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(54) **Title**: POLY(*N*-VINYL CAPROLACTAM-*CO*-ACRYLAMIDE) MICROPARTICLES FOR CONTROLLED RELEASE APPLICATIONS

(57) **Abstract**: The present invention relates to Poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymers prepared by free radical emulsion polymerization from acrylamide (AAm) and *N*-vinyl caprolactam (VCL) monomers with *N,N'* -methylene bisacrylamide (NNMBA) as the crosslinking agent. In particular, the present invention provides the application of this copolymer in the controlled release of drugs. Methods for generating copolymers by emulsion polymerization performed in aqueous media along with ingredients like crosslinking agent and the initiator to make candidate materials for controlled release (CR) of drugs are provided. The use of poly(*N*-vinyl caprolactam-*co*-acrylamide) microparticles inpreparing controlled release formulations of drugs or bioactive molecules are provided.

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TITLE OF THE INVENTION:

POLY(*N*-VINYL CAPROLACTAM-*CO*-ACRYLAMIDE) MICROPARTICLES FOR CONTROLLED RELEASE APPLICATIONS.

CROSS REFERENCE TO RELATED APPLICATIONS:

This application claims benefit of provisional Indian Application No. 1366/MUM/2008, filed June 30, 2008 which is hereby incorporated in its entirety by reference.

FIELD OF THE INVENTION:

The present invention relates to the development of a novel process to prepare biopolymers and their uses as micro particles for the controlled release of drugs. In particular, the invention relates to a novel process of making micro particles of poly (*N*-vinyl caprolactam-*co*-acrylamide) copolymers that are cross-linked with *N'*,*N'*-methylene bisacrylamide.

BACKGROUND OF THE INVENTION

Conventional methods of encapsulating drugs of interest include that of simple physical blending of the drug with the chosen polymer(s) along with other ingredients. This method suffers from many drawbacks such as burst release, which allows large quantities of drug to be released from the device quickly and thus failing to maintain the therapeutic index. Other methods of drug encapsulation include solvent evaporation, phase separation and in some cases, spray drying of drug loaded solutions (S. Freitaset. et. al., J. Controlled Release, 102 (2005) 313-332). Of these, the solvent evaporation method has poor encapsulation efficiency for water-soluble drugs, while phase separation method often produce irregularly shaped particles with trace amount of solvent in the final formulations, which would add to the toxicity of the final product. Spray drying method has the disadvantage of not producing sub-micron level particles in addition to having low yields; also, frequently the drug gets adhered to the surface of the formulated particles, often with a sudden burst release of the drug from the device. Alternatively, in recent years, super critical fluid technology has been employed (Supercritical Fluid

Technology in Materials Science and Engineering Syntheses, Properties, and Applications Edited by Ya-Ping Sun, ISBN: 0-8247-0651-X, 2002) to produce drug-loaded particles without recourse to the use of solvents. Again this method suffers from not being able to produce sub-micron size particles of regular shape with low encapsulation efficiencies. Also, the method requires expensive instrumentation and many process controls are needed for its successful operation. Thus, there is clearly a need for developing improved methods for preparing drug loaded particles. The present invention relates to the development of an emulsion polymerization technique in which drug is loaded during the process of polymerization.

Many drugs are difficult to administer because they must be delivered slowly over a prolonged period to have a beneficial effect. Controlled release (CR) technology has proven to be efficient in delivering drugs in a controlled manner to avoid the side effects. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is combined with a drug or other bioactive agent in such a way that the active agent is released from the material in a pre-designed manner. Release of the active agent may be constant over an extended period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. In any case, the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing.

Other advantages of using CR systems include the maintenance of drug concentrations within desired range (therapeutic window), the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. While these advantages can be significant, the potential disadvantages cannot be ignored: the possible toxicity or non-biocompatibility of the materials used, undesirable by-products of degradation, any surgery required to implant or remove the system, the chance of patient discomfort from the delivery device, and higher cost of CR systems compared with the traditional pharmaceutical formulations.

Even though CR technology is a well established science for the release of drugs, research activities in this area are accelerating at a rapid pace due to the availability of a large number of polymers for encapsulating many drugs of interest (R.L. Dunn and R.M.

Ottenbrite, Polymeric Drug and Drug Delivery Systems, American Chemical Society, Washington, DC, 1991; M.A. El-Nokaly, et. al., Polymeric Delivery Systems, American Chemical Society, Washington, DC, 1993). Further, CR technology could provide a sound basis for delivering drugs in a controlled manner to avoid many disadvantages of side effects. Previously, many biodegradable and biocompatible polymers have been employed as drug delivery devices. Such devices have many advantages over synthetic polymers because they can be easily degraded by the body mechanism and CR dosage formulations from such polymers would offer the desired therapeutic effects and maintain the maximum dose regimen with minimum side effects (N. Isiklan, J. Appl. Polym.Sci. 99, 2006, 1310–1319). The release of any active drug from such delivery devices would occur due to the transport of drug to the surrounding media crossing the polymeric barrier by molecular diffusion and/or erosion of the matrix. The CR systems have many advantages over conventional dosage forms, including improved efficacy, reduced toxicity, improved patient compliance and convenience (S. Vaithiyalingam et al., J. Pharma. Sci. 91, 2002, 1512-1522; V. Ramesh Babu et. al., J. Appl. Polym.Sci., 99, 2006, 2671-2678; J. Shi et. al., Macromol. Biosci. 2006, 6, 358–363).

This technology has gained immense attention and the focus has been mainly on developing ideal polymers to encapsulate the drug. The ideal drug delivery system should be inert, biocompatible, mechanically strong, and comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, easy to fabricate and sterilize. To be successfully used for developing CR formulations, the polymer must be chemically inert, free of leachable impurities, must have an appropriate physical structure, with minimal undesired aging, and be readily processable.

Multiple materials have been explored and employed for the CR of drugs and other active agents. The range of polymers that have been previously studied were originally intended for non-biological uses and were selected for control release of drugs based on their desirable physical properties (K.S. Soppimath, A.R. Kulkarni and T.M. Aminabhavi, J. Controlled Release, 75 (2001) 331-345; T.M. Aminabhavi, K.S. Soppimath, A.R. Kulkarni and W.E. Rudzinski, J. Controlled Release, 70(1-2) (2001) 1-20). These include poly(urethanes) for elasticity, poly(siloxanes) or Silicones for insulating ability,

poly(methyl methacrylate) for physical strength and transparency, poly(vinyl alcohol) for hydrophilicity and strength, poly(ethylene) for toughness and lack of swelling, and poly(vinyl pyrrolidone) for suspension capabilities.

Some of the specific polymers that are currently being used or studied for controlled release of drugs include the poly(2-hydroxy ethyl methacrylate), poly(N-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), and poly(methacrylic acid).

In recent years, other biopolymers designed primarily for medical applications have entered the area of controlled release (Mundargi, et al., J. Controlled Release, 125(2008)193-209). Many of these materials are designed to degrade within the body; among them are polylactides (PLA), polyglycolides (PGA), poly(lactide-*co*-glycolides) (PLGA), polyanhydrides and polyorthoesters.

All previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research is being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove the CR system after release of the active agent has been completed. The greatest advantage of these biodegradable polymers is that they are broken down into the biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways. However, biodegradable polymers produce the degradation by-products that are tolerated with little or no adverse reactions within the biological environment. These degradation products—both desirable and potentially nondesirable—are required to be tested thoroughly, since there are a number of factors that will affect the biodegradation of the original polymers. The chemical, structural and processing properties are important features that affect the biodegradability of the CR device.

Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer into biologically acceptable and progressively smaller compounds. In some cases—as for example, polylactides, polyglycolides, and their copolymers—the polymers will eventually break down to lactic acid and glycolic acid, enter the Kreb's cycle, and be

broken down further into carbon dioxide, water and excreted through the normal processes. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically. For some degradable polymers, notably polyanhydrides and polyorthoesters, degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the CR system.

