Formulation, Optimization and Characterization of Microspheres using Biomaterial Arabinoxylan

JOHAR SHUJAAT[1], ALIA ERUM[1], UME-RUQIA TULAIN[1],
SOBIA NOREEN[2], AND ASMARA ISMAIL[1]

[1] *College of Pharmacy, University of Sargodha, Sargodha, Pakistan*

[2]*Department of Chemistry, University of Sargodha, Sargodha, Pakistan*

ABSTRACT

Microspheres of new biomaterial arabinoxylan loaded with levosulpiride a model drug were formulated using ionotropic gelation technique. Microspheres were optimized by using different reaction parameters like cross linkers type and concentrations, concentration of biomaterial arabinoxylan, effect of reaction temperature and reaction time and drug loading method. Nine different formulations were formulated. It was observed that best microspheres were formulated using 10 % $CaCl_2$, 3 % arabinoxyaln at 60^0C temperature and 15 minutes reaction time for curing. Compatibility of arabinoxylan with levosulpiride was tested by using XRD and FTIR techniques. Microsphere formulations were evaluated for percentage yield, flow properties, swelling index, solvent regain and drug release studies. Formulations possessed excellent flow properties as indicated by their angle of repose, Carr's index, Hausner's ratio, tapped density and bulk density. SEM showed that microspheres were almost spherical and had rough surface morphology. Levosulpiride release from biomaterial arabinoxylan followed Korsmeyer–Peppas model showing drug release by diffusion and erosion of polymer. So this study provides a scientific evidence for the use of arabinoxylan as an effective alternative to sodium alginate for microsphere formulation.

KEYWORDS: *Arabinoxylan, Microspheres, Optimization, Levosulpiride, Ionotropic gelation method,*

INTRODUCTION

While a variety of devices have been used for controlled release drug delivery, biodegradable polymer microspheres are one of the most common types and hold several advantages. They are generally biocompatible, can provide

J. Polym. Mater. Vol. **34,** No. 4, 2017, 809-821

Correspondence author e-mail:alia_erum@hotmail.com

high bioavailability, and are capable of sustained release for long periods of time [1]. The microencapsulation technique, an ideal approach to encapsulate and deliver bioactive molecules, and cells can provide a protective covering for live cells, cytokines, small proteins and other bioactive compounds [2]. Medication is released from a microsphere by drug leaching from the polymer or by degradation of the polymer matrix [3].

Alginates, which are naturally occurring substances, found in brown algae have received much attention as a polymer for microspheres. The Drop wise addition of aqueous alginate solution to the aqueous solution containing calcium ions and or any other divated and polyvalent cations cause spherical gel formation. The dried alginate beads have the property of reswelling and thus they can act as controlled release system. This property is susceptible to pH, which protects the acidsensitive drug from gastric juice [4]. Other polymers for microspheres include chitosan, polyethylene and polystyrene.

Ispaghula (*Plantagoovata*) is indigenous in South Asia including Pakistan and has wide pharmacological and pharmaceutical uses [5]. Arabinoxylans (AX) are important non-starch polysaccharides extracted from husk and seed of *Plantago ovata.* AX has the ability to form gels by covalent linking. Arabinoxyaln has been used as release retardant in matrix tablet as well as in microspheres as coating agent along with sodium alginate. Unsuccessful microencapsulation of Aspirin by using arabinoxylan of *Plantago ovata* had been previously reported [6]. In this study attempt was made to formulate microspheres of arabinoxylan by ionotropic gelation method, optimization of various reaction parameters was also the part of study.

Levosulpiride (-)-enantiomer of sulpiride, a central antidopaminergic activity, antiemetic and antidyspeptic drug was loaded as model drug in arabinoxylan microspheres and its release characteristics were studied.

MATERIALS AND METHODS

Materials

Levosulpiride was obtained from Pacific Pharma Lahore Pakistan. Sodium hydroxide, hydrochloric acid, calcium chloride, barium chloride, aluminum hydrogen sulphate, benzene and acetone were from Sigma Aldrich Germany. All other chemicals and solvents used were of analytical and pharmaceutical grade. Arabinoxylan was isolated from *Plantago ovata* husk.

Method

Extraction of arabinoxylan from *Plantagoovata* husk

About 50 g of *Plantagoovata* husk was soaked in distilled water (seed husk: water 1:50, w/v). The pH was adjusted to 12 by the addition of aqueous NaOH solution (2.5%). The dispersion was stirred for 2-3 min and then the gel was separated from the husk by vacuum. Concentrated acetic acid was used for coagulation of sample. To adjust constant pH, distilled water was used to wash the gel for many times over a period of 3-4 days and then drying of gel was done in oven at 40°C.

