# Preparation and characterization of Slow Release Formulations of Trifluralin based on alginate Modified Chitosan

L. A. Nnamonu[a,*], R. Sha'Ato[a], I. Onyido[b]

lami.nnamonu@gmail.com; +234(0)805 447 5757

[a]Department of Chemistry, Centre for Agrochemical Technology, University of Agriculture, Makurdi.
[b]Department of Chemistry, Nnamdi Azikiwe University, Awka.

## ABSTRACT

*The aim of this work was to encapsulate the herbicide trifluralin in chitosan-alginate beads and characterize the resulting slow release formulations (SRFs) using scanning electron microscopy (SEM), and Fourier Transform infrared spectroscopy (FTIR). Two sets of formulations were made by extrusion into 0.25 M calcium chloride solution: chitosan core/alginate beads (SCTRF) and alginate core/chitosan beads (SCTRFA). Results showed highly porous spherical beads: the chitosan core/alginate beads were bigger and more porous than the alginate core/chitosan beads with diameters of 2.18±0.01 and 1.78±0.01 mm; porosity of 74.16±0.2% and 16.63±0.2% and swelling of 73.19±0.2% and 14.14±0.2% respectively. Both sets of beads exhibited reduced crystallinity of trifluralin. FTIR revealed a shift to longer wavelength of the band at 1528 cm$^{-1}$ which is due to the asymmetric stretching vibration of -NO$_2$, suggesting that encapsulation of trifluralin in chitosan decreases photolability of the herbicide.*

## INTRODUCTION

Slow release (SR) of pesticides is an interesting approach in Integrated Pest Management (IPM) which improves pesticide delivery by optimizing pesticide activity profile while limiting its contact with the environment. In this setting, the amount of pesticide immediately available for undesirable losses is minimized through its application as a material adsorbed on a matrix or carrier. Generally, the advantages of slow release formulations are satisfactory effect at a constant active ingredient level for a longer period of time (residual effect), smaller dosage (less active ingredient is needed to maintain effective biological activity) by elimination of repeat applications and therefore reduced cost (safety and resource-saving), and longer application interval (labour-saving). Other advantages include reduced risk of mammalian toxicity of highly toxic substances, reduced phytotoxicity, decreased leaching and evaporative losses[1], decreased environmental degradation, reduced contamination or pollution of the environment, improved convenience and ease of handling by conversion of liquids to solids/powders, masking of odours[2],

minimizing residues on foodstuff and decreasing dermal toxicity[3].

Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N,N*-p-toluidine) is a selective pre-emergence dinitroaniline herbicide widely used in the control of annual grasses and broad-leaf weeds in horticultural and agricultural crops[4]. It is registered in more than fifty countries for use on more than eighty crops[5]. Trifluralin is a persistent soil contaminant - due to its strong adsorption to soil particles - and in spite of its low water solubility, it has been reported as a surface water pollutant[6]. Even though it has low leachability, trifluralin has a very high bio concentration factor. It is photolabile, a serious drawback for field application since the quantity and frequency of application must be increased to ensure its effectiveness. These factors make trifluralin (TRF) a good candidate for slow release formulation.

Chitosan, a cationic polysaccharide, is the deacetylated derivative of chitin (*N*-acetyl-d-glucosamine), a water insoluble polymer present in insect and crustacean exoskeletons and fungal cell walls. Chitosan has received enormous interest for medical and pharmaceutical applications due to its nontoxic, odourless nature, as well as its biodegradable properties and biocompatibility in animal tissues[7]. Blending of chitosan with other polymers is a convenient and effective method of improving its physical and mechanical properties for practical applications[8].

For agricultural applications, chitosan has been used for controlled release of N-P-K compound fertilizer[9] and of urea and atrazine[10]. Similarly, alginate gels are used as matrices for SR in agricultural applications because of their biodegradability and the ease of incorporation of pesticides using an aqueous system at ambient temperatures. TRF has been encapsulated in starch xanthide[11], spray dried aqueous dispersions of starch[12] and polypropylene pellets[13] and the formulations only evaluated for their herbicidal efficacy and release in water.

