

Formulation and Evaluation of Colon Targeted Drug Delivery System of Levetiracetam using Pectin as Polymeric Carrier

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ABSTRACT

The aim of the present work was to develop and evaluate colon specific sustained release tablet using levetiracetam (LEV), microbially degradable polymeric carrier (pectin), coating material and matrix forming polymers. The colon targeted tablet was prepared by wet granulation technique using different percentage of pectin as matrix carrier, starch mucilage as a binding agent, HPMC K-100 as swellable polymer and coated with Eudragit polymers. Pectin, drug and physical mixture were evaluated for incompatibility study by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). All the batches of matrix tablet (F₁-F₄) were subjected for *in-vitro* dissolution in various simulated gastric fluids for suitability for colon specific drug delivery system. Tablets were evaluated for micromeritic properties of granules, physical properties, drug content, water uptake and erosion characteristics. F₂ was optimized and subjected to coating based on evaluation results. The dissolution study of F₂ revealed, in simulated intestinal fluid (SIF) release was 40.48% at the end of 6h and in simulated colonic fluids (rat caecal content) was 102.88% after degradation at the end of 8h. The colon targeted matrix tablet of LEV showed no change either in physical appearance, drug content or dissolution pattern after performing stability study for 3 months. The studies confirmed that, the designed formulation could be used potentially for colon delivery by controlling drug release in stomach and the small intestine.

INTRODUCTION

Oral ingestion has long been the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, least sterility constraints and flexibility in the design of the dosage form. Hydrophilic polymers are becoming very popular in formulating oral controlled release tablets. As the dissolution medium or biological fluid penetrates the dosage form, the polymer material swells and drug molecules begin to move out of the system by diffusion at a rate determined by the nature and composition of the polymer as well as formulation technology (Sinha *et al.*, 2002). The purpose of designing tablet dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behavior due to unit to unit variation

(Chein *et al.*, 2002), change in gastro luminal pH and enzyme population. Several polysaccharides like, pectin, chondroitin sulphate, amylase, guar gum, xanthan gum and chitosan are being investigated as carriers for colon specific drug delivery. In pharmaceutical formulations, pectin is used as a binder, disintegrant, suspending agent, thickening agent and stabilizing agent (Sarasija *et al.*, 2000).

Pectin and guar gum are reported to be potential carriers for colon specific drug delivery (Kshirsagar *et al.*, 2000). Colon specific drug delivery systems are potential for not only for delivering various drugs to combat the local diseases for colon such as crohn's disease, ulcerative colitis, constipation and colon cancer but also for delivering some drugs for the systematic absorption for treating some diseases such as rheumatoid arthritis, nocturnal asthma, hypertension which possess circadian rhythms in their symptoms (Abrahamsson *et al.*, 1996). There are several strategies being followed for targeting drugs specifically to the colon. Some of them are, pH dependent, time- controlled, prodrug-controlled, microbially triggered drug release (enzyme controlled),

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redox sensitive polymer approach and polysaccharide as carrier. The pH approach has been shown to lack site-specificity because of inter/intra subject variation and the similarity of the pH between the small intestine and the colon. Some of the natural polysaccharides which have already been studied for their potential as colon specific drug carrier systems are chitosan, pectin, chondroitin sulphate, cyclodextrin, dextrans, guar gum, insulin, amylase and bean gum. Approaches being used for new anticonvulsant drugs include the search for agents that block specific cationic channels in neuronal membranes, agents that enhance the activity of the inhibitory neurotransmitter amino butyric acid (GABA), and agents that are capable of inhibiting the activity of the excitatory neurotransmitters glutamic and aspartic acids (Erkoboni *et al.*, 2003). The rationale for the development of polysaccharide based delivery system for colon is the presence of large amounts of polysaccharidases in the human colon as the colon is inhabited by a large number and variety of bacteria which secrete many enzymes e.g. D-glucosidase, D-galactosidase, amylase, pectinase, xylanase, D-sylosidase, dextranase, etc (Ghebre-Sellassie *et al.*). Major approaches utilizing polysaccharides for colon specific delivery are fermentable coating of the drug core, embedding of the drug in biodegradable matrix, formulation of drug saccharine conjugate (Vyas SP *et al.*, 2007). The potential of pectin as carriers for colonic drug delivery has been demonstrated previously (Hiorth *et al.*, 2006). Pectin is heterogeneous polysaccharides composed mainly of galacturonic acid and its methyl ester (Mura *et al.*, 2003). They are refractory to host gastric and intestinal enzymes, but are almost completely degraded by the colonic bacterial enzymes to produce a series of soluble oligogalacturonates. Depending on the plant source and preparation, they contain varying degrees of methyl ester substituent. The degree of methoxylation determines many of their properties, especially solubility and requirements for gelation. High methoxy pectins (HM) are poorly soluble and require a minimum amount of soluble solids (Macfarlane *et al.*, 1990). Levetiracetam, an antiepileptic drug used for the treatment of epilepsy is selected as a model drug. The aim of the present study was to prepare colon targeted tablets of levetiracetam using pectin as matrix polymer that offers protection to the drug until it leaves the stomach which is provided by pH dependent polymer (Singh *et al.*, 2007). Eudragit S 100 and major drug release in small intestine is avoided by providing pH independent coating of Eudragit polymer (Momin *et al.*, 2004). The objective of the present study was to develop a controlled release colon targeted drug delivery system of levetiracetam for the treatment of epilepsy.