Arabinoxylan microspheres formulation

In order to formulate the microspheres of AX, ionotropic gelation technique was used. The polymer AX was dispersed vigorously in 5% NaOH solution with magnetic stirring, until homogenous dispersion was formed. Air bubbles were removed using sonicator. Syringe (#22) was used to disperse AX into cross linker solution drop wise. AX microspheres were formed and were allowed to remain in cross linker solution for some time. Then the microspheres were filtered and washed by acetone-water mixture (50:50). The Drying of microspheres was done in oven at 40°C for 6 hours [7].

Optimization of microspheres

Effect of type and concentration of cross linker on AX microsphere formulation

In order to study the effect of type and concentration of cross linker on AX microsphere formulation, microspheres were prepared using AX as polymer, $CaCl_2$, $BaCl_2$ or Al_2SO_4 as cross linking agents. Nine different formulations of microspheres were prepared using three different concentrations of $CaCl_2$, $BaCl_2$ and Al_2SO_4 (5%, 10% and 15) as shown in Table 1.

TABLE 1. AX Microsphere formulations with different type and concentration of cross linker

Formulation	Cross linker	Conc. of cross linker (%)	Polymer conc. (%)	Temperature (^{0}C)	NaOH conc. (%)
1		5	3	35	5
2	$CaCl_2$	10	3	35	5
3		15	3	35	5
4		5	3	35	5
5	$BaCl_2$	10	3	35	5
6		15	3	35	5
7		5	3	35	5
8	Al_2SO_4	10	3	35	5
9		15	3	35	5

Effect of concentration of polymer on AX microsphere formulation

Effect of concentrations of polymer on properties of AX microspheres was studied by preparing different formulations of microspheres using different concentrations of polymer as shown in Table 2. Factorial design (2 × 3) was used to prepare different batches of microspheres [8].

Effect of temperature and reaction time on AX formulation

These parameters were studied by preparing different batches of microspheres at two different temperatures

TABLE 2. AX Microsphere formulations using different concentrations of polymer

Formulations	Polymer conc. (%)	$CaCl_2$ conc. (%)	NaOH conc. (%)	Temperature (^{0}C)	Reaction time (min)
F1	2	5	5	35	15
F2	2	10	5	35	15
F3	2	15	5	35	15
F4	2.5	5	5	35	15
F5	2.5	10	5	35	15
F6	2.5	15	5	35	15
F7	3	5	5	35	15
F8	3	10	5	35	15
F9	3	15	5	35	15

i.e. 35^{0}C and 60^{0}C and reaction time was taken as 15 minutes and 30 minutes. Four different microspheres formulations were prepared by using 2×2 factorial design as shown in Table 3.

TABLE 3: Microsphere formulations with different temperature and reaction time

Formulations	Temperature (%)	Reaction time (min)	$CaCl_2$ conc. (%)	Polymer conc. (%)
F1	35	15	10	3
F2	35	30	10	3
F3	60	15	10	3
F4	60	30	10	3

Drug (Levosulpiride) loading of AX microspheres

For drug loading in microspheres two methods were tried to select the one with better encapsulation efficiency.

Pre formulation loading

In this method for the loading of drug in microspheres homogeneous mixture of drug was obtained by stirring 20g of drug in $CaCl_2$ solution. Now the polymer solution was added in $CaCl_2$ solution drop wise with syringe with slight stirring. Microspheres were filtered and drying was done at room temperature.

Post formulation loading

About 20g of drug was dissolved homogeneously in 100mL of distilled water to prepare 20% w/v solution of Levosulpiride. Microspheres prepared earlier by ionic gelation method were stirred at slow speed in solution of drug for about 2 hours. Microspheres were filtered and dried at room temperature.

Drug entrapment efficiency

To calculate the amount of drug loaded on microspheres by both loading methods the entrapment efficiency was measured [9]. For this purpose prepared microspheres (50mg) were crushed and the powder was allowed to suspend in 10mL of phosphate buffer (pH 7.4) to extract the drug present in the microspheres. After 24 hours, the solution was filtered and then the filtrate was diluted and analyzed for the drug content present in it by measuring its absorbance in UV visible spectrophotometer. Percentage entrapment efficiency of drug was calculated from the following formula:

$$\frac{\textit{Absorbance of sample solution}}{\textit{Absorbance of standard solution}} \times 100$$

The absorbance of standard solution of drug was also measured by UV visible spectrophotometer.

