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Original research paper

A two pulse drug delivery system for amoxicillin: An attempt to counter the scourge of bacterial resistance against antibiotics

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Bearing in mind the present scenario of the increasing biological tolerance of bacteria against antibiotics, a time controlled two pulse dosage form of amoxicillin was developed. The compression coating inlay tablet approach was used to deliver the drug in two pulses to different parts of the GIT after a well defined lag time between the two releases. This was made possible by formulating a core containing one of the two drug fractions (intended to be delivered as the second pulse), which was spray coated with a suspension of ethyl cellulose and a hydrophilic but water insoluble agent as a pore former (microcrystalline cellulose). Coating of up to 5 % (*m*/*m*) was applied over the core tablet, giving a corresponding lag of 3, 5, 7 and 12 h. Increasing the level of coating led to retardation of the water uptake capacity of the core, leading to prolongation of the lag time. Microcrystalline cellulose was used as a hydrophilic but water insoluble porosity modifier in the barrier layer, varying the concentration of which had a significant effect on shortening or prolongation of the lag time. This coated system was further partially compression coated with the remaining drug fraction (to be released as the first immediate release pulse) with a disintegrant, giving a final tablet. The core tablet and the final two pulse inlay tablet were further investigated for their *in vitro* performance.

Keywords: amoxicillin pulsatile drug release, two pulse, lag time, ethyl cellulose, microcrystalline cellulose

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Controlled release formulations have many advantages over immediate release formulations enumerated in the literature, such as less frequent drug administration, lower plasma peak concentration to avoid adverse effects, and improved patient compliance (1). Principles of delayed release delivery have lately been applied in developing a newer version of drug delivery, popularly known as chronotherapeutic drug delivery system. Such systems are developed based on the concept of biological/circadian rhythm

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or chronopharmacological behavior of symptom manifestation of specific conditions such as asthma, hypertension and allergic rhinitis (1–7). The concept of delivering the drug in this fashion was earlier restricted to the development of single pulse delivery systems for achieving maximum concentration in dependence on the symptoms (8). The concept of delivering the drugs after a well defined lag phase leads to the development of more than one pulse (multiple pulses) delivery systems. The multiple pulse delivery system offers advantages over biological resistance to antibiotics. Spore forming bacteria in the dormant phase are more prone to getting killed (5). The bacterial killing rate *via* antibiotics varies at concentrations of around 4 to 5 times the MBC (minimum bactericidal concentration); high concentrations will not kill bacteria faster than lower concentrations at the MIC (minimum inhibitory concentration) value (9–12).

A multiple pulse drug delivery system differs from single pulse chronotherapeutic delivery in the number of pulses delivered concomitantly with the corresponding number of lags. The first pulse is an immediate release pulse that begins to dissolve in the stomach. The subsequent pulses may be designed to provide a lag time followed by rapid release/delayed release pulses, which may be formulated using sustained or controlled release polymers, depending on the solubility of the drug or the dissolution properties of the core (4, 6).

EXPERIMENTAL

Materials

Amoxicillin trihydrate (Zest Pharma, India), ethyl cellulose 20 cps (S.D. Fine Chemicals Ltd., India), croscarmellose sodium (Ranbaxy Research Labs., India), sodium starch glycollate (Ranbaxy Research Labs.), Vivapur PH 102 (directly compressible binder, MCC grade) (Jubiliant Organosys Noida, India), and magnesium stearate (Central Drug House, India) were used. All other reagents were of analytical grade and were used as received.

Formulation of the two pulse release tablet

The two pulse tablet consisted of the following parts: (*i*) a core containing drug and disintegrant (second pulse release), (*ii*) an intermediate coating layer composed of ethyl cellulose along with a hydrophilic but water insoluble pore former (microcrystalline cellulose) to delay drug release (barrier layer to provide the lag), (*iii*) an outer compression coated layer of the second drug fraction over and above the core (first pulse release).

Upon administration *via* oral route of the two pulse tablet, the first pulse would be released on reaching the stomach and the next pulse is expected to be released in the intestine due to the barrier layer.

