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Evaluation of Venlafaxine Loaded Sodium-Alginate Interpenetrating Network Beads

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ABSTRACT

In this paper an attempt is made to prepare IPN beads of the drug by taking alginate solution in combination with a polymer (PVA/Kennel powder /Guar gum). The prepared copolymer solution is dropped in to 2% $CaCl_2$. The beads are hardened by cross linking with a common cross-linking agent, glutaraldehyde. The beads are characterized by Fourier transform infra-red spectroscopy and scanning electron microscopy. Additionally quality control tests such as swelling index, bead water uptake, entrapment efficiency and drug release studies are performed. The extent of cross-linking is studied in terms of the size and release characteristics of the beads. Most of the formulations indicated erosion based release pattern. The size of the beads ranged from 250 to 400 µm. The entrapment efficiency of the formulation ranged from 73.6% to 94.50%.

Keywords: Sodium alginate, Venlafaxine, Interpenetrating network, dissolution

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INTRODUCTION

Hydrogels are used as materials in the controlled release (CR) of drugs [1-3]. The extraordinary physical characteristics of hydrogels have lead to a special interest in their utility in drug delivery applications. By regulating the cross linking density in gel matrix the highly porous structure of hydro gels can be tuned to an extent[4]. High water content of the materials contributes to their biocompatibility. In the CR applications of drugs and pesticides new polymers are very useful and are also reported[5-12]. Sodium alginate (Na-Alg) is one of such polymer that has been used in CR applications [13-16]. It is a bio-erodible polymer and it form strong gels in aqueous media. Interpenetrating network polymers (IPNs) contain two polymers in a network form; these can be cross-linked in the presence of each other forming a three-dimensional network structure producing free volume for the easy encapsulation of drugs. A report on the preparation of IPNs of Na-Alg with three other biodegradable polymers, such as kennel powder, guar gum (GG) and polyvinyl alcohol (PVA) is focused in this paper. These matrices are used to study the CR of Venlafaxine. It is an antidepressant belonging to the serotonin norepinephrine reuptake inhibitor class. The biological half-life of Venlafaxine (Figure-1) is 4.0 h. By using the new IPNS developed in this research short half life of Venlafaxine can be increased. In earlier studies [17-20], Venlafaxine extended release systems are prepared using matrix type tablet technology. However, to the best of our knowledge, no previous studies have been made on the IPNs of Na-Alg with PVA, kennel powder or GG for the CR of Venlafaxine. The in-vitro release data of Venlafaxine from the IPN beads are presented. Their release characteristics in terms of encapsulation efficiency, polymer morphology, drug diffusion coefficients and the extent and time of cross-linking have been investigated. Physical stability is performed by using a RP-HPLC method that was developed & validated as per ICH guidelines with a slight modification of performing the analysis at 237 nm.

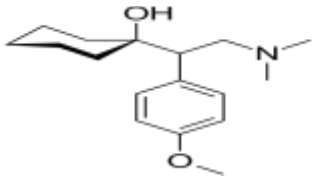

Figure – 1: Structure of Venlafaxine

MATERIALS AND METHOD

Solvents and Chemicals

Venlafaxine Medoximil (purity 99.80 % w/w) is obtained as a gift sample from M/s Vijayasri Organics Pvt Ltd. Methanol HPLC Grade is purchased from Merck (Mumbai, India), Sodium

Alginate, Calcium chloride, GG, and PVA were purchased from Loba Chemie (Mumbai, India), Kernel powder is obtained from a local ayurvedic pharmacy outlet and is of the best grade available. 25% Glutaraldehyde (GA), Orthophosphoric acid are purchased from Merck Ltd (Mumbai, India). Deionized water is processed through a Mille-Q water purification system (Millipore, USA). All other chemicals and reagents are of the highest grade available.

Instrumentation

A Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector constituted Chromatographic system. SCL-10Avp System controller is used to control all the components of the system. LC Solutions software version 1.23 is used in the data acquisition.

Double beam UV-Visible Spectrophotometer (Lab-India Analytical Instruments, India) synchronized to a computer work station using UV-Win software, DS-8000 model; 8-basket dissolution test apparatus(Lab-India Analytical Instruments, India) and magnetic stirrers (REMI Instruments, India) are used in the present study.

Preparation of the beads

The Na-Alg and its IPN beads are prepared by the procedure published earlier [21]. The beads are prepared by taking various ingredients as mentioned in Table-1.

