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Development of Novel Azobenzene Diacetic Acid Allyl Ester Polymers for Colon Specific Drug Delivery

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ABSTRACT

A novel polymer based on azobenzene-4,4'-diacetic allyl ester as core linker polycondensated with methyl methacrylate and n-butyl methacrylate was conveniently synthesized for the purpose of targeting colonic drug delivery. The cross linker was characterized by infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy. The synthesized polymers (PMB) showed good film forming. Comparative study of synthesized polymers was done using *In-vitro* drug release pattern of budesonide from polymer coated capsule formulation in colonic media. Budesonide capsules coated with PMB1:1:2: B and PMB1:1:3: B showed maximum drug release in colon in a sustained release manner.

Keywords: colon specific drug delivery, azoreductase, diazo linker, allyl ester, budesonide.

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INTRODUCTION

In the last few years, much attention has been paid to the selective oral delivery of drugs to the colon[6,20] with the view to reduce GI side effects by medical treatments for colon inflammation[7] and colon motility, enhancing drug absorption, treating diseases of the large bowel with newer anti-inflammatory drugs[16], only a small dose of the drug is required, which subsequently results in fewer adverse drug reactions disorders by local delivery of drugs[6,7,20,23] Colon targeting can be achieved by one of the following approaches, 1) Use of dosage forms coated with a pH sensitive coating 2) use of polymer coatings that are degradable in the large bowel[20]. The ability of microflora to reduce azo groups has been known for many years[3,10,19,24]. It was shown that polymeric azo dyes, designed for application as food dyes, can be degraded in the colon[2,4,11]. This approach was first described by Saffran and co-workers who cross linked copolymers of styrene and 2-hydroxyethyl methacrylate with various azo compounds and used them for oral delivery of insulin[21,22]. As a logical sequel, polymers containing azo groups in the backbone have been prepared for use as site selective degradable coatings. Looking towards obtaining a novel and ideal biodegradable azopolymer for colonic drug delivery, the present study describes our recent contribution to the design of polymeric systems based on azobenzene-4,4'-diacetic acid allyl ester(5) (CLA) cross linked with acrylate monomers like methyl methacrylate (M1) and n-butyl methacrylate (M2) in varying proportion. Further these polymers were coated on capsules containing drug like budesonide by deep coating method, characterized and evaluated for organoleptic, disintegration and dissolution studies. The results were analyzed by applying zero order, first order, Higuchi and korsmayer Peppes models for drug release pattern study.

MATERIALS AND METHOD

All the chemicals and solvents used were purchased from Modern Scientific Labs, Nashik (M.S.), India and were used without previous purification. Budesonide was kindly provided as gift sample from Glenmark R and D centre, Sinner, Nashik (M.S.), India.

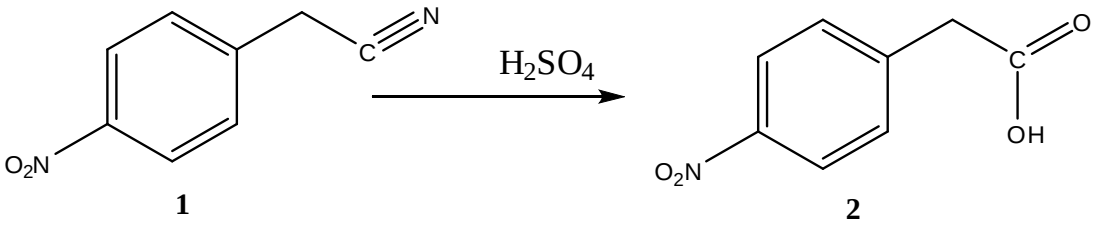
Instrumentation

H-NMR spectrum was recorded on VARIAN 300 MHz instrument (TMS as the internal standard) and chemical shifts (δ) are reported in ppm. Mass spectra were recorded on a thermometer scientific Cerus 800 DSQ-II mass spectrometer at 70 eV. Fourier transform infrared (FTIR) spectra were recorded on Shimadzu 8400S FTIR spectrometer (Japan) with sodium chloride optics and are measured in cm^{-1}. Analytical weighing balance (Shimadzu AUX 220, UniBloc, Japan) was used for weighing. UV spectra were recorded on UV-Visible Double beam

Spectrophotometer, (UV-2501 PC, Shimadzu, Japan). ProgRes CT3 Microscope (Olympus) was used for taking microscopic photographs of polymer coatings. Dissolution data of capsule formulation was recorded on USP Dissolution test apparatus (Lab India 2000). Disintegration data was obtained on Disintegration test apparatus (Shital Scientific, India).