We hereby report simple and direct encapsulation of TRF in SR beads using chitosan modified by cross-linking with alginate, and the characterization of same. The method cuts out multi-step conversions and does not involve use of sophisticated equipment. The use of alginate not only helps bead formation but strengthens the structure of the matrices. SEM was used to determine bead shape, diameter, overall morphology and distribution of the trifluralin herbicide in the slow release formulation (SRF). In addition to spectroscopic characterization of the SRFs, information on intermolecular interactions was obtained using FTIR.

## MATERIALS AND METHODS

### Materials
Chitosan (high molecular weight, Brookfield viscosity 800 000cps, deacetylation degree minimum 85.0%), trifluralin, calcium chloride dihydrate and sodium alginate were obtained from Sigma Aldrich UK and used as received. Sodium hexametaphosphate was purchased from VWR Prolabo (BDH) International N/America and Europe. Glacial acetic and sodium hydroxide are the other chemicals used. High purity water used all through this work was obtained from a purification unit by Millipore® Corporation.

### Methods
Two types of gel beads were prepared, both by extrusion into calcium chloride solution: one set with chitosan core and alginate coating was code named SCTRF, and the other with alginate core and chitosan coating, named SCTRFA. The bi-polymeric beads formed immediately the slurry droplets hit the calcium chloride solution.

### Chitosan core/alginate beads (SCTRF)
An aqueous dispersion containing 3% (w/w) of sodium alginate, 2% (w/w) of trifluralin and 15% (w/w) chitosan was added drop wise by extrusion through a silicone tubing (internal diameter 3mm) with the aid of a peristaltic pump (Pharmacia LKB pump P-1), at a flow rate of 48 droplets/min, to a 200 ml gellant solution of 0.25 M $CaCl_2.2H_2O$, as demonstrated[14]. The distance from the orifice to the surface of the gellant solution was 240 mm to allow good droplet penetration, the beads were allowed to set for 25 minutes, suction-filtered through a coarse frit Buchner funnel, washed twice with distilled water and air-dried. Blank beads were prepared in the same way but without herbicide.

### Algin core / chitosan beads (SCTRFA)
A set of SR formulation was prepared having a sodium alginate core in chitosan coating. An aqueous dispersion of 1.5 g (3%) sodium alginate and 2% TRF in 5 ml methanol was added drop wise, into 100 ml aqueous solution of 0.5% (v/v) acetic acid, 4% (w/w) $CaCl_2.2H_2O$ and chitosan (100 mg) with pH adjusted to 5.0 using 0.5 M NaOH solution. The colorless glassy alginate-chitosan beads were washed with milli-Q water followed by 4% (w/v) sodium hexametaphosphate to harden the chitosan coat and prevent adhesion of beads during drying.

### Swelling
Swelling studies of the beads were conducted by the water evaporation method straight after separation from the gellant solution. A sample of the beads was blotted with Kimberly-Clark® professional medical wipes to remove excess water and weighed immediately in a weighing boat. The sample was air-dried in the fume cupboard and weighed until constant dry mass was attained, which took between 24 to 48 hrs. The percent equilibrium swelling of the beads was computed from the mass changes.

### Porosity
Two techniques were employed in determining the porosity of beads; the reported values are averages of both methods.

(a)    The volume/density method. A 10 ml measuring cylinder was weighed empty, beads poured in and the sides of the cylinder gently tapped until the beads were well packed. The total volume ($V_t$) was noted and the cylinder and contents weighed. The volume occupied by the

beads, $V_b$ was calculated using the density of the matrix ($\rho$)

Volume of beads, $V_b$ = mass / $\rho$

Pore volume and percent porosity were deduced from these.

Pore volume, ($V_p$) = Total volume ($V_t$) – Volume of beads ($V_b$)

% Porosity = $\dfrac{\text{Pore volume } (V_p)}{\text{Total volume } (V_t)}$ x 100

Density (g/ml) of matrices: Algin 1.73; Chitosan 0.6-0.8 (high density)

(b)     *The water evaporation method.*
A fully hydrated sample of freshly prepared beads, (excess water wiped off) was weighed. The beads were left to reach constant dry weight at room temperature.