Table – 1: Composition of the IPN beads prepared with alginate

Sr. No	Weight taken in mg					
	4 % Alginate Solution	PVA	Kernel Powder	Guar Gum	2% Cal. Chloride Solution	Drug
F1	25 ml	-	-	-	100	100
F2	25 ml	100	-	-	100	100
F3	25 ml	200	-	-	100	100
F4	25 ml	500	-	-	100	100
F5	25 ml	-	100	-	100	100
F6	25 ml	-	200	-	100	100
F7	25 ml	-	500	-	100	100
F8	25 ml	-	-	100	100	100
F9	25 ml	-	-	200	100	100
F10	25 ml	-	-	500	100	100

Accordingly a 4% Na-Alg solution in distilled water or its IPN with PVA, kennel powder or guar gum is prepared by gentle heating to obtain a pregelation liquid. A weighed amount of Venlafaxine is then added to the pregelation liquid and mixed homogeneously using a magnetic stirrer. The polymer solution containing Venlafaxine is then added drop-wise into methanol containing 1% GA and 1% of 1 N HCl using a 25 ml hypodermic syringe through a needle

(number 21) under constant stirring. Experimental conditions, such as the distance between the syringe and water level, number of drops/min and temperature is maintained constant. The beads thus formed are removed after 10 min; the beads are washed with water and then allowed to dry. When Na-Alg solution is dropped into methanol (non-solvent), the beads are hardened by cross-linking with GA.

Drying rate study of the beads

A known amount of the beads is placed in open glass bottles and kept in an incubator maintained at 37^{o}C. The beads are removed at predetermined intervals of time for up to 12 hrs. These measurements are continued until constant mass indicating the complete equilibration is attained. Using an electronic Citizen microbalance (Model CX220, Japan) all the masses are measured within an accuracy of ±0.01 mg.

Swelling of the beads

The percentage of water uptake by the beads is measured at a pre-selected time interval. The beads are incubated with distilled water on a watch glass. Then, the mass of all the beads is measured using a Citizen microbalance (Model CX220, Japan) with an accuracy of ±0.01 mg and the average value is calculated. In this process, the swollen beads should be handled carefully in order to avoid any mass loss due to breaking or erosion of the beads.

Assay content and entrapment efficiency

A known mass of the beads are incubated with 5 ml of water for complete swelling then are evaluated for venlafaxine content. A homogeneous solution is prepared by crushing the swollen beads with a pestle. The solution is sonicated for 2 min at 60 MHz of frequency. To precipitate Na-Alg, 20 ml of methanol is added and the precipitate formed is removed from methanol by using a high-speed centrifuge (Remi, R24, India) for 5 min at a rotation speed of 5000 rev./min. Venlafaxine is analyzed by a double beam UV-Visible spectrophotometer (UV 3000, Lab India Analytical Instruments, India) at a λmax value of 237 nm. The percentage entrapment efficiency is calculated.

Dissolution/In Vitro release study

Dissolution experiments are conducted at 37 ^{o}C using a dissolution tester (DS 8000, Lab India, Mumbai, India) equipped with 8 paddles at a paddle speed of 50 rev. / min. A 900 ml solution of 0.1N HCl is used as a dissolution medium and a 5 ml aliquot is used for analyzing the Venlafaxine content at a fixed interval of time. Whenever necessary, the samples are diluted before assaying Venlafaxine. The dissolution media is always replenished with a fresh stock solution. The Venlafaxine released is analyzed by a UV spectrophotometer.

Fourier transform infra-red measurements (FTIR)

FTIR measurements are taken at ambient temperature using a Bruker, Model Alpha T. About 10 mg of the samples are grounded thoroughly with KBr and pellets are formed under a hydraulic pressure of 8-10 Tons.

Scanning electron microscopy (SEM)

The sample is deposited on a brass holder and sputtered with gold. The SEM photographs are then taken at the required magnification at room temperature. These experiments are performed on JSM-6610 scanning electron microscope at Advanced Instrumentation Lab, Andhra University, Visakhapatnam, India

