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Research Article

POLYMER CROSS LINKED SILK PROTEIN AS A PHARMACEUTICAL EXCIPIENT

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ABSTRACT
Silk proteins are natural proteins in silk cocoons. Silk cocoons contain 25% - 30% of sericin, remaining is constituted by fibroin. The present study aims to extract the sericin and cross link it with suitable polymers. And study the influence of cross linked sericin on tablet properties. Sericin was extracted and was cross linked with Gelatin, Gurgum, PVP, Acrylamide. Cross linked sericin was evaluated for binding property of tablets. It was found that sericin loses its binding efficiency by cross linking it with selected polymer. Presence of cross linked sericin in tablet formulation did not influence the dissolution of either the water soluble drug (propranolol HCl) or water insoluble drug (indomethacin). However sericin cross linked with acrylamide retarded drug release. It may be due to formation of Inter Penetrating Polymer Network (IPN). Cross linked sericin could not satisfy the requirements of pharmaceutical excipient for oral solid dosages form. Cross linked sericin with acrylamide exhibited swelling property can further explored for topical controlled release.

INTRODUCTION
Excipients are the additives to convert pharmacologically active compounds into pharmaceutical dosage form suitable for administration to patients. New and improved excipients will continue to be developed to meet the needs of conventional drug delivery systems, and to meet the needs of advancing tablet manufacturing technology. A number of newer excipients are polymeric. It is predicted that the majority of future excipients will be polymers, due to their ability to produce a wide range of materials and properties according to molecular structural alterations[1-4]. The majority of these polymers and polymer derivatives are of natural origin. Biopolymers or natural polymers are an attractive class of biodegradable polymers since they are: Derived from natural sources, easily available, relatively cheap, Qualified for a number of chemical modifications, Biodegradable. A majority of investigations of natural polymers in drug delivery systems have centered on proteins and polysaccharides. Silk proteins are natural polymers and are biodegradable with reactive functional groups that open up possibility to be cross-linked with other polymers to be used in controlled delivery[6-9]. Cross linked gelatin has been used for pH sensitive drug delivery system. Earlier study on sericin reveals that it can be used as binding agent, comparable with conventional binding agent starch. The present study is to investigate the modification of sericin and its subsequent use as a pharmaceutical excipient. Modification of sericin can be done by various cross-linking method like: Cross linking by radical polymerization, Cross linking by Chemical Reaction of Complimentary Group, Cross linking with aldehydes, Cross linking by addition reactions, Cross linking by condensation reactions, Cross linking by High Energy Radiation, Cross linking using enzymes[11, 12].
Polymerization: Polymerization occurs through a series of chemical reactions by which the macromolecule or the polymer is formed from a large number of molecules known as monomers.
The polymer is made up of one or several simple, recurring, structural units, consisting of the individual monomer structure. These monomer units are connected to each other along the polymer chain by covalent bonds. Polymerization is a repetitive intermolecular reaction that is functionally capable of proceeding indefinitely. Because any chemical compound possessing a molecular weight in excess of

5000 is considered to be macromolecules, most polymer molecules can be described as macromolecules. In some instances, the molecular weight of the polymer molecule can be as high as 50 million. Silk cocoons are made up of two proteins sericin and fibroin. Sericin constitutes 25-30% of silk proteins, remaining is constituted by fibroin. Sericin binds together fibroin or silk threads in silk cocoons, showing that it has the property of binding[13]. Both sericin and fibroin have been recently explored in the field of drug delivery systems. Using Fibroin controlled release tablets, gels and micro spheres have been prepared. Further, fibroin has been explored as a biomaterial for various applications. Sericin gels and Nanoparticles have been prepared. Silk proteins are natural polymers and are biodegradable with reactive functional groups that open up possibility to be cross-linked with other polymers.

MATERIALS AND METHODS

Propranolol HCl was taken from ZydusCadila, Bangalore. Sericin & Fibroin was Prepared In House. Glutaraldehyde was purchased from Rolex chemical industry, Mumbai.. Acrylamide was purchased from S.D Fine chemicals Mumbai. Guargum & Gelatin was purchased from Loba chemie, Mumbai. All other materials were of analytical grade.

Method of cross linking

Cross linking with aldehydes is one of the most commonly used methods and is being used for the present study. Earlier study on sericin revealed that sericin is retarding the drug release from Micro particles and Matrix granules. Based on this Propranolol HCl (Water soluble) was chosen for the present study. Drug release studies, from the tablet dosage forms containing various cross linked sericin, were carried out.

