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Der Pharmacia Lettre, 2017, 9[7]:1-9
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Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Formulation and Evaluation of Bilayer Tablets of Nitazoxanide

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ABSTRACT

The aim of present investigation was to formulate bilayer tablet of Nitazoxanide by using the wet granulation method. In this formula one layer provided the loading dose by immediate drug release and another layer provided maintenance dose up to 12hrs by controlled release. The drug excipient compatibility study was carried out by FTIR study, there was no interaction found. Sodium stach glycollate was used as superdisintegrant in immediate release layer and controlled release fraction was formulated by using HPMC E 15 polymer. The prepared granules were evaluated for angle of repose, bulk density, tapped density and compressibility index which showed satisfactory result. The prepared bilayer tablets were evaluated for thickness, hardness, friability and in-vitro release studies. In-vitro dissolution study was carried out for 12 hours using USP dissolution apparatus I using phosphate buffer of 1.2 pH and 7.4 pH as dissolution medium. The bilayer tablet showed initial burst release to provide the loading dose of drug followed by controlled release up to 12 hours. Concentration of polymer & superdisintegrant ratio influenced drug release profile. As the polymer concentration was increased in controlled release layer the % drug release decreased whereas on increasing superdisintegrant concentration in immediate release the % drug release increased. The batch F9 showed maximum release of 95% upto 12 hrs and was selected as best formulation. Stability study was carried out for the optimized formulation at 40ºC/75% RH for 3 months; the result shows that there was no significant change in physical and chemical attributes of the tablet.

Keywords: *Nitazoxanide, Bilayer tablet, HPMC E15, DSC, Immediate release*.

MATERIALS AND METHODS

Women's Nitazoxanide (NTZ)—of the thiazolide family—is a safe and inexpensive broad-spectrum, FDA approved, drug. NTZ is traditionally utilized for the treatment of anaerobic intestinal parasites Giardia lamblia and Cryptosporidium parvum, and has also been found efficacious in the treatment of other anaerobic bacteria and parasites residing in the human bowel [1]. Studies of protozoa and anaerobic bacteria have shown that NTZ inhibits pyruvate-ferredoxin oxidoreductase (PFOR), an essential enzyme for anaerobic energy metabolism [2]. The pharmacological effects of NTZ are not restricted to antiparasitic activities. In fact, NTZ has also been successfully used to promote HCV elimination by improving interferon signaling and promoting autophagy [3]. The drug, Nitazoxanide shows variable absorption in different dosage formulations. The higher dose of nitazoxanide produces side-effect in the intestinal tract that could be overcome by developing a new optimized dosage formulations i.e. Bilayer tablets. Bilayer tablets increases bioavailability of nitazoxanide, enhances the absorption and also increases the therapeutic efficacy while reducing the frequency of dosage intake.

The layered tablet concept has been utilized to develop controlled-release formulations [4, 5]. Such a tablet is considered as a biphasic delivery system that is designed to release the drug at two different rates and is usually composed of a fast-release layer combined with sustained-release layers [6]. Generally, conventional controlled-release dosage forms delay the release of drugs and do not provide a rapid onset of action after oral administration. Hence, the layered tablets offer a pharmacokinetic advantage over conventional controlled- release dosage forms, as the drug is quickly released from the fast-release layer, leading to rapid rise of drug plasma concentration followed by continuation of drug release from the sustained-release layer [7].

Reports have been made for use of sodium starch glycolate (SSG), Cross carmellose, cross povidone, and Hydroxy propyl methyl cellulose (HPMC), chitosan, Xanthan Gum [8] in the formulation of bilayer tablets as superdisintegrants and sustained release polymer, respectively, in the formulation of bilayer tablets [9].

In The present study, bilayer tablet of nitazoxanide in which one layer of immediate release and second layer of sustained release of NTZ was designed. The sustained release layer was formulated using NTZ in combination with HPMC E15. The immediate layer was formulated using sodium starch glycolate as superdisintegrant.

MATERIALS AND METHODS

Materials

Nitazoxanide was received as a gift sample by Ind- Swift Laboratories Limited, Samba, India. Hydroxypropyl methyl cellulose and Magnesium stearate were procured from C.D.H. New Delhi. Sodium starch glycollate, Silicon dioxide and Microcrystalline cellulose were procured from S. D Fine Chemicals, Mumbai, India. HPMC E 15 was procured from Ajanta Pharma Ltd. Aurangabad. All other chemicals and reagents used were of analytical grade, and were used as received.

