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PREPARATION AND *IN-VITRO* EVALUATION OF CHITOSAN-ALGINATE MICROCAPSULES FOR COLON TARGETED DRUG DELIVERY OF METRONIDAZOLE

***HARISH GOPINATH, ABEDA AQTHER, SYED PEER BASHA, VAZRAM, K SIVA, FAROOQ AHMED**
Dept. of pharmaceutics, Nimra College of pharmacy, Jupudi,Ibrahimpatnam, Vijayawada-521 456
***Corresponding author**: E Mail: harishgopinath4u@gmail.com

ABSTRACT

Drug delivery selectively to the colon through the oral route has been the subject of new research initiatives. In recent years there has been considerable research activity within the field of colonic drug delivery. In the present study, the colon specific delivery of drug metronidazole by using xanthan gum, pectin and guar gum along with chitosan and sodium alginate used as polymer. All the formulation found to be stable and has good physio-chemical properties. Metronidazole microcapsules decreased with increasing the concentration of the coating polymer. The chitosan alginate microcapsules containing metronidazole as a model drug were prepared by cross linking method using three different polymers (i,e. pectin, xanthan, guar gum) in three different ratio such as 0.3%,0.4% and 0.5% and estimation was done by U.V spectroscopy. The drug and polymer compatibility studies were determined by I.R spectroscopy, which shows no interaction between polymer and drug. The formulated batches showed a better *in-vitro release* rates.

KEY WORDS: Metronidazole, Chitosan-alginate, Microcapsules, Colon specific.

1. INTRODUCTION

In the recent years considerable attention has been focused on the development of new drug delivery systems. The therapeutic efficacy and safety of drugs administered by conventional methods can be improved by more precise spatial and temporal placement with in the body through a controlled drug delivery. Controlled release refers to the use of delivery (Ramprasad,1996) device with the objective of releasing the drug in to the patient body at a predominated rate, or at a specific time or with specific release profiles. . Controlled drug delivery systems have been introduced to overwhelm the drawback of fluctuating drug levels associated with conventional dosage forms. Drug delivery (Milojevic,1996) (Ashford,1994) selectively to the colon through the oral route has been the subject of new research initiatives. In recent years there has been considerable research activity within the field of colonic drug delivery. The delivery of drugs to the colon for local effect is valuable in a variety of conditions like inflammatory bowel diseases. (E.g.ulcerative colitis and crohn's disease), infectious diseases and colon cancer. The advantages of targeting drugs specifically to a diseased organ includes Reduced incidence of systemic adverse effects, the ability to cut down the required dose, delivery of drugs in its intact form as close as possible to the target site. The targeting of orally administered drugs to the colon is accomplished by Prodrug, Coating with pH dependent polymer, Coating with pH independent biodegradable polymer, Matrices of polysaccharides. Microspheres are solid approximately spherical particle ranging in size from 1 to 1000µm, made of polymeric waxy or other protective materials**.** Microcapsule**s** should be reserved for reservoir type devices where Microspheres are monolithic or matrix type microparticles. Metronidazole is an Antiprotozoal, Antibacterial agent and it is completely and promptly absorbed after oral intake. Metronidazole is a broad spectrum of protozoal and antimicrobial activity. It shows antibacterial action against all anaerobic coci, anaerobic gram negative bacilli including bacteroides species and anaerobic spore forming gram positive bacilli. It is very effective in infections due to *Entamoeba histolytica, Giardia lambia* and *Trichomomiasis*. It shows selective toxicity to anaerobic microorganisms. Chitosan-alginate microparticles were prepared to control the release characteristics and physicochemical properties of drugs. Chitosan complex microparticles have also been used to immobilize cell culture. Chitosan microparticles can be prepared by complex conservation and other suitable methods (Wakerly,1996).

2. MATERIALS AND METHODS

2.1 Materials: Metronidazole was received as a gift sample from Sun Pharmaceutical Ltd., Sodium Alginate was received as a gift from Loba Chemicals, Chitosan was procured from Central Institute of Fisheries, Cochi, Pectin and Guar gum from Hi Media laboratories Pvt.Ltd., Xanthan from Fluka laboratories Pvt. Ltd., Calcium Chloride was received from Ranbaxy laboratories Pvt.Ltd., NaOH from Ranbaxy laboratories Pvt. Ltd., Glacial Acetic Acid and Acetone was procured from Hi-Pure Fine Chem.Ltd.