MATERIALS AND METHOD

Materials

Levetiracetam was provided as a gift sample by Hikal Ltd, (Hyderabad, India). Pectin (viscosity of 1% aqueous dispersion is 4000 cps, particle size < 50 μ) was obtained from Biodeal Laboratories Pvt. Ltd. (Wadhavan G.I.D.C, India), HPMC and HPC were purchased from Chemdyes Chemicals, Rajkot,

India, Eudragit L 100 and Eudragit S 100 were purchased from Loba chemicals, Mumbai. Other materials used in the study such as microcrystalline cellulose (Avicel PH 101), starch, magnesium stearate and talc were of pharmacopoeial grade. All the other chemicals were of analytical grade. Double distilled water was used throughout the study.

METHOD

Preparation of Levetiracetam and pectin tablet

Step - I

Matrix tablet of Levetiracetam-pectin was prepared by the wet granulation technique using 10% w/v starch paste. The compositions of different matrix tablet formulation used in the study containing LEV are shown in Table 1. Compression was done using pectin as a mucopolysaccharide carrier. Pectin was included in the formulations in various proportions after sieving (sieve no 60) separately and mixed with LEV (sieve no. 100) and HPMC K 100M (sieve no. 60). The powders (F1-F4) were blended and granulated with 10% w/v starch paste. The obtained wet mass was pass through sieve number 16 (mesh size: 1000 μ m) and the granules were dried at 50° C for 2h. The dried granules were pass through sieve no. 25 (mesh size: 650 μ m) and were lubricated with mixture of talk and magnesium stearate in definite proportion. The lubricated granules were compressed using 10 stations Cadmach Mini Rotary Tablet Press (Cadmach Machinery Co Pvt. Ltd) (Liu *et al.*, 2003).

Table 1: Composition of Tablet formulations (F₁ – F₄).

Ingredients	Quantity (mg) present in each tablet			
	F ₁	F ₂	F ₃	F ₄
Levetiracetam	500	500	500	500
Pectin	80	120	160	200
HPMC K100 M	40	30	20	10
Microcrystalline cellulose	50	50	50	50
Lactose	25	25	25	25
Mucilage of starch	40	35	30	25
Magnesium stearate	7	7	7	7
Talc	3	3	3	3

Step – II

The optimized formulation of tablet was coated using a combination of Eudragit L 100 and S100 by using a fluidized bed coating apparatus. Coating solution was prepared by dissolution of 500 mg of Eudragit polymers (L-100 and S-100; 1:1) in ethanol: acetone (2:1) to give 10% coating. PEG 4000 (1% w/v) was used as a plasticizer. Coating solution was applied until there is no drug release in simulated gastric fluid. A 10% w/w increase in the coating level was selected as an optimum coating percentage level (Cheng *et al.*, 2004).

Preformulation studies

Differential scanning calorimetry

The DSC curves of levetiracetam, pectin and physical mixture of levetiracetam and pectin were obtained using differential scanning calorimeter (Perkin Elmer, Japan) at increasing heating rate at 10° C/min and heated over a temperature

range of 50° C to 250° C in an atmosphere of nitrogen (20ml/min). Accurately twelve mg of sample was taken in a hermetically sealed, flat bottom aluminum sealed pan and placed at sample stage and thermograms were recorded.

Fourier transforms Infrared spectroscopy

FT-IR spectra of levetiracetam, pectin and physical mixture of levetiracetam and pectin were recorded at room temperature condition using KBr pellet technique. KBr pellets were prepared by applying a pressure of 5-7 tons. IR spectrum was recorded using Perkin Elmer Spectrum GX FT-IR, measured at the maximum at 4000 cm⁻¹ using methanol as a blank.

Evaluation of granules

Determination of bulk density and tapped density

An accurately weighed quantity of the granules (W), was carefully poured into the graduated cylinder and the volume (V₀) was measured. Then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 100 taps and after that, the volume (V_f) was measured and continued operation till the two consecutive readings were equal. The bulk density, and tapped density were calculated using the formulae (Ashutosh *et al.*, 2008),

$$\text{Bulk density} = W/V_0$$

$$\text{Tapped density} = W/V_f$$

Compressibility index

The compressibility index of the granules was determined by Carr's compressibility index.

$$\text{Carr's index \%} = (V_0 - V_f) / V_0 \times 100$$

Hausner's ratio

Hausner's ratio was measured by the ratio of tapped density to bulk density.

$$\text{Hausner's ratio} = \text{Tapped density} / \text{Bulk density}$$

Angle of repose

Angle of repose was determined using funnel method. The height of the funnel was adjusted in such a way that the tip of funnel just touches the heap of the blends. Accurately weighed blends are allowed to pass through the funnel freely on to the surface. The height and diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$$\tan\theta = h / r$$

Where, θ = Angle of repose, h = height of the pile, r = radius of plane surface occupy by the powder.

Evaluation of tablets

Thickness and hardness

Prepared matrix tablets were evaluated for thickness by using vernier calipers. Hardness of the tablets was evaluated using Monsanto hardness tester, which is expressed in kg/cm² (Fukui *et al.*, 2000).

Friability

Friability of tablets was determined using Roche friabilator. Twenty tablets were weighed and placed in a chamber. The friabilator was operated at 25 rpm for four minutes (per 100 revolutions) and the tablets were subjected and the tablets were subjected for combined effect of abrasion and shock because the plastic chamber carrying the tablets drops them at a distance of six inches with every revolution (Hausner *et al.*, 1967). The tablets were then dusted and reweighed and the percentage of friability was calculated by using the following formula,

$$F = W_i - W_f / W_i \times 100$$

Weight variation

Weight variation test was performed according to USP 2004, twenty tablets were taken and their weight was determined individually and collectively on a digital weighing balance. The percentage deviation was calculated and checked for weight variation (Carr and Hausner *et al.*, 1995).