Stability of the beads

Stability studies for Venlafaxine beads are carried out as per ICH guidelines [22, 23]. The samples are stored at 25 ^{o}C, 60% relative humidity (RH) and 40 ^{o}C, 70% RH, in stability chambers for a period of 6 months, samples are drawn at regular interval for stability analysis. At the end of 6 months assay is carried out using HPLC-UV method. Chromatographic separations are accomplished using a Grace C_{18}, 3 µm, 50 mm×4.6 mm column. The mobile phase consists of a mixture of 50 parts of Methanol and 50 parts of 0.1 % Orthophosphoric acid. The detector is set at a wavelength of 237 nm. The mixture is filtered through 0.22 µm membrane (Millipore, Bedford, MA, USA) under vacuum and then degassed by using an ultrasonicator for 5 min. The mobile phase is pumped isocratically at a flow rate of 1.0 ml/min during analysis, at ambient temperature. The rinsing solution consists of a mixture of 50:50 % v/v of methanol: HPLC Grade Water. A representative chromatogram of the Venlafaxine is shown in Figure-10. This experiment is done to find out if there is any interaction between the drug and other ingredients of the formulation upon storage.

RESULTS AND DISCUSSION

Morphology &SEM analysis

Surface and cross-sectional morphologies of beads are examined with a Scanning Electron Microscope. Beads are mounted on metal grids using double-sided tape, gold coated under vacuum. The surface and cross-sectional details of the beads are shown in figure- 2-4. The beads are spherical and the average size of the beads ranged from 250-400 µm.

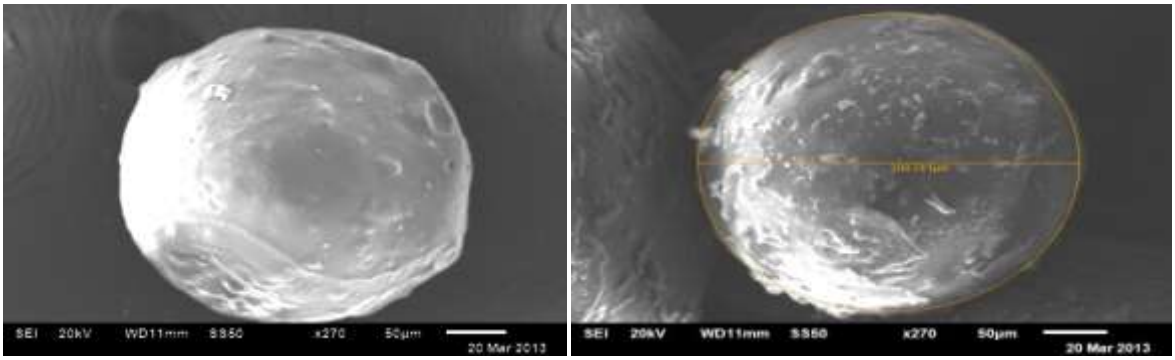

Figure 2: SEM photograph of alginate bead IPN with PVA

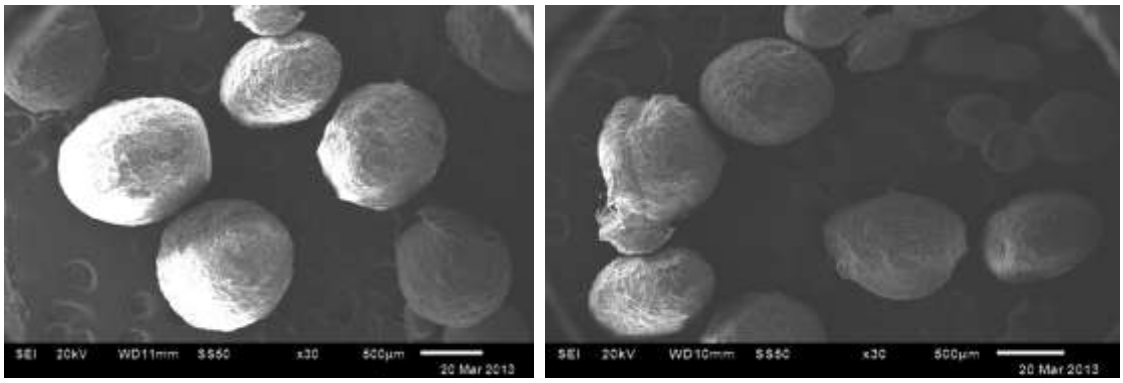

Figure 3& 4 SEM photograph of alginate bead with kennel powder and GG.

Entrapment Efficiency

The drug content is estimated using the double beam UV-Visible spectrophotometer. The results of the entrapment efficiency are demonstrated in Table-2. The entrapment efficiency of the formulation ranged from 73.6% to 94.50%.

