[7] The stub was then coated with fine gold dust. The gel beads were then observed with the scanning electron microscope (JEOL Model JSM-6390LV) at 15kv (Figure 2).

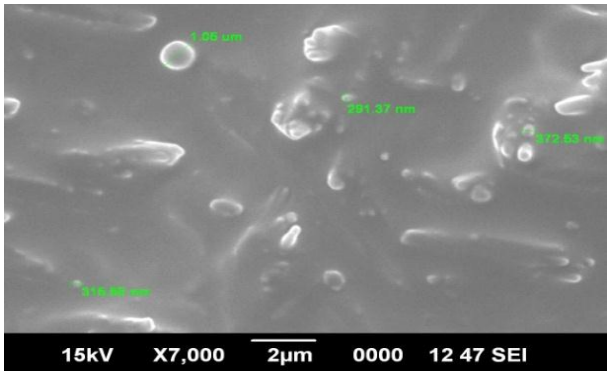


Figure 2-Scanning Electron Microscopy

Measurement of Buoyancy property

For the evaluation of floating property, approximately 100 microspheres were counted and pasted on one side of the glass slide secured to the USP disintegration apparatus. The apparatus was run for 5 hrs and at predetermined time interval (30 mins), the slide was taken out and the number of microspheres still adhering to the slide was counted.[8] (Table 2).

Table 2- Physicochemical properties of the glipizide ethyl cellulose microspheres

Ethyl cellulose emulsification Microspheres of Glipizide					
Code	**% Yield**	**%Entrapmen± S.D**	**Shape**	**Color**	**Buoyancy (%) ± S.D**
GEC1	95.0	89.8 ±0.15	Spherical	White	86.0 ± 1.8
GEC2	89.2	90.1 ±0.09	Spherical	White	83.2 ± 0.3
GEC3	95.2	87.3 ±0.08	Spherical	White	73.2 ± 1.8
GEC4	96.3	89.7 ±0.26	Spherical	White	75.1 ± 1.9
GEC5	94.2	80.0 ±0.15	Spherical	White	69.2 ± 1.6
GEC6	88.1	94.8 ±0.11	Spherical	White	65.1 ± 1.5
GEC7	85.5	82.7 ±0.09	Spherical	White	62.0 ± 0.7

In vitro release studies

a. Preparation of standard plot in Methanol

Glipizide (50 mg) was dissolved in 100 ml of methanol. It was then suitably diluted for graded solutions in range of 0-50 µg/ml. the absorbance was read using an UV spectrophotometer at 276 nm-λ max (Table 3, Figure 3).

Table 3- Glipizide Pure drug absorbance in methanol by UV spectrophotometer

S. No	Concentration (µg/ml)	Absorbance at 276 nm.
1	0	0
2	5	0.115
3	10	0.238
4	15	0.368
5	20	0.482
6	25	0.599
7	30	0.714
8	35	0.824

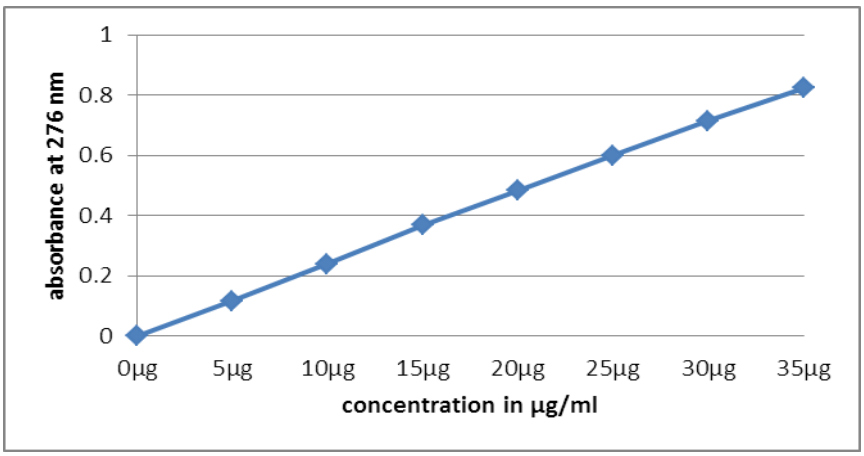


Figure 3- Standard plot of Glipizide in methanol

b. In vitro release of microspheres

The in vitro release studies were carried out at 37°C ± 0.5°C and at 100 rpm by buffer change method using 0.1 N HCl (1 h), 4 pH (1 h), 6 pH (3 h), 6.8 pH (3 h) and 7.4 pH (2 h) phosphate buffers (200 ml) in sink conditions using a Diffusion cell. Accurately weighed sample of prepared microspheres were added to the donor cell. At pre-set time intervals; 5 ml of aliquots are withdrawn and replaced by an equal volume of fresh dissolution medium. The aliquots were analyzed UV spectrophotometrically at 276 nm- λ max after proper dilution (Table 4, Figure 4).