Methods

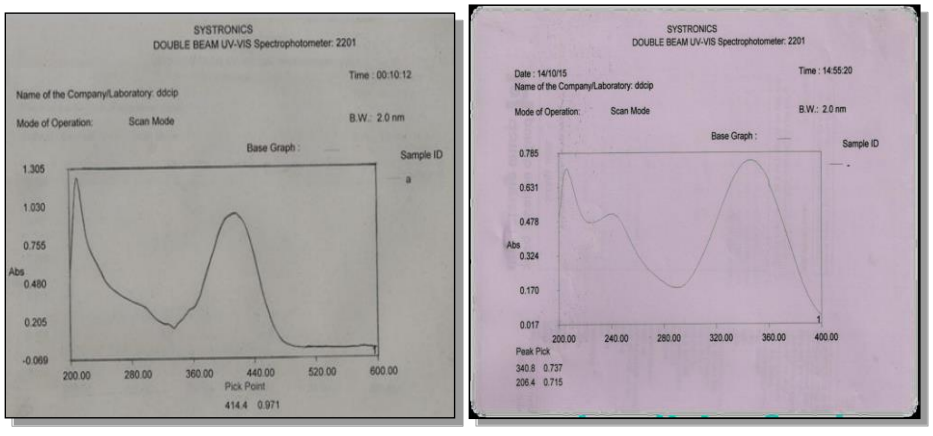
Pre-formulation Study

Confirmation of Drug

Confirmation of drug was carried out by using Infrared spectroscopy, differential scanning Calorimetry (DSC) and UV spectroscopy.

UV Spectrophotometric Study

Nitazoxanide was scanned at 400 to 200nm. The λmax was found to be 414.4 nm and 340.8 nm in phosphate buffer 7.4 and 0.1N HCL respectively (Figure 1).



(A) (B)

Figure 1: UV spectrum of NTZ solution in (A) pH 7.4 phosphate buffer (B) 0.1 N HCL.

FTIR Spectra

The IR absorption spectrum of the pure drug was taken in the range of 4000 to 600 cm−1 using FTIR spectrophotometer (Bruker, USA). The major peaks were reported for Evaluation of purity. Observed peaks are similar to reported peaks of drug (Figure 2, Table 1).

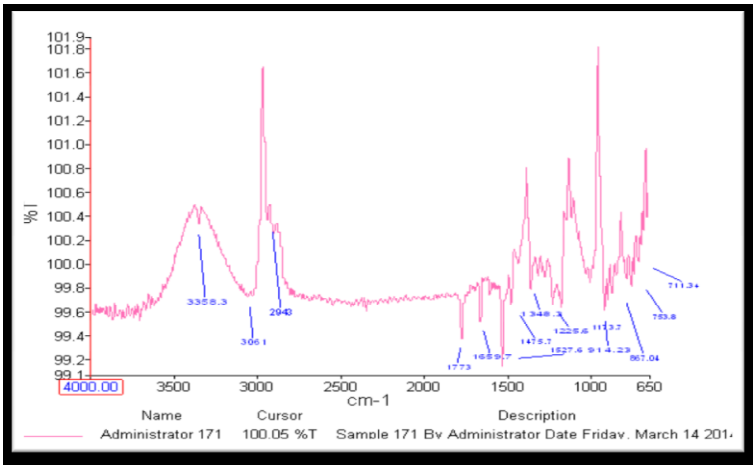


Figure 2: FTIR spectra of pure drug Nitazoxanide

Table 1: Interpretation of IR spectra of pure drug Nitazoxanide

S.No	Functional Group	Range (cm-1)	Observed Frequency (cm-1)
1.	Carbonyl group ester linkage amide linkage	1690-1760 1700-1680	1773 1659.7
2.	Nitro group	1500-1350	1527.69
3.	=CH stretch	2960-2850	3061

Drug excipients compatibility studies

These studies were performed in order to confirm the drug excipient interaction. These studies mainly include FTIR spectroscopy. FTIR spectra of pure drug and physical mixture was recorded on FTIR spectrophotometer (Bruker, USA). The scanning range was from 4000 to 600 cm−1, and the resolution was 1 cm−1. The scans were evaluated for presence of principal peaks of drug, shifting and masking of drug peaks, and appearance of new peaks due to excipient interaction (table 2). This spectral analysis was employed to check the compatibility of drugs with the excipients used [10].