Table 2: Percentage Yield, Percentage drug entrapment of Venlaeaxine beads

Sr. No	Formulation Code	% Yield	% Drug Entrapment
1	F1	93.65	87.00
2	F2	92.06	86.60
3	F3	74.21	77.18
4	F4	93.83	90.46
5	F5	94.97	84.86
6	F6	95.63	73.61
7	F7	84.92	94.50
8	F8	93.71	79.32
9	F9	96.74	82.55
10	F10	93.89	83.87

Drying rate of the beads

In comparison to the simple alginate beads, the IPN beads are found to have a longer drying time. Similar results are reported by Sezer and Akbuga[24] with chitosan treated alginate beads. This is attributed to the increased rigidity of the wall polymer[25].

In - vitro drug release studies:

The in-vitro dissolution studies of the formulations are carried out using USP dissolution test apparatus II (paddle method). The paddle of USP dissolution test apparatus II, each containing an amount of beads equivalent to 20 mg Venlafaxine, are rotated at 50 rpm in 900 ml of 0.1N HCl, maintained at 37 °C ± 0.5 °C. An aliquot of 5 ml of the solution is withdrawn at predetermined time intervals and replaced by fresh dissolution medium. The withdrawn samples are analyzed for Venlafaxine content spectrophotometrically at 237 nm. The results expressed are the mean of two experiments.

To study the various patterns of drug dissolution from the beads [26], samples are collected at pre-determined intervals from the dissolution apparatus and tested using the double beam UV-Visible spectrophotometer. The results are tabulated in Table - 3. The following plots are derived from the data obtained for the beads:

Table – 3: % Drug release over time

Time	%Drug release									
(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	6.74	4.91	2.66	2.90	2.16	3.34	1.96	2.90	3.63	2.54
0.5	28.67	10.18	5.63	5.12	3.96	7.15	3.33	4.16	6.68	4.37
0.75	37.35	13.67	7.53	7.82	6.76	9.16	4.71	6.17	9.22	7.00
1	43.14	22.56	9.52	11.86	10.87	11.75	7.64	10.11	12.10	10.72
2	50.09	26.22	14.12	18.61	18.17	18.90	12.09	13.04	34.57	16.26
3	61.24	33.40	23.15	20.07	19.34	23.29	29.01	24.76	38.53	20.22
4	74.13	40.87	24.76	22.85	34.28	27.25	27.69	40.43	42.92	24.61
5	87.01	44.24	26.22	24.76	49.66	36.04	30.03	50.68	47.31	29.01
6	94.25	45.70	30.18	34.28	56.54	33.25	30.32	58.01	54.05	34.57
7		50.68	38.53	39.40	62.40	39.40	44.24	61.52	61.52	38.38
8		68.11	42.92	47.31	68.11	42.92	53.03	67.82	68.70	42.77
9		79.83	53.03	53.47	67.67	51.56	57.71	72.65	72.66	47.75
10		92.43	61.52	61.96	87.01	53.03	72.80	82.47	82.32	53.03
11		96.82	73.09	63.28	90.82	54.49	76.17	85.25	83.64	60.20
12		96.97	74.71	63.57	91.40	55.66	76.61	85.57	83.79	60.35

a. Zero order plot – cumulative % drug released Vs. time. (Figure-5)
b. First order plot – log of cumulative % drug released Vs. time (Figure-6)
c. Higuchi model – cumulative % drug released Vs. square root of time (Figure-7)
d. Erosion model – cube root of fraction unreleased Vs. time (Figure-8)
e. Korsmeyer–Peppas model – log fraction released Vs. log time (Figure-9)

The slope of the best fit line is then plotted in the Korsmeyer–Peppas graph which indicated that the slope is less than 0.5 indicating a fickian type of diffusion for the formulations.

