ISSN : 0974 - 7532 Volume 8 Issue 3

Research & Reviews in

BioSciences

Regular Paper

RRBS, 8(3), 2014 [117-124]

Application of polyhydroxyalkanoates as a novel tablet disintegrator in fast dissolving tablets and acute toxicity studies

Anjali Mohan Nair[1]*, K.Karthik[2], K.S.G.Arul Kumaran[2], Sandeep George Simon[2], Alex Thomas[2], Shanthi Kuppusamy[1]

[1]Department of Environmental Science, PSG College of Arts and Science, Coimbatore-641014, (INDIA)

[2]Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore-641014, (INDIA)

E-mail : anjali.mohannair86@gmail.com

ABSTRACT

The study was conducted to investigate the application of Polyhydroxyalkanoates as pharmaceutical excipients (disintegrating agent), and to study its effect on the drug release rate of Diclofenac Sodium (Model Drug I) and Bambuterol Hydrochloride (Model Drug II) tablets. Polyhydroxyalkanoates are biopolymers produced by bacteria like *Alcaligenes eutrophus, Ralstonia eutropha*, and *Bacillus subtilis* as intracellular granules and these find a wide range of applications as drug carriers and for making tissue engineering materials. The tablets were manufactured with various concentrations of Polyhydroxyalkanoates and evaluated for preformulation and post compression parameters. The results of preformulation studies showed excellent flow property for the formulations made with Polyhydroxyalkanoates in different concentrations. Polyhydroxyalkanoates was added with Model Drug I, and compressed to find out whether it could be used as a polymeric carrier. For comparison purpose, in another batch Hydroxy Propyl Methyl Cellulose was added and compressed. *In vitro* disintegration studies showed that Polyhydroxyalkanoates was disintegrating faster (45 seconds) than Hydroxy Propyl Methyl Cellulose (310 seconds), so we concluded that Polyhydroxyalkanoates cannot be used as a carrier and may be used as a disintegrant. For evaluating the disintegrant property, Polyhydroxyalkanoates was compared with a batch of high surfactant (Sodium Lauryl Sulphate) and the results showed that Polyhydroxyalkanoates disintegration (45 seconds) was comparable with Sodium Lauryl Sulphate disintegrating (210 seconds) as both the formulations disintegrated faster than with Hydroxy Propyl Methyl Cellulose. Later, formulations were made for Fast Dissolving Tablets with Model Drug II, in the presence of Polyhydroxyalkanoates; it was found that the disintegration of Model Drug II with Polyhydroxyalkanoates was 43 seconds which was comparable with that of cross povidone used as super disintegrant (16seconds). The dissolution rate for Diclofenac tablets made with Hydroxy Propyl Methyl Cellulose, Polyhydroxyalkanoates and Sodium Lauryl Sulphate were 82.98%, 73.57% and 65.94% and the dissolution rate for Bambuterol hydrochloride tablets made with Polyhydroxyalkanoates and cross povidone were 48.97% and 59.13% respectively. Based on the results of pre formulations and post compression studies, Polyhydroxyalkanoates can be considered as a potential drug carrier.

KEYWORDS

Fast dissolving tablets;
Bambuterol hydrochloride;
Cross povidone;
Polyhydroxyalkanoates.

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INTRODUCTION

Polyhydroxyalkanoates (PHA) are a family of bio polyesters produced by numerous bacteria such as *Ralstonia eutropha, Bacillus subtilis,* and *Alcaligenes latus* as intracellular carbon reserve under unfavourable growth conditions. PHA are classified into 3 categories: short chain length PHA (3carbon to 5carbon), medium chain length PHA (6carbon to 14carbon), and long chain length PHA (more than C14)[1].

The molecular weight ranges from $2X10^5$ to $3X10^6$ Daltons and the types of bacterium and growth conditions determine the chemical composition of PHAs. PHA has so many applications; but the cost of production makes them commercially less available. This in turn opens way to the researchers to focus on the use of economic substrates for easy production of PHA[2] and thus attempts had been taken to produce PHA from wastewaters and agro industrial by products[3].