Drug content

The matrix tablets were tested for their drug content following crushing and powdering five tablets from each batch separately. The amount of powder equivalent to 500 mg of the drug was weighed and dissolved in 100mL of distilled water. After 10 minutes of centrifugation, aliquots of 1mL were taken from this solution and diluted to 100mL with water (10µg/mL). The absorbance of resulting solutions was measured in a UV-spectrophotometer at 210nm. Simultaneously, a 10µg/mL of levetiracetam standard solution was prepared in the same medium and the absorbance was recorded. Drug content was calculated.

Water uptake and erosion study

For conducting water uptake studies, the dissolution jars were marked with the time interval of 0.5, 1, 2, up to 9 h. One tablet was placed in each dissolution jar containing 900 ml of phosphate buffer pH 7.4 at 37°C ± 0.5°C and the apparatus was run at 100 rpm using paddle. The tablets were taken out after completion of the respected stipulated time span as mentioned above and weighed after the excess of water at the surface had been removed with filter paper. The wetted samples were then dried in an oven at 40°C up to constant weight. The increase of the weight on the tablet reflects the weight of the liquid uptake. It was estimated according to following equation.

$$Q = 100 (W_w - W_i) / W_w$$

Where Q is the percentage of the liquid uptake and W_w and W_i are the masses of the hydrated samples before drying and the initial starting dry weight, respectively.

The degree of erosion (expressed as percentage erosion of the polymer content, E) was determined using following equation.

$$E = 100 (W_i - W_f) / W_i$$

Where W_f is the final mass of the same dried and partially eroded sample. The entire process was repeated to get three values for each time point and the average was calculated.

In vitro drug release studies

The release studies of all the matrix tablets were performed using a USP type I dissolution test apparatus (paddle apparatus, 100 rpm, $37 \pm 0.5^\circ\text{C}$) in 900 mL of dissolution medium (SGF). 5 ml samples were withdrawn with pipetting syringe at appropriate time intervals and filtered through whatmann filter paper. Samples were estimated for drug using UV spectrophotometer (Simadzu, 1800) at suitable wave length 210 nm. Sink conditions were adjusted with the addition of an equal volume of fresh dissolution medium at the same temperature throughout the test. The pH of the dissolution medium was kept 1.2 for 2h then, pH of the dissolution medium was adjusted to 7.4 (SIF- simulated intestinal fluid) and maintained up to 24h (Swarbrick *et al.*, 2000).

Preparation of 4% w/v rat caecal content

Albino rats weighing 150 to 200 g were kept on a normal diet and administered 1 ml of 1% w/v solution of the selected polysaccharides in water. This treatment was continued for 7 days to induce the specific enzyme responsible for degradation of polysaccharide *in vivo*. Thirty minutes before the drug release studies began, the anesthetized rats were sacrificed, the rat abdomen was opened, ligatures were made before and after the caecum and the caecum was removed under anaerobic conditions. The caecum bag was opened and its contents were weighed and homogenized, then suspended in phosphate buffer saline (pH 7.4) to give the 4 % concentration of caecal contents. The suspension was centrifuged at 2000 rpm for 10 min at 4°C to disrupt the bacterial cells followed by sonication. The resultant mixture was centrifuged at 2000 rpm for 20 min. Because the caecum's environment is naturally anaerobic, all the operations were performed in a CO₂ atmosphere.

In vitro release study in rat caecal medium

The drug release studies were performed in a slightly modified dissolution apparatus which consist of a 250 ml beaker containing 200 ml of rat caecal content medium was suspended using iron string into the original jars containing water at $37 \pm 0.1^\circ\text{C}$. The tablets were placed in the 200 ml dissolution medium containing 4% w/v rat caecal contents. The studies were performed for 12-24 h, samples were diluted appropriately with phosphate buffer saline (pH 7.4) and centrifuged at 2000 rpm for 10 min. The supernatant was filtered through whatman filter paper, and the filtrate was analysed for drug content using UV spectrophotometer at 210 nm. All experiment was performed in triplicate.

Stability studies and storage condition

The selected formulation of tablets were stored in amber colored glass bottles at $45^\circ\text{C} + 75\% \text{RH}$ for a period of 3 months as per ICH tripartite guideline for stability testing of new drug

substances and product framed by European agency for the evaluation of medicinal products and was observed for any changes in color, odour and percentage drug content and cumulative drug release in various simulated gastric fluids (SGF, SIF and SCF) (Kotwal *et al.*, 2007).

Kinetic modelling of drug release profiles

The drug release kinetic data were subjected to zero order, first order, Higuchi model (Higuchi *et al.*, 1963), Korsmeyer model and Peppas model for analyzing the mechanism of drug release and release kinetics from the dosage form using MS Excel 2007. The model with the highest correlation coefficient was considered to be the best fitting one (Dorozynski *et al.*, 2004).

Zero-order release kinetics

Zero-order release kinetics, cumulative amount of drug released vs time and the release rate data are fitted to the following equation: $C = K_0 \cdot t$

First-order release kinetics

First-order release kinetics, log cumulative percentage of drug remaining Vs time and the release rate data are fitted to the following equation:

$$C = 100 \times (1 - e^{-Kt})$$

Higuchi release model

The Higuchi release, cumulative percentage of drug released vs square root of time and the release rate data are fitted to the following equation: $Q = Kt^{1/2}$

Where, K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time (Hixson *et al.*, 1931).

