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VIEWS

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Photocatalytic Decontamination Of Halosulfuron-methyl Residues On Soil Surface Using Fe Doped Tio_2 Nanoparticles Under Direct Sun Light

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ABSTRACT

The decontamination of halosulfuron-methyl (HSM) on soil suface under direct sunlight was studied using Fe doped TiO_2 nanoparticles ($FeTiO_2$) as catalyst. $FeTiO_2$ nanoparticles are synthesized and characterized by X-ray diffraction (XRD), Scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDX) and Transmission electron microscopy (TEM). The effect of concentration of $FeTiO_2$, pH of soil and humic acid content on the decontamination of halosulfuron-methyl in photocatalysis was investigated. The kinetic parameters such as rate constant (k), DT_{50} (2.6 days), DT_{90} were calculated. The $FeTiO_2$ accelerated the decontamination of HSM on soil surface significantly with the rate constant (k) varying from 52.6 x 10^{-3} day^{-1} to 42.6 x 10^{-3} h^{-1}. The effective amount of $FeTiO_2$ was 0.6% (w/w). The $FeTiO_2$ nanoparticles were observed to be excellent decontaminating catalyst for halosulfuron-methyl in soil under direct sun light. The residues of halosulfuron methyl and its Metabolite Chlorosulfonamide acid are quantified by high performance liquid chromatography (HPLC) and confirmed by LC-MS-MS technique. In the absence of catalyst the compound persists several days.

Key words: Fe doped TiO_2, Halosulfuron-Methyl (HSM), Decontamination, Photocatalysis

Introduction

Halosulfuron-methyl is a pyrazole group of sulfonylurea class of herbicide. It has been used as selective herbicide for post-emergence control of annual broad-leaved weeds and nutsedge specious in a range of crops including turf, maize, grain sorghum, rice and sugarcane in all over the season. It is a popular weed control agents due to their biochemical activity at low effective concentration approximately ten to thousand times less than the conventional herbicides apart from low mammalian toxicity. Halosulfuron-methyl belongs to herbicide resistance (HRAC) classification B due to the inhibition action of acetolactate synthase (ALS) enzyme in the weed plants [1]. Halosulfuron-methyl is known to biodegradable only in those soils which contain high moisture content and low pH (pH 6 and less than that) at high temperature. Residues of HSM are strongly sorbed and weakly desorbed in those soil which have high organic matter and low pH HSM after application and would cause phytotoxicity to non-target succeeding crops especially in multiple cropping system area [2]. This long persistence nature results in metabolites formation, which are toxic than HSM and contaminate ground water through leaching. The residual contamination is always a threat to non-target organism in the ecosystem and also to human when enter in to food chain through.

In the recent years the use of heterogeneous photocatalyst nano Fe doped TiO_2 ($FeTiO_2$) in the degradation and mineralization of herbicide, insecticide, N-heterocyclic compounds, saturated fatty acids, different organic dyes in water and gaseous pollutant in air using UV and visible-light has gained wide attention due to its low cost preparation, low toxicity, high stability and effectiveness than TiO_2 [3-5] When $FeTiO_2$ nanoparticles are subjected to UV, VIS or solar light, it gains energy from light and promote electrons (e^-) from the valence band (VB) of TiO_2 to the conduction band (CB) leaving a positive hole (H^+). Fe in $FeTiO_2$ trap electrons (e^-) and positive holes (H^+) from TiO_2 since the energy levels of Fe^{2+}/Fe^{3+} lies close to that of Ti^{3+}/Ti^{4+}, and reduce the recombination of photo-generated electron and hole pair in TiO_2 and enhance the availability of electrons (e^-) and positive holes (H^+) in $FeTiO_2$ [6]. These electrons (e^-) and positive holes (H^+) are

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involving in the degradation of organic molecules by oxidation / reduction process. Destroying of Escherichia coli bacteria in water have been achieved completely within an hour by deposited Escherichia coli bacteria in water on $FeTiO_2$ thin film and irradiating it in visible radiation [7]. The wide use of herbicide RS-2-(4-chloro-o-tolyloxy) propionic acid (mecoprop), 4-chloro-2-methylphenoxy acetic acid (MCPA) and 3,6-dichloropyridine-2-carboxylic acid (clopyralid) for selective control of some perennial and many annual weeds have been decomposed by using $FeTiO_2$ in visible light irradiation. The study indicated the activity of $FeTiO_2$ for clopyralid decomposition was high [8]. A 15 times higher rate of mineralization was achieved for insecticde monocrotophos in water using TiO_2 and Fe^{3+} [19]. There is no need to dope Fe in TiO_2 evenly to achieve good catalytic activity, Song and Yanshan 2009 have proven that uneven distribution of Fe in TiO_2 increased the catalytic activity of $FeTiO_2$ than even distribution [10] Buddee, et. al., 2009 have recovered $FeTiO_2$ after used for the degradation of methylene blue dye in water and then reused ten times with same effect [11]. Based on the information present study was conducted to investigate the decontamination of Halosulfuron-methyl on four different soil surfaces using $FeTiO_2$ as catalyst under natural climatic conditions in solar light.

Materials and Method

Preparation of $FeTiO_2$ nanoparticles

The TiO_2 nanoparticles were prepared by the drop wise addition of 4 mL of $TiCl_4$ (sigma Aldrich) in 100 mL distilled water containing 1% HCl (E. Merck) at 5°C and ultrasonicated for 1 hour at 80°C and kept for 16 hours at 80°C in a thermostat controlled oven ($TiCl_4 + 2H_2O$ $TiO_2 + 4HCl$). The obtained white precipitate was washed in distilled water eight times by using refrigerated centrifuge and finally washed with methanol. The methanol was then decanted and the precipitate (TiO_2 nanoparticles) was dried at 120°C for 4 hours [12]. A 100 mL boiling solution of Iron nitrate (sigma Aldrich) was added drop wise to the boiling distilled water containing 2 g of TiO_2 nanoparticles. The solution was sonicated at 100æ%C for about 30 min, according to the following chemical equation:

$$TiO_2(aqua) + Fe(NO_3)_3 \cdot 9H_2O(aqua) \longrightarrow Fe–TiO_2$$

The obtained brown colour $FeTiO_2$ nanoparticles were washed with distilled water five times by using refrigerated centrifuge and finally washed with methanol. The $FeTiO_2$ nanoparticles dried at 120°C for 4 hours after decanted the methanol (E. Merck) [13].

Photocatalytic studies

The photocatalytic study was conducted under sunlight in natural climatic conditions at International Institute of Bio-technology And Toxicology, (IIBAT), Padappai – 601 301, Kancheepuram District, Tamil Nadu. The soils (from 10 cm depth) of Sandy loam, Loamy sand, Sandy clay and Clay were collected at IIBAT farm and sieved through 2mm sieve after drying in hot air oven at 120°C were mixed thoroughly with an aqueous suspension of $FeTiO_2$ nanoparticles to obtain a homogeneous 0.6 % (w/w) of the $FeTiO_2$ in soil. Three sets (AR1, AR2 and AR3) of $FeTiO_2$ loaded soil in triplicates were spread by hand to get 5 mm height in a glass tray. Aqueous solution of HSM 75% WG (supplied by Nissan Chemical Industries, Ltd., Japan) at 7.5 mg a.i./ Kg was sprayed on AR1 and AR2 set of soil by using 1L high density polyethylene bottle sprayer. The first set AR1 of soil was exposed to sun light from morning 8 AM to evening 5 PM in the month of June and the second AR2 was kept in oven at dark at simulate natural condition without light for the measurement of non photocatalytic degradation of HSM in soil. The third set AR3 of soil ($FeTiO_2$ loaded) and $FeTiO_2$ unloaded soil (AR4-Control) sprayed with distilled water were also exposed to sun light.

To investigate the effect of the $FeTiO_2$ concentration on the decontamination of HSM on soils, studies were conducted by varying the concentration of the $FeTiO_2$ (0.2, 0.4, 0.6, 0.8, and 1.0 %) in soils without adding humic acid and without changing the pH of soil.

The effect of humic acid content, on photocatalytic decontamination of HSM in soil was studied by varying the humic acid concentration in soil (0, 10, 20, 30 and 50 mg/Kg) without varying the concentration of the $FeTiO_2$ and without changing pH of soil. The effect of pH, on the photocatalytic decontamination of HSM in soil was conducted by varying the pH of soil (pH 4.0, 6.5, 8.0 and 9.7) using an aqueous solution of H_2SO_4 or an aqueous solution of NaOH with constant $FeTiO_2$ concentration without the addition of humic acid in soils.

The photolysis of HSM in soil without catalyst was also conducted on unloaded $FeTiO_2$ soil samples sprayed with aqueous solution of HSM 75% WG at 7.5 mg a.i./ Kg (AR5) [14].

HPLC Analysis

The quantification of the residues of HSM and its metabolite CSA were done using Shimadzu HPLC equipped with prominence LC-20 AT binary pumps, DGU-20A3 degasser, CTO-20A column oven and SPD-20A UV-VIS detector connected with LC solution software. Detector wavelength was set at 245nm. A binary gradient mobile phase (Acetonitrile: Milli-Q-Water pH 3.0 adjusted with H_3PO_4) at 1mL/min flow rate was used to achieve a good separation of the HSM (RT 12.6 min) and CSA (RT 8.9 min) [2].

Results and Discussion

Description of $FeTiO_2$ nanoparticles

The X-ray diffraction (XRD) of the synthesized $FeTiO_2$ was performed to investigate its crystalline phase by

scanning from 3° to 50°. The **Fig.1** indicates (peak at 27° or 31°) the rutile phase due to three (rutile, anatase and brookite) naturally occurring phases of titania and **Fig. 2** Scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDX) for the quantitative determination and elemental composition of Fe/Ti ratio indicates the Fe content was 0.5 % and Ti, O and Fe are the elemental compositions. The size of the particle was observed to be 15-18 nm by transmission electron microscopy (TEM) as shown in **Fig. 3**.

Photocatalytic Studies

Results of photocatalytic studies are presented in **Table 1** and **Fig.4.** The data clearly demonstrate that the decontamination of HSM follows pseudo-first-order kinetics in 0.6 % (w/w) $FeTiO_2$ loaded soil (AR1) and unloaded $FeTiO_2$ soil (AR5).

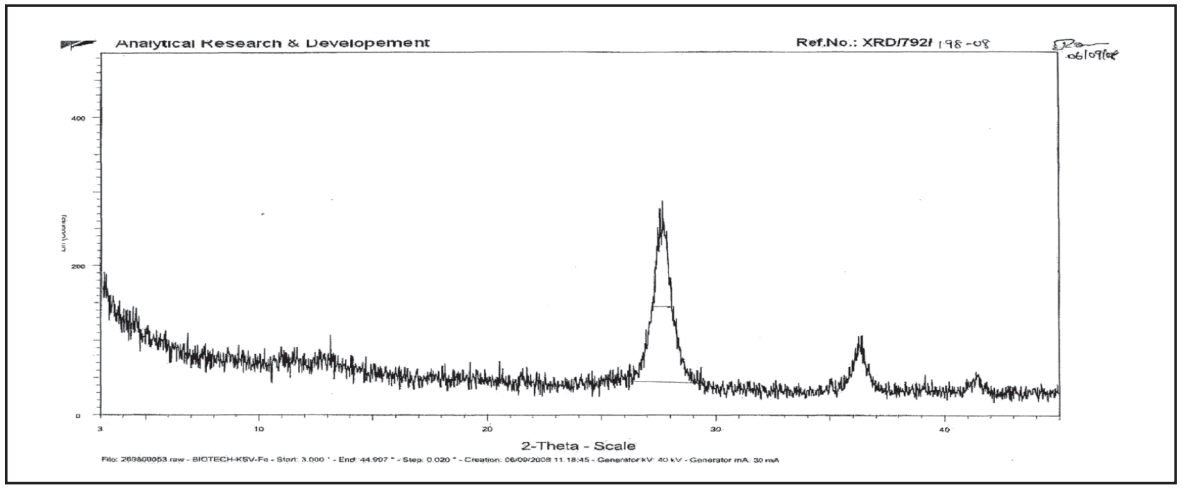

Fig. 1: XRD spectrum of $FeTiO_2$ nanoparticles

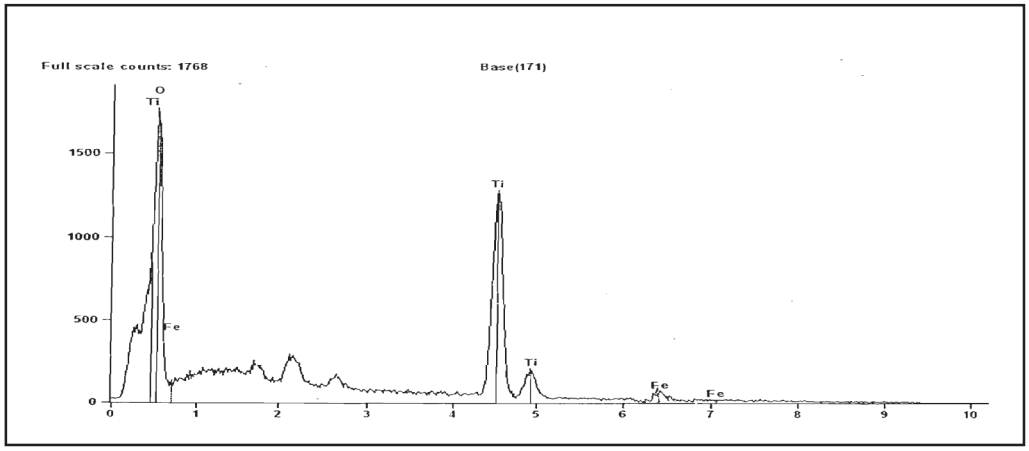

Fig. 2: EDX analysis of $FeTiO_2$ nanoparticles

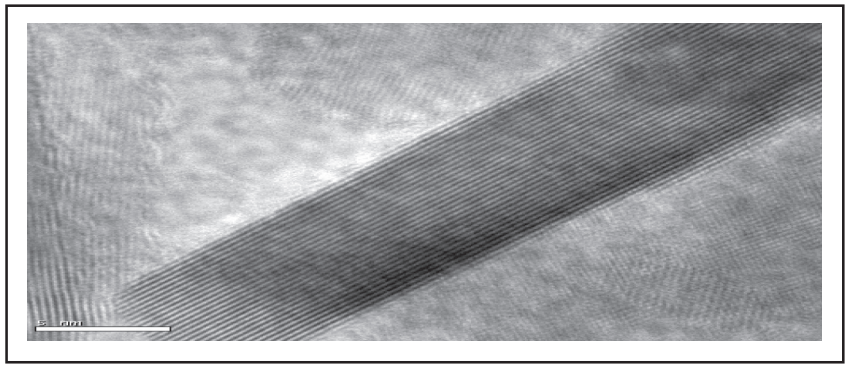

Fig. 3: TEM Image of $FeTiO_2$ nanoparticles

The pseudo-first-order kinetics form is:

$$-\ln \frac{C_t}{C_0} = kt \quad \ldots (1)$$

$$t_{1/2} = DT50 = \frac{\ln 2}{k} = \frac{0.6931}{k} \quad \ldots (2)$$

$$k = \frac{2.303}{t} \times \log_{10} \frac{C_0}{C_t} \quad \ldots (3)$$

Where C_0 and C_t are the HSM concentration at times zero and t, respectively, and k is the rate constant [17]. The pseudo-first-order rate constants (k) of HSM in AR5 set soils were 52.6 x 10^{-3}, 53.9 x 10^{-3}, 55.9 x 10^{-3} and 58.2 x 10^{-3} (day^{-1}) respectively; the AR1 set soil has 42.6 x 10^{-3}, 39.4 x 10^{-3}, 41.2 x 10^{-3} and 43.2 x 10^{-3} (h^{-1}) respectively, and presented in **Table 2**.

