FORMULATION AND EVALUATION OF ENTERIC COATING TABLETS BY WET GRANULATION METHOD

B.Shibu[1]*, S. Suresh[1], M.Purushothaman[2], C.Saravanan[2], C.J. Lissy Joice[3]

[1]PGP College of Pharmaceutical Science and Research Institute, Namakkal-637207, Tamilnadu, India.
[2]Aadhi Bhagawan College of Pharmacy, Rantham, Cheyyar-604407, Tamilnadu, India.
[3]Padmavathi College of Pharmacy, Periyanahalli, Dharmapuri – 632205, Tamilnadu, India.

ABSTRACT

Serratiopeptidase is derived from bacteria belonging to genus Serratia. Serratiopeptidase tablets used in the treatment of viral diseases and hepatitis. Serratiopeptidase were formulated using HPMC phthalate as enteric coating polymer in different concentrations to optimize delayed drug release profile and to target the drug release in the small intestine regions. The present work was made to develop enteric coated tablets containing Serratiopeptidase tablets were made by direct compression method. The tablets were evaluated for physical characterization, in vitro release study and stability studies. Results of in vitro release profile indicated that formulation F1 was the most promising formulation as the extent of drug release from this formulation was optimum and match with the In-house Specification when compared to other formulations.

Keywords: Enteric coated tablets, Serratiopeptidase, Hydroxylpropylmethylcellulose, *In-vitro* release studies.

INTRODUCTION

Enteric coatings are those which remain intact in the stomach but will dissolve and release the contents once it reaches the small intestine. Their prime intension is to delay the release or drugs which are inactivated by the stomach contents or may cause nausea or bleeding by irritation of gastric mucosa. Cracking of the film either during application or on storage will result in a loss of enteric properties. Therefore consideration must be given to the mechanical properties of the applied film. Cracking problems can be effectively overcome by plasticization. Plasticizer can also be used to reduce the permeability of the polymer film to water vapor. The choice of suitable plasticizer is restricted to non-water soluble material because these are likely to be most effective.

An evaluation in made of the solubility parameters of species together with an assessment of the intrinsic viscosity of dilute solutions of the polymer on the plasticizers. This determines the maximum interaction between polymer and plasticizer and indicates which plasticizer is likely to be most effective. A general rule to follow is to use 1 part plasticizer to 10 parts polymer one

should also consider viscosity of the plasticizer its influence on the final coating solution its effect on film permeability tackiness flexibility solubility and taste and its toxicity compatibility with other coating solution components and stability of the film and the final coated product [1-4].

The aim and objective of this work is to develop small intestine targeting tablets of Serratiopeptidase [5-8] enteric coated tablets-conventional standard coated technique. The present study is to develop a pharmaceutically stable, cost effective and quality improved formulation of Serratiopeptidase enteric coated tablets.

MATERIALS AND METHODS

Serratiopeptidase was received as a gift sample from the Sun Pharmaceuticals Pvt. Ltd. Various grades of Hydroxylpropylmethylcellulose were obtained as gift from Pushkar Pharma Pvt. Ltd.., Lactose, Maize starch, Methyl paraben sodium, Propile paraben sodium, Magnesium state, Propylene glycol, Isopropyl Alcohol, Iron red oxide

Corresponding Author: - B.S.Shibu Email: - shibubs03@gmail.com

were purchased from Nice Chemicals Pvt. Ltd, Cochin.

