Hydrogel Based Formulations of *Imazethapyr*: Development and Appraisal of Release Behaviour

इमाज़ेथेपायर की हायड्रोजेल आधारित फार्मूलेशंस : विकास एवं निर्मुक्ति व्यवहार

By

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This is to certify that the thesis entitled "**Hydrogel Based Formulations of *Imazethapyr*: Development and Appraisal of Release Behaviour**" submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of Master of Science in Agricultural Chemicals, embodies the results of bona fide research work carried out by **Mr. Vikas Kumar**, under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma. The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by her.

(Dr. (Mrs.) Anupama)

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Chairperson, Advisory committee Place: New Delhi, India

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Place: New Delhi *Vikas Kumar*

Dated: 30 June, 2014

Dedicated to Anupama Ma'am & my Parents…

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INTRODUCTION

One of the most serious bottlenecks in agricultural productivity is weed management. Uncontrolled weeds reduce crop yields through their competition for light, nutrients, and moisture. Both grasses and broad leaf weeds like *Achyranthus aspera, Desmodium diffusum, Commelina bengalensis, Phyllanthus niruri, Cynotis axillaris and Dinebra retraflexan, Cyperus rotundus, Echinochloa colonum, Dactyloctenium aegyptium, Digitaria sanguinalis, Trianthema portulacastrum, Parthenium hysterophorus, Flaveria australasica, Amaranthus viridis, Cynodon dactylon, Dactyloctenium aegyptium, Echinochloa crusgalli, Eclipta alba, Corchorus olitorious, Chenopodium album, Cleome monophylla etc.* predominate the weed population across India and pose a real challenge for some viable solutions.

Competition between crops and weeds generally does not begin at the early stages after emergence of the crop. If the weeds are checked during this period, the crop gets an advantage. *Imazethapyr*, an imidazolinone group of herbicide shows longer persistence and broad spectrum for weed control. It kills weeds by inhibiting the enzyme acetohydroxy acid synthase (AHAS). It is applied as both pre and post emergence. In recent studies conducted at the Division of Agronomy, Indian Agricultural research Institute, it has been found that the pre emergence application of *imazethapyr* in soybean, has also been found effective in weed control. Controlled release formulation technology can be very effective in this context. There is thus a need to develop carrier based formulation(s) of *imazethapyr* that can serve as regulated release reservoir of the chemical at an early stage of crop growth period and continue releasing the same afterwards. Adequate soil moisture is important for optimum activity of *imazethapyr*. Thus the choice of carrier is an important factor in developing controlled release formulation of the herbicide. Water absorbing cross linked polymers referred to as hydrogels with versatile network properties offer the desired advantages in this context. Hydrogels have been established to possess superior water and agrochemical retention-

release behavior in soil and water. Formulation(s) of *imazethapyr* based on hydrogel chemistry is likely to provide useful leads in pre emergent weed management strategies.

Liquid formulations of *imazethapyr* available for post emergence application involve use of toxic solvents, surfactants, wetting agents and other adjuvants etc. There is thus a need to replace these with ecofriendly options that mitigate or do not require the use of these adjuvants/excipients. Core shell nanogels offer a potential alternative in this context. Once exposed to water these nanosized gel networks form micro/nano-suspensions and release the loaded *a.i.* in water. These hydrogels when exposed to water form nano-suspension and possess the ability to entrap water insoluble organic molecules, thus enhancing their solubility.

It is therefore proposed to develop hydrogel carrier based novel formulations of *imazethapyr* with the following objectives:

1. To develop core shell nanogels and porous hydrogel composites as carrier materials to formulate *imazethapyr*.
2. To evaluate developed *imazethapyr* formulations for their active ingredient release behaviour in water and bioefficay against prevalent weeds of kharif/rabi season.

BACKGROUND

2.1 General

Agriculture continues to play a major role in Indian economy, contributing approximately 13.7% of the total national gross domestic production (GDP) (Economic survey of India, 2013-14). In modern agriculture, use of agro-chemical inputs has become highly relevant for crop production and protection particularly in view of the fast changing climate, shrinking natural resources and pest resistance, misuse of agrochemicals is continuously increasing their toxic levels in food, feed and the environment. Weeds management is an important component of crop protection since farmers continue to experience heavy losses in crop yield due to weed interference. Weeds are generally unwanted hardy plant species that compete very efficiently with crop plants for same resources namely, nutrients, water, space and light for growth and development and thus adversely affect the crop growth and yield. Herbicides based weed management is most widely used approach to combat this problem worldwide.

Imidazolinone herbicides are being increasingly reported as efficient weed control tool for herbicide resistance management. *Imazethapyr* is an imidazolinone herbicide applied as both pre and post emergence in soybean, groundnut and other legumes. It kills weeds by inhibiting the enzyme acetohydroxy acid synthase (AHAS). Weeds normally controlled by pre-emergence application of *imazethapyr* are *Acanthospermum australe, Acanthospermum hispidum, Amaranthus deflexus, Amaranthus hybridus, Amaranthus thunbergii, Chenopodium album, Galinsoga parviflora, Commelina benghalensis, Cyperus rotundus, Portulaca oleraceae, Xanthium strumariumr, Tribulus terrestris*.

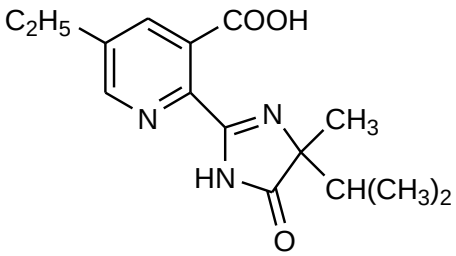

Figure 1: Chemical structure of *Imazethapyr*

Table 1: *Imazethapyr* description

Molecular Formula	$C_{15}H_{19}N_3O_3$
Molecular Weight	289.3
Chemical Abstracts Name	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3 pyridine carboxylic acid
IUPAC name	(R,S)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid.
Product	Pursuit®
Formulations	10% SL, 70%WG (WDG), 70%WP

In recent studies conducted at the Division of Agronomy, Indian Agricultural Research Institute, it has been found that top soil surface application of liquid formulation of *imazethapyr* as pre-emergence application also controls the weed. When applied as pre followed by post emergence herbicide in same crop, it controls *Chenopodium album, Datura ferox, Commelina benghalensis, Physalis angulata, Cleome monophylla*, *Datura ferox etc and other several weed species* besides weed species killed by only pre emergence application. In India, only liquid formulation is available which has inherent constraints such as fast dissipation of *a.i.*, use of surfactants etc. Present study aims at development of novel formulations of *imazethapyr* based on hydrophilic polymer carriers and their evaluation for pre and post-emergence bioefficacy of *imazethapyr*.

Cross linked hydrophilic polymers, the superabsorbent hydrogels (SAP) and superporous hydrogels (SPH) are reported as potential carriers of drugs and agrochemical inputs (Chen *et al.*, 1999; Lee and Kim 1997; Singh *et al.*, 2010). The actual use of hydrogel in agricultural sector was visualized in 1950s by United States Department of Agriculture (USDA). This led to development of water soluble polymers such as polyvinyl acetate (PVA), polyethylene glycol (PEG) and polyacrylamide (PAM) to function as soil conditioners followed by the introduction of water-swellable polymeric hydrogels in the early 1980s (Zohuriaan-Mehr and Kabiri, 2003). The most important difference between SAP and SPH is their swelling characteristics mainly related to elasticity of the network, the presence of hydrophilic functional groups (such as -OH, -COOH, $-CONH_2$, $-SO_3H$) in the polymer chains, the extent of cross-linking, and porosity

of the polymer. Additionally, the physical characteristics of hydrogels including their swelling ratio also depend on the balance between attractive and repulsive ionic interactions and solvent mediated effects (Barbieri *et al.,* 1998; Bhalerao *et al.,* 1998). In early 1990s, superporous hydrogels (SPHs) were introduced as another category of water-absorbent polymer systems (Omidian and Park, 2002). SPHs are hydrogels that, regardless of their size, swell to their equilibrium in aqueous media in shorter duration than the conventional SAPs. Initially, SPHs were developed as gastric retention devices due to fast swelling irrespective of size, large surface area and fast mass transfer (Chen *et al.,* 1998). Due to the presence of interconnected pores in SPH matrix, water can be rapidly absorbed by capillary attraction forces within the pores and these polymers swell to their maximum volume very quickly (Chen *et al.,* 1999; Lee and Kim, 1997). SPHs are generally prepared by copolymerization/ cross linking of co-monomers or crosslinking of linear polymers by irradiation or by chemical compounds (Satish *et al.,* 2006). Gas blowing techniques are also reported to synthesize SPHs (Omidian *et al.,* 2005).

SPHs are divided into three different generations. The first generation SPHs are characterized by fast swelling, high swelling ratio and weak mechanical properties. Limitations of SPHs mainly the poor mechanical strength and loading capacity has prompted the researchers to develop the second generation of hydrogels known as Superporous hydrogel composites (SPHCs), characterized by fast swelling, medium swelling ratio and improved mechanical properties, been explored to develop controlled release formulations of soil applied pesticides and nutrients / fertilizers and other plant growth additives. The third-generation SPHs (SPH hybrids) possess elastic properties that can be highly useful in the development of gastrointestinal devices, as well as in other pharmaceutical and biomedical applications (Omidian *et al.,* 2005).

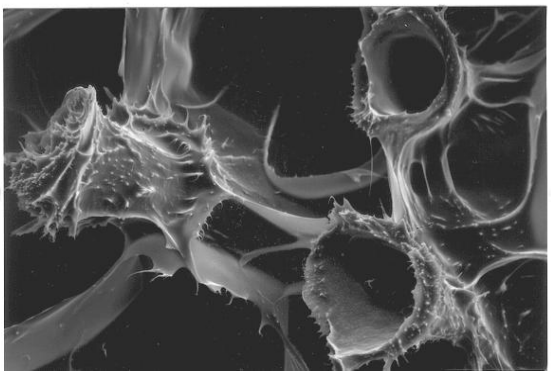

Figure 2: SEM image of a typical superporous hydrogel

Polymeric materials have been popularly used as carriers for sustained release (Kost and Langer, 2001). Biopolymeric microspheres of sodium alginate and starch using $CaCl_2$ as a cross linker are used as carriers for the controlled release of the pesticides, nutrients and microbes (Roy and Bajpai, 2009). Controlled release formulations of various insecticides, herbicides, pheromones and other bioactive molecules entrapped in the superabsorbent and superporous hydrogel microbeads have been developed through inverse phase suspension polymerization (Chavda and Patel, 2010). Polyelectrolytic SAPs and SPHs exhibit swelling response to the external pH and ionic strength and this property can be used as the switch for controlled release delivery of nutrients and crop protection chemicals. In our laboratory, matrix properties of crosslinked hydrogels (SAP) and hydrogel composites (SAPC) have been utilized to develop bioformulations (Singh *et al.,* 2010) of an entomopathogenic nematode *Steinernema thermophilum* (Ganguly *et al.,* 2008), a plant growth promoting rhizobacteria (PGPR) with improved shelf life characteristics (Anupama *et al*., 2009), integrated formulations of root extracts of *Tagetes* sp. and secondary plant nutrients (Adaka, 2011) and controlled release nutrient (Prithu, 2012) formulations based on hydrogels (Anupama *et al.,* 2006).

The carrier used in these kinds of polymeric formulations of nutrient and crop protection agent, can only be utilized for pre-emergence herbicide applications. Thus a novel approach of post-emergence *imazethapyr* formulation based on hydrogel was developed using nanogel technology. Micro and nano sized polymeric gels are being manipulated for their use in liquid form since they easily form micro and nano suspension

form. Size of hydrogels when manipulated to be reduced to 1-500 nm the resulting materials are termed as nanogels (Li *et al.*, 2012). Various types of stimuli responsive nanogels have been reported as effective carriers of drugs with stimuli responsive release properties (Bhagat *et al.*, 2013).

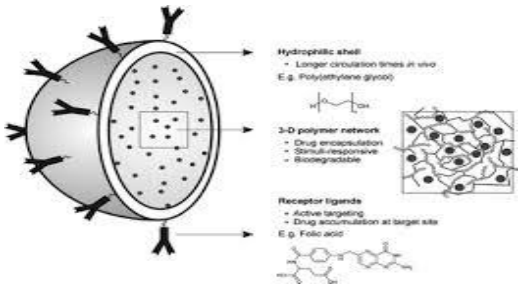

Fig 3: Structure of nanogel

Encouraged by these findings, the present work was undertaken to develop novel formulations of *imazethapyr* based on hydrogels with the following objectives:

1. To develop core shell nanogels and porous hydrogel composites as carrier materials to formulate *imazethapyr*.

2. To evaluate developed *imazethapyr* formulations for their active ingredient release behavior in water and bioefficay against prevalent weeds of kharif/rabi season.

This dissertation embodies results of the investigations carried out to achieve the objectives.

2.2 Research Area I (Objective I)

Development of porous hydrogel composites and core shell nanogels as carrier materials to formulate *imazethapyr*

Imazethapyr is a board spectrum herbicide imidazolinone group recommended primarily for post emergence application in crops particularly soybean (Habimana *et al.*, 2013) although its pre-emergence application has also been reported (Jeffrey *et al.*, 2001).

Formulations commercially available are soluble liquid which requires use of emulsifiers and hazardous solvents. One such formulation under name Pursuit® is extensively reported for post-emergence weed control in India. Pre-emergence weed management of controlled release formulation (CRF) approach is of much interest because of regulated release of herbicides in the weed root environment and thus showing affectivity for longer time. In this context cross linked hydrogels are of major interest for development of *imazethapyr* formulation for pre-emergence weed control. Hydrogels are three-dimensional macromolecular networks that can absorb water many times their dry mass and significantly expand in their volume (Aouada *et al.,* 2006; Moura *et al.,* 2006; Aouada *et al.,* 2009*a*). In spite of tremendous potential due to versatile matrix and fluid absorption properties, large scale use of superabsorbent polymers and superporous hydrogels in agriculture and allied areas has been limited mainly due to high cost, biodegradation concerns, high rates of application etc. (Singh *et al.,* 2010). In view of the environmental concerns all over the world, the importance of replacing the synthetics by "greener" options is being felt (Zohuriaan-Mehr and Kabiri, 2008). One of the most popular approaches explored for the purpose is introduction of polysaccharides/ carbohydrates through a suitable mechanism. Chitin, cellulose, starch and natural gums (such as xanthan, guar and alginates) are finding extensive application because of their low cost, natural abundance and availability. In view of the inherent hydrophilic characteristics of guar gum (GG) coupled with their low cost and environment friendly structural properties, guar gum based hydrogels are gaining prominence in worldwide (Peterson and Oksman, 2006). Guar gum (GG) is a polysaccharide originating from the seed endosperm of the plant *Cyamopsis tetragonolobus.* It is a galactomannan, which consists of a (1→4) linked β-mannopyranosyl backbone partially substituted at O-6 with α-d-galactopyranosyl side groups, with the ratio of mannose to galactose ~1.6–1.8:1(Cheng *et al.,* 2002). It has been extensively used in a number of applications such as thickening agents, suspending agents, ion-exchange resins, surfactant and dispersing agent). Owing to the hydrophilic properties of guar gum due to the presence of hydroxyl groups, preparation of guar gum based superporous hydrogel composites (SPHCs) and nanogels is being considered a promising approach (Peterson and Oksman, 2006; Chen *et*

al., 2000; Kim and Park, 2004). Guar gum based superporous hydrogel (GG-SPHs) was reported first from our laboratory (Chandrika *et al.*, 2014).

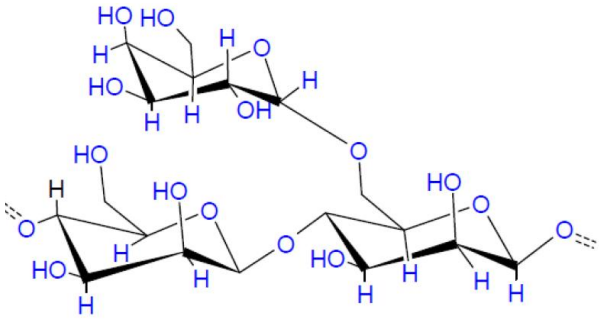

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Figure 4: Structure of guar gum

For post-emergence weed control, formulations are applied in the form of suspension by diluting them with excess water. For this purpose most of the liquid formulations employ emulsifiers. In this context the nanogels hold lot of potential as they are naturally water loving and don't require any emulsifier. The size of nanogel particles are of size ranges 1-500 nm (Daoud-Mahammed *et al.*, 2009). Nanogels are being extensively used now-a-days as control release vehicles of drugs for human health (Chiang *et al.*, 2012). Various types of stimuli responsive nanogels for drug delivery have been reported (Chiang *et al.*, 2012). Some of them employ natural polymers like chitosan, sodium alaginite, starch etc. But no one has reported nanogels based on guar gum. Present work employs the guar gum based SPHCs and nanogels as carriers for development of *imazethapyr* formulations for pre emergence and post emergence evaluation.

2.3 Research Area 2 (Objective II)

Evaluation of developed *imazethapyr* formulations for their active ingredient release behaviour in water and bioefficay against prevalent weeds of kharif/rabi season

Controlled release formulations (CRF) approach is of tremendous interest among the formulation chemists because of scope of manipulation of carrier properties to achieve zero order release kinetics. Many CRF of herbicides have been developed and extensively reported (Adak *et al.*, 2012; Kumar *et al.*, 2010). *Imazethapyr* is a comparatively newer type of herbicides available primarily as a liquid formulation and

applied both for pre and post emergence (Habimana *et al.*, 2013). The hydrogel based *imazethapyr* formulations prepared in present work are investigated for their release behavior in water and release kinetic parameters were studied. Mostly, *imazethapyr* is known to control weeds in soybean. Of late, work on its evaluation for herbicidal activity in garlic crop has been initiated in IARI, New Delhi. Present work aims at evaluation of SPHC and nanogel based formulations in garlic crop under field conditions.

MATERIALS AND METHODS

Enhancement of crop productivity and quality is of paramount importance in modern agriculture. Weed management is an integral component of modern agriculture and herbicides comprise a major agro-input in this context.

Uncontrolled weeds reduce crop yields through their competition for light, nutrients, and moisture. Both grasses and broad leaf weeds like *Achyranthus aspera, Desmodium diffusum, Commelina bengalensis, Phyllanthus niruri, Cynotis axillaris and Dinebra retraflexan, Cyperus rotundus, Echinochloa colonum, Dactyloctenium aegyptium, Digitaria sanguinalis, Trianthema portulacastrum, Parthenium hysterophorus, Flaveria australasica, Amaranthus viridis,Cynodon dactylon, Dactyloctenium aegyptium, Echinochloa crusgalli, Eclipta alba, Corchorus olitorious, Chenopodium album, Cleome monophylla etc.* predominate the weed population across India and pose a real challenge for some viable solutions (Sandhu *et al.,* 1997). Most commonly employed herbicides to control these weeds are Fluazifop-P, oxadiazon, oxyfluorfen, pendimethalin, *imazethapyr*, (Sandhu, 1997; Pandey and Velasco, 2002).

Imazethapyr, ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3 pyridine carboxylic acid) an imidazolinone herbicide applied primarily as post emergence and kills weeds by inhibiting the enzyme acetohydroxy acid synthase (AHAS). In recent studies conducted at the Division of Agronomy, Indian Agricultural research Institute, it has been found that the pre emergence application, which comprises spreading the water herbicide mixer on the top soil surface, also controls the weeds. Weeds normally controlled by *imazethapyr* are *Acanthospermum australe, Acanthospermum hispidum, Amaranthus deflexus, Amaranthus hybridus, Amaranthus thunbergii, Chenopodium album, Galinsoga parviflora, Commelina benghalensis, Cyperus rotundus, Portulaca oleraceae, Xanthium strumariumr, Tribulus terrestris, Datura ferox, Commelina benghalensis, Physalis angulata, Cleome monophylla, Datura ferox etc.* In most of the reported studies, *imazethapyr* is used for post emergence weed control (Jha *et al*., 2013; Meena *et al.,* 2011). Very few pre

emergence control studies have been reported (Jeffrey and Eric, 2001). *Imazethapyr* formulation registered in India is SL form (10% *a.i.*). In order to ensure its efficient application, the formulation preparation requires use of surfactants and adjuvants such as ammonium solution, a question solution of polydimethyl siloxane and polyoxyethylene stearate additionally cyspread and cyboost are added at time of application, which are hazardous to applicators. Therefore an alternative to such hazards is imperative. Fast dissipation and degradation of *imazethapyr* in water is a challenge that necessitates exploring effective alternatives to existing formulations. In terms of *a.i.* release, ease of application and reduced rate of application (*a.i.*/ha).

Carrier base formulation approach is extensively reported for drugs as well as agrochemicals.

Water insoluble crosslinked hydrophilic polymers that possess versatile network due to which they serve as controlled release systems for regulated release of the agrochemicals. In order to reduce the load of acrylic polymers of petrochemical origin, more emphasis is being given nowadays to introduce biopolymer backbones and inorganic fillers like clay minerals (Wang *et al.*, 2007; Chandrika and Anupama, 2013).

Guar gum (GG) derived from the seeds of guar plant, *Cyamoposis tetragonolobus* (Leguminosae), is a natural nonionic branched polymer with β-D-mannopyranosyl units linked (1–4) with single membered α-D-galactopyranosyl units occurring as side branches. Guar gum is a low cost, easily available, non-toxic and biodegradable polysaccharide. Its potential in hydrogel chemistry and new generation formulation technology still lay less exploited as compared to other backbones like starch, cellulose, chitosan, xanthan gum etc. Novel porous hydrogels based on guar gum have been reported from our laboratory (Chandrika and Anupama, 2013). Use of inorganic fillers has been very investigated in development of superabsorbent composites but very less work has been focused for superporous composites. The present work envisages development and appraisal of novel said formulations of *imazethapyr* employing Guar gum based superporous composites (GG-SPHC) as carriers for pre emergence application.

A new generation of hydrogels, the nanogels where particle size is controlled during synthesis in the range of 1-500 nm are of tremendous interest in delivery of hydrophilic drugs. Their interesting features, such as instant miscibility in water without requiring any extraneous adjuvant, effective loading potentials of hydrophilic drug and scope of manipulation at synthesis level to prepare ecofriendly biopolymeric forms qualify them for new generation agrochemical formulations.

Materials used and methods employed to achieve the objectives of the present investigation are briefly described below:

3.1 Materials

3.1.1 Reagents for synthesis of hydrogels

3.1.1.1 Superporous hydrogel composites

Guar gum (LR), acrylamide (LR), N, N'- methylene bis acrylamide and a per sulphate initiator were purchased from Thomas Baker Pvt Ltd., Mumbai, India. N,N,N',N'-Tetramethyl ethylene diamine (TEMED) GR, surfactant GR (Chemworth) and organic acid were purchased from Merck Specialties Pvt. Ltd., Mumbai, India. Sodium bicarbonate (99.5% minimum assay), acetic acid were procured from SD Fine Chem. Pvt. Ltd., Mumbai, India. Bentonite, was courteously provided by from Earth international Pvt. Ltd., New Delhi.

3.1.1.2 Core *shell* hydrogels

Guar gum (LR), N, - methylene bis acrylamide, Hexane, and a per sulphate initiator were purchased from Thomas Baker Pvt. Ltd., Mumbai, India. Surfactant GR (chemworth) (99% assay) was purchased from Merck Specialities Pvt. Ltd., Mumbai, India. *N*-Isopropylacrylamide (NIPAm) and N-vinyl pyrrolidone (VP) was received from Aldrich chemicals Co. limited, USA.

3.1.1.3 *Imazethapyr* (Herbicide)

Imazethapyr technical Grade, 96% was obtained through the courtesy of BASF India limited and commercial formulations of imazethapyr (Pursuit®) and pendimethalin (Stomp®) were procured from local market.

3.1.1.4 Water absorbency experiments

Distilled water (pH 7.0, EC 0.001 Mhos/cm) was used for swelling tests. For salt/fertilizer experiments, ammonium sulphate (AS), potassium nitrate (PN), ammonium nitrate (AN), sodium chloride (SC) and urea (U) was procured from SD Fine Chem. Pvt. Ltd., Mumbai, India.

