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Design and comparative *in-vitro* and *in-vivo* evaluation of starch-acrylate graft copolymer based salbutamol sulphate sustained release tablets

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ABSTRACT

The present work deals with the development of controlled release tablets of salbutamol sulphate (SS) using graft copolymers of methyl methacrylate (St-g-PMMA and Ast-g-PMMA) on starch and acetylated starch. Formulations were evaluated for physical characteristics like hardness, friability, drug release, drug content and weight variations, which fulfilled all the official requirements of tablet dosage form. The release rates from formulated matrix tablets were studied at SGF (pH 1.2) followed by SIF (pH 6.8). Drug release from the graft copolymer based tablets was found to be sustained upto the 14 h with >75% drug release. The *in-vitro* release study showed that the graft copolymer based matrix formulations (F3 & F4) exhibited highest correlation value (r^2) for higuchi kinetic model and Korsmeyer's model with *n* values between 0.61 and 0.67 proved that release mechanisms were governed by both diffusion and erosion mechanism. There was no significant difference in the pharmacokinetic parameters (t_{max}, C_{max}, AUC, K_e, and $t_{1/2}$) of the graft copolymers matrices and HPMC K100M matrix tablets, indicating their comparable sustained release effect. The potential of graft copolymers to sustain the drug release is well supported by *in-vivo* pharmacokinetic studies and their adequate physicochemical properties make them promising excipients for controlled drug delivery system.

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1. Introduction

Controlled release technology has rapidly emerged over the past few decades as a new interdisciplinary science that offers novel approaches for delivery of bioactive agents into systemic circulation at a predetermined rate, achievement of optimum therapeutic responses, prolonged efficacy and decreased toxicity [1]. Salbutamol Sulphate (SS), a directly acting sympathomimetic drug, is a good candidate for controlled release formulations since its short half life (2–4 h) which necessitates frequent administration to maintain constant therapeutic drug levels, but it is challenging because of its high water solubility [2]. In recent years, use of natural polymers such as starch, cellulose, chitosan etc. as carriers in controlled drug delivery applications has attracted the attention of investigators because of their inherent biocompatibility, biodegradability and biosafety [3–5]. But natural polymers share some common disadvantages like poor flow properties, inadequate compression behavior, thermal liability and enormous swelling owing to their hydrophilic nature. Hydrophilicity results in premature release of drug in the stomach/upper intestine, and therefore they should be protected while gaining entry into stomach and small intestine. This can be achieved by the modification of polysaccharides, such as cross linking, addition of protective coating, or grafting using acrylic monomers [6–8]. Among the currently available graft copolymers, starch-based graft copolymers has drawn considerable attention due to their potential value as directly compressible excipients for controlled release matrices and methyl methacrylate was chosen for grafting because of its known biocompatibility and non-toxic behavior, together with its hydrophobicity and ease of polymerization [9,10]. In terms of controlled release formulations, reservoir and matrix type tablets are most commonly used for modified release formulation. Especially matrix tablets, in which drug particles are embedded in the matrix core of the retardant polymeric material, formulated with direct compression technique, one of the best method to sustain the release rate effectively over a period of 10 h [11]. The afore mentioned facts, directed our interest to design oral controlled release matrix tablets of SS using starch-based graft copolymers as hydrophobic inert matrix. Evaluation of tablets for various physical characteristics, *in-vitro* release study and *in-vivo* bioavailability studies in rabbits upon oral administration were performed. Release kinetics are studied in depth by fitting dissolution profile in various kinetic models (viz. zero order, first order, Higuchi, Hixon–Crowell and Korsmeyer–Peppas models) and *in-vitro-in-vivo correlation* (IVIVC) is established by using the Wagner–Nelson method.

2. Materials and methods

2.1. Materials

Maize starch (St) was obtained from Universal Starch Chem Allied Ltd., (Mumbai India). Solvents of analytical grade were obtained from Merck Ltd., Germany. Gift sample of salbutamol sulphate was received from Micro Labs Limited Ltd., (Mumbai, India). Sprayed dried lactose and magnesium sulphate were obtained from Micro Labs Limited Ltd., (Mumbai, India). Hydroxypropylmethyl cellulose (HPMC K 100) was obtained from Micro Labs Limited Ltd., (Mumbai, India).

2.2. Method

2.2.1. *Synthesis of acetylated starch and graft copolymers [starch grafted poly (methyl methacrylate) (St-g-PMMA) & acetylated starch grafted poly (methyl methacrylate) (Ast-g-PMMA)]*

Acetylated starch (Ast) and graft copolymers were prepared in laboratory, based on information provided in our recent published research article [12]. Here on starch back bone, methyl methacrylate was grafted via redox reaction. Wherein, Ce(IV) ion was reduced to Ce(III) ion and made an active site on starch back bone for grafting of methyl methacrylate. Synthesized samples were used for further study.