PHA can be used in different applications, such as making of films, moulds, and personal hygiene products and adhesives[4]. Different composites of PHA are used in the synthesis of optically active compounds[5] and in biomedical applications like making scaffolds, sutures or biomedical implants and Controlled Drug Delivery System[6]. The Bio-absorbable and biocompatible nature makes them attractive towards the application in medicinal fields[7].

Designing Fast Dissolving Tablets (FDT) is a promising drug delivery mechanism as they easily dissolve in the mouth of patients, who have swallowing or chewing difficulty and hence it can be used by children or elderly people[8]. These drugs when placed in mouth get dissolved rapidly in saliva without any liquid to assist in swallowing and will have systemic effects reaching different parts of the body via blood stream.

The main aim of this study is to use PHA as a tablet disintegrator for mouth dissolving tablets and evaluation of the parameters (pre formulation and post compression).

MATERIALS AND METHODS

Diclofenac sodium (Model Drug I) and Bambuterol Hydrochloride (Model Drug II) were obtained as gift samples from Sun Pharmaceuticals Ltd., Vadodara, Gujarat. Cross-povidone (Loba Chemicals) and all other chemicals/ Solvents used were of analytical grade.

Test material (PHA)

In this present study, *Bacillus subtilis* was isolated from soil and used for PHA production using Sodium Hypochlorite method[9].

Acute toxicity studies (LD50)

Swiss Albino mice (25-30g) were grouped (n=4) and used for this experiment. Before the experiment, animals were fasted for 18 hours with free access to water. Multiple doses (500mg/kg to 4000mg/kg) of PHA were prepared as suspension in 1% Carboxy Methyl Cellulose (CMC) and administered orally (as per OECD 425 guidelines) to all groups of mice as shown in TABLE 1. Animal mortality was recorded after a period of 72 hours. Besides the number of deaths, parameters such as alertness, sedation, dyspnea, urination, convulsions, spontaneous motor activity, postural reflex, were observed for 14 days.

The studies were approved by the institutional animal research committee, and were conducted in accordance with the internationally accepted principles for laboratory animal use and care [Proposal number: KMCRET/Ph.D/04/2013-2014].

TABLE 1 : Acute Toxicity Studies (LD50)

Markings	Group I (500mg/kg)		Group II (1000mg/kg)		Group III (2000mg/kg)		Group IV (4000mg/kg)	
	Wt(g)	Dose administered/ animal	Wt(g)	Dose administered/ animal	Wt(g)	Dose administered/ animal	Wt(g)	Dose administered/ animal
Head	27	13.5	28	28	27	54	28	112
Body	28	14	29	29	28	56	29	116
Tail	29	14.5	27	27	29	58	30	120
No Marking	30	15	30	30	30	60	27	108

Wt-Weight, g-grams, Mg/kg-Milligram per Kilogram

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Preparation of mixed blend of drug and excipient[11,12]:

Required quantity of each ingredient was taken for each specified formulation (listed in the TABLE 2 and 3 for Model drug I and II) and they were co-ground using a mortar and pestle and all the Ingredients were passed through mesh number 60. The powdered blend was evaluated for flow properties such as Bulk density, Tapped density, compressibility index, and Hausner's ratio.

TABLE 2 : Composition of Diclofenac sodium (Model Drug I) in mg

Ingredients	Batch I	Batch II	Batch III	Batch IV
Model drug I	100	100	100	100
Hydroxy Propyl Methyl Cellulose	100	90	50	0
Sodium Lauryl Sulphate	0	10	0	0
Polyhydroxyalkanoates	0	0	50	100
Anhydrous Lactose	32.5	32.5	32.5	32.5
Aspartame	10	10	10	10
Talc	2.5	2.5	2.5	2.5
Magnesium stearate	2.5	2.5	2.5	2.5
Pineapple Flavour	2.5	2.5	2.5	2.5

