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STUDIES ON HYDROLYSABLE NATURE OF DIFFERENT POLYSACCHARIDE DRUG CARRIERS FOR COLON SPECIFIC DRUG TARGETING

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ABSTRACT

The reason for the development of a polysaccharide based delivery system for colon is the presence of large amounts of polysaccharidases in the human colon as the colon is inhabitant of a large number and variety of bacteria which secrete many enzymes e.g. D-galactosidase, xylanase, D-xylosidase, amylase, pectinase, dextranase, D-glucosidase etc. The aim of the present studies was to evaluate Various natural polyisaccharides for their hydrolysable nature in presence of colonic bacteria. Natural polysaccharides such as guar gum, pectin, sodium alginate, xanthane gum, tragacanth, were studied, the polymers were used in combination of agar and estimated for microbial colony forming unit per ml (CFU/ML). Study revealed that pectin was found to be more hydrolysable and undergo highest microbial degradation compared to other polysaccharides under study and showed 82 CFU/ML.

Introduction:

Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug to the colon[1]. The use of materials that are degraded exclusively by the colonic bacterial enzymes is found promising because of their site specificity. These materials include prodrug, azopolymers and polysaccharides. Natural polysaccharides such as xylen[2], pectin[3], guargum[4], xanthan gum, sodium alginate, etc. are not digested in human stomach or small intestine, but are degraded in the colon resident bacteria. This formed the basis to investigate the usefulness of polysaccharides as a carrier for colonic drug delivery.
A large number of aerobic and anaerobic bacteria are present throughout the length of gastrointestinal tract. The colon has over 300-400 distinct bacterial species. Most of them are anaerobes e.g., bacterioids and clostridium. Others are facultative anaerobes e.g. *Escherichia coli*. Among them 20-30% are bacterioids. The bacterial count colony forming unit per milliliter (CFU/ml) in different regions of the gastrointestinal tract is $0\text{-}10^3$ CFU/ml in stomach, $0\text{-}10^5$ CFU/ml in jejunum, $10^3\text{-}10^7$ CFU/ml in ileum and $10^{11}\text{-}10^{12}$ CFU/ml in colon[5].

The various metabolic reactions accomplished via bacterial enzymes in colon include: Hydrolysis, Reduction, Dealkylation, Deamination, Esterification, Decarboxylation, Heterolytic ring fusion and Nitrosamine formation. The bacterial count (CFU/ml) in colon is found to be $10^{11}\text{-}10^{12}$ (CFU/ml) [6].

The bacterial enzymes of colon degrade the carrier polymer in a well defined way and release the contents for localized colonic delivery or systemic absorption through colon. The gelling properties of pectin offer several advantages, including formation of viscous diffusional barriers and fermentability in the large intestine, which are useful attributes in colon-specific drug delivery.

Present study was aimed to evaluate the best polysaccharide carrier for colon specific drug delivery which is evident by highest growth of colonic microbes.

Materials:

Agar, Guar Gum, Pectin, Sodium Alginate, Xanthan gum purchased S.D Fine-chem Ltd Mumbai India, Tragacanth LOBA Chemie laboratory India, Peptone, Beef extract, Sodium Chloride, Yeast extract Sigma-Aldrich Co.

Method:

Preparation of sterile media of pectin and other polymers used in combination with agar:
Accurately weighed quantities of different ingredients are given in Table-1 were dissolved in 1000 ml of water. Final pH was adjusted to 7.2±0.2 with 10N NaOH and sterilized in an autoclave at 15 lb for 20 minutes[7].
Media M_1 being used as standard agar media and media M_2, M_3, M_4, M_5 and M_6 are various test media with different hydrolysable polymers in combination with agar.

Isolation of resident colonic bacteria from the ceacal contents of Albino rat:

A healthy male albino rat weighing about 225 g was anesthetized by using petroleum ether. The abdominal region was trimmed to remove hairs and dissected by using surgical blade. The large intestine was identified and tied at both the ends with thread and was excised at a distance 1 cm away from the thread. To remove the extraneous material, intestine was washed with distilled water. The lower end was untied and the contents were transferred into a clean beaker containing normal saline solution by gently pressing and inverting intestine serosally[8].
The above normal saline solution containing resident colonic bacteria was further used as a source for inoculation.