The herbicide was observed to be degraded by direct sunlight, forming metabolites and therefore, the extent of photolysis of HSM in soil and the formation of the metabolites were studied in the absence of the $FeTiO_2$ in the soil. Results were clearly indicate that the rate constant was high when the $FeTiO_2$ was present in soil than in absence of soil and no degradation of herbicide in AR2 set

Table 1

Photocatalytic decontamination of HSM and on four different soils

Sampling intervals (hours)	Residues (µg/g)			
		HSM		
	Sandy loam	loamy sand	Sandy clay	Clay
		AR1		
0	0.221	0.219	0.223	0.218
9	0.167	0.174	0.174	0.162
18	0.109	0.116	0.112	0.105
27	0.071	0.077	0.075	0.069
45	BDL	BDL	BDL	BDL
		AR2		
0	0.221	0.219	0.223	0.218
9	0.221	0.219	0.223	0.218
18	0.221	0.219	0.223	0.218
27	0.221	0.219	0.223	0.218
45	0.221	0.219	0.223	0.218
		AR3		
0	BDL	BDL	BDL	BDL
9	BDL	BDL	BDL	BDL
18	BDL	BDL	BDL	BDL
27	BDL	BDL	BDL	BDL
45	BDL	BDL	BDL	BDL
Days		AR4		
0	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL
20	BDL	BDL	BDL	BDL
30	BDL	BDL	BDL	BDL
40	BDL	BDL	BDL	BDL
		AR5		
0	0.207	0.212	0.215	0.214
10	0.112	0.106	0.112	0.106
20	0.081	0.084	0.076	0.072
30	0.04	0.038	0.037	0.035
40	BDL	BDL	BDL	BDL

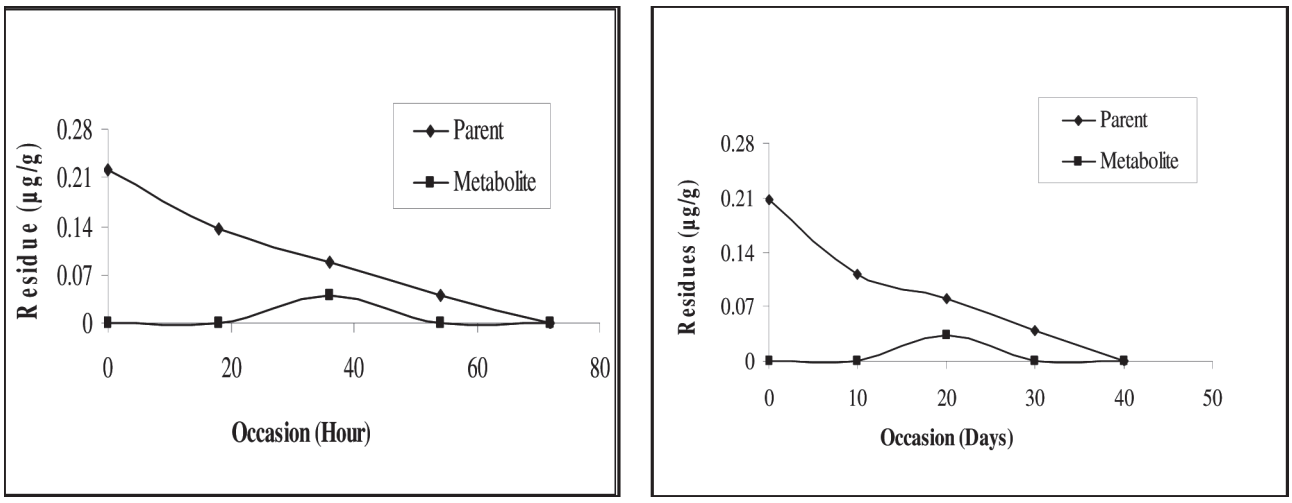

Fig. 4: Photocatalytic and photolytic decontamination of HSM and CSA in Loamy sand soil

of soil which were kept in dark. The decontamination was fast when studied under sunlight in presence of $FeTiO_2$ in soil due to the formation of electrons (e^-) and positive hole (h^+) in TiO_2. The absorbed energy from sun light and the availability of electrons (e^-) and the positive holes (h^+) pairs which were contributing the simultaneous oxidation and reduction of HSM in soil was enhanced by Fe in $FeTiO_2$. This was confirmed by the no degradation of herbicide in dark. Absence of HSM residues and metabolites were also observed in water sprayed 0.6 % (w/w) $FeTiO_2$ loaded soil (AR3) and unloaded soil (AR4) because of no HSM was applied.

Further an acceleration of photocatalytic decontamination rate was observed with increase in concentration of catalyst over the range 0.2 to 0.6 wt.% and beyond this limit no increase in the rate was observed

Effect of humic acids

The effect of the concentration of humic acids on the photocatalytic decontamination of HSM on soil surface was studied with concentrations ranging from 0 to 50 mg Kg^{-1}. It can be concluded from results that the presence of 0 to 20 mg Kg^{-1} was accelerate the photocatalytic decontamination of HSM in the presence of $FeTiO_2$. The absorption of light by humic acid might have contributed to rapid photosensitized reactions of HSM via energy transfer from molecules in its triplet state. There was no enhancement in the decontamination with concentrations more than 20 mg kg^{-1} of humic acids which could be due to the shielding effect of Humic acids from the photons or photo-generated (e^-) and (h+).

Effect of soil pH

The pH of the soil plays an important role in the studies on the photodegradation of HSM, since it influences the surface-charge properties of the catalyst TiO_2 thus governing the rate of reaction. It can be concluded from results that the rate of the decontamination of HSM appeared to be greater at soil pH 4.0 and pH 9.7 than at soil pH 6.5 and pH 8.0.

The $FeTiO_2$ surface is positively charged in acidic media (pH < 6.5), whereas it is negatively charged under alkaline conditions (pH > 8.0). Under acidic or alkaline conditions, more H^+ or OH^- ions are produced in soil, and these ions would be able to facilitate the photocatalytic reactions of HSM.

Table 2

Kinetic parameters for the photocatalytic and photolytic decontamination of HSM in four different soil

Soil type	Photocatalysis				Photolysis			
	DT50 (hours)	DT90 (hours)	k ($1x10^{-3}$ h^{-1})	R2	DT50 (day)	DT90 (day)	k ($1x0^{-3}day^{-1}$)	R2
Sandy Loam	22.75	75.56	30.5	0.9784	13.19	43.81	52.6	0.9511
Loamy Sand	23.38	77.68	29.6	0.995	12.86	42.72	53.9	0.9324
Sandy Clay	22.37	74.31	31	0.9807	12.23	40.63	56.7	0.9378
Clay	20.66	68.64	33.6	0.9577	11.91	39.57	58.2	0.9231

Confirmation of residues by LC-ESI-MS/MS

The residues of HSM and its metabolites were confirmed by LC-ESI-MS/MS analysis on 0, 36 and 72 hours. The data obtained by the LC-MS-MS analysis presented in **Fig.5** shows the formation of metabolites due to the degradation of HSM by photons.

Conclusion

The photocatalytic decontamination of HSM in the presence of $FeTiO_2$ nanoparticles on soil surfaces in sunlight under natural climatic conditions followed pseudo-first-order kinetics. Addition of humic acid at concentrations from 10 to 20 mg Kg^{-1} was also found to increase the decontamination of HSM and its major metabolite in the presence of $FeTiO_2$ nanoparticles. In conclusion, Photocatalysis using $FeTiO_2$ nanoparticles in sunlight under natural climatic conditions appear to be attractive for the decontamination of surface soils contaminated with persistent herbicide.

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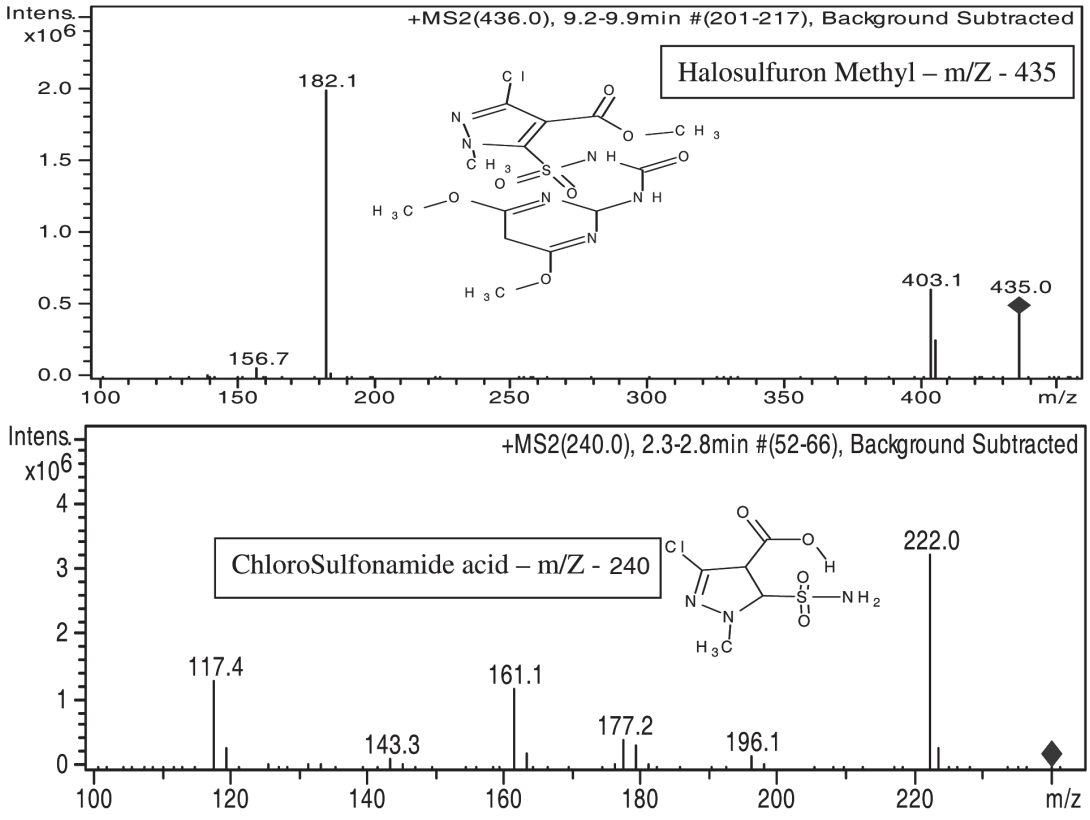

Fig. 5: MS2 Spectra of HSM and CSA

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Determination of Dithiocarbamate Residues in Aquatic Tox Medium by Gas Chromatography Electron Ionisation Mass Spectrometric Method

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ABSTRACT

A simple and sensitive gas chromatography electron ionization mass spectrometry method in selective ion monitoring (GC-MS, SIM) was developed for the determination of dithiocarbamates (mancozeb, zineb and propineb) residues in different aquatic tox mediums. The test mediums are blended water for fish, M4 Medium for Daphnia magna, OECD TG 201 medium for Alga and 20XAAP Medium for lemna. The dithiocarbamates estimation as CS_2 involves the reduction of dithiocarbomate moiety under strong acidic conditions in presence of stannous chloride ($SnCl_2$) as reducing agent. The trapped CS_2 in n-Heptane was quantified by GC-MS in SIM mode. The calibration curve was linear over the range of 0.01 mg/L to 100 mg/L concentration. The recovery from spiked samples at 0.02 and 2 mg/L of dithiocarbamates in different mediums were in the range 95-102%, with relative standard deviations 0.87 to 2.8% (n=5). The proposed validated method can be applied successfully for the determination of dithiocarbamate residues in different aquatic solutions having pH range 5-9 and high ionic concentration.

Key words: Dithiocarbamates, Carbon disulfide (CS_2), Aquatic Tox medium, GC-MS.

Introduction

The dithiocarbamate fungicides used in crop protection services are the complex metal salts which contains manganese (maneb), iron (ferbam), zinc (zineb, mancozeb, propineb) etc., The dithiocarbamates posses the potential fungicide activity for the treatment of broad spectrum of pathogens of more than 400 species over 70 crops [1]. They were characterized based on their potential activity against different plant pathogens. In combination with modern systemic fungicides, they were also used to manage resistance and to broaden the spectrum of activity. The so called "maneb group" (zineb, maneb, mancozeb, propineb, metiram) fungicides are most frequently detected chemicals in several of the export commodities like grapes, and that this group also had the highest frequency in exceeding maximum residue limits (MRLs) [2]. Which lead to the use of an array of different methods for the analysis of their residues in different substrates.

The general methods of analysis are based on the decomposition of dithiocarbamates to liberate carbon disulfide (CS_2) using lead acetate or by hot mineral acid to the amines. The liberated CS_2 is subsequently trapped in a digestion solvent and the active ingredient is determined by iodometric titrations [3-10]. Several of these published methods are titrimetric methods and suffer from practical difficulties due to the interference associated with the samples. The draw back of the method is the CS_2 liberation may not be complete or it may leak while trapping or the reverse flow may contribute to the negative results forcing the analyst to do multiple sample analysis.