Since density of water = 1g/ml, the difference (in grams) between the weight of wet beads and that of the dry beads is equal to the volume of water removed from the sample which is exactly equal to the pore volume.

Pore volume = mass of wet beads – mass of dry beads.

Porosity (%) = $\dfrac{\text{Pore volume}}{\text{Vol. of wet beads}}$ x 100

Diameter was an average of measurements taken with Vickers M17 Industrial microscope and the scanning electron microscope.

*Scanning electron microscopy*

The shape, external morphology and internal structure of the SR formulations were examined by scanning electron microscopy. SEM images (at different magnifications) of dry whole beads and their cross sections were captured on an FEI/PHILIPS XL 30 ESEM microscope after the samples were deposited on brass stubs (using double sided carbon tapes) and sputter coated with gold (20 nm) on an Edwards Pirani 501 Scan Coat Six sputter coater. The samples were exposed to accelerated voltage beam strength of 20.0 KV.

## RESULTS AND DISCUSSION

*Formation of beads*
In this work, it was necessary to modify chitosan with alginate because it has been reported that the mechanical strength of chitosan beads is low, thus limiting its usage as a pharmaceutical dosage form[8]. The alginate matrix, which consists of an open lattice structure, forms porous beads[14] and it was used to modify chitosan in a polyelectrolyte complex[15]. This approach was therefore employed here. The structure of chitosan is modified by cross linking with alginate via ionic interaction between the carboxyl residues of alginate and the amino terminals of chitosan[14]. A conceptual representation of this is depicted in Figure 1. This complexation reduces the porosity of the alginate beads and decreases the leakage of the encapsulated substances. In addition, chitosan acquires a higher level of mechanical strength with the support of the alginate gel mass. Formulation characteristics are displayed in Table 1.

Table 1 Formulation Characteristics

| Characteristic | SCTRF | SCTRFA |
|---|---|---|
| Diameter (mm) | 2.18 ± 0.01 | 1.78 ± 0.01 |
| Porosity (%) | 74.16 ± 0.2 | 16.63 ± 0.2 |
| Swelling (%) | 73.19 ± 0.2 | 14.14 ± 0.2 |