Characterization of microspheres

Flow properties of microspheres

According to USP guidelines [10] flow properties of microspheres were checked by measuring the angle of repose, tapped density, bulk density, Hausner's ratio and Car's index.

Swelling index of microspheres

The behavior of polymeric microspheres in different solutions was evaluated by checking the property of swelling index. Three different physiological solutions i.e. 0.1N HCl, phosphate buffer having pH 6.8 and distilled water were used and accurately weighed amount of microspheres (50mg) was separately immersed in each medium for 24 hours. The microspheres are filtered and then washing was done. Following equation was used to calculate swelling [12].

$$A = (W2-W1)/W1$$

Where; α is the swelling index, W1 is weight of microspheres before swelling, W2 is weight of microspheres after swelling.

Shape and surface morphology

Scanning electron microscopy was used to check the shape and surface morphology of microspheres. The samples were made ready by spreading powder of microspheres on a double sided carbon dust, which was positioned on a sample carrier in the cylindrical shape. After the samples were fixed on stub, the microspheres were seen under scanning electron microscope [11].

Fourier transforms infrared analysis

Fourier transform infrared analysis (FTIR) measurements of levosulpiride drug, arabinoxylan unloaded microspheres and drug loaded arabinoxylan were obtained on JASCO V5300 FTIR (Tokyo, Japan). The pellets were prepared on KBr- press under hydraulic pressure of 150 kg/cm^{-2}. The spectra were scanned over the wave number range of 3,600 to 400 cm^{-1} at ambient temperature [13].

In-vitro drug release studies

To check the release behavior of drug from microspheres USP-XXII paddle apparatus was used. The dissolution study was conducted for 2 hours in 1000mL of 0.1 N HCl solutions at 37°C ± 0.5°C at 100 rpm. After one hour 5mL of aliquots from the dissolution medium was withdrawn and were replaced with fresh dissolution medium after each withdrawal. The aliquots were analyzed by U.V spectrophotometer for levosulpiride content at 282.4 nm. After 2 hours these microspheres were shifted to PH 7.4 buffer medium. After one hour, interval 5ml of aliquots from dissolution medium were withdrawn and were analyzed by U.V spectrophotometer for levosulpiride content at wavelength 282.4 nm. The process was continued for 10 hours [15].

Drug release kinetics

To determine Levosulpiride release kinetics different kinetic models were applied to interpret release mechanisms by using DD solver software.

RESULTS AND DISCUSSION

Yield of arabinoxylan

The yield was 22.5 g (45% related to the weight of seed husk) of arabinoxylan (AX) which was quite satisfactory yield and was in accordance with the research conducted by Shazia et al.[16]. This yield is more than the one obtained with water extraction as reported by Saeed et al. (2011)[17] which was 35%. Methods for the extraction of gel from *Plantago ovata* can be broadly classified into water and alkali extraction methods. Extraction with hot water is though most commonly used method, it has a number of disadvantages for e.g. certain pigments are present as impurities Thus, the method used in this study was alkali extraction which is economical and produces salt free and refined colourless gel .

Optimization of microsphere formulation

Effect of type and concentration of cross linker of AX formulation

The shape and stability of the microspheres depends on the cross linker type and concentration. It was found that best spherical and stable microspheres were manufactured by using $CaCl_2$ as cross linker at 10% concentration as shown in Table 4. At high concentration (15%) somehow less spherical particles and at low contents amorphous polymer aggregates were observed. This might be because smaller and irregular microspheres were produced by increasing the Ca^{2+}concentration. This result was in agreement with study conducted by Singh and Kumar [18]. The chemical structures and concentrations of the crosslinking agents affected both the swelling ratio and the porosity of the networks. It also had been reported that

better sustained release activity was shown by microspheres prepared with $CaCl_2$. $BaCl_2$ and Al_2SO_4 impart very little cross linking. $BaCl_2$ behaved as mild cross linking agent at concentration 15%.

Effect of concentration of polymer on AX microsphere formulations

The study was conducted to check at what extent the morphology, stability and release characteristics of microspheres were affected by the polymer arabinoxylan concentration. It was observed that the stability and percentage entrapment of the microspheres was increased by increasing the concentration of polymer arabinoxylan to 3%. As the polymer concentration is increased the porosity of prepared microspheres is decreased and hence the release of the drug would also become sustained by increasing the polymer concentration. These results were in accordance with previous finding regarding polymer concentration on microspheres as reported [19].