2.2 Method of preparation of chitosan alginate microcapsules: All the formulations were prepared using 20ml of sodium alginate solution containing 200mg of Metronidazole and 100 ml of chitosan solution (prepared in 25% v/v acetic acid) containing 2000mg calcium chloride. p^H was adjusted to 5.5 with 10% sodium hydroxide solution.20 ml of sodium alginate solution was loaded into syringe fitted with 26G needle. Calcium chloride solution was taken in a Petri dish and alginate-metronidazole solution is syringed in to the chitosan calcium chloride solution. Microcapsules were formed which are kept in solution for 10-20 min filtered and washed with distilled water and hardened with acetone(Muzzarelli,1998)**.**

2.3 Preparation of outer coat solutions: Three different outer coat solutions such as pectin, xanthan and guar gum at concentration 0.3%, 0.4% and 0.5% containing 2.5% concentration of calcium chloride is prepared and the prepared chitosan-alginate microcapsules are added to the outer coating solution and kept for 30 min. This results in forming a layer around the microcapsule; the excess coating solution is removed, washed with water and dried (Krishnaiah,1998).

2.4 Drug Content Analysis: Drug was extracted from the microcapsules with phosphate buffer pH 6.8 and absorbance was measured using UV spectrophotometer at 320 nm. The amount of Metronidazole in the microcapsules estimated with the help of standard graph (Krishnaiah,2002).

2.5 Determination of shape and size of microcapsules: This was determined by using sieving method and by Scanning electron microscope, Particles having size range between 50-1500μm are estimated by sieve analysis. This size is expressed by sieve, which describes a diameter of sphere that passes through the sieve aperture as the asymmetric particle (Chourasia,2010).

2.6 Micromeritic properties of chitosan-alginate microcapsules: The microcapsules are characterized by their micromeritic properties such as bulk density, true density, porosity, Hausner's ratio and flow property (Krishnaiah,1998).

2.7 *In-vitro* evaluation studies: The prepared microcapsules of Metronidazole were evaluated for their integrity in the physiological environment of stomach and small intestine under conditions mimicking mouth to colon transit (Krishnaiah,2001).

2.8 *In-vitro* evaluation study without rat caecal content: 50 mg were taken in a hard gelatin capsules tested for drug release for 2 hrs in 0.1N HCl (750 ml) Then the basket is placed in pH 7.4 phosphate buffer (750ml) and tested for drug release for 3 hrs. At the end of the period, two samples each of 1ml were taken suitably diluted and analyzed spectrophotometrically. Then the basket is replaced with pH 6.8 phosphate buffer. The drug release studies were carried out for 24 hrs (usual colonic transit time is 25-35 hrs) and 1ml samples were taken at different time and replaced with 1 ml of pH 6.8 phosphate buffer .The samples are diluted and analyzed spectrometrically. (Chourasia,2007)

2.9 *In-vitro* evaluation using rat caecal content: The drug release studies were carried out using USP dissolution rate test apparatus (apparatus 1, 75 RPM, 37^{o}C) with slight modification (beaker containing 200 ml of dissolution medium which was placed in the water bath of the apparatus). The capsules were placed in the basket of the apparatus and immersed in the dissolution medium containing rat caecal contents. The drug release studies were carried out for 24 hrs (colon transit time is usually 25-35 hrs) and 1ml samples were taken at different time intervals and replaced with 1ml of 6.8 pH phosphate buffer to maintain a constant volume and pH. The samples were diluted and analyzed spectrophotometrically.

3. RESULTS

The chitosan alginate microcapsules containing Metronidazole as a model drug were prepared by cross linking method using three different polymers (i,e. pectin, xanthan, guar gum) in three different ratio such as 0.3%,0.4%,0.5% and estimation was done by U.V spectroscopy. The drug and polymer compatibility studies were determined by I.R spectroscopy, which shows no interaction between polymer and drug.

3.1 Shape and Size of microcapsules: The average particle size and average thickness of outer coat of microcapsules containing Metronidazole were determined by the image software under 10X magnification.

3.2 Scanning electron microscope: Chitosan alginate microcapsules containing Metronidazole were observed in SEM which shows that the particles were spherical and smooth enough which can be shown in fig 1, 2, 3.