Dithiocarbamates are practically insoluble in organic and inorganic solvents. Indirect methods for determination of dithiocarbamates including spectrophotometer [11-13], capillary electrophoresis with UV detection [14], liquid chromatography with UV detection [15], reversed phase ion-pair chromatography [16] and gas chromatography [17] were reported in literature. In this particular study the work describes in detail the evaluation of dithiocarbamates in aquatic media solutions prepared according to regulatory guideline [18-21], OECD 201 (Alga medium), OECD 202 (Daphnia medium), OECD 203 (Fish medium) and OECD 221 (Lemna medium) with a duration of 5-8 hours to as low as 0.02 mg/L. The analytical method is based on the principle of decomposition of dithiocarbamates by hot

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mineral acid to amines and carbon disulfide (CS_2). The decomposition process is catalyzed by a reducing agent (stannous chloride II) in a sealed crimp top vial. n-Heptane was selected as CS_2 trapping solvent. The trapped CS_2 was analyzed by using GC-MS in Selective Ion Monitoring (SIM) mode [22-25].

Experimental

Instrumentation

GC-MS conditions for the determination of Dithiocarbamates

The configuration of GC–MS system used includes a GC-17A (Shimadzu, Tokyo, Japan) gas chromatograph coupled with QP-5050A Mass-Selective Detection (MSD) and GC Solution software, the detector was set in selective ion monitoring mode (SIM). The target ion used for the measurement was the ion at m/z 76. The CS_2 peak separation was obtained on a special Supelco SPB-1 capillary column (30 m length, 0.32 mm internal diameter, 4.0 µm film thickness). The injection system was operated in split mode with a split ratio of 10:1. The injector and the transfer line temperatures were 250ºC and 300ºC, respectively. The oven temperature program was 30ºC, held constant for 4.5 min and ramp at 70ºC /min to 120ºC, held constant for 3.0 min. The carrier gas used was helium (Purity 99.999%) at a flow rate of 2.0 mL /min and the sample volume injected onto the column was 1.0 µL. A Shimadzu GCMS solution Chromatography Software was used for acquisition of data and calculation of peak area.

Analytical standards, Reagents and Solutions

The analytical standards Mancozeb (Purity 82.10%) and Zineb (Purity 76.80%) was obtained from Indofil chemicals Ltd, and Propineb (Purity 98.9%) was purchased from Sigma Aldrich. The hydrochloric acid, ethylene diaminetetraacetic acid disodium salt (EDTA) used were Analytical Reagent grade. HPLC grade methanol, acetonitrile and isooctane were purchased form Merck Darmstadt, Germany. Stannous (II) chloride (Purity 98%) was obtained from Aldrich Chemicals, Mumbai. Milli-Q water was obtained from Millipore India Ltd, Bangalore, India.

Analytical standard solutions of dithiocarbamates were prepared by dissolving aliquots in 0.2 M EDTA. Purity correction and CS_2 conversion factor was incorporated in the preparation of analytical standard solutions. For each pesticide a stock solution of 500 mg/L was prepared, which was serially diluted to produce working standard solutions. The working standard solutions were prepared freshly and used.

Stock solutions of EDTA (100 g/L) and 8 N HCl was prepared in Milli-Q water. 3% stannous (II) chloride solution was prepared in 8 N hydrochloric acid.

Aquatic Tox medium

The test medium is a mixture of different macro nutrients, salts and vitamins. This helps in the survival of different organisms during exposure of different compounds. A mixture of well water and reverse osmosis water in the ratio of 1:1.7 liters was used as a blended water [26]. The M4 Medium was prepared in combination of different trace elements, marco nutrients and vitamins . The OECD TG 201 medium and the 20X AAP medium contain nutrients and other useful salts that help the growth and multiplication of aquatic organism .

Digestion procedure

To a known volume of standard/sample taken in 250 ml crimp top vial, 5mL of 10% EDTA solution in boiled distilled water was added, swirled the contents to mix, then 15 ml of HCl/$SnCl_2$ [8 N / 3%(w/v)] mixture was added

Table 1
Determination of dithiocarbamates in different spiked samples

Medium	Fortification level (mg/L)	Mancozeb		Zineb		Propineb	
		Mean Recovery (%)*	% RSD	Mean Recovery (%)*	% RSD	Mean Recovery (%)*	% RSD
Blended water	0.02	98.12	2.36	98.25	2.73	102.12	2.56
	2	97.56	2.02	98.42	0.98	98.61	2.12
OECD TG 201	0.02	96.58	1.58	96.4	1.64	98.27	1.35
	2	95.87	2.14	95.76	1.21	95	0.87
M4	0.02	96.75	1.45	100.18	2.32	97.65	1.96
	2	97.95	1.69	98.62	1.28	96.28	1.48
20XAAP	0.02	97.65	1.84	97.45	1.75	99.12	2.31
	2	95.99	2.78	98.24	2.03	98.42	1.98

* Average of five replicate determinations.

along with 20 ml of n-Heptane and immediately capped the crimp top vials. The vials were placed in a hot air oven at 95° C for one hour. After digestion period the n-Heptane layer was collected in GC vials and injected into GC-MS.

Method validation

The method for the determination of dithiocarbamate residues in aquatic tox medium was validated in terms of method specificity, linearity, assay accuracy, precision, limit of determination and quantification. The results are expressed in term of its CS_2 content.

Results and Discussion

Specificity

Specificity was confirmed by injecting the n-Heptane trap of medium [Fish Medium (1:1.7 Reverse osmosis water and well water), Daphnia (M4 Medium), Algae medium (OECD – TG201 Medium)]. There were no matrix peaks in the chromatograms to interfere with the analysis of CS_2 shown in **Fig. 1**. Furthermore, the retention time of CS_2 was relatively constant at 5.0 ± 0.2 min **Fig. 2**.

Linearity

A series of calibration solutions were prepared by diluting the stock solution into 10 mL volumetric flasks, and brought to volume with DMSO. The individual calibration solutions prepared in 10 mL volumetric flasks were pipetted, in triplicate, into separate clean 250mL capacity crimp top vials giving rise to a series of solutions containing 0.01, 0.10, 1, 5, 10, 50 and 100 µg. All the standard solutions were processed and qualitative analysis was based on the retention time (5.0 min) of carbon disulfide by injecting carbon disulfide solutions made in n-Heptane.

Calibration curve constructed for concentration against instrument response was found to be linear. The following linear equation with regression coefficient R^2=0.999 was indicative of good correlation of concentration over

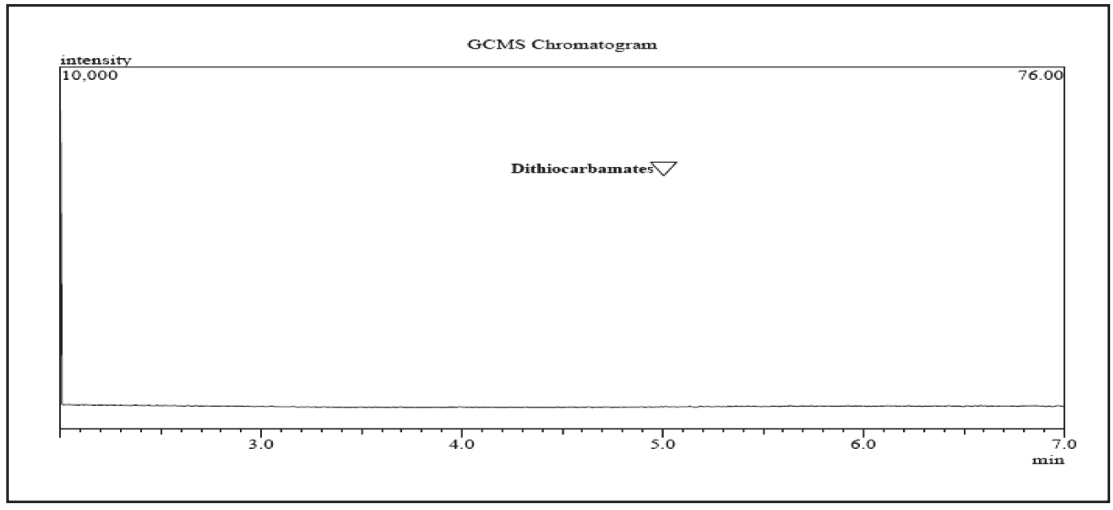

Fig.1: Representative GC-MS Chromatogram of n-Heptane trap of Daphnia (M4 medium)

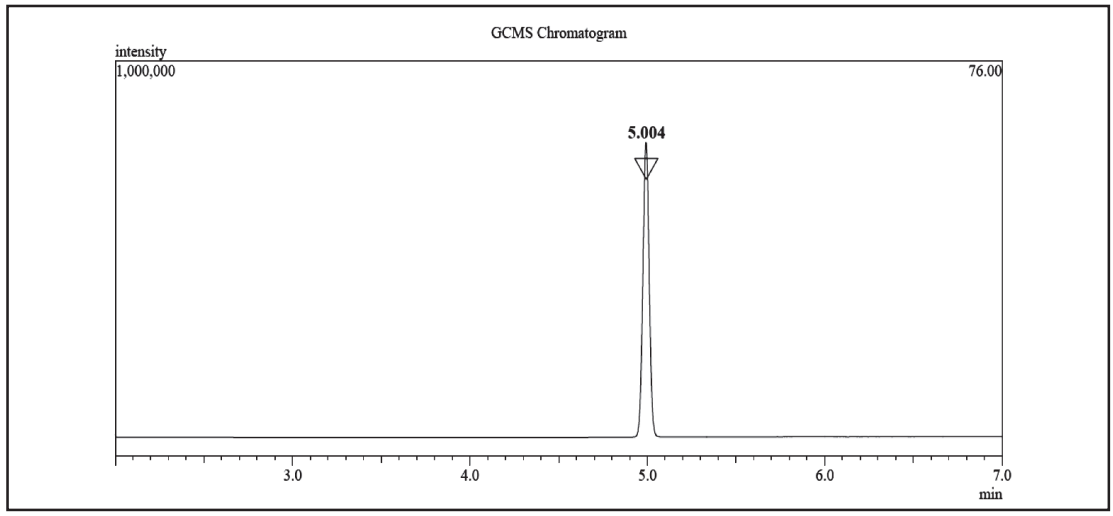

Fig. 2: Representative GC-MS Chromatogram of CS_2 Standard (5 µg/mL)

instrument response for dynamic range 0.01 to 100 mg/L. Linear equation y = -3.260+12164x.

Assay accuracy and precision

Recovery studies were carried out at 0.02 and 2 mg/L fortification levels for mancozeb, zineb and propineb (n=5 for each at two fortification levels) by spiking 10 mL (aquatic mediums meant for fish, daphnia, alga and lemna) samples with the appropriate volumes of standard solutions. After spiking, samples were handled and processed as described.

The recovery of mancozeb, zineb and propineb were > 95%. The method was validated over the fortification level 0.02 – 2.0 mg/L. The repeatability of the method is satisfactory (RSDs <5 %) for five replicate analysis. The recovery data and relative standard deviation values for various mediums are shown in **Detection and Quantification Limits**

The limit of quantification was determined to be 0.02 mg/L. This quantification limit was defined as the lowest fortification level evaluated at which acceptable average recoveries were 96-102%, RSD <3%. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at a level approximately 10 times the baseline noise in the chromatogram.

The limit of lowest detection was determined to be 0.006 mg/L at a level of approximately three times the back ground of control injection around the retention time of the peak of interest. The LOD and LOQ values were 0.006 mg/L and 0.02 mg/L **Fig.3**.

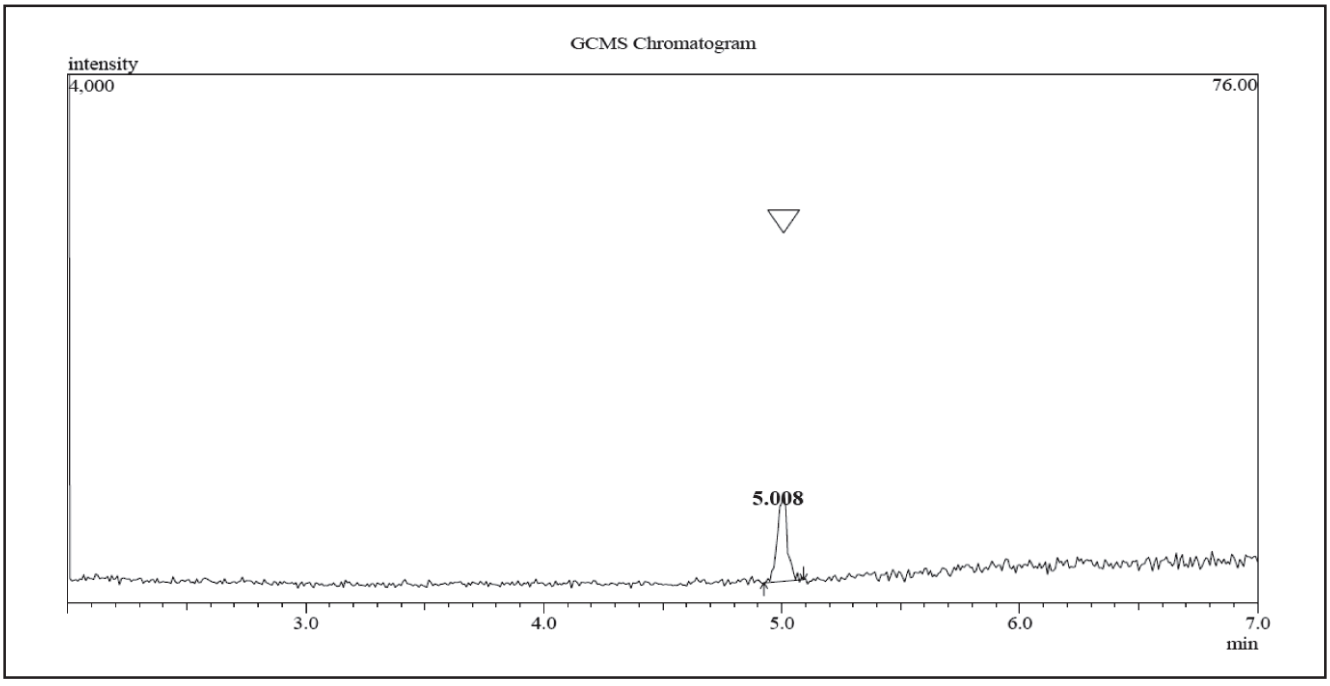

Fig. 3: Representative GC-MS Chromatogram at fortification level of 0.02 mg/L in M4 medium

Conclusion

This validated analytical method provides fast and accurate results in quantification of dithiocarbamate residues and has greater advantage over classical methods such as UV-VIS method and GC-FPD in terms of precise quantification of CS_2 over a wide range from 0.01 mg/L to 100 mg/L.