Effect of temperature and reaction time on AX microspheres

Four different formulations of microspheres were prepared to investigate the effect of temperature and reaction time on the properties of microspheres. Microspheres formulations were produced at two temperatures i.e. 35°C and 60°C and reaction time was taken as 15 minutes and 30 minutes. The temperature affects the stability and production of microspheres because it might increases the entry of calcium ions into the AX gel and also by increasing the temperature the viscosity of the solution of AX decreases. It was observed that by increasing the temperature to 60°C the stability and morphology of microspheres were improved. The results were in accordance with the work done by Fundueanu *et al*. [20] They observed the effect of temperature on the production of microspheres. With the increase in reaction time the cross linking between AX gels would also increase which resulted in less spherical geometry and also small degree of swelling and hence less drug loading capacity. So as far as reaction time is concerned 15 minutes reaction time produced microspheres with desirable properties as shown in Table 6.

Effect of drug loading method on encapsulation efficiency

Encapsulation efficiency was greater i.e. almost 70% in case of post loading method as compared to almost 33% in preloading method. It is observed that the drug loading method has a substantial impact on drug content of the microspheres. The post loading process using same quantity of drug increased the content of levosulpiride in microspheres. Similar results were reported previously in case of drug load into microcapsules of alginate/poly-L-lysine/ chitosan ternary complex [21].

Characterization of microspheres

Flow properties

Flow properties were checked for each formulation of microspheres. Different features like bulk density, tapped density, Hausner's ratio, Carr's index and angle of repose were determined. The concentration and type of cross linker as well as the concentration of AX affect the flow characteristics of microsphere formulations. The results showed that the best flow characteristics were shown by the formulations coded with F5 (having 10% $CaCl_2$

TABLE 4. Effect of type and concentration of cross linker on AX microsphere formulation

Formulation	Cross linker	Conc. of cross linking agent (%)	Polymer conc. (%)	NaOH conc. (%)	Results
1		5	3	5	Amorphous polymer aggregates, only few particles
2	$CaCl_2$	10	3	5	Good stable microspheres
3		15	3	5	Less stable particles
4		5	3	5	No formation of microspheres
5	$BaCl_2$	10	3	5	Colloidal aggregates
6		15	3	5	Less stable microspheres
7		5	3	5	No microspheres formation
8	Al_2SO_4	10	3	5	No microspheres formation
9		15	3	5	Amorphous polymer aggregates

TABLE 5. Effect of concentration of polymer on microsphere formulation

Batch	Polymer conc. (%)	$CaCl_2$ Conc. (%)	NaOH conc. (%)	Temperature (%)	Reaction time (mint)	Results
F1	2	5	5	35	15	Colloidal aggregates
F2	2	10	5	35	15	Distorted microspheres
F3	2	15	5	35	15	Amorphous polymer aggregates, only few particles
F4	2.5	5	5	35	15	Less stable microspheres
F5	2.5	10	5	35	15	Good stable microspheres
F6	2.5	15	5	35	15	Less stable microspheres with distorted shape
F7	3	5	5	35	15	Only few particles
F8	3	10	5	35	15	Best stable microspheres
F9	3	15	5	35	15	Microspheres with distorted shape

TABLE 6. Effect of temperature and reaction time on microsphere formulations

Formulation	Temperature (ºC)	Reaction time (min)	$CaCl_2$ Conc. (%)	Polymer conc. (%)	Results
F1	35	15	10	3	Almost spherical microspheres with good stability
F2	35	30	10	3	Spherical and stable microspheres
F3	60	15	10	3	Best stable and spherical microspheres with best drug loading capacity
F4	60	30	10	3	Almost spherical microspheres but less stable

and 2.5% arabinoxylan) and F8 (having 10% $CaCl_2$ and 3.5% arabinoxylan) as shown in Table 7 which was most probably due to more spherical microspheres as compared to others. As the concentration of arabinoxylan was decreased the flow properties became less efficient due to irregular geometry of microparticles.

Percentage yield

The percentage yield of the tested formulations ranged from 65% to 85%. Formulations F8 and F5 showed the best yield having arabinoxylan 3% and 2.5% respectively and $CaCl_2$(10%) as cross linker. The percentage yield decreased by decreasing the concentration of $CaCl_2$ to 5% because there would be no enough cross linker to form the polymeric framework for the development of AX shell of microspheres.