Process Flow Chart

```
┌─────────────────────────────────┐
│ Dispensing and reciept of       │
│ raw Materials.                  │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Shifting of Raw Materials.      │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Binding Agent                   │
│ Preperation.                    │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Mixing                          │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Wet Granulation.                │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Drying                          │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Shifting & Milling              │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Blending                        │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Compression                     │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Enteric Coating                 │
└─────────────────────────────────┘
              ↓
┌─────────────────────────────────┐
│ Evaluation                      │
└─────────────────────────────────┘
```
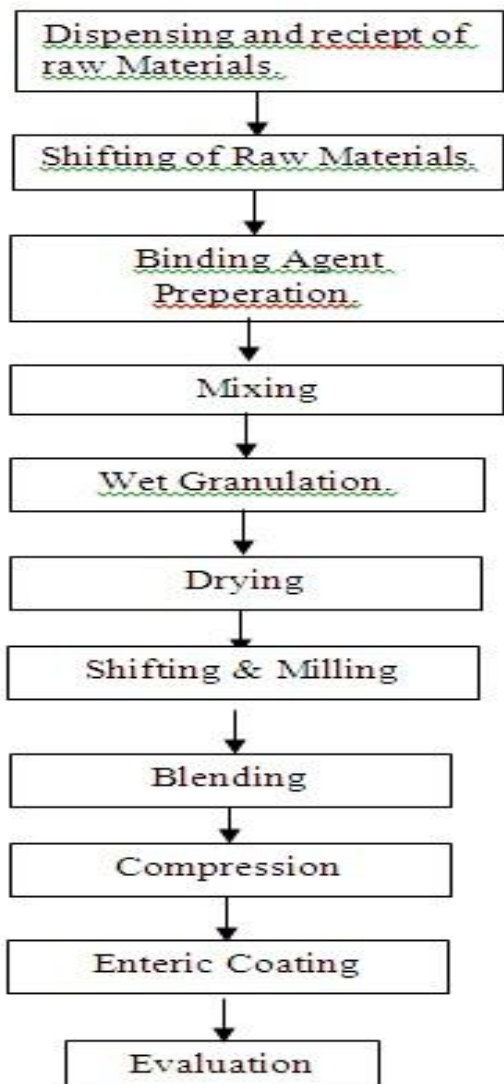
PREFORMULATION STUDIES

Preformulation activities range from supporting discovery's identification of new active agents to characterizing physical properties necessary for the design of dosage form. Critical information provided during preformulation can enhance the rapid and successful introduction of new therapeutics entities for humans. Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage form [9-16].

1. Physical appearance

A small quantity of serratiopeptidase powder was taken in butter paper and viewed in well illuminated place. Finally the colour, odour and texture were observed.

2. Solubility

A semi-quantitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute or vice versa. After each addition, the system vigorously shaken and examined visually for any undissolved solute particles. The solubility was expressed in terms of ratio of solute and solvent.

3. Determination of bulk density and tapped density

It refers to a measurement to describe packing of particles and also used to determine the amount of drug that occupies the volume in mg/ml before tapping and after tapping an accurately weighed quantity of the powder (W), was carefully poured into the graduated cylinder and the volume (Vo) 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 (Vf) was measured and continued operation till the two consecutive readings were equal. The bulk density and tapped density were calculated using the following formula.

$$\textbf{Bulk density} = \textbf{W} / \textbf{V}_o$$
$$\textbf{Tapped density} = \textbf{W} / \textbf{V}_f$$

Where,

W = weight of the powder,
VO = initial volume,
VF = final volume

4. Loss on drying

This is employed in EP, BP and USP. Although, the loss in weight in the samples so tested, principally is due to water, small amount of other volatile materials will a contribute to the weight loss. The moisture balance combines both the drying process and weight recording, it is suitable where large numbers of samples are handled and where a continuous record of loss in weight with time is required. The results were given in Table No: 21.(LIMIT: Not more than 0.5% W/W).

5. Angle of repose

This is the maximum angle possible between the surface pile of powder and horizontal plane. The frictional forces in the lose powder can be measured by angle of repose. The tangent of angle of repose is equal to the co-efficient friction (μ) between the particles. Hence the rougher & more irregular the surface of particles the greater will be angle of repose.

$$\theta = \tan^{-1} h/r$$

Where,

h = height of the pile
r = radius of the pile

6. Compressibility Index

The compressibility index is indirectly related to relative flow rate, cohesiveness and particle size of the

powder. The compressibility index of material can be estimated from the tapped and bulk density of power.
%Compressibility index = [(T.D – B.D) / T.D] x 100
Where T.D and B.D are bulk density and tap density respectively.