Among the various types of polymers employed in pharmaceutical applications, hydrophilic biopolymers such as sodium alginate, chitosan etc., (V. Ramesh Babu et. al., Carbohydrate Polymers 71, 2008, 208-217; K. Hosoya, et. al., J. Chromatogr. A, 979, 2002, 3-10; H. Kanazawa, et. al., Anal. Chem. 68, 1996, 100-105) have been widely used (H. Vihola et. al., Eur. J. Pharm. Sci. 16, 2002, 69-74), since these are found to have many advantages over the synthetic polymers. Particularly, in the treatment of deadly diseases like cancer, AIDS, rheumatoid arthritis, etc., a considerable therapeutic advantage can be gained if drugs are be delivered in a selective and controlled manner to the target site. However, the development of efficient, compliant and reliable therapy requires that the drug to reside as long as its therapeutic action is required at the site of action (by systemic absorption, binding, inhibition, etc.). This concept has led researchers to develop various types of polymer-based CR dosage formulations as dispersible matrices, coated tablets, nanoparticles or microparticles (Babu et al., Expert Opinion in Drug Delivery, 5(2008):403-415). However, the development of an appropriate delivery system requires a proper consideration between the properties of the drug, the disease and destination in the body.

The most exciting opportunities in controlled drug delivery lie in the arena of responsive delivery systems which make it possible to deliver drugs through implantable devices in response to measured blood level or to deliver drug precisely to the targeted site. Much of the development of novel CR polymers is focusing on the preparation and use of these responsive polymers with specifically designed macroscopic and microscopic structural and chemical features. Such systems include copolymers with desirable hydrophilic/hydrophobic interactions, block or graft copolymers, complexation networks responding via hydrogen or ionic bonding, dendrimers or star polymers as nanoparticles

for immobilization of enzymes, drugs, peptides, or other biological agents (Babu et al., Expert Opinion in Drug Delivery, 5(2008)403-415).

More recently, new biomaterials-tailor-made copolymers with desirable functional groups are being created by researchers, who envision their use not only for developing innovative drug delivery systems, but also as potential linings for artificial organs, as substrates for cell growth or chemical reactors, as agents in drug targeting and immunology testing, as biomedical adhesives and bioseparation membranes, and as substances able to mimic biological systems. Successfully developing these novel formulations require assimilation of a great deal of emerging information about the chemical nature and physical structure of these new materials. There has been tremendous growth in the stimuli-responsive polymers that are now being intensely studied due to the ability to change their physical states under the influence of external environment such as temperature, pH, ionic strength, light illumination, etc. Recently, chromatographic (M. T. Lugo et. al., Macromolecules 32, 1999, 6646-6651; Y. E. Kirsh et. al., Sep. Purif. Technol. 22-23, 2001, 559-565), drug delivery (J. F. Hester et. al., J. Membr. Sci. 208, 2002, 375-388; J. P. Lederhos et. al., Chem. Eng. Sci. 51, 1996, 1221-1229), membrane technology (D.C. Coughlan et. al., J. Control. Rel. 98, 2004, 97-114; H. G. Schild, Prog. Polym. Sci. 17, 1992, 163-249) and kinetic inhibition (Y. E. Kirsh et. al., Eur. Polym. J. 35, 1999, 305-316) applications of such systems have been well documented (Babu et al., Expert Opinion in Drug Delivery, 5(2008)403-415).

Since biodegradable polymers have been the lead development in this area, there is still a need for developing an efficient process of preparation of these copolymers so that residual chemicals do not pose problems during the regulatory process.

OBJECTIVE OF THE INVENTION

It is an object of the present invention to provide a biodegradable copolymer prepared from *N*-Isopropylacrylamide and *N*-vinyl caprolactam monomers.

It is an object of the present invention to provide an environmentally friendly process for the preparation of microparticles of copolymer POLY(*N*-VINYL CAPROLACTAM-*CO*-ACRYLAMIDE).

It is an object of this invention to provide detailed characterization of the microparticles of POLY(*N*-VINYL CAPROLACTAM-*CO*-ACRYLAMIDE).

It is an object of the present invention to provide a process of preparation of microparticles of POLY(*N*-VINYL CAPROLACTAM-*CO*-ACRYLAMIDE) by *in situ* emulsion polymerization technique.

It is an object of the present invention to provide a single step method involving emulsion polymerization, producing microparticles and encapsulating the drug simultaneously.

It is an object of the present invention to provide POLY(*N*-VINYL CAPROLACTAM-*CO*-ACRYLAMIDE) copolymeric matrices with improved purity profile using N,N'-methylenebisacrylamide as the crosslinking agent.

It is an object of the present invention to provide a single step process for the production of microparticles that are free of toxic solvents, thus making it cost effective and commercially viable on an industrial scale.

It is an object of the present invention to demonstrate the use of potassium persulfate as the initiator in emulsion polymerization.

It is an object of the present invention to demonstrate the efficiency of the synthesized copolymer in the CR of drugs and/or active agents.

It is an object of the present invention to demonstrate the environmentally benign nature of the process employed.

SUMMARY OF THE INVENTION

The present invention relates to new tailor-made biodegradable copolymers prepared from the monomers: *N*-isopropylacrylamide and *N*-vinyl caprolactam. Considerable therapeutic advantage is gained as the process is environmentally benign. The present invention provides an environmentally friendly process for the preparation of microparticles from the copolymer: POLY(*N*-VINYL CAPROLACTAM-*CO*-

ACRYLAMIDE) and its application in the CR of bioactive molecules. The systems developed in this invention are temperature sensitive and hence, pulsatile release of drug can be achieved by varying the temperature from ambient to body temperature. The entire process is carried out in aqueous media thus avoiding toxic organic solvents.

The present disclosure provides a method to prepare poly(*N*-vinyl caprolactam-*co*-acrylamide) cross-linked with *N,N'*-methylenebisacrylamide. More particularly, the invention aims to prepare the microparticles from the above-mentioned copolymer and propose their potential applications for the CR of any drug, particularly anti-cancer drugs, such as 5-flurururacil (5-FU).

In one embodiment, the present invention provides a process for developing microparticles of poly(*N*-vinyl caprolactam-*co*-acrylamide) that are cross-linked with *N,N'*-methylene-*bis*-acrylamide in an aqueous media. In one embodiment, the invention provides a process for the preparation of copolymer by free radical emulsion polymerization using varying amounts of monomers viz., acrylamide (AAm), *N*-vinyl caprolactam (VCL) and crosslinking agents like *N,N'*-methylene-*bis*-acrylamide (NNMBA) along with potassium persulfate as the initiator.

In one embodiment, the present invention provides microparticles of the copolymer. In one preferred embodiment, the present invention provides microparticles in the size range of 16-34 μm.

In one embodiment, the present invention provides drug encapsulation in the copolymer matrix. In one preferred embodiment, the present invention provides anti-cancer drugs such as 5-FU encapsulated into these microparticles.

In one embodiment, the present invention provides characterization of these microparticles by the most widely used experimental techniques. In one embodiment, the microparticles are characterized by differential scanning calorimetry (DSC) to investigate drug dispersion in the polymer matrix, while scanning electron microscopy (SEM) is used to understand the surface morphology of microparticles. The size of microparticles is measured by zeta sizer that is based on the principles of dynamic light scattering.

In one embodiment, the present invention provides a convenient method of in *situ* polymerization and release kinetics of the drug-loaded microparticles. In one embodiment, the present invention provides valuable information on the release kinetics of 5-FU in phosphate buffer solution (pH 7.4) at two temperatures viz., 25° and 37°C i.e., ambient and body temperatures, respectively.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, the inventions of which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 shows the DSC thermograms of (A) pure 5-FU, (B) plain poly(*N*-vinyl caprolactam-*co*-acrylamide) as placebo, and (C) 10 wt.% of 5-FU-loaded poly(*N*-vinyl caprolactam-*co*-acrylamide) microparticles.

Figure 2 shows the Scanning electron micrographs of poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing 10 wt.% of 5-FU.

Figure 3 shows the Particle size distribution curve (measured by dynamic light scattering method) of poly(*N*-vinyl caprolactam-*co*-acrylamide) microparticles dispersed in aqueous media containing 10 wt.% of 5-FU.