Acknowledgement

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Design And Study of Darifenacin Oral Extended Release Tablets

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ABSTRACT

The objective of current research work is development and evaluation of Darifenacin hydrobromide extended release tablets and compared with the innovator product (ENABLEX). The Darifenacin ER Tablets were developed with two grades of controlled release polymers such as hydroxypropylmethylcellulose K4M & K100M by direct compression method. The developed tablets were film coated with opadry white. Drug excipient compatability studies was determined by DSC.The developed Darifenacin ER Tablets were evaluated for physiochemical parameter and all physical parameters were found to be satisfactory and drug release was similar with innovator product (ENABLEX) with hydroxypropylmethylcellulose K4M & K100M at 45% w/w and 5%w/w respectively. The compatability study found that there was no chemical interaction between drug and excipients.The Darifenacin ER Tablets developed succesfully and drug release obtained upto 24 hours at controlled manner.

Keywords: Darifenacin Hydrochloride, Extended release tablets, Hydroxypropylmethylcellulose.

Introduction

High patient compliance and flexibility in designing dosage forms attracted the oral drug delivery systems to be the most convenient mode of drug administration when compared to other dosage forms. Of these, matrix systems have gained widespread importance in controlled drug delivery due to cost-effective manufacturing technology. Matrix drug delivery systems are of two types: diffusion/swellable systems and dissolution systems. In diffusion systems, drug release is mainly governed by the hydration of matrices followed by diffusion of the drug molecules from the hydrated layer to the surrounding bulk solution, and sometimes, partially by erosion/dissolution [1]

Darifenacin works by blocking the M3 muscarinic acetylcholine receptor, which is primarily responsible for bladder muscle contractions. It thereby decreases the urgency to urinate. It should not be used in people with urinary retention Darifenacin hydrobromide is a white to almost white, crystalline powder, with a molecular weight of 507.5, it is sparingly soluble in methanol and soluble in methylene chloride. The oral bioavailability of 7.5 mg ER tablets is very low (15%) due to the hepatic first pass metabolism, protein binding is 98%, steady-state volume of distribution (Vss) is 163 L, clearance is 40 L/h – 32 L/h and elimination half-life is approximately 13-19 hours.

Hydroxypropyl methylcellulose (HPMC) has been widely used for pharmaceutical applications because of its nontoxic property, ease of handling, small influence of processing parameters, and direct compressible manufacturing[2, 3]. A swellable and hydrophilic property of HPMC has been paid considerable attention for the preparation of matrix tablet as sustained release formulations of various drugs[2, 5].

The purpose of this study was to formulate Darifenacin extended release matrix tablets using HPMC as ER polymer. The developed ER tablets were evaluated for physical parameteres and the drug release profiles and mechanism of drug release from HPMC matrix tablet were investigated in 0.01 M Hcl for 24 h. The in vitro dissolution profile of developed tablets were compared with innovator product (ENABLEX manufactured by Novartis Pharma, Puerto Rico) and drug excipient compatibility study carried out by DSC.

Materials

Darifenacin Hydrobromide was obtained from MSN Pharmachem Pvt.Ltd, Hyderabad, Lactose anhydrous

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purchased from DMV Fonterra excipients, Dibasic calcium phosphate (DCP) purchased from Signet chemical corporation Pvt. Ltd, Mumbai, Methocel K4M, K100M were purchased from Colorcon Asia Pvt Ltd, Magnesium stearate purchased from Sunshine organics Pvt. Ltd. ENABLEX purchased from Warner Chilcott USA.

Methods

Preparation of Darifenacin HBr ER tablets

Tablet formulations were prepared by direct compression method. Proportion of excipients with drug was as given in Table 1. All ingredients were sifted through sieve no. 40 and magnesium stearate sifted through sieve no. 60. Darifenacin Hbr, Lactose anhydrous, DCP, Methocel K4M, Methocel K100M loaded into Rapid Mixer Granulator (RMG) and mixed for 15 mins, after 15 min added magnesium stearate and mixed again for 5 min. Tablets were compressed using Rotary tablet machine with 8.00 mm standard concave punch with 200 mg tablet weight followed by film coating with opadry white.

Evaluation of granules:

Bulk Density

Both poured (or fluff) bulk (Do) and tapped bulk densities (DF) were determined by Tapped density apparatus USP (Electrolab ETD 1020), according to the method reported by Raghuram et al[5] whereby a quantity (35 g) of granules from each formula, previously lightly shaken to break any agglomerates formed, was introduced into a 100-mL measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2-second intervals. The tapping was continued until no further change in the volume was noted [6].

Hausner's Factor

Hausner found that the ratio DF /DO was related to interparticle friction and, as such, could be used to predict powder flow properties [6].

Compressibility Percentage

The compressibility index of the granules was determined by Carr's compressibility percentage[6].

Compressibility % = (DF - DO)/DF . 100

The physical properties of granules were shown in Table 2.

Evaluation of Tablets

Thickness

The thickness of the tablets was determined using a

Table 1.
Composition of Darifenacin oral extended-release tablets

S.No	Name of the Ingredient	F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)	F6(mg)
1	Darifenacin HBr	17.5	17.5	17.5	17.5	17.5	17.5
2	Lactose anhydrous	108.5	98.5	88.5	41.5	40.5	39.5
3	DCP (DC Grade)	80	80	80	40	40	40
4	Methocel K4M	170	180	190	80	90	100
5	Methocel K100M	20	20	20	10	10	10
6	Magnesium stearate	4	4	4	2	2	2
	Total Weight	**400**	**400**	**400**	**200**	**200**	**200**
	Film coating (%)	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**

Table 2.
Evaluation data of Darifenacin ER granules

Formulation Code (LBD) gm/ml	Loose bulk density (TBD) gm/ml	Tapped bulk density index (%)	Compressibility	Hausner ratio
F1	0.44	0.54	18.52	1.23
F2	0.51	0.63	19.05	1.24
F3	0.54	0.67	19.4	1.24
F4	0.47	0.56	16.07	1.19
F5	0.49	0.59	16.95	1.2
F6	0.52	0.64	18.75	1.23

thickness gauge (Mitutoyo, New Delhi, India). Ten tablets from each batch were used. Thickness values were reported in millimeters. Mean and SD were calculated.

Average Weight of the Dosage Unit

To study weight variation, 10 tablets of each formulation were weighed using an electronic balance (Mettler Toledo, Basel, Switzerland). Weight values were reported in milligrams. Mean and SD were calculated.

Hardness Test

For each formulation, the hardness of 6 tablets was determined using a hardness tester (VK 200, Vankel, Varian Inc, Palo Alto, CA). Hardness values were reported in kilograms (kg). Mean and SD were calculated.

Friability Test

For each formulation, 6 tablets were weighed. The tablets were placed in a friabilator (Campbell Electronics, Mumbai, India) and subjected to 100 rotations in 4 minutes. The tablets were then dedusted and reweighed. The friability was calculated as the percentage weight loss.

Content Uniformity:

20 tablets were weighed and finely powdered. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of Darifenacin Hbr to a 500-ml volumetric flask and add 50 ml of 0.01 M hydrochloric acid, and sonicate to dissolve it. Shake by mechanical means for 10 min, dilute with water to volume, mix, and pass through a filter having a 0.5¼m or finer porosity. Drug content was determined by using UV Visible Spectrophotometer at 286nm.

Compatability Studies:

Compatability studies were carriedout by Differential Scanning Calorimetry (DSC Q10 V9.0 Build 275). The Pure sample along with individual excipients were analysed for DSC to know the compatability of exciepients with drug.

***In-Vitro* Drug Release study:**

In-vitro drug release studies were carried out for extended release Darifenacin Hbr formulations using 0.01M HCl as dissolution medium using USP Apparatus-I (Basket) at 100 rpm (Electrolab, 2000) and the temperature was maintained at 37±0.5°C. The dissolution was continued for 24 hours while samples of 5 ml were withdrawn at regular interval and replaced with equal volume of fresh dissolution medium to maintain the volume constant. The samples were filtered, diluted and analyzed for drug content. The amount of drug released was determined by UV spectrophotometer at 286nm. Drug release at specified time points was calculated. The obtained results were compared with the Innovator results for the calculation of similarity factor [7] using the formula

$$f2 = 50 * \log \{[1+ (1/n) \text{“}t=1 * n (Rt-Tt)^2]^{-0.5} *100\}$$

Kinetic Data analysis:

To study the release kinetics and mechanism for drug release, *In-Vitro* release data was fitted in to various kinetic models (Zero order, First order, Higuchi and Korsmayer peppas) [8],[9]. The zero order rate Eq. (1), describes the systems where the drug release rate is independent of its concentration[10]**.** The first order Eq. (2) describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix reservoir as a square root of time dependent process based on Fickian diffusion Eq. (3)[11]

$$C = K_0 t \quad \text{..... (1)}$$

Where, K_0 is zero-order rate constant expressed in units of concentration/time and ‘t’ is the time.

$$\text{Log } C = \text{Log } C_0 - Kt / 2.303 \quad \text{..... (2)}$$

Where, C_0 is the initial concentration of drug and **‘K’** is first order constant.

$$Q = Kt_{1/2} \quad \text{..... (3)}$$

Table 3

Physical properties of Darifenacin oral extended-release tablets

Formulation Code	Weight (mg) (mm)	Thickness (kg/cm²)	Hardness (%)	Friability (%)	Drug Content
F1	400±4	4.3±0.05	10±1	0.05	99.1±1.0
F2	400±4	4.3±0.05	10±0.5	0.06	99.5±0.65
F3	400±4	4.3±0.05	10±1	0.04	98.7±1.3
F4	200±2	3.7±0.05	8±0.5	0.05	100.5±0.75
F5	200±2	3.7±0.05	9±0.5	0.04	99.8±1.0
F6	200±2	3.7±0.05	10±1	0.05	101.2±0.63

All values represent Mean±SD

Where, **'K'** is the constant reflecting the design variables of the system. minutes, dilute with water to volume, mix, and pass through a filter having a 0.5¼m or finer porosity. Drug content was determined by using UV Visible Spectrophotometer at 286nm.

Mechanism of drug release:

Korsmeyer et al derived a simple relationship which described drug release from a polymeric system Eq. (4). To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model[12].

$$Mt / M\ 8 = Kt^n \quad \text{..... (4)}$$

Where Mt / M8 is the fraction of drug released at time t, K is the rate constant and '**n**' is the release exponent. The **'n'** value is used to characterize drug release mechanism from the cylindrical shaped matrices. The following plots were made: cumulative % drug release vs. time (zero order kinetic model); log % drug remain to be released vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model) and log cumulative % drug release vs. log time (Peppas model).

Stability studies:

The prepared tablets were subjected to stability studies. The stability conditions include Long-term (ICH) 25^0C/ 60%RH, Intermediate (ICH) 30^0C/65%RH and Accelerated (ICH & WHO) 40^0C/75%RH.

Results

The pre-formulation studies of granules of Darifenacin oral extended release tablets were shown in Table 2 The bulk density was in the range of 0.44 – 0.54 g/ml,Hausner ratio 1.19-1.24, and compressability index 16.07-19.4. The tablet physical parameters were shown in Table-3**.** The tablet hardness was found tobe 8 -11 Kg/cm^2,Friability 0.04-0.06% and content uniformity 98.7-100.5%.

The extended drug release pattern obtained with HPMC K4M(45% w/w) and 5 % of HPMC K100M.

Compatability Studies:

The DSC reports of Pure Darifenacin HBr and Drug with individual excipients shown in Figure 1-4 The compatability studies found that the main thermic peak of pure drug found at 232^0C.When it mixed with excipients DCP, HPMC K 100, HPMC K4M. The main endothermic peak of Darifenacin was found that 230^0C – 233^0C. It observed that there is no significant change in Darifenacin main endothermic peak so it indicate there is no interaction between Darifenacin and DCP, HPMC K 100 M & HPMC K4M.It compatible with these excipients.