Serial dilution technique for resident colonic bacterial suspension:

Sterile test tubes (cotton plugged) were arranged in a test tube stand and labeled as 10^{-1}, 10^{-2}, 10^{-3} till 10^{-7} dilutions,4.5 ml of sterile saline solution (0.85% NaCl) was distributed to each tube aseptically,0.5ml of the above bacterial suspension was transferred to the first tube and mixed by vortexing (dilution 10^{1}),0.5 ml from first tube was taken using a sterile pipette and transferred to the 2nd tube and mix by vortexing (dilution 10^{2}), Serial dilution from one to next was repeated till the last tube (dilution 10^{7}) **[9]**.

Note: The dilutions are in the order of 10^{1}, 10^{2}, 10^{3}, 10^{4} till 10^{7} and the numbers of cells present in these tubes comparing the above bacterial suspension are in order of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} till 10^{-7}.
A dilution having concentration 10^{7} was selected for Inoculation into standard agar media and all the other test media. At this dilution number of colonies lies between 30-300 per plate.

Inoculation and incubation for resident colonic bacterial suspension in various types of media by pour plate method:

25 ml of standard agar media (M_1) was liquefied in a tube by heating it in water bath, Tube is cooled to 45ºC and held at this temperature until ready to pour into the plate, Label the tube and corresponding petridish, 0.5ml of serial dilution (10^{7}) of bacterial suspension was mixed with standard agar media (M_1) by gentle rotation, The contents of tubes were poured into the labeled petriplate and allowed to solidify, Plate was incubated for different time intervals 12, 24, 36, 48 and 72 hours at 37ºC and growth was measured.

Similar procedure was repeated for media M_2, M_3, M_4, M_5 and M_6 given in Table-1 and growth was measured.

Study of Microbial Growth–Colony forming unit per milliliter (CFU/ml):

After incubation each organism grows, reproduces and forms visible mass in the form of colonies and was estimated. Depending on facilities available in the department, the method used was colony forming units per milliliter (CFU/ml)**[10]**.

Colony forming unit per milliliter	=	No. of colonies counted on plate	X	Dilution of sample

The colonies were counted manually on each petriplates containing media M_1, M_2, M_3, M_4, M_5 and M_6 by using large magnifying glass at different incubation period i.e. 12, 24, 36, 42 and 72 hours. By illuminating them from below (dark field illumination), so that they are easily visible. The counting was also carried out by using electronic colony counters and subjected to CFU/ml. The CFU/ml value of different media M_1, M_2, M_3, M_4, M_5 and M_6 are given in table-2 and figure-1 and figure-2.

Results and Discussion:

The present study was aimed at finding the usefulness of various biodegradable drug carriers for colon specific drug delivery. It was earlier reported that guar gum, pectin, sodium alginate, xanthan gum and like polysaccharide polymers embedded in the matrix system is not digested in human stomach but are degraded by microbial flora of the colon, which makes these biodegradable polymers ideal natural drug carriers for the colon site specific drug delivery.

Media containing different biodegradable polymers used in combination of agar as given in table-1 were subjected to bacterial growth i.e. colony forming unit per milliliter (CFU/ml). The result revealed that all the media gave maximum growth of colonies at 36th hour, were as Agar media (M_1) produced 48 CFU/ml, Agar-guar gum media (M_2) produced 68 CFU/ml, Agar-Pectin media (M_3) produced 82 CFU/ml, Agar-Sodium alginate media (M_4) produced 37 CFU/ml Agar-xanthan gum media (M_5) gave 52 CFU/ml, Agar-Tragacanth media (M_6) produced 35 CFU/ml. Among all the media, agar-pectin media (M_3) showed the highest microbial growth i.e., 82 CFU/ml as shown in table-2, figure-1 and figure-2.