Synthesis of p-Nitrophenyl acetic acid (2)

Into a 100ml round-bottomed flask was placed a mixture of *p*-nitrobenzyl cyanide (0.05 mol, 1) concentrated sulfuric acid (0.435 mol) in 22.6 ml. of water. The mixture was shaken well, until the solid was all moistened with the acid. The flask was attached to a reflux condenser, then set for reflux without shaking and heated until the mixture boils. The boiling was continued for fifteen minutes and cooled to 0°C with addition of equal volume of water. The solution was filtered; the precipitate was washed several times with ice water and then dissolved in 1600 ml. of boiling water. This solution was filtered as rapidly as possible in hot condition and cooled. The product was recrystalized from boiling water. Yield:-93%, Melting point - 153^0C.



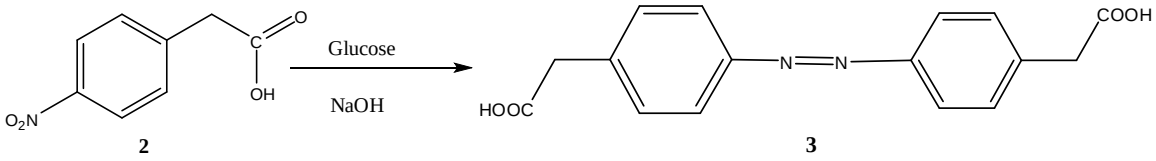
1 **2**

Scheme 1: Synthesis of p-Nitrophenyl acetic acid (2)

Synthesis of azobenzene-4, 4' –di acetic acid (3)

4-nitrophenyl acetic acid(2) (0.079 mol) was heated in a solution of sodium hydroxide (0.125 mol) in 25 ml of water at 50^0C. A solution of glucose (0.056 mol) in 15 ml of water was added slowly at this temperature with occasional shaking. The reaction mixture was then cooled to ambient temperature and aerated for 8 hr with vigorous stirring until orange-colored crystals were formed. The mixture was acidified, filtered, washed with water and dissolved in hot potassium carbonate solution to get an orange-colored solution. This solution was concentrated to get orange-colored crystals of potassium salt of diacid. On acidification rose-colored Azobenzene-4, 4' -diacetic acid (3) was obtained. Yield: (82%). Melting point: above 360^0C.

IR: v cm^{-1}: 3056 (C-H stretching aromatic), 3367 (O-H stretching), 1680 C=O stretching (aryl conju.), 2916(CH-CH stretching aliphatic), 1652 (C=C stretching aromatic), 1450 (N=N stretching), 1290 (Aryl conjugated C-O stretching), 1255 (O-H bending).



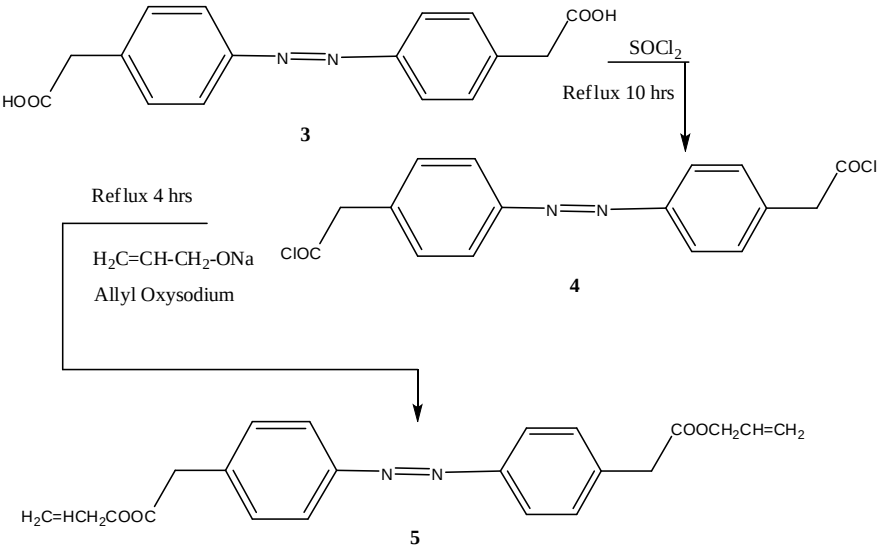
Scheme 2: Synthesis of Azobenzene- 4, 4'-di acetic acid (3)