3.2 Methods

3.2.1.1 Synthesis of superporous hydrogels composites

In situ solution polymerization was used to synthesize superporous hydrogels clay intercalated composites taking various ratios of backbone, vinyl monomer/s, cross linker, initiator, foam stabilizer ratio, porogen in a definite volume of polar solvent/s at a particular temperature for a definite time period. The reaction parameters were standardized using sequential procedure following factorial completely randomized block design.

3.2.1.2 Synthesis of Core *shell* nanogels

In situ solution polymerization and microemulsion polymerization was used to synthesize core *shell* nanogel taking various ratios of backbone, vinyl monomer/s, cross linker, initiator in a definite volume of polar solvent/s or mixture of polar-apolar solvent at a particular temperature under nitrogen atmosphere or under ambient conditions for a definite time period. The reaction parameters were standardized using sequential procedure following factorial completely randomized block design.

3.3 Characterization of superporous hydrogel composites (GG-SPHCs), superporous hydrogel (GG-SPH), core *shell* hydrogel (GG-CSH) their formulation and analysis of active ingredient

Micro Fourier transform infrared spectroscopy (Bruker, ALFA, 0.5 Kv), Indian Agricultural Research Institute, New Delhi, India; Scanning electron microscopy (ZEISS EVO Series Scanning Electron Microscope (EVO 50) with resolution of 2.0 nm @ 30 kV, Indian Agricultural Research Institute, New Delhi, India; X-ray diffraction technique (PHILIPS PW1710 diffractometer control equipped with PHILIPS PW1728 X-ray

generator, Indian Agricultural research Institute, New Delhi, India; Zetatrac TM, Indian Agricultural research Institute, New Delhi, India; Transmission electron microscopy (Jeol , JEM-1011, 100Kv), Indian Agricultural research Institute, New Delhi, India techniques were used to characterize the prepared materials synthesized in the present work.

3.4 Water absorption study of GG-SPHC and GG-SPH in distilled water, tap water and hard water through gravimetric method

The accurately weighed hydrogel (GG-SPHC) (0.1 g, particle size 100-240 mesh size) was immersed in excess of distilled water (pH 7.0, EC 0.001 Mhos/cm) in triplicate and kept temperatures, 35°C until equilibrium was attained. The surface water was filtered through a nylon sieve (200 mesh size), gel allowed to drain on sieve for 10 minutes and weighed. The water absorption capacity (Q_{H_2O}) was calculated using the following equation:

$$Q_{H_2O} = (w_2\text{-}w_1)/ w_1,$$

Where w_1 is the weight of dried absorbent and w_2 is the weight of swollen absorbent at equilibration. Q_{H_2O} was calculated as grams of water absorbed per gram of dry sample. Water absorption in distilled water was studied as function of time duration (15mi -60 min) i.e., rate of absorption and temperature (5°C, 35°C and 50°C).

3.5 Absorption study of GG-SPHCs and GG-SPHs in different salt and pH solutions

Salt solutions of the salts- ammonium sulphate (AS), potassium nitrate (PN), ammonium nitrate (AN), sodium chloride (SC) and one fertilizer urea (U) each of strength 5 mM, 10 mM, 15 mM and 20 mM were prepared and used for evaluation of swelling behavior of prepared hydrogel. Buffer solutions of pH 4, 7 and 9 were prepared and used for absorption studies.

3.6 Absorption under load study

Laboratory customized assembly was used to determine the absorbency under load of GG-SPHCs and GG-SPH. A plastic beaker (diameter 4.2 cm, height 5.6 cm) with

sintered bottom was placed in a Petri dish, and a weighed, dry sample (0.3±0.01 g) of hydrogel (GG-SPHC or GG-SPH) was uniformly placed on the surface of polyester gauze placed on the sintered bottom of beaker. A solid weight to attain load of 68.77 psi, that could slip freely through the sintered beaker was placed on the gel particles. Distilled water was added in petri dish to ensure that the liquid level was at half height level of the porous beaker. Increase in height of swollen particles was measured periodically.

3.7 *Imazethapyr* formulations

Imazethapyr solutions (142 and 171 ppm) were prepared by dissolving the required quantity of *imazethapyr* (96.0 % purity) in distilled water. A specific weight of dry GG-SPHC powder was added to a particular volume of *imazethapyr* solution in a beaker at room temperature and subjected to constant agitation for 120 min to ensure proper mixing and maximum expansion of carrier particles. Loaded GG-SPHCs were dried by lyophilization to get dry GG-SPHCs formulation of *imazethapyr*.

GG-CSH-*imz* formulations were prepared by drop wise addition of methanolic solution of herbicide into aqueous suspension of nanogel. Stirring was continued for 2 hours followed by membrane filtration. The suspension was lyophilized to get dry powder.

3.8 Determination of loading capacity and encapsulation efficiency

The loading capacity and encapsulation efficiency of developed core *shell* hydrogels and superporous hydrogels were determined by extraction of *a.i. i.e. imazethapyr* using solvent replacement method with minor modification. Weight percent loading was calculated based on the amount of *imazethapyr* detected into the formulation over the amount of test carrier (core *shell* hydrogels and superporous hydrogels). It can be represented as:

Loading capacity (Lc, %) = (Amount of *imzazethapyr* extracted from formulation/Amount of test carrier used) × 100 (1)

Encapsulation efficiency was calculated based on the concentration of *imazethapyr* extracted from the formulation over the initial concentration of *imazethapyr* added to make the formulation.

Encapsulation efficiency (Ee, %) = (Amount of *imzazethapyr* extracted from formulation/Amount of *imazethapyr* added) × 100 (2)

3.9 Characterization of carriers and carrier based formulations

Micro Fourier transform infrared spectroscopy (Bruker, ALFA, 0.5 Kv), Indian Agricultural Research Institute, New Delhi, India; Scanning electron microscopy (ZEISS EVO Series Scanning Electron Microscope (EVO 50) with resolution of 2.0 nm @ 30 kV, Indian Agricultural Research Institute, New Delhi, India; X-ray diffraction technique (PHILIPS PW1710 diffractometer control equipped with PHILIPS PW1728 X-ray generator, Indian Agricultural research Institute, New Delhi, India; Zetatrac TM, Indian Agricultural research Institute, New Delhi, India; Transmission electron microscopy (Jeol , JEM-1011, 100Kv), Indian Agricultural research Institute, New Delhi, India techniques were used to characterize the prepared materials synthesized in the present work.

3.10 Release Kinetics of *Imazethapyr* in water

An accurately weighed quantity 0.1 g SPHC *imazethapyr* formulation, 0.02 g core *shell* nanogel *imazethapyr* formulation and Pursuit® (10% SL) 80 µl was added (in triplicate) to 30 ml water in a plastic beaker. Plastic beaker were kept at 35 ^{0}C. At different time intervals (0, 1, 3, 5, 7, 10, 15 and 30 days), aliquots of 1 ml were removed for determination of *imazethapyr* by HPLC. Recovery of *imazethapyr* from different developed formulations varied from 38.7 to 50.5%.

3.10.1.2 HPLC analysis

3.10.1.2.1 Chromatographic condition

A reverse phase high performance liquid chromatography (HPLC) technique was used for quantitative analysis. A Hewlett Packed HPLC instrument (series 1100) equipped with degasser, quartenary pump, photo diode-array detector connected with rheodyne injection system (20 µL loop) and a computer (model Vectra) was used for analysis. The stationary phase consisted of Lichrospher on C-18 packed stainless column (250 mm x 4

mm i.d). Chromatogram was recorded in a Windows NT based HP chemstation programme. Acetonitrile:acidic water with gradient elution at 1 mL/min. flow rate was used as mobile phase. HPLC analysis was performed at wavelength of 250 nm, which was detected for absorption maxima using photodiode array. Each run was repeated thrice and the detector response was measured in terms of peak areas. Calibration curve was prepared by plotting concentration of imazethapyr in µg on x-axis against average peak area on y-axis. Limit of detection of imazethapyr was 0.01µg/ml.

$$Y= (D\times C\times V/B) \qquad (3)$$

Where, Y, concentration of herbicide in water (µg); D, peak area of sample; B, peak area of standard; C, concentration of standard solution (µg g^{-1}), V, final volume of the water (ml).

3.10.1.2.2 Analysis of release data

To describe the kinetics of *imazethapyr* release from developed formulations, release data was analyzed with the semi-empirical power law equation as suggested by Ritger and Peppas (1987).

$$M_t/M_o = Kt^n \qquad (4)$$

Where M_t/M_o is the fraction of active ingredient released at time t, K is a rate constant that incorporates characteristics (porosity, tortuosity) of the macromolecular network system and the active ingredients, and n, a diffusion parameter which is indicative of the transport mechanism. The model was fitted by taking logarithm on both sides of equation. The Values of *K* and *n* were determined from *imazethapyr* release data. The same semi-empirical power law equation ($M_t /M_o = Kt^n$) can be used to calculate the time taken for release of 50% of initial *a.i.* from the formulations under study.

At time taken for release of 50% the Eq. (4) will be,

$$0.5 = K (t_{1/2})^{n}$$

$$t_{1/2} = (0.5/K)^{1/n} \qquad (5)$$

The data were analysed using Sigma Plot software (Version 12.0, Systat Software, Inc).

3.11 Bio efficacy evaluation

3.11.1 Experimental Site

The experiment was conducted at the Research Farm of Division of Agronomy, Indian Agricultural Research Institute, New Delhi. The field had an even topography and good drainage system.

3.11.2 Climate and Weather

New Delhi is situated at 28°35‘N and 77°12’ E longitude and an altitude of about 228.61 m above mean sea level. It has a semi-arid and sub-tropical climate with dry summers and severely cold winter. The mean maximum temperature in June, which is the hottest month of the year, ranges from 40 to 45^0C, while the mean minimum temperature in the coldest month of January is low as 1.9^0C.

3.11.3 Soil characteristics

Soil of experimental field belong to the order inceptisol, mahauli series having sandy loam texture in upper 30 cm layer and loam below that. Water table remains below 3.5 m deep from soil surface during crop growth period. Soil of the experimental farm was sandy loam, pH 7.9, organic carbon 0.52%, and medium in terms of available N (272.6 kg/ha), P (18.4 kg/ha) and K (191.6 kg/ha).

3.11.4 Experimental Details

3.11.4.1 Treatments

Eight weed control treatments (Table 1) were adopted during winter season in Garlic crop.

Table 1: Treatment description of bioefficacy experiment

Code	Type	Test carrier/formulation	Dose
GG-SPHC-*imz*-75	Pre emergence	Superporous hydrogel	*Imazethapyr* @ 75 g / ha
GG-SPHC-*imz*-100	Pre emergence	Superporous hydrogel	*Imazethapyr* @100 g / ha
Pursuit-Stomp	Pre emergence	Pursuit® and Stomp®	*Imazethapyr* @ 100 g/ha Pendimethalin @ 1 kg/ha
Pursuit	Pre emergence	Pursuit®	*Imazethapyr* @ 100 g/ha
GG-CSH-*imz*-75	Post emergence	Core *shell* hydrogel	*Imazethapyr* @75 g / ha
GG-CSH-*imz*-100	Post emergence	Core *shell* hydrogel	*Imazethapyr* @100 g / ha
HW	--	Hand weeding(Weed free check)	--
AC	--	Absolute Control	--

3.11.4.2 Treatment description

The commercial formulation pursuit and stomp mixture were applied pre emergence by mixing in recommended volume of water and spraying on the soil in the crop area one day after sowing. The hydrogel composite (GG-SPHC) based formulation were applied in dry powered form by mixing the required amount in 250 grams dry finely sieved sand and broad casting in the crop area of each plot space. The post emergence application of nanogel based formulation was achieved by mixing the dry formulation in one liter water and spraying on the plot at 35 DAS. Weed free check plots were maintained throughout the experiment by hand weeding. Population and dry weight of weeds were recorded at 45 and 90 DAS of crop by placing a quadrate of 0.5 x 0.5 m^2 from three places in each plot.

Plot size: Plot size: 2 m × 2 m

Variety: Garlic: G-41

Design: Randomized complete Block Design (RBD)

Replication: 3

3.12 Pendimethalin

Pendimethalin [*N*-(1-ethylpropyl)-2, 6-dinitro-3, 4-xylidine; Stomp; Prowl; Pendilin; (stomp®)] is a derivative of the 2, 6-dinitroanilines and is formulated mainly as emulsifiable concentrates. It is a highly selective, broad-spectrum and pre-emergence herbicide and effective against a number of annual grasses and broad-leaved weeds. It can also be used as pre-plant incorporation in soybean, maize, onion and potato. It is a germination inhibitor and susceptible weeds are affected during germination or seedling emergence.

3.13 Agronomic practices

3.13.1 Land preparation

In order to ensure good germination, a pre-sowing irrigation was given to entire field prior to sowing of garlic. The field, when came to condition, was tilled, using a tractor drawn disc plough followed by harrowing and planking to have a seed bed of fine tilth.

3.13.2 Fertilizer application

A uniform recommended dose of 120 kg N/ha, 60 kg P_2O_5/ha and 50 kg K_2O/ha was applied in the field. Half of the recommended dose of N was applied basally through broadcasting and mixed with soil before sowing of garlic along with the full dose of P and K. The remaining N was applied at 40 days of growth. Nitrogen, P and K were given in the form of urea, diammonium phosphate (DAP) and muriate of potash (MOP), respectively throughout the experiment.

3.13.3 Sowing of seeds

Garlic cloves were planted manually. Cloves were sown at 4-5 cm depth in 15 cm row. Seed rate of 500 kg/ha of garlic clove was used for sowing.

3.13.4 Irrigation

A total of four irrigations was given.

3.13.5 Thinning

Seeds germinated uniformly, so no thinning was needed.

3.13.6 Harvesting and drying

Well-matured cloves were dug out manually by using spade and then sun dried.

3.14 Observations Recorded

3.14.1 Weeds

Species-wise weed distribution in the field was assessed at 45 and 90 DAS from all the experimental plots using a quadrate of size 0.5 m x 0.5 m.

3.14.2 Weed population and dry weight

Weed population was recorded at 45 DAS and 90 DAS and expressed as number of weed plants per m^2. Weeds collected from 0.25 m^2 area from each plot were first dried under open air conditions and finally kept in a hot air oven at 70°C till constant weight. Dry weights of each were expressed as gram per square meter (gm^{-2}).

3.14.3 Weed control efficiency (WCE)

In order to assess the efficiency of developed formulations in comparison commercial weed control efficiency was calculated as follows: (Das, 2008)

$$WCE= (WP_C\text{-}WP_T/WP_C) \times 100 \qquad (6)$$

Where, WP_C is the weed population (number/m^2) in weedy check plot and WP_T is the weed population (number/m^2) in treated plot.

3.14.4 Weed control index (WCI)

Weed control index (WCI) was calculated as follows: (Das, 2008)

$$WCI= (W_C\text{-}W_T/W_C) \times 100 \qquad (7)$$

Where, Wc is the dry weight of weeds in un weeded check and W_T is the dry weight of weeds in treated plots.

3.14.5 Phytotoxicity rating in crop and yield

Visual scoring for weed control and phytotoxic rating in garlic crop was done on 45th days after pre emergence herbicidal application.

3.15 Statistical Analysis

Data on different parameters of polymer, crops and weeds were subjected to statistical analysis following. Significance of difference between means across treatments was tested through variance ratio (i.e., *F* test), and least significant difference (LSD) was worked out where variance ratio was found significant for treatment effect at 5% probability level. Data on garlic and weeds during rainy season were analyzed using randomized complete block design (RCBD).

RESEARCH PAPER -I

Characterization and Swelling Properties of Fast Swelling Novel Guar gum-*G*-Polyacrylate/Bentonite Superporous Composites

Abstract:

A novel series of biopolymer bentonite based superporous hydrogel composites based on guar gum have been prepared by *in-situ* graft copolymerization of acrylamide in the presence of bentonite and cross linker. The structures of prepared materials was confirmed using FT-IR spectroscopy. Morphology of the samples was examined by scanning electron microscope which exhibited extensive porosity in the gel matrix. Presence of micro to nano sized pores was established by TEM analysis. The reaction variables i.e. monomer, crosslinker, clay contents and initiator concentration were optimized to achieve porous hydrogel composite with maximum possible swelling capacity. The results of absorbency under load showed that the superporous composites (GG-SPHC) possessed better mechanical strength than the corresponding clay free gel (GG-SPH). The effect of various salt media and solution of different pH etc on swelling of GG-SPHC *vis-à-vis* GG-SPH is also presented.

Key words: guar gum, superporous, composite, swelling

4.1 Introduction

Hydrogels are cross-linked hydrophilic polymers with a network structure consisting of acidic, basic, or neutral monomers which are able to imbibe large amounts of water (Omidian *et al.,* 2002). Because of their network structures and the possibility of rearrangements of hydrophobic/hydrophilic domains during the swelling process, including entanglements and crystalline regions, these polymers are water insoluble (Choi *et al.,* 2007).

Basically hydrogels are of two types with respect to swelling: conventional hydrogels and new generation of hydrogels (Chavda *et al.,* 2010). The most important difference between these two groups is their swelling characteristics (Kim *et al.,* 2004).

Among the new generation hydrogels, superporous hydrogels are lightly crosslinked hydrophilic polymers that can absorb aqueous solutions up to hundreds of times their own weight in shorter duration (Hyojin *et al.,* 2006). The fast swelling of these polymers can be related to capillary wetting of interconnected open pores (Yin *et al.,* 2007). Of late, biopolymeric superporous hydrogels are of interest due to their multifarious applications in domains.

Polysaccharides such as starch, chitosan, cellulose, and their derivatives (guar gum, sodium alginate, etc.) have been utilized to useful super absorbent and super porous hydrogel materials for bio-applications (Gils *et al.,* 2009; Zhang *et al.,* 2007; Chen *et al.,* 2000. Guar gum, a non-ionic galactomannan polysaccharide derived from *Cymaposis tetragonolobus*, has also been used to develop superabsorbent polymers (SAP) and super absorbent polymer composites (SAPC) with pH-sensitive characteristics (Zhang *et al.,* 2006). Its use as backbone to prepare porous hydrogels has been reported for the first time in our laboratory (Chandrika, 2013). It is well known in the literature that superporous hydrogels are mechanically fragile and superporous hydrogel composites (SPHCs) present a better alternative (Chavda and Patel, 2010). The present study reports for the first time GG-*g*-polyacrylate/bentonite superporous composites, their detailed characterization and fluid absorbency behavior. The swelling behaviour of the prepared GG-SPHC as a function of external stimuli namely, pH, salts and fertilizers and water quality has been compared with GG-SPH (clay free corresponding gel).

4.2 Materials and methods

4.2.1 Chemicals

Commercial guar gum (purity 95%), acrylamide, were purchased from SD Fine (Pvt) Ltd., Mumbai, India. Surfactant and foaming aids were purchased from Merck, (Pvt) Ltd. Mumbai, India. Bentonite was received M/S Earth international Pvt. Ltd., India. All the reagents were used as such without further purification.

4.2.2 Synthesis of Guar gum-*g*-*cl*-polyacrylate/bentonite super porous hydrogel composites (GG-SPHCs)

The super porous hydrogels composite were prepared by free radical polymerization technique employing redox initiator system. A general method of preparation followed in earlier reports (Chen *et al.,* 1999; Guo and Gao, 2007; Park *et al.,* 1993), involves sequential addition of monomer, cross linker, initiator, foam stabilizer and the foaming aid in distilled water (Figure 1). A procedure comprised dissolution of all the reagents including the backbone in distilled water in a particular sequence at a particular pH followed by start of initiation reaction. The time for harmonizing gelation and foaming reactions was standardized. The solution was kept undisturbed for a specific period. The gel obtained were treated with alkali of particular strength for a particular period of time, washed, dehydrated and finally dried. The process of preparation of SPHCs in the present work will be protected under IPR.

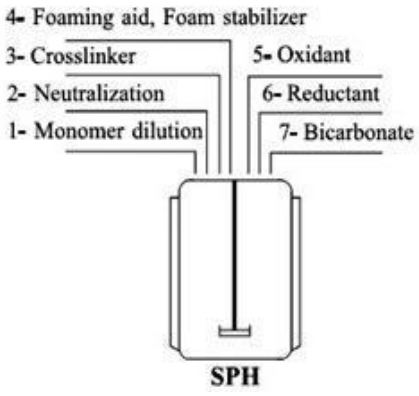


Figure 1: General steps involved in SPH/SPHC synthesis

The prepared composites were characterized by FT-IR spectra recorded in KBr disc carbons on a Bruker Fourier Transform Infrared Spectrophotometer under dry air at room temperature. Scanning electron microscopy images were obtained with a Zeiss EVO series scanning electron microscope (EVO 50) with resolution of 2.0 nm at 30 kV. X ray Diffraction patterns of clay and GG-SPHC were obtained using a Philips PW1710 diffractometer control equipped with Philips PW1728 X-ray generator. The scanning range was 3–20^0 2θ, with a scanning rate of 1.2^0 2θ/min.

4.2.3 Water absorbency measurements

A sample weighing 0.1 g (particle size 100–240 mesh size) was immersed in the excess of distilled water (pH 7.0, EC 0.001 mhos/cm) in triplicate and kept at room temperature,

until equilibrium was attained. Free water was filtered through a nylon sieve (200 mesh size), gel allowed to drain on sieve for 10 min, and finally weighed. The water absorbency (Q_{H_2O}) was calculated using the following equation: Q_{H_2O} (g/g) = w_2-w_1/w_1; where w_1 is the weight of xerogel (dry absorbent) and w_2 is the weight of swollen gel. Swelling behavior of GG-SPHC and GG-SPH exhibiting maximum absorption in distilled water was further evaluated in different salt solutions and water of different qualities.

4.2.4 Salt solution absorbency measurements

GG-SPHC and GG-SPH showing maximum absorption in distilled water were milled to achieve particles 100–240 mesh size. Range solutions of different strengths (5, 10, 15, and 20 mM) of four salts namely ammonium sulphate (AS), ammonium nitrate (AN), potassium nitrate (PN), sodium chloride (SC), and a fertilizer namely urea (U) were prepared. The dried and milled sample was immersed over night in salt solution of strength at room temperature. The swollen gel was weighed after 24 hours and Q_{H_2O} was computed using same equation as above. Similar experiment was repeated in tap water (pH 7.87, EC 1.099 mhos/cm), hard water, and aqueous solutions of pH 4, 7, and 9. Hard waters of three different strengths were prepared in the laboratory according to CIPAC standard method (Daasch, 1947) and labelled as hard water A (hardness 20 ppm, pH 5–6, and Ca : Mg ratio 50 : 50), hard water B (hardness 20 ppm, pH 8–9, and Ca : Mg ratio 80 : 20), and hard water C (hardness 500 ppm, pH 7–8, and Ca : Mg ratio 80 : 20).

4.2.5 Absorbency (AUL) under load measurements

A plastic beaker (diameter 4.2 cm, height 5.6 cm) with sintered bottom was placed in a Petri dish, and a weighed, dry sample (0.3±0.01 g) of hydrogel (GG-SPHC or GG-SPH) was uniformly placed on the surface of polyester gauze placed on the sintered bottom of beaker. A solid weigh to attain load of 68.77 psi, could slip freely through the sintered beaker was placed on the gel particles. Distilled water was added in petri dish to ensure that the liquid level was at half height level of the porous beaker. Increase in height of swollen particles was measured periodically.

4.2.6 Statistical analysis

The experiments on effect of various parameters on water absorbency values were conducted using completely randomized design. To identify the best treatment combinations, the data were analysed by one-way classified analysis using PROC GLM procedure of SAS package (SAS Institute, Cary, NC).