7. Moisture Content (Or) Water by Kf
Take around 50ml of methanol in titration vessel of Karl Fischer titrator and titrate with Karl Fischer reagent to end point. In a dry mortar grind the pellets to fine powder .Weigh accurately about 0.5 g of the sample, transfer quickly to the titration vessel, stirr to dissolve and titrate with Karl Fischer reagent to end point.

$$Moisture\ content = \frac{V\ X\ F\ X\ 100}{Weight\ of\ Sample\ in\ Mg}$$

Where,
F= factor of Karl Fischer reagent.
V= volume in ml of Karl Fischer reagent consumed for sample titration.

8. Assay
Standard preparation
Standard serratiopeptdise 100mg into 50ml volumetric flask. Make up the volume with 0.1 NaoH. From this solution take 25ml into 50ml volumetric flask and make up the volume with 5N Hcl.

Sample Preparation
100mg serratiopeptdise into 50ml volumetric flask and make up the volume with 0.1N NaoH. Take 25ml into 50ml volumetric flask and make up the volume with 5N Hcl.

Procedure
Take 10ml sample and standard to 50ml volumetric flask + 5ml 20% NaoH cool on ice for 10min + 5ml 5% Na2co3 + 2ml folins reagent(folins reagent dilution 1ml to 10ml with water).Measure the obsorbants of both the solutions at 660nm.

Calculation:

$$A = \frac{A_T}{A} \times \frac{W_S}{100} \times \frac{2}{100} \times \frac{100}{W_T} \times \frac{100}{2} \times P$$

AT = Absorbance of the sample preparation.
AS = Absorbance of the standard preparation.
WS = Weight of the standard taken in mg
WT = Weight of the sample taken in mg
P = Purity of the standard

EVALUATION OF TABLETS
To design tablets and later monitor tablet production quality, quantitative evaluation and assessment of tablet chemical, physical and bioavailability properties must be made.

The important parameters in the evaluation of tablets can be divided into physical and chemical parameters [9-16].

Physical parameters
General appearance
The general appearance of tablets its visual identity and over all elegance is essential for consumer acceptance. The control of general appearance of tablet involves measurement of number of attributes such as tablet size, shape, color presence or absence of odor, taste, surface texture and consistency of any identification marks.

Tablet size and thickness
Control of physical dimensions of the tablets such as size and thickness is essential for consumer acceptance and tablet-tablet uniformity. The diameter size and punch size of tablets depends on the die and punches selected for making the tablets. The thickness of tablet is measured by Vernier Calipers scale. The thickness of the tablet related to the tablet hardness and can be used an initial control parameter. Tablet thickness should be controlled within a ±5%. In addition thickness must be controlled to facilitate packaging.

Average weight of Tablets
Take randomly 20 tablets and weigh accurately 20 tablets and calculate the average weight.

$$Average\ weight = \frac{Weight\ of\ 20\ tablets}{20}$$

Weight variation test
It is desirable that all the tablets of a particular batch should be uniform in weight. If any weight variation is there, that should fall within the prescribed limits:

±10% for tablets weighing 130mg or less
±7.5% for tablets weighing 130mg-324mg
±5% for tablets weighing more than 324mg

The test is considered correct if not more than two tablets fall outside this range. If 20 tablets are taken for the test and not more than 1 tablet fall outside this range if only 10 tablets are taken for the test. The difference of weight in tablets can lead to variation in doses. For carrying out this test 20 tablets at random are taken and weighed. The weights of individual tablets are then compared to equal to average weight.

Friability
This test is performed to evaluate the ability to withstand abrasion in packing, handling and transporting. Initial weight of 20 tablets is taken and these are placed in the friabilator, rotating at 25rpm for 4min. the difference in

the weight is noted and expressed as %. It should be preferably between 0.5 to 1.0%.

Dissolution by UV VISIBLE SPECTROSCOPY
Dissolution Parameters
Type of apparatus: U.S.P. Type II (paddle)
Medium: 0.1N HCL for 2Hrs,Phosphate buffer pH 6.8 for 45 min.
RPM: 100
Volume of Medium:900mL
Sampling intervals:10min,20min,30min,45min,60min
Sampling volume : 10mL
Method of analysis: UV Spectrometric
Wave length:660nm.