Mechanism of drug release

To evaluate the mechanism of drug release from pectin tablet, data for the first 80% of drug release were plotted in Korsmeyer *et al.*'s equation, as log cumulative percentage of drug released vs. log time, and the exponent n was calculated through the slope of the straight line. $M_t / M_\infty = Kt^n$

Where M_t/M_∞ is the fractional solute release, t is the release time, K is a kinetic constant characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers (Korsmeyer *et al.*, 1983). For matrix tablets, if the exponent $n = 0.45$, then the drug release mechanism is Fickian diffusion, and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release (Siepmann *et al.*, 2001).

Statistical analysis

The cumulative percentage release of levetiracetam from tablets in different medium was compared and the statistical significance was tested using student's t-test. A value of $p < 0.05$ was considered statistically significant.

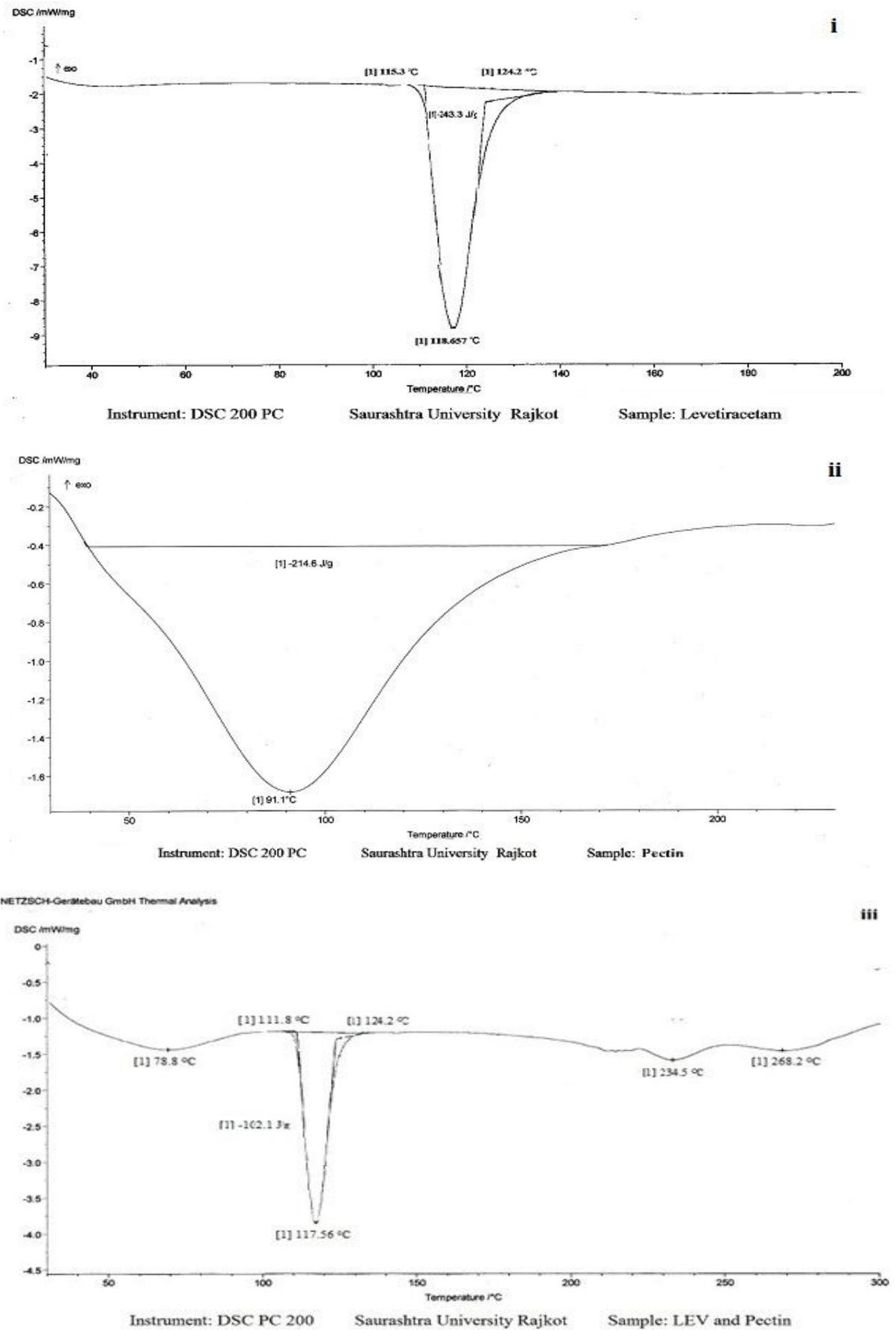


Fig. 1: DSC thermogram of Levetiracetam (i), Pectin (ii) and physical mixture (iii).