In-Vitro **drug release Studies:**

In-vitro drug release profiles of all the six formulations of extended release tablets of Darifenacin and the marketed product (Innovator)were shown in Figure 5. The percentage

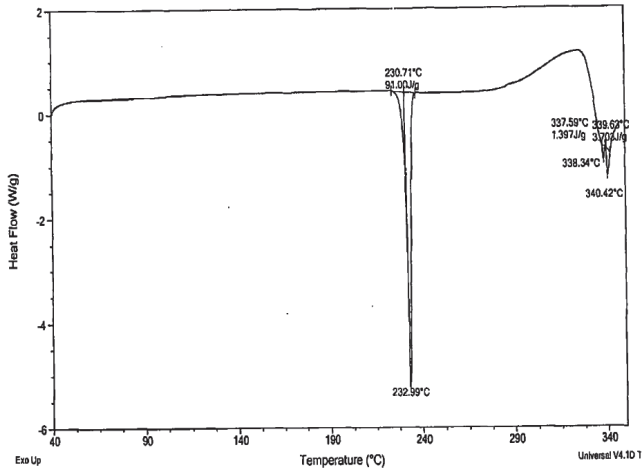

Figure 1. Darifenacin HBr DSC report

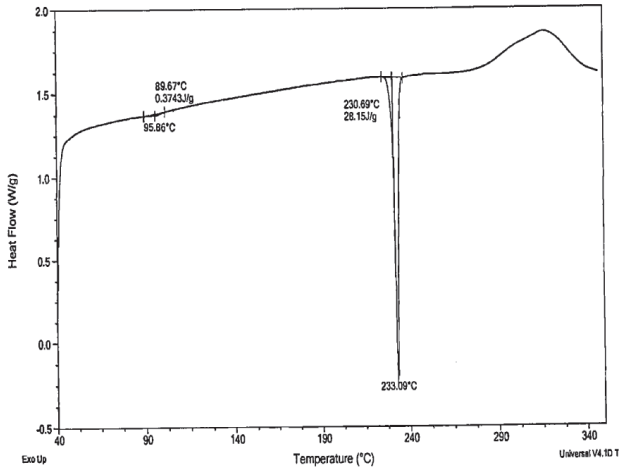

Figure 2. Darifenaci HBr + DCP DSC report

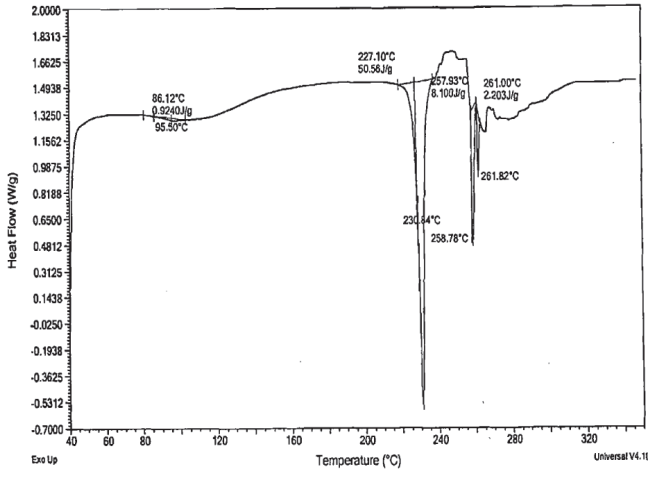

Figure 3. Darifenacin HBr + HPMC K100M DSC report

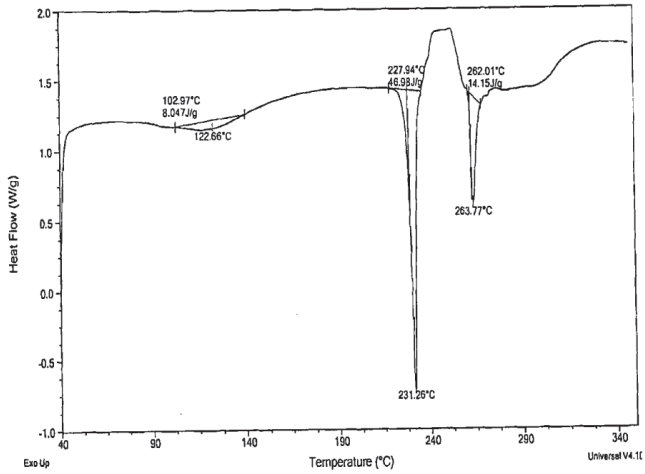

Figure 4. Darifenaci HBr + HPMC K4M DSC report

drug release of formulations F1-F6 was 87%, 88%, 85%, 96%,100% and 86% respectively at the end of 24hrs. From the above results the formulation F5 releases high % drug release and it has similarity factor f2=62, it is more than the other formulations. The extended drug release pattern obtained with HPMC K4M(45% w/w) and 5 % of HPMC K100M.

The drug release kinetic data shown in Table-4. The correlation coefficients(r^2) for zero order 0.8 – 0.94,first order 0.79 – 0.99 and Higheni model 0.92 – 0.99.The release exponent(n), correlation coefficient values for peppars model were found 0.32 – 0.58 & 0.92 -0.99 respectively.

Discussion

The HPMC which is commonly used in hydrophilic matrix drug delivery system,is mixed alkyl hydroxy alkyl cellulose ether containing methoxy and hydroxy propyl groups.The hydration rate of HPMC increase in the hydroxy propyl content.The solubility of HPMC is pH independent [13]. From the pre-formulation studies,it was concuded

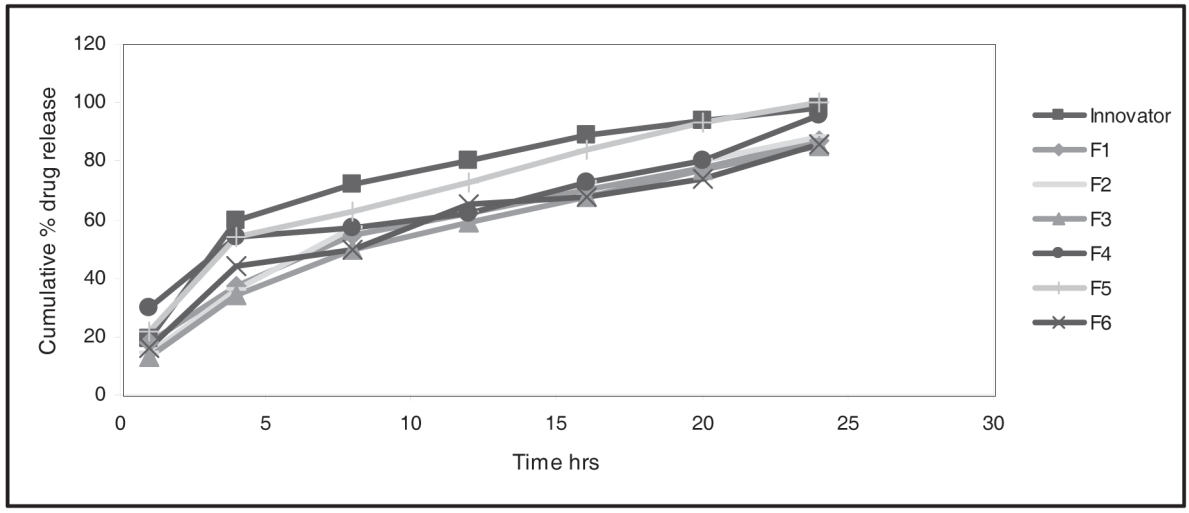

Figure 5. *In-Vitro* Drug release profile Darifenacin ER tablets

Table 4

***In-vitro* release kinetics of Darifenacin oral extended-release tablets**

Formulation Code	ZeroOrder	First Order	Higuchi model	Peppas model	
				n	r
F1	0.94	0.98	0.99	0.49	0.99
F2	0.92	0.98	0.99	0.58	0.98
F3	0.95	0.99	0.99	0.58	0.99
F4	0.93	0.79	0.94	0.32	0.95
F5	0.91	0..96	0.98	0.46	0.97
F6	0.9	0.95	0.97	0.5	0.97
Innovator	0.8	0.97	0.92	0.49	0.92

that all physical parameters of blend analysis,the flow of granules were free flowing property and tablet parameter like weight variation, thickness variation, hardness, friability were shown acceptable pharmaceutical properties and complied with the in-house specifications. Content uniformity results indicated that the drug content is uniform to tablet to tablet and within the specification.

In F1 -F3 total tablet weight was 400 mg. and the drug release slow when compared with innovator. We changed total tablet weight from 400 mg to 200 mg to improve the dissolution. In F4 formulation the drug release was fast. In F5 the drug release was compiled with innovator product and also maximum drug release obtained 45 % of HPMC K4M and 5 % of HPMC K100 M were required for optimum drug release compared with innovator and also the weight of total tablet also affecting dissolution profile when weight decreses the % drug release increased.

In the present study HPMC K100M & HPMC K4M were used as hydrophilic matrixing agent because it forms a strong viscous gel on contact with aqueous media,Which may be useful in controlled delivery of water soluble drugs.

In present study direct compression technique was better compared with matrixes prepared by wet granulation of physical mixture of the polymer with the drug.

In invitro dissolution study initial burst release within 1 hr, after drug release was controlled manner .The initial burst release due to the dissolution of drug from the surface of the tablet and this burst release pattern helpful for initiation of therapeutic effect controlled release pattern of drug release helpful for maintainance of therapeutic activity upto 24 hours.

The correlation coefficients for First order is high compared to Zero order,so the drug release was First order kinetics and mechanism of drug release was diffusion controlled .From pappers model the n values was in range of 0.32 – 0.58 it indicated that the drug release from matrix tablet following non-fickian (anomalous diffusion) diffusion.

In the present work, formulation F5 was selected as optimized formulation because in this formulation drug release pattern was initially rapid and then contolled manner upto 24hrs and more ever drug release was similar with the innovator product. In the formulations F1-F3 and F6 the drug release was low compared with Innovator product and in formulatio F4 the drug release was good but it is dissimilar with Innovator.

Conclusion

The Darifenacin HBr oral extended release tablets were prepared by direct compression method showed satisfactory results for various physical parameters like tablet hardness, weight variation, content uniformity and *in-vitro* drug release. Formulation, F5 was better than other formulations and it is similar with innovator product (ENABLEX) (f2 = 62.97).

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A Study on Antistress Activity of *Vitis Vinifera* and *Chicorium Intybus* in Albino Mice

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ABSTRACT

Vitis vinifera and *Chicorium intybus* are medicinal plants used in the treatment of many clinical conditions in India. Their antistress activity have been investigated in this study using albino mice. The ethanolic extracts of *Vitis vinifera* seed and *chicorium intybus* roots were prepared and subjected to preliminary qualitative phytochemical screening. Acute toxicity studies were carriedout in albino mice. The ethanolic extracts did not show the lethal effect up to the dose of 5gm/Kg body weight with no signs of abnormalities or any mortality observed for 14 days period under observation after single dose of extract administration. The parameters studied were swimming endurance and drug induced narcosis in mice. Geriforte was used as a standard anti-stress agent which is a commercial preparation of Himalaya Drug Company. The results indicate that pretreatment with ethanolic extract of *Vitis vinifera* seed and *Chicorium intybus* root exhibited significant antistress activity at the tested doses of 200 mg/Kg and 400 mg/Kg body weight. On the basis of results, it was concluded that *Vitis vinifera* and *Chicorium intybus* possess antistress activity.

Key words : Anti-stress, *Vitis vinifera*, *Chicorium intybus.*

Introduction

Dr. Hans Seyle defined stress as the sum of all nonspecific responses of the body to any external stimuli action upon it [1]. Homeostatic mechanism attempts to counteract stress. When they are successful, the internal environment remains within normal physiological limits. If stress is extreme, unusual or long lasting, the normal mechanism may not be enough [2]. Some medicinal plants have been demonstrated to induce a state of non-specific increase of resistance in experimental animals as well as human beings, the most prominent being *Panax ginseng* [3]. The present study was therefore, undertaken to explore antistress activity of *Vitis vinifera* and *Chicorium intybus* in mice.

Vitis vinifera also called as common grape or wine grape or European grape belongs to the family Vitaceae, which is one of the fruit crops most widely grown throughout the world [4]. Grape seed is a complex matrix containing approximately 40% fiber, 16% oil, 11% protein and 7% complex phenols including tannins, in addition to sugars, minerals, salts etc [5]. Proanthocyanidins of grape seed are a group of polyphenolic bioflavonoids, which are known to possess broad pharmacological activities and therapeutic potentials [6]. Some of the pharmacological activities reported on *Vitis vinifera* are antibacterial and antioxidant [7-9], cardioprotective action [10, 11], Hepatic and Brain lipid preoxidation [12], antidiabetic [13], antithrombotic [14] and antilisterial [15].

Chicorium intybus is an erect perennial plant belongs to family asteraceae, with phytochemical constituents of Inulin, sesquiterpene, lactones, phytosterols, triterpenoids, flavonoids, coumarins (including cichoriin), caffeic acid derivatives, vitamins, tannins, pectins and fats [16,17]. Traditionally this plant is used in disordered menstruation, liver and spleen disorders, fever and inflammation. some of the reported pharmacological activities are tumor inhibitory activity [18], antimalarial activity [19], hepatoprotective activity [20], antidiabetic activity [21], antibacterial activity [22], anti and pro-oxidant activity [23], Gastro protective activity [24], postcoital contraceptive

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activity [25], anticonvulsant activity [26], antilithiatic activity [27] and immunomodulatory activity [28].

While reviewing the reported pharmacological activities and clinical uses in Ayurveda for *Vitis vinifera* and *Chicorium intybus*, it was found several diseases that are postulated to be induced by stress. This prompted us to study the antistress activity of *Vitis vinifera* and *Chicorum intybus* in stress induced animal model.

Materials and Methods

Plant materials:

Vitis vinifera (grapes) and roots of *Chicorum intybus* were collected from Bangalore market. The collected material were identified and authenticated by botanist. The voucher specimen was deposited in the laboratory for future reference.

Extraction of *Vitis vinifera:*

Grape seeds were finger pressed to separate seeds from pulp and skin. The seeds were washed twice with tap water and then left to dry in open air away from direct sun light for one week. The seeds were ground into powder using coffee grinder and passed through 40 mesh to obtain a particle size of less than 0.4 mm. The powdered grapes seeds were extracted in soxhlet extractor with petroleum ether at 60^0 C for 6 hrs to remove fatty materials. The defatted grape seed powder was re-extracted in a soxhlet extractor with 95% ethanol at 65^0 C for 24 hrs. The ratio of seed powder to solvent was 1:7.5. The extract was concentrated in a rotary evaporator under reduced pressure at a temperature of 35^0 C to get a viscous liquid. Finally it was evaporated on water bath to a dry residue and kept it in a dessicator. The yield of grape seed extract was 12.31% w/w.

Extraction of *Chicorium intybus*

Chicorium intybus roots were air dried away from direct sun light for 15 days. The dried slices of *Chicorium intybus* were ground into powder using mill. The powder was extracted in soxhlet extractor with 95% ethanol at 70^0 C for 20 hrs. The extract was concentrated in a rotary evaporator under reduced pressure at a temperature of 40^0 C to get a brown viscous liquid that was finally dried. The yield of extract was 14% w/w.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed as per standardized procedure [29] and various phytoconstituents present in the extract were identified.

Experimental Animals

Adult Albino mice of either sex, weighing between 22 to 26 gm were used for the study. Animals were housed in a clean polypropylene cage under standard conditions of temperature (25 ± 2^0 C), humidity (50-55%) and light (dark/light-12/12 hrs cycle). Animals were allowed to take standard laboratory feed and water ad libitum. All the animals were acclimatized for seven days to the laboratory environment. Animal experiment was carried out according to the guidelines and approval of the Animal Ethics Commitee (Reg No. 131/99/CPCSEA).

Acute toxicity study

The acute toxicity study was carried out as per guideline set by Organization for Economic Co-operation and Development (OECD guideline no. 425) received from CPCSEA. Starting dose was selected to be 2000 mg/Kg body weight and finally a dose of 5000 mg/Kg body weight was evaluated for toxicity. Mortality within 48 hrs was recorded in overnight fasted Albino mice. The animals were observed for a further 14 days for any signs of delayed toxicity.

Antistress activity

Effect of extracts on swimming endurance of mice

Albino mice of either sex were divided into following groups of six animals each.

Group I (stress control): Animals received only 1% w/v Acacia as a vehicle.