Preparation of core tablets. – Direct compression was applied as the primary tabletting method for compressing the core. Various excipients belonging to the different functional categories were used during optimization of the formulation composition. Croscarmellose sodium was used as a disintegrant based on the swelling index. Core tablets (composition in Table III) containing one of the drug dose fractions (core tablet dose, 200

mg amoxicillin), Ac-Di-Sol (5 %, *m*/*m*), magnesium stearate (0.5 %, *m*/*m*) and Aerosil (0.5 %, *m*/*m*) were prepared by the direct compression method. Weighed amounts of each ingredient (other than lubricants) were uniformly mixed after sifting through a 0.5 mm sieve. After 15 minutes of mixing, lubricating agents (pre-sieved through 0.17 mm) were added and the mixing continued for a further 5 minutes. This tablet mix was transferred to a sixteen-punch tablet press (Cadmach Machinery, India) having an 8-mm diameter standard concave punch and die set. The desired tablet mass was achieved by adjusting the fill volume of the die. The most appropriate compression force was obtained by adjusting the dial setting on the tabletting press. Finally, the compression force that was applied for each tablet formula was determined by performing hardness tests immediately after fabrication of a few preliminary tablets. Hardness of each tablet was kept within a range of 58.8–68.8 N. The tablets were then stored in an airtight polyethylene bag and protected from moisture until they were coated.

Preparation of coating solution. – The coating solution was prepared by dissolving ethyl cellulose in isopropyl alcohol (IPA) and dichlomethane (DCM) as solvent in a ratio of 4:6. Ethyl cellulose was slowly added in the agitating solvent mixture to obtain a clear solution. Microcrystalline cellulose (MCC) and dibutylphthalate (as a plasticizer) were further added to the clear polymer solution. MCC is insoluble in the solvent mixture was kept suspended throughout the coating operation with constant stirring through a mechanical shaker. The composition of coating solution has been given in Table I.

Table I. Coating solution composition

Ingredient	Coating solution composition (%, *m*/*m*)
Ethyl cellulose 20 cps	2.25
Vivapur PH 102	0.75
Dibutyl phthalate	0.5
Isopropyl alcohol (40 %)	qs
Dichloromethane (60 %)	qs

Coating of core tablets. – The core tablets were coated in a conventional rotating pan and was done as per the conditions given in Table II. The coating pan was set at an angle of 45° from the horizontal surface. After continuosly drying the tablets in the rotating coating pan under hot air, the tablets were further dried in the coating pan for 15 min at 40 °C. The tablets were then placed in a hot air oven for 2 h at 40 °C to remove the solvent traces.

Optimization of lag time of the two pulse drug release system and in vitro *analysis.* – The desired lag time of the formulation between the two pulses was set at 3 h. The lag time of the core tablet was optimized by coating the core with different levels of the coating solution (1, 2, 3, 4 and 5 %, *m*/*m*) and *in vitro r*elease behavior was studied. Dissolution testing was performed in a USP type II apparatus (paddle type) (13) with 75 rpm in 900

Table II. Coating conditions employed for pan coating of the core tablet

Parameter	Condition
Pan rotation speed (rpm)	25
Inlet air temperature (°C)	45
Bed temperature (°C)	30–34
Atomizing air pressure (Pa)	1.2×10^5
Flow rate (mL min^{-1})	3
Nozzle diameter (mm)	1

Table III. Layout of intact two pulse tablet

Composition of the layer	Ingredient	Mass (mg per tablet)
Core tablet (inner layer)	Amoxicillin	200
	Ac-Di-sol	10
	Magnesium stearate	1
	Aerosil	1
Water barrier layer (middle layer)[a]	Ethyl cellulose 20 cps	2.25
	Vivapur PH 102	0.75
	Dibutyl phthalate	0.5
Compression coating blend (outer layer)	Amoxicillin	300
	Sodium starch glycolate	15
	Magnesium stearate	2
	Aerosil	2
	Amaranth	0.5

mL phosphate buffer, pH 7.4, at 37 ± 0.5 °C (n = 3). Samples (5 mL) were taken at predetermined time intervals and the quantity of drug released was assayed using a UV-spectrophotometer (Shimadzu UV-1601, double beam, Japan) at 229 nm.

Effect of pore former concentrations. – The concentration of pore former was studied by coating core tablets keeping the EC concentration of 2.25 % (m/m) and the core tablet mass build up of 2% as a constant. In addition to the one mentioned above (Table I), three other coating solutions were prepared by changing the MCC concentration and are given in Table I.