Cross Linking of Sericin with Acrylamide

Acrylamide (10mM) was added to 20 ml of 2% w/v sercin solution containing potassium persulfate (1mM) and methylene bisacrylamide (0.4mM). Solution was mixed for 2 hrs. 1% glutaraldehyde solution was added. Polymer obtained is washed with water and dried at room temperature.

Cross linking of Sericin with different Polymers-Gelatin, Guargum, and PolyVinyl Pyrolidone

Sericin solution was added to polymer solutions in varying amounts. Mixture was stirred at 50 rpm for 2 hour. 1% glutaraldehyde solution was added and further stirred for 1 hour. Polymer is washed with water and dried at room temperature. (As shown in table 1)

EVALUATION OF CROSS LINKED SERICIN AS A BINDER

FT-IR analysis

The prepared cross- linked product of sericin were subjected to FT-IR analysis by KBr pellet method using Fourier- transform Infrared (FT-IR) spectrophotometer, Perkin Elmer (spectrum-100), Japan. This was employed to ascertain the confirmation of cross- linking of sericin with polymer.

Preparation of binder solutions

Aqueous solutions of sericin, SAC, SGU, SGE, SPVP, PVP, Guargum, Gelatin in the concentrations of 5% w/w were prepared with the aid of heat.

Formulations of tablets

Tablets were prepared by wet granulation technique. All the ingredients were passed through sieve no 40 weighed and mixed in a polybag. To this the binder solution was added, mixed well. Wet dough was passed through sieve. No. 16 and granules were dried for 30minutes at 50^{0} C. Granules were weighed and calculated amount of Talc and Magnesium stearate were added and mixed well. The granules were punched to tablets using ten station automatic tablet punching machine. (Shown in Table. 2)

EVALUATION

Weight variation

Twenty tablets were randomly picked up and weighed individually and the average weight was calculated. The individual weights were then compared with the average weight. The tablets pass the test if not more than two tablets fall outside the percentage limits and none of the tablets differ by more than the double the percentage limit given below. Average weight of tablets was above 260 mg. The percentage deviation allowed was ±5%.

$$\% \text{ deviation} = \frac{\text{Difference between average weight and individual tablet weight x100}}{\text{Average tablet weight}}$$

Hardness uniformity test

The hardness of tablets was measured by using Monsanto hardness tester. The lower plunger was placed in contact between the tablet and the upper plunger was forced against a spring by turning a threaded bolt until the tablet fractures and the force for the fracture was recorded. The results were expressed in Kg/cm^2.

Thickness uniformity test

The thickness of the formulations was measured by vernier calipers. The results were expressed in mm

Friability

Twenty tablets were accurately weighed and placed in the Roche friabilator, and operated for 100 revolutions. The tablets were then dedusted and reweighed. The tablets that loose less than 0.5% to 1.0% weight were considered to be acceptable.

$$\% \text{ Friability} = [1 - w/w_0] \times 100$$

Where, w_0 = Initial weight

w = Final weight of tablet

Disintegration test

Disintegration assembly consists of six test tubes. Six tablets were taken, and introduced into each test tube. Disc was placed in each test tube. Assembly was suspended in the beaker containing 0.1 N HCl. Maintaining temperature at 37^{0} C $\pm$ 2^{0} C Apparatus was operated for specified time. Assembly was removed from the beaker. The tablets pass the test if all of them will disintegrate in specified time.

The following are specifications for the tablets.

Uncoated tablets	Film coated tablets	Other coated tablets
Up to 15 minutes	Up to 30 minutes	Up to 60 minutes

Influence of cross linked sericin on dissolution of drug

Formulation of Propranolol HCl tablets containing cross linked sericin

Tablets were prepared by wet granulation technique. All the ingredients were passed through sieve no 40, weighed and mixed in a polybag. To this the binder solution was added, mixed well. Wet dough was passed through sieve. No. 16 and granules were dried for 30minutes at 50^{0} C. Granules were weighed and calculated amount of Talc and Magnesium stearate were added and mixed well. The granules were punched to tablets using ten station automatic tablet punching machine. Result was shown in Table 3.

Evaluation

Content uniformity test

Five tablets were accurately weighed and powdered in mortar using pestle until uniform powder was obtained. The powder equivalent to 100mg of Propranolol HCl was transferred to 100ml volumetric flask and it was dissolved, solution was made up to 100ml with 0.1NHCl. Solution was suitably diluted and absorbance was measured at λ_{max} of 291nm. Drug content is expressed in mg.