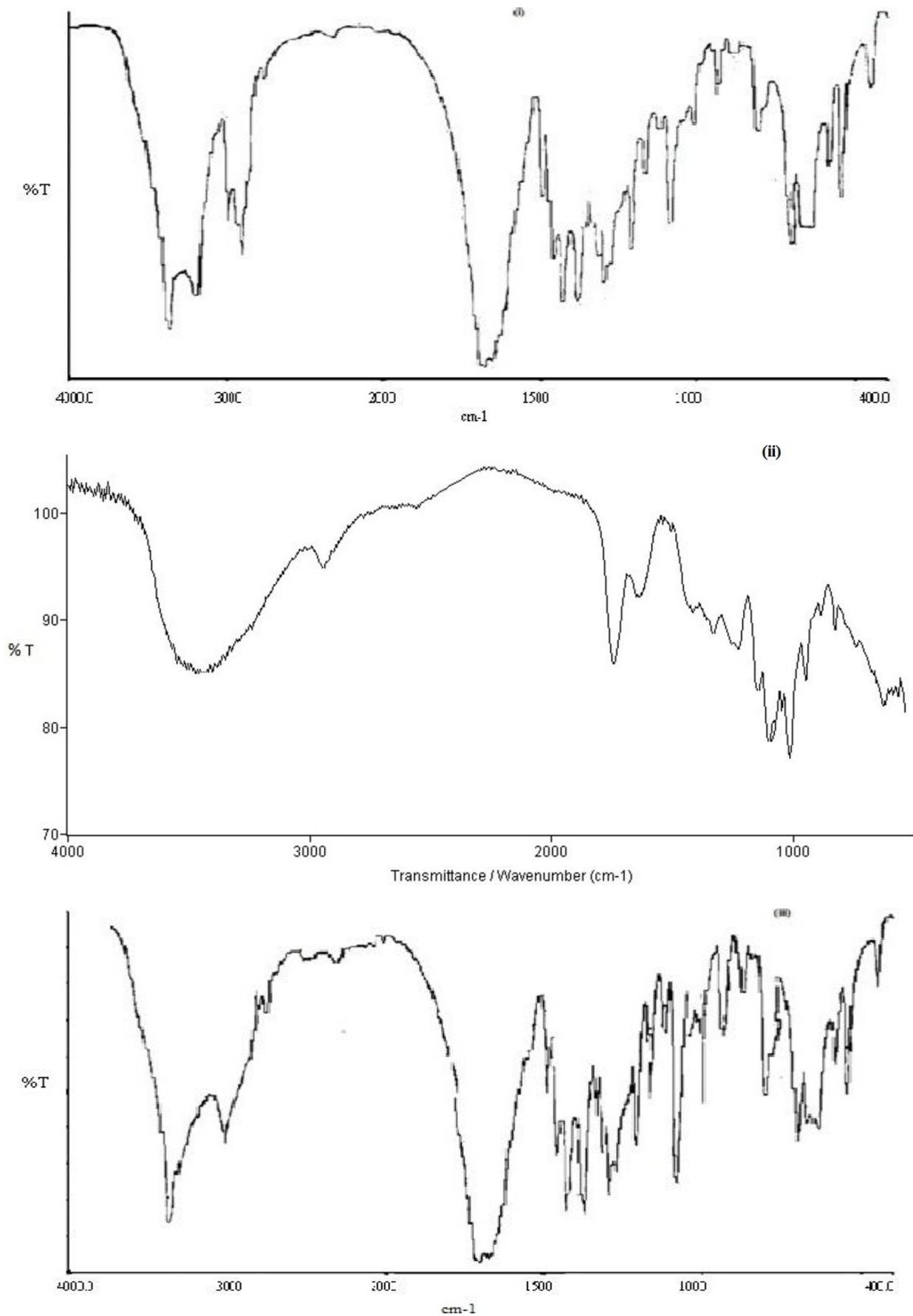


Fig. 2: FT-IR graph of Levetiracetam (i), Pectin (ii) and physical mixture (iii).

Table .2: Physical and micromeritic properties of Levetiracetam pectin tablet.

Test	F ₁	F ₂	F ₃	F ₄
Bulk density	0.539	0.452	0.681	0.759
Tapped density	0.649	0.519	0.742	0.891
Carr's index	18.29	14.51	19.18	20.80
Hausner's ratio	1.20	1.18	1.21	1.22
Angle of repose	17°28'	16°26'	18°58'	19°61'
Thickness (mm)	6.5 ± 0.06	6.5 ± 0.04	6.6 ± 0.03	6.6 ± 0.09
Hardness Kg/cm ²	4.5	4.5	5.0	5.0
Friability (%)	0.69 ± 0.03	0.61 ± 0.03	0.70 ± 0.03	0.67 ± 0.03
Weight variation (mg)	771.1 ± 0.02	745.25 ± 0.01	794.9 ± 0.01	819.9 ± 0.02
Drug content (% w/w)	99.12 ± 1.10	99.18 ± 1.21	99.78 ± 1.51	99.2 ± 0.84

RESULTS AND DISCUSSION

DSC studies

DSC thermogram of Levetiracetam, pectin and mixture are depicted in (Figure 1-a, 1-b and 1-c), respectively. The thermogram of pure drug exhibited a sharp endothermic peak at 118.65° C corresponding to its melting point, while the pectin exhibited a broad endothermic peak at 91.1° C owing to its amorphous nature, while the thermogram of physical mixture of levetiracetam and pectin was 117.5° C. The DSC thermogram of pectin and levetiracetam mixture showed identical peaks corresponding to pure drug indicated the absence of well defined chemical interaction between the drug and the pectin.