Group II (Test 1 and stress): Animals received Vitis *vinifera* seed extract (200mg/Kg body weight)

Group III (Test 2 and stress): Animals received Vitis *vinifera* seed extract (400mg/Kg body weight)

Group IV (Test 3 and stress): Animals received *Chicorium intybus* root extract (200mg/Kg body weight)

Group V (Test 4 and stress): Animals received *Chicorium intybus* root extract (400mg/Kg body weight)

Group VI (Standard and stress): Animals received Geriforte (100 mg/Kg body weight)

Vehicle, extract and Geriforte were given to mice once daily for a period of 10 days orally. On tenth day all the mice were subjected to swimming stress by keeping them in cylindrical vessel (30cm X 25cm) containing water at room temperature. The mice were allowed to swim till they got exhausted. The end point was taken when the animals drowned and death occured. Swimming survival time for each animal was noted and mean swimming survival time for each group was calculated. The data obtained were subjected to statistical analysis.

Effect of extracts on drug induced narcosis in mice

Albino mice were deprived of food for 24 hrs before experiment. Animals were divided into following groups of six animals each.

Group I: Animals received 1% w/v Acacia as a vehicle 30 minute prior to the pentobarbitone sodium (50 mg/Kg body weight, i.p.)

Group II: Animals received *Vitis vinifera* seed extract

(200 mg/Kg body weight) 30 minute prior to the pentobarbitone sodium (50 mg/Kg body weight, i.p.).

Group III: Animals received *Vitis vinifera* extract (400mg/kg body weight) 30 minute prior to the pentobarbitone sodium (50 mg/Kg body weight, i.p.).

Group IV: Animals received *Chicorium intybus* root extract (200 mg/Kg body weight) 30 minute prior to the pentobarbitone sodium. (50 mg/Kg body weight, i.p.).

Group V: Animal received *Chicorium intybus* root extract (400mg/kg body weight) 30 minute prior to the pentobarbitone sodium. (50 mg/Kg body weight, i.p.).

Group VI: Animal received Chlorpromazine (3mg/kg body weight, i.p.) 30 minute prior to the pentobarbitone sodium. (50 mg/Kg body weight i.p.).

Group VII: Animal received Chlorpromazine (3 mg/kg body weight i.p.).

Mice were treated with vehicle, pentobarbitone sodium, *Vitis vinifera* extract, *Chicorium intybus* extract and chlorpromazine in the above order. Then Narcosis time was recorded in each animal.

Narcosis time:- this is the time elapsed between the loss of righting reflex and recovery of the same reflux. This interval was expressed in minute.

Statistical Analysis

All the results were expressed as mean ± SEM and subjected to one way Analysis of variance followed by Dunnet's test for comparision between the groups. A difference in the mean p value < 0.05 was considered as significant.

Results

Preliminary phytochemical screening revealed the presence of carbohydrate, proteins and phenolic compound in *Vitis vinifera* seed extract, whereas in *Chicorium intybus* root extract carbohydrate, protein, phenolic compound and phytosterol were present.

Acute toxicity study

The animals did not show significant autonomical and behavioural changes observed for 14 days. There were no lethal effect upto the doses of 5000 mg/kg. Thereby showing the safety of *Vitis vinifera* and *Chicorium intibus*.

Effect of extracts on swimming endurance of mice

As shown in table-1 the survival time of swimming mice was significantly (P < 0.01) increased in the animals treated with extract of *Vitis vinifera* and *Chicorium intybus* at both the tested doses of 200 and 400 mg/Kg body weight as compared to the untreated animals. The extract effects were at par with that of the standard drug (Geriforte) and it is dose-dependent.

Effect of extract on drug induced narcosis in mice

As shown in table-2 the narcosis time of mice was significantly (P < 0.01) increased in the animals treated with extract of *Vitis vinifera* and *Chicorium intybus* at both the tested dose of 200 and 400 mg/Kg body weight as compared to group untreated with extract. The extract effects were at par with that of the standard drug (Chlorpromazine) and it is dose dependent.

The extract effect were approximately equi-effective as standard drug whereas *Vitis vinifera* (400 mg/Kg) exhibited a higher antistress activity.

Discussion

Adaptogens are pharmacological agents that induce a state of non-specific increase of resistance of organisms (SNIR) to aversive stimuli that threaten to perturb internal homeostasis [3]. During 1958, medicinal substance causing SNIR were named as ADAPTOGENS [30]. A rational approach was made to evaluate antistress activity. For this various stressful situations were induced in animals. Rodents when forced to swim in a restricted space from which they

Table-1: (Effect of extracts on swimming endurance of mice)

Sl. No.	Group	Treatment	Dose	Swimming survival time (min) Mean±SEM
1	Stress control	Vehicle (1% w/v Acacia)	0.3 ml	187.33 ± 7.365
2	Test 1 and stress	*Vitis vinifera* seed extract	200mg/kg body weight	227.33 ± 5.469*
3	Test 2 and stress	*Vitis vinifera* seed extract	400 mg/kg body weight	243.5 ± 9.291**
4	Test 3 and stress	*Chicorium intybus* root extract	200 mg/kg body weight	223.66 ± 7.706 *
5	Test 4 and stress	*Chicorium intybus* root extract	400 mg/kg body weight	236.66 ± 8.131**
6	Standard and stress	Geriforte	100 mg/kg body weight	248 ± 12.056**

Values are expressed as mean ± SEM. n= 6. when compared with stress control *P<0.01, **P<0.001

Table- 2: (Effect of extract on drug induced narcosis in mice)

Sl. No.	Group	Treatment	Dose	Narcosis time (min) Mean± SEM
1.	Control (pentobarbitone sodium)	Pentobarbitone sodium	50mg/kg body weight	32.5 ± 2.262
2.	Test 1 and pentobarbitone sodium	Vitis vinifera seed extract and pentobarbitone sodium	200mg/kg body weight + 50mg/kg body weight	49.5 ± 4.448*
3.	Test 2 and pentobarbitone sodium	Vitis vinifera seed extract and pentobarbitone sodium	400mg/kg body weight + 50mg/kg body weight	58.16 ± 5.300***
4.	Test 3 and pentobarbitone sodium	Chicorium intybus root extract and pentobarbitone sodium	200mg/kg body weight + 50mg/kg body weight	51 ± 5.079**
5.	Test 4 and pentobarbitone sodium	Chicorium intybus root extract and pentobarbitone sodium	400mg/kg body weight + 50mg/kg body weight	52.83 ± 5.558**
6.	Standard and pentobarbitone sodium	Chlorpromazine and pentobarbitone sodium	3mg/kg body weight + 50mg/kg body weight	61.83 ± 4.094***
7.	Standard	Chlorpromazine	3mg/kg body weight	Nil

Values are expressed as Mean ± SEM. n = 6 when compared with control. *P<0.02, **P<0.01, ***P<0.001

cannot escape become immobile after an initial period of vigorous activity. It has been suggested that the observed immobility signifies behavioural "despair" resembling a state of mental depression [31] and has been used to screen antidepressants. It is now recognised that this behavioural depression is fairly a common consequence of stress [32]. It is also evident that the animal ability to cope with the stress largely influence the neurochemical consequences of stress. Thus exposure of rats to inescapable and severe stress leads to depletion of central noradrenaline and serotonin [33], postulated to be the cause of endogenous depression.

Greater swimming endurance has reported in mice when pretreated with antistress agents [34] and the test has been utilized to investigate the adaptogenic activity of different agents, based on the fact that swim endurance reflects physical endurance [35].

The swimming endurance test results indicate clearly that the extract of *Vitis vinifera* and *Chicorium intybus* have the properties whereby they increased the physical endurance as well as the overall performance in mice. Recent research has confirmed that adaptogens have various effects on organism. Generally, they seem too able to protect the organism from the unfavourable stress conditions i.e. physical, biological and mental [36]. It proposed that the mechanism by which these adaptogens act appears to involve stimulation of the inhibitory synapse in the brain [37]. One of the mechanisms that could explain the adaptogenic effect is their capacity to depress the CNS. This can evaluated by an investigation of the ability of the extracts to synergise with known CNS depressors such as barbiturates [38]. This study evaluates the effect of these adaptogenic extracts on the righting reflex in mice, as the means to evaluate the effect of these adaptogens on the CNS. *Vitis vinifera* and *Chicorium intybus* extract have a clear synergism with pentobarbitone sodium in depressing the mouse CNS response.

Conclusion

The present study clearly indicate that *Vitis vinifera* and *Chicorium intybus* could increase the non specific resistance of the organism against stress, thereby providing a promising adaptogen and further in depth studies are required to elucidate its mechanism of action.

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Design and Evaluation of Aceclofenac Embedded Eudragit S 100 Granules Filled in HPMCP Coated Capsules for Colon Targeting

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ABSTRACT

The purpose of this research was to develop a novel colon specific drug delivery system for aceclofenac using pH dependent polymers HPMCP and Eudragit S100. The capsules were evaluated for physico-chemical properties, drug content, moisture uptake and *in vitro* drug release studies. The amount of aceclofenac released from the capsule at different time intervals was estimated by UV spectrophotometric method at 275nm. The optimized formula showed, no release in stomach, in small intestine it showed 19.6% and in colon it was 90.04% in controlled manner. Hydroxy propyl methyl cellulose phthalate prevented release of the Aceclofenac in the physiological environment of stomach and Eudragit S 100 controlled the release in intestine. The FTIR and DSC results revealed the compatability of drug with the excipients. The *In-vitro* dissolution studies shown that capsules were promising for target delivery of the drug. The findings of the present study conclusively state that the capsules are promising for colon targeting of aceclofenac to synchronize the chronobiological symptoms for effective treatment of rheumatoid arthritis.

Keywords: Aceclofenac, Eudragit S100, Colon target, Hydroxy propyl methyl cellulose phthalate.

Introduction

Oral route is one of the most convenient routes for administration of drugs. The administered drugs normally dissolve in the acidic/basic gastric fluids and gets absorbed easily. Oral route becomes a limitation when drug has to be concentrated on particular area of the GIT for localized action or when side effects occurs due to release of the drug in the initial stages of the gastro intestinal tract. Colon offers many advantages like near neutral pH, long transit time and reduced activity of digestive enzymes. Colon as a site is suitable for both local and systemic administration of drugs [1 - 3]. To reach the colon, the dosage forms must be formulated taking into account the obstacles of the gastrointestinal tract (GIT). Various methods used for colon targeting include, pH dependent drug release, metabolism by microbial flora, enzyme metabolism and increasing transit time**.** CDDS can be used for the treatment of diseases that shows peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis [4]. Due to a comparatively longer transit time of colon than the stomach, absorption of poorly absorbed drugs can be improved by colon targeting [5]. The colonic site is being investigated as a potential site for the delivery of proteins, peptides, vaccines, and other drugs such as nifedipine, theophylline, and isosorbide [6, 7].

NSAIDS are widely used in the treatment of chronic inflammatory diseases like ulcerative colitis, inflammatory bowel syndrome, rheumatoid arthritis etc. Aceclofenac is a cyclo-oxygenase inhibitor which reduces the prostaglandin synthesis resulting in inhibition of inflammatory response [8]. It is practically insoluble in water and freely soluble in acetone and has a half life of 4 hrs [9].

The purpose of this study was to target Aceclofenac to colon using a pH sensitive polymer, Eugragit S 100 for the treatment of Rheumatoid Arthritis. It is an anionic copolymer of Methacrilic acid and Methyl methacrylate in

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1:2 ratio [10]. It is poorly soluble in acidic medium while readily soluble in neutral to weakly alkaline conditions (pH 6-7), thus it can be used for bypassing the stomach for targeting of drugs to colon region [11].

A novel approach of coating the capsules with Instacoat EN-HPMCP for bypassing the stomach was also performed. HPMCP is a phthalic half ester of hvdroxy propyl methyl cellulose, which is insoluble in gastric fluid while swells readily in upper intestinal fluid. Moreover Instacoat EN-HPMCP has got an advantage over other conventional enteric coating materials like Cellulose acetate phthalate since the tablets coated with the former has considerably less disintegration time [11]. Compared with small intestine where the pH value is higher than that of colon the rate release of drug from pH controlled systems is higher in small intestine. The small intestinal transit is fairly constant at 3-4 hrs in most individuals but gastric emptying is highly dependent on whether the dosage form is ingested in fasted or fed state. The pH dependent dosage form was enteric coated to prevent the release of drug in stomach; this represents a potentially suitable system for colon-targeted drug delivery.

Materials

Aceclofenac was purchased from Rachita Pharma Ltd, Avicel pH 101 was purchased from Accent microcel, Eudragit S 100 was purchased from Degussa Ltd., Instacoat EN-HPMCP was purchased from Ideal Cure Pvt Ltd. Other ingredients and solvents were of analytical grade.

Methods

Preparation of Granules

Aceclofenac granules were prepared by wet granulation method using Eudragit S 100 as binder. Aceclofenac and MCC pH 101 were passed through sieve no.40 and mixed using a Planetary Mixer (Kenwood, Chef Titanium), Eudragit S 100 dissolved in Isopropyl alcohol was added to the mixture as granulating agent. The wet granules were air dried and then at 60^0 C in a tray dryer for 20 min and then passed through sieve no.20. The granules were then mixed with magnesium stearate and talc which had been passed through sieve no. 60.

Filling of Capsule

The blend was filled in size 2 hard gelatine capsule using manual mapsule filling machine (Pam MF 30). The machine had a maximum loading capacity of filling 300 capsules. Empty capsules were loaded into the holes of loading tray, cap up and body down .The capsules were then separated and the pre-weighed blend was filled from the powder tray into the body. Using the teflon powder dispenser the powder was spread until the required weight gain was obtained. The caps were then placed back over the body.

Coating of Capsules

Base coat

The coating solution was prepared by dispersing Opadry white in water. The capsules were then charged into the pan of coating machine (Ideal cures). The coating solution was sprayed over the capsule using 1.5mm air nozzle with an atomizing air pressure of 3-4 atm. The pan speed was maintained at 30 RPM. The inlet and Outlet temperature were maintained at 40^0C and 35^0C respectively. The coating was performed until required weight gain was obtained.

Functional coat

The coating solution was prepared by dispersing Instacoat EN-HPMCP in a 1:3 mixture of Isopropyl alcohol and Dichloromethane. The base coated capsules were then loaded into the coating pan. The coating solution was sprayed over the capsule using 1.5mm air nozzle with an atomizing air pressure of 3-4 atm. The pan speed was maintained at 30 RPM. The inlet and outlet temperature were maintained at 35^0C and 30^0C respectively. Hot air was passed during the application of coat. Caution was taken to maintain the bed temperature not to exceed 32^0C. The coating was performed until required weight gain was obtained.