Swelling index

The swelling index of different formulations of microspheres is shown in Figure 2. The results showed that degree of swelling and ratio of water uptake was less in 0.1 N HCl, as compared with that in phosphate buffer. In phosphate buffer 6.8, erosion and break down of microsphere occur due to maximum water uptake. These

TABLE 7. Flow properties of microspheres

Formulation code	Bulk density (g/mL)	Tapped density (g/mL)	Hausner's ratio	Carr's index Carr's index	Angle of repose
F2	0.279 ± 0.02	0.301 ± 0.03	1.2 ± 0.13	7.8 ± 0.11	37 ± 0.01
F3	0.493 ± 0.01	0.56 ± 0.01	1.12 ± 0.04	10.2 ± 0.12	31 ± 0.02
F4	0.473 ± 0.02	0.567 ± 0.11	1.19 ± 0.03	9.7 ± 0.02	33 ± 0.02
F5	0.681 ± 0.05	0.778 ± 0.02	1.11 ± 0.01	12.4 ± 0.04	28 ± 0.11
F6	0.312 ± 0.04	0.456 ± 0.01	1.29 ± 0.12	7.5 ± 0.02	36 ± 0.12
F7	0.412 ± 0.03	0.527 ± 0.03	1.18 ± 0.04	9.4 ± 0.11	35 ± 0.03
F8	0.689 ± 0.02	0.727 ± 0.01	1.09 ± 0.04	14.7 ± 0.05	26 ± 0.11
F9	0.398 ± 0.03	0.47 ± 0.01	1.29 ± 0.03	7.9 ± 0.01	37 ± 0.03

results suggested that slight swelling of microspheres would occur in stomach as these are subsequently transferred to upper intestine, where Levosulpiride is to be absorbed, the swelling became rapid and hence the release. So the given microspheres would act as the controlled and delayed release dosage form. Maximum swelling was shown by the formulation F8 containing 10% $CaCl_2$ showing swelling index of 1.35 in phosphate buffer 6.8 and very low swelling index in 0.1N HCl.

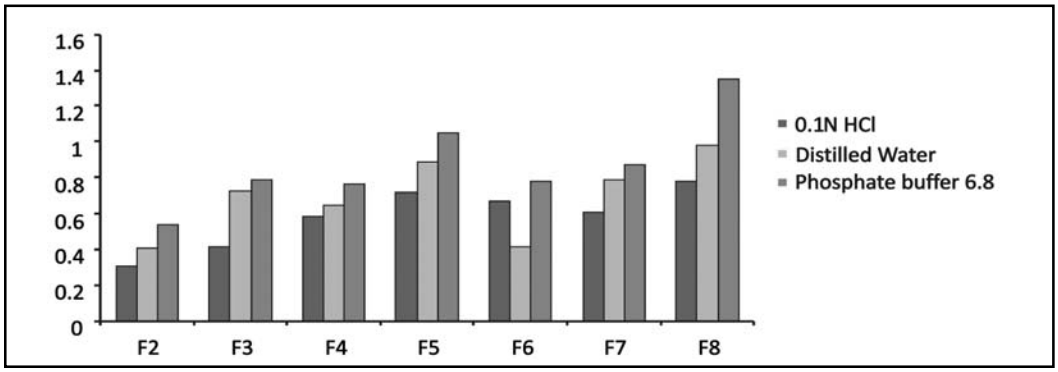

Fig. 1. Swelling index of AX microspheres formulations

Shape and surface morphology

As reported by previous studies it was observed in SEM (Fig. 2) that the shape and surface of microspheres was irregular. Senthil *et al.* [22] reported that even in the pH region where anions interact with AX, irregular particles were obtained in the case of conventional emulsification and inotropic gelation method. The SEM photographs revealed that the hardening after crosslinking was responsible for irregular points on the surface of microspheres. The $CaCl_2$ concentration also affected the shape and size of microspheres. The increasing the amount of cross linker would increase the cross linking density thereby inducing the shrinkage of polymeric gel.