IR studies

Drug polymer interaction when studied by FT-IR, showed no drug: excipient interaction. From the FTIR spectral interpretation the following result were obtained. The FTIR of levetiracetam shows the intense band at 3362.7 cm⁻¹, 1678.1 cm⁻¹ and 1491.1 cm⁻¹ corresponding to the functional groups -NH₂, -CONH₂, and methylene C-H bend. The peak observed in FTIR of physical mixture of levetiracetam and pectin was found to be at 3361.5 cm⁻¹, 1678.5 cm⁻¹ and 1491.3 cm⁻¹. From the above interpretation it is understood that there is no major shifting in the frequencies of above said functional groups of levetiracetam was identified which indicates that there is no chemical interaction between levetiracetam and pectin which were used in the formulations. It is given in figure 2 (i, ii, iii).

Micromeritic properties

The micromeritic properties of all the formulations were compared and it was found that F₂ was optimal and within specified limits. The micromeritic properties of various formulations are given in table 2. Different formulations of tablets were formulated using wet granulation and compression for which the granules were subjected to various micromeritic parameters. The optimum value of Carr's index (%) and Hausner's ratio should be upto 15% and 1.20 respectively (Aulton *et al.*, 1988). Values for flow behavior less than or equal to 25 reveals free flowing characteristics of the material. All the formulation possessed good flow properties. Low value of angle of repose, Carr's index and Hausner's ratio (Table 2) revealed good micromeritic behavior of the granules. Since, the flow properties of the powder mixture are important for the uniformity of dose of the tablets; F₂ was found to

be the best among all the tablet formulations due to low Hausner's ratio, Carr's index and angle of repose.

Physical properties

The hardness increased from 4.5 to 5.0 kg/cm², which showed that hardness increased gradually with increasing pectin concentration. The tablets of different formulation showed varied thickness and hardness, 6.5 ± 0.04 to 6.6 ± 0.09 and 4.5 to 5.0, respectively. The friability and weight variation of different tablet formulations were found in compendial limits, i.e. 0.61 ± 0.03 to 0.70 ± 0.03 and 745.25 ± 0.01 to 819.9 ± 0.02 respectively. The drug content was found to be uniform in the different formulations (F₁-F₄). Thus various concentration of pectin did not influence the physical characteristics of the tablets, but swelling behavior and erosion are highly influenced by the pectin concentration.

Percentage swelling and erosion of tablet

The percentage of swelling increased with an increase in the concentration of the polymer as evident from the results which showed that the rate of swelling was 12.11 % (F₁) in 30 min and it increased gradually up to 32.41 % (F₄). The percent swelling increased up to 3rd h for all the formulations and it decreased there after till the end of the study. At the end of 10 h the percentage swelling was 8.02 % for F₁ and it increased up to 24.21% for F₄. The results of percentage swelling are given in figure 3. The percentage erosion was 16.21 % for F₁ at 30 min and it decreased towards the final formulations as it was 2.12% due to increasing concentrations of polymer. The percent erosion was increased with the increase in the time period so that it was 98.33% for F₁ batch at 8th h and likewise it decreases due to increased polymer concentration and was 26.22% for F₄ batch for the same time period. The results for percentage erosion for F₁ to F₄ tablets are given in figure 4. Polymer swelling and erosion studies demonstrated a linear increase of these parameter upto 2h. The tablet prepared with high concentration of pectin showed a lower rate of erosion and a faster rate of swelling, as compared with the tablets containing lower concentration of pectin. This effect may be due to an increase water uptake in the presence of large amount of the pectin and elastic mass formation of tablet. After 4h linear mechanism of water uptake is altered due to the high erosion percentage of the formulations. The percentage erosion was measured as the weight loss from matrix tablets immersed in dissolution media as a function of time.

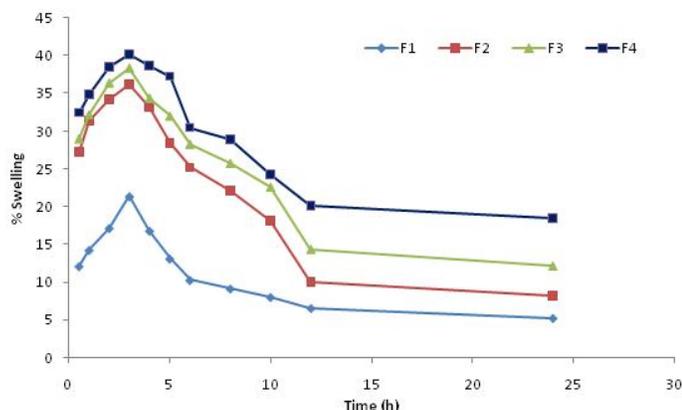


Fig. 3: Percentage swelling of levetiracetam and pectin tablets.

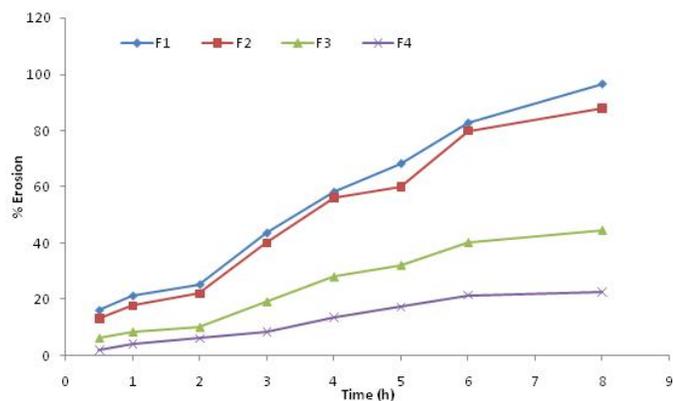


Fig. 4: Percentage erosion of F₁ to F₄ tablets in SCF with enzyme.