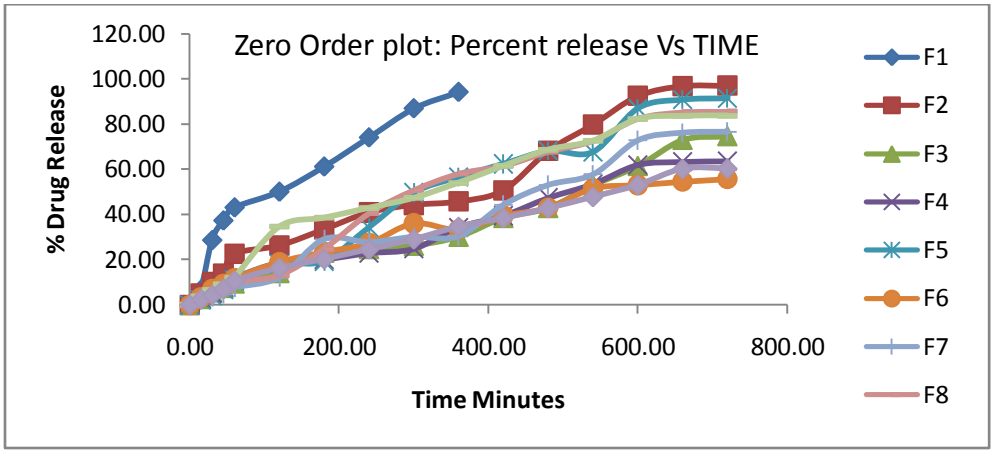

Figure – 5: Zero order plot – cumulative % drug released Vs. time

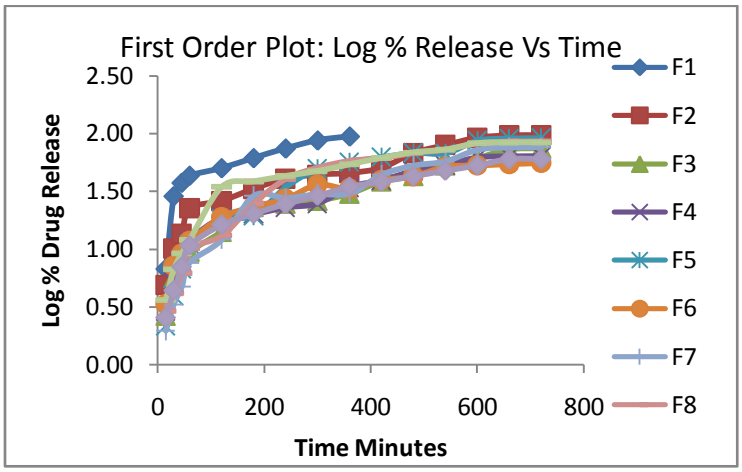

Figure – 6: First order plot – log of cumulative % drug released Vs. time

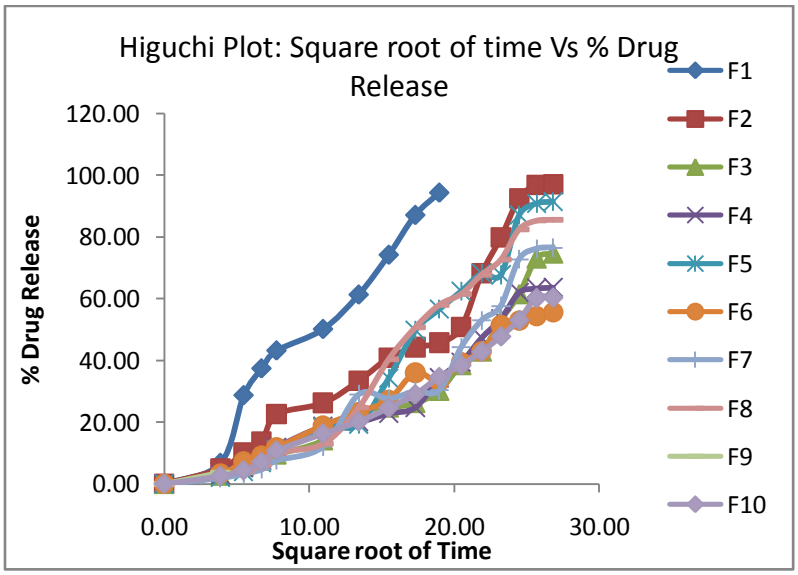

Figure – 7: Higuchi model – cumulative % drug released Vs. square root of time

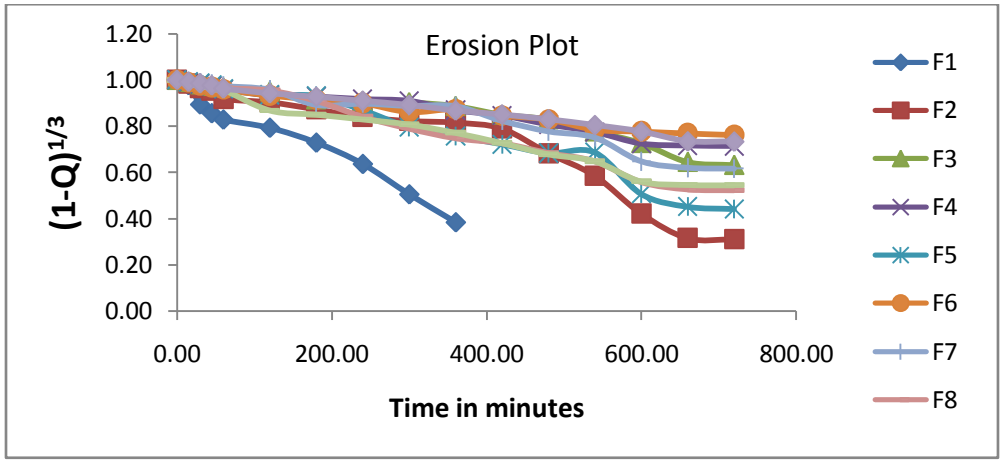

Figure – 8: Erosion model – cube root of fraction unreleased Vs. time

***Q-Fraction released**

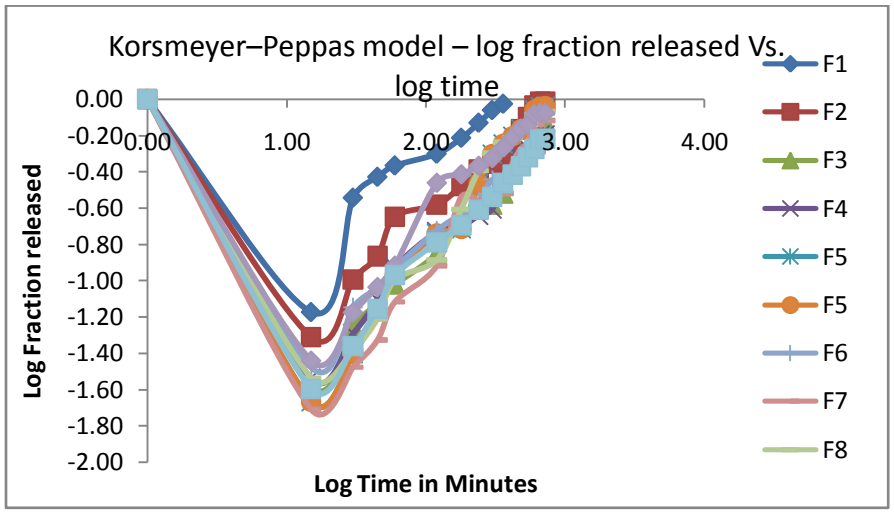

Figure – 9: Korsmeyer–Peppas model – log fraction released Vs. log time

Stability & FTIR studies

To assess the stability, samples are subjected to 6 months exposure as per ICH guidelines. At the end of the evaluation period, all the samples are shown to have good physical stability. To remove the interferences caused by degradation products during quantification, a developed & validated RP-HPLC method is used. A