In the early incubation stage, the dissolution rates of Glipizide were slightly faster especially during the first hour. This was due to the fast dissolution of the drug present on the surface of the microspheres and the rapid penetration of aqueous solution into the microspheres, which is also called burst effect.

Table 4- In vitro release kinetic equation data of glipizide ethyl cellulose microspheres (D: P- 1: 0.5 to 1:2), n=3

Code	Zero order	First order	Higuchi	Korsmeyer Peppas	Hixson Crowell
	R_0	R_1	R_H	R_K	R_{HC}
GEC1	0.979	0.862	0.979	0.773	0.929
GEC2	0.972	0.952	0.972	0.776	0.968
GEC3	0.979	0.962	0.978	0.800	0.975
GEC4	0.973	0.968	0.974	0.816	0.974
GEC5	0.955	0.970	0.950	0.848	0.971
GEC6	0.965	0.962	0.963	0.894	0.964
GEC7	0.969	0.968	0.970	0.867	0.970

R= regression coefficient value.

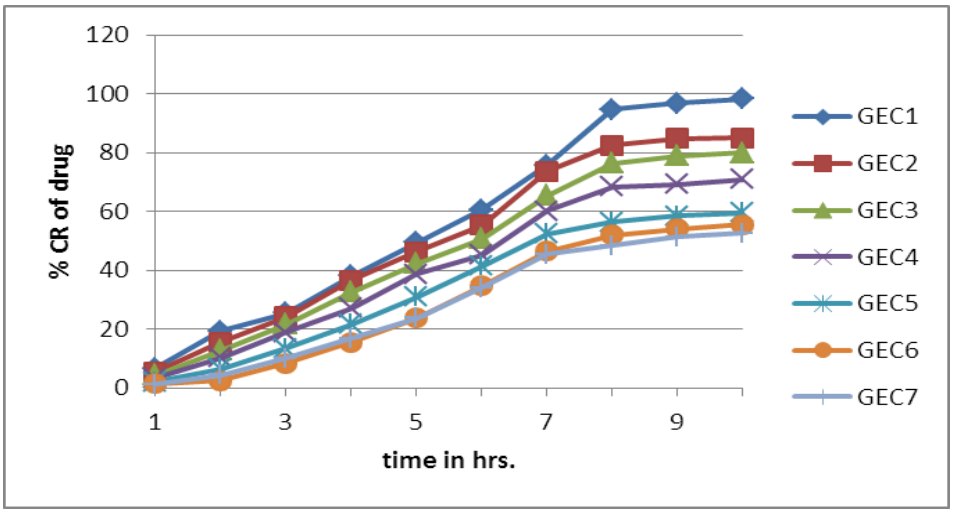


Figure 4- In-vitro release graph GEC1-GEC7

Differential Scanning Calorimetry (DSC) of Glipizide formulations

DSC was carried out to determine the possible interaction between drug and polymer.[9] No interaction was observed and individual peaks of the polymer and Drug were present in DSC graphs. Additional peaks if observed are those of the impurities or solvent residues in the formulations (Figure 5).