mg-Milligrams

TABLE 3 : Composition of Bambuterol Hydrochloride (Model Drug II) in mg

Ingredients	Batch I with Cross Povidone	Batch II with 50% PHA	Batch III with PHA
Model Drug II	10	10	10
Mannitol	50	50	50
Microcrystalline cellulose	159	159	159
Cross-povidone	10	5	0
Polyhydroxyalkanoates	0	5	10
Cabosil	1	1	1
Magnesium Stearate	5	5	5
Aspartame	10	10	10
Talc	5	5	5
Pineapple Flavour	2.5	2.5	2.5

mg-Milligrams

Compression of tablets

All the ingredients listed in TABLE 2 & 3 (except magnesium stearate & Talc) were mixed homogenously and co-grinded in a mortar and pestle for making the formulations. Finally, magnesium stearate and Talc were added and mixed for 5 minutes. The mixed blend of drug and excipients were compressed carefully using 12 station rotary punch tablet machine (RIMEK ROTARY PRESS-MINI PRESS II MT) to produce convex faced tablets. A minimum of 50 tablets were prepared for each batch.

Post compression studies of the tablets prepared from model drug I and II

The tablets were evaluated for weight variation, thickness, friability, hardness, disintegration time (DT), content uniformity, drug content and *in vitro* dissolution study as mentioned below. The results were tabulated accordingly.

Weight variation[13]

For weight variation, 20 tablets were randomly selected from each batch and weighed individually on an electronic balance and mean weight was taken. The standard deviation in weight was calculated for each batch. The weight variation was carried out according to USP 2000.

Thickness

The thickness of each tablet was measured using Vernier Calipers and expressed in mm.

Friability[14]

Friability test was performed using a Roche friability testing apparatus. Pre-weighed tablets were (n=10) placed in friabilator which is then operated at a speed of 100 revolutions/minute. The tablets are then dusted and re-weighed. The percentage friability was measured using following formula:

$$\% F = [(W - W_0)/W_0] \times 100$$

Where; %F = Friability in percentage, W = Initial weight of tablet, W_0 = Weight of tablet after test

Hardness[15]

The Hardness was measured using a tablet hardness tester (Monsanto Hardness Tester). The strength of tablet is expressed as tensile strength (Kg/cm^2).

Disintegration time

Disintegration Time for the compressed tablets was determined by using disintegration test apparatus (campbell electronics, Mumbai). Each tablet was placed in the basket, with an up and down movement for 30 times

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per minute in a phosphate buffer of pH 6.8 at37±2ºC. The time taken for complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured and recorded.

In vitro dissolution studies[16,17]

The *in vitro* dissolution study was carried out in USP dissolution test apparatus Type 2 (Electrolab, India). The dissolution medium consisted of phosphate buffer (pH 6.8) 900 ml at 37±0.5 ºc with stirring speed of 50 RPM. Tablets was placed in the dissolution buffer and samples were withdrawn at 1 to 10 minutes, at the time interval of 1 minute by replacing with same dissolution medium and samples were analyzed by measuring the absorbance at 265 nm by UV spectrophotometer.

Assay

Tablets were crushed in mortar and transferred in 100 ml flask. The powder was dissolved in 30 ml methanol and made up to 100ml with distilled water. Sample is sonicated for 30 minutes, and filtered through Whatman filter paper (pore size, 0.45microns). The filtered solution after appropriate dilution (1 to 10 ml) with distilled water was analysed by spectrophotometrically at 265nm.

Compatibility studies

The drug excipient compatibility study was done using SHIMADZU FTIR spectrometer. The IR spectra for pure drug, pure PHA and a mixture of drug and PHA in the ratio of 1:1 with KBr pellets were studied for checking the compatibility of PHA with the ideal drug.

RESULTS AND DISCUSSION

Acute toxicity studies (LD50)

No mortality was observed after PHA administration up to 4000mg/kg. No significant changes were seen in the wellness parameters used for evaluation of toxicity in the mice. Skins, fur, eyes, mucous membrane, behavioural pattern, salivation, sleep of the treated animals were found to be normal till 14 days. Tremors, lethargy, diarrhoea and coma did not occur in any of them. Thus PHA is considered to be non toxic.