Figure 4 shows the % Cumulative release of 10 wt.% of 5-FU from poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles having different ratios of acrylamide:vinyl caprolactam monomers at the physiological temperature of 37°C: different symbols in the diagram represent different % wt. ratios as: (×) 100:00; (■) 90:10; (♦) 80:20 and (▲) 70:30.

Figure 5 shows the % Cumulative release of 10 wt.% of 5-FU from poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing different weight % ratios of acrylamide:vinyl caprolactam monomers at 25°C: symbols represent the % ratios as: (■) 90:10; (♦) 80:20 and (▲) 70:30.

Figure 6 shows the % Cumulative release of 10 wt.% of 5-FU through poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing different amounts of crosslinking agent at 37°C: symbols are representative of different amounts (weight %) of crosslinking agent used viz., *N,N'*-methylene bisacrylamide: (■) 1 %, (•) 2% and (▲) 3%.

Figure 7 shows the % Cumulative release of 10 wt.% of 5-FU from poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing different weight % of 5-FU with respect to weight of the copolymer at 37 °C: symbols for different weight % of drug loaded in the matrix system: (■) 5 %, (•) 10 % and (▲) 15 %.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

The term "copolymer" as used herein refers to the copolymer of *N*-vinyl caprolactam and acrylamide monomers. The abbreviation "*co*" is used in between the names of the two monomers to represent the derived polymer as "copolymer". Different monomer ratios used are described in all the diagrams.

The term "5-FU" as used herein refers to "5-fluorouracil", which is available in a crystalline form.

The term "drug loading" means the amount of drug (5-FU) loaded in weight percent units with respect to weight of the polymer during the preparation of microparticles from the copolymer solution.

The term "encapsulation" means the amount of drug encapsulated in the delivery device, such as copolymer, as described in this embodiment. It is estimated in % units. The scope of micro-encapsulation techniques are summarized or exemplified in "Novel drug delivery and its therapeutic application" L. F. Prescott & W. S. Nimmo, Ed. John Wiley & Sons, which is hereby incorporated by reference in its entirety. The term "encapsulating" is intended to cover any means of incorporation of a drug in a delivery device including, but not limited to, embedding, dispersing, forming a solid solution, coating, associating, covalent or non-covalent bonding and the like.

The term "cumulative release" means the amount of drug released in a definite time interval controlled in the *in vitro* experiments.

The term "swelling" means the extent of increase in the size of the delivery matrix. In the present art, it is calculated by measuring the extent of weight gain of the microparticles in the medium of interest.

The term, "microparticle" means that particles having diameters in the micron size range. These can be prepared from different types of polymers such as homopolymer, copolymers, graft polymers or graft copolymers, etc. Microparticles of different types as prepared by oil-in-water phase, methods of their preparation and drug encapsulation are disclosed in several US patents (Nos. 4,690,825 (Won), 4,873,091 (Jankower et al.), 5,073,365 (Katz et al.), 5,135,740 (Katz et al.) and 5,145,675 (Won et al.)).

The term "delivery device" means a device in which drug is embedded, dispersed, coated or encapsulated inside the matrix system and it protects the drug from the surroundings. Delivery devices are obtained from biodegradable or biocompatible polymers as solid, water-insoluble, water-soluble, etc., forms.

Within the meaning of the present invention the expression "controlled release" means any formulation technique wherein release of the active substance from the dosage form is modified to occur at a slower rate than that from an immediate release product, such as a conventional swallow tablet or capsule. The term "controlled release" includes formulations exhibiting a slow release, delayed release, sustained release, pulsed release or comparable release profiles.

Microparticles are spherical with the average diameters ranging from about 1 μm to about 500 μm. Their average diameter can be determined by dynamic light scattering technique, called zeta-sizer.

Other terms or nomenclatures for all the chemicals used in process development include:

AAm- acrylamide monomer,

VCL-vinyl caprolactam monomer

NNMBA-, *N,N'*-methylenebisacrylamide, the crosslinking agent

Poly(VCL-*co*-AAm)- copolymer of vinyl caprolactam and acrylamide, collectively written as poly(*N*-vinyl caprolactam-*co*-acrylamide)

Semi-IPN- is a system prepared by two different polymers, of which one is crosslinked in the presence of another polymer.

Drugs include therapeutic compounds suitable for the treatment of diseases including, but not limited to cancer, AIDS, auto-immune diseases such as arthritis, and the like.By "drug" or "pharmacologically active compound" suitable for controlled-release formulations according to the invention, it shall be understood an agent causing a valuable effect in vivo, such as a bioactive effect, a therapeutic effect, or the like. A pharmacologically active compound can be any organic, inorganic or living agent that is biologically active. It can be a protein, a polypeptide, a polysaccharide (e.g. heparin), an oligosaccharide, a mono-or disaccharide, an organic compound, an organometallic compound or an inorganic compound containing any element. It can be a living or dead cell, bacterium, a virus or a part thereof. It can be a biologically active molecule such as a hormone, a growth factor, a growth factor producing virus, a growth factor inhibitor, a growth factor receptor, an integrin blocker (e.g. a IIa/IIIb inhibitor) or a complete or partial functional gene in sense or antisense orientation in a suitable expression vector or in any other expression vector construct for local delivery of therapeutically active agents. Pharmacologically active agents include those especially useful for long-term therapy, such as hormonal treatment, for example contraception and hormone replacement therapy, and for treatment of diseases such as osteoporosis, cancer, epilepsy, Parkinson's disease and pain. The suitable biologically active agents may be, e.g. anti-inflammatory agents, anti-infective (e.g. antibiotics and antiviral agents), analgesics and analgesic combinations, antiasthmatic agents, anticonvulsants, antidepressants, antidiabetic agents, antineoplastics, anticancer agents, antipsychotics, agents used for cardiovascular diseases.

Drug substances suitable for the treatment of cancer include antimetabolites (including nucleoside analogs), platinum-based agents, alkylating agents, tyrosine kinase inhibitors, anthracycline antibiotics, vinca alkloids, proteasome inhibitors, macrolides, and topoisomerase inhibitors. These include chemotherapeutic agents including, but not

limited to, carboplatin, navelbine® (vinorelbine), anthracycline (Doxil), lapatinib (GW57016), Herceptin, gemcitabine (Gemzar®), capecitabine (Xeloda®), alimta, cisplatin, 5-fluorouracil, epirubicin, cyclophosphamide, avastin, velcade®, etc.

Anti-cancer drugs are well known and include: Acivicin®; Aclarubicin®; Acodazole Hydrochloride®; Acronine®; Adozelesin®; Aldesleukin®; Altretamine®; Ambomycin®; Ametantrone Acetate®; Aminoglutethimide®; Amsacrine®; Anastrozole®; Anthramycin®; Asparaginase®; Asperlin®; Azacitidine®; Azetepa®; Azotomycin®; Batimastat®; Benzodepa®; Bicalutamide®; Bisantrene Hydrochloride®; Bisnafide Dimesylate®; Bizelesin®; Bleomycin Sulfate®; Brequinar Sodium®; Bropirimine®; Busulfan®; Cactinomycin®; Calusterone®; Caracemide®; Carbetimer®; Carboplatin®; Carmustine®; Carubicin Hydrochloride®; Carzelesin®; Cedefingol®; Chlorambucil®; Cirolemycin®; Cisplatin®; Cladribine®; Crisnatol Mesylate®; Cyclophosphamide®; Cytarabine®; Dacarbazine®; Dactinomycin®; Daunorubicin Hydrochloride®; Decitabine®; Dexormaplatin®; Dezaguanine®; Dezaguanine Mesylate®; Diaziquone®; Docetaxel®; Doxorubicin®; Doxorubicin Hydrochloride®; Droloxifene®; Droloxifene Citrate®; Dromostanolone Propionate®; Duazomycin®; Edatrexate®; Eflornithine Hydrochloride®; Elsamitrucin®; Enloplatin®; Enpromate®; Epipropidine®; Epirubicin Hydrochloride®; Erbulozole®; Esorubicin Hydrochloride®; Estramustine®; Estramustine Phosphate Sodium®; Etanidazole®; Etoposide®; Etoposide Phosphate®; Etoprine®; Fadrozole Hydrochloride®; Fazarabine®; Fenretinide®; Floxuridine®; Fludarabine Phosphate®; Fluorouracil®; Flurocitabine®; Fosquidone®; Fostriecin Sodium®; Gemcitabine®; Gemcitabine Hydrochloride®; Hydroxyurea®; Idarubicin Hydrochloride®; Ifosfamide®; Ilmofosine®; Interferon Alfa-2a®; Interferon Alfa-2b®; Interferon Alfa-n1®; Interferon Alfa-n3®; Interferon Beta-I a®; Interferon Gamma-I b®; Iproplatin®; Irinotecan Hydrochloride®; Lanreotide Acetate®; Letrozole®; Leuprolide Acetate®; Liarozole Hydrochloride®; Lometrexol Sodium®; Lomustine®; Losoxantrone Hydrochloride®; Masoprocol®; Maytansine®; Mechlorethamine Hydrochloride®; Megestrol Acetate®; Melengestrol Acetate®; Melphalan®; Menogaril®; Mercaptopurine®; Methotrexate®; Methotrexate Sodium®; Metoprine®; Meturedepa®; Mitindomide®; Mitocarcin®; Mitocromin®; Mitogillin®; Mitomalcin®; Mitomycin®; Mitosper®; Mitotane®; Mitoxantrone Hydrochloride®;