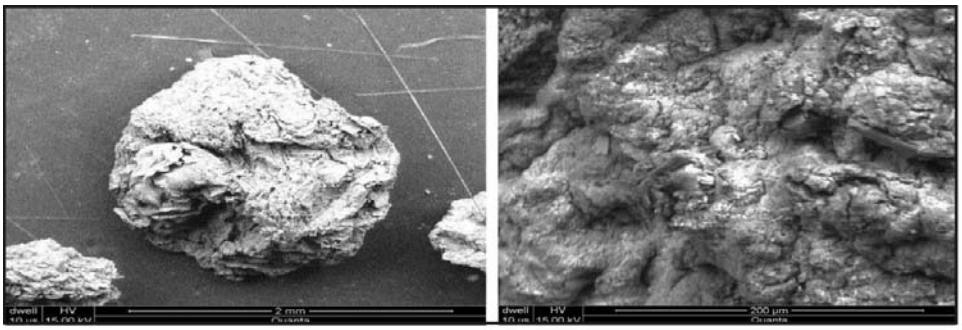

Fig. 2: SEM image of levosulpiride loaded AX microsphere.

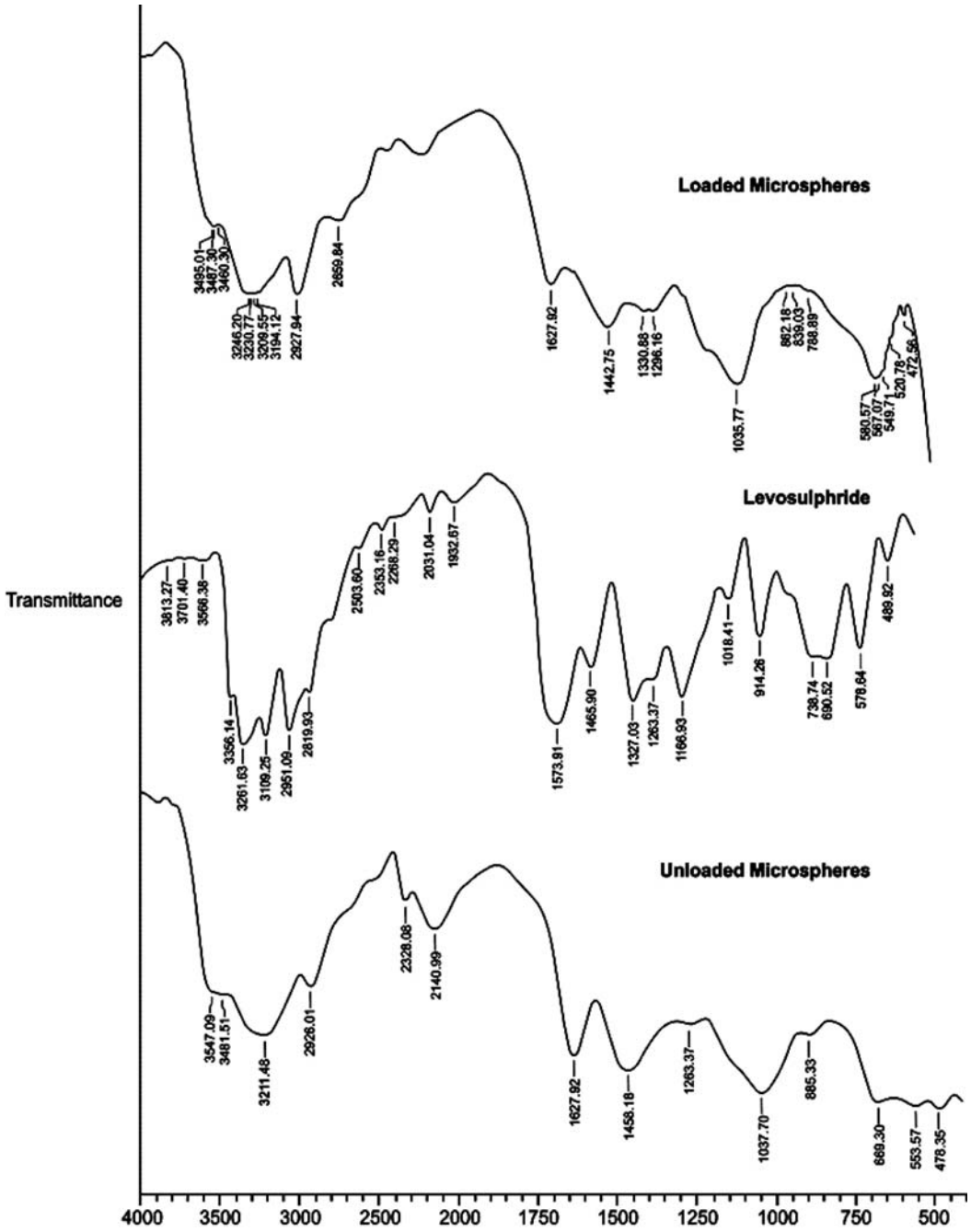

Fig. 3 : Wave number (1/cm)