Determination of bulk density and tapped density

It is a measurement of packing of particles and also determines the amount of drug that occupies the volume in mg/ml before tapping and after tapping. An accurately weighed quantity of the powder (W), was carefully poured into the graduated cylinder and the volume(Vo) was measured, then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 100 taps and after that, the volume (Vf) was measured and continued operation till the two consecutive readings were equal. The bulk density and tapped density were calculated using the following formula:

$$\textbf{Bulk density} = W / Vo$$
$$\textbf{Tapped density} = W / Vf$$

Where, W = weight of the powder

Vo = intial volume

Vf = final volume

Compressibility index

Compressibility index is indirectly related to the relative flow rate, cohesiveness and particle size of the powder. The compressibility of a material can be estimated from the tap and bulk density measurements. Compressibility was calculated from the powder density using the following formula:

$$\% \text{ Compressibility} = 100 \times (Pt - Po)/Pt$$

Where, Pt= Tapped density

Po = Bulk density

Hausner's ratio

Tapped density and bulk density were measured and the Hausner's ratio was calculated using the formula,

Hausner's ratio = Pt/Po

Where, Pt = Tapped density

Po = Bulk density

Determination of moisture uptake

The moisture absorption behavior of each filling formula **(F1–F6)** was studied using the weight gain method [12]. Five grams of the formula were accurately weighed and transferred to a pre-weighed petridish. The dishes were then stored at room temperature in desiccators at constant relative humidity. Three values of relative humidity were used for each blend, 45%, 65% and 75% obtained using saturated solutions of potassium carbonate, sodium citrate and sodium chloride, respectively [13]. The petri dishes were weighed every two days for a period of 8 days and the percentage moisture uptake by each formula was calculated according to the following equation 2 [12]:

Moisture uptake = 100 (Wt – Wo)/Wo

Where, Wt is the weight of the formula at time t and Wo is the initial weight of the formula. The calculated percentages were plotted against time to obtain the moisture absorption isotherm for each formula.

Determination of drug content

Aceclofenac blend was taken from the capsule and was grinded using mortar and pestle and a quantity of powder equivalent to 50 mg of Aceclofenac was accurately weighed and transferred to 100 ml volumetric flasks containing 50 ml of 6.8 pH buffer. The flasks were then shaken to solubilize the drug. The volume was made up with 6.8 pH buffer and mixed thoroughly. The solutions were filtered through a 0.22 mm membrane filter and analyzed for the content of Aceclofenac using the UV–Visible Scanning Spectrophotometer at λ = 275nm.

Weight variation

Twenty capsules from each formulation were individually weighed and mean weights of each capsule was calculated. Then the weight variation was assessed for each capsule.

Content uniformity

Ten randomly selected capsules from each formulation were assayed for drug content uniformity. The content of the capsule was dissolved in 25 ml 6.8 pH buffer, vortexed for 5 min to dissolve and the volume was diluted to 100 ml with 6.8 pH buffers. The ultraviolet absorbance of the solution was measured at 275nm after filtration and appropriate dilution [14].

Compatibility studies

Fourier transformed infrared spectroscopy (FT-IR)

IR spectroscopy of Aceclofenac capsules were taken using fourier transform infrared spectrophotometer (Perkin Elmer-Spectrum 100). The drug and KBr was mixed and grounded into fine powder using mortar & pestle. Then the mixture was compressed at 20 psi for 10 min on KBr press to make it into a disc form. The spectrum was obtained in a range of 4000 - 400 cm^{-1} and the characteristic peaks of IR transmission spectra of Aceclofenac pure and physical mixture were recorded.

Diffential scanning calorimetry(DSC)

Thermograms of Aceclofenac blend was obtained using a DSC instrument (DSC Q10 V9.0 Build 275) equipped with an intra-cooler. Powder samples were hermetically sealed in perforated aluminum pans and heated at a constant rate. Purge gas-nitrogen at a flow rate 50 ml/min and heating temperature of 100^0C was used to maintain inert atmosphere. In this technique the difference in energy

Table 1:

Composition of Aceclofenac Colon target Capsules

Name of Ingredient	F1	F2	F3	F4	F5	F6
Aceclofenac	100	100	100	100	100	100
Eudragit S 100	25	25	30	35	40	45
Avicel pH 101	111.35	111.35	106.35	101.35	96.35	91.35
Talc	1.6	1.6	1.6	1.6	1.6	1.6
Magnesium stearate	0.8	0.8	0.8	0.8	0.8	0.8
Isopropyl alcohol	q.s	q.s	q.s	q.s	q.s	q.s
Aerosil	1.25	1.25	1.25	1.25	1.25	1.25
Coating ingredients						
Opadry White	3%	3%	3%	3%	3%	3%
Instacoat EN-HPMCP	5%	8%	8%	8%	8%	8%

*All quantities in mgs

input into a substance and reference material was measured as a function of temperature as the specimens were subjected to controlled temperature program.

In-Vitro Release studies

The *In-Vitro* drug release study was carried out using USP Paddle type dissolution test apparatus. The study was conducted at 37± 0.5^{0}C, 50 rpm for 2 hours in 900 mL 0.1 N HCl buffer (simulated gastric fluid pH 1.2). Then, the dissolution medium was replaced with 900 mL of phosphate buffer (simulated small intestinal fluid pH 7.4) and release was obtained for 3 hrs. Then, the dissolution medium was replaced with 900 mL phosphate buffer (simulated colon condition pH 6.8) and release was obtained for 24 hrs. 5mL of samples were withdrawn at different intervals and an equal amount of the medium was replaced. The aliquots were suitably diluted and drug release was calculated spectrophotometrically at 275 nm using reagent blank. The concentration of drug in sample solution was determined from calibration curve.

Results And Discussion

Micromeritics:

The pre-formulation studies of granules of Aceclofenac colon target capsules were found to be satisfactory. The bulk density (0.44 - 0.54 gm/ml), hausner ratio (1.22 – 1.25) and compressibility index (18.18 - 20.31) of all formulations confirmed good flow property shown in Table 2. The physical properties of capsules, such as weight variation, drug content and content uniformity were determined by using standard protocols. The results obtained were shown in Table 3. It was observed that the content uniformity 98.5 – 100.26% of labeled amounts that reflect good distribution and homogeneity of drug. Weight of the filled capsule was within the specifications. The drug content of all formulations was observed in the range of 99.05 – 99.61.

Moisture uptake by filling formula

The moisture absorption isotherms shown in **Figures 1-3** illustrate the percentage moisture uptake of different filling formula stored at room temperature under different relative humidities. For all formulae, the moisture uptake was found to increase on increasing the percentage of relative humidity. It was also clear that there were marked variations in moisture absorption between the investigated formulae at the same relative humidity indicating marked differences in the formula hygroscopicity. The percentage moisture uptake of the formulae and consequently their hygroscopicity, was found to be in the following descending order: F1, F2, F3, F4, F5 and F6 decreasing. This showed that hygroscopicity of the formulation is directly proportional to concentration of the filler (Avicel pH 101).

Table: 2

Evaluation data of Aceclofenac granules

FormulationCode	Loose bulk density (LBD) gm/ml	Tapped bulk density (TBD) gm/ml	Compressibility index (%)	Hausner ratio
F1	0.44	0.54	18.51	1.22
F2	0.51	0.63	19.04	1.23
F3	0.51	0.64	20.31	1.25
F4	0.54	0.67	19.40	1.24
F5	0.53	0.66	18.18	1.24
F6	0.53	0.65	18.46	1.22

Table: 3

Physical properties of Aceclofenac Colon target Capsules

Formulation Code	Weight (mg)	Drug content (mg)	Content uniformity
F1	300 ± 5	99.61	99.61 ± 0.65%
F2	300 ± 5	99.23	99.23 ± 0.73%
F3	300 ± 5	99.05	99.05 ± 0.35%
F4	300 ± 5	99.34	99.34 ± 0.54%
F5	300 ± 5	99.12	99.12 ± 0.48%
F6	300 ± 5	99.43	99.43 ± 0.37%

All values represent Mean ± SD.

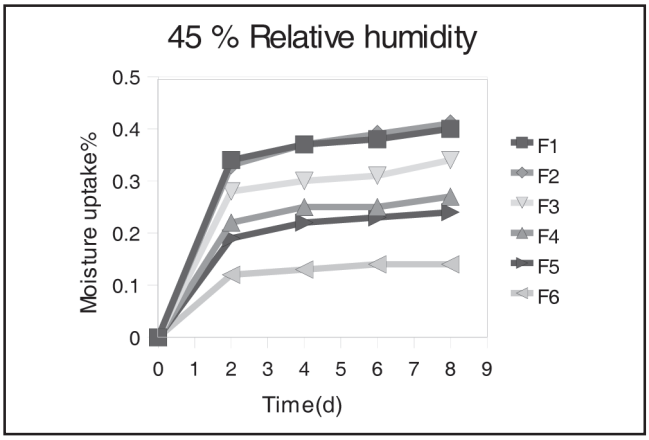

Fig.1 Moisture uptake at 45% RH

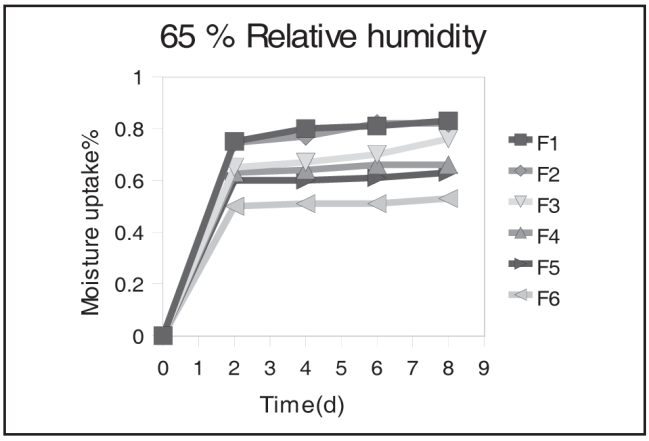

Fig.2 Moisture uptake at 65% RH

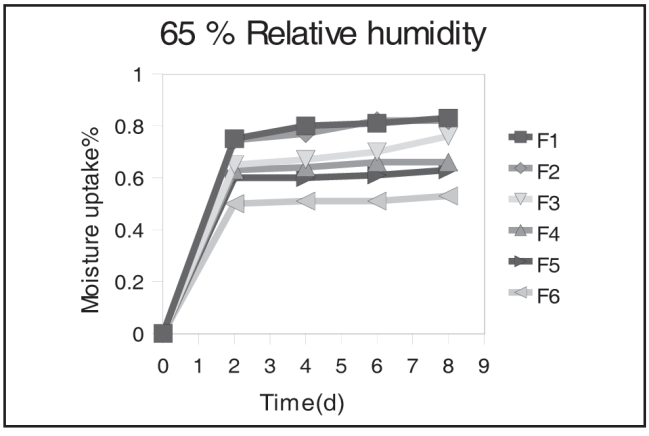

Fig.3 Moisture uptake at 75% RH

Compatibility studies

Fourier transformed infrared spectroscopy (FT-IR)

FTIR spectroscopy was used to ensure that no chemical interaction occurred between the drugs and polymers used. From the FTIR spectral interpretation the following result were obtained. The FTIR of aceclofenac shown intense bands at 1771.47 cm^{-1}, 1716.89 cm^{-1}, 1589.53 cm^{-1} and 1055.9 cm^{-1} corresponding to the functional groups C=O, COOH, NH and OH bending. The peaks observed in FTIR of physical mixture of aceclofenac and excipients was found to be at 1771.62 cm^{-1}, 1716.76 cm^{-1}, 1589.84 cm^{-1}, 1055.88 cm^{-1} respectively. The IR spectrum is shown in **Figure 4**. From the above interpretation it was understood that there was no major shifting in the frequencies of above said functional groups of aceclofenac, which indicates that there was no [* Object too big for pasting as inline graphic. | In-line.EMF *]chemical interaction between aceclofenac and polymers which were used in the formulations.

Differential scanning calorimetry(DSC)

DSC provides information about the physical properties of the sample as crystalline or amorphous nature and demonstrates a possible interaction between drug and polymers in formulations. According to the thermograms, aceclofenac presented a sharp endothermic peak at 153.96ºC corresponding to the melting point of the drug in the crystalline form. DSC of Pure drug and drug with physical mixer shown in Figures 5, 6 respectively. While the thermogram of physical mixture of aceclofenac and excipients was 154.33ºC. It conforms that there was no interaction between aceclofenac and polymers which were used in the formulations.ss

In Vitro Release studies

Cumulative percentage drug release in 0.1 N HCl

The percentage of the drug release was analyzed in 0.1 N HCl medium. The percentage release of drug at the end of 2 hrs was found to be nil for all formulations (F2-F6), except in case of F1- 17.46±0.32%. This was due to the insufficient coating of HPMCP used in the formulation, i.e 5%.

Cumulative percentage drug release in phosphate buffer pH 7.4

The percentage release of the drug in phosphate buffer pH 7.4 was analyzed. The percentage release of drug at the end of 3 hrs was found to be 84.07±0.03%, 63.08±0.47%, 58.34±0.67%, 45.59±0.62%, 19.60±0.99%, 12.25±0.12% for F1, F2, F3, F4, F5 and F6 respectively. The percentage release was found to be decreasing with increasing concentration of Eudragit S 100.

Cumulative percentage drug release in phosphate buffer pH 6.8

The release of the drug was analyzed in phosphate buffer pH 6.8. The percentage release of drug at the end of 24 hrs was found to be 100.00% for formulations F1-F3 and 94.25±0.16%, 90.04±0.16%, 68.02±0.97% for F4, F5 and F6 respectively. The release of drug was found to decrease with increase in the polymer concentration.