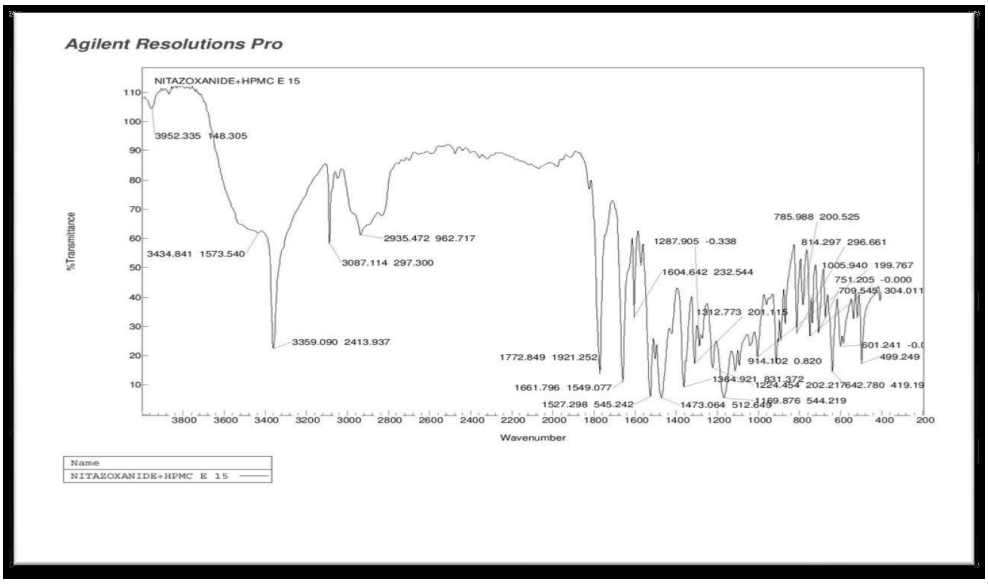


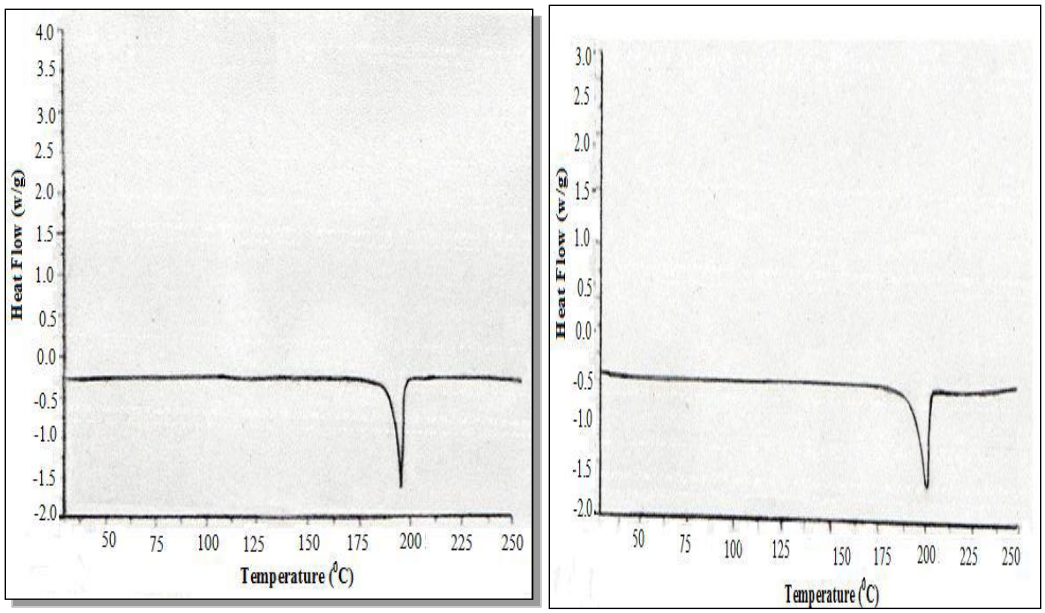
Figure 3: FTIR spectra of pure drug (Nitazoxanide) + HPMC E 15 polymer