Procedure
The *In-vitro* dissolution study was carried out with the USP dissolution test apparatus.900ml of dissolution medium (6.8 phosphate buffer) was taken in covered vessel and the temperature was maintain at 37 ± 0.5^{o}C.The speed of the paddle was set at 100rpm.Sampling was done every 10min interval. For each sample 10ml of dissolution medium was withdrawn and the same amount of dissolution medium at 37°C was replaced. The sample withdrawn was filtered with what man filter paper and diluted with 6.8 phosphate buffer and then analyzed in the UV-spectrophotometer. The absorbance was measured at 660nm and percentage drug release was calculated.

Hardness test
This is to force required to break a tablet in diametric compression. Hard ness of the tablet is determined by Stock's Monsanto hardness tester which consists of a barrel with a compressible spring. The pointer moving along the gauze in the barrel which the tablet fractures. Hardness of 5 kg considered as suitable for handing the tablet.

Disintegration test
For most tablets the first important step toward solution is break down of tablet into smaller particles or granules, a process known as disintegration. This is one of the important quality control tests for disintegrating type tablets. Six tablets are tested for disintegration time using USP XXII apparatus. Disintegration type sustained release tablets are tested for disintegrating time.

RESULTS AND DISCUSSION
Coating parameters
Pan Rpm: 11-12
Inlet temperature:50-60°C
Outlet temperature:45-55°C
Air pressure:3-4kg/sq.cm.

Preformulation Characteristics of All Formulations
The value of precompression parameters were found to be within the prescribed limits and indicated good free flowing property and the results were given in the table.

Chemical Evaluation
The assay of all formulations is found to be between 90% - 114%.

Standard graph of Serratiopeptidase
Series of dilutions are made from standard working solution with 6.8 pH phosphate buffer to get concentrations ranges from 10 -60mcg and the absorbance was measured at 660nm and the values are listed below.

Physical Evaluation
Postcompressional parameters
The data obtained of postcompressional parameters such as weight variation, thickness, friability and hardness, disintegration time were shown in the table. Hardness was found to be the range of NLT 3.0kg/cm^{2} in all the formulations indicating good mechanical strength with an ability to withstand physical and mechanical stress conditions were handling. In all the formulations the friability values NMT 1.0% giving an indication that the tablets formulated are mechanically stable. All the tablets passed weight variation test at the percentage weight variation was within the I.P limits. All the tablets passed disintegration test NMT 15min as per I.P limits.

Dissolution Studies of All Formulations
By comparing the all formulations F1 formulation showed better percentage drug release.

Table 1. Formulation table

S.No	INGREDIENTS (kgs)	F1	F2	F3	F4	F5	F6
	Dummy Granules						
1.	Lactose	16.10	16.10	16.10	16.10	16.10	16.10
2.	Maize Starch	4.21	4.21	4.21	4.21	4.21	4.21
3.	Methyl Paraben Sodium	0.03	0.03	0.03	0.03	0.03	0.03
4.	Propyl Paraben Sodium	0.01	0.01	0.01	0.01	0.01	0.01
5.	Purified Water(Lit)	5.50	5.50	5.50	5.50	5.50	5.50
6.	Serratiopeptidase	2.00	2.00	4.00	6.00	4.00	2.00
7.	Magnesium Stearate	0.35	0.35	0.35	0.35	0.35	0.35

	Sub Coating Materials						
8.	HPMC 15cps	0.45	0.45	0.45	0.45	0.45	0.45
9.	Propylene Glycol	0.12	0.12	0.12	0.12	0.12	0.12
10.	Methylene Chloride (Lit)	7.80	7.80	7.80	7.80	7.80	7.80

Table 2. Enteric coating material

S.No	INGREDIENTS (kgs)	F1	F2	F3	F4	F5	F6
11.	HPMC Pthalate	1.83	3.66	1.83	3.66	5.49	5.49
12.	Cetyl Alcohol	0.12	0.24	0.12	0.24	0.36	0.36
13.	Diethyl Pthalate	0.035	0.070	0.035	0.070	0.105	0.105
14.	Iron Red Oxide(gm)	22.3	22.3	22.3	22.3	22.3	22.3
15.	Titanium Dioxide	0.035	0.070	0.035	0.070	0.105	0.105
16.	Acetone (lit)	27.0	27.0	27.0	27.0	27.0	27.0
17.	Isopropyl (lit) Alcohol	6	6	6	6	6	6