***In vitro* dissolution studies**

In vitro dissolution studies were carried out using Veego tablet dissolution test apparatus (USP XXII, apparatus type-II). 900 ml of 0.1NHCl was used as a dissolution medium, paddle was rotated at 50 rpm with bath temperature being 37 ± 0.5^{0}C.The studies were carried out for 1 hour. Aliquots were withdrawn at regular interval of time with the help of gurded pipette. The same volume was replaced with 0.1N HCl. The absorbance of these samples was measured atλ_{max} 319nm..

RESULTS AND DISCUSSION

Extraction of sericin

Extraction procedure for sericin was developed and optimized. Ethanol was used to precipitate sericin, % yield was found to increase with the increase in the volume of ethanol (95%). Maximum yield was found to be with 10% (v/v) ethanol (95%). (Shown in Table 4)

Extraction of fibroin

Fibroin was extracted by Ajisawa's method of dissolution. (Shown in Table 5)

FT-IR analysis

FT-IR Analysis by KBr pellet method was carried out for sericin, Gelatin, Guargum, PVP and acrylamide in their pure state and cross linked with sericin, The FT-IR spectra obtained are given in Figures (Fig. 1).

Cross linked sericin as a binder

Nine tablets formulation containing Propranolol hydrochloride were prepared using each of sericin-OD, potato starch, Gelatin, Guargum. PVP, SAC, SGU, SGE, SPVP as binders. The binding efficiency of Cross linked sericin was compared with conventional binding agent like Gelatin, Guargum, PVP, and starch. Tablets were evaluated for its various other tablet property. (Results are shown in Table 6.

Content uniformity of Proparnolol HCl in formulations F1-F6

Content uniformity studies were carried out in 0.1 NHCl. The data are given in (Table 7) the results obtained showed that the drug content in all the formulations were within I.P limit.

***In-vitro* dissolution test**

The results of *in vitro* dissolution data of formulations F1 – F6 shows that formulation F3 is retarding the drug release Dissolution study was extended for 8 hours for Formulation (F-3), results are shown in Fig.2 & 3.

DISCUSSION

The precipitate of sericin obtained using ethanol took 3 to 5 days for drying, prone for microbial and fungal contamination causing sericin to decompose. The duration of drying was reduced to 16 hours for oven drying. In FT-IR study showed that At 2867 cm^{-1} new peak appear, which indicate unconjugation. i.e C=C=C, Peaks at 2524 cm^{-1}(S-H group), 2426 cm^{-1} (C=C), and 2191 $cm^{-1}$are missing from acrylamide., Peaks at 1885 cm^{-1} (C=O group) & 1922 cm^{-1}, 1280 cm^{-1}(C-O or C-C or C-N) are missing from acrylamide, 1545 cm^{-1} (C=C or C=N or N-H) new peaks found on conjugation., Peak at 1241 cm^{-1}, 1403 cm^{-1}(O-H or C-H or C-C) are missing from sericin on conjugation This was employed to ascertain the confirmation of cross- linking of sericin with polymer. The above result showed that there were no interaction between the drug and other exipieants.

Results show that the hardness of tablet has decreased with cross linked sericin compared to conventional binding agent. The hardness of tablet formulations varies in the following order with respect to the binders, Potatostarch >Gelatin > Guargum >PVP >SAC >SGU >SGE >SPVP > sericin. Friability was more in case of formulation prepared using SPVP and less with SGE. Disintegration time is more for tablet containing gelatin as a binder and less for tablet containing sericin as a binder. Results indicate that sericin is losing its binding property after cross linking

Content uniformity studies showed that all the formulations were within I.P limit. So that drug was equally distributed in all the formulations.

The results of *in-vitro* dissolution data of all the formulations showed that formulation F3 is retarding the drug release Dissolution study was extended for 8 hours for Formulation (F-3), which indicated that this method can be used for extended release formulation.

CONCLUSION

Method of extraction of sericin from cocoons was developed and optimized for higher yield. Fibroin was extracted from degummed silk by Ajisawa's method of dissolution of fibroin. Sericin was cross linked by aldehyde method. Cross linking was confirmed by FT-IR study. Evaluation of cross linked sericin as a binder showed that cross linked sericin loses its binding property. Presence of cross linked sericin in tablet dosage form has not influenced the dissolution of water soluble drug (Propranolol HCl). Drug release from cross linked sericin with acrylamide is retarding due to formation of Inter Penetrating Network (IPN) by acrylamide and sericin.