Table 2: Interpretation of IR spectra of pure drug + HPMC E 15 polymer

S. No	Functional Group	Range (cm-1)	Observed Frequency (cm-1)
1.	Carbonyl group ester linkage amide linkage	1690-1760 1700-1680	1772 1661.7
2.	Nitro group	1500-1350	1527.29
3.	=CH stretch	2960-2850	3087.11

Differential Scanning Colorimetery (DSC)

DSC provides information about all physical properties of sample and demonstrates the possible interaction between Drug and other Polymers. The thermal behavior of Nitazoxanide and physical mixture of drug & polymers are shown in (Figure 4), according to thermogram, Nitazoxanide produced sharp Endothermic peak at 197.5oC which conformed crystalline form of the drug. DSC curves of the drug and Physical mixture of drug & polymers exhibited an endothermic peaks at 201.5oC, which has been attributed to the evaporation of water. The thermogram of the physical mixture of Drug and Polymers showed that there was no interaction between drug and polymer [11].



(A) (B)

Figure 4: DSC Spectra of pure drug (a) Nitazoxanide and (b) physical mixture of pure drug Nitazoxanide +HPMC E 15.

Method of preparation of immediate release granules of Nitazoxanide

Granules of immediate release layer were formulated by dissolving HPMC in water and allow to stand overnight. Measured quantities of Nitazoxanide and Lactose as per formula given in table 3 were uniformly mixed by slowly adding above solution. The wet damp mass was passed through fine sieves and the granules were dried in hot air oven for 6 to 8 hours. Then sodium starch glycollate, silicon dioxide and microcrystalline cellulose were added and mixed thoroughly, finally magnesium stearate was added and mixed thoroughly before compression of granules.

Method of preparation of controlled release granules of Nitazoxanide

Granules of controlled release layer were formulated by preparing a solution of HPMC with water which was allowed to stand overnight. Then, the measured quantity of Nitazoxanide was taken and the above solution was added slowly in to it. The wet damp mass was passed through fine sieve and the granules were dried at hot air oven. HPMC E15, calcium carbonate, silicon dioxide as per formula given in table 3 were added and mixed thoroughly. Finally magnesium stearate was added and mixed thoroughly before compression.

Table 3: Formulation table of different batches of bilayer tablet of Nitazoxanide.

S.No	**Composition (mg)**	**Formulation code**								
	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
	Immediate release layer									
1.	Nitazoxanide	125	125	125	125	125	125	125	125	125
2.	Lactose	50	50	50	50	50	50	50	50	50

3.	HPMC	5	5	5	5	5	5	5	5	5
4.	Sodium starch glycollate	7	7	7	8	8	8	9	9	9
5.	Silicon dioxide	2	2	2	2	2	2	2	2	2
6.	Microcrystalline cellulose	23	23	23	23	23	23	23	23	23
7.	Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	Controlled release layer									
8.	Nitazoxanide	375	375	375	375	375	375	375	375	375
9.	HPMC	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
10	HPMC E15	90	100	110	120	130	140	150	160	170
11	Calcium carbonate	102. 5	102.5	102.5	102.5	102.5	102.5	102.5	102.5	102.5
12	Silicon dioxide	4	4	4	4	4	4	4	4	4

Characterization of Granules

Prior to compression, granules were subjected to pharmacotechnical characterization. They were evaluated for tapped density, Carr's index and angle of repose. Carr's compressibility index was calculated from the bulk and tapped densities [12] using a digital tap density apparatus (Electro lab Ltd, India).

Compression of Bilayer Tablets

The bilayer tablet of Nitazoxanide was prepared using a Rotary Mini tablet press (Karnavati Pvt. Ltd. India) equipped with 11 mm beveled, flat punches. The die was initially filled with the weighed amount of controlled release portion and was lightly compressed. Over this compressed layer, the required quantity of the immediate release granules was placed and compressed to obtain hardness of the tablet 6–7kg/cm2. It was observed that table compressed at this force did not show any layer separation [13].