Mycophenolic Acid®; Nocodazole®; Nogalamycin®; Ormaplatin®; Oxisuran®; Paclitaxel®; Pegaspargase®; Peliomycin®; Pentamustine®; Peplomycin Sulfate®; Perfosfamide®; Pipobroman®; Piposulfan®; Piroxantrone Hydrochloride®; Plicamycin®; Plomestane®; Porfimer Sodium®; Porfiromycin®; Prednimustine®; Procarbazine Hydrochloride®; Puromycin®; Puromycin Hydrochloride®; Pyrazofurin®; Riboprine®; Rogletimide®; Safingol®; Safingol Hydrochloride®; Semustine®; Simtrazene®; Sparfosate Sodium®; Sparsomycin®; Spirogermanium Hydrochloride®; Spiromustine®; Spiroplatin®; Streptonigrin®; Streptozocin®; Sulofenur®; Talisomycin®; Taxol®; Taxotere®; Tecogalan Sodium®; Tegafur®; Teloxantrone Hydrochloride®; Temoporfin®; Teniposide®; Teroxirone®; Testolactone®; Thiamiprine®; Thioguanine®; Thiotepa®; Tiazofurin®; Tirapazamine®; Topotecan Hydrochloride®; Toremifene Citrate®; Trestolone Acetate®; Triciribine Phosphate®; Trimetrexate®; Trimetrexate Glucuronate®; Triptorelin®; Tubulozole Hydrochloride®; Uracil Mustard®; Uredepa®; Vapreotide®; Verteporfin®; Vinblastine Sulfate®; Vincristine Sulfate®; Vindesine®; Vindesine Sulfate®; Vinepidine Sulfate®; Vinglycinate Sulfate®; Vinleurosine Sulfate®; Vinorelbine Tartrate®; Vinrosidine Sulfate®; Vinzolidine Sulfate®; Vorozole®; Zeniplatin®; Zinostatin®; Zorubicin Hydrochloride®, 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta-lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole;

carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflomithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-I receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone;

mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anti cancer compound; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista;

suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Drug agents suitable for the treatment of HIV/AIDS are broadly classified into three categories, namely: (1) Nucleoside Reverse Transcriptase Inhibitors (NRTI), which include lamivudine, zidovudine, didanosine, abacavir, stavudine, and zalcitabine. (2) Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI), which include nevirapine, efavirenz, and delavirdine. (3) Protease Inhibitors (PI), which include indinavir, ritonavir, nelfinavir, saquinavir and amprenavir.

In the arena of pharmaceutics/pharmacokinetics, prior-art indicates the use of a variety of copolymers, blends, grafts, etc., to develop drug-loaded CR devices. 5-FU containing formulations have been developed previously by other researchers (P.L. Ritger et. al., J Control Rel 5,1987, 37-42; S.B. Harogoppad and T.M. Aminabhavi, Macromolecules 24, 1991, 2598-2605) using acrylamide and poly(methyl methacrylate) polymers.

Biodegradable copolymers prepared from vinyl caprolactam and acrylamide are potentially useful biomaterials used in CR of hydrophilic drugs like 5-FU. The *in vitro* release data of the copolymers of vinyl caprolactam and acrylamide, cross-linked with NNMBA are influenced by the copolymers, extent of cross-linking agent, amount of drug loading as well as the temperature.

The present invention is a novel approach, in the sense that the copolymers are new to the art and are used for the first time as delivery matrices for 5-FU. They can also be useful

for similar type of other drugs. Microparticles developed with different copolymer compositions in yields of 80-85 %.

Formulations of these microparticles were well characterized by a variety of techniques. For instance, DSC confirmed the uniform distribution of 5-FU in microparticles and SEM suggested spherical nature of the microparticles with rough surface morphologies.

The *in vitro* drug release data indicated that particle size and release kinetics are dependent on copolymer composition, amount of cross-linking agent used and amount of 5-FU loaded in microparticles. These formulations can be used for delivering other types of anticancer drugs.

It is demonstrated that the release of 5-FU can be extended up to 10 h. It is visualized that biomaterials of the present invention would offer significant advantages over other polymers for delivering 5-FU.

Previously, the core shell microparticles were designed and developed following another type of emulsion polymerization method in which the core is formed with methylmethacrylate, while the shell consists of acrylamide (V. Ramesh Babu et. al., Inter. J. Pharma. 325, 2006, 55-62). 5-FU is encapsulated in both the core and the shell. Drug loading was done by two techniques viz., *in situ* polymerization and absorption/adsorption methods. The *in situ* method showed longer release rates than the absorption/adsorption method.

Further it has been reported that the microparticles prepared from acrylamide monomer using the dispersion polymerization method (M. Sairam et.al., Inter. J. Pharma. 320, 2006, 131-136) in the size range of 5 μm with high encapsulation efficiency of 80 % for 5-FU. The 5-FU was released in 10 h in a desired slow release manner.

According to Sairam *et al.*, two different cross-linking agents viz., *N',N'*-methylene bisacrylamide (NNMBA) and ethyleneglycol dimethacrylate (EGDMA) were used to produce these microparticles. NNMBA produced microparticles with higher hydrophilicility than the hydrophobic EGDMA. The overall hydrophilic nature of NNMBA resulted in high encapsulation efficiency of 5-FU up to 82 % when compared to 66 % by the microparticles prepared using EGDMA. The release of 5-FU from these

hydrophilic microparticles was faster than the hydrophobic matrix prepared using EGDMA.

K.M. Reddy et al., (J. Applied Polymer Sci., 107, 2008, 2820-2829) teaches that semi-interpenetrating polymer networks (semi-IPNs) were prepared using biodegradable polymer such as sodium alginate (NaAlg) and incorporating it with *N*-isopropylacylamide (NIPAAm) to generate the semi-IPN structure. The 5-FU was encapsulated into these microparticles and its *in vitro* release in gastric media was continued up to 12 h. The matrix exhibited temperature-sensitive behavior and the release of 5-FU was investigated at temperatures of 25° and 37°C.

Considerable therapeutic advantage can be gained if an anticancer drug like 5-FU can be formulated to deliver in a controlled manner. An important feature of this invention is that the methods of preparing poly(vinyl caprolactam-*co*-acrylamide) copolymer matrices are completely free from toxic solvents that are generally used in producing microparticles by solvent evaporation and other similar methods. These copolymers are developed for the first time and are prepared in aqueous media, thus eliminating the costly and toxic organic solvents. In addition, microparticles are prepared by *in situ* free radical polymerization technique without recourse to the conventional methods of preparing microparticles. The advantage of the method is that it involves a single step of preparing polymers, producing the microparticles and simultaneously encapsulating the drug thereby obviating the disadvantages associated with conventional methods. This method is equally applicable to other drugs, including anti-cancer drugs.