Preparation of the final two pulse release tablet. – After optimizing the core tablet and attaining the desired release profile, the final two pulse tablet was prepared in the form of an inlay or a dot tablet design. The tablet was prepared in a semi automatic manner by compression coating. The composition of the outer blend (first pulse) release fraction is given in Table III.

Table IV. Coating solution composition (change in former pore concentration)

Ingredient	Composition 1 (%, *m*/*m*)	Composition 2 (%, *m*/*m*)	Composition 3 (%, *m*/*m*)	Composition 4 (%, *m*/*m*)
Ethyl cellulose (20 cps)	2.25	2.25	2.25	2.25
Vivapur PH 102	0.5	0.75	1.5	2.0
Dibutyl phthalate	0.5	0.5	0.5	0.5
Isopropyl alcohol (40 %)	qs	qs	qs	qs
Dichloromethane (60 %)	qs	qs	qs	qs

To prepare inlay tablet, the bottom of the die cavity was filled with a blend of pre--weighed drug (300 mg) and disintegrant, sodium starch glycollate (5 %) and the core was placed upon it. Due to pushing of the core tablet, the blend got displaced from the bottom and rearranged in the clearances between the core tablet and die wall, resulting in coverage of the core tablets from all the sides, except for the top surface. The hardness of the final tablet was kept in the range of 147–157 N. The pattern of tablet cleavage during the hardness test was monitored. The tablet cleaved from the centre without separation of the core and the surrounding layer. The core tablet (pan coated) and the final two pulse inlay tablet are displayed in Fig. 1. Drug dose was divided into two parts: core tablet (200 mg), compression coated drug dose (300 mg). Compression coating over the core for the two pulse design as high drug loading may not be possible with pan coating alone. In yet another modification of such a system, the core tablet and the outer drug fraction blend can be put inside a hard gelatin capsule shell and administered in the form of a two pulse capsule. A full compression coat (complete coating from all sides) of the core with the drug fraction could have been applied (instead of coating the core partially/incompletely). This was not done to avoid an additional step in the tablet making process. For complete compression coating, an additional blend fraction needs to be weighed for covering the core from the upper side as well.

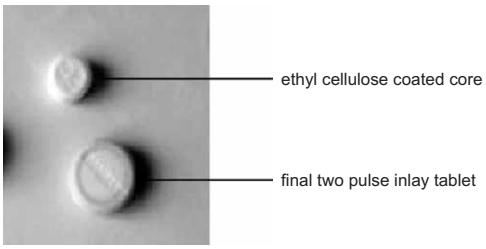

Fig. 1. Photograph showing the amoxicillin ethyl cellulose coated core and the final two pulse inlay tablet

The tablet when ingested has to face changing body conditions in relation to the shear, the supply of medium and the pH of the medium. The in-house prepared tablet being a combination of an immediate release and delayed release component intended for a specific purpose, was desirable to study the effect of change in the dissolution pH

Table V. Protocol for analysis of amoxicillin two pulse tablets

Chronology time	Medium	pH	Simulates
1 h	0.1 mol L^{-1} HCl (900 mL)	1.2	Stomach (fasting)
+ 15 min	Phosphate buffer (500 mL)	6.5	Proximal jejunum
+ 15 min	Phosphate buffer (500 mL)	6.8	Distal jejunum
+ 30 min	Phosphate buffer (500 mL)	7.2	Proximal ileum
+ 120 min	Phosphate buffer (500 mL)	7.5	Distal ileum
Rest of the study	Phosphate buffer (500 mL)	6.5	Ascending/transverse colon

simulating the body conditions. A protocol was designed for that purpose (Table V) with a set time interval for each dissolution medium in which the tablet was supposed to stay simulating the body conditions (14).