Pre formulation of model drugs I and II

When formulations were made with PHA for Model Drug I and II, their properties were found to be excellent.

With Model Drug I the Hausner's ratio was 1.08, Compressibility index was 7.5 and the angle of reponse was 23.31, which showed excellent flow property. With Model Drug II the Hausner's ratio (1.06), compressibility index (6.25%), and the angle of reponse (28.52) made also showed excellent flow property[19]. The results are shown in TABLE 4 and 5.

TABLE 4 : Pre-formulation studies for Model Drug I

Formulations	Bulk Density	Tapped Density	Compressibility index %	Hausner's Ratio	Angle of reponse
Batch I	0.57	0.59	3.07	1.03	24.83
Batch II	0.5	0.52	5.33	1.05	25.67
Batch III	0.41	0.43	3.33	1.03	21.96
Batch IV	0.46	0.50	7.5	1.08	23.31

%-Percentage

TABLE 5 : Pre-formulation studies for Model Drug II

Formulations	Bulk Density	Tapped Density	Compressibility index %	Hausner's Ratio	Angle of reponse
Batch I	0.46	0.57	18.75	1.23	30.52
Batch II	0.41	0.48	13.88	1.16	30.76
Batch III	0.46	0.5	6.25	1.06	28.52

%-Percentage

Post compression studies

Model drug I

The friability of the tablets of Batch IV was 0.79 and the *in vitro* disintegration time was 45 seconds. The low disintegration time of PHA suggested that it can be used in FDT. The disintegration time of Batch II containing SLS showed less disintegration time of 290

TABLE 6 : Results of Post Compression Parameters of Model Drug I

Evaluation	Batch I	Batch II	Batch III	Batch IV
Weight variation (mg)	251.7±1.6	248.8±2.4	250.8±2.2	249.2±1.4
Thickness (mm)	4.05	4.16	4.12	4.21
Friability (%)	0.79	0.65	0.62	0.79
Hardness (Kg/cm^2)	2.8	2.5	2.6	2.5
Disintegration Time (sec)	310sec	290sec	420sec	45sec
Assay (%w/w)	96.45	98.72	97.62	98.12

Mg-milligrams; mm-Millimetre; %-percentage; Kg/cm^2 – Kilogram per square centimetre; Sec-Seconds; %w/w-Percentage of weight by weight

seconds, since SLS acts as a disintegrator. The results are shown in TABLE 6.

Model drug II

The friability of tablets (Batch III) was 0.71 and the disintegration time was 43 seconds as shown in TABLE 7.

TABLE 7: Results of Post Compression Parameters of Model Drug II

Evaluation	Batch I	Batch II	Batch III
Weight variation (mg)	248.1±2.6	250.7±1.4	249.4±2.1
Thickness (mm)	4.11	5.09	4.86
Friability (%)	0.247	0.59	0.71
Hardness (Kg/cm2)	2-3kg/cm^2	2-3kg/cm^2	2-3kg/cm^2
Disintegration Time (sec)	16sec	13sec	43sec
Assay (%w/w)	97.56	94.68	96.82

mg-milligrams; mm-Millimeter; %-percentage; Kg/cm^2 – Kilogram per square centimetre; Sec-Seconds; %w/w-Percentage of weight by weight

Dissolution studies

From the results of the dissolution studies (Figure 1 and 2), it was observed that the dissolution rate of Model Drug I with Hydroxy propyl methyl cellulose, Polyhydroxyalkanoates and Sodium Lauryl Sulphate were 82.98%, 73.57% and 65.94% respectively. From the results it was found that PHA was showing comparable and faster dissolution to SLS. Since Model Drug I was not required to formulate FDT, we tried the remaining formulation with Model Drug II. It was found that the dissolution rate for Bambuterol hydrochloride tablets made with Polyhydroxyalkanoates and cross povidone were 48.97% and 59.13% respectively, which was comparable.