Synthesis of azobenzene-4, 4' –di acetic acid allyl ester(5) (CLA)

Azobenzene-4, 4' -diacetic acid (0.01007mol) was refluxed with excess of thionyl chloride for 10 hrs. The excess of thionyl chloride was distilled off to get Azobenzene-4, 4' -diacetic acid-chloride (4). Then Azobenzene-4, 4' -diacetic acid-chloride (4) was dissolved in 1,4-dioxane and refluxed with equimolar quantity of sodium salt of allyl alcohol for 3-4 hrs. The precipitate was filtered and filtrate was concentrated to get solid product (5). Progress of reaction was monitored by using precoated TLC plates and structure was confirmed with IR spectroscopy. Yield: (67%). Melting point: above 360 ^{0}C

IR v cm^{-1} : 3045 Aromatic C-H stretching, 2966,2918 C-H stretching, 1724 Aryl conjugated vinyl ester C=O stretching, 1649 C=C stretching (aromatic), 1444 N=N stretching, 1369 C-N stretching, 1290 Aryl conjugated C-O stretching, 1257 C-O str. Acetates, M$^+$: 378.

δH: 0.80-0.83 (M , 1H -CH2 (Ha)) , 1.21-1.24 (S 2*1H -CH2 (Ha1, Ha2)) 3.33-3.426 (S 2*5H =CH2 (b, b'), -CH (c ,c'),-CH2 (d, d')) , 6.56-6.58 (M,1H =CH2) , 7.26-7.67 Multiplate, 2*4H Ar–CH (e, e')



Scheme 3: Synthesis of Azobenzene- 4, 4'-di acetic acid allyl ester

Polymerization of Azobenzene- 4, 4'-di acetic acid allyl ester with acid monomers

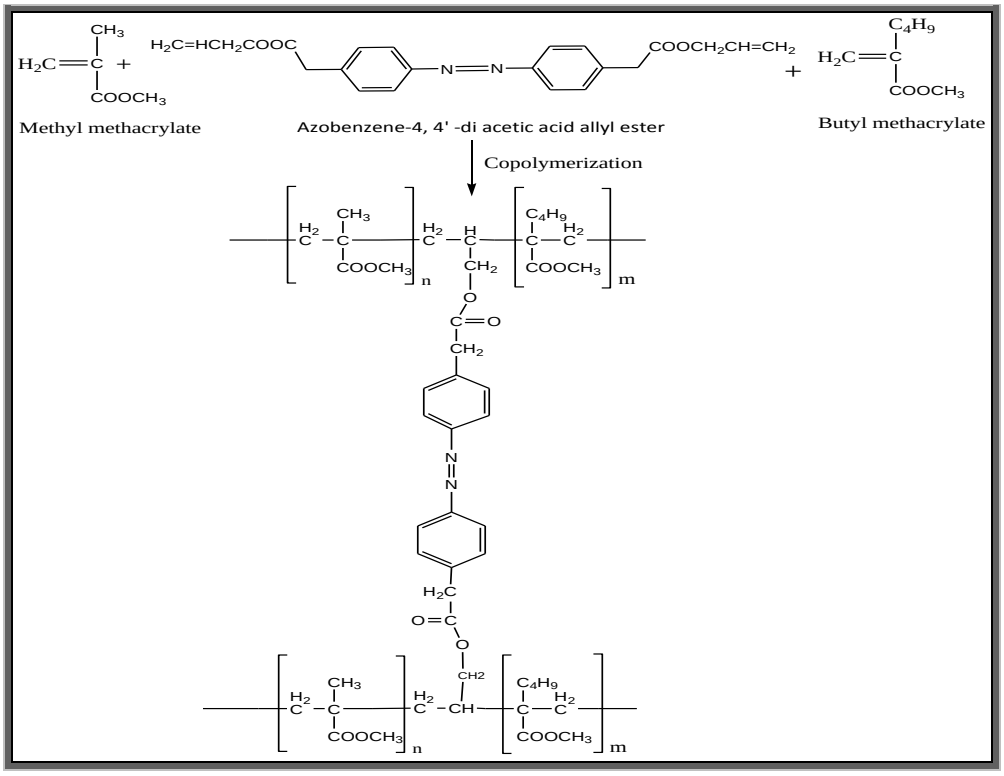
Polycondensation of azobenzene -4,4'-diacetic acid allyl ester(CLA)(5) with methyl methacrylate (M1) and n-butyl methacrylate was carried out by solution polycondensation technique in dry 1,4-