3.3 Pre-formulation properties: The pre-formulation properties such as angle of repose, Hausner's ratio, bulk density and true density of microcapsules were studied. To determine the flow nature, angle of repose, Hausner's ratio were calculated. The results are tabulated in table 8. The obtained value angle of repose (θ)

ranges between 20-30, Hausner's ratio below 1.1 indicating good flow properties. The above micromeritic studies shows that the prepared microcapsules were spherical, non-aggregated and uniform size.

3.4 *In-vitro* release studies: The formulations targeted to the colon should not only protect the drug from being released on the physiological environment of stomach and small intestine, but also release the drug in the colon after enzymatic degradation by colonic bacteria. Hence invitro drug release studies were carried out in pH 6.8 phosphate buffer containing 4% w/v of rat caecal contents. At the end of the 24 hr of testing this includes testing in simulated gastric fluid (Hcl) and intestinal fluid (Phosphate buffer pH7.4 The percentage of drug released at different time intervals from Metronidazole microcapsules coated with coat formulation F1-F9 in 0.1 NHCl and pH 7.4 phosphate buffer (3hrs) and pH 6.8 phosphate buffer containing 4% w/v rat ceacal contents (24hrs) were shown in the table 8, 9 and 10. F1, F2, and F3 formulation containing 0.3%, 0.4% and 0.5% of pectin released 15.85%, 13.65% and 11.85 % of the drug (Table 9). In formulation F4, F5, and F6 containing 0.3%, 0.4%, and 0.5% of xanthan gum the drug release was 16%, 13.95%, 12.25%,of the drug(Table 10) and in formulation F7, F8 and F9 containing 0.3%, 0.4%, and 0.5% of guar gum release was 16.23%, 14.25%, and 12.95% of drug respectively (Table 11). From the above results, it was found that the rate of drug released from Metronidazole microcapsules decreased with increasing the concentration of the coating polymer. In these formulations the coats were much degraded by the rat ceacal contents in the dissolution medium, since the polymer content and thickness of the coat was less as compared to the coat of the other formulations such as F2, F3, F5, F6, F8 and F9. So, a fast release of drug is obtained in F1, F4 and F7 respectively. Finally, from the above observations we can say that in presence of calcium chloride pectin has got much gelling property than xanthan, and xanthan has got much gelling property than guar gum.

4. CONCLUSION

The method of preparation of chitosan alginate microcapsules containing Metronidazole was found to be simple and reproducible. Polymers used are easily available and biocompatible. These polymers can be successfully used to protect the drug from being released under conditions mimicking mouth to colon transit. Drug release from the microcapsules takes place at a highly retarded rate till the microcapsules coat is digested by the micro flora of the colon. Thus chitosan alginate microcapsules of Metronidazole gave better colon specific delivery

Table: 1 Formula for chitosan alginate (C.A) microcapsule

S.No	Ingredients	Concentration
1	Metronidazole	2%
2	Sodium alginate	2.5%
3	Chitosan	0.4%
4	Calcium chloride	2%

Table: 2 Formula for xanthan gum coated chitosan alginate microcapsule

Formulation code	Concentration of calcium chloride	Concentration of xanthan gum
F4	2.5%	0.3%
F5	2.5%	0.4%
F6	2.5%	0.5%

Table: 3 Formula for guar gum coated chitosan alginate microcapsules

Formulation code	Concentration of calcium chloride	Concentration of guar gum
F7	2.5%	0.3%
F8	2.5%	0.4%
F9	2.5%	0.5%

Table: 4 Formula for pectin coated chitosan alginate microcapsules

Formulation code	Concentration of calcium chloride	Concentration of pectin
F1	2.5%	0.3%
F2	2.5%	0.4%
F3	2.5%	0.5%

Table: 5 Microcapsule size distribution determinations by sieve analysis

Sieve no	Particle size Range(µm)	Amount of spheres retained (mg)			%weight Fraction			Cumulative %Retained		
		F1	F2	F3	F1	F2	F3	F1	F2	F3
20/22	840-710	150	185	230	7.5	9.25	11.5	7.5	9.25	11.5
22/30	710-590	550	680	890	27.5	34	44.5	35	43.25	56
30/35	590-500	1200	1055	800	60	52.75	40	95	96	96
35/40	500-240	100	80	80	5	4	4	100	100	100