In vitro release data are affected by varying copolymer composition, amount of crosslinking agent and amount of drug. Microparticles with different copolymer compositions are obtained in yields up to 80-85 % with encapsulation efficiencies ranging from 69 to 79%. DSC data indicated uniform distribution of 5-FU particles in the matrix and SEM confirmed the spherical nature of particles with rough surface morphology.

The *in vitro* drug release data indicated that particle size and release kinetics are influenced by copolymer composition, amount of crosslinking agent and amount of 5-FU present in the microparticles.

The following examples are included to demonstrate the preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples, which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments, which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES ***Materials***

Vinyl caprolactam (VCL) was purchased from Aldrich Chemicals, Milwaukee, WI, USA. Acrylamide (AAm), *N,N'*-methylenebisacrylamide (NNMBA), sodium laurylsulfate, potassium persulfate and calcium chloride were all purchased from S.D. Fine Chemicals (Mumbai, India). 5-FU was purchased from MP Biochemicals, Eschwege, Germany.

EXAMPLE 1: Preparation of poly(*N*-Vinyl caprolactam-co-acrylamide) microparticles

Sodium laurylsulfate (1 g) was dissolved in 80 mL of water taken in a three-necked round bottom flask equipped with a mechanical stirrer, a condenser and a gas inlet to maintain the inert nitrogen atmosphere. The flask was immersed in an oil bath with a thermostatic control to maintain the desired temperature accurately within the precision of ± 1°C. The solution was stirred at 800 rpm speed until it became clear and 100 mg of potassium persulfate initiator was added. In the preparation, calculated amounts of AAm, VCL, crosslinking agent (NNMBA) and 5-FU were used on weight basis. These were all dissolved separately in 5 mL water. Individual solutions of each were then mixed in a beaker to form 20 mL of the total volume of the solution. This solution was added to the emulsion mixture of sodium lauryl sulfate prepared above (80 mL) in a drop-wise manner using a dropping funnel. The 100 mL total solution was taken in a three-necked round bottom flask and the mixture was heated to 70°C for 80 h to obtain nearly 70-80 % yield of the final product. After 8 h, the above reaction mixture was added drop-wise to 1 % calcium chloride solution to break the emulsion

Particles were then isolated by centrifuging the product at the rotor speed of 12,000 rpm, washed with water and dried under vacuum at 40^0C for 24 h. Eight different batches of formulations were prepared by varying the amounts of monomer as well as drug and crosslinking agent. The composition of all the formulations viz., VCL-AAm-1 to VCL-AAm-8 are given in Table I.

Table I. Formulation data of poly(VCL-*co*-AAm) microparticles

Code	Weight % VCL	Weight % AAm	Weight % NNMBA	Weight %5-FU	%EE ± SD	Size (μm) ± SD
VCL-AAm-1	20	80	1	5	71 ± 1	29 ± 6
VCL-AAm-2	20	80	1	10	75 ± 2	31 ± 8
VCL-AAm-3	20	80	1	15	79 ± 2	34 ± 6
VCL-AAm-4	20	80	2	10	76 ± 9	28 ± 4
VCL-AAm-5	20	80	3	10	72 ± 8	16 ± 2
VCL-AAm-6	10	90	1	10	69 ± 6	30 ± 4
VCL-AAm-7	30	70	1	10	72 ± 5	24 ± 1
VCL-AAm-8	0	100	1	10	73 ± 1	22 ± 8

EE = Encapsulation efficiency was calculated using formula (2) below

SD = Standard deviation calculated at 95 % confidence limit

EXAMPLE 2: CHARACTERIZATION OF CR PARTICLES

A) Differential scanning calorimetric (DSC) studies. Differential scanning calorimetric (DSC) curves were recorded on a Rheometric scientific differential scanning calorimeter (Model-DSC SP, UK). The instrument was calibrated using indium as the standard. Samples were heated in sealed aluminum pans between 30° and 400°C at the heating rate of 10°C/min under an inert nitrogen purge gas at the rate of 20 mL/min. DSC tracings of pure 5-FU, drug-loaded microparticles and plain microparticles are displayed in **Figure 1**. The onset-melting peak of 5-FU was observed at 285.16°C. However, no characteristic peak of 5-FU was observed in the DSC curves of the drug-loaded microparticles, suggesting that drug is molecularly dispersed in the polymer matrix.

B) Scanning electron microscopic (SEM) studies. Morphology of the microparticles was studied by SEM. Micrographs of the dry micro particles in powder form are recorded using Leica 400, Cambridge, UK instrument. **Figure 2** displays the SEM photograph,

wherein the morphology of micro particles can be observed. The microparticles are spherical with diameters around 10 μm; their surfaces are smooth, but some micro particles have microporous surface structures. The initial burst release of 5-FU as observed in Figures 4 to 6 is attributed to the quick diffusion of 5-FU through the water swollen microporous matrix structures. The observed differences in the release patterns are due to the differences in surface morphologies of the particles.

C) Particle size and encapsulation efficiency

Size distribution of microparticles was further confirmed by particle size analyzer (Mastersizer 2000, Malvern Instruments, UK) equipped with dry accessory system. Results of mean particle size with standard deviations and encapsulation efficiency for different formulations are presented in **Table I.** Initial drug loading of the matrix was 5, 10 and 15 wt. % with respect to weight of the copolymer. Cross-linking agent (NNMBA) was varied as 1, 2 and 3 % with respect to weight of the copolymer. These variations are attempted to establish optimum release of 5-FU over an extended time up to 10 h. Other variations were tried by varying the monomer ratios. These details are given in **Table I.** In the case of VCL-AAm-1 to VCL-AAm-5, the VCL monomer was maintained at 20 wt. % with the remaining of 80 wt. % of AAm. For formulations VCL-AAm-6 and VCL-AAm-7, the quantities of VCL and AAm were varied by keeping the cross-linking agent and 5-FU as constant. In formulation VCL-AAm-8, no VCL was used, but pure (100 %) acrylamide was polymerized using 1 wt. % of the cross-linking agent and 10 wt. % of 5-FU. In all cases and with all the above-mentioned variations, encapsulation efficiency varied from 69 to 79%, whereas the particle size ranged between 22 and 34 µm, except in case of VCL-AAm-5, for which particle size was smaller i.e., 16 µm. This is possibly due to the rigid nature of the polymer matrix at higher (3 wt. %) amount of cross-linking agent.

The size distribution curve of VCL-AAm-5 formulation according to **Figure 3** is broad and volume mean diameter of the microparticles is 16 μm. There were differences in particle size of the formulations containing different amounts of cross-linking agent (i.e., 1, 2 and 3 %) are, respectively 34, 28 and 16. This trend suggested the possibility of increasing chain rigidity of the copolymer with a reduction in the size of microparticles at

higher concentration of cross-linking agent. It is postulated that the decrease in particle size with increasing amount of cross-linking agent is due to the formation of a rigid matrix of the copolymer.

The % encapsulation efficiency (see Eq. 2) is the extent of drug remaining after complete processing of the formulations. These results depend upon the nature of the delivery device as well as the rigidity or flexibility of the copolymer. More flexible polymers generally incorporate higher amount of drug than the rigid ones. The encapsulation efficiency data given in **Table I** vary from 69-79 %, depending upon the initial loading of 5-FU. For instance, higher initial loading (15 wt. %) of 5-FU offered higher encapsulation efficiency and vice versa. For formulations VCL-AAm-1, VCL-AAm-2 and VCL-AAm-3, the percentage of encapsulation efficiency increased systematically i.e., 71, 75 and 79, respectively with increasing drug content viz., 5, 10 and 15 wt. % of the matrices. For higher cross-linking agent (i.e., 2 or 3 wt. % of NNMBA) used in the matrix, the % encapsulation efficiency is low, due to the rigid nature of the copolymer matrix. However, the highest % encapsulation efficiency of 79 is observed for VCL-AAm-3 formulation (20 wt. % of VCL and 80 wt % AAm) that contained 15 wt. % of 5-FU with only 1 % of NNMBA. The lowest encapsulation efficiency (69 %) found in case of VCL-AAm-6 formulation showed the mean particle size of 30 μm.