Present study reveals that cross linked sericin fail to satisfy the properties of conventional pharmaceutical excipient for solid oral dosage form. Sericin cross linked with acrylamide showed swelling properties cam further be explored for topical control release.

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REFERENCES

1. Hennink WE, Nostrum CF: Novel crosslinking methods to design hydrogels. Adv, Drug Deliv. Review. 2002.54:13-36.
2. Masanobu N, Rie O, Yasuo G: Structures and Physical Properties of Cross-Linked Sericin Membranes. J of insect Bio. and Sericology. 2001.70:149-153
3. Mingzhong LI, Silk-based biomaterials. Biomaterials,. 2003: 24, 357-365.
4. Moy RL, Lee A, Zalka A. Commonly used suture materials in skin surgery. *Am. Fam. Physician* 1991, *44*: 2123–2128.
5. Altman GH, Diaz F, Jakuba C, Calabro T, Horan, RL. Silk-based biomaterials. *Biomaterials* 2003, *24*: 401–416.
6. Tanaka K, Kajiyama N, Ishikura K, Waga S, Mizuno S. Determination of the site of disulfide linkage between heavy and light chains of silk fibroin produced by *Bombyx mori*. *BBA Protein Struct. M* 1999, *1432*: 92–103.
7. Zhou CZ, Confalonieri F, Medina N, Zivanovic Y, Esnault, C, Yang, T. *et al.* Fine organization of Bombyx mori fibroin heavy chain gene. *Nucleic Acids Res.* 2000, *28*: 2413–2419.
8. Simmons A, Ray E, Jelinski LW. Solid-state C-13 NMR of *Nephila-Clavipes* dragline silk establishes structure and identity of crystalline regions. *Macromolecules* 1994, *27*, 5235–5237.
9. Simmons AH, Michal CA, Jelinski LW. Molecular orientation and two-component nature of the crystalline fraction of spider dragline silk. *Science* 1996, *271*: 84–87.
10. Vollrath, F, Knight, DP. Liquid crystalline spinning of spider silk. *Nature* 2001, *410*, 541–548.
11. Vollrath, F. Strength and structure of spiders' silks. *J. Biotechnol.* 2000, *74*: 67–83.
12. Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC, Kaplan DL. Silk matrix for tissue engineered anterior cruciate ligaments. *Biomaterials* 2002, *23*: 4131–4141.
13. Freed LE, Vunjaknovakovic G, Biron RJ, Eagles DB, Lesnoy, DC. Biodegradable polymer scaffolds for tissue engineering. *Biotechnology* 1994, *12:* 689–693.

Table 1: Concentration of sericin and polymers used for cross linking

	SGE	SGU	SPV	SAC
Sericin	2%	2%	2%	2%
Gelatin	2%	-	-	-
Guargum	-	2%	-	-
Pvp	-	-	2%	-
Acrylamide	-	-	-	10(Mm)
Glutaraldehyde	1%	1%	1%	1%
Potassium persulfate	-	-	-	1(mM)
Methylene biacrylamide	-	-	-	0.4(mM)

SGE- Sericin cross linked with gelatin, SGU – Sericin cross linked with guargum, SPV – Sericin cross linked with PVP, SAC - Sericin cross linked with Acrylamide

Table 2: Formulation of Propranolol HCl tablets containing varying binders

INGREDIENTS	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Propranolol HCl	20mg	20mg	20mg	20mg	20mg	20mg	20mg	20mg	20mg
Potato starch (5%)	-	-	-	-	-	-	-	-	12.5mg
Sericin(5%)	12.5mg	-	-	-	-	-	-	-	-
SAC (5%)	-	12.5mg	-	-	-	-	-	-	-
SGU (5%)	-	-	12.5mg	-	-	-	-	-	-
SGE (5%)	-	-	-	12.5mg	-	-	-	-	-
SPV (5%)	-	-	-	-	12.5mg	-	-	-	-
PVP (5%)	-	-	-	-	-	12.5mg	-	-	-
Guargum(5%)	-	-	-	-	-	-	12.5mg	-	-
Gelatin (5%)	-	-	-	-	-	-	-	12.5mg	-
Potato starch (8%intragranular)	20mg	20mg	20mg	20mg	20mg	20mg	20mg	20mg	20mg
Talc (4%)	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg
Mag.stearate (1%)	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg
Lactose	185mg	185mg	185mg	185mg	185mg	185mg	185mg	185mg	185mg