4.3 Results and Discussion

4.3.1 Characterization of GG-SPH and GG-SPHC

4.3.1.1 FT-IR analysis

The FT-IR spectra of native guar gum, bentonite and GG-SPHC and GG-SPH and are shown in Figure 2. The absorption bands of guar gum (GG) at 1655 cm^{-1} assigned to H-OH bending and at 1440 cm^{-1} assigned to C-OH bending vibration almost disappeared in FTIR of the clay free and hydrogel composite (Figure 2), where as new bands at 1685-1655 cm^{-1} (C=O stretching of COOH groups), 1458-59 cm^{-1} (O-H bending) and ≈ 1320 cm^{-1} (C-O stretching of carboxylic acid group appeared. Absence of characteristic peaks of $CONH_2$ group of acrylamide and appearance of characteristic peaks of $–COO^-$ group confirm hydrolysis of amide to $–COO^-$ group. In GG-SPHC, presence of peak at 1040 cm^{-1}, which can not be seen in corresponding free clay gel confirms the presence of bentonite in the GG-SPHC net work chains.

4.3.1.2 Scanning Electron Microscopy analysis

Morphology of a representative GG-SPH/GG-SPHC examined by SEM at various magnifications is given in Figure 3. It is clearly visible that while GG-SPH contains macro sized pores and irregularly distributed channels, in case of the clay composite GG-SPHC, a highly uniform micro to nano porosity uniformly distributed across the hydrogel network is observed. Hydrogel contains large number of pores of almost same sizes. Pore size was estimated from the images by averaging the diameter of ten cells.

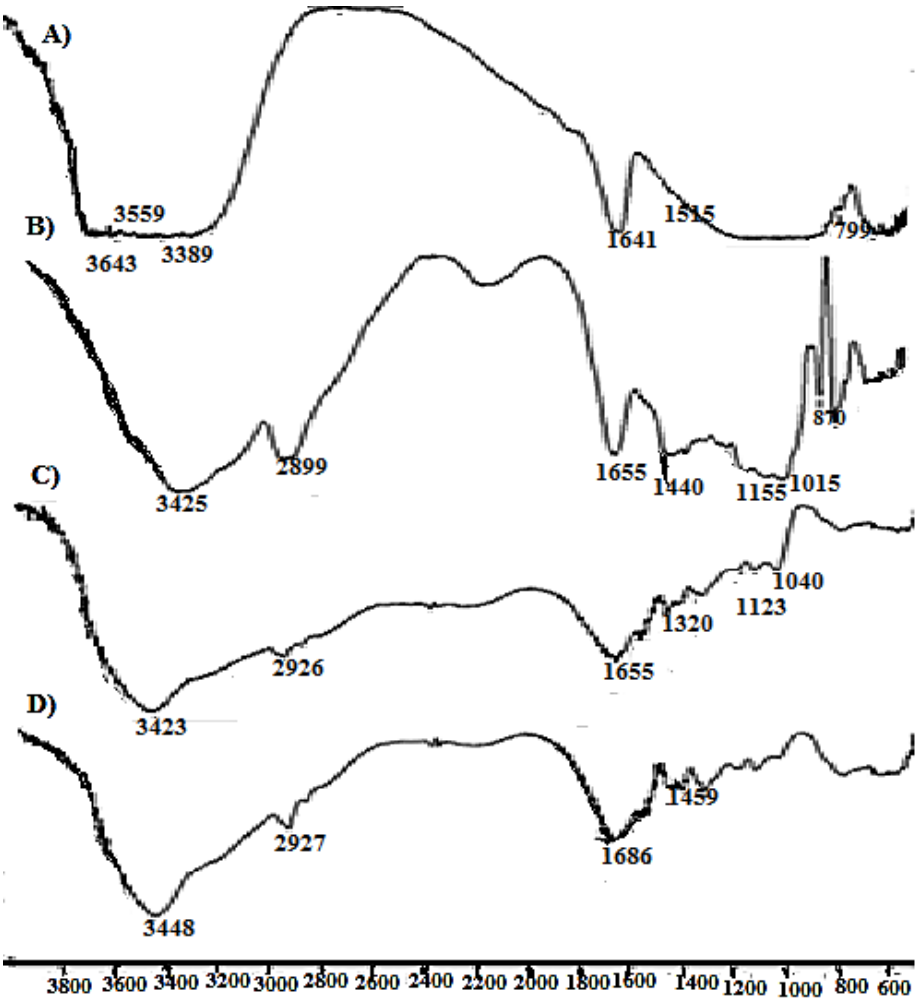


Figure 2: FT-IR spectra of bentonite (A), guar gum (B), GG-SPHC (C) and GG-SPH (D)

4.3.1.4 XRD analysis

Powder X-ray diffraction pattern recorded for pure bentonite and the porous guar gum *g*-polyacrylate/bentonite composite is depicted in Figure 4. The characteristic 001 reflection of the major montmorillonite (MT) phase of bentonite which appeared at 2θ=6°, has shifted to 5° in the composite. This points out towards intercalation of growing polymer chain into the interlayer spaces of montmorillonite phase. On the basis of Bragg's law nλ= 2d sinθ, the interplanar distances in bentonite and the composite are given in Table 1.

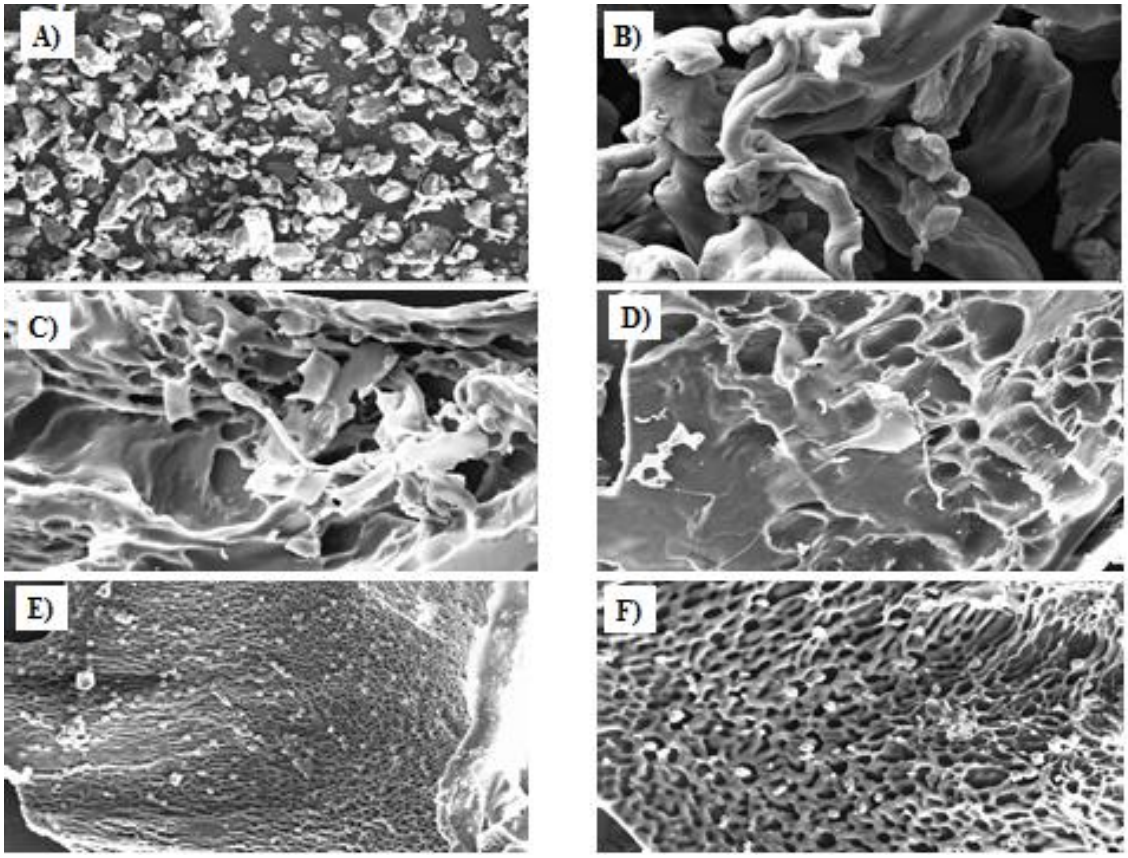


Figure 3: SEM images of bentonite (A), guar gum (B), GG-SPH (C,D) and GG-SPHC (E,F)

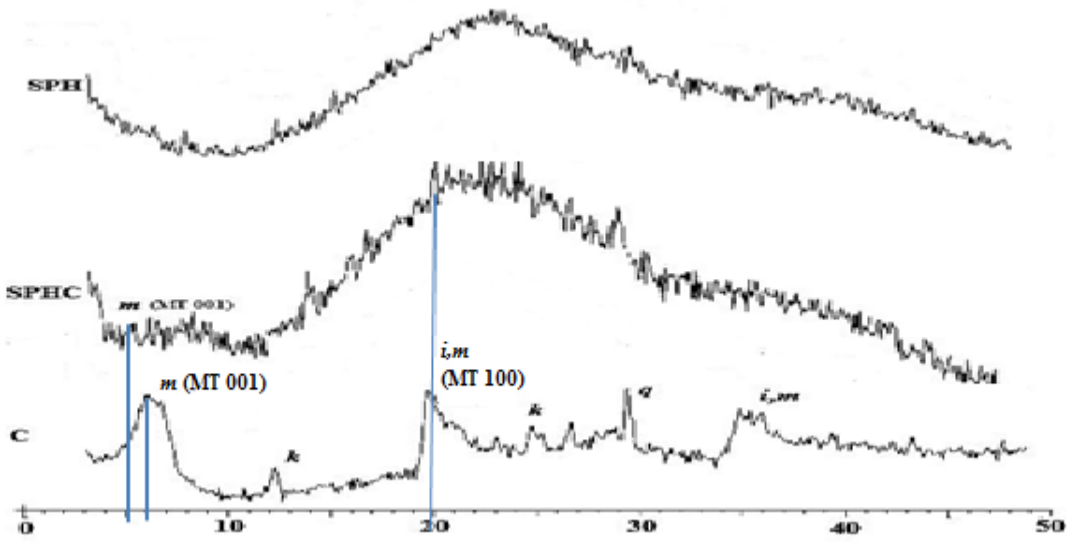


Figure 4: Powder X-ray diffractogram of bentonite (C), guar gum (B), superporous hydrogel (GG-SPH) and super porous hydrogel composite (GG-SPHC)

Table 1: Value of interlayer spacing in bentonite and GG-SPHC

Material	**Spacing (Å)**	**2θ**
Bentonite	14.70, 4.4	6, 20
GG-SPHC	17.65, 4.4	5, 20

4.3.2 Superporous Hydrogel composite (GG-SPHC) synthesis and effect of reaction factors on water absorbency behaviour

Major reactions steps involved in the preparation of GG-SPHCs are gelation and foaming. In order to generate uniform pores in the matrix of the composite, harmonization between the two processes is the key element. As described by Omidian *et al.*, 2005, the whole process can be divided into three stages. Stage-II involving synchronized gel and pore formation is most critical. Therefore sequence of addition of various reactants plays determinant role in the preparation of GG-SPHs. A general synthetic procedure reported in most of the studies reported is shown in (Figure 1).

$$\text{R- COOH} + \text{NaHCO}_3 + \text{Foaming aid}$$

$$\downarrow$$

$$\text{R- COONa} + \text{CO}_2 + \text{H}_2\text{O}$$

Figure 5: Scheme of reaction responsible for pore generation in superporous hydrogels

The process conditions standardized by Chandrika *et al.,* (2013) to prepared Guar gum-*g*-SPH gels were employed to synthesise GG-SPHCs in the present work with minor modification in the sequence of reagent addition and backbone- monomer ratio. The details of the process will be covered under IPR. Cross linker plays an important role in influencing the SPH properties (Kabiri *et al.,* 2003). Effect of cross linker content on the water absorbency of GG-SPHC is shown in Table 2. As can be seen from Figure 5, foaming aid reacts with sodium bicarbonate, carbon dioxide gas bubbles start evolving.

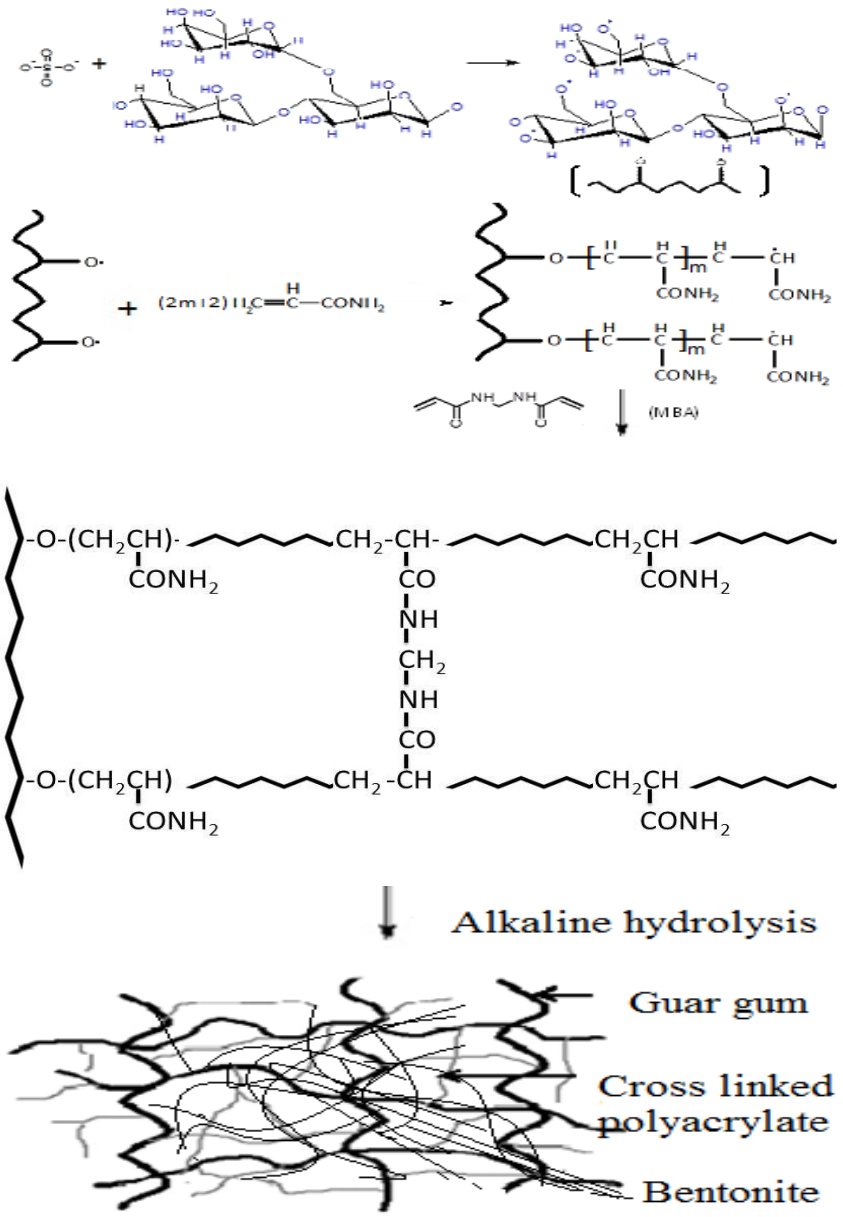


Figure 6: Diagrammatic representation of formation of a typical cross linked guar gum - *g*-polyacrylate/bentonite hydrogel composite

Efficiency of trapping the gas bubbles during gelation determines the porosity of GG-SPHC. As is evident from Table 2, increase in cross linker concentration reduced water absorbency. The decrease in swelling capacity can be attributed to generation of tighter network as results of increase in cross linker concentration (Gupta and Shivakumar, 2010). Concentration of the porogen significantly influences the swelling properties of the hydrogels. As is clear from the Table 2, under all the experiment

conditions employed in the present study increase in porogen concentration in the range of 0.388-3.745 wt %, resulted in the SPHCs with increasing swelling ratios. At higher concentrations, consistently low swelling behaviour was exhibited. Under acidic pH conditions employed (pH 4 to 5). SBC reacts with acid to generate CO_2 gas bubbles. Beyond an optimum concentration of the porogen, the pH of the reaction medium tends to rise which adversely affects the gelation process. This effect is reflected in inferior water absorbency values of the resulting SPHC. Similar observation on the performance of pH-sensitive SPH has been reported earlier (Chen *et al.,* 2000).

Table 2, shows an increase in swelling capacity of GG-SPHC with increase in monomer content upto 96.8 wt % monomer in feed. This behavior is ascribed to increase in concentration $–COO^-$ groups on the grafted chain. Beyond 96.8 wt %, decrease in water absorbency can be explained in terms of increase in non-polymer content at fixed cross linker, clay and initiators contents (Zhang *et al.,* 2006; Singh *et al.,* 2010). In most of published reports on effects of bentonite on swelling behaviours of hydrogels decrease in water absorbency with increase in clay content has been presented. In present work an initial increase from 0.019 to 0.194 wt % swelling ratio increase which then starts falling with further clay content increase. The initial increase can be ascribed to the participation of hydrophilic clay in the network formation leading to materials of optimum level of cross linking.

Table 2: Effect of various synthesis parameters on water absorbency of GG-SPHC

Monomer Concentration (wt %)	**Water absorption (g/g)**	**Clay concentration (wt %)**	**Water absorption (g/g)**	**Cross linker concentration (wt %)**	**Water absorption (g/g)**	**Porogen concentration (wt %)**	**Water absorption (g/g)**
92.593	208.3^{G}	0.019	311.6^{E}	0.022	440.0^{A}	0.388	300.0^{D}
93.985	220.0^{F}	0.039	321.7^{D}	0.220	400.0^{B}	1.908	350.0^{B}
94.937	243.3^{E}	0.117	331.7^{C}	0.439	280.0^{C}	3.745	410.0^{A}
95.628	253.3^{D}	0.194	340.0^{B}	0.873	210.0^{D}	7.220	330.0^{C}
96.154	311.6^{B}	0.388	351.7^{A}	1.304	160.0^{E}	10.453	280.0^{E}
96.899	368.3^{A}	1.154	321.7^{D}	1.732	143.3^{F}	1.908	250.0^{F}
97.403	318.3^{B}	1.908	271.7^{F}				
97.598	295.0^{C}	3.198	233.3^{G}				
97.765	257.0^{D}						
LSD at 5 %	7.21		8.29		13.6		17.79
CV	1.52		1.54		2.73		3.12
F value	458.4		200.6		838.2		96.0

Table 3: Effect of clay content and equilibrium swelling ratio (Q_{H_2O}, g/g)

Type of gel	Monomer (wt %)	Clay (wt %)	Cross linker (wt %)	Porogen (wt %)	Q_{H_2O} (g/g)
GG SPH	97.65	0.000	0.389	0.387	430
GG SPHC-M	97.65	0.389	0.389	0.387	368
GG SPHC-B	97.65	1.150	0.389	0.387	351

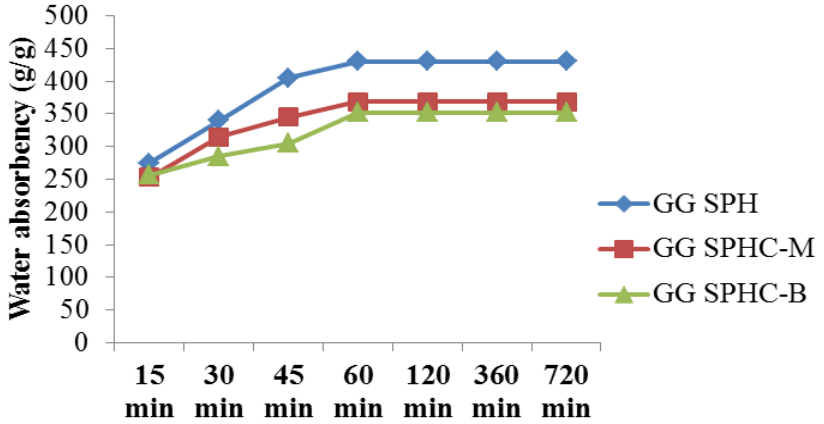


Figure 7: Rate of swelling as a function of clay content

4.4 Swelling rate measurements

4.4.1 Effect of external environment on water absorbency

A comparison of temperature of the swelling medium on the water absorbency of GG-SPHC and GG-SPH is shown in Figure 8. Rise in temperature of swelling medium exhibited increase in Q_{H_2O} in both GG-SPHC and GG-SPH. The effect was more pronounced in case of GG-SPHC. Rise of temperature from 5° to 35°C led to ~40% swelling enhancement in GG-SPHC as compared to 8% in GG-SPH from 35 to 50 °C, however only GG-SPHC swelled more by 8% only GG-SPHC did not show significant variation in swelling as a function of temperature of swelling medium. Maximum swelling is observed at 50°C. This behaviour can be due to the extensive entanglement or expansion of the macromolecular chains at higher temperature.

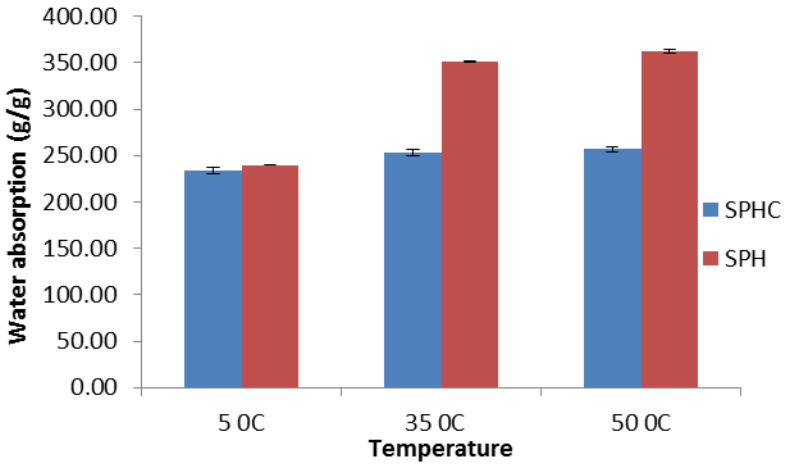


Figure 8: Effect of temperature on water absorbency of GG-SPHC vs GG-SPH

The swelling behaviour of GG-SPHC vs. GG-SPH in different pH environments is presented in Figure 9. At pH 9, the hydrogel shows maximum swelling. This can be explained in terms of ionization of the amide and -COOH groups to $-COO^-$ groups. The electrostatic repulsion between the COO^- groups leads to macromolecular expansion and the resulting enhancement of Q_{H_2O}. In acidic pH, protonation of the $-CONH_2$ and $–COO^-$ groups leads to decrease in extent of hydrogen bonding with water molecules and thus the decrease in water absorbency. Similarly findings have been confirmed in previous studies on SPHs (Singh *et al.*, 2010; Chandrika *et al.*, 2014). At acidic pH, the GG-SPHC and GG-SPH swelled to similar extent but at pH 7 and pH 9, the Q_{H2O} of GG-SPHC significantly suspended the GG-SPH. The results can be explained in terms of

extensive porosity in GG-SPHC as compared to irregularly shaped and fractured pores in GG-SPH.