***In vitro* drug release studies**

The matrix tablets were subjected to *in vitro* drug release study in SGF, SIF and SCF.

In SGF (pH 1.2 buffer)

The F₁ batch of tablets started release at 15 min and a maximum release of $7.07 \pm 0.88\%$ of the drug at the end of 2 h of the study. This may be due to less percentage of pectin (22%) added in the F₁ batch. The F₂ batch started to release the drug from 15 min onwards. However at the end of 60 min, it released $3.70 \pm 0.99\%$ and at the end of 120 min, the release was only $4.81 \pm 0.81\%$. F₃ batch of tablets started to release the drug at 30 min and at the end of 120 min it was only $2.65 \pm 0.87\%$. While the F₄ batch of tablets had started its release from 45 min and a maximum of $2.31 \pm 0.47\%$ obtained at the end of 120 min of the study. Thus from the above results it has been understood that as the percent of pectin increased from the F₁ to F₄, the percentage of drug release decreased gradually. This is due to the protective effect of added percentage of the pectin which swelled, and formed a barrier for drug to release outside.

In SIF (pH 7.4 buffer) and SCF (pH 7.4 buffer) with 4% w/v of rat caecal enzyme

In SIF, F₁ showed $43.59 \pm 0.46\%$ of drug release at the end of 6th h the tablet remained intact during the dissolution but showed very loose gel like appearance at the end of the 6th h.

However the release percentage was up to $102.22 \pm 0.99\%$ at the end of 10th h in SCF. The amount of drug released from F₂ matrix tablets at the end of 6th h was found to be $35.25 \pm 0.68\%$ in SIF and $82.99 \pm 0.77\%$ at the end of the 6th h in the SCF with enzymes. The release percentage was up to 103.22 ± 0.70 at the end of 24 h in SCF. The percentage of drug released from the F₃ tablets in the SIF was only half to that of F₁ batch and found to be $20.17 \pm 0.66\%$ at the end of 6 h of the study and it was about $49.43 \pm 0.83\%$ in the colonic fluid with enzyme at the end of 6th h. The drug release percentage was up to 80.22 ± 1.00 at the end of 24 h in SCF, where the amount of drug released from the F₄ tablets in the SIF was found to be $14.33 \pm 0.10\%$ at the end of 6th h of the study it was about $34.50 \pm 0.67\%$ in the dissolution medium containing 7.4 buffer with enzyme for the same period. However for F₄ the drug release percentage was up to 76.23 ± 1.21 at the end of 24h. From the above results it is understood that the drug is released upto 6% for F₁ batch and 4.10%, 2.83%, 2% for F₂ F₃ and F₄ batch in SGF at 120 min respectively. From this it is understood that F₃ and F₄ batch are not releasing much amount of drug in the stomach medium. From the dissolution studies conducted for F₁, F₂, F₃ and F₄ in simulated intestinal medium it has been understood that F₁ and F₂ tablets which were containing 11% and 16% of pectin are not protecting the drug release in the stomach media. Upto 43.60 ± 0.47 and $35.25 \pm 0.68\%$ of drug have been released from the F₁ and F₂ the batches at the end of the studies. But the F₃ (20%) and F₄ (25%) batch containing pectin was found to be successful to protect the drug release at the end of 6th h. Thus, in the present study an attempt was done to develop a colon targeted drug delivery system of levetiracetam using pectin as a protective agent to retard the drug release in the stomach and intestinal pH.

In colon, a simulated condition was developed by the addition of 4% w/v of rat caecal contents. In the presence of microbial enzymes, the pectin is degraded as if degradation done by microbial enzymes produced by colonic bacterial flora, and the drug was released. Since the batches F₁ and F₂ are releasing 96.55 ± 0.90 and 83.00 ± 0.78 percentage of the drug in the colonic fluid, they are considered as suitable batches for colon targeting. To prevent the drug release from F₁ and F₂ batches in the stomach and intestinal media, a suitable enteric coating can be given. So that the drug release will be completely prevented in the SGF. Pectin was added in 11, 16, 20 and 25% w/w to tablet weight to get F₁ to F₄ formulations. By observing the above results it was found that, though F₁ and F₂ batches released 7.07 ± 0.88 and $3.70 \pm 0.99\%$ of the drug in simulated stomach fluid and 43.60 ± 0.47 and 35.25 ± 0.68 in the simulated intestinal fluid. They release up to 96.55 ± 0.90 and $83.00 \pm 0.78\%$ at end of the 6th h in the simulated colonic medium. If they were given an enteric coating, the drug release in the stomach and intestine can be completely prevented. So the F₁ batch which released a maximum percent of drug i.e. 96.55% in colonic medium was selected to given eudragit S 100 coating to prevent the drug release in acidic medium and to start release the drug at the ileocaecal junction at the threshold value of pH in dissolution of eudragit S 100 is 7 or above 7.0. The results for F in SIF and SCF were given in figure 5 and 6 respectively.