Evaluation of Tablets

The prepared matrix tablets were evaluated for hardness, weight variation, thickness, friability, and drug content. Hardness of the tablets was tested using a Strong-Cobb hardness tester (Tab-machine, Mumbai, India). Friability of the tablets was determined in a Roche friabilator (Campbell Electronics, Mumbai, India). The thickness of the tablets was measured by vernier caliper. Weight variation test was performed according to the official method [14].

Durg content: Ten tablets were powdered in a mortar. An accurately weighed quantity of powder tablet (100mg) was extracted with phosphate buffer 7.4 (100ml) and the solution was filtered through 0.45µ memberane. Each extract was suitably diluted and analysed by spectrophotometer at 414.4nm (UV-2201, systronics).

In-Vitro Dissolution Study: Bilayer tablets were subjected to in vitro drug release studies in simulated gastric and intestinal fluids to assess their ability in providing the desired controlled drug delivery. Drug release studies were carried out using USP dissolution test apparatus I at 100 rpm, 37±0.5°C, and pH 1.2 phosphate buffer for 2 hours, since the average gastric emptying time is about 2 hours. The dissolution medium was replaced with pH 7.4 Phosphate buffer and experiment continued for upto 12

hours. At different time intervals, 5ml of the samples were withdrawn and replaced with 5ml of drug-free dissolution medium. The samples withdrawn were analysed after suitable dilutions, for drug release and release kinetics, using UV spectrophotometer.

Stability of Bilayer Tablets

A three month accelerated stability test was carried out for best formulation, in which sample were kept in humidity chamber at a temperature of 40 oC ± 2 oC and a relative humidity of 75 ± 5%. The release profile of optimized fomulation was determined at the end of 1, 2 and 3 months, respectively [15, 16].

Result and Discussion

Nitazoxanide was formulated into bilayer tablet form comprising of immediate release layer for loading dose and controlled release layer as maintenance dose. Total nine batches were designed with varying concentrations of superdisintegrants and rate controlling polymers. Prior to compression, granules were subjected to pharmacotechnical characterization. It was found that all the batches had good compressibility. Flow properties also found to be good for all batches (angle of repose between 28-30 indicated good flow). Hausner's ratio (less than 1.20) for all batches which indicated good flow properties. Drug excipients compatibility study was carried out by FTIR spectrophotometer and there was no interaction found among drug and excipients as shown in Figure 3. Post compression parameter of bi-layer tablet like weight variation, thickness, hardness, friability, and drug content uniformity represented in Table 4. The drug content of all the formulations varied between 88.61 % to 98.98 %. All the formulations exhibited friability values ranging between 0.13% w/w to 0.81% w/w.

Table 4: Post compression characteistics of Nitazoxanide bilayer tablet

Batch No.	Diameter(mm)	Hardness(kg/cm2)	Thickness(mm)	Friability (%)	Drug Content (%)
F1	11.80	3.67±0.23	7.32±0.04	0.81±0.08	88.61±1.05
F2	12.00	5.30±0.32	7.27±0.02	0.70±0.02	90.61±0.42
F3	12.10	5.03±0.33	7.28±0.01	0.76±0.02	91.53±0.23
F4	11.95	5.63±0.50	7.31±0.03	0.52±0.06	91.39±0.26
F5	11.99	9.00±0.50	7.32±0.01	0.27±0.03	92.86±0.33
F6	12.00	7.40±0.21	7.30±0.01	0.27±0.02	97.31±0.10
F7	12.00	7.60±0.29	7.29±0.02	0.14±0.01	98.35±0.71
F8	12.00	7.00±0.08	7.31±0.01	0.13±0.13	98.79±0.81
F9	12.00	7.40±0.21	7.33±0.02	0.14±0.03	98.98±0.73

Bilayer tablets were subjected to in vitro drug release studies in simulated gastric and intestinal fluids to assess their ability in providing the desired controlled drug delivery. In-vitro drug release study was performed initially in 1.2 pH buffer for 2 hours. Then buffer was replaced with 7.4 pH phosphate buffer. F9 formulation shows maximum release in 2 hours (24%) due to increase in amount of superdisintegrants, whereas F1 formulation showed only 10% release in first 2 hours due to less amount of superdisintegrant as shown in table 3. The in-vitro dissolution of Controlled release layer was studied further for 12 hours in 7.4 pH phosphate buffer. F1 formulation showed complete release of 94% up to 8 hours; F3 formulation showed 93% release up to 12 hours (Figure 5). Further F5-F9 released 92-95% drug release within 12 hours as shown in table 5.