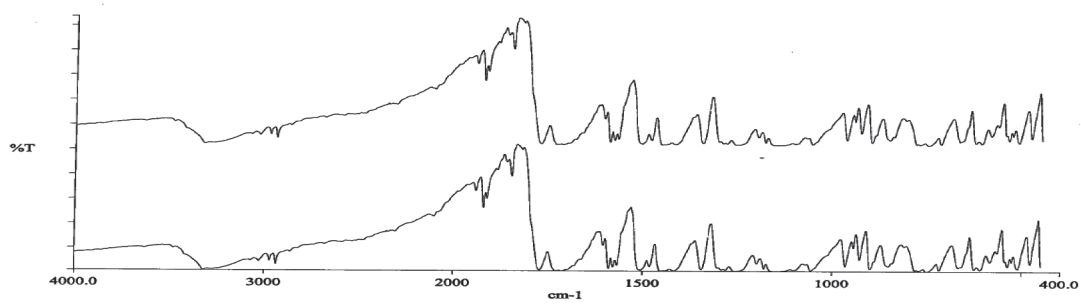

Fig.4: FT-IR spectrum of Pure Aceclofenac and Aceclofenac with physical mixture

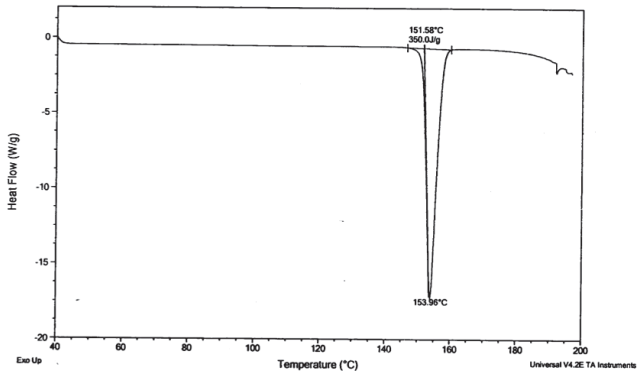

Fig.5: DSC of Aceclofenac pure drug

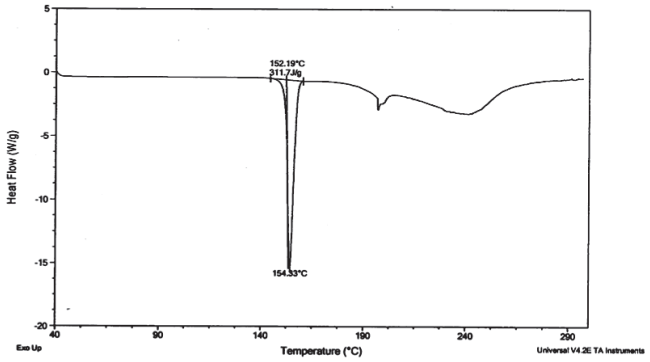

Fig.6: DSC of Aceclofenac and physical mixture

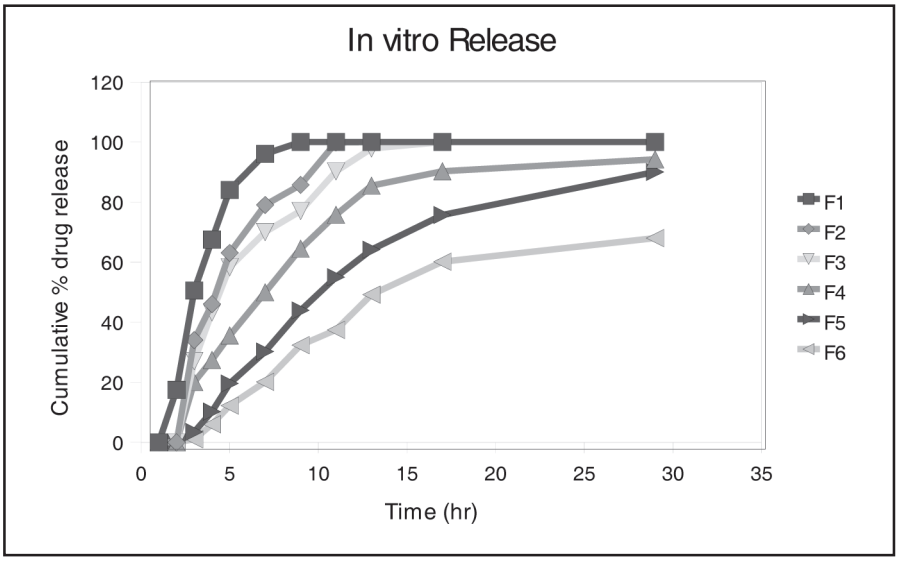

Fig. 7 *In-vitro* release of Aceclofenac

The drug release of the Formulation F1 in 0.1 N HCl was more due to insufficient coating of Instacoat EN-HPMCP i.e 5%. Whereas in the later formulations Instacoat EN-HPMCP coating of 8% was sufficient to bypass the gastric environment. In phosphate buffer pH 7.4 the HPMCP coating eroded and the capsule shells separated. In colon environment, i.e, Phosphate buffer pH 6.8, the drug released from the granules in a controlled fashion and was optimum in formulation F5 and F6

All the formulations shown good release in colon environment but formulations F1 – F4 were failed to target the colon. F6 showed a much slower release pattern in all mediums compared to F5 formulation. *In-vitro* drug release pattern in different mediums is shown in **Figure 7**.

Conclusion

The study was carried out to investigate the ability of HPMCP and Eudragit S 100 for targeting the drug to colon. From results obtained in the study, it was concluded that concentration of Eudragit S100 was optimum in formulation F5 for colon targeting. Instacoat EN-HPMCP coating of 8% was found sufficient enough to bypass the gastric environment. The *in vitro* studies shown that this formulation successfully delivers the maximum amount of drug in intact form to the colon. Formulations when subjected to stability studies indicated no significant change in physical appearance, drug content, and *in vitro* release pattern. Furthermore, no physical and chemical interaction was evident from DSC and FTIR studies, indicating stability of Aceclofenac in the capsules.

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Synthesis and Antimicrobial Activity of Some New 6-Acetyl (N substituted Aryl) 5-hydroxy, 4-methyl, Coumarine Derivatives

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ABSTRACT

6-acetyl,5-hydroxy-4 methyl coumarine 1 has been prepared by the reaction of 2,4 dihydroxy aceptophenone with ethyl acetoacetate using pachman condensation. Different schiff's bases 2 were synthesized by the reaction of compound 1 with various aromatic amines. The resulting schiff's bases were characterized by chemical and spectral methods. The synthesized coumarine derivatives 2_{a-i} have been screened for antimicrobial activity against various bacteria and fungi.

Keywords: 2,4 dihydroxy aceptophenone, antibacterial activity, antifungal activity.

Introduction

Coumarines are well known naturally occurring oxygen containing heterocyclic compounds isolated from various plants [1-3] owing to their diverse bioactivities, antibacterial, antifungal [4] anticoagulant [5,6] and other effects, natural, semi-synthetic and synthetic coumarines are useful substances in drug research. Some coumarine derivatives can be utilized as beneficiaries for the synthesis of valuable heterocyclic ring systems[7].

It is suggested that the compounds having antimicrobial activity may act either by killing the microbes or blocking their active sites [8-10]. Some coumarin derivatives possessing carboxamide moiety are found to have diuretic [11], analgestic, myorelaxant [12], antifungal [13] and anthelmentic activities. We have synthesized 10 derivatives of coumarine and screened for their antimicrobial activity.

Experimental

All the melting points have been determined in an open capillary and are uncorrected. IR spectra were recorded on Perkin Elmer 377 spectrophotometer and ^{1}HNMR spectra on AMX 400 MHz in DMSO-d_6 using TMS as an internal standard. Elemental analysis of the newly synthesized heterocycles were carried out on a Calro Elba 1108 analyzer and results were found with in the range of the theoretical value.

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Synthesis of 6-acetyl, 5-hydroxy, 4-methyl, coumarine I

The mixture of 2,4 dihydroxy aceptophenone (1.58g, 0.01 mol) with ethyl acetoacetate (2.8ml, 0.02 mol) was added slowly, with stirring, to 20 ml of precooled conc. H_2SO_4. After the addition, stirring continued for about 30 min and the reaction mixture was poured into crushed ice, the product separated was filtered, washed, dried and recrystallized from hot methanol as pale yellow needles m.p. 270ºC.

Synthesis of 6-Acetyl(N substituted Aryl) 5-hydroxy, 4-methyl coumarine 2

6-acetyl, 5-hydroxy, 4-methyl coumarine (2.38g, 0.01 mol) derivative of compound **1** was dissolved in ethanol (50 ml) and then (0.06 mol) aromatic amine was added. Refluxed for about 5 hrs. It was cooled and product separated was filtered, washed with cold water, dried and recrystallized from ethanol. Compound **2a**, yield 75%, m.p. 250. IR (KBr, cm^{-1}); 3325 (Ar-OH), 3075 (Ar-H), 2909 (Ali-H); 1675 (C=O); 1575 (C=C);1465 (C=N); 1182 (C=O); 1460 (in plane bending), 822 (out of plane bending) ^{1}HNMR ($CDCl_3$ in δ ppm); 6.518 (Sym m benzene ring); 9.012 (s, O-H); 2.431 (s, CH_3); 6.416 (unsym, m, methoxy substituted coumarine ring).

Results and Discussion

Compound 1 has been prepared from resacetophenone and ethyl acetoacetate in ethanol in the presence of conc. H_2SO_4 according to reported procedure. The synthesized heterocyclic compounds 2 have been prepared from compound 1 in 30 ml ethanol and (0.06 mol) of substituted amines were added and refluxed for 5 hrs. The structure

Reaction Sequence of the synthesized Coumarine derivatives;

Table-1:

Physical and Analytical Data of the Synthesized Coumarine derivatives 2a-j

Comp.	R	MP °C	Yield (%)	Mol. Formula	Mol Weight	Elemental analysis					
						C%		H%		N%	
						Calc	Found	Calc	Calc	Calc	Found
II_a	C_6H_{-5}	250	55	$C_{17}H_{12}O_3N$	278.23	73	72.9	8	7.8	5	4.8
II_{-b}	$2\text{-}C_6H_5$ Cl	280	60	$C_{17}H_{11}O_3NCl$	315.34	60	64.88	7.2	7	5	4.8
II_c	$3\text{-}C_6H_5$ Cl	250	62	$C_{17}H_{11}O_3NCl$	315.87	65	64.9	7.2	7	5	4.8
II_d	$4\text{-}C_6H_5$ Cl	270	64	$C_{17}H_{11}O_3NCl$	315.59	65	64.9	7.2	7	5	4.8
II_e	$2\text{-}C_7H_7O$	260	65	$C_{18}H_{15}O_4N$	174.39	49	48.9	17.2	17	8	7.8
II_f	$C_{14}H_{14}N$	240	66	$C_{37}H_{29}O_3N$	561.81	79	78.6	10	9.5	3	3.8
II_g	$C_6H_4NH_2$	225	65	$C_{19}H_{15}O_3N$	320.76	71	70.4	10	9.6	4.5	4.1
II_h	$C_{10}H_5NH_2$	220	63	$C_{22}H_{15}O_3N$	256.67	74	73.1	9	8.5	4	3.8
II_i	$2\text{-}C_6H_4O_2N$	235	61	$C_{18}H_{15}O_5N$	306.87	71	70.2	10	9.5	4.6	4.2
II_j	$3\text{-}C_6H_4O_2N$	233	60	$C_{18}H_{15}O_5N$	322.29	67	66.3	8.6	8.4	4.5	4.4

Table 2:

Antibacterial activity table of the synthesized coumarine 2a-j dervatives

Comp. code	*B. fragitis Conc ppm*		*B. vulgatus Conc ppm*		*B. ovatus Conc ppm*		*E. lentum Conc ppm*	
	100	**500**	**100**	**500**	**100**	**500**	**100**	**500**
2:00 AM	-	+	-	++	-	+	+	++
2-_b	++	+++	++	+++	++	+++	+++	+++
2_c	+++	+++	+	+++	++	+++	++	+++
2_d	+++	+++	++	+++	+++	+++	+++	++++
2_e	-	+	+	++	+	+	+	++
2_f	+	+	+	++	-	+	+	+
2_g	+++	++++	+++	+++	++	+++	++	+++
2_h	+++	++++	++	+++	++	++++	+++	+++
2_i	+	++	-	+	+	+	-	++
Std	++++	++++	+++	++++	+++	++++	+++	++++

Std. Streptomycin inhibition diameter in mm

(-) 0-4; (+) 5-10; (++) 11-15; (+++) 15-19; (++++) 19-24

Table3:

Antifungal activity table of synthesized coumarine derivatives

Comp. code	*F.oxysporum* *Conc ppm*		*A niger* *Conc ppm*		*A flavus* *Conc ppm*		*T viridae* *Conc ppm*	
	100	**500**	**100**	**500**	**100**	**500**	**100**	**500**
2_a	-	+	-	-	+	+	+	++
2_b	++	+++	+++	+++	++	+++	+++	+++
2_c	++	+++	+++	+++	++	++	++	+++
2_d	+++	++++	+++	+++	++	+++	++	+++
2_e	-	++	-	++	+	+	+	++
2_f	+	++	+	+	+	+++	+	+++
2_g	+	++	+	++	++	++	+	+++
2_h	+	+	-	+	+	++	+	+
2_i	-	-	-	-	+	++	+	+
Std	+++	++++	+++	++++	++++	++++	++++	++++

Griseofulvin used as standard drug, inhibition diameter in mm
(-) 0-4; (+) 5-10; (++) 11-15; (+++) 15-19; (++++) 19-24

of the 2a has been confirmed by its spectral and analytical studies as discussed below: the pmr spectrum of 2a has displayed a symmetrical multiplet at δ 6.158 due to benzene ring: the two singlet at δ 9.012 & 2.431 due to the presence of OH and CH_3 group respectively

The IR spectrum has revealed the presence of C=N group exhibiting a strong absorption at 1465 cm^{-1} and absorption at 3325 and 3075 cm^{-1} due to the presence of OH and Aro (C-H) stretching & a strong absorption at 1675 cm^{-1} due to the presence of C=O stretching. The synthesized Coumarine derivatives have been screened for antibacterial activity against *B fragitis, B vulgatus, B ovatus and E. lectum* at two different concentration at (100 & 500) ppm respectively by filter paper disc plate method and antifungal activity against *Aspergilus niger, Fusarium oxysporum, Aspergilus flavus and Trichoderma viridae* by filter paper disc plate method at two different concentrations (100 and 500ppm). Standard drug Streptomycin was used for antibacterial and Griseofulvin was used for antifungal activity have been screened under the similar condition for comparison.

Synthesized coumarine derivatives 2_b, 2_c, 2_d, 2_g, 2_h are highly active against selected bacteria and fungi and rest of the coumarine derivatives have shown good to moderate activity. Which have been shown in table 2 & table 3 respectively

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