2.2.2. *Tabletting*

2.2.2.1. Blend preparation. HPMC K100M was chosen for comparison with graft copolymers for its controlled release properties. Various combinations were selected and blend was prepared for salbutamol sulphate using starch/acetylated starch (Ast)/St-g-PMMA/Ast-g-PMMA/HPMC K100M, spay dried lactose, and magnesium stearate. These were physically blended until a homogenous mixture was obtained (composition as shown in Table 1). No more additives were included in order to get intrinsic information of the polymeric material itself.

2.2.2.2. Angle of repose. Angle of repose was derived for the powder blend as an indicator for flowability characteristics. This was determined by a fixed funnel and free standing cone method [13]. The blend was poured through a funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of the heap (r) was measured and the angle of repose (θ) was calculated by using the formula:

$$\theta = \tan^{-1}(h/r)$$

Table 1 – Composition of salbutamol sulphate controlled release tablets.

S. No.	Ingredients	Formulation code				
		F1	F2	F3	F4	F5
		Qty (mg/tablets)				
1	Salbutamol sulphate	8	8	8	8	8
2	Maize starch	159	–	–	–	–
3	Acetylated starch (Ast)	–	159	–	–	–
4	St-g-PMMA	–	–	159	–	–
5	Ast-g-PMMA	–	–	–	159	–
6	Hypromellose (HPMC K 100 M PREMIUM)	–	–	–	–	159
7	Spray dried lactose	30	30	30	30	30
8	Magnesium stearate	3	3	3	3	3
	Total weight	200	200	200	200	200

2.2.2.3. *Formulation of tablets*. Tablets were prepared by direct compression method. The homogenous mixtures of different blends with required flow properties were shifted through #60 sieve. This was manually fed into the die and compressed in hydraulic press using an 8 mm flat faced punch, with the crushing force of 70–80 N. The compressed tablets were flat and round shaped, with average weight of 200 mg. Physicochemical properties for various parameters were assessed on 50 tablets.

2.2.3. *Evaluation of tablets*

2.2.3.1. *Physical testing*. The physical testing on tablets of each batch was performed after a relaxation period of at least 24 h. Weight variation test was performed on 20 individually weighed (Citizen CY204 Analytical Balance, Minnesota, USA) tablets according to the official method of United State Pharmacopoeia. The thickness and diameter of ten tablets were measured individually using vernier caliper (Edutek Instrumentation, Ambala, India) and average value of thickness was calculated. The crushing strength (Newton) of prepared tablets was determined using Monsanto hardness tester (MHT-20, Campbell Electronics, Mumbai, India). Tablet friability was calculated as the percentage of weight loss occurred due to attrition during revolution of plastic chamber in 4 min span at the rate of 25 rpm, performed on 20 tablets using Roche friabilator (C-FT-20, Pharma Chem Machineries, Mumbai, India).

2.2.3.2. *Drug content*. Ten tablets were weighed individually and crushed into a fine powder by mortar and pestle. Crushed powder was weighed with quantity equivalent to 10 mg of the drug; this was transferred in five volumetric flasks separately. Distilled water was poured into each conical flask and these flasks were further subjected to sonication. Drug was extracted from 50 ml solution prepared previously using bath sonicator, with sonication time of 2 min. The actual drug content was determined using high performance liquid chromatography system of Adept series CECIL CE 4201 with UV/Visible detector at 277 nm and the drug concentration was determined using the standard calibration curve, covering the drug concentration from 5.0 to 50.0 μg/ml.

2.2.4. *In vitro and in vivo studies*

2.2.4.1. *In vitro release studies*. *In-vitro* release study was carried out using USP dissolution type II apparatus, with rotation speed of 50 rpm to provide information regarding *in-vitro* drug release information. This study was performed in simulated gastric fluid (0.1N HCl) for 2 h followed by same volume of simulated intestinal fluid (PBS, pH 6.8) for next 12 h. The dissolution media (900 ml) was maintained at 37 ± 0.5 °C throughout the study. At predetermined time intervals of 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 h, 5 ml sample was withdrawn with syringe using 0.45 μm syringe filter and the media was replaced with the fresh media to maintain an ideal sink condition. The percentage content was calculated by validated RP-HPLC method and used to calculate the percentage release on each time of dissolution profile. The cumulative percentage of drug released was plotted against time in order to obtain the release profile [14].

The column used for separation was octadecyl silane (C_{18}) with length 250 mm and internal diameter 4.6 mm (Phenomenex). Mobile phase used for analysis was composed of acetonitrile, methanol and water in the ratio of 60:20:20 (v/v), with pH adjusted to 2.8 using orthophosphoric acid, with flow rate of 0.5 ml/min. Sample of 20 μl was injected manually and peaks were monitored at 277 nm.