Compatibility studies

The IR spectra for pure drug (Model Drug II), pure PHA & in combination of Drug and PHA in1:1 ratio mixture was shown in the Figure 3, 4 and 5. The spectrum of the mixture was found to be a mere summation of the individual spectrum of the drug and PHA which suggests that there were no interactions between drug and excipients and was compatible with each other.

DISCUSSION

In the present work, we used PHA and HPMC as carriers for making Diclofenac sodium tablets and it was found that the disintegration time was 45 seconds with PHA and 310 seconds with HPMC. The tablets made with SLS showed disintegration time of 290 seconds. This suggested the use of PHA in oral tablets which needs faster disintegration (within 60 seconds).

Later when Bambuterol Hydrochloride (FDT) was used, it was found that the disintegration Time was 43 seconds with PHA and 16 seconds with Cross Povidone.

Thus the disintegration Time in both the cases was less than 60 seconds, which is an important factor in making FDT.

This ensures that the completely biodegradable and biocompatible PHA can be used as excipient (super disintegrant) in making fast dissolving tablets.

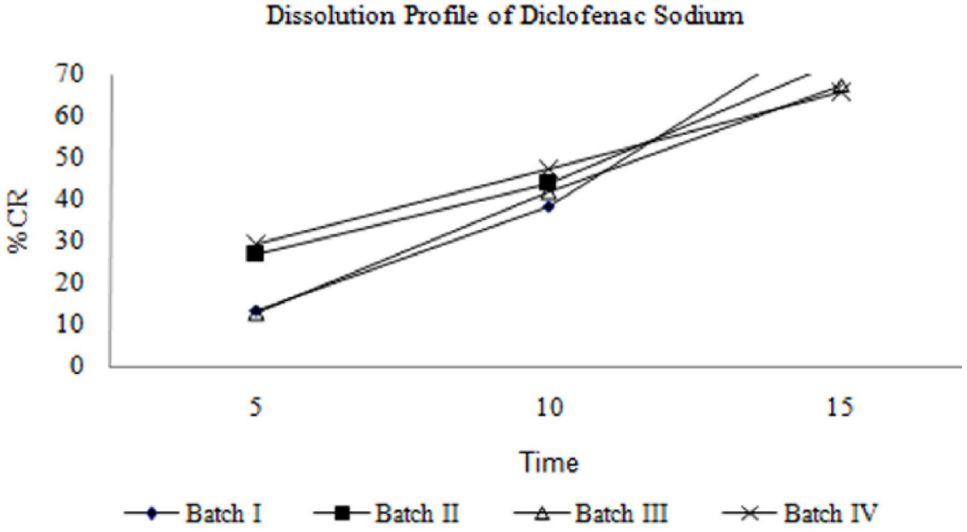

Figure : 1 Dissolution profile of Model Drug I

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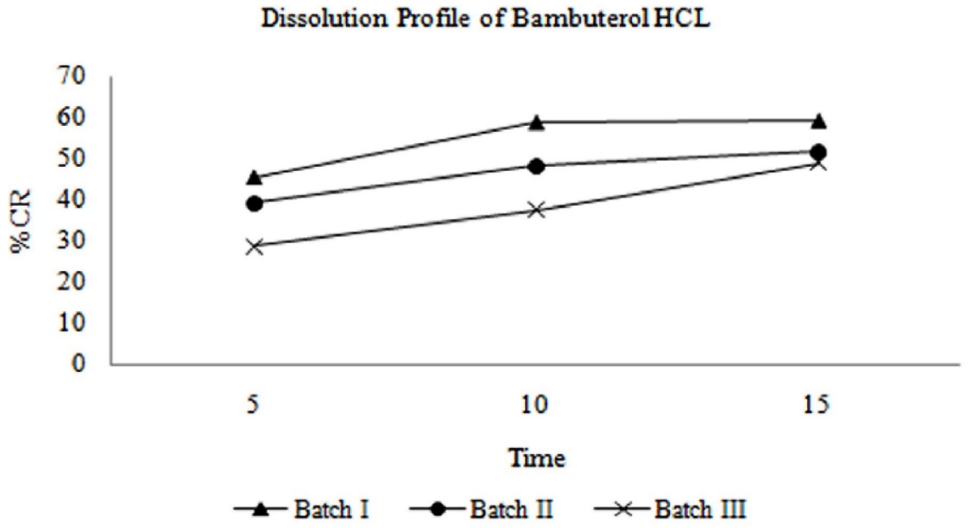