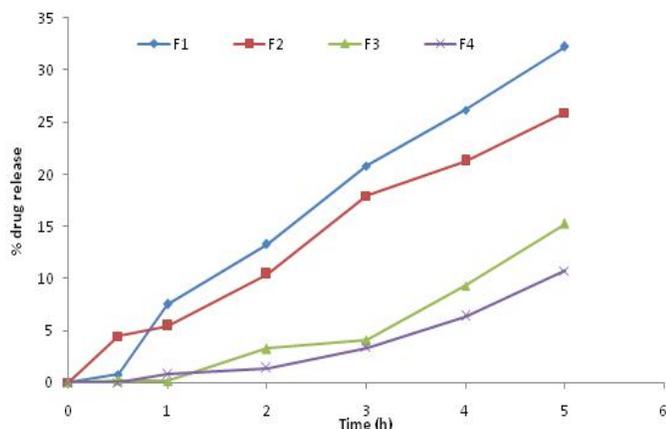


Fig. 5: Cumulative percentage drug release of F1-F4 in SIF.

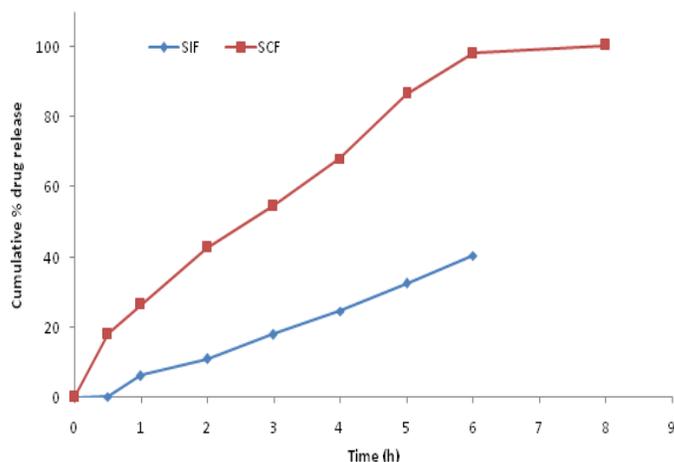


Fig. 7: Cumulative percentage drug release of E-F1 in SIF and SCF.

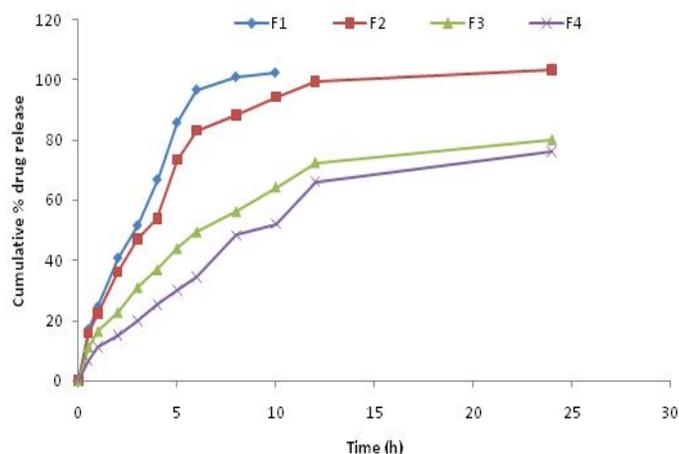


Fig. 6: Cumulative percentage drug release of F1-F4 in SCF.

Step II

Based on physical properties, micromeritic properties, erosion and swelling behavior and *in vitro* drug release characteristics, F₁ was selected as optimized batch and was given pH dependent polymeric coating as described under the general methodology until to get a weight increase of 10 % w/w to the tablet weight and dried and subjected to *in vitro* dissolution studies in SGF, SIF and SCF.

In SIF and SCF

There was no drug release obtained for the pH polymeric coated tablets in SGF, while drug release in SIF was very less. The drug release was found to be significantly higher than in SIF. The release data for percentage drug release in SIF and SCF were given in figure 7. The coating polymers were Eudragit S-100 and Eudragit L-100, dissolves above pH 7.0, thereby protecting the drug release from the core before reaching the colonic region. Once the enteric coating dissolves, it is believed that drug release would then be controlled by pectin in the target area.

In vitro release kinetics

The mechanism of drug release from matrices containing swellable polymers is complex and not completely understood. Some systems may be classified as either purely diffusion or erosion controlled, while most system exhibit a combination of these mechanisms. In this study, drug release kinetics data were evaluated and the optimized for F₁ followed by zero order kinetics and diffusion as mechanism. Further the value of ‘n’ from Korsmeyer-Peppas equation indicated n = 0.6455 (i.e. 0.4 < n < 0.8) indicates the release followed non-fickian diffusion which might be due to the combination of swelling and erosion of the tablets and diffusion of the drug to the dissolution fluid. The results are given in the table 3.

Stability studies

The result of accelerated stability studies, carried out according to ICH guidelines, indicated that there was no significant change in physical parameters (colors, friability and hardness) organoleptic characteristics and percentage drug content, during the study period. Study was performed at a raised temperature of 45°C and 75 % RH for 6 month. The content was found above 97% at the end of 180 days. This indicated that E-F₁ tablet exhibited good physical stability and acceptable potency at accelerated storage condition for 6 months.

CONCLUSION

The prepared tablets met the compendia limits in terms of physiochemical parameters and dissolution studies. HPMC and pectin as mucoadhesive polymer are best suitable in colon targeted drug delivery system to provide necessary drug release of levetiracetam to be absorbed in colon and protect it from SGF and SIF. As a result, colon delivery of levetiracetam appeared to be a promising alternative to traditional drug administration routes.

Table. 3: Release kinetic studies of tablet.

S. No.	Formulation code	Zero order		First order		Higuchi		Korsmeyer Peppas	
		K	R ²	K	R ²	K	R ²	N	R ²
1	Pectin tablets F ₁	22.54	0.9531	1.799	0.7948	87.06	0.9533	0.6455	0.9909

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