To study the mechanism of drug release from the optimized formulation of matrix tablets, *in vitro* release profile were plotted and correlated with various kinetic models like zero order (i.e. cumulative amount of drug released vs time), first order (log cumulative percentage of drug remaining vs time), Higuchi model (cumulative percentage of drug released vs square root of time) Korsmeyer–Peppas (log cumulative percentage of drug released vs log time) and Hixson–Crowell (cube root of concentration of drug remaining vs time) equations.

2.2.4.2. *In vivo studies*. Tablets prepared from graft copolymers (F3 and F4) with acceptable physical characteristics and optimum drug release behavior, were chosen for the *in-vivo* study. Pharmacokinetic parameters of graft copolymer formulations were compared against tablets prepared by native starch (F1), HPMC K 100M (F5) and commercial sustained release tablets (Asthalin SA-8 mg).

Male albino rabbits weighing 2.5–3.0 kg were randomly selected for the bioavailability studies. The animals were divided into five groups and each group comprised of six rabbits. Each group received one of the tested formulas namely F1, F3, F4, F5 and Asthalin SA-8 mg. The animals were fasted over night (before administering tablets) and during the course of experiment, animals under trial had free access to water. Tablets were kept behind the tongue to avoid its destruction due to biting followed by sufficient amount of water for easy swallowing. Blood samples (about 1 ml from each animal) were collected from orbital sinus, before dosing (zero time) and afterwards at different intervals post dosing *viz.* at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16,18, 20, 22, and 24 h. Samples were collected in microcentrifuge tubes containing 50 μl of 10% w/w disodium EDTA as an anticoagulant. The collected samples were immediately centrifuged at 10000 rpm for 10 min and plasma was separated and stored at −20 °C until analysis.

A high performance liquid chromatography system of Adept series CECIL CE 4201 with UV/Visible detector was used for analysis. The data was recorded by using the software "*power stream*". The column used for separation was octadecyl silane (C_{18}) with length 250 mm, internal diameter 4.6 mm (Phenomenex) and particle size 5 μ. Chloramphenicol was used as an internal standard. In 0.5 ml of plasma sample, 20 μl of internal standard (100 μg/ml) was added, drug was extracted from plasma using 5 ml methanol. Mobile phase used for analysis was composed of acetonitrile, methanol and water in the ratio of 60:20:20 (v/v), with pH adjusted to 2.8 using orthophosphoric acid, with flow rate of 0.5 ml/min. Sample of 20 μl was injected manually and peaks were monitored at 277 nm. Quantification of salbutamol sulphate was obtained by plotting SS to the internal standard peak area ratio as a function of its concentration.

2.2.5. *Assay method validation*

The developed method was validated following bioanalytical guidelines [15]. Bioanalytical method validation required the

Table 2 – Angle of repose as an indicator of powder flow property (All values are expressed as mean ± SD, $n = 3$).

Formulation code	Angle of repose	Type of flow
F1	32.34 ± 0.11	Passable
F2	30.41 ± 0.23	Passable
F3	25.73 ± 0.17	Good
F4	23.45 ± 0.19	Good
F5	19.11 ± 0.20	Excellent

determination of selectivity, linearity, LOD, LOQ, accuracy, recovery and precision respectively.

2.2.6. *In-vivo data analysis*

The plasma kinetic data were assessed with Kinetica® software (version 5). The maximum SS concentration in serum (C_{max}) and corresponding peak time (t_{max}) were determined by the inspection of the individual serum drug concentration–time profiles. The elimination rate constants (Ke) were obtained from least square fitted terminal log-linear portion of the serum concentration–time profile. The elimination half life ($t_{1/2}$) was calculated as 0.693/Ke. The area under the plasma concentration–time curve $[AUC]_{0-24}$ was determined by linear trapezoidal rule until last measurement point.

2.2.7. *In-vitro-in-vivo correlation (IVIVC) study*

In order to establish a level A IVIVC, the Wagner–Nelson method [16,17] was used to calculate the percentage of the drug absorbed:

$$F_{(t)} = C_{(t)} + k_e AUC_{(0-t)}$$

where $F_{(t)}$ is the amount absorbed. The fraction absorbed is determined by dividing the amount absorbed at any time by the plateau value, $k_e AUC_{(0-\infty)}$:

$$\text{Fraction absorbed}\left(\text{Fa}\right) = \frac{C_{(t)} + k_e AUC_{(0-t)}}{k_e AUC_{(0-\infty)}}$$

In-vitro fraction of drug released at each time points was subjected to Weibul model fit options in IVIVC tool kit of WinNonlin v 5.3. Level A IVIVC was developed by drawing a plot between the percentage drug absorbed (along y-axis) of a formulation and its percentage drug dissolved (along x-axis) followed by the regression analysis of each curve to evaluate the strength of correlation determining whether the curve is linear or non-linear [18,19]. The closer the value of determination coefficient to 1, the stronger is the correlation and linear is the curve. The correlation coefficient, prediction error of C_{max} and AUC were calculated to validate the predictability of IVIVC.