Table-1 Preparation of sterile media of pectin and other polymers used in combination with agar:

Composition	Media Code					
	M_1	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**
Agar (gm)	2.00	15.0	15.0	15.0	15.0	15.0
Guar Gum (gm)	--	5.0	--	--	--	--
Pectin (gm)	--	--	5.0	--	--	--
Sodium Alginate (gm)	--	--	--	5.0	--	--
Xanthan gum (gm)	--	--	--	--	5.0	--

Tragacanth (gm)	--	--	--	--	--	5.0
Peptone (gm)	5.0	5.0	5.0	5.0	5.0	5.0
Beaf extract (gm)	1.5	1.5	1.5	1.5	1.5	1.5
Sodium Chloride (gm)	5.0	5.0	5.0	5.0	5.0	5.0
Yeast extract (gm)	1.5	1.5	1.5	1.5	1.5	1.5
Water (ml)	1000	1000	1000	1000	1000	1000

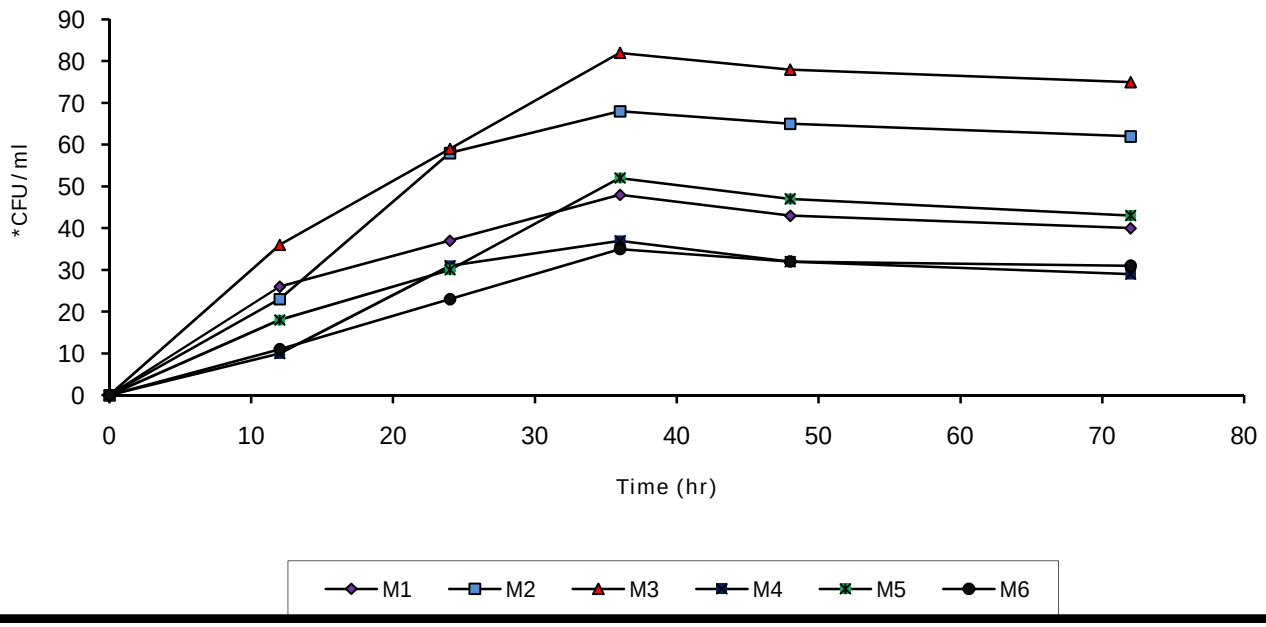
M_1 Agar media (standard media)
M_2 Agar-Guar gum media (test media)
M_3 Agar-pectin media (test media)
M_4 Agar-sodium alginate media (test media)
M_5 Agar-xanthan gum media (test media)
M_6 Agar-tragacanth media (test media)

Table-2

Comparative bacterial growths mean values of different media (M_1, M_2, M_3, M_4, M_5 and M_6)

Sl. No.	Time (hr)	Media Code					
		M_1	M_2	M_3	M_4	M_5	M_6
1.	12	26	23	36	10	18	11
2.	24	37	58	59	31	30	23
3.	36	48	68	82	37	52	35
4.	48	43	65	78	32	47	32
5.	72	40	65	78	32	47	32

Figure-1: Mean Comparative Bacterial Growth Values of Media M_1, M_2, M_3, M_4, M_5, M_6, M_7, M_8 and M_9