Table: 6 Results of Microcapsules size distribution determination by sieve

Sieve no	Particle size Range(µm)	Amount of spheres retained (mg)			%weight Fraction			Cumulative %Retained		
		F4	F5	F6	F4	F5	F6	F4	F5	F6
20/22	840-710	125	150	210	6.25	7.5	10.5	6.25	7.5	10.5
22/30	710-590	400	640	860	20	32	43	26.25	39.5	53.5
30/35	590-500	1105	930	730	55.25	46.5	36.5	81.5	86	90
35/40	500-240	370	280	200	18.5	14	10	100	100	100

Table: 7 Results of Microcapsules size distribution determination by sieve

Sieve no	Particle size Range(µm)	Amount of spheres retained(mg)			%weight Fraction			Cumulative% Retained		
		F7	F8	F9	F7	F8	F9	F7	F8	F9
20/22	840-710	160	190	240	8	9.5	12	8	9.5	12
22/30	710-590	525	725	865	26.25	36.25	43.25	34.25	45.75	55.25
30/35	590-500	1140	970	800	57	48.5	40	91.25	94.25	95.25
35/40	500-240	175	115	95	8.75	5.75	4.75	100	100	100

Table: 8 Pre-formulation properties

Code	Bulk density	True Density	Porosity	Hausner's Ratio	Angle of repose $\theta=\tan^{-1} h/r$
F1	0.66	0.68	0.033	1.031	$24^o 9'$
F2	0.64	0.66	0.032	1.030	$26^o 5$
F3	0.61	0.63	0.030	1.030	$27^o 1$
F4	0.64	0.68	0.064	1.062	$25^o 4$
F5	0.61	0.64	0.046	1.040	26^o
F6	0.60	0.63	0.045	1.046	$27^o 7$
F7	0.62	0.64	0.031	1.024	$25^o 2$
F8	0.61	0.63	0.030	1.030	26^o
F9	0.60	0.62	0.030	1.031	$27^o 1$

Table.9 Cumulative percentage release of Metronidazole on various concentrations of pectin

Time in hrs	Cumulative percentage release of Metronidazole on various concentrations of pectin		
	F1	F2	F3
2	-	-	-
5	-	-	-
8	4.01	2.95	1.83
10	4.95	3.15	2.85
12	6.52	4.95	4.05
14	7.12	5.75	5.05
16	8.99	7.05	5.96
18	10.55	7.95	7.15
20	11.9	11.05	9.95
24	15.85	13.65	11.85

Table 10 Results of Cumulative percentage release of Metronidazole on various concentrations of xanthum

Time	Cumulative percentage release of Metronidazole on various concentrations of xanthan

in hrs	F4	F5	F6
2	-	-	-
5	-	-	-
8	4.18	3.05	2
10	5.01	3.55	3
12	6.75	4.75	4.25
14	7.55	6	5.25
16	9	7.5	6.25
18	10.85	8.5	7.75
20	12.75	11.5	10.5
24	16	13.95	12.25

Table 11 Results of Cumulative percentage release of Metronidazole on various concentrations of Guar Gum

Time in hrs	Cumulative percentage release of Metronidazole on various concentrations of Guar Gum		
	F7	F8	F9
2	-	-	-
5	-	-	-
8	4.28	3.15	2.01
10	5.25	3.86	3.11
12	6.9	5.5	4.55
14	7.89	6.15	5.62
16	9.21	7.92	6.89
18	11.05	9.68	8.3
20	13.65	12.54	11.76
24	16.23	14.25	12.95