2.2.8. *Statistical analysis*

Data were subjected to analysis of variance (ANOVA) (Graph Pad Instat software v 3.06, CA, USA). Significant differences between formulations were analyzed using *student newmann keuls* multiple comparison test and obtained p values of <0.05 were considered to be statistically significant.

3. Results and discussion

3.1. *Flow property (angle of repose)*

Prior to compression, blends (F1–F5) were evaluated for their flow property. From the values of angle of repose, it is evident that blends having graft copolymers showed better flow properties than that of blends having acetylated starch and native starch (Table 2). For formulation containing maize starch (F1) and acetylated starch (F2), flow property was falling in the passable range, in which values of angle of repose were found to be 32.34° ± 0.11 and 30.41° ± 0.23 respectively (31–34° is said to be a passable range). While formulation with St-g-PMMA (F3) and Ast-g-PMMA (F4) had good flow property, wherein values of angle of repose were 25.73 ± 0.17 and 23.45°± 0.19 respectively. Hypromellose (F5) containing blend depicted excellent flow property (wherein angle of repose was 19.11°± 0.20).

3.2. *Standard physical test of tablets*

Formulations of salbutamol sulphate (F1–F5) with mentioned array of excipients had thickness ranging from 3.19 to 3.41 mm (Table 3). Drug content was found to be uniform among different batches of the tablets and was found between 100.98 and 104.21%. Hardness and percentage friability of the tablets of all the batches were found amid 3.27–4.01 kg/cm^2 and 0.53–0.84 %, respectively. Tablets with all the aforesaid compositions passed USP criteria for friability (<1.00% w/w). Outcome from friability assessment revealed good mechanical strength of the tablets. The percentage of weight variation of individual tablet to that of average weight was found within ±5% w/w, which fits in USP criteria for weight variation.

3.3. *In-vitro release study*

The *in-vitro* release profile of salbutamol sulphate matrix tablets in SGF followed by SIF is shown in Fig. 1. Study was conducted for the period of 14 h and a higher percentage of drug release was observed for matrices containing native starch (F1) compared with the ones having acetylated starch (F2) and graft copolymers (F3 & F4). The release of drug was

Table 3 – Physical properties of formulated salbutamol matrix tablets using graft copolymers and HPMC K 100 M as release retardants. (All values are expressed as mean ± SD, $n = 3$).

Formulation code	Weight deviation (mg)	Hardness (kg/cm^2)	Friability (%)	Thickness, mm	% Drug content
F1	204 ± 2.1	3.27 ± 0.57	0.75 ± 0.24	3.24 ± 0.22	102.47 ± 2.6
F2	206 ± 2.1	3.35 ± 0.87	0.53 ± 0.21	3.26 ± 0.52	100.98 ± 2.3
F3	203 ± 1.9	3.49 ± 0.92	0.69 ± 0.17	3.37 ± 0.47	101.57 ± 2.8
F4	205 ± 2.3	4.01 ± 0.53	0.77 ± 0.29	3.19 ± 0.38	104.21 ± 2.9
F5	204 ± 2.7	3.76 ± 0.73	0.84 ± 0.19	3.41 ± 0.29	103.61 ± 3.1

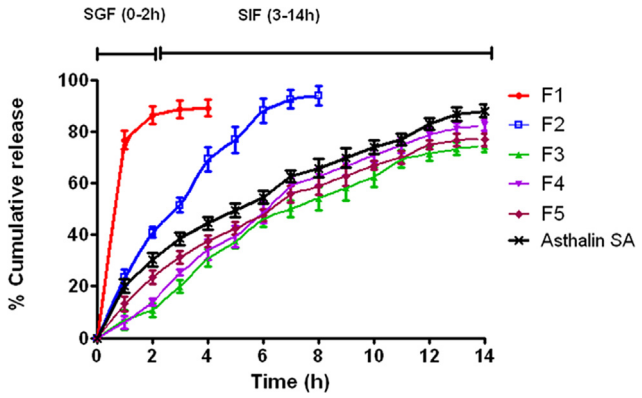

Fig. 1 – Release profiles of model drug salbutamol sulphate (SS) in SGF followed by SIF at 37 °C, with starch (F1), acetylated starch (F2), St-g-PMMA (F3), Ast-g-PMMA (F4), HPMC K 100 M (F5) as carriers, and commercial sustained release tablets (Asthalin SA-8 mg). Vertical bars represents mean ± SD ($n = 3$).

prolonged up to 14 h for matrices containing graft copolymers (F3 & F4) and up to 6 h for matrices containing acetylated starch (F2) whereas in case of starch >85% drug was released within 2 h. Significant retardation in drug release behavior of graft copolymer matrices could be attributed by a better swelling property of the polymers, while a fast drug release behavior of starch was attributed by the burst release tendency of starch causing tablets to break after immersing in dissolution media.