dioxane. benzoyl peroxide was used as the initiator. Acrylic monomers(M1 and M2) and CLA(5) with predetermined quantity(Table 1)were introduced in Quickfit test tubes containing 5 ml of dry 1,4-dioxane. The reaction vessels were sealed and placed in a thermo stated water bath at 70^0C. Vessels were uncapped after 24 hr and the polymeric networks were retrieved in the form of rods. The materials were pulverised in boiling methanol for 12 hr in order to remove any residual monomer and/or initiator and washing was done. The washed samples were dried under reduced pressure at 70^0C for 24 hr.

Table 1: Quantities of monomers, cross-linkers, initiator taken for Polymer synthesis

Cross linker (gm)		Polymer Code	Initiator (gm)	Monomer (gm)	
				M1	M2
CLA (5)	0.1454 (2%)	PMB 1:1:2:B	0.121	3	4.27
	0.2181 (3%)	PMB 1:1:3:B			
	0.2908 (4%)	PMB 1:1:4:B			
	0.5816 (8%)	PMB 1:1:8:B			

Scheme 4: Synthetic pathway for the titled polymers



Characterization of Polymers

Organoleptic evaluation and Solubility analysis[13]

The polymers were visually evaluated for nature, colour, state and percent practical yield of polymer. Solubility was tested in series of blend of solvents (2 ml each) in screw-capped test tube by taking 50 mg polymer and shaking the contents at constant speed at room temperature.

Casting of films

2%w/v solutions of polymer containing different percentages of dibutyl phthalate (3, 4 or 5%w/v as plasticizer) in ethanol were casted in a petri dish. After complete evaporation of solvent under controlled conditions, films were removed and dried to constant weight at 30^0 C and stored in desiccators.

Effect of microbial flora of colon on polymers

Glass cover slips were coated with polymer, by dipping the cover slips in polymer solutions (2% w/v) containing 5% w/v (based on polymer weight) dibutyl phthalate (plasticizer) and then dried aseptically. These polymer coated glass cover slips were inoculated in 3 different media; freshly prepared pH 6.8 buffer (Control), freshly obtained rat caecal content (2% w/v) in freshly prepared pH 6.8 buffer (Negative Control) and freshly obtained rat caecal content (2% w/v) in freshly prepared pH 6.8 buffer along with 2 ml liquid paraffin (to ensure anaerobic conditions). These test tubes were incubated at 37 ± 0.5^0C for 4 days.

Pre-formulation study of Budesonide[1,9,17]

This was done by analyzing organoleptic properties, melting point, UV- spectroscopy and infra red spectroscopic methods. Wavelength maxima was determined in three mediums like pH 6.8 phosphate buffer with Tween 20 (1%), pH 1.2 acid buffer with Tween 20 (1%) and in pH 6.8 phosphate buffer with Tween 20 (1%) containing 2% rat caecal content. The solubility of Budesonide was determined in water, pH 6.8 phosphate buffer and pH 6.8 phosphate buffer+ 1% tween 20 at room temperature.

Formulation of Colon Targeted Drug Delivery Using Budesonide

Budesonide and spray dried lactose were passed through sieve # 60 and blended thoroughly in PVC bags for 10 min. The resulting powder was filled in Size No. 2 hard gelatin capsules to get strength of 9mg of budesonide in each capsule. The prepared capsules were coated with the 2%w/v newly synthesized polymer solution in ethanolic solution of 5% dibutyl phthalate as plasticizer using dip coating method.The capsules were dipped six times in this polymer solution to form uniform coating and finally dried in dessicator.

Evaluation of Budesonide Formulation

Organoleptic evaluation

All Capsules were tested for appearance, colour and odor of coating. The Capsules were evaluated for diameter and height of uncoated and coated capsules (Vernier Caliper, Japan)

Disintegration test

One capsule was placed in each of the six tubes of the basket containing hydrochloric acid buffer pH 1.2 maintained at 37 ± 2^0C as the immersion fluid. After 2 hour the capsules were observed for evidence of disintegration, bursting and softening. The process was continued using pH 6.8 phosphate buffer, maintained at 37 ± 2^0C as the immersion fluid, for 1 hr. and capsules were observed for sign of disintegration. Similarly di-azo polymer coated capsules were tested.