RESULTS AND DISCUSSION

Influence of the barrier layer on lag time. – Ethyl cellulose was the polymer of choice for coating along with MCC as a pore former because the combination forms an excellent film suitable for the desired release profile. A significant alteration of the lag time of drug release from the coated tablets was observed with five levels of coating. Lag time gets prolonged on increasing the coating level/thickness of core tablets. There was a significant alteration in the pulse release after the lag phase in case of coating levels above 3 %. The release was abrupt or pulse release (or equivalent to immediate release) at 1 % of coating with no significant lag phase. However, after a lag time of 3 h, the release was also abrupt with 2 % of coating, but as the coating level increased up to 3 % and above, the release after the lag phase got slowed down giving a typical controlled release (Fig. 2a). The formulation with 2 % (*m*/*m*) coating gave the desired lag time of 3 h. The rationale of getting the desired lag time of 3 h is that designed two pulse tablets are given four times a day (each 6 h). This means that the first pulse is immediate release like the conventional release profile and the expected time to release the drug is about 1.5 h (300 mg, the first dose to maintain the plasma drug concentration up to MIC and before the concentration falls below MIC); the next pulse of drug release (lag time, 3 h) would be furnished in 1.5 h (200 mg). Therefore, the overall time to accomplish the drug release for one tablet is (1.5 + 3 + 1.5), *i.e.*, 6 h.

Effect of the pore former concentration. – Microcrystalline cellulose was used as a pore former owing to its properties mentioned above and complementing exactly to the objective of the study. The concentration of MCC pore former affected drug release, as it is evident from the release profiles (Fig. 2b). An evident decrease in the lag phase and a corresponding increase in the drug release were observed with increasing concentrations of MCC (0.5 %, *m*/*m*) in the coating solution. This is attributed to fact that MCC forms fine pores/channels over the tablet surface on contact with water and hence early exit of the drug from the channels might be possible at higher concentration. However,

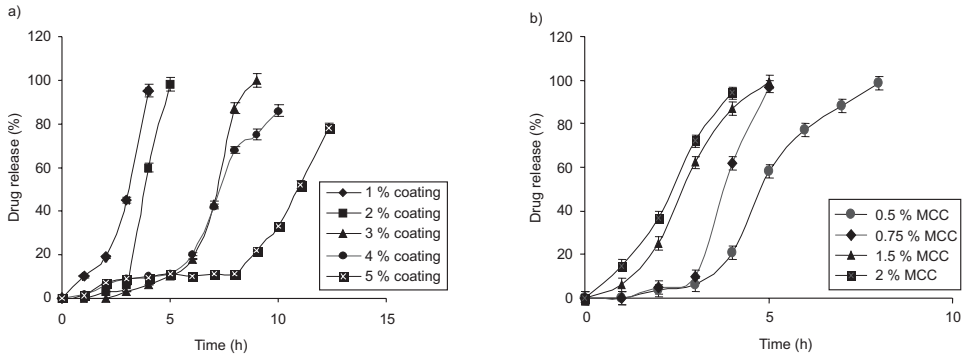

Fig. 2. Drug release profile with different: a) coating concentrations; b) pore former concentrations in the coating solution with constant ethyl cellulose coating level of 2 % (*m*/*m*), in phosphate buffer, pH 7.4 (mean ± SD, $n = 3$).

while visibly monitoring each tablet during the dissolution, it was observed that in all the release studies there was no case of premature rupture of the coat from the core and the core contents squirting out. The integrity of the coat was maintained even when the contained a hydrophilic component such as MCC. This may be attributed to the water imbibing capacity of water insoluble MCC.

Rationale for the use of excipients in the coating solution as per the objective of the study: ethyl cellulose is a pH independent inert polymer, insoluble throughout the GI tract. It has, however, excellent film forming capabilities and is often used in a variety of tablets and pellets for providing extended or delayed release coatings. Its controlled or delayed release function is attributed to its substantially water impermeable nature, since it allows very little solvent entry in the core not by its dissolution but through inter-particle pores in the plastic film. In the present study, the desired lag time to be applied to the core tablet had to be sufficient to prevent the core from breaking in the initial phase of dissolution in the GIT. For this purpose, EC was the polymer of choice for providing delayed release characteristics to the tablet.

Since solvent entry in the EC film is very low, to allow the solvent to enter the core, MCC was used as a pore former due to its special property of being hydrophilic as well as water insoluble. MCC employed in the concentration mentioned above meets this requirement. The time that the solvent takes to enter inside the core through MCC and the time that the inner disintegrant (Acdisol) in the core takes to swell to attain the threshold swellability of Acdisol are just sufficient enough to rupture the outer already loosened layer of EC and MCC is the time that forms the lag phase. Some low viscosity HPMCs (Methocel E-5 and E-15) were evaluated for playing the same role as MCC, but could not be screened since they solubilized and started releasing the core contents prematurely before the desired lag.