In SGF media, a remarkable decrement in drug release was observed in graft copolymer matrices, when St-g-PMMA was used as a carrier then 10.7% of the drug was released in initial 2 h, 13.8% of the drug was release in case of Ast-g-PMMA whereas tablets containing HPMC K 100M and commercial sustained release tablets (Asthalin SA-8 mg) showed % drug release 23.6% & 30.2% respectively. Comparison of drug release pattern of graft copolymers matrices (F3 and F4) with HPMC K 100M containing tablets & commercial sustained release tablets (Asthalin SA-8 mg) in SIF media revealed that formulation F3 and F4 released upto 74.4% and 82.5% of the drug in successive 12 h while at same time point HPMC K 100M tablets released 76.8% and Asthalin SA-8 mg released 87.8% of the drug. Retardation in drug release rate for graft copolymer formulations (F3 & F4) was almost equivalent to that of commercially used controlled release polymer i.e. HPMC K 100M and commercially available sustained release tablets (Asthalin SA-8 mg). Thus it can be inferred based on release profile of graft copolymer and commercially available formulation that graft copolymer has a potential application as a control release excipient in modified drug delivery.

3.4. *Analytical method validation*

The developed method was validated following ICH guidelines [20]. The *in-vitro* release study was validated to salbutamol tablets through the determination of specificity, linearity, precision and accuracy.

3.4.1. *Specificity*

The specificity of the *in-vitro* release test was evaluated through the analysis of placebo tablets (Fig. 2A). The specificity test by HPLC demonstrated that the excipients from tablets do not interfere in the drug peak because retention times of placebo (3.02 min) and salbutamol sulphate (5.22 min) are quite different (Fig. 2B).

3.4.2. *Linearity*

Linearity of the method was evaluated at five concentration levels ranging from 2 to 10 μg/ml ($y = 30874x - 6522$) with correlation coefficient of 0.9968.

3.4.3. *Precision*

The precision of the *in-vitro* release study were evaluated by analyzing intra-day precision and inter-day precision. The % RSD for intra-day precision is 1.68% and inter-day precision is 0.98%. According to ICH norms, in all condition %RSD was <2 which shows method is precise.

3.4.4. *Accuracy*

Recovery studies were performed to validate the accuracy of developed method. To the preanalysed sample solution, a definite concentration of standard drug was added and then its recovery was analyzed. The method was found to be accurate with % recovery of 98.42–101.53%.

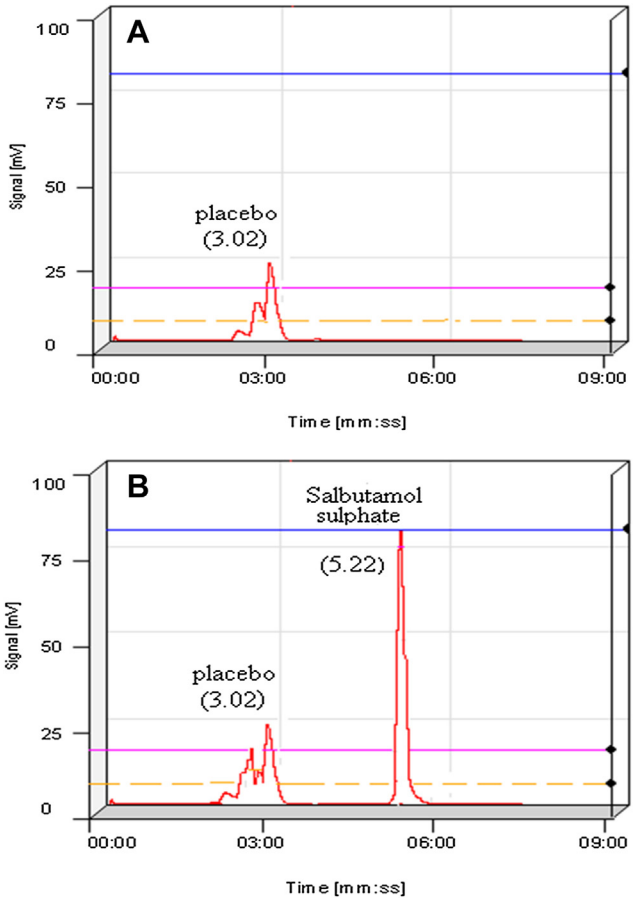

Fig. 2 – (A). Chromatogram for placebo (Specificity study). (B). HPLC chromatograms of salbutamol sulphate in graft copolymer matrix.