representative chromatogram of the sample is represented in Figure-10. As compared to the reference samples (pure drug alone), the samples showed good assay values ranging from 94.3 – 97.5 % stability.

To study the interaction of the drug with the polymers, a 1:1 ratio of the polymer along with the drug are prepared and subjected to Infrared spectral analysis using KBr pelletization method. By comparative analysis, it can be inferred from the overlap spectrum that drug does not interact with the polymer. FTIR spectrum of venlafaxine HCl showed a characteristic stretching band (Figure-11) of O-H at 3500 cm^{-1}, aromatic C-H stretching at 1609cm^{-1}, C-O stretching at 1500

cm^{-1} and C-N stretching at 1176 cm^{-1} wave number. These characteristic stretching bands are retained after pre-formulation study which revealed no chemical interaction.

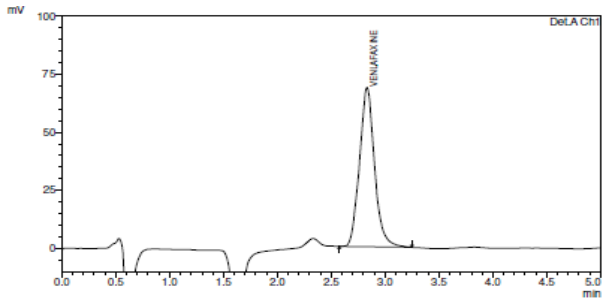

Figure – 10: Representative chromatogram of Venlafaxine.