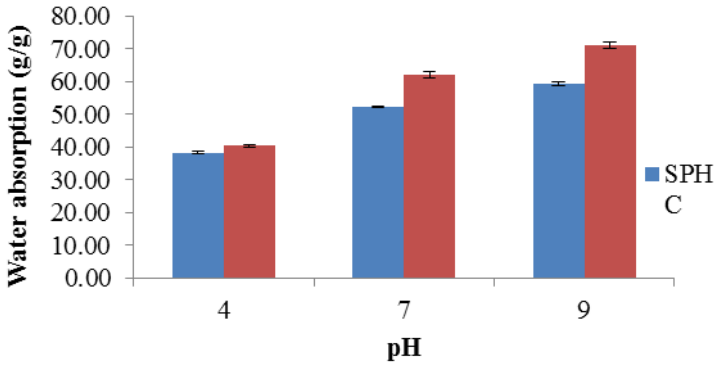
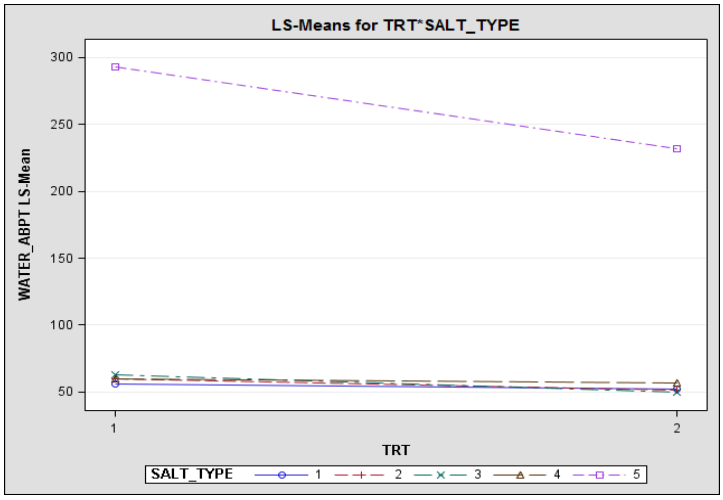


Figure 9: Effect of pH on water absorbency of GG-SPHC vs GG-SPH

Swelling behaviour of the GG-SPHC *vs.* GG-SPH in different salt solutions namely ammonium sulphate (AS), ammonium nitrate (AN), potassium nitrate (PN), sodium chloride (SC) and fertilizer urea solution each of strengths 5 mM, 10 mM, 15 mM and 20 mM is depicted in Table 4. It is clear from the Table 4, that except in urea solution, a drastic reduction in swelling capacities of GG-SPHC as well as GG-SPH was observed in all the salt solution. The best performance of both materials was seen in urea solution (Figure 11), although in urea solutions of all the test strengths, GG-SPH performed better than GG-SPHC (Figure 10). Similar observation has been reported by Gils *et al.*, 2009. The cations in the salt solutions screen the polar groups causing reduction in the hydrogen bonding with H_2O molecules. Also, in the salt solutions, the osmotic pressure resulting from the difference in mobile ion concentration between the gel matrix and the surrounding aqueous phase decreases, adversely affecting absorbency. In urea solution also, the absorbency exhibited fall with increase in its concentration from 5 mM to 20 mM, though the overall reduction in Q_{H_2O} was lesser than that in salt solutions.

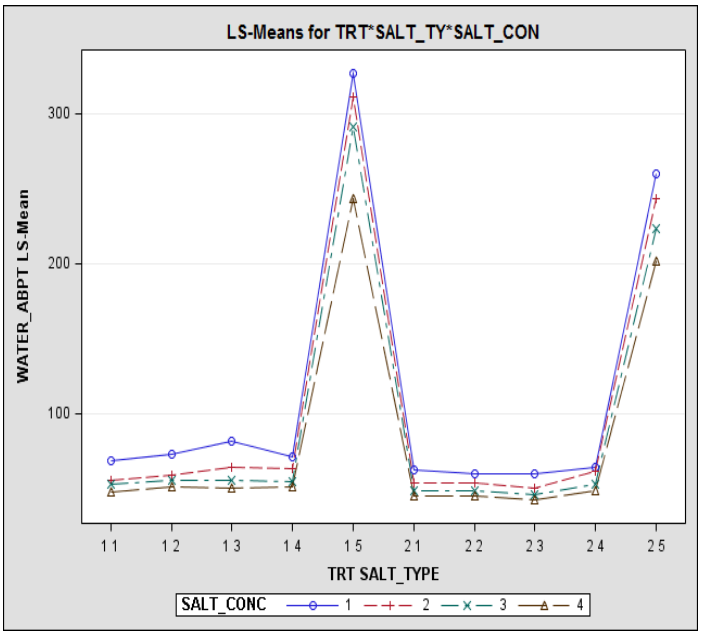
Table 4: Effect of salt/fertilizer type and strength on equilibrium swelling ratio of GG-SPH and GG-SPHC

Salt/Fertilizer type	GG-SPH				GG-SPHC			
	5 mM	**10 mM**	**15 mM**	**20 mM**	**5 mM**	**10 mM**	**15 mM**	**20 mM**
$(NH_4)SO_4$	68.7^{J}	55.3^{NO}	53.3^{OPQ}	$47.3S^{TUV}$	62.3^{KI}	53.7^{OPQ}	48.7^{RSTU}	45.3^{UVW}
NH_4NO_3	72.7I	58.7^{MN}	55.7^{NO}	51.0^{POR}	60.0^{LM}	53.7^{OPQ}	48.3^{RSTUV}	45.0^{VW}
KNO_3	81.3^{H}	64.0^{K}	55.3^{NO}	$50.3Q^{RS}$	60.0^{LM}	$50.7Q^{RS}$	46.0^{TUV}	42.3^{W}
NaCl	71.0^{IJ}	63.0^{KI}	54.3^{OP}	51.0^{PQR}	64.3^{K}	61.7^{KLM}	53.0^{OPQ}	49.0^{RST}
NH_2CONH_2	326.7^{A}	313.3^{B}	291.3^{C}	243.3^{E}	260.0^{D}	243.3^{E}	223.3^{F}	201.7^{G}
LSD at 5%	3.56				3.56			
CV	2.25				2.25			
F value	9.85				9.85			



Salt type	
1	$(NH_4)SO_4$
2	NH_4NO_3
3	KNO_3
4	NaCl
5	NH_2CONH_2

Figure 10: Interaction mean plot of hydrogel type and salt type for water absorption



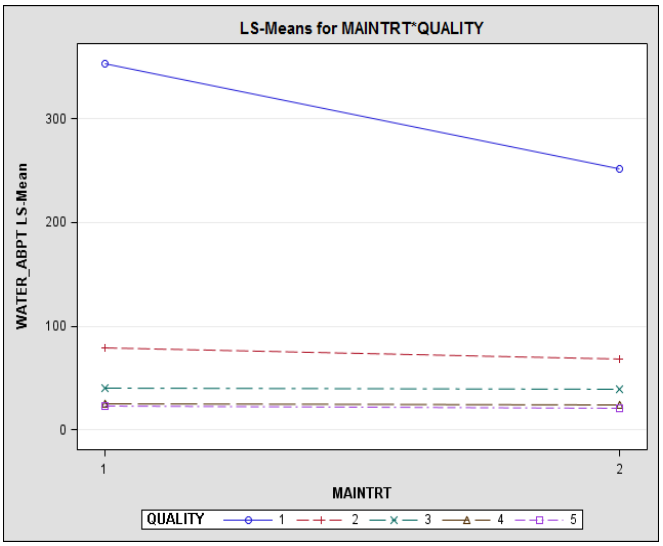
11	GG-SPH	$(NH_4)SO_4$
12		NH_4NO_3
13		KNO_3
14		NaCl
15		NH_2CONH_2
21	GG-SPHC	$(NH_4)SO_4$
22		NH_4NO_3
23		KNO_3
24		NaCl
25		NH_2CONH_2

Figure 11: Interaction mean plot of hydrogels, salt type and salt concentration for water absorption

In our previous work Singh *et al.*, 2010 reported that water quality also influences the swelling performance of hydrogels. The effect of water quality on the swelling behaviour of GG-SPHC and GG-SPH is shown in Table 5 and Figure 11. As compared to distilled water, absorbency is significantly reduced in different aqueous environments. The observed behaviour may be attributed to the reduction in osmotic potential between gel and the surrounding water containing cations of varying strengths (Pourjavadi *et al.*, 2008).

Table 5: Effect of water quality on equilibrium swelling ratio of GG-SPH and GG-SPHC

Quality of water	Water absorption Q_{H_2O} (g/g)	
	GG-SPH	GG-SPHC
Distilled water	353.3^{A}	251.3^{B}
Tap water	78.7^{C}	68.3^{D}
Hard water –A	40.7^{E}	38.7^{E}
Hard water –B	25.0^{F}	$23.7F^{G}$
Hard water –C	$22.7F^{G}$	20.3^{G}
LSD at 5%	3.36	3.36
CV	2.14	2.14
F value	743.1	743.1



Along X-axis	
1	GG-SPH
2	GG-SPHC

Water quality

1	Distilled water
2	Tap water
3	Hard water A
4	Hard water B
5	Hard water AC

Figure 12: Interaction mean plot of hydrogel type and quality for water absorption

4.5 Measurement of water absorbency under load

Absorbency under load was determined as shown in Figure 13 and was calculated by equation (1). The specific work (Joules) done by the developed hydrogels during absorbency under load (68.77 psi) was calculated by equation (2).

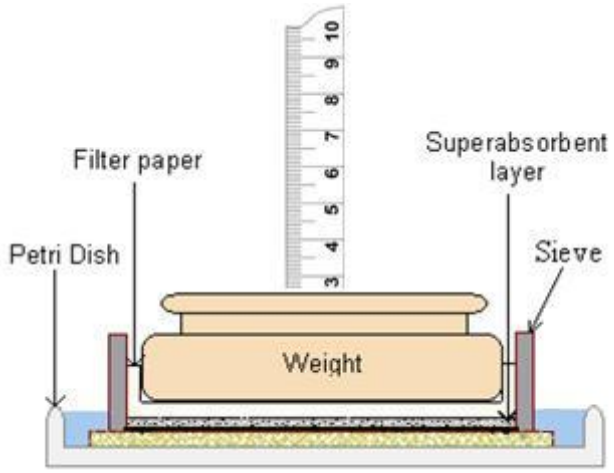


Figure 13: Diagrammatic representation of device used for the AUL measurement

$$\text{AUL (g water / g dry gel)} = (\pi x r^2 x h x d)/a \qquad (1)$$

Where,

r, the radius of the cylinder (cm)

h, the height attained after swelling of hydrogel at each time interval (cm)

d, density of test solution i.e. water (g/cm^3)

a, amount of dry gel (g)

$$\text{Specific work (Joules)} = (l \, x d x 9.8 \ m/s^2)/a \qquad (2)$$

Where,

l, the weight of load (Kg)

d, density of test solution i.e. water (g/cm^3)

a, amount of dry gel (g)

AUL was used as a marker of mechanical strength of porous material in present work. In general the rate of swelling under pressure is related to the particle size and particle size distribution, specific surface area and density of the superabsorbent polymer **(**Lu *et al.*, 2003). When a compressive load is employed to the swelling superabsorbent particles, it is forced to change shape, so dimension of pores with increasing the pressure is decreased. In general, where as the AUL value, increased as a function of degree of cross linking, the water retention capacity decreases as a reciprocal of the same (Barghi., 2009). The degree of the formation is related to on

the initial cross linking density and is measured in in the extent of swelling capacity. A high value of AUL depends on high hydrogel strength and the high hydrogel strength can maintain a more hydrogel mass during swelling.

In the present work, AUL of GG-SPHCs and GG-SPHs of varying cross linking densities was investigated periodically over 15 days (Figure 14). AUL of GG-SPHs increased with time till 10^{th} day for all the super absorbents and then it reached equilibrium. In the present study superporous hydrogel composites (GG-SPHC) showed higher AUL than superporous hydrogel (GG-SPH) at all the crosslinker concentrations. As expected, AUL increased with increase in crosslinker concentration in both the series of GG-SPHC and GG-SPH. The presence of inorganic mineral provides additional network points in polymerization reaction that result in higher crosslinking density and simultaneously higher mechanical strength. Improved mechanical strength of GG-SPHC as compared to GG-SPH, by addition of clay filler material as a function of cross linker content, cross linking density can be further explained in terms of higher specific work done by GG-SPHC and GG-SPH of higher cross linking densities (Figure 15). The point worth discussing here is superior performance of all the test guar gum based superporous composites as compared to Pusa Hydrogel, a cellulosic superabsorbent.

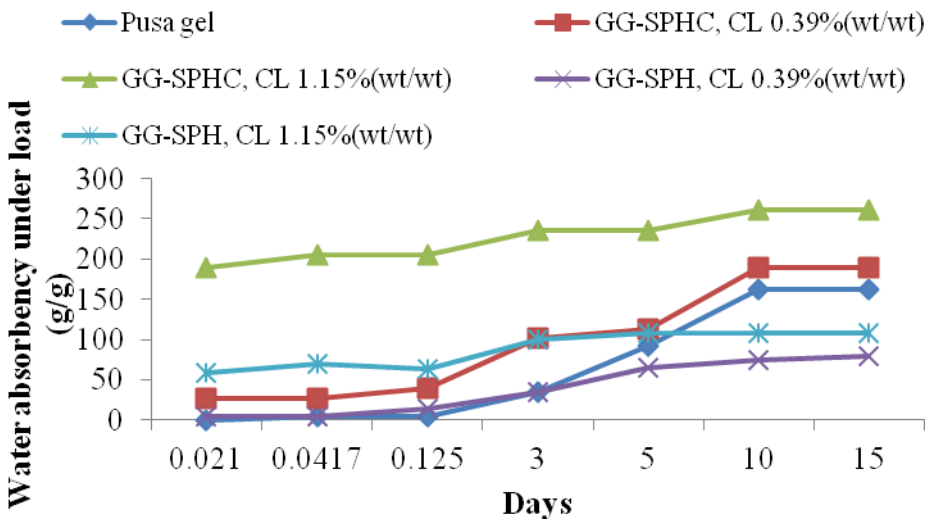


Figure 14: The AUL of developed hydrogel under applied load

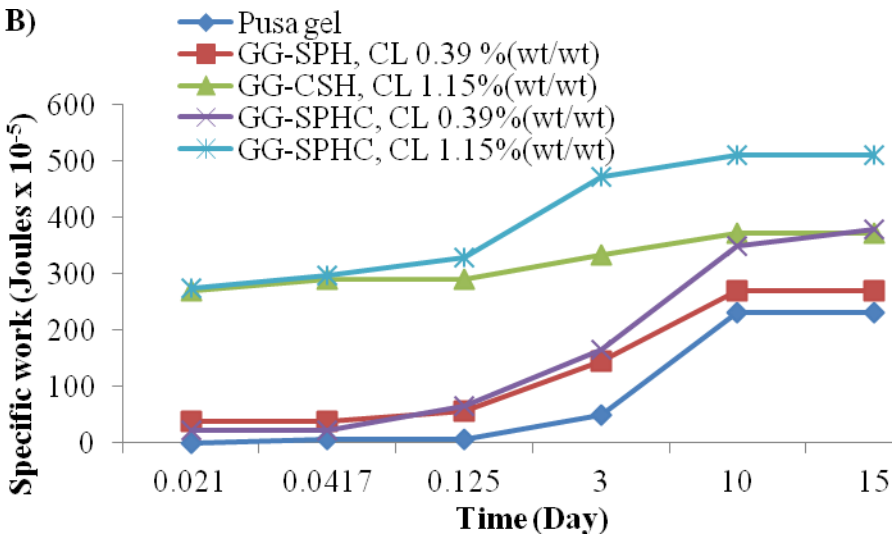


Figure 15: The Specific work by developed hydrogels under applied load

4.6 Conclusion

Novel super porous composites GG-*g*-polyacrylate/bentonite were prepared and investigated for their swelling behaviour in different environments. The prepared composites exhibited superior swelling in all the aqueous environments as compared to related composites reported in the literature. Absorbency measurements of clay free gel, GG-SPH and the composite under load confirmed the higher mechanical strength of GG-SPHCs relative to GG-SPHs. SEM characterization revealed extensive porosity while XRD imaging pointed out towards formation of intercalated bentonite based superporous composite. Their fast swelling behaviour and the established use of related materials in controlled release application, the porous composites synthesized in the present work will be evaluated as carriers of agrochemicals such as herbicides.

RESEARCH PAPER -II

Guar gum Based Novel *Core Shell* Nanogels: Synthesis and Characterization

Abstract

Three series of *core shell* nanogels employing guar gum and two techniques of polymerization i.e. free radical precipitation polymerization and inverse emulsion technique was synthesized and characterized in the present work. PNIPAm-*core*-guargum-*shell*, PVA-*core*-guargum-*shell*, GG-PVA-*core*-PNIPAm-*shell* nanogels were synthesized. In free radical polymerization technique, the surfactant free process conditions were standardized. The effect of reaction factors namely monomer (N-isopropylacrylamide) content, crosslinker (N, N-methylenebisacrylamide) content and guar gum content on the particle size of the nanogels was studied. Structures of prepare nanogels were confirmed by FT-IR spectroscopy. Particle size, expressed here as hydrodynamic diameter, D_h determined by DLS technique confirmed the formation of naogels. TEM analysis showed formation of spherical shaped nanogel particles with *core shell* morphologies. Amongst two techniques employed, the inverse emulsion polymerization produced uniform sized and shaped nanoparticles as compared to solution polymerization technique.

Key words: Guar gum; *core shell*; nanogel

5.1 Introduction

Hydrogel (*core shell*) nanoparticles have gained considerable attention in recent owing to their unique potentials via combining the characteristics of a hydrogel system with nanoparticles (e.g., very small size). Several polymeric *core shell* nanogels based on both natural and synthetic polymers have been prepared and characterized and are widely used in drug release system (Motornov *et al.*, 2010), biosensors (Kim and Park, *2004*), emulsion and suspension stabilizers (Brugger and Mikhail, 2008; Atta and Al-Shafey, *2013*). The term nanogel usually refers to a nanoparticle composed of chemically or physically crosslinked synthetic polymers or biopolymers, sizes ranging from tens to hundreds of nanometers, with controllable properties, such as swelling, degradation and

chemical functionality (Hayashi *et al.,* 2004). They are gaining importance primarily because of advantages such as efficient loading of drugs, controlled and sustained drug release at the target site and improved efficiency of pesticides. Drug loading in nanogels can be achieved without chemical reactions, an important factor for preserving the drug/agrochemical activity. Recently, ionic micro and nanogels have received wide attention because of their unique hybrid structures, having cohesive properties of solids and diffusive properties of liquids (Sannier, 2006; Li *et al.,* 2012). This is due to their well-defined morphology, size, and surface, as well as to their unique physical and chemical properties (Sundberg and Durant, 2003). In controlled release formulation strategies for agrochemicals, Nano carriers like amphiphillic polymers (Kumar *et al.,* 2010), nanoclays (Datta *et al.,* 2008), nanocomposites (Hussein *et al*, 2005) have been used. *Core shell* nanogels potentially unexploited in the agrochemical formulation technology. Poly (N-isopropylacrylamide) (PNIPAm) is a widely investigated thermo responsive polymer (Atta and Al-Shafey, 2013; Schmidt et al., 2010). The amphiphilic nature of PNIPAm is attributed to the presence of both amide groups and isopropyl groups. Solution properties of Poly NIPAm nanogels such as surface charge and colloidal stability strongly depend on the location of the functional groups inside the nanogels (Su *et al.,* 2000; Sheikholeslami *et al.,* 2012). PNIPAm based nanogels are widely investigated and reported for their use in drug delivery applications (Subhash *et al*, 2011). Polyvinyl alcohol is another polymer which is non toxic, semi-crystalline, biodegradable and water soluble and is gaining lot of interest in controlled release devices (Shah *et al.,* 1997). Generally, the monodispersed nanogel particles are prepared in the presence of surfactant because of convenience. In the present work, we report a novel surfactant free approach of preparation of PVA and PNIPAm based *core shell* nanogels by manipulating the dispersant and hydrophilic properties of guar gum. The effect of reaction factors *viz.* the crosslinker content, monomer content and guar gum/feed ratio on particle size has been investigated . Surfactant free synthesis of nanogels has been tried in generation of copolymeric nanogels (Zhang *et al.,* 2009). Based on our previous work on guar gum based polyacrylate macro hydrogels (Chandrika *et al*, 2014), this article aims to prepare nanogels based on crosslinked *N*-isopropylacrylamide (NIPAm)/PVA and guar gum in the absence of surfactants. Presence of PNIPAm in the *shell* component of core-*shell*

nanogels can also impart interesting thermo sensitive behavior to the nanoparticles. The second part of the present study therefore aims at synthesis of PNIPAm-*shell*-GG-PVA-*core* nanogels employing GG-PVA nanogels as the seed materials.

5.2 Materials and methods

5.2.1 Chemicals

Guar gum (LR) N, N'- methylene bis acrylamide and a persulphate initiator were purchased from Thomas Baker Pvt Ltd., Mumbai, India. Surfactants Span 80, GR (99% assay), Acetone, Cyclohexane, polyvinyl alcohol (MW 85000-124000) were purchased from Merck Specialties Pvt. Ltd., Mumbai, India. *N*-Isopropylacrylamide (NIPAm) was procured from Aldrich Chemicals Co. USA. All the reagents were used as such without further purification.

5.2.2 Synthesis of guar gum -*core*-PNIPAm-*shell* nanogels

N-isopropylacrylamide (62.5 to 86.9% w/w), N, N-methylene bisacrylamide(1.06 to 3.1% w/w), were taken in water in a three necked round bottom flask, degassed and kept at 60^0C with constant stirring. After 30 minutes of continuous N_2 purging, APS (60 to 70%) was added and reaction was allowed for 30 minutes. Varying ratios of guar gum (8.3 to 21.4% w/w) were added with continuous rapid stirring and reaction was set undisturbed for 6 hr. After 1 hr, the solution turned turbid marking the nanogel formation. The contents of the flask were centrifuged at 20000 rpm for 30 min and supernatant solution was lyophilized. The dried white powder obtained was stored in the sealed vials.

5.2.3 Synthesis of guar gum-*shell*-PVA-*core* nanogel

Polyvinyl alcohol (25 to 62% w/w) was dissolved in 85 ml distilled water in a round bottom flask, stirred at 50 °C till complete dissolution. Acetone (15 ml) was added drop wise with constant stirring and contents were kept at 5 °C for 24 h. The pale yellow solution were degassed in an oil bath maintained at 60 °C , different ratios of guar gum were added with stirring under N_2 atmosphere. To the feed, crosslinker (1.06 to 32.0% w/w) and APS initiator (2.2 to 5.2% w/w) were added and polymerization reaction was allowed for 6 hr with constant purging. The solution turned turbid after one hr of reaction which showed that nanogels were formed. The contents of the flask were centrifuged at

20000 rpm for 40 min, supernatant was lyophilized to get white powder. The dried nanogel was stored in sealed vials.

5.2.4 Synthesis of guar gum PVA-*core*- PNIPAMm-*shell* nanogel

Inverse emulsion method (Oh *et al.*, 2008) was employed as described by Sahiner *et al.*, 2006 with some modifications. GG-PVA *core:* Poly vinyl alcohol (59.1% w/w) was dissolved in 85 ml distilled water in a round bottom flask, stirred at 50°C till complete dissolution. Acetone (15 ml) was added drop wise with constant stirring and kept it at 5 °C for 24 h. The pale yellow solution were degassed in an oil bath maintained at 60°C , different ratios of guar gum were added with stirring under N_2 atmosphere. To the feed, crosslinker (5.9% w/w) and APS initiator (5.3% w/w) were added and polymerization reaction was allowed for 6 hr with constant purging. The solution turned turbid after one hr of reaction which showed that nanogels were formed. The contents of the flask were centrifuged at 20000 rpm for 40 min, the supernatant solution was passed through membrane filter and stored as aqueous phase. Synthesis of *shell:* Aqueous phase (4.7 ml), predetermined quantity of Span 80 in 2.0 ml cyclohexane were taken in three necked round bottomed flask. The solution was purged with N_2 for 15 min. Aqueous solution (15 ml) containing different ratios of NIPAm (84.6 to 88.8% w/w) and crosslinker (2.1 to 10.5% w/w) was added to the oil phase slowly with constant stirring. Temperature of oil bath was raised to 60^0C and after 15 min, initiator was added and the reaction was allowed for 6 hr. Turbid solution appeared after 1 hr which showed formation of nanogels. The content of the flask was centrifuged at 20000 rpm for 30 min, the supernatant was filtered through membrane and excess acetone was added repeatedly to emulsion to ensure complete precipitation. This was followed by acetone:methanol (9:1) addition to precipitate followed by washing in excess water. The solution was lyophilized and dried white powder obtained was stored in the sealed vials.

5.2.5 Characterization

Transmission electron micrographs (TEM) were obtained using a transmission electron microscope (Jeol, JEM-1011, 100Kv). Diluted dispersions were placed onto carbon-coated copper grids, stained with uranyl acetate and dried at room temperature. Zeta sizer (scattering angle 90°) was used to determine the average hydrodynamic diameter (Dh,

nm) of the gel particles. The samples were allowed to equilibrate for 15 min before measurement. FT-IR spectra were recorded in KBr disc carbons on a Bruker Fourier Transform Infrared Spectrophotometer under dry air at room temperature.