Table 4 – Comparative release kinetics parameter of all the batches of controlled release tablets.

Formulation code	Release kinetic parameters									
	Zero order		First order		Higuchi		Hixon–Crowell		Korsemeyer–Peppas	
	r^2	K_0	r^2	K_1	r^2	K_H	r^2	K_{HC}	r^2	n_P
F1	0.324	3.18	0.858	0.109	0.576	17.92	0.649	0.166	0.788	0.33
F2	0.646	5.25	0.979	0.129	0.864	25.67	0.889	0.261	0.83	0.38
F3	0.949	5.71	0.991	0.061	0.995	24.68	0.981	0.156	0.929	0.63
F4	0.951	5.42	0.989	0.051	0.991	23.38	0.987	0.138	0.988	0.67
F5	0.937	5.36	0.993	0.052	0.996	23.33	0.988	0.14	0.992	0.6

3.5. *Kinetics and mechanism of drug release*

The drug release mechanism was determined by fitting the *in-vitro* release profile in various release kinetic models and the values of release exponent (n_P), kinetic constant (K) and regression coefficient are shown in Table 4. Zero order, first order, Higuchi, Hixon–Crowell and Korsmeyer–Peppas are the major models to identify the drug release from sustained release formulations and criteria of selecting the most appropriate model was based on the its goodness of fitting. Starch and acetylated starch tablets (F1 &F2) showed a first order release, with regression value of 0.858 and 0.979 respectively. *In-vitro* release profile of graft copolymers matrix formulations (F3 & F4) and HPMC K 100M containing tablets (F5) are best expressed by the Higuchi model, as the plots showed high linearity with regression value of 0.995, 0.991, and 0.996 followed by first order kinetics with regression value of 0.991, 0.989, and 0.993 respectively. Two factors, however, diminish the applicability of Higuchi's equation to matrix systems. This model fails to explain the influence of swelling of the matrix upon hydration and gradual erosion of the matrix. Therefore, the *in-vitro* release data were also fitted to the well-known exponential Korsmeyer–Peppas equation and value of release exponent (n_P) explains the release mechanism of the drug from the tablets. The observed 'n_P' values for release profiles of formulation F3, F4 and F5 were fall in between 0.50 and 0.89 indicated anomalous release behavior coupled with diffusion and erosion. The release exponents for formulation F1 and F2 are less than 0.5 indicating quasi fickian diffusion mechanism of drug release.

3.6. *In-vivo study*

The results of the plasma drug concentration at different time intervals, after administration of formulation F1, F3, F4, F5 and Asthalin SA-8 mg tablet containing 8 mg of salbutamol sulphate to rabbits, are presented in Table 5. SS was detected and quantified in plasma by using HPLC method and mean plasma concentration curve of formulated tablets and commercial tablets were plotted as depicted in Fig. 3.

Graft copolymer matrices (F3 & F4) and starch matrix (F1) showed significantly different ($P < 0.05$) C_{max} values of about 578.9, 546.7 and 924.1 ng/ml, respectively. Decrement of C_{max} value in case of controlled release tablets indicated and justified sustained and prolonged release potential of graft copolymer. Observed mean plasma [AUC] $_{0-24}$ values for F3 (6460.12 ng h/ml) and F4 (6679.48 ng h/ml) was significantly ($P < 0.05$) higher than F1 (5261.19 ng h/ml) which indicated improvement in relative bioavailability of graft copolymer matrices. The t_{max} value of graft copolymer matrices F3 (6 h) and F4 (7.5 h) was significantly ($P < 0.05$) higher than starch matrix F1 (1.5 h), which indicates the slow absorption rate in graft copolymer tablets due to extended release effect of hydrophobic polymer matrix. When elimination rate constants (Ke) for above mentioned formulations were compared, it was found that formulation F1 has Ke value was 0.127, this Ke value significantly went down in F3 (Ke = 0.057) and F4 (Ke = 0.053), indicating slow elimination rate of the drug from body in graft copolymer formulations. The elimination half life ($t_{½}$) of the F3 (12.15 h) and F4 (13.07 h) was more than F1 (5.45 h), which confirmed prolonged availability of SS in body. However there was no significant difference in the pharmacokinetic parameters (t_{max}, C_{max}, AUC, Ke, and $t_{1/2}$) for graft copolymers matrices, HPMC K100M matrix tablets and commercial tablets. Results revealed that the graft copolymer matrices provided comparable sustained and prolonged effect to that of HPMC K 100M matrix tablets and Asthalin SA-8 mg (commercial tablets), so graft copolymers can be used as potential excipients in controlled drug delivery system.

Table 5 – Pharmacokinetic parameters of formulated (F1, F3, F4 & F5) and marketed tablets (Asthalin SA) of salbutamol sulphate in rabbits (All values are expressed as mean ± SD, $n = 3$).