Fourier transforms infrared spectroscopy

The FTIR spectra recorded for Levosulpiride, drug loaded microspheres and AX unloaded microspheres is shown in Figure. 3. FTIR spectrum of pure drug was compared with the spectrum of drug loaded microspheres. It was observed that the drug have characteristic peaks of groups at 3000 cm^{-1} (due to benzamide group), 1627.3 cm^{-1} (due to carbon-carbon stretching vibrations in the aromatic ring), 1327.3 (due to C–H in-plane bending) and 1013.4 cm^{-1} (due to C=C bending) as major peaks which are also present in drug loaded microspheres. These groups may be pharmacophores of Levosulpiride responsible for showing the therapeutic effect and hence should be remain in intact form after encapsulation in polymeric chains.

The FTIR study revealed no additional peaks and brands observed, the drug and polymer would have no interaction between them. So it was concluded that Levosulpiride was compatible with arabinoxylan which was used as polymer. Also the results ensured the drug presence and identity in the loaded AX microspheres as reported by Manakal *et al.,* 2011 [23].

Drug release studies

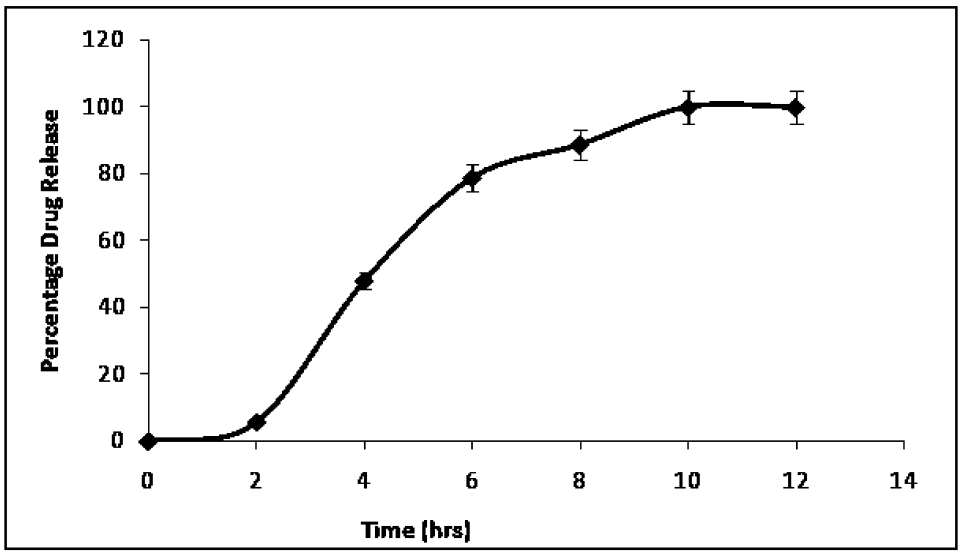

Fig. 4. Dissolution profile of formulation F8

Dissolution profile of formulation F8 (best in term of percentage yield and stability) is shown in Figure 6. Drug release was minor in 0.1 N HCl but it was satisfactory in phosphate buffer 6.8. The release of entrapped drug was up to 12 hours. In phosphate buffer, initial slow release then fast release was observed. The slow release is an imbibition period. As in ispaghula husk, uronic acid content may be cross linked by calcium chloride that may

increase the total cross linked content of the beads[25]. Once the phosphate got imbibed, the gel structure could be destroyed by ion exchange between the gel forming Ca^{++} ions and Na^{+} ions of the dissolution medium. This release of ions creates electrostatic repulsion which causes erosion of polymeric shell causing release of drug. So the results confirmed that the AX microspheres loaded with Levosulpiride behaved as good controlled release dosage form and release the drug in alkaline pH of intestine.

To investigate the drug release kinetics, DD solver software was used in order to study different kinetic models. Korsemeyer-Peppas model was best fit to the formulation as indicated by the values of R^2. Non-fickian transport and super case II transport were followed for the drug release according to this model.