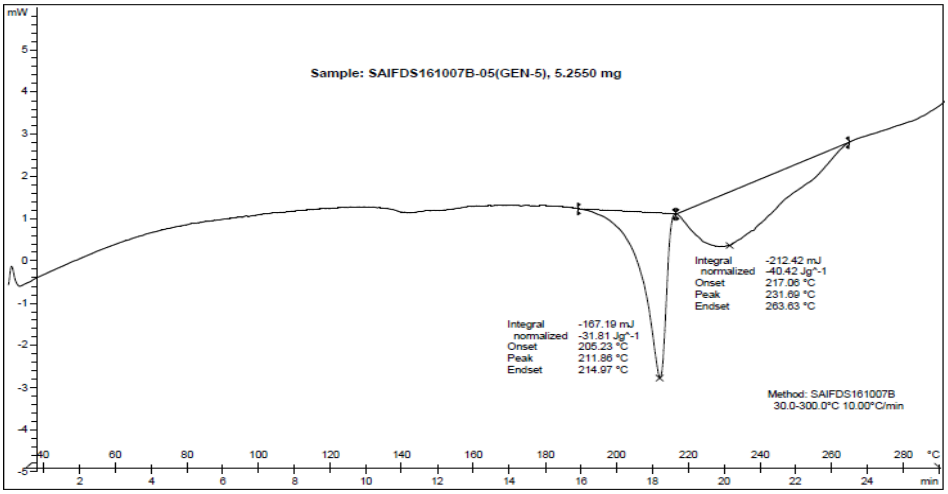


Figure 5- DSC graph of glipizide ethyl cellulose microspheres (GEC-6)

Stability studies of Glipizide formulations

Stability testing is an integral part of the formulation development. It generates information about shelf life of drug substances and their produced formulations and recommends appropriate guidelines for storage.[10] In the glipizide formulation there was no significant change observed on stability. Loss of drug content was seen in formulations with low polymer concentrations.

Morphological characteristics and release profile

EC-Glipizide microspheres were stable throughout course of study of 3 months (90 days)[11] and no significant morphological changes were observed. Slight changes observed at the elevated temperature that caused for degradation of polymers. Microspheres prepared by ethyl cellulose were highly stable at all storage conditions with no change in morphology or release characteristics. At accelerated stability studies conditions, no significant changes in the data were observed.

RESULT AND DISCUSSION

The anti-diabetic, water insoluble drug; Glipizide was formulated as microspheres using various microencapsulation techniques. The polymer used for the process was hydrophobic in nature, ethyl cellulose. The effects of process variables on the various physicochemical, morphological and in vitro release characteristics were studied. The results of the analytical methods used are given in (figure 3 and 4). The methods obeyed Beer's law in the range of 0-35 µg/ml and were suitable for the study. The low coefficient of variations showed that the methods are highly reproducible.

Glipizide-ethyl cellulose microspheres were prepared by emulsification solvent evaporation method. Round and small sized discrete microspheres were obtained. Effect of various factors like stirring rate, concentration of polymer etc. was studied. Larger microspheres showed greater drug loading while smaller microspheres showed rapid drug release. For preparation of microspheres by this method, a constant speed of 800 rpm was chosen which aided in the evaporation of solvent from polymer solution. While increase the speed, size of microspheres also decreased.

The polymer concentration directly affected the morphological and release characteristics of formulations. Low polymer concentration lead to uneven while high polymer concentrations lead to smooth surfaces and even sized microspheres. The formulations were white, spherical and small in size. Polymer ethyl cellulose is insoluble in all physiological pH values but swells at all pH conditions.

The polymer to drug ratio was varied to find out the effect of polymer on the size and surface characteristics. It was found that with the increase in polymer concentration, larger microspheres were produced because of the increase in the viscosity of the phase.[12-13] Due to this increased viscosity and continuous stirring, the smaller droplets coalesce to form larger droplets.[14] It was seen that with the increase in percentage of Span 80 used, the mean particle size decreased.

The methods obeyed Beer's law in the range of 0-35 µg/ml and were suitable for the study. The low coefficient of variations showed that the methods are highly reproducible.[15-16]

The dissolution of microspheres carried out in simulated gastrointestinal media showed an initial burst effect may be due to migration of drug towards periphery.[17,18] Release GEC1 (fig: 4 table: 4) of 1:0.5 concentration of drug and polymer respectively was high within 8-9 hrs. When the concentration of polymer increased, release rate of drug from microspheres decreased.[19] The drug was released from pores generated by ethyl cellulose entity.[20,21]

CONCLUSION

A new sustained release system of ethyl cellulose microspheres were designed and formulated by an emulsification method. It's morphological and release characteristics were studied. The microspheres were easy to prepare and the mean diameter of microspheres increased with increase in the amount of the span 80 surfactant phase. The pore size of emulsified microspheres was affected by concentration of the ethyl cellulose. The microspheres showed excellent sustaining properties as compared to the conventional microspheres. Thus, drug entrapment technique is a useful tool for the development of multi-particulate system even for a water-insoluble drug like glipizide.