5.3 Results and Discussion

5.3.1 Synthesis of nanogels

A novel surfactant free method for nanogel synthesis was used to give nanogels based on i) guar gum and PNIPAm and ii) guar gum and PVA. Major constraint in PNIPAm based micro/nanogels is their sensitivity to temperature. Above LCST, the polymer turns hydrophobic and expels water while below LCST remains in the expanded swollen (Blackburn and Lyon, 2008). PNIPAm based micro/nanogels have been, in most of the reported studies prepared by simultaneous copolymerisation of monomers and crosslinking agents. This method usually requires use of surfactants such as sodium dodecyl sulphate. Presence of surfactants shift the LCST to higher value (>32^0C). Due to amphiphilic nature of surfactant, *shell* is created around the PNIPAm chains that results in increase in LCST. Removal of surfactant and aggregation instability limit the role of PNIPAm gels in CRF applications. It is well reported that structural stability can be improved not only by the presence of surfactant or ionic groups but also by introduction of hydrophilic polymer chain into nanogel structures (Mendrek, 2006). The obtained aggregates become stable in water above the LCST without addition of surfactant so the combined *core shell*-PNIPAm surrounding by hydrophilic corona leads to preparation of well defined particles with *core shell* morphology.

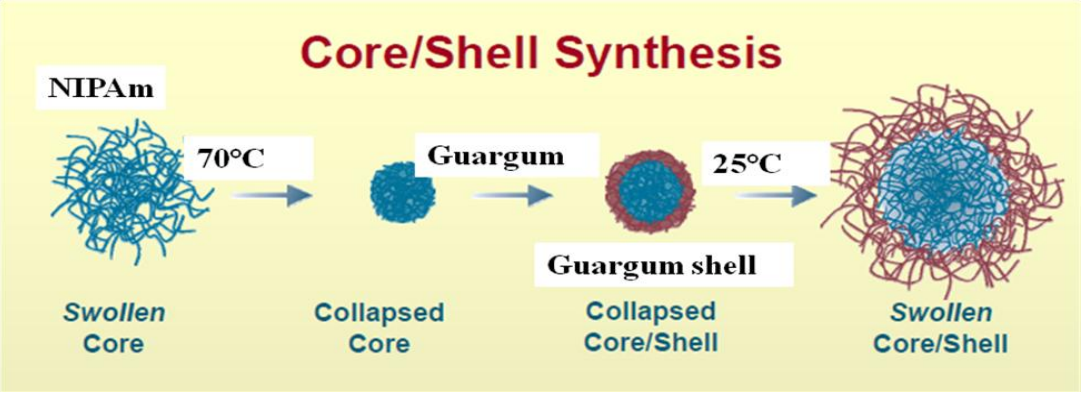


Figure 1: Scheme of formation of PNIPAm-*core*-guar gum-*shell*-nanogel

The hydrophilic free chains surrounding the crosslinked polymer *core* act as steric stabilizers leading to thermal stability. Guar gum is a naturally occurring cationic hydrophilic polymer containing polar OH groups on its polysaccharide chains. In view of its surfactant properties in the colloidal system, our approach of its use in preparation of *core shell* nanogels led to formation of nanosized hydrogels with definite spherical structures.

The inverse mini emulsion technique is often used to confine the particle size of growing networks through restriction induced by oil soluble surfactant. The technique is well adpted to prepare controlled release products (Dorwal, 2012). In the present work, the PNIPAm-*shell*-GG-PVA-*core* nanogels were synthesized by combining solusion and emulsion polymerization techniques. The first step that involved reaction between guar gum and polyvinyl alcohol in the presence of crosslinker led to GG-*shell*-PVA-*Core* nanoparticles. These nanoparticles were taken in aqueous phase and were made to react with PNIPAm and crosslinker in oil phase in presence of surfactant and initiator. The aqueous droplets containing the seed nanoparticles, monomer and crosslinker, in the presence of initiator at 60^0C, led to generation of PNIPAm-*shell* around GG-PVA-*core*.

5.3.2 Characterization of nanogels

5.3.2.1 Particle size analysis

The particle size analysis by zetasizer revealed that increase in crosslinker concentration and monomer concentration the reaction feed significantly influenced the particle size. In case of GG-*shell*-PNIPAm-*core* nanogels, the particle hydrodyanamic diameter increased with increase in crosslinker content from 1.06 to 3.1%. In most of the published reports on PNIPAm based nanogels, crosslinker content had inverse relation with particle size which can be understood in terms of restriction on the growing polymer networks due to high crosslink density. The anomaly was reported in chitosan-alginate crosslinked nanoparticles also by (Motwani *et al.,* 2008). The observed trend can be explained in terms of increase in grafting of PNIPAm network the guargum as the crosslinker concentration was increased resulting in generation of larger sized nanoparticles. Similar trend was observed by Soni *et al.,* 2011, during synthesis of sodium alginate based nanogels. GG-*shell*-PVA-*core* series and PNIPAm-*shell*-GG-PVA-*core* nanogels

followed the expected behavior. Increase in crosslinker concentration registered decrease in particle size in both the series.

Increase in monomer content led to generation of polymer particles of larger sizes in all the three series. Higher monomer content generates scope for expansion of network chains at a fixed crosslinker concentration, leading to increase in particle size. This finding is in conformity with earlier published reports (Blackburn and Layon, 2008).

The basis of synthesis of GG-*shell*-PNIPAm-*core* series was to exploit hydrophilic characteristics and stabilizing properties of guar gum. Therefore guar gum content was varied in the feed to assess the effect on particle size. The analysis showed that the particle size of nanogels decreased with increase in guar gum content. This is a well known fact that increase in surfactant concentration leads to decrease in particle size (Blackburn and Lyon, 2008). Guar gum, because of its stabilizing properties seems to have played same role here.

Table 1: Effect of monomer, cross linker and guar gum content on particle size of GG-*shell*-PNIPAm-*core* nanogels

Factor	**Amount used (wt %)**	**D_h (nm)**
Crosslinker	1.06	13.7
	1.5	90.9
	2.6	163.6
	3.1	211.3
NIPAm (monomer)	62.5	48.9
	78.9	90.1
	83.3	163.1
	86.9	265.5
Guar gum	8.3	94.4
	15.3	81.3
	21.4	37.2

Table 2: Effect of monomer, cross linker content on particle size of GG-*shell*-PVA-*core* nanogels

GG *shell* PVA *core*		
Crosslinker (w/w %)	GG (w/w %)	Mean (nm)
6.2	31.0	313.5
25	31.0	108.5
34	31.0	62.2

5.3.2.2 FT-IR analysis

The FT-IR spectra of NIPAm, Poly vinyl alcohol, GG-*shell*-PNIPAm-*core* nanogel and GG-*shell*-PVA-*core* nanogel are shown in Figure 2. The amide peaks of the NIPAm units at 1648 cm^{-1} (C = O stretching), 1527cm^{-1} (N-H bending), and around 3438 cm^{-1} (N-H stretching) can be seen. The absorption bands of guar gum (GG) at 1661 cm^{-1} assigned to H-OH bending and at 1439 cm^{-1} assigned to C-OH bending vibration have almost disappeared showing the grafting of PNIPAm onto guar gum in the *shell* component. In case of GG-*shell*-PVA-*core* nanogel, band at 3394 cm^{-1} can be attributed to OH stretching of PVA and at 2940 cm^{-1} to the methylene vibrations.

Table 3: Effect of monomer, cross linker content on particle size of GG-PVA-*core* PNIPAm-*shell* nanogels

Monomer (w/w %)	**Crosslinker (w/w %)**	**Mean (nm)**
	3.9	90
	5.5	33
84.6	10.5	23
	2.7	196
	3.8	44
86.0	7.7	64
	2.1	337
	3.0	260
88.8	5.9	80

5.3.2.3 TEM analysis

TEM micrographs of the nanogels of all the three series reveal successful generation of spherical particles While GG-*shell*-PNIPAm-*core* TEM depicts poly dispersity, GG-*shell*-PVA *core* image shows distinct core shell morphology. As compared to these two nanogels synthesized by solution polymerization, the particle size of PNIPAm–*shell*-GG-PVA-*core* nanogels synthesized by inverse emulsion technique appears to have been controlled resulting in mono disperse particles. The same observation is supported by zetasizer analysis (Appendix).

5.4 Conclusion

TEM micrographs of the polymer particles prepared in the present study confirm the generation of spherical nanogels with *core shell* morphologies. The *core shell* structure is more pronounced in case of GG-*shell*-PVA-*core* nanogels. Particle size estimation by zeta sizer depicted the effect of reaction factors monomer content, crosslinker content and guar gum content on the particle size of different nanogel series. Use of guar gum led to generation of *core shell* nanogels of spherical geometery employing surfactant free procedure. This is a new finding that would help in exploiting guar gum as a stabilizer in synthetic operations in nanopolymer chemistry.

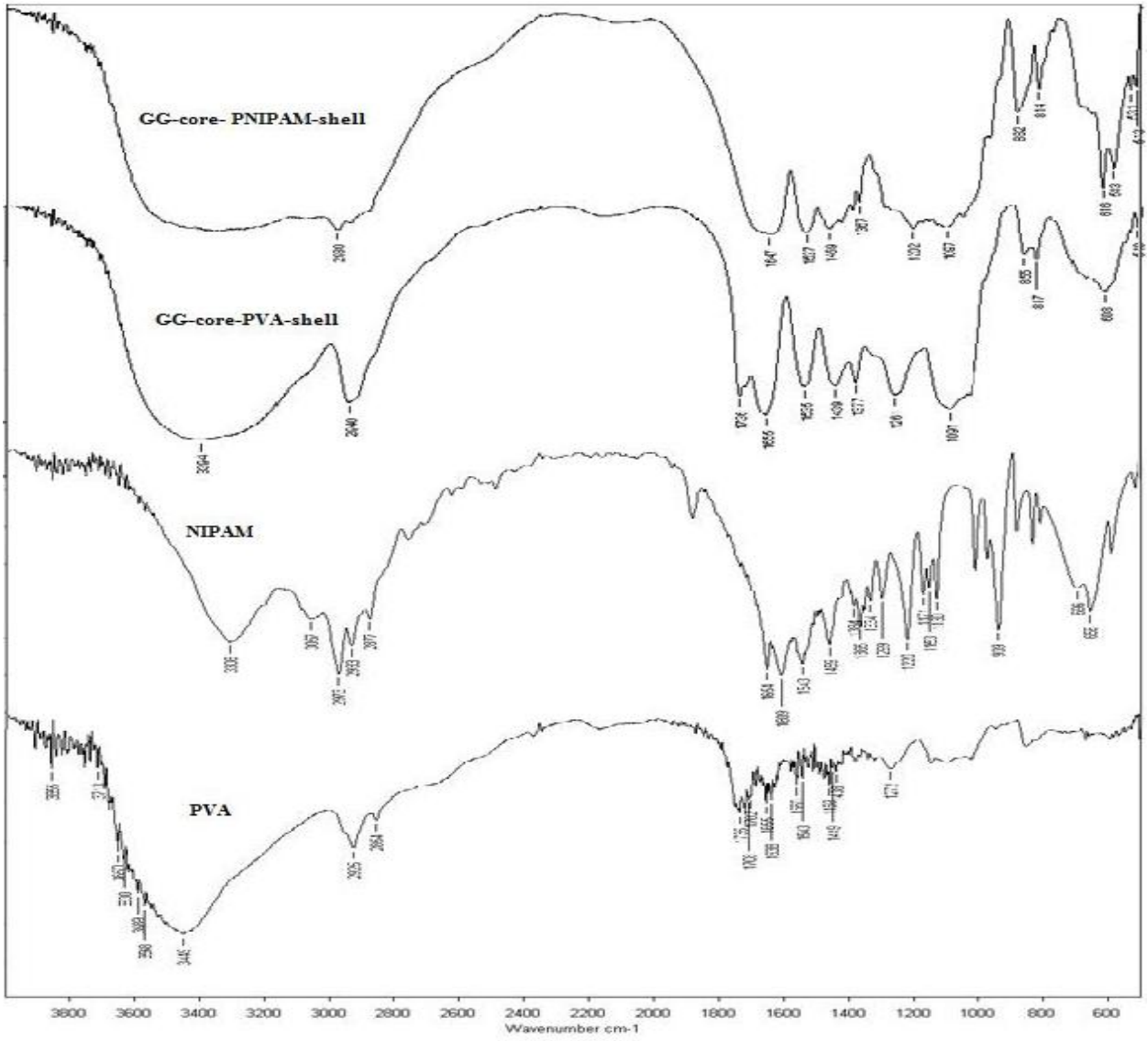


Figure 2: FT-IR spectra of NIPAm, Poly vinyl alcohol (PVA), GG-*shell*-PNIPAm-*core* nanogel and GG-*shell*-PVA-*core* nanogel

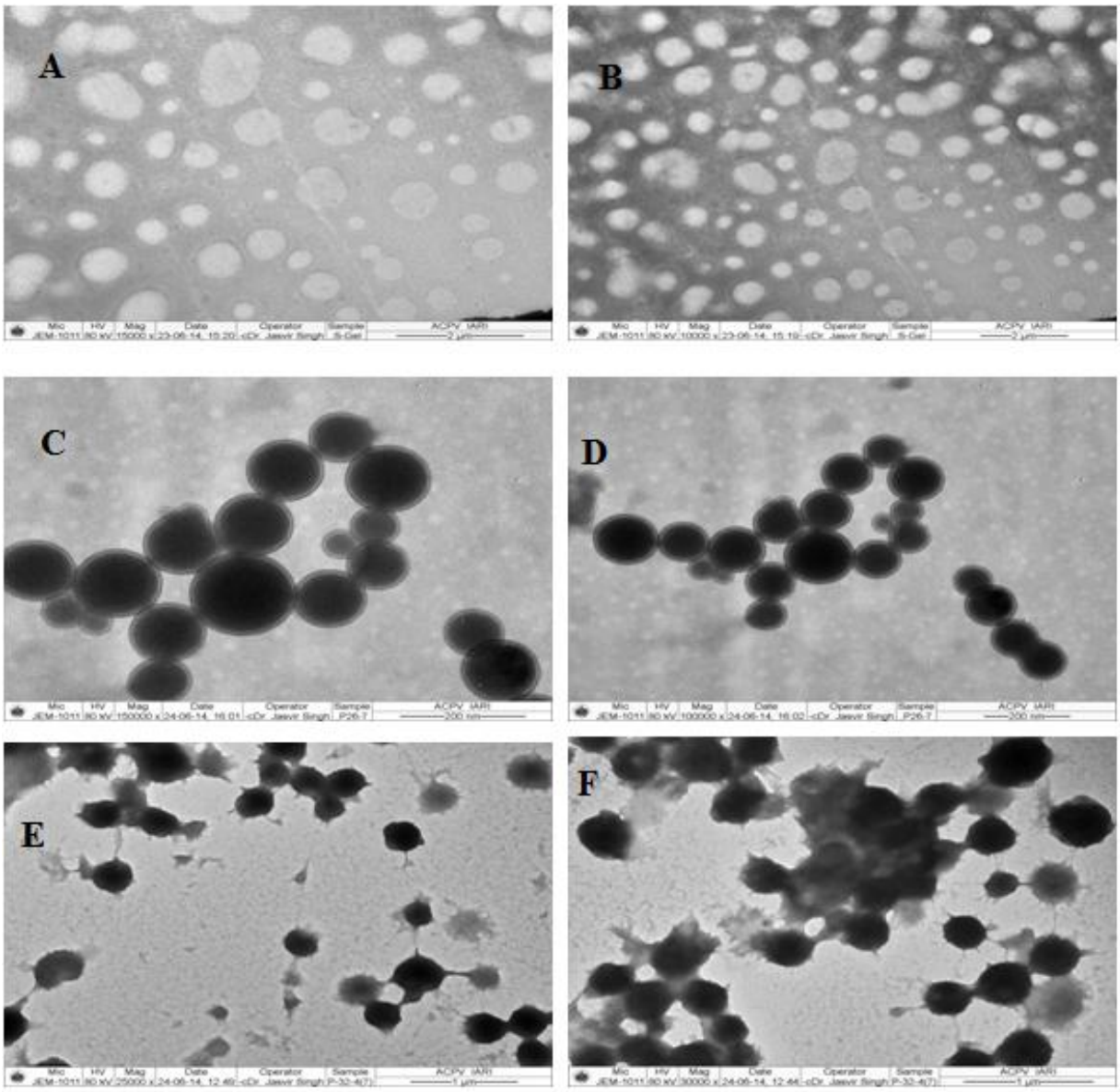


Figure 3: TEM micrographs of GG-*shell*-PNIPAm-*core* nanogel (A, B); GG-*shell*-PVA-*core* (C, D); PNIPAm-*shell*-GG PVA-*core* nanogel (E, F)

RESEARCH PAPER-III

Biopolymeric Hydrogel and Nanogel Based Novel Formulations of *Imazethapyr* for Pre and Post Emergence Application: Release Behavior and Bio Efficacy Evaluation

Abstract

Novel controlled release (CR) formulations of *imazethapyr* herbicide were prepared in the present work employing guar gum based superporous hydrogel clay composite (GG-SPHCF) and core shell nanogel (GG-CSHF). Impregnation of *imazethapyr* in carrier matrix/core was confirmed by FT-IR analysis of formulations. The encapsulation efficiency (%) and loading capacity (%) of developed formulations were estimated with the help of HPLC. The release kinetics of *imazethapyr* was studied in water. *a.i.* release was faster from commercial formulation than from prepared formulations. The time taken for release of 50 % loaded *a.i.* ranged between 0.06 to 4.8 days in case of GG-CSHF formulations and 4.4 to 12.6 days for GG-SPHCF formulations. The diffusion exponent (n) value of *imazethapyr* in water showed that the predominant mechanism of release was fickian diffusion in all the formulations. The prepared formulations were evaluated under field conditions for their bio efficacy activities against weeds prevalent in garlic crop. Weed control index (WCI) of both formulations GG-SPHC-*imz* and GG-CSH-*imz* showed which is at par with the WCI of the commercial formulations and improved efficacy around the crop growth period.

Key words: Core shell nanogels, superporous hydrogel composite, guargum, imazethapyr, garlic, weed control

6.1 Introduction

Weeds pose one of the major crop production constraints all over the world. Judicious use of broad spectrum herbicides to manage this problem is an important component in modern agricultural practices. *Imazethapyr* (±-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3 pyridine carboxylic acid) belonging to imazdazoline group is a promising broad spectrum herbicide recommended in number of

crops. It is applied at both pre and post emergence stages in crops such as soybean, groundnut and other legumes (Habimana *et al*., 2013) applied although mostly as post emergence, its pre emergence application has also been evaluated (Masson and Webster, 2009 ; Jeffrey *et al.,* 2001). Weeds normally controlled by *imazethapyr* are *Acanthospermum australe, Acanthospermum hispidum, Amaranthus deflexus, Amaranthus hybridus, Amaranthus thunbergii, Chenopodium album, Galinsoga parviflora, Commelina benghalensis, Cyperus rotundus, Portulaca oleraceae, Xanthium strumariumr, Tribulus terrestris, Datura ferox, Commelina benghalensis, Physalis angulata, Cleome monophylla, Datura ferox etc.* In most of the reported studies, *imazethapyr* is used for post emergence weed control (Meena *et al*., 2011). The commercially available liquid formulation of *imazethapyr* used in India is 10 % Soluble liquid (SL). Soluble liquid formulations or other formulation of similar type suffer from certain inherent limitations such as fast dissipation of *a.i.*, fast degradation, requirement of use of toxic adjuvants etc. (Meena *et al.,* 2011). Controlled release formulations are thus being viewed as a potential strategy (Tai and Zhu, 2008). Hydrogels, the cross linked hydrophilic polymers with network structures containing polar functional groups are versatile carriers extensively used in controlled drug delivery and as controlled release devices in agriculture (Blodgett *et al.,* 1993). The macro sized hydrogel particles are amorphous solids, where as nano-sized gels, the nanogels form nano suspension in water. Nanogels have sizes ranging from tens to hundreds of nanometers, with controllable release properties (Hayashi *et al.,* 2004).

In the present work, a novel (hitherto unknown so far in literature) attempt has been made to develop guar gum based formulations of *imazethapyr* aimed at both pre and post emergence application. Guar gum based polyacrylate clay superporous hydrogel composites were used as test carriers for development of solid formulation. For post emergence application, biopolymeric nanogels have been utilized.

6.2. Materials and methods

6.2.1 Materials

Imazethapyr technical Grade, 96.0 %, m/m, Commercial formulations Pursuit® and Stomp® through the courtesy of BASF India limited.

For routine work, laboratory grade and for HPLC analysis, analytical grade chemicals and solvents were employed.

The test carriers guar gum based super porous hydrogel clay composites (GG-SPHC) were prepared as per method described in chapter 4 and used as such without further purification. The core shell nanogels (GG-*shell*-PNIAm-*core*) were prepared per as method described in chapter 5 and was used as such without further purification.

For HPLC analysis, analytical grade chemicals and solvents were used while for routine work laboratory grade quality of the same was used.

6.2.2 Preparation of *Imazethapyr* formulations

Imazethapyr solutions (142 and 171 ppm) were prepared by dissolving the required quantity of *imazethapyr* (96.0% purity) in distilled water. A specific weight of dry GG-SPHC powder was added to a particular volume of *imazethapyr* solution in a beaker at room temperature and subjected to constant agitation for 120 min to ensure proper mixing and maximum expansion of carrier particles. Loaded GG-SPHCs were dried by lyophilization to get dry GG-SPHCs formulation of *imazethapyr*.

GG-CSH-*imz* formulations were prepared by drop wise addition of mathanolic solution of herbicide into aqueous suspension of nanogel. Stirring was continued for 2 hours followed by membrane filtration. The suspension was lyophilized to get dry powder. Codes and description of various test formulations prepared for the present work is given in Tables 1.

Table 1: Description of *imazethapyr* formulations prepared for controlled release study

Sl no.	Code	Description (wt %)
1	GG-CSHF-1	Guar gum -Core shellhydrogel, Crosslinker- 0.0018
2	GG-CSHF-2	Guar gum- Core shellhydrogel, Crosslinker- 0.029
3	GG-CSHF-3	Guar gum- Core shellhydrogel, Crosslinker- 0.039
4	GG-SPHCF-1	Guar gum-Superporous hydrogel composite- Cross linker 0.17, bentonite 0.056
5	GG-SPHCF-2	Guar gum-Superporous hydrogel composite- Cross linker 0.28, bentonite 0.056
6	GG-SPHCF-3	Guar gum-Superporous hydrogel composite- Cross linker 0.057, bentonite 0.056
7	GG- SPHCF-4	Guar gum-Superporous hydrogel composite- Cross linker 0.057, bentonite 0.170
8	GG -SPHCF-5	Guar gum-Superporous hydrogel composite- Cross linker 0.057, bentonite 0.280

6.2.3 Characterization of carriers and carrier based formulations

Micro Fourier transform infrared spectroscopy (Bruker, ALFA, 0.5 Kv), Indian Agricultural Research Institute, New Delhi, India; Scanning electron microscopy (ZEISS EVO Series Scanning Electron Microscope (EVO 50) with resolution of 2.0 nm @ 30 kV, Indian Agricultural Research Institute, New Delhi, India; X-ray diffraction technique (PHILIPS PW1710 diffractometer control equipped with PHILIPS PW1728 X-ray generator, Indian Agricultural research Institute, New Delhi, India; Zetatrac TM, Indian Agricultural research Institute, New Delhi, India; Transmission electron microscopy (Jeol , JEM-1011, 100Kv), Indian Agricultural research Institute, New Delhi, India techniques were used to characterize the prepared materials synthesized in the present work.

6.2.4 Loading capacity and Encapsulation efficiency

The loading capacity and encapsulation efficiency of GG-SPHCF test carriers were determined as follows: The *a.i.* was extracted from the carrier matrix by solvent replacement method. In case of GG-CSHF formulations, Lc and Ee were determined after separating nanogels from supernatant after centrifugation at 9000 rpm for 30 minutes. The process was repeated three times.