All our findings suggest that in order to develop an efficient delivery system with the best possible optimum results, it is very necessary to control all process parameters as explained above.

EXAMPLE 3: FACTORS INFLUENCING PARTICLE PROPERTIES

A) Estimation of drug loading and encapsulation efficiency.

Loading efficiency of 5-FU by the microparticles was determined UV spectrophotometer. About 10 mg of the drug-loaded microparticles were placed in 10 mL of buffer solution and stirred vigorously for 48 h to extract the drug from the microparticles. The solution was filtered and assayed by UV spectrophotometer at the fixed λ_{max} value of 270 nm. The results of % drug loading and encapsulation efficiency were calculated, respectively using Eqs. (1) and (2) and results are compiled in **Table I.**

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in beads}}{\text{Amount of beads}} \right) x100 \quad (1)$$

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}} \right) x100 \quad (2)$$

B) Formation of the copolymer. The yield of microparticles produced was determined gravimetrically. After copolymerization, the latex solution was added to 1 % calcium chloride solution and centrifuged to isolate the particles from the mixture. The microparticles were washed several times successively with water and methanol solvents to remove the remaining monomer, the initiator, and then dried in vacuum oven at 50°C until attainment of constant weight. The % conversion of monomers was calculated as:

$$\% \text{ Conversion} = (W/M) \times 100 \quad (3)$$

where W is weight of the dry copolymer obtained from the latex sample and M is the weight of monomers. The yield of microparticles varied between 80 and 85 % for various formulations prepared in this study.

C) Effect of acrylamide content

Cumulative release data of 5-FU from the formulations prepared by varying different amounts of monomers are displayed in **Figure 4**. These experiments are done to show that higher the amount of AAm in the matrix higher will be the release rates. It is observed that for microparticles that containing lower amounts of AAm, the release of 5-FU is also slower than those that contained higher amount of AAm. As discussed previously, the drug release rates depend upon the size of microparticles as well as the crystallinity of the drug, surface properties, polymer composition, swelling ratio, and encapsulation efficiency. In the systems of this embodiment, it is observed swelling rates of the matrix are influenced by the presence of AAm and both these parameters have affected the drug release rates. The rapid release of > 98% of 5-FU from the formulation containing (10 wt. %) of AAm is due to hydrophilic nature of the copolymeric matrix.

D) Effect of temperature

Microparticles of this invention are influenced by change in temperature from 25° to 37°C. At 25°C (i.e., in the swollen state), the release of 5-FU and the total amount of 5-FU released are considerably higher than those observed at 37°C (i.e., in a collapsed state of the copolymer). Under these conditions, 5-FU particles that are entrapped inside the polymer network will diffuse out of the microparticles, since they are quickly hydrated in the swollen state, thus allowing the transport of more of 5-FU from the delivery system. In contrast, at 37°C, the network structure is collapsed, thus exhibiting a lesser tendency to uptake water molecules or buffer solution, leading to a decrease in drug diffusion from the matrix.

Temperature has thus shown distinctive effect on the release of 5-FU as shown by the cumulative release data displayed in **Figures 4 and 5**, respectively for microparticles prepared by varying VCL. The 5-FU was released slower at 37°C (above the LCST of 32°C), but its release was much faster at 25°C (below the LCST than at 37°C). The release of 5-FU from the devices thus varied, depending upon the monomer ratios of the copolymer. As shown in **Figure** 5, the release of 5-FU decreased considerably at increaseing amount of VCL in the copolymer, resulting in the slow release of 5-FU. For formulations prepared by taking 100 % AAm, the release of 5-FU much faster and is higher than those formulations prepared using copolymers. For all formulations, 5-FU release is completed in about 10 h.

E) Effect of crosslinking agent

Previous studies indicated an effect of crosslinking agent on the formulated products, especially in terms of affecting their release patterns. In the present embodiment, it is demonstrated that the presence of cross-linking agent has also has shown an affect on the release of 5-FU and these results are displayed in **Figure 6**. Similar findings on other polymers were reported previously for the encapsulation of 5-FU (M. Sairam et. al., Inter. J. Pharma. 320, 2006, 131-136). Notice that at higher concentration of NNMBA in the copolymer, crosslink density of the matrix increased due to increased rigidity of the final product by slowly releasing 5-FU. At lower concentration of the crosslinking agent, fast and higher cumulative release of 5-FU is observed.

F) Effect of drug loading

Extent of drug loading has an effect on encapsulation efficiency of the matrix. Such details are explained in (V. Ramesh Babu et.al., Inter. J. Pharma. 325, 2006, 55-62,. In the present invention, release profiles of formulations prepared from poly(VCL-*co*-AAm) microparticles are displayed in **Figure 7** for different loadings of 5-FU. Notice that in the first hour, the release is quite fast for all formulations, but at later stage, it becomes slow. These findings follow similar trends to those published previously for 5-FU-loaded microparticles prepared from poly(acrylamide) and also the copolymers of acrylamide with methyl methacrylate (M. Sairam et. al., Inter. J. Pharma. 320, 2006, 131-136; V. Ramesh Babu et. al., Inter. J. Pharma. 325, 2006, 55-62). From the examination of release data, the formulations containing maximum amount of 5-FU (i.e., 15 wt. %) displayed higher release rates than those containing minimum amounts of 5-FU (i.e., 10 and 5 wt. %). The slow release is persistent also for the formulation containing lower amount of 5-FU (i.e., 5 wt. %).

EXAMPLE 4: DRUG RELEASE PROFILES

A) *In-vitro* release study

Dissolution was carried out using a tablet dissolution tester (LabIndia, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37°C under 100 rpm speed. Drug release from the microparticles was studied in 7.4 pH phosphate buffer solution. Aliquot samples were withdrawn at regular time intervals and analyzed by UV spectrophotometer as explained before.

B) Drug release and diffusion kinetics

In vitro release kinetics of 5-FU was assessed by studying the time-dependent cumulative release. There is enough prior evidence about the analysis of such data (see for instance, P.L. Ritger and N.A. Peppas, J Control Rel 5, 1987, 37-42). The analysis of release data was done using the empirical relationship given in Eq. (4).

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \qquad (4)$$

In Eq. (4), the quantity M_t/M_∞ represents the fractional amount of 5-FU release at time, *t;* *k* is the kinetics rate constant that is characteristic of the formulation and *n* is an empirical parameter, whose values suggest the nature of the release mechanism. Using the least-squares fittings and analysis of the release data from log (M_t/M_α) vs log t plots, the values of *n* and *k* have been calculated with the statistically estimated errors for all eight formulations of this invention. It is known that, if $n = 0.5$, then drug diffuses and releases out of the microparticles due to Fickian trend. If $n > 0.5$, then anomalous or non-Fickian transport is observed. For $n = 1$, non-Fickian or more commonly called Case II release kinetics is operative. In formulations of this invention, the values of *n* ranging between 0.5 and 1 indicate anomalous type transport through the delivery device. These observations are similar to those published (*See* S.B. Harogoppad and T.M. Aminabhavi, Macromolecules, 24, 1991, 2598-2605).

The results of *k* and *n* calculated from Eq. 4 are dependent on the concentration of the cross-linking agent used, % drug loading and AAm content of the final product. Thus, the values of *n* for microparticles prepared by taking 90, 80 and 70 wt. % of AAm monomer in microparticles, keeping 5-FU (10 wt. %) and 1 wt. % NNMBA constant, ranged from 0.56 to 0.76, suggesting the anomalous transport. These findings are in agreement with the work published previously on similar type of studies (V. Ramesh Babu et. al., J. Appl. Polym. Sci. 99, 2006, 2671-2678) in which the effects of monomer ratios in the copolymer have shown an effect on dissolution kinetics. The kinetic rate constants (*k*) are quite small for drug-loaded microparticles, suggesting insignificant interaction of 5-FU with the delivery matrix containing varying amounts of AAm. With the placebo matrices (having no drug), water is transported, giving lower values of *k*. It is likely that hydrophilic 5-FU will attract more water molecules due to the Donnan equilibrium effect, resulting in the rapid diffusion of 5-FU from the matrices as result of polymer chain relaxation (T.K. Lee et. al., Science, 213, 1981, 233-235).