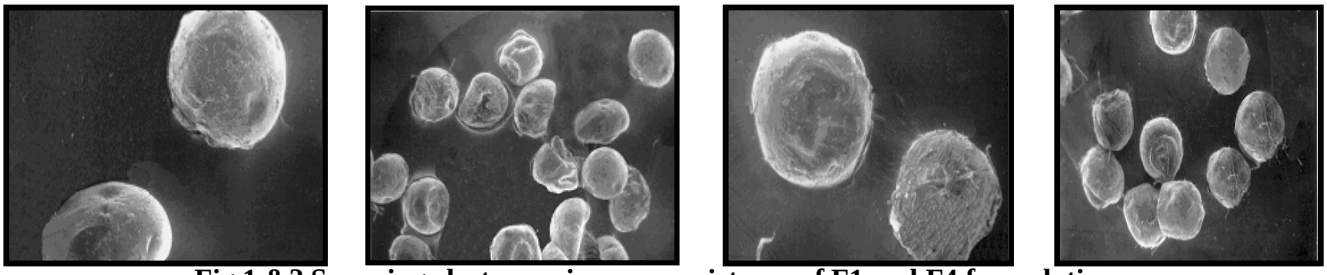

Fig.1 &2 Scanning electron microscope pictures of F1 and F4 formulations

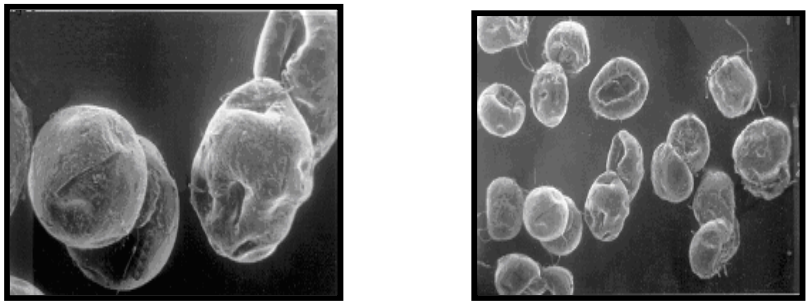

Fig.3 Scanning electron microscope pictures of F7 formulation

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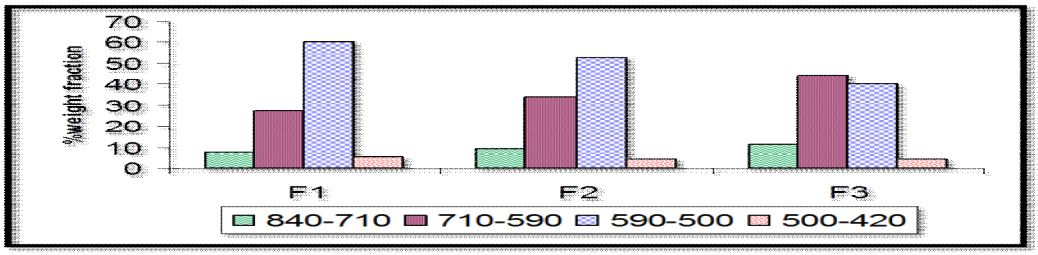

Fig.4 size distribution of pectin coated chitosan-alginate Microcapsules by sieving method