Loading capacity (Lc, %) = (Amount of *imzazethapyr* extracted from formulation/Amount of test carrier used) × 100

Encapsulation efficiency (Ee, %) was calculated on the basis of concentration of *imazethapyr* extracted from the formulation over the initial concentration of *imazethapyr* added to make the formulation.

Encapsulation efficiency (Ee, %) = (Amount of *imzazethapyr* extracted from formulation/Amount of *imazethapyr* added) × 100

6.2.5 Release kinetics of *imazethapyr* from developed formulations in water

6.2.5.1 Release of *imazethapyr* in water

The release of *imazethapyr* from the developed formulations and the commercial formulation (Pursuit®) was studied in water under laboratory conditions (room temperature 35 ±2°C) as per literature with minor modifications (Kumar *et al.*, 2010).

The term ‘release’ refers to amount of active ingredient in water at a given time. GG-SPHC-*imz* formulation (dry powder, 0.1g) was taken in a nylon bag (5 cm×5cm) was immersed in 30 ml distilled water and incubated at 35°C. GG-CSH-*imz* formulation (dry power, particle size 10 to 250 nm, 0.02g) was taken in a parchment paper sachet and placed in 30 ml distilled water in a stoppered flask at 35°C. An aliquot of 0.5 mL were drawn from each flask periodically (0, 1, 3, 5, 7, 15 and 30 Day) and subjected for *imazethapyr* determination by HPLC. The injector volume for the sample was 20 μl.

6.2.5.2 HPLC conditions

A reverse phase high performance liquid chromatography (HPLC) technique was used for quantitative analysis. A Hewlett Packed HPLC instrument (series 1100) equipped with degasser, quaternary pump, photo diode-array detector connected with rheodyne injection system (20 µL loop) and a computer (model Vectra) was used for analysis. The stationary phase consisted of Lichrospher on C-18 packed stainless column (250 mm x 4 mm i.d). Chromatogram was recorded in a Windows NT based HP chemstation programme. Acetonitrile:acidic water with gradient elution at 1 ml/min. flow rate was used as mobile phase. HPLC analysis was performed at wavelength of 250 nm, which was detected for absorption maxima using photodiode array. Each run was repeated thrice and the detector response was measured in terms of peak areas. Calibration curve was prepared by plotting concentration of imazethapyr in µg on x-axis against average peak area on y-axis. Limit of detection of imazethapyr by HPLC was 0.01µg/ml.

6.2.5.3 Analysis of release data

In order to describe the kinetics of *imazethapyr* release from developed formulations, the release data was analyzed with the semi-empirical power law equation as suggested by Ritger and Peppas (1987).

$$M_t/M_o = Kt^n \qquad (1)$$

Where M_t/M_o is the fraction of active ingredient released at time t, K is a rate constant that incorporates characteristics (porosity, tortuosity) of the macromolecular

network system and the active ingredients, and n, a diffusion parameter which is indicative of the transport mechanism.

The Values of *K* and *n* were determined from *imazethapyr* release data. The same semi-empirical power law equation ($M_t / M_o = Kt^n$) can be used to calculate the time taken for release of 50% of initial *a.i.* from the formulations under study.

At time t corresponding to release of 50% *a.i.* the Eq. 1 will be,

$$0.5 = K (t_{1/2})^{n}$$

$$t_{1/2} = (0.5/K)^{1/n} \qquad (2)$$

The data were analyzed using Sigma Plot software (Version 12.0, Systat Software, Inc).

6.2.6 Bioefficacy evaluation

A field experiment was conducted at IARI farm in rabi garlic crop in 2013-14. The objective of the experiment was to evaluate performance of newly prepared formulations in comparison with commercial formulation against weeds in garlic crop. Garlic crop was taken under a larger objective to explore the suitability of *imazethapyr* for weed management in garlic variety (G-41). The garlic seeds were procured from National Horticulture Research Development Foundation, Karnal (Haryana). The experiment was conducted in completely randomized block design. Soil was sandy loam, pH 7.9, organic carbon 0.52% and medium in terms of available N (272.6 Kg/ha) A uniform recommended dose of 120 kg N/ha, 60 kg P_2O_5/ha and 50 kg K_2O/ha was applied in the field. Half of the recommended dose of N was applied basally through broadcasting and mixed with soil before sowing of garlic along with the full dose of P and K. The remaining N was applied at 40 days of plant growth. Nitrogen, P and K were given in the form of urea, diammonium phosphate (DAP) and muriate of potash (MOP), respectively through out the experiment. The crop was sown on 2nd December 2013; with a seed rate of 500 kg/ha with row spacing of 20 cm and plant to plant spacing 10 cm. The size of each plot was 4 m^2 (2 m x 2 m). A total of four irrigations were given during the experiment. The commercial formulation pursuit and stomp mixture were applied at pre-emergence by mixing in recommended volume of water and spraying on the soil in the crop area one day after sowing. The hydrogel composite (GG-SPHC) based formulation

were applied in dry powered form by mixing the required amount in 250 grams dry finely sieved sand and broad casting in the crop area of each plot space. The post emergence application of nanogel based formulation was achieved by mixing the dry formulation in 1000 mL water and spraying on the plot at 35 DAS. Weed free check plots were maintained throughout the experiment by hand weeding. Population and dry weight of weeds were recorded at 45 and 90 DAS of crop by placing a quadrate of 0.5 x 0.5 m^2 randomly from three places in each plot.

Table 2: Treatment description for field study: formulation types, carrier and doses used for weed control

Code	Type	Test carrier/formulation	Dose
GG-SPHC-*imz*-75	Pre emergence	Superporous hydrogel	*Imazethapyr* @ 75 g / ha
GG-SPHC-*imz*-100	Pre emergence	Superporous hydrogel	*Imazethapyr* @100 g / ha
Pursuit-Stomp	Pre emergence	Pursuit® and Stomp®	*Imazethapyr* @ 100 g/ha Pendimethalin @ 1 kg/ha
Pursuit	Pre emergence	Pursuit®	*Imazethapyr* @ 100 g/ha
GG-CSH-*imz*-75	Post emergence	Core shell hydrogel	*Imazethapyr* @ 75 g / ha
GG-CSH-*imz*-100	Post emergence	Core shell hydrogel	*Imazethapyr* @100 g / ha
HW	Hand weeding(Weed free check)	-	-
AC	Absolute Control	-	-

6.2.7 Agronomic observations

6.2.7.1 Weeds

Species-wise weed distribution in the field was assessed at 45 and 90 DAS from all the experimental plots using a quadrate of size 0.5 m x 0.5 m.

6.2.7.2 Weed population and dry weight

Weed population was recorded at 45 DAS and 90 DAS and expressed as number of weed plants per m^2. Weeds collected from 0.25 m^2 area from each plot were first dried under open air conditions and finally kept in a hot air oven at 70°C till constant weight. Dry weight of each was expressed as g/m^2.

6.2.7.3 Weed control efficiency (WCE)

In order to assess the efficiency of developed formulations in comparison to commercial weed control efficiency was calculated as follows: (DAS, 2008)

$$WCE= (WP_C\text{-}WP_T/WP_C) \times 100 \quad (3)$$

Where, WP_C is the weed population (number/m^2) in weedy check plot and WP_T is the weed population (number/m^2) in treated plot.

6.2.7.4 Weed control index (WCI)

Weed control index (WCI) was calculated as follows: (DAS, 2008)

$$WCI= (W_C\text{-}W_T/W_C) \times 100 \quad (4)$$

Where, Wc is the dry weight of weeds in un weeded check and W_T is the dry weight of weeds in treated plots.

6.2.7.5 Phytotoxicity rating in crop and yield

Visual scoring for weed control and phytotoxic rating in garlic crop was done on 45^{th} day after herbicidal application.

6.2.8 Statistical Analysis

Data on different parameters of formulations, crops and weeds were subjected to statistical analysis following the procedures described by Gomez and Gomez (1984). Significance of difference between means across treatments was tested through variance ratio (i.e. *F* test), and least significant difference (LSD) was worked out where variance ratio was found significant for treatment effect at 5% probability level. Data on garlic and weeds during rainy season were analyzed using randomized complete block design (RCBD).

6.3 Results and Discussion

6.3.1 Loading capacity and encapsulation efficiency of developed formulations

Loading capacity and encapsulation efficiencies of all the test formulations determined for all the formulations are presented in the Table 3. The loading capacity of the GG-SPHCF formulations ranged from 3.15 to 4.12%, whereas for GG-CSHF formulations it

was 45.32 to 53.94%. The encapsulation efficiencies of GG-SPHCF formulations were in between 75.99 to 98.96% and of GG-CSHF formulations the values between 67.98 to 71.78.90%. The highest encapsulation efficiency and loading capacity were observed for the In the GG-SPHCF-1 series, the formulation GG-CSHF-3 corresponding to crosslinker content 0.039 % w/w exhibited highest Ee. This can be explained in terms of the tightly crosslinked network of nanogel particles leading to more efficient entrapment of the active ingradient as compared to that in the loosely crosslinked network. Similarly, high clay content and high cross link content in SPHC carriers favoured effective impregnation of *a.i.* in the hydrogel matrix.

Table 3: Loading capacity and encapsulation efficiency of test formulations

Formulations	Loading capacity(Lc%)	Encapsulation efficiency(Ee%)
GG-CSHF-1	45.32	67.98
GG-CSHF-2	47.86	71.78
GG-CSHF-3	53.94	80.90
GG-SPHCF-1	4.12	98.96
GG-SPHCF-2	3.66	87.84
GG-SPHF-3	3.22	77.48
GG-SPHCF-4	3.63	88.45
GG-SPHCF-5	3.15	75.79

6.3.2 Characterization of prepared formulations

6.3.2.1 FT-IR

The characteristic bands in the FT-IR of imazethapyr are: 3248 cm^{-1} (-CONH- stretching), 2972 cm^{-1} (-OH stretching of -COOH groups), 1745 cm^{-1} (C=O stretching of –COO- group) and 1648 cm^{-1} (-NH bending) (A. Fig. 1). The FT-IR of GG-HG (hydrogel carrier) exhibits 2926 cm^{-1} (CH stretching) at 1648 (-C=O stretching of unhydrolyzed $CONH_2$ groups), 1123 cm^{-1} (C-O-C stretching). (B. Fig. 1). The FT-IR of GG-HGF (guargum hydrogel imazethapyr formulation) exhibits similar bands as of hydrogel carrier due to masking effect of carrier as concentration of imazethapyr is only 1%. (C. Fig. 1). In case of GG-NG (nanogel) bands at 3391 cm^{-1} can be attributed to free OH stretching of guar gum, 1680 cm^{-1} (-C=O stretching) and 1636 cm^{-1} due to –NH bending PNIPAm, 1105 cm^{-1} (C-O-C stretching of guar gum). Presence of bands along with characteristics bands of imazethpyr (2972 cm^{-1} and 1745 cm^{-1}) in the FT-IR of GG-NGF

formulation confirms encapsulation of the imazethapyr in the matrix/core of nanogel particles.

6.3.2.2 Transmission Electron Microscopy (TEM)

TEM images (Figure 2) clearly depict the actual size and shape of core shell hydrogel formulations and the porous structure of superporous hydrogel composite formulations. TEM confirmed the spherical shape of core shell hydrogel formulations and micro to nano size of nanogel particles. Similarly in case of superporous hydrogel composite formulations, nano sized pores can be seen.

6.3.3 Release of *imazethapyr* from developed formulation in water

6.3.3.1 GG-CSHF formulation

Periodic release of *imazethapyr* in water from three GG-CSHF test formulations is presented in Figure 3. The rate of release of *a.i.* from all the three formulations was significantly slower than that from commercial formulation (Pursuit®). As compared to 99.8% release of *a.i.* from Pursuit on the 1st day of release study, GG-CSHF formulations released the *a.i.*in the order GG-CSHF-1 (56.7%) >GG-CSHF-2 (35.4%) > GG-CSHF-3(14.08%).

The same pattern continued till 30th day of study (Figure 3). PNIPAm based nanogels are well reported as thermo sensitive nanogels with low critical solution (31-32°C). At temperatures below LCST, they are in expanded from and keep releasing the *a.i.* so that diffusion is the predominant release mechanism. At temperature > LCST, they turn hydrophobic and burst open so that burst kinetics operates. In view of the field application requirement of agrochemical formulations, the study was done at incubation temperature of 35°C. Therefore, in order to sustain the *a.i.* release from nanogel, GG-shell stabilized PNIPAm nanogels were taken as carriers in the present study. As is clear from the release data (Table 3 and figure 2), the release remained diffusion controlled inspite of incubation temperature > LCST.

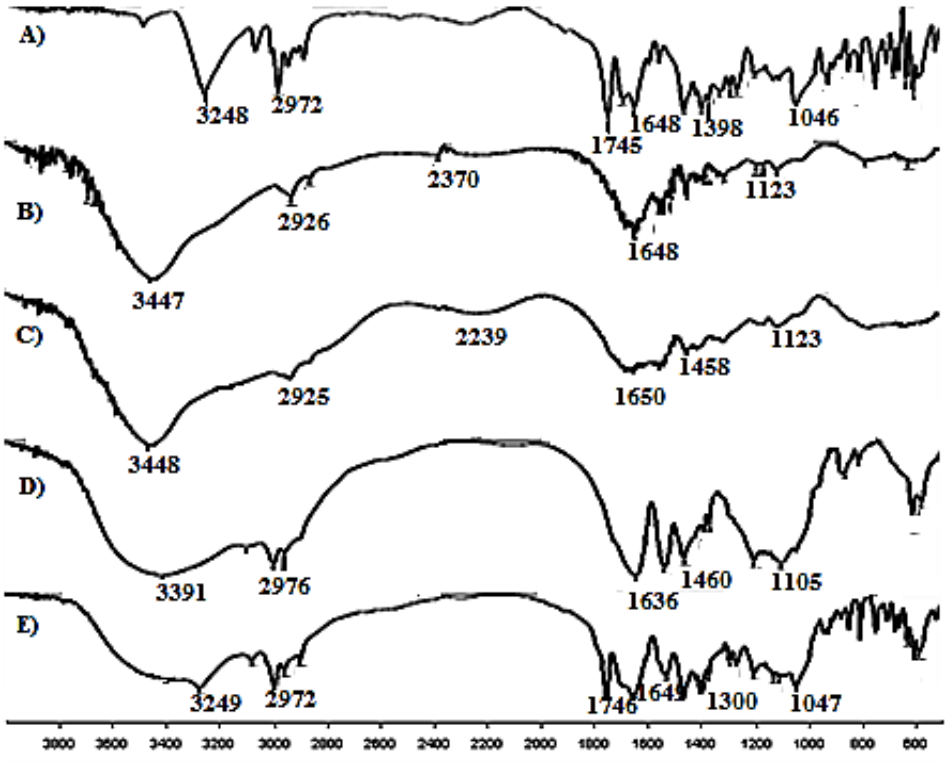


Figure 1: FT-IR spectra of *Imazethapyr* (A), GG-SPHC carrier (B), GG-SPHCF formulation (C), GG-CSHG carrier (D) and GG-CSHGF formulation (E).

6.3.3.2 GG-SPHCF formulations

Periodic release of *imazethapyr* in water from the superporous hydrogel composite formulations is depicted in Figure 4. Like GG-CSHF formulation, release of *a.i.* from the GG-SPHCF was much slower than the commercial formulation (Pursuit®). On the first day of release study, maximum release was observed from the commercial formulation (99.8%), which was followed by GG-SPHCF-1 (34.03%) > GG-SPHCF-2 (30.63%) > GG-SPHCF-3 > (16.5%) ≈ GG-SPHCF-4 (16.6%) ≈ GG-SPHCF- 5 (16.5%). From 5th day onwards, no further increase in *a.i.* content was recorded in solution rather slight decline was observed during further investigation. Due to presence of inter connected pores (Figure 2), the super porous hydrogels/composites swell very fast due to which release of *a.i.* is also expected to be very fast.

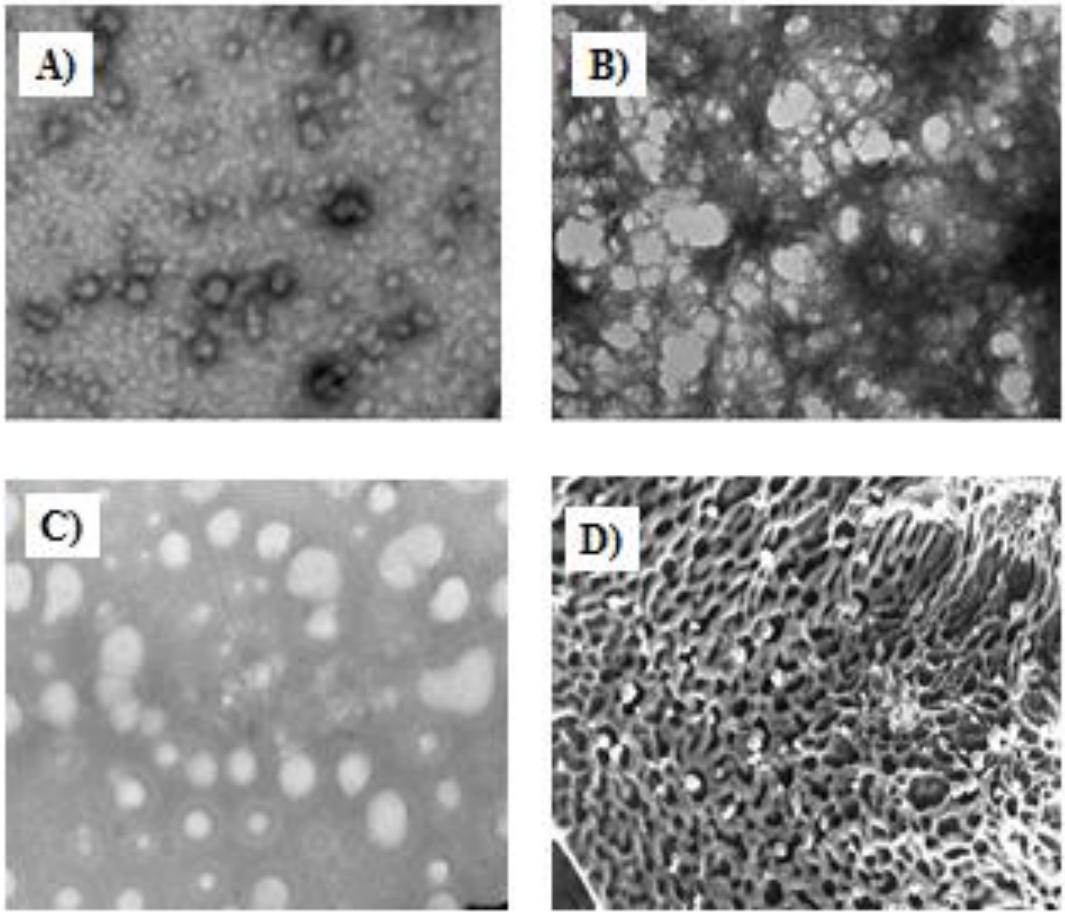


Figure 2: TEM images of GG-CSHF formulation (A), GG-SPHCF formulation (B), and TEM image of GG-CSH carrier(C), SEM image of GG- SPHC carrier (D)

The cross link density and the clay content play determinant role in the release kinetics from these carriers. Slower release at higher cross linker content in the present study supports this observation. Kumar *et al.,* 2010 also reported the similar findings on drug release as a function of cross linker content. Role of clay can be understood in terms of increased gel strength at higher clay content that leads to decrease in swelling and thus release of *a.i.* from the matrix. (Aguzzi *et al.,* 2007*;* Garrido *et al.,* 2010). Maximum amount of *imazethapyr* in water (38.74 to 50.53 %) was observed between 3rd to 5th day. Release trend was similar for all the SPHC formulations. There was a steep increase in release in release rates till 3rd to 5th days depending on the test carrier used.

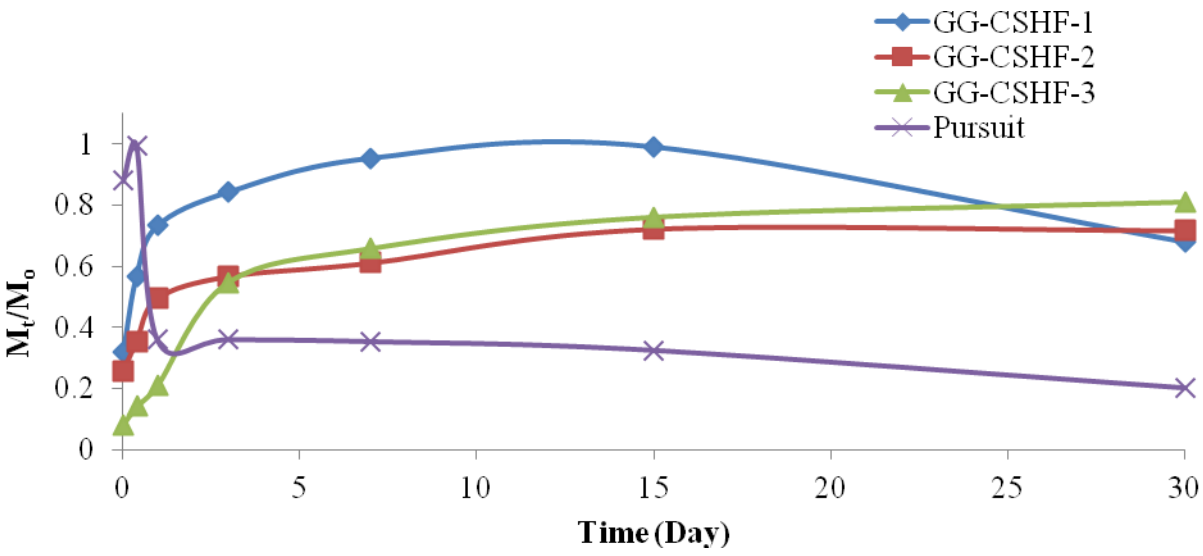


Figure 3: Release of *imazethapyr* in water from GG-CSHF formulations

6.3.3.3 Kinetics analysis of release behavior of *imazethapyr* from developed formulations

To describe the mechanism of *imazethapyr* release from developed hydrogel formulations, release data was analyzed according to Korsmeyer-peppas model (Ritger and Peppas, 1987). As is clear from Table 4, release of imazethapyr from GG-CSHF and GG-SPHCF formulations followed korsmeyer-peppas model (Ritger and Peppas, 1987).

For the case of spherical swelling hydrogel/nanogels 0.43 = *n* corresponds to a Fickian diffusion mechanism, 0.43<*n*<0.85 to non-Fickian transport and *n* = 0.85 to Case II (relaxational) transport, and *n* > 0.85 to super case II transport (Ritger and Peppas, 1987). The *n* values ranged from the 0.10 to 0.31 for test GG-CSHF formulations implying Fickian diffuion release of *a.i.* from the carrier matrix. K values obtained from the Ritger–Peppas model ranged from 0.24 to 0.66 for developed formulations concluding formation of controlled release *imazethapyr* formulations.

The calculated $t_{0.25}$, $t_{0.50}$ and $t_{0.90}$ of release of *a.i.* from developed controlled release formulations and commercial formulation is shown in the Table 3. The value of $t_{0.5}$ of release of *imazethapyr* in water from various test formulation was ranged from 0.068 to 12.61 days. In order of increasing $t_{0.5}$ value of release of *imazethapyr* from various formulations revealed in the following order: GG-CSHF-1< GG-CSHF-2 < GG-

SPHCF-5< GG-SPHCF-1 < GG-CSHF-3 < GG-SPHCF-4 <GG-SPHCF-2 <GG- SPHCF-3.