F1 –Sericin, F2- SAC, F3- SGU, F4- SGE, F5- SPVP, F6- PVP, F7- Guargum, F8- Gelatin, F9 – Starch

Table 3: Formulation of tablets containing various Cross linked Sericin

INGREDIENTS	F-1	F-2	F-3	F-4	F-5	F-6
Propranolol HCl	20mg	20mg	20mg	20mg	20mg	20mg
Potato starch (5%)	12.5mg	12.5mg	12.5mg	12.5mg	12.5mg	12.5mg
Sericin(25%)	-	62.5mg	-	-	-	-
SAC (25%)	-	-	62.5mg	-	-	-
SGU (25%)		-	-	62.5mg	-	-
SGE (25%)		-	-	-	62.5mg	-
SPV (25%)		-	-	-	-	62.5mg
Potato starch (8%intragranular)	20mg	20mg	20mg	20mg	20mg	20mg
Talc (4%)	10mg	10mg	10mg	10mg	10mg	10mg
Mag.stearate (1%)	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg	2.5mg
Lactose	185mg	122.5mg	122.5mg	122.5mg	122.5mg	122.5mg

Starch is used as binder

F1- Conventional Tablet, F2 – Sericin as a Polymer, F3- SAC as a Polymer
F4- SGU as a Polymer, F5- SGE as a Polymer, F6- SPVP as a Polymer

Table 4: Percentage yield of sericin

Quantity of cocoon shells used (g)	Method of drying	Amount of % sericin extracted (g) (Mean ± SD)*
100	At 40^0c	14.75 ± 0.25

* Standard deviation n = 3

Table 5: Percentage yield of Fibroin

Quantity of degummed silk used (g)	Amount of Fibroin extracted (g) (Mean ± SD)*	%
10	8 ± 0.5	80

* Standard deviation n = 3

Table 6: Various tablet property using sericin, cross linked sericin and conventional binding agent

	% Weight variation	Hardness	Friability	D.T	Thickness	Diameter
F1	-5.73 - 4.43	2kg/cm^2	0.3600	0.5mins	9mm	2.4mm
F2	-5.73 - 4.91	4kg/cm^2	0.161	4 mins	9mm	2.5mm
F3	-4.87 - 6.91	0.161	0.161	4mins	9mm	2.6mm
F4	-6.04 - 1.2	3kg/cm	0.080	8mins	9mm	2.6mm
F5	-6.17 - 5.34	2kg/cm^2	0.620	4mins	9mm	2.5mm
F6	-4.87 - 4.06	5kg/cm^2	0.451	6mins	9mm	2.6mm
F7	-6.17 - 5.76	8kg/cm^2	0.080	5mins	9mm	2.5mm
F8	-7.17 - 3.18	8kg/cm^2	0.211	10mins	9mm	2.5mm
F9	-5.17 - 4.76	5.34kg/cm^2	0.84	3.6mins	9mm	2.5mm

F1 – Sericin as a binder, F2- SAC as a binder, F3- SGU as a binder, F4- SGE as a binder
F5- SPVP as a binder, F6- PVP as a binder, F7- Guargum as a binder, F8- Gelatin as a binder,F9- Starch as a binder

Table 7: Content uniformity data of F1- F6 formulations of Propranolol HCl

Formulation No	Content uniformity (mg)			Mean±SD*
	Trial -I	Trial –II	Trial -III	
F-1	18	19	19	18.66±0.57
F-2	18	19	18	18.33±0.57
F-3	17	19	17	17.66±1.15
F-4	18	19	19	18.66±0.57
F-5	17	17	18	17.33±0.57
F-6	19	18	18	18.33±0.57