***In vitro* drug release study[15]**

The Budesonide capsules were evaluated for *In-vitro* drug release using USP Type I Dissolution Apparatus at 37±0.5°C temperature with constant stirring rate of 50 rpm. The dissolution studies were carried out in a pH progression media in which 1% v/v Tween 20 was added to maintain sink conditions. As per IP, Acid buffer pH 1.2 (900 ml) was used for next 2 hr after which phosphate buffer of pH 6.8 (900 ml) for 3 hr and 200 ml of phosphate buffer pH 6.8 containing 2% w/v rat caecal contents was taken for further period of 19 hr. In order to imitate anaerobic conditions of colon, drug release studies in rat caecal media** were carried out under continuous supply of Nitrogen. In a predetermined manner an aliquots of 5 ml were withdrawn and analyzed immediately for Budesonide using UV spectrophotometer 2501PC. After each sampling, an equal volume of respective buffer containing Tween 20 (0.5% v/v) was replaced. All dissolution studies were repeated on three capsules.

Preparation of rat caecal content medium[14,18]

Rats weighed (150–250 g) and maintained on a normal diet were used. The rats were sacrificed fourty-five minutes before the drug release experiment using cervical dislocation. After opening the abdomen the caecum was traced, ligated at both ends, and immediately transferred into phosphate buffered saline (PBS, previously bubbled with nitrogen to maintain anaerobic conditions) pH 6.8 after dissection. The caecal bags were opened; their contents were weighed and suspended in pH 6.8 PBS to give a final caecal content concentration of 2% w/v.

Acute Toxicity study in Rats (LD_{50} determination)

Up and Down procedure was used for determination of acute toxicity study in minimizing the number of animals required to estimate the acute toxicity of a chemical in estimating its median lethal dose.

Procedure

Individual animals were administered dosage at the interval of 24 h. The polymers **PMB 1:1:2:B, PMB 1:1:3:B, PMB 1:1:4:B and PMB 1:1:8:B** was given with minimum doses to the animals by mixing polymer in rats feed and observed for next 24 h by recording percentage mortality. If no mortality was seen then the dose was increased. The polymers were administered in doses 25, 30,

35, 40, 45 and 50 µg/kg orally . During the first 1 h after the drug administration, the rats were observed for gross behavioral changes. After administration of oral dose of 50 µg/kg of polymer, the rats were observed for various parameters (Irwin et al., 1968.). The parameters observed for morbidity and mortality.

RESULTS AND DISCUSSION

The synthesized azobenzene dicarboxylic acid allyl ester polymers can be conveniently synthesized using the adopted route with good yield and purity (Table 2).

Table 2: Characterization of synthesized polymers

Polymer code	Appearance	% Practical yield
PMB 1:1:2:B	Whitish orange sticky opaque crystals	26.80
PMB 1:1:3:B	Yellowish orange sticky opaque crystals	32.90
PMB 1:1:4:B	Orange sticky opaque crystals	35.80
PMB 1:1:8:B	Dark red orange sticky opaque crystals	28.20

Following are the results obtained for the film forming property of the synthesized polymers.

Table 3: Film forming properties Ethanol (Solvent with Dibutyl phthalate Plastisizer

Polymer code	3 %w/v)	(4 %w/v)	(5%w/v)
PMB 1:1:2:B	+	++	++
PMB 1:1:3:B	_	+	++
PMB 1:1:4:B	_	+	++
PMB 1:1:8:B	_	+	++

negligible film formation, + : non-continuous film formation, ++ : continuous film formation.

Solubility study

This study revealed that the newly developed polymers possess significant solubility in different polar solvents. This property can help in developing the novel formulation with the most promising polymers (Table 5).

Table 4: Solubility study of polymers in various solvents

Polymer Code	Solubility										
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}
PMB 1:1:2:B	+	++	++	+	+	++	+	++	+	++	++
PMB 1:1:3:B	+	++	++	+	+	++	+	++	+	++	*
PMB 1:1:4:B	-	+	+	+	+	+	+	++	+	++	*
PMB 1:1:8:B	-	+	+	+	+	+	*	+	+	*	*

S_1: Acetone, S_2: ethanol, S_3: Methanol, S_4: Ethyl acetate, S_5: Methylene dichloride, S_6: Methylene dichloride: Ethanol (50:50), S_7: Toluene, S_8: Dioxane, S_9: Chloroform, S_{10}: Dimethyl sulfoxide, S_{11}: Dimethyl formamide

insoluble, + : partially soluble, ++ : completely soluble, * : Swelling.