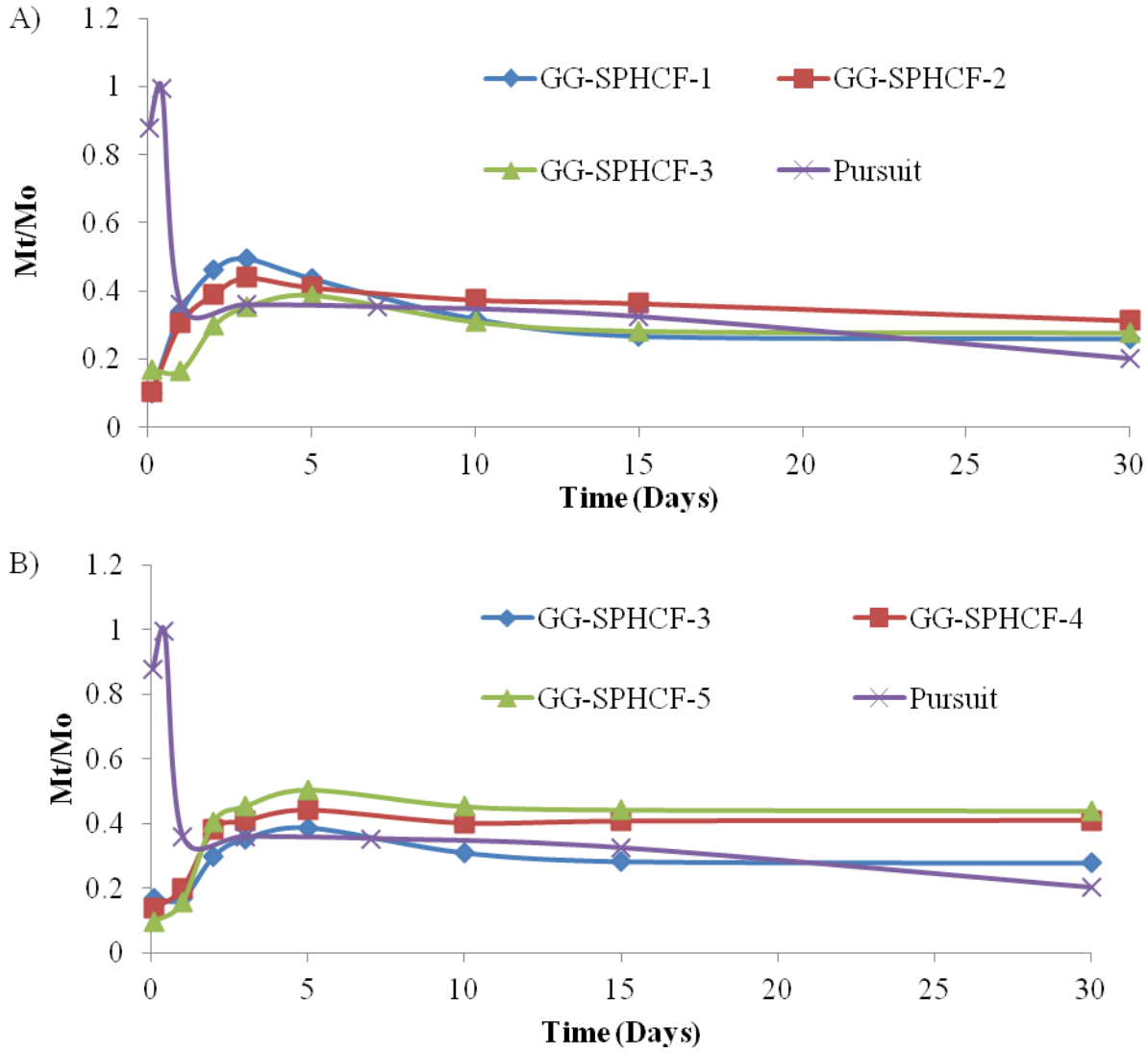


Figure 4: Release of *imazethapyr* in water from GG-SPHCF formulations: Effect of (A) crosslinker content and (B) clay content

6.4 Field evaluation

6.4.1 Effect on species-wise weed population and dry weight

It was observed till 25th DAS that all the herbicide treated plots were weed free. The relative composition of weed flora in different plots at 45 DAS and 90 DAS was recorded. The predominant weeds observed in the experimental plot were: *Coronopus*

didymus, Melilotous indica, Chenopodium album (broad laef) and *Avena ludoviciana, Phalaris minor* (Monocot leaf).

Table 4: *In-vitro* release kinetics parameters of *imazethapyr* in water

Formulation	**K**	**n**	**R^2**	**Mechanism**	**$t_{0.25}$**	**$t_{0.5}$**	**$t_{0.9}$**
GG-CSHF-1	0.66	0.10	0.75	Fickian diffusion	0.0001	0.0687	19.4483
GG-CSHF-2	0.45	0.15	0.98	Fickian diffusion	0.0204	1.8857	87.4449
GG-CSHF-3	0.30	0.31	0.96	Fickian diffusion	0.5370	4.7754	30.4636
GG-SPHCF-1	0.32	0.29	0.90	Fickian diffusion	0.4037	4.4468	34.0132
GG-SPHCF-2	0.29	0.29	0.94	Fickian diffusion	0.5952	6.3910	47.8399
GG-SPHCF-3	0.24	0.29	0.90	Fickian diffusion	1.1687	12.610	94.7883
GG-SPHCF-4	0.26	0.34	0.95	Fickian diffusion	0.8486	6.2987	34.4713
GG-SPHCF-5	0.24	0.49	0.95	Fickian diffusion	1.0710	4.3030	13.9945
Pursuit®	0.49	2.30×10^{-9}	0	--	--	--	--

All weed control treatments adopted in the study resulted in the significant reduction in the population of weeds (Tables 5 and 6) and their dry matter content (Table 7 and 8) as compared to absolute control. The maximum suppression of weeds in terms of total weed population at 45 DAS was observed (37.3/m^2) in treatment Pursuit-Stomp which also showed lowest weed population at 90 DAS (24.0/m^2). Commercial *imazethapyr* formulation (Pursuit®) treated plot showed weed population of 41.3/m^2 at 45 DAS but after 90 DAS the weed population increased to 54.2/m^2. The treatment GG-SPHC-*imz*-75 containing 75 g a.i /ha exhibited average population of 49.3 weeds/m^2 at 45 DAS and 32.4/m^2 at 90 DAS. The corresponding pre emergence treatment, GG-SPHC-*imz*-100 containing 100 g a.i/ha exhibited 42.7weeds/m^2 and 37.8/m^2 at 45 and 90 DAS respectively. Improved weed control over commercial formulation was clearly due to controlled release characteristics of GG-SPHC-imz formulation and GG-CSH-*imz* formulation, GG-CSH-*imz*-75 and GG-CSH-*imz*-100 led to significantly weed controlled although this was lesser as compared to that exerted by Pursuit®, Pursuit- stomp® and GG-SPHC-*imz* treatments. GG-CSH-*imz*-75 treated plot registered 63.3 and 35.6 weeds/m^2 at 45 and 90 DAS where as GG-CSH-*imz*-100 registered 67.7 and 49.3 weeds/m^2 respectively at 45 and 60 DAS. GG-SPHC-*imz*-75 and GG-SPHC-*imz*-100 exerted significantly higher effect on growth of weeds as compared to GG-CSH-*imz* formulations and performed almost at par with Pursuit formulation w.r.t. average dry weed weight /m^2 (Table 7 and 8). Pursuit-Stomp combined treatment performed best

amongst all the treatments applied in the present study. The overall comparative performance of a herbicidal formulation is expressed more efficiently in terms of weed control efficiency (WCE) and weed control index (WCI).

Table 5: Effect of test formulations on weed population (no/m^2) at 45 DAS

Treatment	***Coronopus didymus***	***Melilotus indica***	***Avena ludoviciana***	***Phalaris minor***	***Chenopodium album***	**Total weeds**	**WCE (%)**
GG-SPHC-*imz*-75	16.0	12.0	9.3	6.7	5.3	49.3	61.8
GG-SPHC-*imz*-100	17.3	10.7	5.3	4.0	5.3	42.7	67.0
Pursuit-Stomp	16.0	10.7	4.0	4.0	2.7	37.3	71.1
Pursuit	16.0	12.0	4.0	5.3	4.0	41.3	68.0
GG-CSH-*imz*-75	24.0	16.0	6.7	9.3	7.3	63.3	51.0
GG-CSH-*imz*-100	20.0	14.3	12.0	16.0	5.3	67.7	47.7
HW	0.0	0.0	0.0	0.0	0.0	0.0	100.0
AC	44.0	36.0	20.0	17.3	12.0	129.3	0.0
SEM (Mean Standard Error)	1.5	3.8	1.9	1.2	1.6	10.1	
CD (Critical Difference) = 0.05	4.5	11.1	5.5	3.6	4.7	29.4	

Table 6 : Effect of test formulations on weed population (no/m^2) at 90 DAS

Treatment	***Coronopus didymus***	***Melilotus indica***	***Avena ludoviciana***	***Phalaris minor***	***Chenopodium album***	**Total weeds**	**WCE (%)**
GG-SPHC-*imz*-75	10.0	8.0	2.3	6.8	5.3	32.4	92.2
GG-SPHC-*imz*-100	8.3	13.3	2.7	8.1	5.3	37.8	91.0
Pursuit-Stomp	5.3	6.0	2.7	4.7	5.3	24.0	94.3
Pursuit	6.7	24.0	8.0	12.9	2.7	54.2	87.0
GG-CSH-*imz*-75	4.0	16.0	4.7	8.2	2.7	35.6	91.5
GG-CSH-*imz*-100	13.3	13.3	9.3	12.0	1.3	49.3	88.2
HW	0.0	0.0	0.0	0.0	0.0	0.0	100.0
AC	206.7	44.0	30.7	93.8	42.7	417.8	0.0
SEM (Mean Standard Error)	3.8	5.0	1.9	3.6	2.5	16.8	
CD (Critical Difference) = 0.05	11.2	14.7	5.4	10.5	7.3	49.2	

Table 7: Effect of test formulations on weed dry weight (g/m^2) at 45 DAS

Treatment	*Coronopus didymus*	*Melilotus indica*	*Avena ludoviciana*	*Phalaris minor*	*Chenopodium album*	Total weeds	WCI (%)
GG-SPHC-*imz*-75	0.73	0.44	0.25	0.32	0.27	2.01	83.55
GG-SPHC-*imz*-100	0.73	0.46	0.19	0.30	0.26	1.93	84.18
Pursuit-Stomp	0.49	0.21	0.16	0.22	0.13	1.21	90.13
Pursuit	0.74	0.38	0.35	0.27	0.20	1.94	84.10
GG-CSH-*imz*-75	1.70	1.00	0.80	0.59	0.43	4.52	63.01
GG-CSH-*imz*-100	1.02	0.70	0.47	0.34	0.23	2.76	77.41
HW	0.00	0.00	0.00	0.00	0.00	0.00	100.00
AC	5.53	2.27	1.74	1.63	1.05	12.22	0.02
SEM (Mean Standard Error)	0.074	0.209	0.101	0.033	0.040	0.46	
CD (Critical Difference) = 0.05	0.218	0.613	0.294	0.096	0.117	1.34	

Weed control efficiency (WCE) and weed control index (WCI) of different weed control treatments is shown in Table 5, 6, 7 and 8. According to the equation 3 and 4, lower weed density results in higher weed control efficiency and lower weed biomass will lead to higher weed control index, respectively. As dry weight of weed population gives better measurement of weed control efficiency of herbicidal formulation, WCI is preferred over WCE. It is clear from Table 5, 6, 7 and 8 that WCI of GG-SPHC-*imz* formulations was at par with Pursuit throughout observation period where as GG-CSH-*imz* formulations initially showed lower WCI at 45 DAS, but at 90 DAS, the WCI of all the test formulations came par with both commercial formulation treatments (Pursuit-Stomp and Pursuit). The observed bioefficacy results undoubtedly can be attributed to slow release characteristics of hydrogel based formulations.

Table 8: Effect of test formulations on weed dry weight (g/m^2) at 90 DAS

Treatment	*Coronopus didymus*	*Melilotus indica*	*Avena ludoviciana*	*Phalaris minor*	*Chenopodium album*	Total weeds	WCI (%)
GG-SPHC-*imz*-75	3.18	0.67	0.51	0.28	0.79	5.43	98.56
GG-SPHC-*imz*-100	1.36	0.42	0.40	0.26	0.88	3.32	99.12
Pursuit-Stomp	0.76	0.51	0.29	0.20	0.39	2.16	99.43
Pursuit	1.22	1.41	0.17	0.87	0.20	3.86	98.98
GG-CSH-*imz*-75	0.57	5.93	1.88	3.02	0.93	12.33	96.73
GG-CSH-*imz*-100	0.36	3.71	3.90	1.20	0.50	9.67	97.43
HW	0.00	0.00	0.00	0.00	0.00	0.00	100.00
AC	224.78	9.13	34.31	77.57	31.21	377.00	0.00
SEM (Mean Standard Error)	0.916	0.284	0.317	1.097	0.451	0.613	
CD (Critical Difference) = 0.05	2.679	0.830	0.928	3.209	1.321	1.793	

Collet and Moreton in 2004 ascribed the efficacy of SCL to the rapid initial release of active ingredient, followed by decrease to maintain effective level for a prolonged for a prolonged period of time as compared to conventional liquid formulations. The same behaviour has been displayed by the formulations developed in the present work. Kok *et al*., (1999) reported controlled release behaviour of aldicarb from CMC-lignin based hydrogel microspheres where release into water was almost 100 % in 24 hr and release was fickian diffusion driven. However, when applied and evaluated in soil, the delayed release effect was observed in case of prepared gel formulations as compared to commercial formulation. Our findings are also in conformity with these reports.

6.4.2 Phytotoxicity in garlic

Ironically, potential phytotoxicity in garlic crop was observed in all the *imazethapyr* treated plots irrespective of formulation type and/or dose of *imazethapyr*. Maximum damage was caused by commercial formulation treatments followed by GG-SPHC-imz formulations. GG-CSH–*imz* formulations also induced damage but to lesser extent.

6.5 Conclusion

In the present study biopolymeric hydrogel and nanogel based formulations of *imazethapyr* were developed. The controlled release study suggests the formation of controlled release systems. The results suggest that depending on the test carrier, the herbicide release can be manipulated. The kinetics of *imazethapyr* release from two tyres of test carriers although was not similar in water but displayed significantly high bioefficacy almost at par with commercial formulations The present study brings out certain interesting facts that are undoubtedly researchable issue for future.

i) *Imazethapyr* is not suitable to garlic crop in general and test variety (G-41) in particular

ii) Post emergence application of *imazethapyr* in slow release formulated from (GG-CSH-*imz* in present study) is less damaging than the pre emergence application.

iii) New formulations developed in the present study possess potential herbicidal activity.

GENERAL DISCUSSION

Weed management is an important component of crop protection in modern agriculture. Productivity loss due to weed interference is a matter of serious concern among researchers and crop growers. Weeds are generally unwanted hardy plant species that compete very efficiently with crop plants for same resources namely, nutrients, water, space and light for growth and development and thus adversely affect the crop growth and yield. In the present scenario of labour shortage and increasing wages, farmers perceive chemical approach i.e. herbicide application as only viable alternative.

Over the past few years, imidazolinone herbicides have come out as efficient molecules for weed management. *Imazethapyr* is a popular imidazolinone herbicide because of its broad spectrum features and is applied as both pre and post-emergence in various crops like, soybean, groundnut and other legumes. It kills weeds by inhibiting the enzyme acetohydroxy acid synthase (AHAS). Weeds normally controlled by pre-emergence application of *imazethapyr* are *Acanthospermum australe, Acanthospermum hispidum, Amaranthus deflexus, Amaranthus hybridus, Amaranthus thunbergii, Chenopodium album, Galinsoga parviflora, Commelina benghalensis, Cyperus rotundus, Portulaca oleraceae, Xanthium strumariumr, Tribulus terrestris* (Jeffrey *et al.*, 2003).

In India, its liquid formulation in the form of soluble liquid (SL) is largely used whose preparation involves the use of surfactants, emulsifiers and inorganic salts. This, coupled with fast dissipation and general limitations associated with liquid formulations (Sopeña *et al*., 2009) in water necessitates the development of controlled release formulations of *imazethapyr* that are simple to prepare, possess slow release properties and involve minimal or no use of toxic adjuvants. Controlled release formulations of herbicides are thus being viewed as potential alternative approach (Tefft and Friend 1993; Sopena *et al*., 2009, Fernández-Pérez *et al*., 2011). A new generation carrier approach in this context is use of a special class of polymers called hydrogels. Hydrogels are of two types based on porosity and swelling rates, nonporous superabsorbent polymers (SAP) and superporous hydrogels (SPH). When

synthetic procedures are manipulated w.r.t. particle size upto nano level, the resulting hydrogels are termed nanogels.

Guar gum based novel super porous hydrogels composites (GG-SPHC) and *core-shell* nanogels (GG-CSH) were developed in the present work and used as carrier to develop *imazethapyr* herbicide based formulations.

The guar gum based superporous hydrogel composites (GG-SPHC) were prepared by standardising various reaction parameters like monomer: backbone concentration and cross linker concentration. The composites exhibited the optimised hydrogel based on water absorbency was studied under different temperatures, time periods, pH and water quality and also in salt and fertilizer solutions. Increase in cross linker concentration reduced water absorbency due to generation of tighter network as results of increase in cross linker concentration (Gupta and Shivakumar, 2009). Increase in porogen concentration in the range of 0.056-0.56 wt %, resulted in the SPHCs with increasing swelling ratios. At higher concentrations, consistently low swelling behaviour was exhibited. Under acidic pH conditions employed (pH 4 to 5). Porogen reacts with acid to generate CO_2 gas bubbles. Beyond an optimum concentration, the pH of the reaction medium tends to rise which adversely affects the gelation process. This effect is reflected in inferior water absorbency values of the resulting SPHC (Chen *et al.,* 2000). Clay content in the feed influenced the swelling capacity but swelling rate exhibited not much variation.

Rise in temperature of swelling medium exhibited increase in water absorption capacity in both the composite SPHC and the clay free SPH. The effect was more pronounced in case of SPHC. Rise of temperature from 5° to 35°C led to ~40% swelling enhancement in GG-SPHC as compared to 8% in GG-SPH from 35 to 50 °C, Maximum swelling was observed at 50°C. This behaviour could be due to the extensive expansion of the macromolecular chains at higher temperature. Except in urea solution, a drastic reduction in swelling capacities of GG-SPHC was observed in all the salt solutions.

AUL was used as a marker of mechanical strength of porous materials. In general, the rate of swelling under pressure is related to the particle size and particle size distribution, specific surface area and density of the superabsorbent polymer.

The superporous hydrogel composite (GG-SPHC) showed higher AUL than superporous hydrogel (GG-SPH) at all the crosslinker concentrations. AUL increased with increase in crosslinker concentration in both the series of GG-SPHC and GG-SPH. The presence of inorganic mineral provides additional network points in polymerization reaction that result in higher crosslinking density and simultaneously higher mechanical strength.

Three series of *core shell* nanogels namely PNIPAm-*core*-guar gum-*shell,* PVA-*core* guar gum-*shell*, GG-PVA-*core*-PNIPAm-*shell* nanogels were synthesized employing guar gum and two techniques of polymerization i.e. free radical precipitation polymerization and inverse emulsion In the synthesis of namely PNIPAm-*core*-guargum-*shell*, PVA-*core*-guar gum-*shell*, the surfactant free process conditions were standardized. Guar gum is a naturally occurring hydrophilic polymer containing polar -OH groups on its polysaccharide chains. In view of its stabilizer properties in the colloidal system, our novel approach of its use in preparation of *core shell* nanogels led to formation of nanosized hydrogels with definite spherical structures. The inverse mini emulsion technique is often used to confine the particle size of growing networks through restriction induced by oil soluble surfactant. The technique is well adopted to prepare controlled release products (Dorwal, 2012). PNIPAm-*shell*-GG-PVA *core* nanogels were synthesized by combining solution and inverse emulsion polymerization techniques. The process generated uniform sized spherical nanogel particles with *core shell* morphologies.

Crosslinker concentration and monomer concentration in the reaction feed significantly influenced the particle size of nanogel particles in all the three series. In case of GG-*shell*-PNIPAm-*core* nanogels, the particle's hydrodyanamic diameter increased with increase in crosslinker content. In most of the published reports on PNIPAm based nanogels, crosslinker content had inverse relation with particle size which can be understood in terms of restriction on the growing polymer networks due to high crosslink density. The anomaly was reported in chitosan-alginate crosslinked nanoparticles. The observed trend can be explained in terms of increase in grafting of PNIPAm network on guargum as the crosslinker concentration increased resulting in generation of larger sized nanoparticles. Similar trend was observed by Soni *et al*., 2011 during synthesis of sodium alginate based nanogels. GG-*shell*-PVA-*core* series

and PNIPAm-*shell*-GG-PVA-*core* nanogels followed the expected behavior. Increase in crosslinker concentration registered decrease in particle size in both the series. Monomer content increase led to generation of polymer particles of larger sizes in all the three series. The basis of synthesis of GG-*shell*-PNIPAm-*core* series was to exploit hydrophilic characteristics and stabilizing properties of guar gum. Therefore guar gum content was varied in the feed to assess the effect on particle size. The analysis showed that the particle size of nanogels decreased with increase in guar gum content. This is a well-known fact that increase in surfactant concentration leads to decrease in particle size. Guar gum, because of its stabilizing properties seems to have played same role.

The GG-SPHC composite and GG-CSH nanogel with optimized swelling properties and particle size respectively were employed as carriers to prepare *imazethapyr* based formulations. Two rates of herbicide were impregnated i.e. 75 g/ ha and 100 g/ ha. The loading capacity and encapsulation efficiency of GG-SPHCF formulations varied from 3.15 to 4.12% and 38.74 to 50.53% respectively while in case of GG-CSHF formulations, the corresponding values were 45.32 to 53.94%.and 67.98 to 80.90% respectively. The highest encapsulation efficiency and loading capacity were observed for the formulation of GG-CSHF formulation with maximum test crosslinker content which might be due to the higher crosslinker density induced during synthesis. In case of GG-SPHCF formulations, high clay content and high cross link content in GG-SPHC carriers favoured extensive impregnation of *a.i.* in the hydrogel matrix. The rate of release of *a.i.* from all the formulations was significantly much slower than that from commercial formulation (Pursuit®). PNIPAm based nanogels are well reported as thermo sensitive nanogels with low critical solution (31-32°C). At LCST, they are in expanded from and keep swelling and releasing the *a.i.* so that diffusion is the predominant release mechanism. At temperature > LCST, they turn hydrophobic and burst open so that burst kinetics operates. In view of the field application requirement of agrochemical formulations the study was done at incubation temperature of 35 °c. Therefore, in order to sustain the *a.i.* release from nanogel, GG-*shell* stabilized PNIPAm nanogels were taken as carriers in the present study. In the prepared formulations, the release remained diffusion controlled in spite of temperature > LCST.

Unlike GG-CSHF formulations, release of *a.i.* from the GG-SPHCF formulations was also much slower than the commercial formulation (Pursuit®). Due to presence of inter connected pores (Figure 2), the super porous hydrogels /composites swell very fast due to which release of *a.i.* is also expected to very fast. The cross link density and the clay content may play determinant role in the release kinetics from these carriers. Slower release at higher cross linker content in the present study supports this observation. Kumar *et al.,* 2010 also reported the similar findings on drug release as a function of cross linker content. Role of clay can be understood in terms of increased gel strengths at higher the clay content that leads to decrease in swelling and thus release of *a.i.* from the matrix. (Aguzzi *et al.,* 2007*;* Garrido *et al.,* 2010). Maximum amount of *imazethapyr* in water was observed between 3rd to 5th day. Release trend was similar for all the SPHC formulations.

To describe the mechanism of *imazethapyr* release from developed hydrogel formulations, release data was analyzed according to Korsmeyer-Peppas model (Ritger and Peppas, 1987). Release of a.i from GG-CSHF nanogel and GG-SPHCF macrogel formulations followed Korsmeyer-Peppas model (Ritger and Peppas, 1987). The *n* values ranged from the 0.10 to 0.31 for test CSH formulations, and 0.29 to 0.31 in case of SPHC formulations implying Fickian mechanism of release of *a.i.* for the carrier matrix. K values obtained from the Korsmeyer-Peppas model ranged from 0.24 to 0.66 for developed formulations confirming formation of controlled release formulations of herbicide.