*Scanning electron microscopy*
SEM images of cross section of the beads, at magnifications spanning 35X to 1500X, are presented in Plates 1 and 2.
The chitosan core-alginate formulation (Plate 1) beads were rougher and larger than the alginate core-chitosan (Plate 2) formulation beads. The SEM images show the massive and uniform structure, without primary particle, of the formulations. A cross-section of the chitosan core beads (magnification 35X) showed a fibrous internal structure like the longitudinal cross-section of a white cabbage. Higher magnifications revealed a hollow internal structure without visible crystals of TRF.
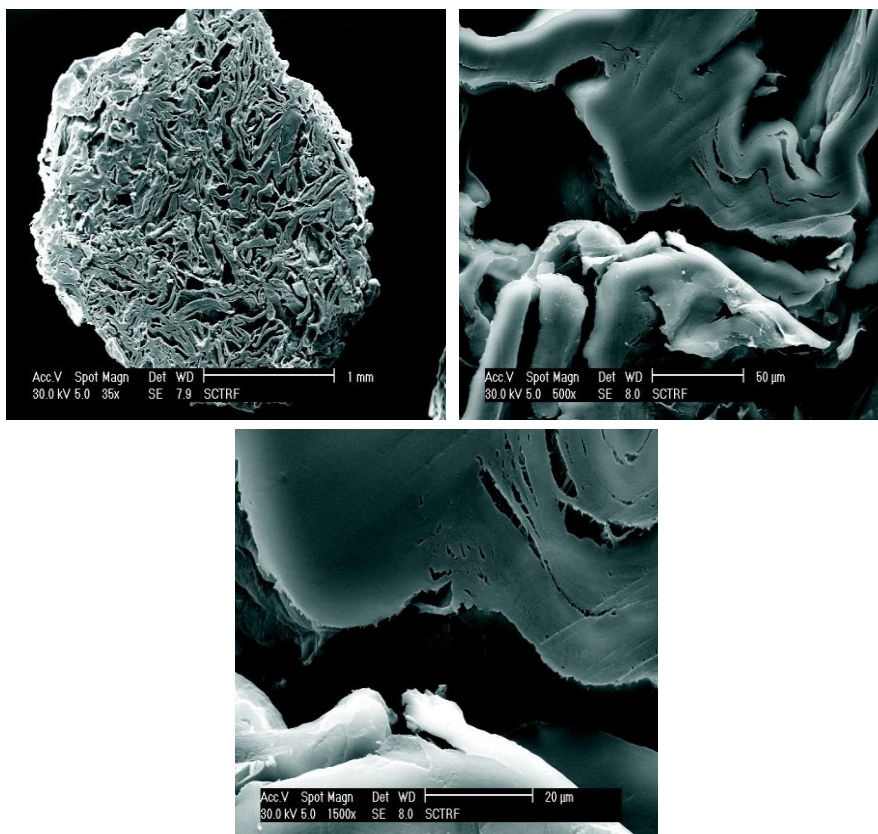
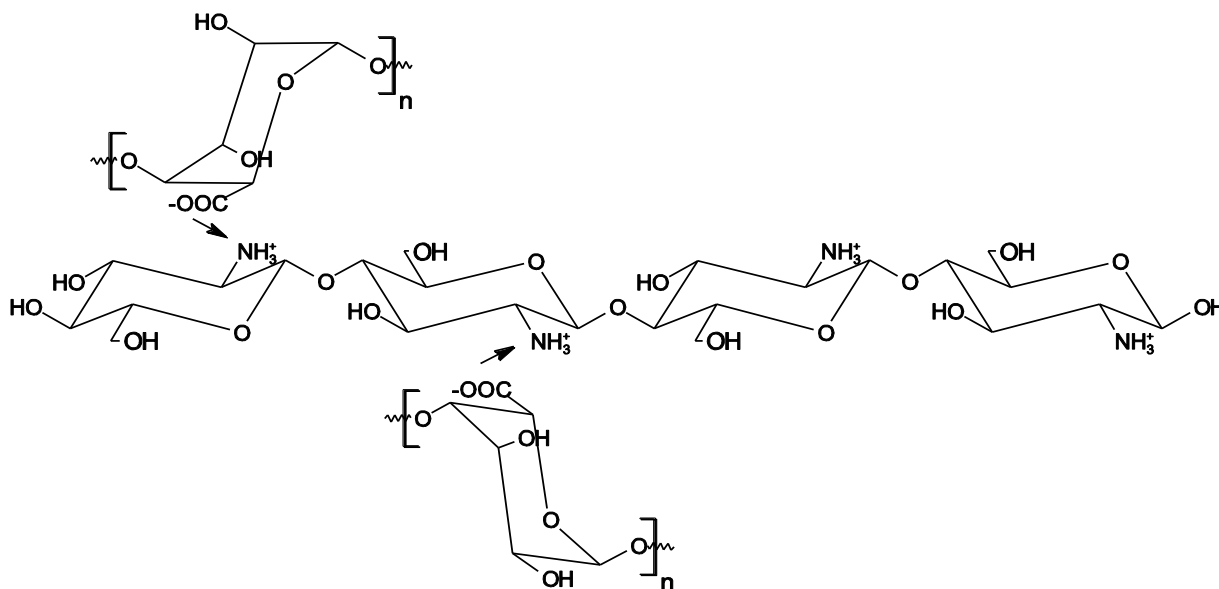Plate 1: Cross section of Chitosan core/Alginate Beads



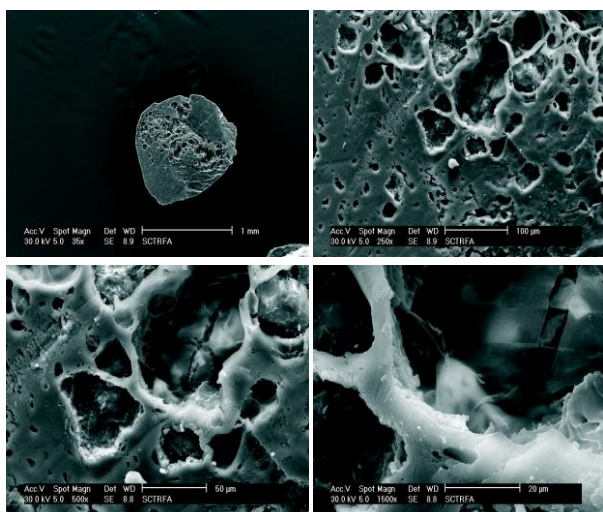Figure 1 Chitosan - Alginate Cross linking Interaction