Pharmacokinetic parameters	F1	F3	F4	F5	Asthalin SA
C_{max} (ng/ml)	924.1 ± 10.1	578.9 ± 9.1[a]	546.7 ± 8.9[a]	598.1 ± 10.7[a]	601.9 ± 6.3[a]
t_{max} (h)	1.5 ± 0.23	6.0 ± 1.3[a]	7.5 ± 1.2[a]	6.2 ± 1.1[a]	7.1 ± 0.75[a]
[AUC] $_{0-24}$ (ng-h/ml)	5261.19 ± 5.1	6460.12 ± 8.3	6679.48 ± 7.5	6276.34 ± 6.8	6592.28 ± 5.8
Ke (h^{-1})	0.127 ± 0.017	0.057 ± 0.011[a]	0.053 ± 0.011[a]	0.049 ± 0.012[a]	0.059 ± 0.014[a]
$t_{1/2}$ (h)	5.45 ± 0.59	12.15 ± 0.91[a]	13.07 ± 1.21[a]	14.14 ± 1.09[a]	14.43 ± 0.87[a]

[a] Significantly different from starch matrix tablets ($P < 0.05$).

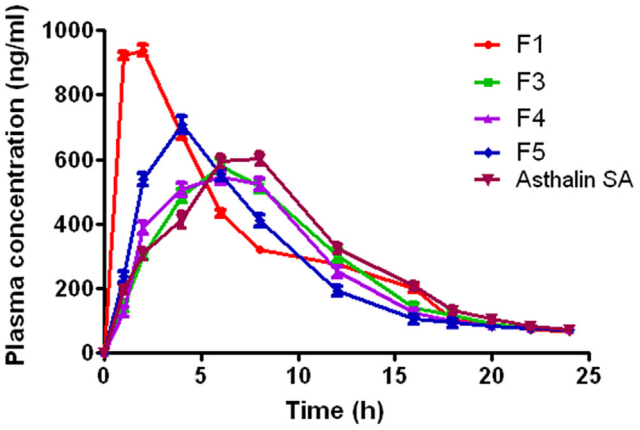

Fig. 3 – Comparative *in-vivo* pharmacokinetic study of tablets having starch matrix (F1), graft copolymer matrices (F3 and F4), HPMC K 100 M matrix (F5) and Asthalin SA. Vertical bars represents mean ± SD ($n = 3$).

3.7. *Bioanalytical method validation*

3.7.1. *Selectivity*

The chromatogram of salbutamol sulphate extracted from plasma is shown in Fig. 4. With the chromatogram it is clear that retention times of plasma (2.03 min) and salbutamol sulphate (5.21 min) are quite different means plasma components are not showing interference with the drug elution.

3.7.2. *Limit of detection (LOD) and Limit of quantification (LOQ)*

LOD and LOQ for *salbutamol sulphate* were 33.97 ng/ml and 101.91 ng/ml, respectively.

3.7.3. *Linearity*

Standard calibration curve in rabbit plasma was found to be linear at concentrations ranging from 100 to 1200 ng/ml ($Y = 0.0318x + 1.4027$) with correlation coefficient of 0.9968.

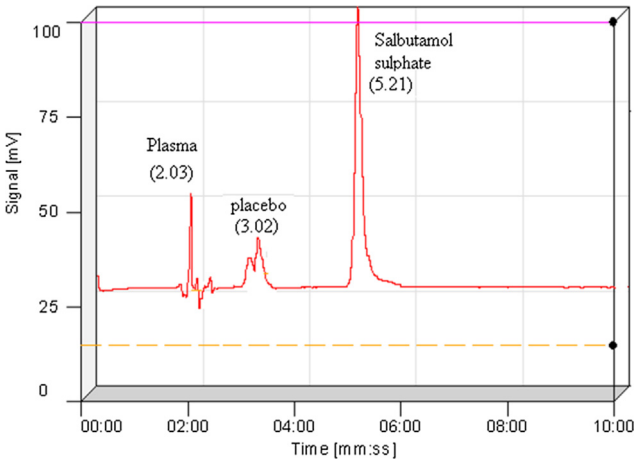

Fig. 4 – HPLC chromatogram of salbutamol sulphate extracted from plasma.

3.7.4. *Accuracy*

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug was added and then its recovery was analyzed. The method was found to be accurate if % recovery is 100 ± 15% and % CV is <15%. The % recovery of drug in plasma found in the range of 89.59–92.41% and coefficient of variation was 2.21–3.81. Since the % recovery and % CV is within the range, it shows accuracy of method.

3.7.5. *Precision*

Precision of the method was assessed by intra-day and inter-day analysis of six replicates for each concentration at 3 different concentration levels (100, 600, and 1200 ng/ml, respectively). The coefficient of variation (CV) at each concentration level was expressed as precision. The method proved to be precise because the % CV for is not more than 5.61% at three different concentration levels.