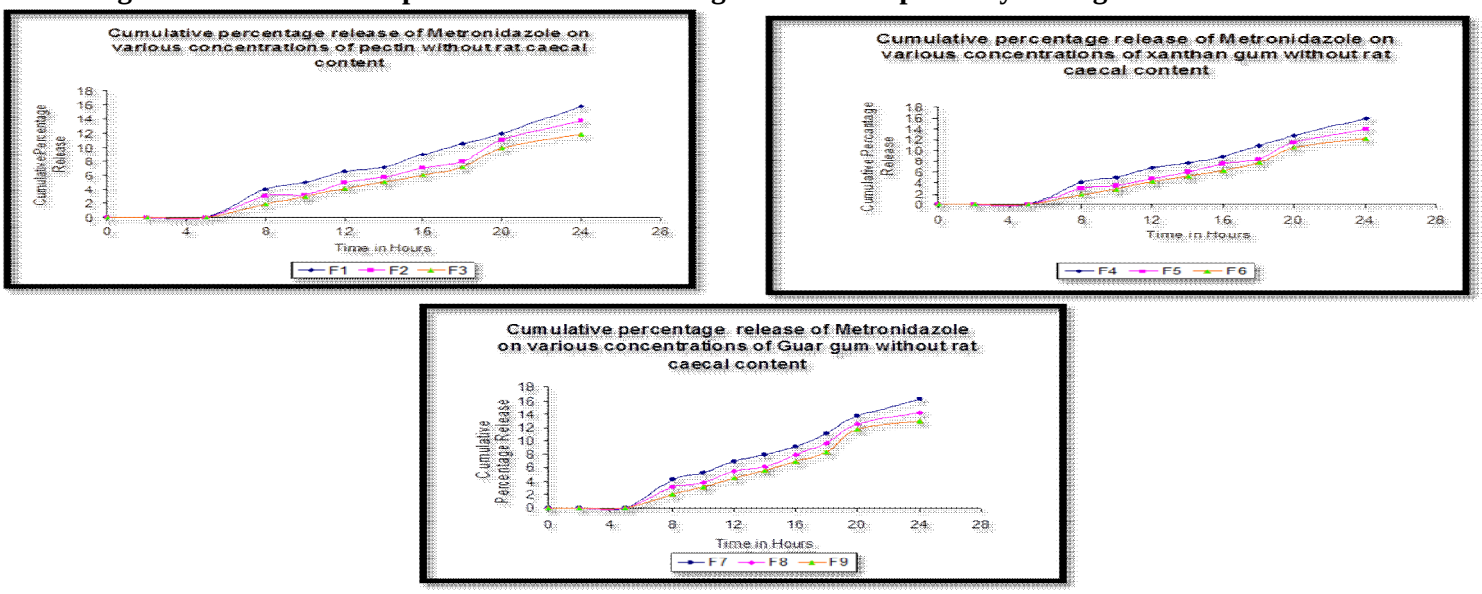

Fig.5 Cumulative percentage release of metronidazole on various concentrations of gums without rat caecal content

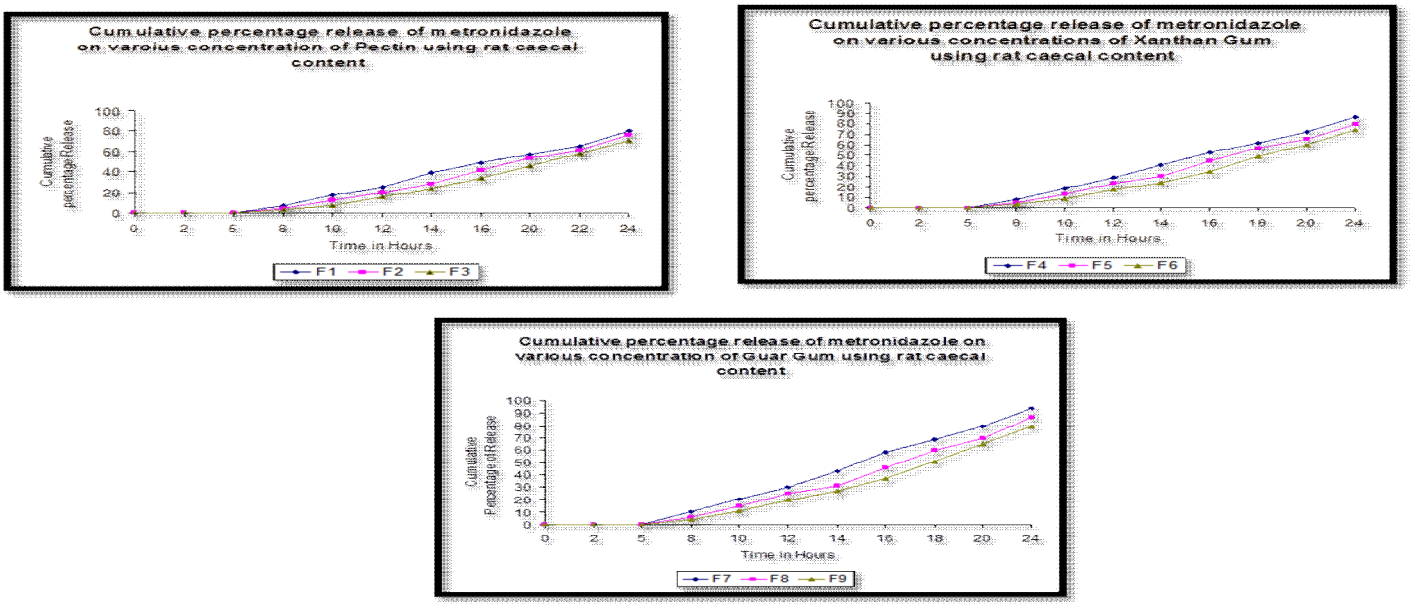

Fig.6 Cumulative percentage release of metronidazole on various concentrations of gums with rat caecal content

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