Effect of dissolution medium pH on lag time

The release obtained in each dissolution medium (0.1 mol L^{-1} HCl, pH 1.2, phosphate buffer, pH 6.5, 6.8, 7.2, 7.5) at the set time intervals was calculated and the plot was obtained from cumulative release calculations (Fig. 3). Two distinct pulse releases can be seen in the profile from the *in vitro* studies. The lag time and hence release profile remained unaffected by altering the pH of dissolution media. However, a better understanding of the concept can be gained after evaluating the developed formulation *in vivo*, calculating the drug concentration in plasma and observing the clinical effects.

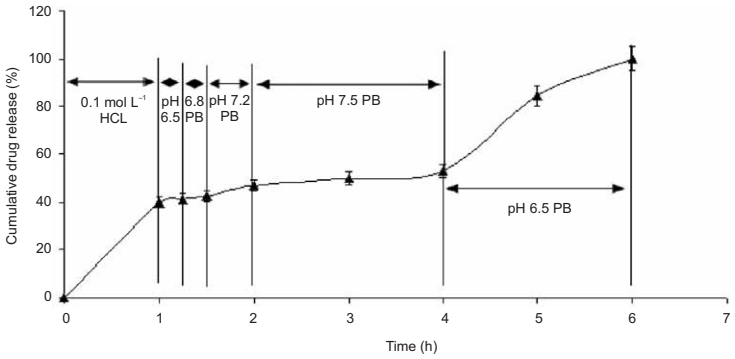

Fig. 3. Drug release from two pulse tablets: outer layer of the tablet constitutes the first pulse, which released the drug in the stomach within 1 h, the second pulse gave delayed release after a preset lag time of 3 h in phosphate buffer (mean ± SD, n = 3).

CONCLUSIONS

The concept of providing two pulses is in its early infancy and promises to go a long way further due to the increasingly serious issue of biological tolerance. The in-house developed formulation successfully demonstrated provision of a two fraction release of amoxicillin separated by a well defined time controlled lag phase.

A two pulse tablet consisting of a coated core tablet (inner layer) having the compression coating drug (outer layer) of well defined lag time with controlled drug release was developed. This delivery system is proposed to counter the serious problem of bacterial resistance against antibiotics. Such type of release in spurts has proved to be more effective in successfully exterminating the microbes with a comparatively low dose compared to the conventional dosage regimens.

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S A Ž E T A K

Dvopulsni sustav za isporuku amoksicilina: Pokušaj sprečavanja bakterijske rezistencije na antibiotike

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Zbog sve učestalije pojave rezistencije bakterija na antibiotike, razvijen je dvopulsni sustav s vremenskom kontrolom za isporuku amoksicilina. Sustav čine slojevite tablete s obloženim slojem dobivenim metodom kompresije, koji omogućavaju isporuku lijeka u dva pulsa u različite dijelove gastrointestinalnog trakta, s utvrđenom odgodom između dva oslobađanja. Ovakav način oslobađanja postignut je s pripravkom koji u jezgri tablete sadrži jednu frakciju lijeka (koja se oslobađa kao drugi puls), a u oblozi drugu. Obloženi dio dobiven je sprejanjem sa suspenzijom etilceluloze i hidrofilnog, ali vodonetopljivog sredstva koji tvori pore (mikrokristalinična celuloza). Oblaganje sa slojem koji čini 1 do 5 % (*m*/*m*) mase jezgre postignut je vremenski odmak drugog pulsa od 3, 5, 7 i 12 h. Povećanjem mase obložnog sloja smanjuje se kapacitet prodiranja vode u jezgru tablete, što produljuje vrijeme drugog pulsa. Mikrokristalinična celuloza uporijebljena je kao hidrofilno, vodonetopljivo sredstvo za kotrolu poroznosti u barijernom sloju. Promjena koncentracije celuloze značajno je utjecala na skraćenje ili produljenje vremenskog odmaka. Obloženi sustav je potom djelomično obložen s preostalom frakcijom lijeka (koja se oslobađa odmah u prvom pulsu) pomiješanom s dezintegratorom. Tableta s jezgrom i dvopulsna slojevita tableta ispitivane su *in vitro*.

Ključne riječi: amoksicilin, pulsativno oslobađanje lijeka, dva pulsa, vremenski odmak, etilceluloza, mikrokristalinična celuloza

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