The formulation F9 was selected as the best formulation as it showed the best drug release up to 12 hours as compared to the other formulation.

The optimized formulations were found to be stable at all the stability conditions. During stability studies, no significant variation (1–4%) in drug release was observed, indicating that formulation batch F 9 was stable over the chosen condition for 3 months.

Table 5: Release profile of Nitazoxanide bilayer tablet formulations (F1-F9)

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
	% Drug Release								
2	10±0.27	14±0.47	12±0.71	17±0.27	15±0.47	18±0.71	20±0.27	22±0.47	24±0.71
4	57±0.27	54±0.71	50±0.71	46±0.27	44±0.47	42±0.71	40±0.47	37±0.71	35±0.27
6	85±0.47	80±0.27	74±0.27	69±0.27	67±0.47	68±0.27	50±0.47	44±0.27	40±0.47
8	94±0.71	89±0.27	85±0.71	82±0.27	80±0.47	75±0.27	70±0.27	64±0.47	60±0.27
10	-	97±0.27	90±0.47	89±0.47	87±0.71	80±0.27	86±0.71	87±0.27	83±0.47
12	-	-	94±0.27	94±0.47	92±0.47	92±0.71	93±0.47	93±0.27	95±0.27

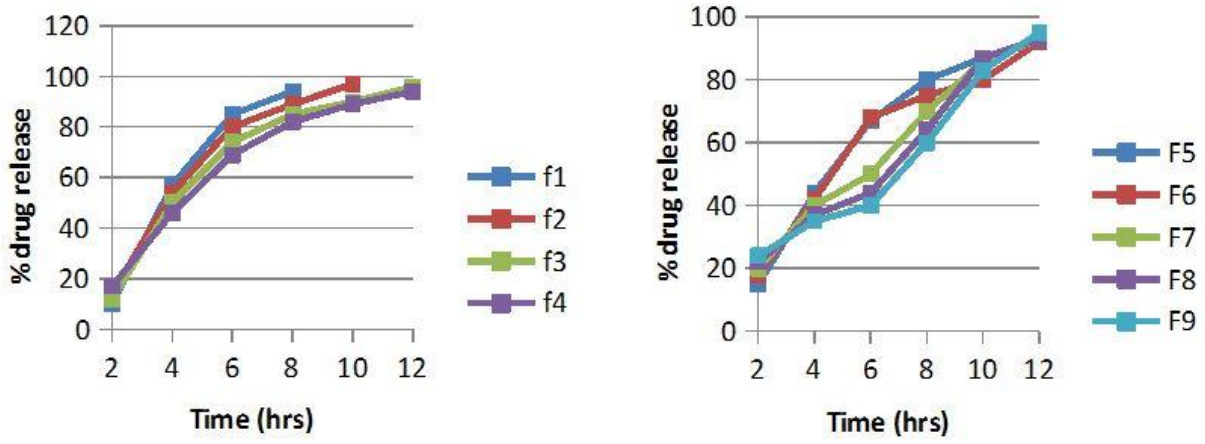


Figure 5: Drug release profile of Nitazoxanide Bilayer tablets (F1- F9)

CONCLUSION

The present research was carried out to developed a bilayer tablet of Nitazoxanide using superdisintegrant sodium starch glycollate for immediate release layer and polymer HPMC E15 for controlled release layer. The micromeritic study of pre compressed granules suggests that on formulation of granules from pure drug the flow behaviour of drug was improved. Concentration of polymer & superdisintegrant ratio influenced drug release profile. As the polymer concentration increases, % drug release decreases whereas as the superdisintegrant concentration increases, % drug release also increases. The bilayer tablet showed initial burst release to provide the loading dose of drug followed by controlled release up to 12 hours. The Formulation

F9 showed maximum controlled release up to 12hr. Formulation F9 bilayer tablet were selected as best formulation for preparation of controlled drug delivery system. The data obtained thus suggest that bilayer tablet can be successfully designed for controlled delivery of Nitazoxanide and to improve patient compliance.

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