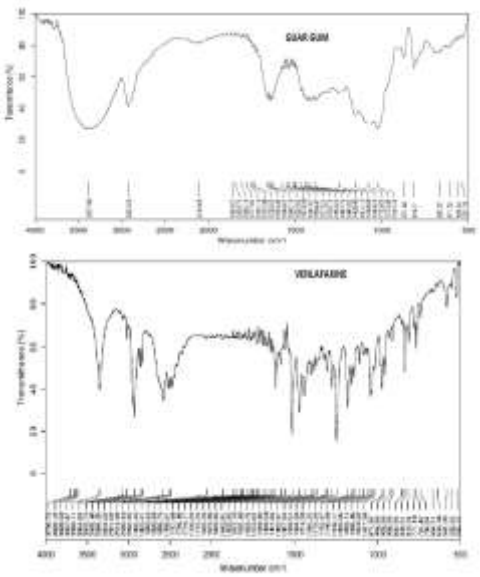

Figure – 11: Overlap IR spectra of Venlafaxine & GGTable-4: Values for coefficient of regression (r^2)

Values of coefficient of Regression											
Release	**Description**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
Zero Order	% Release Vs Time	0.909	0.973	0.978	0.983	0.983	0.966	0.977	0.975	0.957	0.992
1 st Order	Log % Release Vs Time	0.615	0.829	0.869	0.839	0.811	0.792	0.829	0.815	0.747	0.813
Higuchi Model	Sq. Root Time Vs Cumulative % Release	0.974	0.932	0.903	0.938	0.947	0.983	0.917	0.961	0.981	0.967
Erosion Plot	Cube Root of Fraction unreleased Vs Time	0.969	0.904	0.939	0.975	0.965	0.980	0.953	0.991	0.985	0.992
Koresmeyers Peppas Model	Log fraction released vs Log Time	0.062	0.18	0.126	0.119	0.202	0.103	0.162	0.197	0.195	0.118

CONCLUSION

In the literature, beads have been developed to achieve an efficient and site-selective drug delivery [27]. Kim and Lee[28]studied the gel erosion problems associated with Na-Alg beads. In order to circumvent this problem, Murata et al.[29] treated the alginate beads with chitosan and found that chitosan helped to suppress the gel erosion of alginates. Erosion studies on the calcium-induced alginate gel matrix for the release of brilliant blue have also been reported [30].In the present research, egg albumin and gelatin are found to be compatible for forming blends with Na-Alg, which are then cross-linked with GA to give IPNs. Both egg albumin and gelatin are stable, non-antigenic and metabolizable and are capable of encapsulating a wide variety of drugs.GA is also biodegradable and at the same time, it can serve as a good cross-linking agent[31]. Conventionally, micro particles in dosage forms have been prepared by the emulsion/solvent evaporation technique using liquid paraffin as the dispersion medium, followed by rigidization and washing of the micro particles with large volumes of organic solvents [32]. However, the present method avoids the use of any hazardous solvents. The exposure time to GA limits the extent of cross-linking and this was found to be optimized 5±10 min after a series of preliminary experiments. However, increasing the exposure time did not show any significant ($P< 0:01$) effect on particle size and encapsulation efficiency. Polymer swelling and cross-linking are intimately connected to the release kinetics of the drugs from the swollen matrix. A lower cross-link density led to a higher swelling of the matrix, thereby giving a slow release. Thanoo et al.[33] also observed similar effects on the CR of cross-linked chitosan microspheres.

The release rates of Venlafaxine are much faster for the Na-Alg beads than for the IPNs of gelatin or egg albumin. This is due to an increased rigidity of the polymeric beads. Furthermore, the release of Venlafaxine from the kennel & guar gum based IPNs are slower than from the PVA-based IPNs. Diffusion-CR of the drug-loaded beads is intimately related to the molecular transport of drugs through the polymeric matrices. Therefore, in order to understand the type of transport phenomenon, the release data has been analyzed as described earlier [34-35].

New IPNs of Na-Alg with kernel, PVA or guar gum were prepared and used in the CR of venlafaxine. Cross-linking was done using GA, and all the materials used are environmentally friendly. We have demonstrated that it is possible to prepare venlafaxine-loaded beads with high encapsulation efficiencies (up to 94%) and low burst release rates. The method developed is simple, fast and reproducible. From the regression values in Table – 4, it can be inferred that the release predominantly follows an erosion from the surface causing leaching of the drug into the

surrounding fluid.

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