3.8. *In-vitro-in-vivo correlation*

In-vitro-in-vivo correlation was established by plotting the graph of fraction absorbed *in-vivo* against fraction dissolved *in-vitro*. In this study, F_a and F_d data of graft copolymer

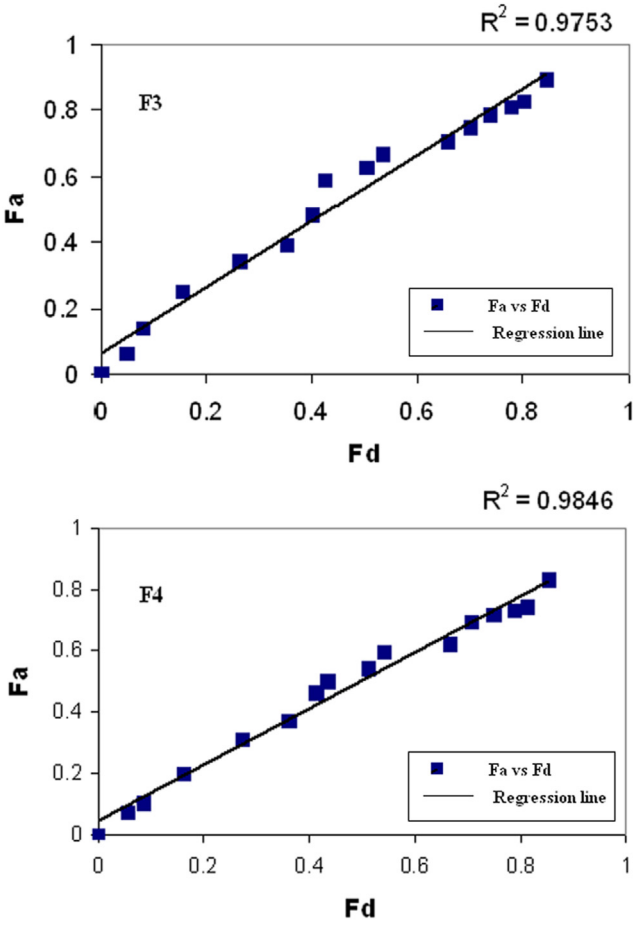

Fig. 5 – Plot of the fraction absorbed *in-vivo* vs. fraction dissolved in-vitro for graft copolymer formulations F3 & F4 respectively.

formulations (F3 & F4) were analyzed and a reliable correlation ($R^2 > 0.97$) was observed between fraction dissolved *in-vitro* and fraction absorbed *in-vivo* shown in Fig. 5. The prediction error of the C_{max} and AUC of the correlation were found to be −9.62% and 12.02%. The low prediction error indicates the reliability of model towards carrying out predictions; hence it can be selected as a bio relevant tool to screen the best formulation and used for waiver approval in future.

4. Conclusion

Release characteristics of salbutamol sulphate was evaluated and assessed using graft copolymer as a carrier matrix and was compared with HPMC K100M containing controlled release matrix system and marketed formulation (Asthalin SA-8 mg). It was revealed that the graft copolymerization improves the flow property of native starch and modified starch. Matrix tablets prepared employing graft copolymers imparted slow release profile of up to 14 h, similar was in case of HPMC K100M matrix formulation.

Statistical model and kinetic data revealed that in graft copolymer based matrices; drug release was governed by diffusion and erosion mechanism. The *in-vitro* release profiles of salbutamol sulphate starch matrix tablets showed >70% drug release within 1 h whereas in graft copolymer matrix tablets same amount of drug was released upto 9 h. This reflects the potential of graft copolymers to sustain the drug release and the results are well supported by *in-vivo* pharmacokinetic studies. Pharmacokinetic parameter i.e. t_{max} (1.5 ± 0.23 h) values of the starch matrix tablets clearly indicates the ability of the formulation to release the drug immediately upon reaching the GIT which is due to the hydrophilic nature and high solubility of starch. The dramatic shift in t_{max} of the graft copolymer matrix tablets [F3 (6.0 ± 1.3) & F4 (7.5 ± 1.2) h] w.r.t starch matrix tablets (1.5 ± 0.23 h) are indicative of the graft copolymer carrier to control the release of the drug even under the gastrointestinal environment. Whereas pharmacokinetic parameters (t_{max}, C_{max}, AUC, Ke, and $t_{1/2}$) of graft copolymers matrices (F3 & F4), HPMC K 100M matrix tablets (F5) and marketed tablets are comparable, indicating that graft copolymers might be a promising vehicle for sustained release preparations in oral therapy. A well *in-vitro-in-vivo* correlation was observed for graft copolymer formulations, which indicates their suitability for the waiver approval in future.

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