Plate 2: Cross section of Alginate core/chitosan Beads



Figure 2: FTIR of Trifluralin

### Fourier Transform Infrared Spectroscopy

FTIR spectra (Spectrum 100 Fourier Transform IR Spectrophotometer) of the herbicide, herbicide incorporated formulation and the blank matrix formulation, are shown in Figs. 2 and 3.

The medium intensity band at 3500 $cm^{-1}$ is characteristic of chitosans, attributed to -$NH_2$ and -OH group stretching vibrations. Partial protonation of the amine group of chitosan explains the band at 1530 $cm^{-1}$ in the spectrum of SCTRF due to one of the –$NH_3^+$ vibrational modes [25]. A band for –$COO^-$ (from alginate) is also seen around 1600 $cm^{-1}$. The two peaks at 1528 and 1549 $cm^{-1}$ in the spectrum of TRF are assigned to the assymmetric stretching vibrations of each of the two $NO_2$ groups [26], which are not identical due to steric reasons (non-planarity with aromatic ring). These peaks are still visible in the FTIR spectra of the SRFs, with the 1528 $cm^{-1}$ peak shifted to longer wave number. The two bands observed between 3000 and 2780 $cm^{-1}$ in the TRF spectrum were present in the spectrum of the SRFs but only weaker and shifted to lower wave numbers. Decreased crystallinity may be due to electrostatic attractions, possibly, between the protonated amine group of chitosan (R-$NH_3^+$) and the negative dipole of the nitro group of trifluralin (Figure 4).

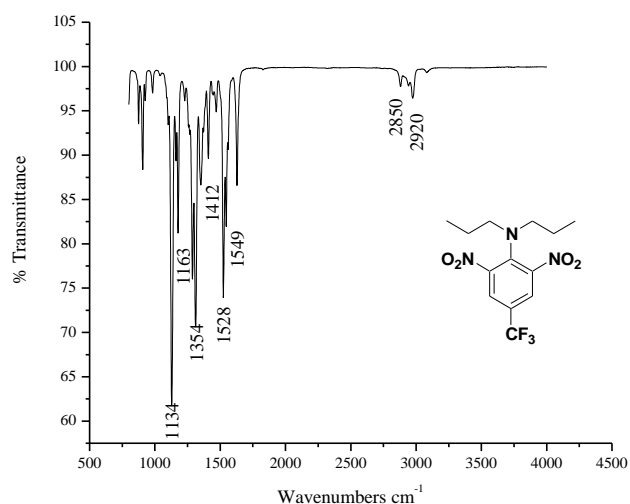The shift to longer wave number of the band at 1528 $cm^{-1}$, which was observed when TRF was complexed with montmorillonite[16], was also observed in the spectrum of the chitosan/alginate/trifluralin SR beads. This suggests that TRF encapsulation in chitosan also decreases its photolability. Similar to the explanation proffered for trifluralin stabilization by adsorption on clay, the -$NO_2$ group involved in cyclization during photodecomposition is that through which the trifluralin molecules are adsorbed to the chitosan. Steric hindrance imposed by the chitosan to the cyclization step of the photochemical reaction (Fig. 5) may be responsible for the photostabilization effect[17].
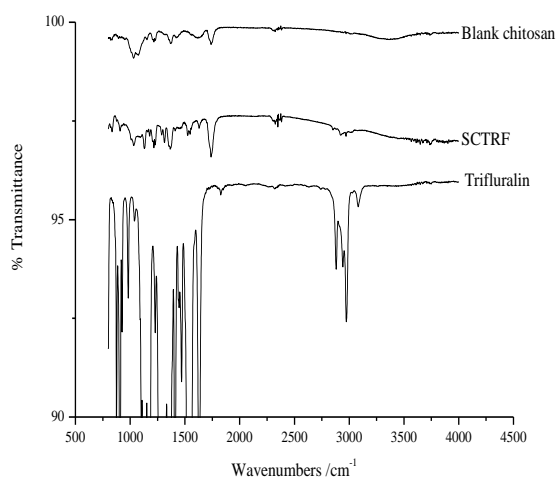