Table 3. Precompressional parameters

S.No	Formulations	Angle of Repose (º)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Compressibility Index (%)	Moisture Content (%)
1	F1	25.4	0.55	0.69	20.0	2.1
2	F2	26.4	0.53	0.67	21.0	2.2
3	F3	28.2	0.57	0.59	23.0	2.4
4	F4	28.4	0.55	0.64	19.0	2.2
5	F5	23.2	0.53	0.62	23.0	2.1
6	F6	29.6	0.56	0.68	26.0	2.4

Table 4. Assay of formulations F1 to F6

Physical parameter	F1	F2	F3	F4	F5	F6
Assay% (w/w)	114	99.03	96	95.01	96	92

Table 5. Absorbance of Serratiopeptidase

Concentration (µg/ml)	Absorbance
10	0.041
20	0.083
30	0.124
40	0.166
50	0.206
60	0.245

Table 6. Postcompressional parameters

S.NO	Physical Parameters	F1	F2	F3	F4	F5	F6
1	Weight Variation (mg)	0.12	0.11	0.12	0.13	0.13	0.11
2	Hardness (kg/cm^2)	3.2	3.1	3.4	3.2	3.5	3.2
3	Thickness (mm)	3.1	3.3	3.3	3.2	3.5	3.1
4	Friability (%)	0.25	0.32	0.22	0.45	0.35	0.47
5	Disintegration Time	4m20s	6m20s	3m20s	8m32s	10m40s	11m23s

Table 7. Specification of Serratiopeptidase

S. No	Tests	Results	Limits
1	Description	Off white powder	Off white to light brown color
2	Loss on drying	5.88%	NMT 7.00w/w
3	Heavy metal	<50ppm	NMT 50ppm
4	Arsenic	< 5ppm	NMT 5ppm
5	Enzyme activity	2.463u/mg	2, 200u/mg to 2, 600u/mg

Table 8. Dissolution studies of F1 to F6

S.No	Dissolution time (min)	F1	F2	F3	F4	F5	F6
1	10	32.71	21.73	39.29	37.10	19.54	5.98
2	20	52.27	33.79	54.93	49.66	25.89	19.00
3	30	70.79	49.64	76.06	62.88	39.08	29.20
4	45	89.30	68.49	84.96	81.37	47.09	45.06
5	60	94.66	78.76	93.93	91.98	57.60	54.98
6	90	99.94	91.97	95.70	97.38	70.81	67.87

Figure 1. Standard graph of Serratiopeptidase
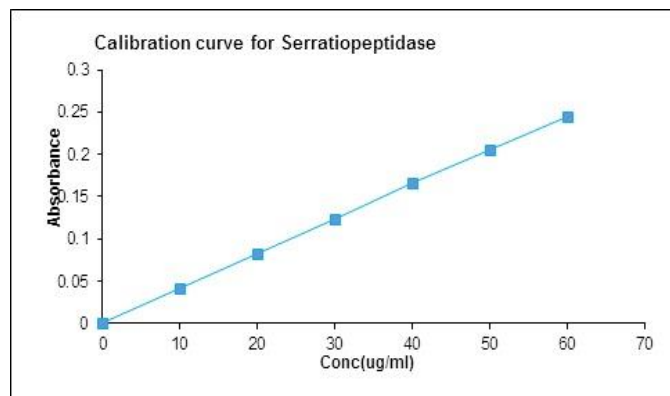
Figure 2. Comparative *Invitro* Dissolution profile of formulations
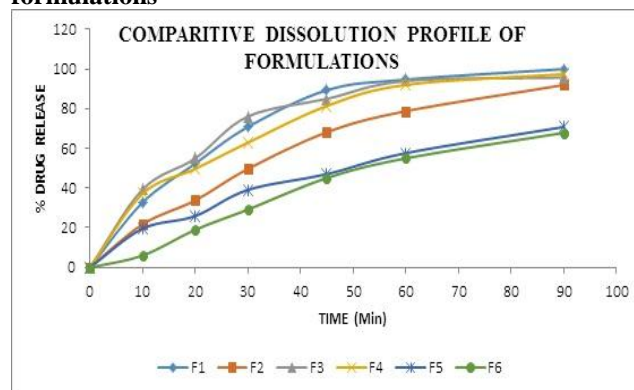
SUMMARY AND CONCLUSION