Diffusion coefficients, *D*, of water through the placebo or drug-containing matrices would aid in understanding transport trends; these were calculated using the equations proposed before (A. R. Kulkarni et. al., J. Control. Rel. 63, 2000, 97-105)

$$D = \left(\frac{r\theta}{6M_\infty}\right)^2 \pi \qquad (5)$$

In the above equation, θ is the slope of the linear portion of the plot of log M_t/M_α vs log $t^{1/2}$, where r is the radius of spherical particles, and M_α is the maximum sorption. Diffusion coefficients calculated assuming the Fickian diffusion are in the range (0.47-0.83) x 10^{-5} cm^2/s, which are dependent on the extent of crosslinking of the matrix. For instance, D values decrease systematically with increased cross-linking of the matrix, which is due to increased rigidity of the network chain.

EXAMPLE 5: BIODEGRADATION PRODUCTS AND TOXICITY PROFILE

PVCL is a homologue of poly(*N*-vinylpyrrolidone) (PVP), another biocompatible polymer that is widely used in the area of pharmaceutics. PVCL has been toxicologically assessed for its suitability in cosmetic preparations. On the basis of the information available at our disposal, provided that the recommended concentrations and fields of application are adhered to, there is no evidence of any toxicological risks associated with its use (BASF Technical Information, July 2005, Supersedes issue, May 2005).

PVCL is also known to be very stable against hydrolysis and is a biocompatible polymer. The amide group in the lactam ring is directly connected to carbon-backbone chain. PVCL does not break easily hydrolytically, but if it is hydrolysed, the carboxylic acid group builds up and small toxic amide compounds will not form, like in case of PNIPAm. Major disadvantage for PVCL is its non-biodegradable nature and is known to absorb numerous organic compounds from water. Even though it is less popular than PNIPAm, the characteristics of PVCL have been studied and some applications of PVCL in the area of biotechnology and biomedicine are available. Enzyme immobilization has been achieved with the help of PVCL, and the stability of enzymes has been increased with PVCL-based hydrogel that will protect enzymes from denaturation by entrapment as an ingredient in wound-healing film (Makhaeva, E et al., Makromol. Chem.Phys. 1996, 197, 1973-1982). PVCL has been utilized in multi-layered glass materials in the 1960's

(Patent, 1968, DE1285124). Commercially, PVCL is available as a hair-fixative excipient under the trade name, Luviskol® Plus by the BASF company. Acrylamide is also a proven material by the FDA that it has no harmful products after degradation. The copolymer of PVCL and AAm are thus regarded to be biocompatible, since its decomposed products include the release of carbon dioxide that is neither harmful nor toxic. However, the copolymers of acrylamide as hydrogels have been used for biomedical applications (Sommadossi et al., Int. J. Biol. Chem. 1982, 257, 8171-8176).All compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure.

While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are chemically or physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. Accordingly, the invention is not limited except as by the appended claims. While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All patents and published or unpublished patent applications and published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

CLAIMS

1. A method for manufacture of drug-containing microparticles of a copolymer by free radical emulsion polymerization *in situ* in an aqueous medium, the process comprising:
providing a first monomer, a second monomer, a cross-linking agent and an initiator, wherein each compound is suspended in water;
providing a drug substance suspended in water;
providing an emulsion comprising a surfactant and water;
adding the aqueous suspensions of the first monomer, the second monomer, the cross-linking agent, the initiator and the drug substance to the emulsion under conditions that allow formation of microparticles comprising the drug substance; and
isolating drug-containing copolymer microparticles.

2. The method of claim 1, wherein the drug-containing copolymer microparticles are controlled release formulations of the drug substance.

3. The method of claim 1 or 2, wherein:
the first monomer is an acrylamide monomer;
the second monomer is a vinyl caprolactam monomer; and
the cross-linking agent is *N.N'*-methylene-*bis*acrylamide.

4. The method of claim 3, wherein the microparticles comprise poly(*n*-vinyl caprolactam-*co*-acrylamide).

5. The method of claim 3, wherein the initiator is potassium persulfate.

6. The method according to any of claims 1-3, wherein the drug substance is a therapeutic agent suitable for the treatment of AIDS, an agent suitable for the treatment of rheumatoid arthritis, an anti-inflammatory agent, an anti-infective, an antibiotic, an antiviral agent, an analgesic or analgesic combination, an antiasthmatic agent, an anticonvulsant, an antidepressant, an antidiabetic agent, an antineoplastic, an anticancer agent, an antipsychotic, or an agent suitable for treating cardiovascular diseases..

7. The method of claim 6, wherein the anti-cancer drug is 5-fluoro-uracil (5-FU).

8. The method according to any of claims 1-7, wherein the formation of the microparticles and encapsulation of the drug substance occur in a single step.

9. A microparticle comprising poly(*n*-vinyl caprolactam-*co*-acrylamide) microparticles containing a drug substance wherein the microparticle is formed by a process comprising:
providing an aqueous suspension of an acrylamide monomer;
providing an aqueous suspension of a vinyl caprolactam monomer;
providing an aqueous suspension of *N.N'*-methylene-*bis*acrylamide, as a cross-linking agent;
providing an aqueous suspension of a drug substance;
adding the aqueous suspensions to an emulsion of a surfactant in water under conditions that allow formation of poly(*n*-vinyl caprolactam-*co*-acrylamide) and encapsulation of the drug substance in a single step.

10. The microparticle of claim 9, wherein the microparticle is a controlled-release formulation suitable for delivery of a therapeutically effective amount of the drug substance.

11. The microparticle of claim 10, wherein the drug substance is selected from the group consisting of a therapeutic agent suitable for the treatment of AIDS, an agent suitable for the treatment of rheumatoid arthritis, an anti-inflammatory agent, an anti-infective, an antibiotic, an antiviral agent, an analgesic or analgesic combination, an antiasthmatic agent, an anticonvulsant, an antidepressant, an antidiabetic agent, an antineoplastic, an anticancer agent, an antipsychotic, and an agent suitable for treating cardiovascular diseases.

12. The microparticle of claim 10, wherein the agent is 5-fluorouracil.

13. The microparticle of claim 10, wherein the controlled-release formulation is suitable for oral, nasal, buccal, ocular, urethral, transmucosal, vaginal, rectal, topical, intraperitoneal, intrathecal, intramuscular, intravitreal or cosmetic delivery of the drug substance.

14. The controlled release formulation of claim 11, wherein the anticancer agent is an antimetabolite, a nucleoside analogs, a platinum-based agent, an alkylating agent, a

tyrosine kinase inhibitor, an anthracycline antibiotic, a vinca alkloid, a proteasome inhibitor, a macrolide, or a topoisomerase inhibitor.

15. The controlled release formulation of claim 11, wherein the therapeutic agent suitable for the treatment of AIDS is selected from the group consisting of:

(1) a Nucleoside Reverse Transcriptase Inhibitor (NRTI), selected from the group consisting of lamivudine, zidovudine, didanosine, abacavir, stavudine, and zalcitabine;

(2) a Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI) selected from the group consisting of nevirapine, efavirenz, and delavirdine; and

(3) a Protease Inhibitor (PI) selected from the group consisting of indinavir, ritonavir, nelfinavir, saquinavir and amprenavir.

16. The microparticle of claim 9, comprising a poly(*n*-vinyl caprolactam-*co*-acrylamide) wherein the vinyl caprolactam and acrylamide are present in a ratio of 1:1 to 1:9 by weight.

17. The microparticle of claim 9, comprising a poly(*n*-vinyl caprolactam-*co*-acrylamide) wherein the *N.N'*-methylene-*bis*acrylamide is between 1-3% w/w.

18. The microparticle according to any of claims 10-13, wherein the drug substance comprises 5, 10 or 15% w/w of the microparticle.