Bio-degradation Studies

The effect of colonic bacteria on polymers PMB 1:1:2:B and PMB 1:1:3:B was better as compared to any other polymers (Table 5). The complete degradation of polymeric films was due to cleavage effect of azoreductase enzyme released by the colonic bacteria on azo bonds of the polymers. The enzyme catalyzed the reduction of the azo bonds leading to formation of amines via amides.

Table 5: Biodegradation study of polymers

Polymer Code	Polymer Degradation		
	pH 6.8 Buffer (Control)	pH 6.8 Buffer + Rat caecal content (Negative Control)	pH 6.8 Buffer + Rat caecal content+ anaerobic conditions
PMB 1:1:2:B	0	0	****
PMB 1:1:3:B	0	0	****
PMB 1:1:4:B	0	0	***
PMB 1:1:8:B	0	0	**

0: No pore formation, **: partial pore formation, ***: complete pore formation, ****: complete film degradation

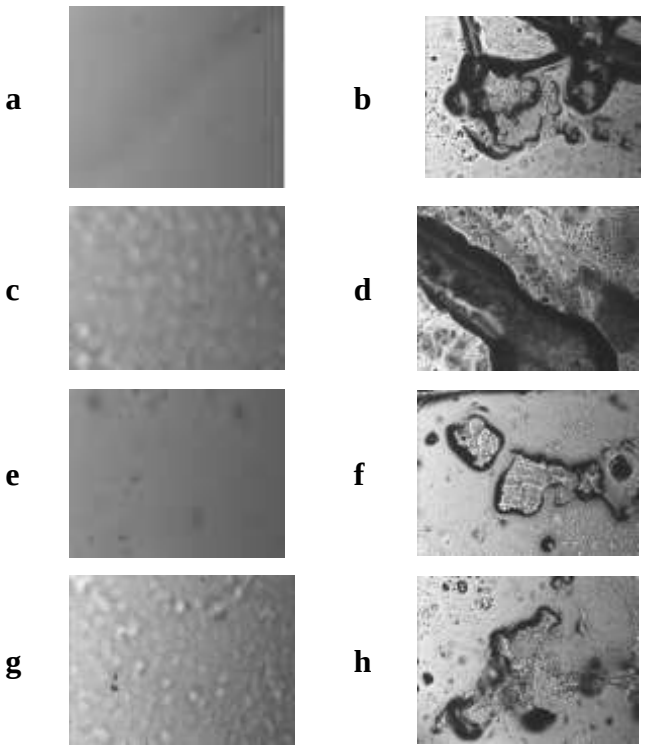


Figure 1: photomicrograph showing polymer film before and after incubation in rat caecal content (X 100)

a, c, e, g: PMB 1:1:2:B, PMB 1:1:3:B, PMB 1:1:4:B and PMB 1:1:8:B respectively before incubation

b, d, f, h : PMB 1:1:2:B, PMB 1:1:3:B, PMB 1:1:4:B and PMB 1:1:8:B respectively after incubation

Evaluation of Uncoated and Coated Budesonide capsules

The budesonide capsules (Outer diameter in mm 6.38 ±0.432 to 6.55 ±0.0423 and Height of capped capsule in mm 18.03 ±0.043 to 18.36 ±0.032, n=3 for uncoated and polymer coated capsules respectively) were evaluated for following parameters;

Disintegration test

All six uncoated budesonide capsules disintegrated in pH 1.2 acid buffer media in 2.10 ±0.102 (min). Also capsules coated with both (PMB 1:1:2:B and PMB 1:1:3:B) azo aromatic polymers had shown no signs of disintegration in acidic as well as phosphate buffer. Thus all capsules i.e. uncoated and coated, pass the disintegrtion test as per IP. Each sample was analyzed in triplicate (n=3)

***In vitro* drug release studies**

The capsules containing 9 mg budesonide were coated with selected polymers and used for the drug release study. The drug release study revealed that the capsules coated with di-azo aromatic polymers PMB 1:1:2:B and PMB 1:1:3:B released 5.79% and 4.34% drug in pH 6.8 phosphate buffer without 2% rat caecal content respectively within 3 Hrs. At the same time the release in media containing pH 6.8 phosphate buffer with 2% rat caecal content was 36.72% and 24.14% drug within 3 Hrs for polymer PMB 1:1:2:B and PMB 1:1:3:B respectively. There was significant difference (P=0.0193) ($P < 0.05$) between cumulative percent drug release (within 3 Hrs) in presence and absence of colonic contents. This confirms polymers released drug only in presence of colonic contents. The azo reductase enzyme present in colonic contents degraded the azo aromatic polymer coat; which facilitated penetration of dissolution media and thereby diffusion of drug.