Figure 3: FTIR of Chitosan Blank, SRF Bead and Trifluralin
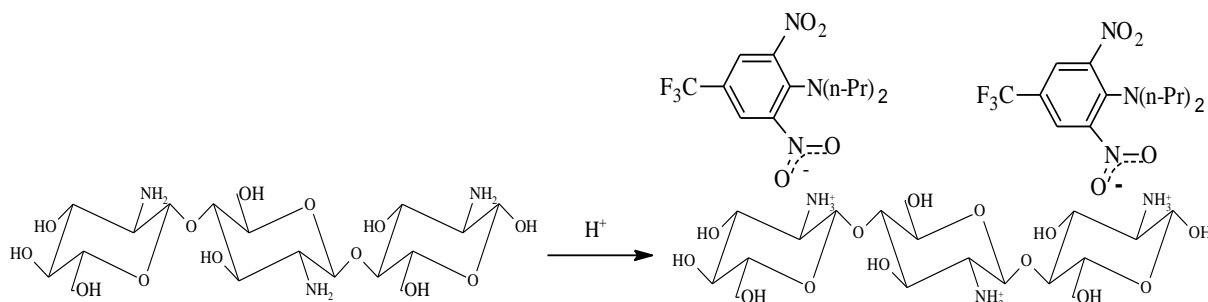
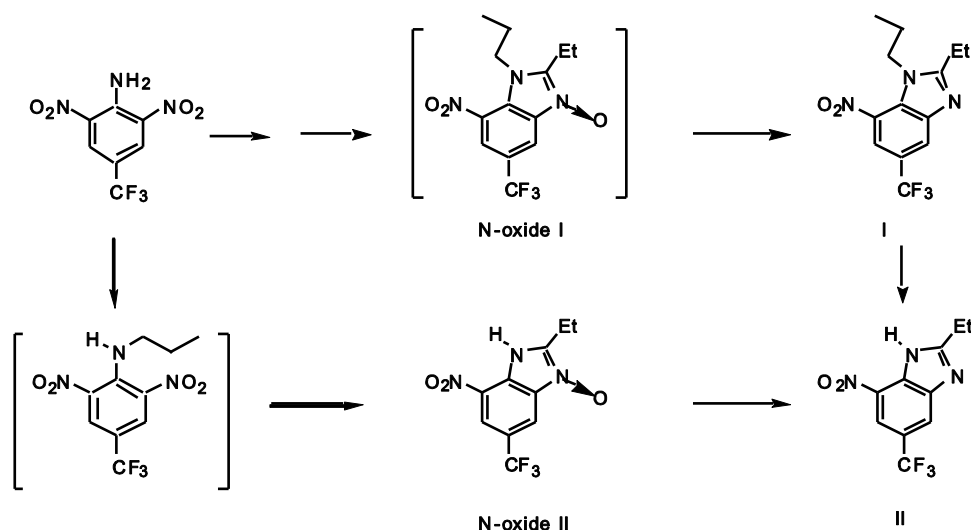Figure 4: Trifluralin/Chitosan Interaction



Figure 5: Photodecomposition of Trifluralin to Benzimidazoles

## CONCLUSIONS

Two sets of formulations encapsulating trifluralin in algin modified chitosan have been synthesized by extrusion into 0.25 M CaCl$_2$ solution. SEM showed that the beads were spherical and porous, with chitosan core/alginate beads (SCTRF) bigger and more porous than the alginate core/chitosan beads (SCTRFA). FTIR of the beads revealed some degree of ionic interaction between chitosan and TRF that makes for good entrapment and subsequent slow release. From the FTIR spectra, it was deduced that the -NO$_2$ group involved in cyclization during photodecomposition of trifluralin is the one through which trifluralin molecules are adsorbed to chitosan. Hindrance of the cyclization reaction makes trifluralin less photolabile.

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