* Standard deviation n = 3

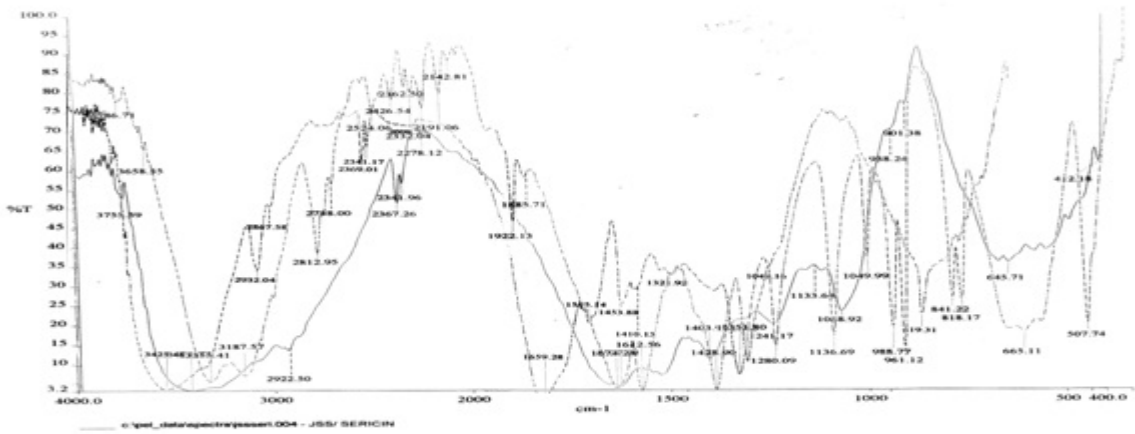


Fig 1: Comparison of FTIR Spectra of sercin, polymers & cross linked sercin to confirm cross linking

Fig 2: : Dissolution data of Propranolol HCl in different Formulation

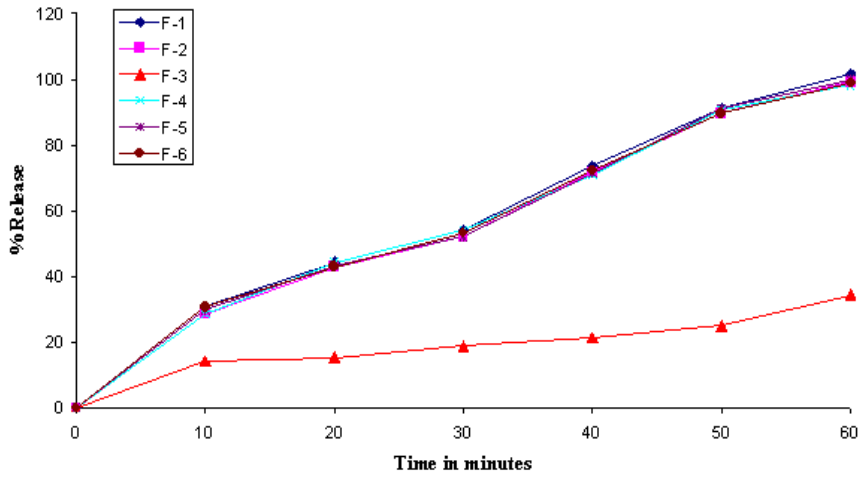
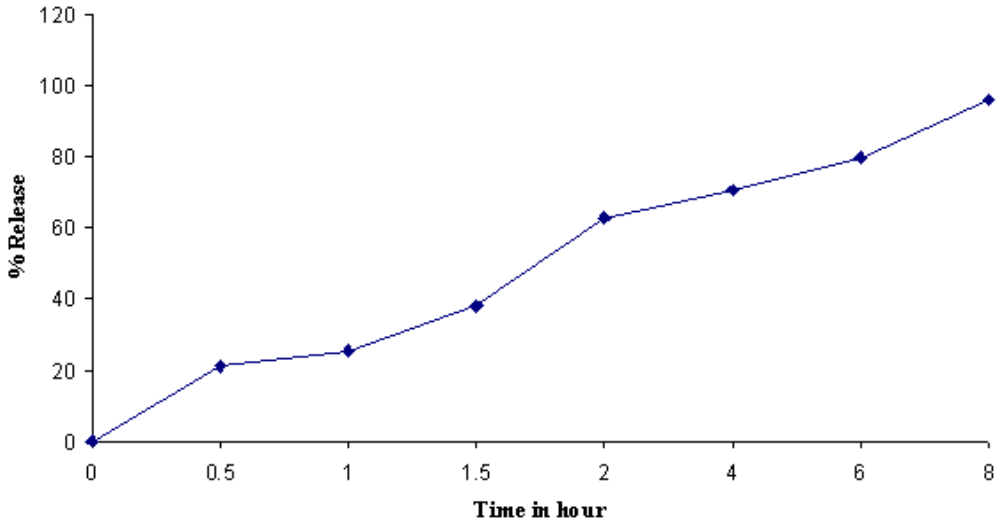


Fig 3: : Dissolution data of propranolol HCl formulation F- 3 For 8 hr.



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