Table 6: Correlation coefficient values for various kinetic models

Capsules coated with	Zero order	First order	Higuchi	Korsmeyer peppas	
	R	R	R	R	N
PMB 1:1:2:B	0.928	0.678	0.892	0.826	1.72207
PMB 1:1:3:B	0.972	0.736	0.894	0.875	1.63671

The correlation coefficient (R) value was used as criteria to choose best fit model to describe the drug release from the polymer coated capsules. Capsules coated with both (PMB 1:1:2:B and PMB 1:1:3:B) azo aromatic polymers shown higher correlation coefficient (R) values for zero order equation, indicating the drug release follows zero order.

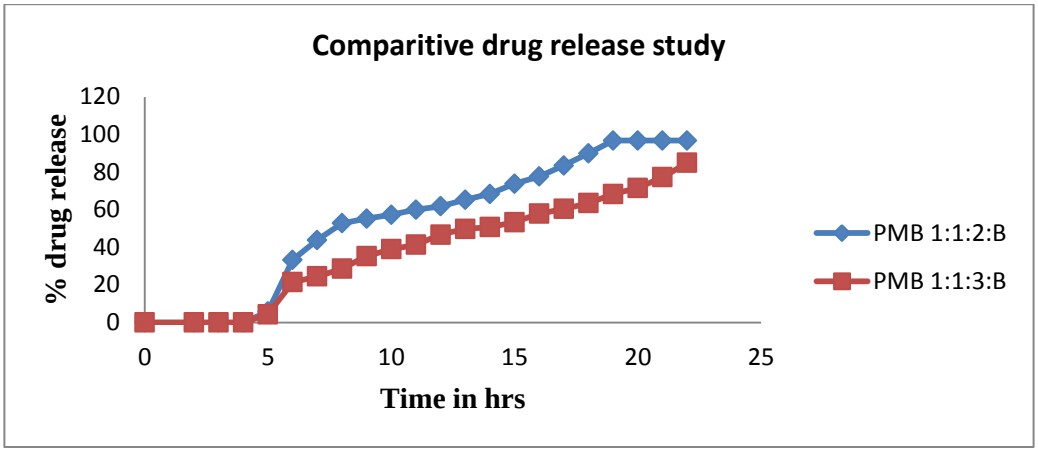


Figure 2: Drug release study of polymer coated capsules

Drug release kinetics

The release data obtained were fitted to Zero order, First order, Higuchi and Korsmeyer peppas equations to determine the corresponding release rate and mechanism of drug release.

Acute Toxicity Study (LD_{50})

Oral administration of PMB 1:1:2:B, PMB 1:1:3:B, PMB 1:1:4:B and PMB 1:1:8:B up to 50 µg/kg did not produce any toxic effects and no mortality was observed in rats. This suggests that LD_{50} of PMB 1:1:2:B, PMB 1:1:3:B, PMB 1:1:4:B and PMB 1:1:8:B was found to be above 50 µg/kg. During this study, no deaths were observed; no significant clinically relevant changes were observed in general behavior and other physiological activities in the present study.

CONCLUSION

The newly synthesized azobenzene diacetic acid allyl ester polymers can be conveniently synthesized using the adopted route with good yield and purity. The drug release kinetics study with formulation of budesonide capsules coated with PMB1:1:2:B and PMB1:1:3:B showed that drug is released maximally in colon in a sustained release form. PMB1:1:2:B and PMB1:1:3:B showed very similar results as that of polymers synthesized from cross linker 4,4'-azobenzenedicarboxylic acid[8]. As a result, colon delivery of budesonide and like drugs is possible with the newly developed polymers appeared to be a promising alternative to traditional drug administration routes in upcoming future. This study shows that polymers synthesized from cross linker 4,4'-azobenzenedicarboxylic acid and cross linker 4,4'-azobenzene diacetic acid with M1 and M2 showed very significant result which are somewhat similar to each other[8].

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