The value of 'n' for the release of Levosulpiride from the formulations studied was higher than 0.5 which suggested the non-fickian diffusion. As reported previously [26], a greater contribution of non-fickian diffusion on Levosulpiride release on increasing the amount of AX was observed which can be considered due to more imbibition of water on increasing the amount of AX polymer. In this case two steps control the drug release process i.e. water entry into the device and then the drug diffusion through the continuously swelling microspheres composed of swollen hydrogel.

CONCLUSION

This study successfully evaluates the use of new biomaterial arabinoxylan in the formulation of microspheres of model drug levosulfiride. However *in vivo* drug release studies further confirm the drug release behavior of micro particles in the body**.**

REFERENCES

1. K.K. Kyekyoon and W.P. Daniel. *BioMEMS and Biomedical Nanotechnology* (2006) 19-50.
2. Y. Man, P.Wang, Y. Guo, L. Xiang, Y. Yang, Y. Qu and L. Deng.. *Biomaterials* 33 (2012): 8802–8811.
3. K.V Neelesh and W.P. Daninel. *Expert opin.Bio.Ther* 4 (2004):35-51.
4. S. S. Kumar, A. K. Saha, K. Kavitha, S. K. Basu. *Der Pharmacia Lettre.* 4 (2012) 33-41
5. P. K. Rout, B. S. Nayakd, *Asian Journal of pharmaceutical and clinical research.* 2 (2009)
6. S. Gupta, G. R. Garg, S. Halder, K. K. Sharma. *Dehli Psychiatry Journal.* 10(2007) 144-146.
7. V.K. Sharma and A. Bhattacharya. *Indian drugs.* 49(2009) :3038-3042
8. D. M. Hariyadi, E. Hendradi, T. Purwanti, C. N. Ramadani. *International Journal of Pharmacy and pharmaceutical sciences.* 6(2014) 469-474
9. E. Alia, A. Sara, S.Uzma, R. Aisha. *J. Polym. Mater.* 33(2016) 759-770.
10. United State Pharmacopeia Chapter (2006). p.616
11. A. Pandey, V.S. Bhadoria. *Pharmacia,* 1(2011) 67-72.
12. K.S. Dada, Y.K. Sudhakar, N.Jayaveera. Research Journal of Pharmaceutical, *Biological and Chemical Sciences,* 2(2011) 489-498
13. N. Mrunalini, S. Parveen and P. Atmara. *Pharma Sci Tech.* 11(2014) 267-27
14. S.K. Mankala, S.K. Nagamalli, R. Rapla. Stamford *Journal of pharmaceutical sciences,* 4(2011) 38-42.
15. A.V. Badrinath, J.R. Reddy, K.M. Rao, C.M. Chetty and K. Gananaprakash . *International Journal* of Chem Tech *Research.* 2 (2010) 361-367

16. S. Shazia , H. Ijaz, K.Andresea and H. Thomas *Carbohydrate polymers.* 74(2010) 309-317.

17. M. S. Iqbal, J. Akbar, M. A. Hussain, S. Saghir, M. Sher. *Carbohydrate Polymers.* 83 (2011) 1218–1225

18. S. S. kumar, A. K. Sahaa, K. kavitha and S. K. Basu. *Der Pharmacia Lettre,* 4 (2012) 33-41

19. K. Neha, M. Ashwani and K.P. Anupam. *IJAP.* 4 (2015) 49-54

20. G.Fundueanu, E. Esposito, D. Mihai , A. Carpov , J. Desbrieres , M. Rinaudo and C. Nastruzzi . *Int. J. Pharm.* 170(1998) 11–21

21. S. B Wang, A. Z Chen, L. J. Weng, M. Y. Chen, and X.L. Xie. Macromolecular bioscience 4(2004) 27-30.

22. S. S. Kumar, A. K. Saha, K. Kavitha, S. K. Basu. *Der Pharmacia Lettre.* 4 (2012) 33-41.

23. S.K. Mankala, S.K. Nagamalli, R. Rapla and R.Kommula R. *Stamford j. pharm. sci.* 4(2011) 38-42.

24. M. Sanchita, K. Senthil, K. Balakrishnam, KB. Sanat. *Brazilian Journal of Pharmaceutical Sciences,* 46(2010) 785-793

25. V.K Sharma and A.Bhattacharya. *Indian drugs.* 46 (2009) 30-38.

26. Sabar, L.Samein and H.B.Sahib. *J. Pharm. Allied Health Sci.* 1(2011) 1–15.

Received: 12-08-2017

Accepted: 12-12-2017