All weed control treatments evaluated in the field bioefficacy study in the study exhibited the significant reduction in the population of weeds and their dry matter contents as compared to absolute control. The maximum suppression of weeds in terms of total weed population at 45 DAS was observed in treatment Pursuit-Stomp (*imazethapyr* + pendimethalin) which also showed lowest weed population at 90 DAS. The treatment GG-SPHC-*imz*-75 containing 75 g *a.i.*/ha and GG-SPHC-*imz*-75 containing 100 g *a.i.*/ha exhibited better weed than commercial formulation, understandably due to controlled release characteristic of GG-SPHC formulation. GG-CSH-*imz* formulation, GG-CSH-*imz*-75 and GG-CSH-*imz*-100 led to significant weed control although this was lesser as compared to that exerted by Pursuit®, Pursuit®- stomp® and GG-SPHC-*imz* treatments. GG-SPHC-*imz*-100 formulations

exerted significantly higher effect on growth of weeds as compared to GG-CSH-*imz* formulation and performed almost at par with Pursuit formulation w.r.t. average dry weed weight /m^2. Pursuit-Stomp combined treatment performed best amongst all the treatments applied in the present study. Weed control index (WCI) of GG-SPHC-*imz* formulations was at par with Pursuit throughout observation period where as GG-CSH-*imz* formulations initially showed lower WCI at 45 DAS, but at 90 DAS, the WCI of all the test formulation came at par with both commercial formulations (Pursuit-Stomp and Pursuit). The observed bioefficacy results undoubtedly can be attributed to slow release characteristics of hydrogel based formulations. In terms of yield parameter in test crop, potential phytotoxic in garlic crop was observed in all the *imazethapyr* treated plots irrespective of formulation type and or dose of *imazethapyr*. Maximum damage was caused by commercial formulation treatments followed by GG-SPHC formulations, GG-CSH–*imz* formulation also induced damage but to lesser degree. These findings imply that The findings suggest that i) *Imazethapyr* is not suitable to garlic crop in general or this variety (G-41) in particular ii) Post emergence application of *imazethapyr* in slow release formulated from (GG-CSH-*imz* in present study) is less damaging than the pre emergence application.

SUMMARY AND CONCLUSION

1. Agriculture continues to play a major role in Indian economy; contributing approximately 13.7% of the total national gross domestic production (GDP).
2. Use of agro-chemical inputs has become highly relevant for crop production and protection particularly in view of the fast changing climate, shrinking natural resources and pest/weed resistance.
3. Weeds management is an important component of crop protection and despite off good efforts made in research and extension in the field of agriculture science, the farmers continue to experience heavy losses in crop yield due to weed interference.
4. Imidazolinone herbicides have come out as efficient weed control tool for herbicide resistance management. *Imazethapyr* is an imidazolinone herbicide applied as both pre and post-emergence in various crops like, soybean, groundnut and other legumes.
5. In India, its liquid formulation in the form of soluble liquid (SL) is largely used Development of its suitable formulations that are simple to prepare, endowed with slow release properties and involve minimal or no use of adjuvants is imperative. Controlled release formulation approach holds lot of potential as an alternative.
6. Hydrogels, the cross linked speciality polymers with versatile network and water absorption characteristics, both micro sized and nano sized have been explored worldwide to serve as controlled carriers of agrochemicals.
7. The objective of present work was to develop hydrogel based formulations of *imazethapyr* for pre and post emergence application, to evaluate their release behaviour in water and bioefficacy against prevalent weeds in a rabi/ kharif crop.
8. Novel biopolymeric superporous hydrogel composites (GG-SPHC) and *core-shell* nanogels (PNIPAm-*core*-GG-*shell*, PVA-*core*-GG-*shell*, GG-PVA-*core*-PNIPAm-*shell*) based on guar gum were prepared to explore their potential as carriers of *imazethapyr*.

9. Swelling response of GG-SPHC was studied in different simulated environments like temperature, time period, pH, salt type and fertilizers at different strengths.
10. The GG-SPHC hydrogels based on water absorbency were characterised by FT-IR, XRD, scanning electron microscope and transmission electron microscope.
11. Water absorbency under load (AUL) of GG-SPHC hydrogel composites was studied and related to gel strength.
12. The GG-SPHC with optimized properties was used as carrier for *imazethapyr*.
13. *Core shell* nanogels (GG-CSH) were characterized by FT-IR, Transmission Electron Microscopy and Zetasizer.
14. TEM analysis showed formation of spherical shaped *core shell* nanogel particles. , PVA-*core*-guar gum-*shell* appeared to have inter particle crosslinking suggesting aggregates of nanogel particles. Particle size analysis exhibited the effect of reaction factors monomer content, crosslinker content and the guar gum content on particle size of gel particle size.
15. Two types of formulations, GG-SPHC based solid formulations and GG-CSH nanogel based nanosuspension concentrate were prepared employing *imazethapyr*. A total of eight compositions were prepared for controlled release study and four compositions containing *imazethapyr* @ 75g *a.i.*/ha and 100 g a.i/ha for field bioefficacy study. The details of the preparation processes of formulations will be protected under IPR.
16. The prepared compositions were evaluated for controlled release behaviour in water under laboratory conditions and for herbicidal activity evaluation under field conditions.
17. The release of *a.i.* was faster from commercial formulation than from prepared formulations. The time taken for release of 50% loaded *a.i.* ranged between 0.06 to 4.8 days in case of GG-CSHF formulations and 4.4 to 12.6 days for GG-SPHCF formulations.
18. The diffusion exponent (n) value of *imazethapyr* in water showed that the predominant mechanism of release was fickian diffusion in all the formulations.

19. Test formulations GG-SPHC-*imz* and GG-CSH-*imz* showed very high weed control index (WCI) almost at par with the WCI of the commercial formulations.
20. All herbicide treatments including commercial formulation showed phytotoxicity to garlic crop (var. G-41), implying that use of *imazethapyr* in this variety of garlic cannot be recommended.
21. It is concluded that hydrogel and nanogel based controlled release formulations of imazethapyr in particular and herbicides in general can be successfully developed for management of weeds at pre- as well as post-emergence stages. The findings particularly w.r.t bioefficacy in different crops other than garlic and *a.i.* release behaviour in soil need to be comprehensively investigated.

Hydrogel Based Formulations of *Imazethapyr*: Development and Appraisal of Release Behaviour

ABSTRACT

Imazethapyr, a systemic broad spectrum herbicide is recommended for soybean, groundnuts and other leguminous crops. Primarily applied as post-emergence, its use is also recommended for pre emergence weed control. In India, its liquid formulation in the form of soluble liquid (SL) is largely used whose preparation involves the use of surfactants, emulsifiers and inorganic salts. This, coupled with fast dissipation in water necessitates the development of controlled release formulations of *imazethapyr* that are simple to prepare, possess slow release properties and involve minimal or no use of toxic adjuvants. The objective of present thesis was to develop hydrogel based controlled release formulations of *imazethapyr* for pre and post emergence application, to evaluate their release behaviour in water and bioefficacy against prevalent weeds in a *kharif*/*rabi* crop.

Guar gum (GG), a galactomannan polysaccharide was employed to prepare novel GG-*g*-polyacrylate/bentonite superporous hydrogel composites (GG-SPHC) and *core shell* nanogels (GG-CSH) as carriers. Reaction factors, the backbone-monomer ratio, cross linker, bentonite and porogen contents were optimized w.r.t swelling characteristics. The prepared hydrogels were characterized by FT-IR, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Swelling behaviour of the representative GG-SPHC was evaluated w.r.t. salt and fertilizer solutions, temperature, water quality and pH. GG-SPHC exhibited very fast swelling (30 minutes), almost similar to the corresponding clay free hydrogel. Higher absorbency under load as compared to the clay free gel showed improved mechanical strength of the composites. The *core-shell* nanogels (GG-*shell*-PNIPAm-*core*; GG-*shell*-PVA-*core*; PNIPAm-*shell*-GG-PVA-*core*) exhibited mean particle size in the range of 11 nm to 490 nm. TEM analysis exhibited *core shell* spherical structures of prepared nanogels.

GG-SPHC and GG-CSH carriers with optimized compositions were used to prepare *imazethapyr* formulations of *imazethapyr* under ambient conditions. The release of *a.i.* from the all the test compositions in water followed Fickian mechanism.

Release was faster in GG-SPHC formulations ($t_{1/2}$ =4.4 to 12.6 days). Field appraisal on bioefficacy of prepared formulations *vis-a-vis* commercial formulations showed very high weed control index of test formulation (96.7- 99.1% on 90 DAS) comparable to that of commercial formulations (98-99.4%). GG-SPHC formulations of *imazethapyr* applied at pre-emergence stage, exhibited significantly higher weed control as compared to other formulation treatments. All the *imazethapyr* formulations exhibited acute phytotoxicity in garlic crop (variety G 41) implying that its use in garlic crop and varietal response to *imazethapyr* and its formulations need further investigations.

इमाज़ेथेपायर की हायड्रोजेल आधारित फार्मूलेशंस : विकास एवं निर्मुक्ति व्यवहार का

सार

इमाज़ेथेपायर, एक सर्वांगी बृहद स्पेक्ट्रम शाकानाशी, की सोयबीन, मूँगफली एवं अन्य दलहन फसलों के लिए संस्तुति की जाती है। प्राथमिक रूप से निर्गमन–पूर्ण अनुप्रयुक्त होता है तथा निर्गमन–पूर्व खर–पतवार नियंत्रण हेतु भी इसके उपयोग की संस्तुति की जाती है। भारत में, घुलनशील द्रव (एस एल) के रूप में इसकी तरल फार्मूलेशन का काफी अधिक उपयोग किया जाता है जिसमें पृष्ठ संक्रियकों, इमल्सीकारकों एवं अकार्बनिक लवणों का समावेश होता है। जल में त्वरित विसरण होने के कारण इमाज़ेथेपायर की नियंत्रित निर्मुक्ति फार्मुलेशंस को विकसित करने की आवश्यकता है जिन्हें तैयार करना सरल है, धीमी निर्मुक्ति का गुण रखती हैं तथा जिनमें विषाक्त सहायकों का उपयोग न्यूनतम होता है या बिल्कुल नहीं होता । प्रस्तुत शोध–प्रबंध का उद्देश्य था, निर्गमन–पूर्व अथवा निर्गमन के बाद अनुप्रयोग हेतु इमाज़ेथेपायर की हायड्रोजैल आधारित नियंत्रित निर्मुक्ति फार्मूलेशंस विकसित करना, जल में उनके निर्मुक्ति–व्यवहार का मूल्यांकन एवं खरीफ/रबी फसल में विद्यमान खर–पतवारों के विरूद्ध उनकी जैव–प्रभाविता का अध्ययन करना।

गुआर गम (जी जी), एक गैलेक्टोमोनन पॉलीसैकराइड का उपयोग, नवीन GG-g-पॉलीएक्रायलेट/बेंटोनाइड सुपरपोरस हायड्रोजैल कंपोजिटस (जी जी– एस पी सी एच) एवं संवाहक के कोर–शैल नैनो जैल्स (जी जी – सी एस एच) को तैयार करने के लिए किया गया। प्रतिक्रिया कारकों, बैक बोन– मोनोमर अनुपात, क्रॉस लिंकर, बेंटोनाइट एवं पोरोजेन अंशों को आकाए में फूलने संबंधी गुणों हेतु इष्टतम बनाया गया। विकसित किए गए इन हायड्रोजैल्स का एफ टी – आई आर, एक्स किरण विवर्तन (एक्स आर डी), क्रमवीक्षण सूक्ष्मदर्शिता (एस ई एम) एवं पारेषण इलेक्ट्रॉन सूक्ष्मदर्शिता की सहायता से अभिलक्षणन किया गया। लवण एवं उर्वरक घोलों, तापमान, जल–गुणवत्ता एवं पी एच मान के सन्दर्भ में प्रतिनिधि जी जी – एस पी सी एच के फूलने संबंधी व्यवहार का मूल्यांकन किया गया। जी जी – एस पी सी एच ने तेजी से फूलना (30 मिनट) दर्शाया जो तद्नुसार मृत्तिका मुक्त हायड्रोजैल के लगभग समान था। मृत्तिका मुक्त जैल की तुलना में भार के अन्तर्गत उच्चतर अवशोषण करना, इन कम्पोजिट्स की उन्नत यांत्रिक शक्ति को दर्शाता है। कोर–शैल नैनोजैल्स (जी जी – शैल – पॉली एन आई पी ए कोर; जी जी शैल – पी वी ए – कोर; पॉली एन आई पी ए – शैल – जी जी – सी एल – पी वी ए कोर) ने 11 नै मी से 490 नै मी की सीमा में औसत कण–परिमाण दर्शाया। टी ई एम –विश्लेषण ने तैयार किए इन नैनोजैल्स की कोर–शैल गोलाकार संरचना को दर्शाया।

परिवेशी परिस्थितियों के अन्तर्गत की फार्मूलेशंस को तैयार करने के लिए इष्टतम कंपोजिट्स के साथ एस पी पी एच एवं सी एस एच संवाहकों का उपयोग किया गया। परीक्षण की गए सभी रचनाओं से जल में सक्रिय तत्व की निर्मुक्ति मे फिकियन क्रिया विधि दर्शायी। एस पी सी एच फार्मूलेशंस हेतु $T_{½}$ 4.4 से 12.6 डी ए एस की सीमा में देखा गया जबकि सी एस एच फार्मूलेशन हेतु $T_{½}$ मान 1.7 घन्टे से 4.8 दिन की सीमा में थे।

खेत में परीक्षण के दौरान वाणिज्यिक फार्मूलेशंस के साथ, तैयार की गई इन फार्मूलेशंस के तुलनातमक प्रदर्शन ने दर्शाया कि विकसित की गई इन फार्मूलेशंस का खर–पतवार नियंत्रण घातांक (90 डी ए एस पर 96.7 से 99.1%) था जो वाणिज्यिक फार्मूलेशंस द्वारा नियंत्रण (98–99.4%) के तुलनीय था। की एस पी सी एच फार्मूलेशंस के निर्गमन–पूर्व अवस्था मे अनुप्रयोग ने दर्शाया कि अन्य फार्मूलेशन उपचारों की तुलना में इन्होंने महत्वपूर्ण रूप से अधिक खर–पतवार–नियंत्रण दर्शाया। सभी इमाज़ेथेपायर फार्मूलेशंस ने लहसून की फसल (किस्म जी 41) में गंभीर पादप विषाक्तता को दर्शाया जिससे सुझाव मिलता है कि लहसुन की फसल में इसके प्रयोग, इमाज़ेथेपायर एवं इसकी फार्मूलेशंन के प्रति किस्मों की अनुक्रिया के संबंध में और अधिक शोध–कार्य की आवश्यकता है।

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Layout of Design

R1	R2	R3
GG-SPHC-*imz*-75	AC	GG-SPHC-*imz*-100
GG-SPHC-*imz*-100	HW	GG-SPHC-*imz*-75
Pursuit-Stomp	GG-CSH-*imz*-100	Pursuit
Pursuit	GG-CSH-*imz*-75	Pursuit-Stomp
GG-CSH-*imz*-75	Pursuit	GG-CSH-*imz*-100
GG-CSH-*imz*-100	Pursuit-Stomp	GG-CSH-*imz*-75
HW	GG-SPHC-*imz*-100	AC
AC	GG-SPHC-*imz*-75	HW

Measurement Results

Graph Type	ID#	File Name	Measurement Type	Date
	20140422145700 1	20140422145700 1.nsz	Particle Size	22 April 2014 14:57:36

20140422145700 1.nsz

Measurement Results

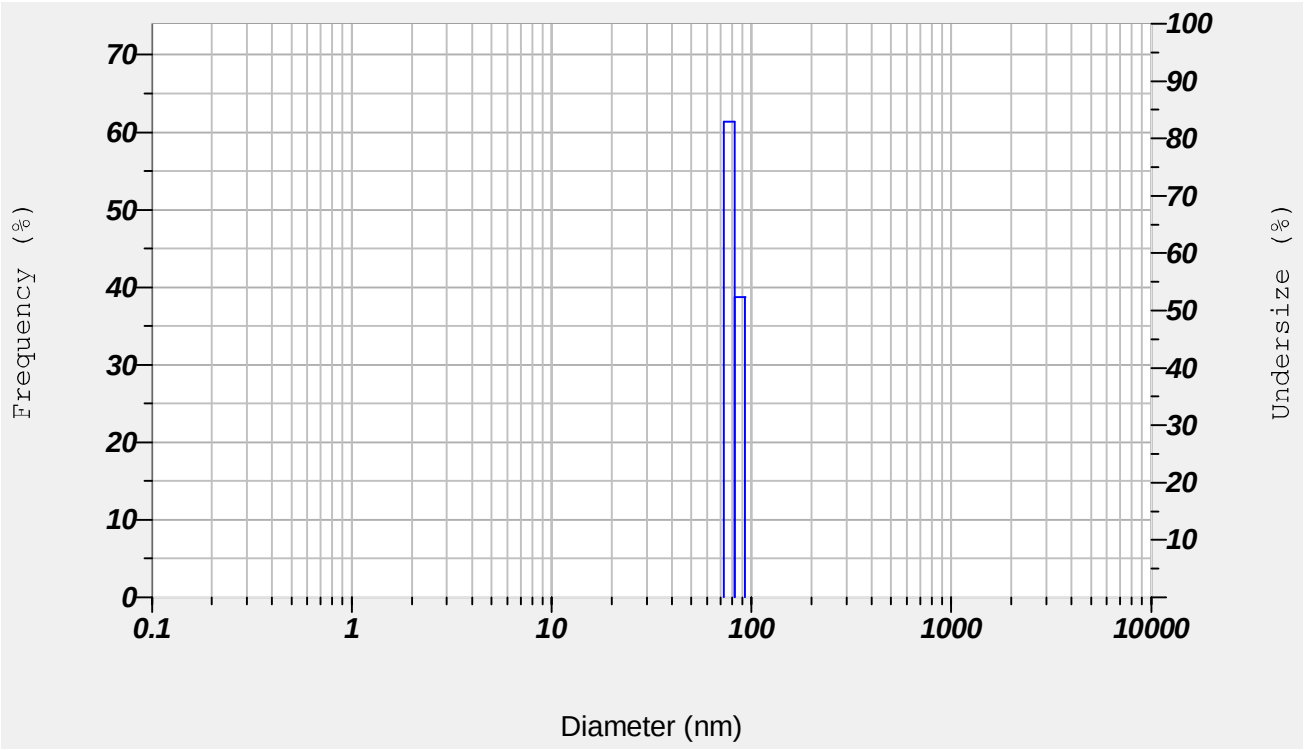
Date	:	22 April 2014 14:57:36
Measurement Type	:	Particle Size
Sample Name	:	p10 2
Material	:	
Scattering Angle	:	90
Temperature of the Holder	:	25.0 deg. C
Dispersion Medium Viscosity	:	0.894 mPa.s
Transmission Intensity before Meas.	:	31175
pH	:	---
Distribution Form	:	|Standard|
Distribution Form(Dispersity)	:	Polydisperse
Representation of Result	:	Scattering Light Intensity
Count Rate	:	45 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	81.3 nm	4.9 nm	80.5 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	81.3 nm	4.9 nm	80.5 nm

Cumulant Operations

Z-Average	:	6608.0 nm
PI	:	4.984



Measurement Results

Graph Type	ID#	File Name	Measurement Type	Date
	20140422150100 2	20140422150100 2.nsz	Particle Size	22 April 2014 15:01:53

20140422150100 2.nsz

Measurement Results

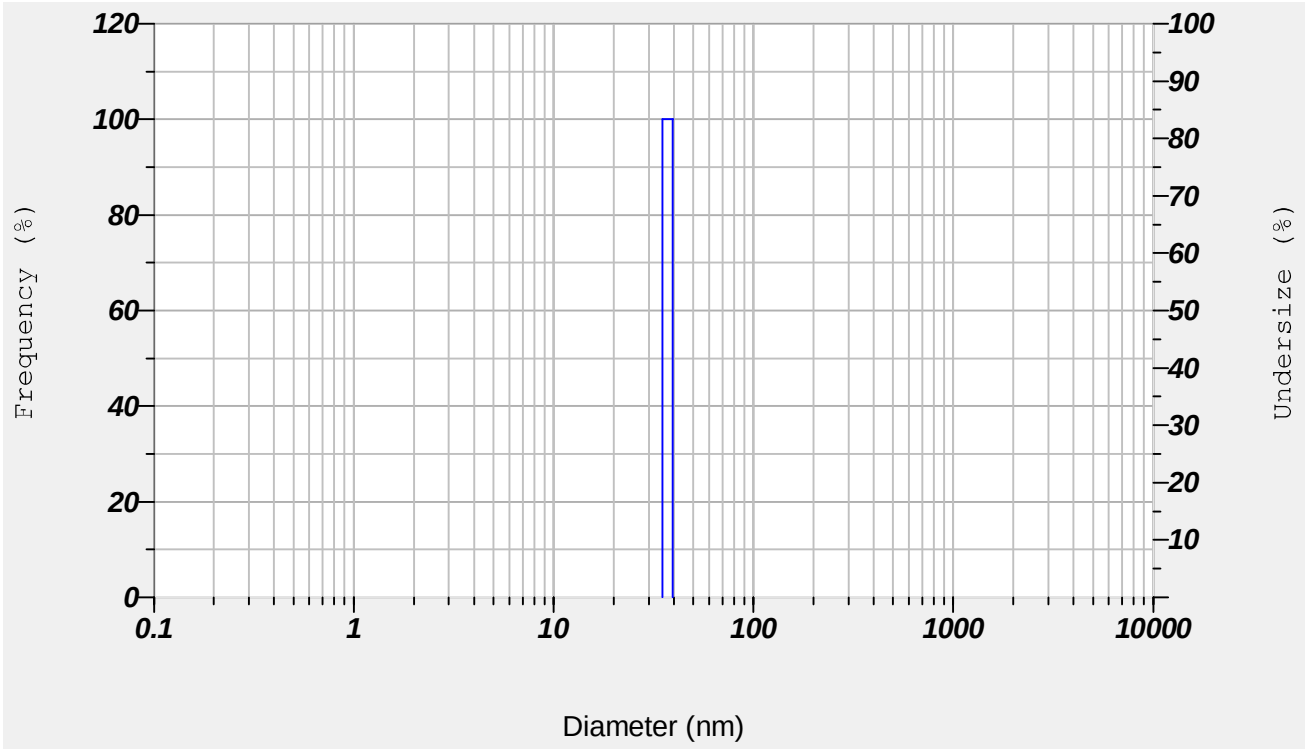
Date	:	22 April 2014 15:01:53
Measurement Type	:	Particle Size
Sample Name	:	p10 3
Material	:	
Scattering Angle	:	90
Temperature of the Holder	:	25.1 deg. C
Dispersion Medium Viscosity	:	0.893 mPa.s
Transmission Intensity before Meas.	:	32080
pH	:	---
Distribution Form	:	|Standard|
Distribution Form(Dispersity)	:	Polydisperse
Representation of Result	:	Scattering Light Intensity
Count Rate	:	50 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	37.2 nm	0.0 nm	37.2 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	37.2 nm	0.0 nm	37.2 nm

Cumulant Operations

Z-Average	:	8806.6 nm
PI	:	7.668



Measurement Results

Graph Type	ID#	File Name	Measurement Type	Date
	20140422154100 9	20140422154100 9.nsz	Particle Size	22 April 2014 15:41:38

20140422154100 9.nsz

Measurement Results

Date	:	22 April 2014 15:41:38
Measurement Type	:	Particle Size
Sample Name	:	p26 6
Material	:	
Scattering Angle	:	90
Temperature of the Holder	:	25.0 deg. C
Dispersion Medium Viscosity	:	0.895 mPa.s
Transmission Intensity before Meas.	:	32655
pH	:	---
Distribution Form	:	|Standard|
Distribution Form(Dispersity)	:	Monodisperse
Representation of Result	:	Scattering Light Intensity
Count Rate	:	29 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	267.5 nm	23.9 nm	267.8 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	267.5 nm	23.9 nm	267.8 nm

Cumulant Operations

Z-Average	:	4082.4 nm
PI	:	1.825