ACKNOWLEDGEMENT

Authors are grateful to Sophisticated Test & Instrumentation Centre (STIC), Cochin University. Kochi, Kerala for providing DSC and SEM facility.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

REFERENCES

1. Mathiowitz, E., Chickering, D., Jacob, J.S. and Santos, C., Bioadhesive drug delivery systems. Encyclopedia of Controlled Drug Delivery 1999; 1: 9-44.
2. Aulton M E. Pharmaceutics; "The science of dosage form design" Churchill Livingstone; 1989; 113-114.
3. Xiaoyun, H., Novel preparation method for sustained-release PLGA microspheres using water-in-oil-in-hydrophilic-oil-in-water emulsion, International Journal of Nanomedicine, 2013; 8: 2433–2441.
4. Brahamankar D M and Jaiswal S B. "Biopharmaceutics and Pharmacokinetics: A treatise" 1995; Ist Edition; Vallabh Prakashan; 347.
5. Jorgensen, L., Moeller E.H., Weert, M., Nielsen, H.M. and Frokjaer, S., Preparing and evaluating delivery systems for proteins. European J. Pharm. 2006; 29(3–4): 174–182.
6. Castellanos, I.J., Cruz, G., Crespo, R. and Griebenow, K.; Encapsulation-induced aggregation and loss in activity of γ-chymotrypsin and their prevention. Journal of Control Release. 2002; 81(3): 307–319.
7. Ceriello, A., The emerging role of post perdanial hyperglycaemic spikes in pathogenesis of diabetes

complications. Diabetec Medicines 1998; 15: 188–93.
8. Deruiter, J., Overview of the Antidiabetic Agents, Endocrine Pharmacotherapy Module, Spring, 2003; 25.
9. Rao, N. M., Development and validation of UV-Spectroscopic method for estimation of Voglibose in bulk and tablets J. Chem. Pharm. Res., 2010; 2(2): 350-356.
10. Priyanka., Mishra, D.N., Singh, S.K. and Purohit, S., Box Behnken Design in optimization and evaluation of Metformin HCl loaded Guar gum Microspheres; 2012; 2012(4): 1-8.
11. Choudhury, P. K., and Kar, M., Preparation of Alginate Gel Beads Containing Metformin Hydrochloride Using Emulsion- Gelation Method, Tropical Journal of Pharmaceutical Research, December 2005; 4(2): 489-493.
12. Choudury, P. K., Kar, M. and Chauhan, C.S., Cellulose Acetate microspheres as floating depot systems to increase gastric retention time of antidiabetic drug: Formulation, Characterisation and in vitro-in vivo evaluation., Drug Development & Industrial Pharmacy, 2008; 122-125.
13. Sarode, S. M., Mittal, M., Magar, R. M., Shelke, A. D., Shrivastava, B. and Vidyasagar, G., Formulation and evaluation of floating microspheres of Glipizide. J. Chem. Pharm. Res., 2011; 3(3): 775-783.
14. Longer, M. A., Cheng, H. S. and Robinson, J. R. Mucoadesive materials for CR dosage forms. J. Pharm. Sci. 1987; (74): 406-410.
15. Mesiha, M. and Sidhom, M. Increased oral absorption enhancement of insulin by medium viscosity hydroxyl propylcellulose. Int. J. Pharmaceutics. 1995; 115: 137-140.
16. Wan, L.S., Heng, P. W. and Wong, L.F., matrix swelling: simple model describing extent of swelling of HPMC matrices. Int. J. Pharmaceutics. 1995; 116: 159-168.
17. Longer, M. A., Cheng, H. S. and Robinson, J. R., Mucoadesive materials for Control Release dosage forms. J. Pharm. Sci. 1987; 74: 406-410.
18. Patel, S. S., Patel, J. K., Patel, G. N., Bharadia, P. D., & Patel, M. M. (2006). Formulation and evaluation of floating chitosan microspheres. *Pharmaceutical formulation and Quality*, *8*(2): 34–36.
19. Rouge, N., Leroux, J. C., Cole, T., Doelker E., & Buri, P. (1997). Prevention of the sticking tendency of floating minitablets filled in hard gelatin capsules. *Eur. J. Pharm. Biopharm.*, *43*: 165–171.
20. Sato, Y., Kawashima, Y., Takechi, H., & Yamamoto, Y. (2003). In vivo evaluation of riboflavin-containing microballoons for floating controlled drug delivery system in healthy human volunteers. *J. Control. Release*, *93:* 39–47.
21. Seth, P. R., & Tossounian, J. (1984). The hydrodynamically balanced system HBS: A novel drug delivery system for oral use. *Drug Dev. Ind. Pharm.*, *10*: 313–339.