*CFU/ml = Colony forming unit per milliliter

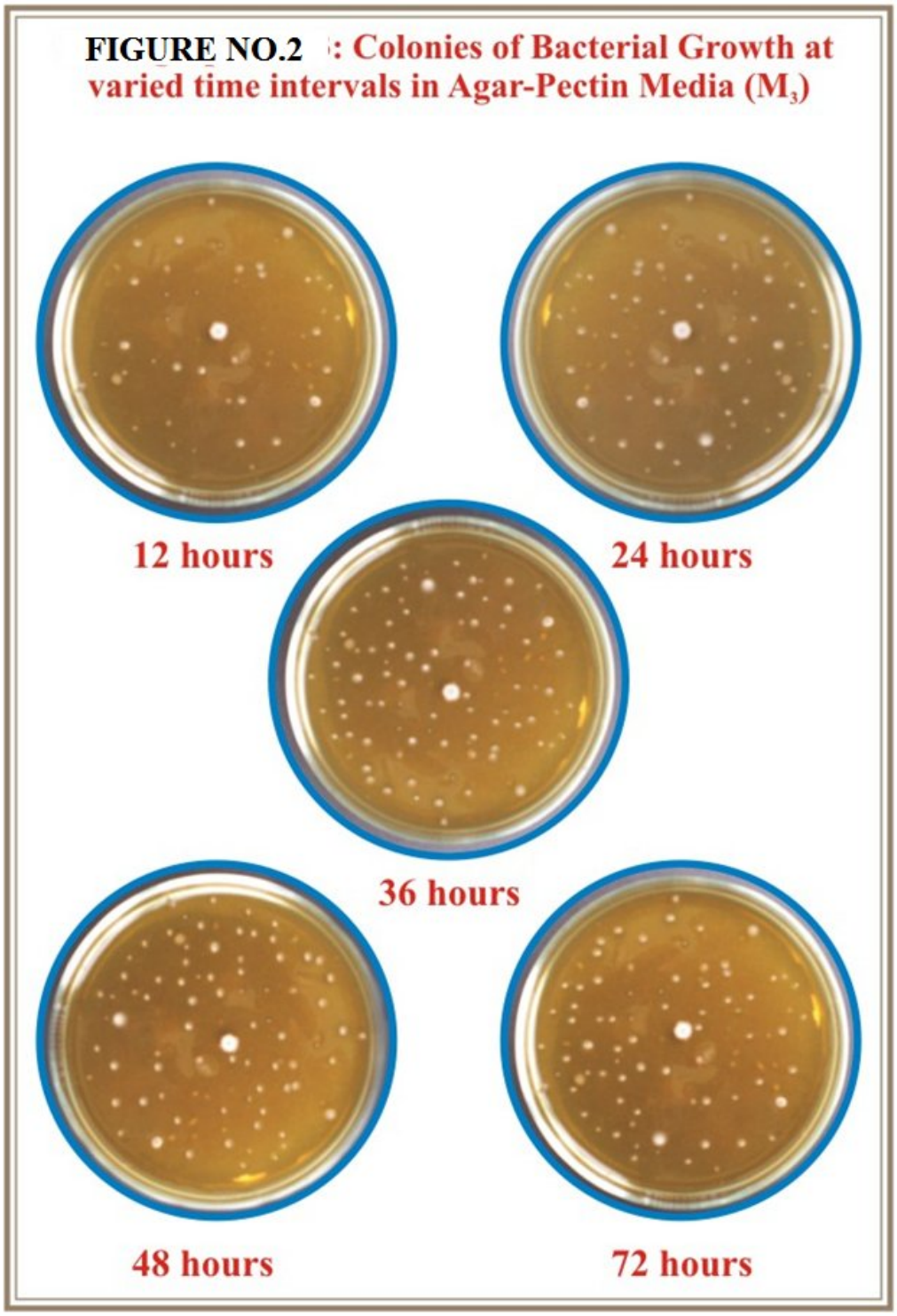


FIGURE NO.2 : Colonies of Bacterial Growth at varied time intervals in Agar-Pectin Media (M_3)

Conclusion:

Following conclusions can be drawn from the estimated results obtained from the present investigation, during the microbiological investigation studies, pectin was found to be the ideal drug carrier when compared to agar, guar gum, sodium alginate, xanthan gum and tragacanth. Furthermore the results suggest that Pectin was is more hydrolysable than other polysaccharides under studies, and hence can be used as a best drug carrier for targeting the drug to colonic site.

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