Serratiopeptidase is derived from bacteria belonging to genus Serratia. Serratiopeptidase tablets used in the treatment of viral diseases and hepatitis. In this study Serratiopeptidase enteric coated tablets were prepared by using HPMC phthalate, HPMC 15cps (Polymer).

Several formulations were made with varying the concentrations of drug polymer and enteric coating tablets were done by wet granulation method. They were tested for normal quality control tests like disintegration, weight variation, hardness and friability. The drug release study is carried out for 2hrs in 0.1N HCl and followed with 1hr in 6.8 phosphate buffer.

The present work of Serratiopeptidase was formulated as delayed release tablet which significantly increase the small intestinal absorption and the drug was targeted to small intestinal regions. This was achieved by enteric coating of tablets by simple standard pan coating method.

Serratiopeptidase were formulated using HPMC phthalate as enteric coating polymer in different concentrations to optimize delayed drug release profile and to target the drug release in the small intestine regions. The present work was made to develop enteric coated tablets containing Serratiopeptidase tablets were made by direct compression method.

While concluding the best formulation among the six F1 formulation shows 99.94% drug release than the all other formulations.

REFERENCES

1. Liberman, lachman and Joseph, Tablet in pharmaceutical dosage form, 2nd edn, 2, 2005,201-339.
2. M.E.Aulton. Tablet in the science of dosage form design, 2nd edn, 397-460.
3. Remington. The science and practice of pharmacy, 20th edition, 1, B.I publications Pvt.Ltd, Noida, 2000, 903-929.
4. Aulton ME. Pharmaceutics- The sciences of dosages form design, 3rd edition, international student Edition, Churchill Living stone, 2007, 419-421.
5. Manish maheshwari. Development of tetracycline serratiopeptidase-containing gel: Formulation and preliminary clinical studies. *AAPS pharm sci Tech*, 2006, 76.
6. T.H. Al khateeb. Effect of the proteolytic enzyme serrapeptase on swelling pain and trismus after surgical extraction of mandibular third molar, *International Journal of Oral and Maxillofacial Surgery*, 37, 2008, 264-265.
7. Deependra singha, serratiopeptidase-loaded PLA microspheres using selected variables, *Journal PDA* (pharmaceutical science and technology), 2009.
8. K.V.Sandya. liposomal formulation of Serratiopeptidase – *in vitro* studies using PAMPA and CACO – to model, Molecular pharmaceutics book, 2008, 92-97.

9. Sumit chakraborty. Formulation development and evaluation of Pantoprazole enteric coated tablet, *International Journal of chem tech Research*, 1, 2009, 663-2666.

10. Murat – turkoglu. Tableting and stability evaluation of enteric coated omeprazole pellets, *European Journal of pharmaceutics and Biopharmaceutics,* 2004, 279-286.

11. J.Michael, Enteric coated pellets: theoretical analysis of effect of dispersion in the stomach on blood level profiles, 1976.

12. Singh, C., Kumar, R., Agarwal, K., Nema, R.K.Development and Evaluation of Enteric Coated Tablet Containing Diclofenac Sodium. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 2(1), 2009,443-445.

13. Nair, A., Gupta, R., Jacob, S. Formulation and evaluation of enteric coated tablets of proton pump inhibitor. *Journal of Basic and Clinical Pharmacy*, 2010, 215-221.

14. Kim, Sang Min et al an enteric, sustained-release tablet comprising paroxetine or a hydrates United States Patent Application 20080292696.

15. Chen, Chih Deshmukh, Abhijit Mukund -ming an enteric coated diltiazem dosage form United States Patent 5830503.

16. Deshmukh, Abhijit Mukund an enteric formulation of Fluoxetin United States Patent Application 20040170688.