Figure : 2 Dissolution profile of Model Drug II

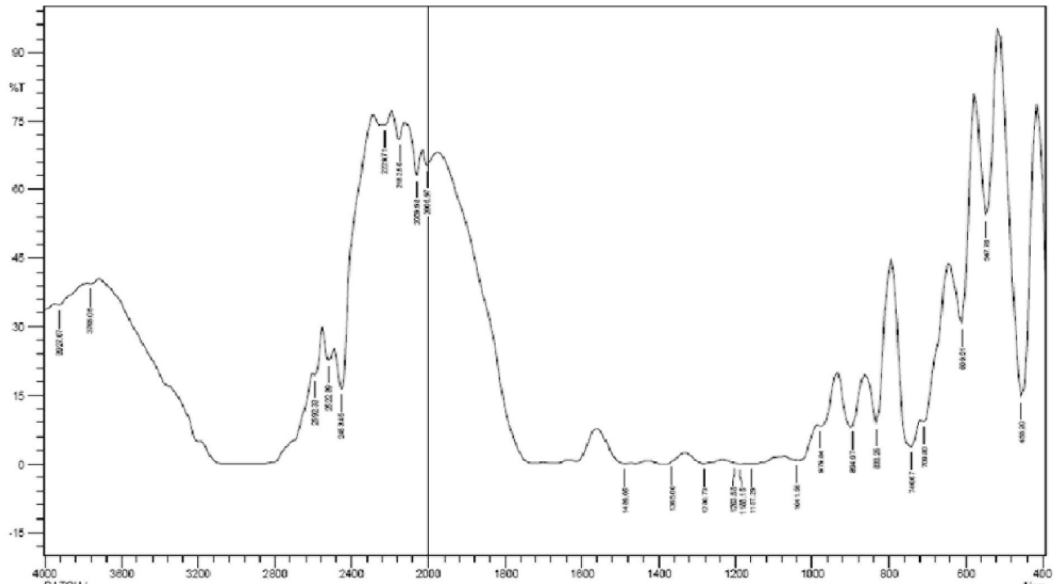

Figure : 3 FTIR of Model Drug II

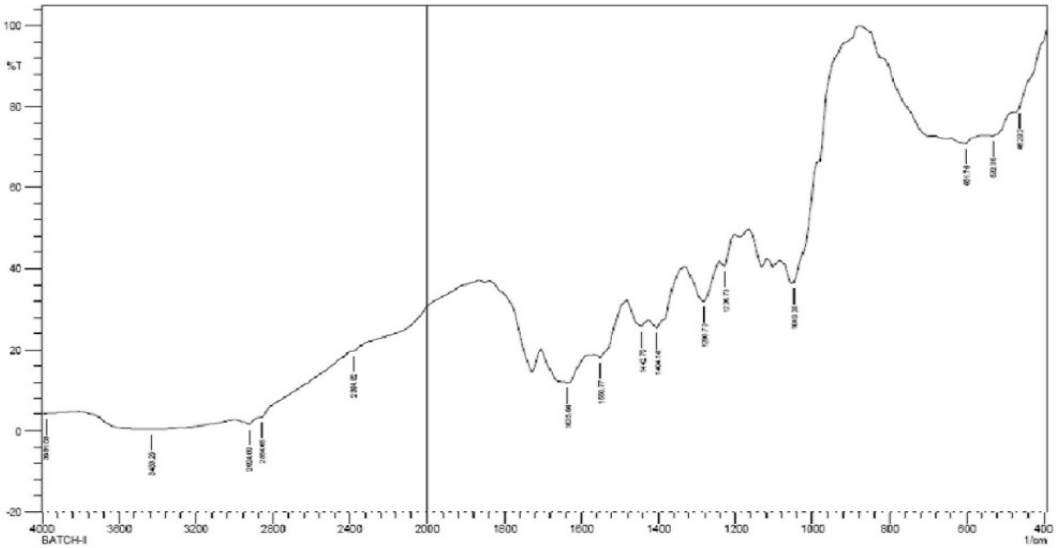

Figure : 4 FTIR of PHA

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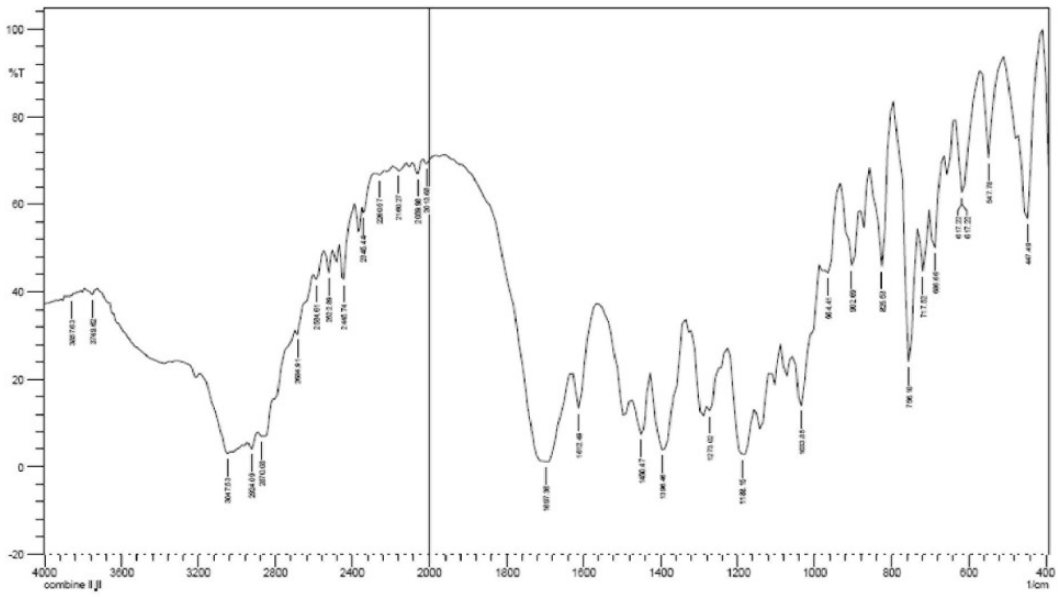

Figure : 5 FTIR of Model Drug II and PHA in 1:1 ratio

CONCLUSION

The study indicates that the tablets made with PHA shows excellent flow property (pre formulation studies) and lesser Disintegration time (post compression studies), which in turn implies that PHA can be used as tablet disintegrator in FDT. The results obtained using PHA as excipient was comparable with that of the tablets made with Cross Povidone (existing super disintegrator).

FDT of Bambuterol HCl can be successfully prepared using PHA, (biodegradable polymer) as super disintegrants in order to improve disintegration/dissolution of the drug in oral cavity and hence better patient's compliance and effective therapy. Thus the study suggested the PHA can also be considered as a super disintegrant.

ACKNOWLEDGEMENTS

The authors are thankful to Dr.C.Sankar, Professor, Dept of Pharmaceutics, and Dr.K.T Mani Senthil, Professor, Dept of Pharmacology, KMCH College of Pharmacy, Kalapatti road, Coimbatore for providing valuable support and necessary guidance during the course of work. The authors are grateful to Sun Pharmaceuticals Industries Ltd., Vadodara, Gujarat, India for providing the drug sample and KMCH College of Pharmacy, Kalapatti road, Coimbatore for providing necessary facilities to conduct the work.

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