19. The microparticle according to any of claims 10-13, wherein the encapsulation efficiency of the microparticle is between 69% and 79%.

20. The microparticle according to any of claims 10-13, wherein the microparticle has a mean diameter between 16 and 34 μm.

21. The microparticle according to any of claims 10-13, wherein the drug substance is molecularly dispersed within the poly(*n*-vinyl caprolactam-*co*-acrylamide polymer matrix.

22. The microparticle and its process of preparation of poly(*n*-vinyl caprolactam-*co*-acrylamide) as claimed above exemplified herein substantially in the examples and figures.

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Figure 1: Figure 1 shows the DSC thermograms of (A) pure 5-FU, (B) plain poly(*N*-vinyl caprolactam-*co*-acrylamide) called placebo, and (C) 10 wt.% of 5-FU-loaded poly(*N*-vinyl caprolactam-*co*-acrylamide) microparticles.

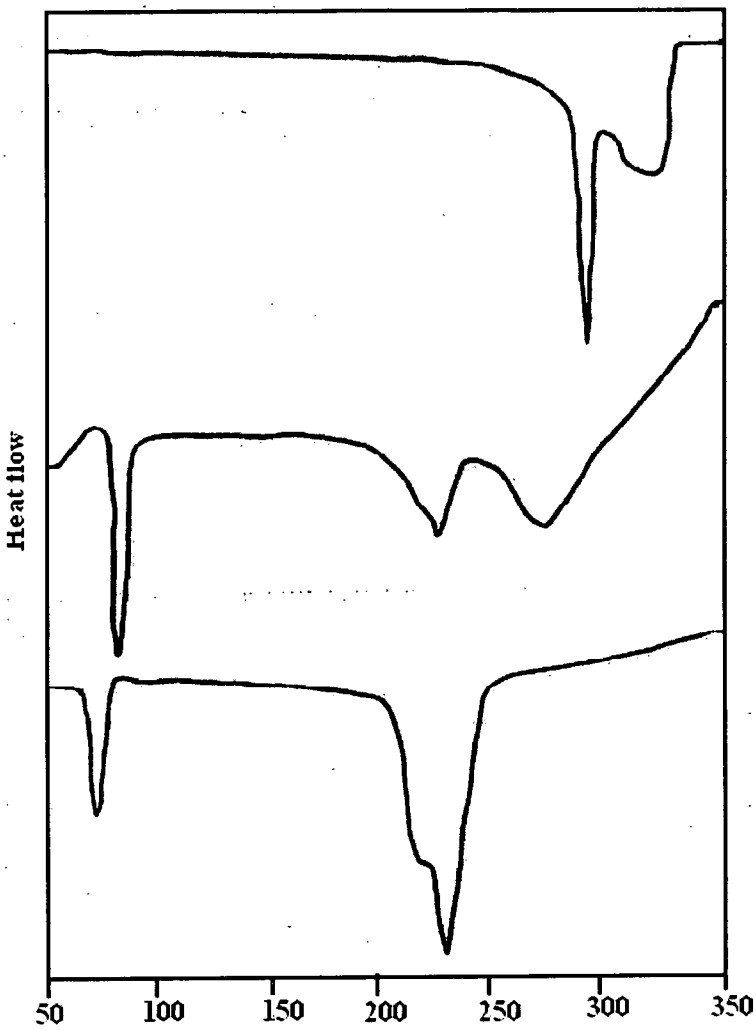

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Figure 2: shows the Scanning electron micrographs of poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing 10 wt.% of 5-FU.

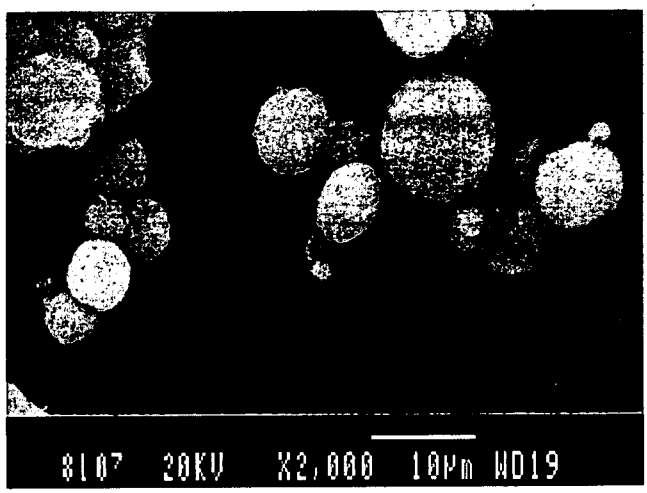

Figure 3: shows the Particle size distribution curve (measured by dynamic light scattering method) of poly(*N*-vinyl caprolactam-*co*-acrylamide) microparticles dispersed in aqueous media containing 10 wt.% of 5-FU.

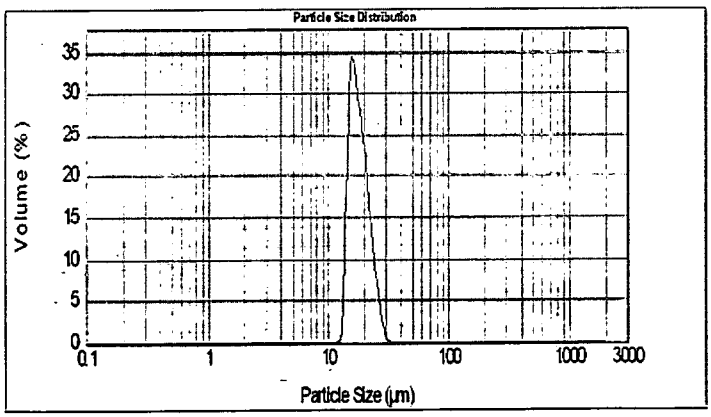

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Figure 4: Figure 4 shows the % Cumulative release of 10 wt.% of 5-FU from poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles having different ratios of acrylamide:vinyl caprolactam monomers at the physiological temperature of 37°C: different symbols in the diagram represent different % wt. ratios as: (×) 100:00; (■) 90:10; (♦) 80:20 and (▲) 70:30.

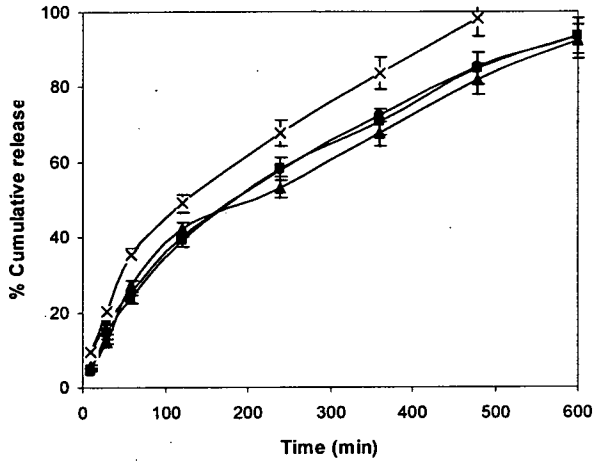

Figure 5: shows the % Cumulative release of 10 wt.% of 5-FU from poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing different weight % ratios of acrylamide:vinyl caprolactam monomers at 25°C: symbols represent the % ratios as: (■) 90:10; (♦) 80:20 and (▲) 70:30.

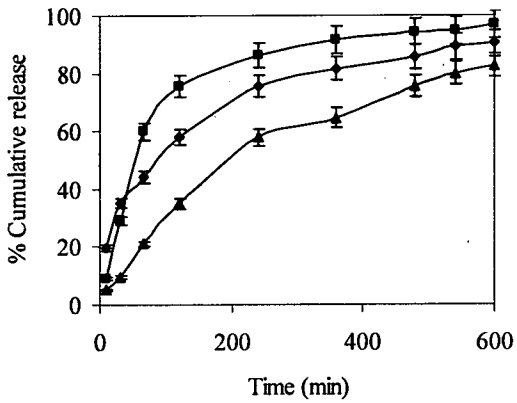

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Figure 6: shows the % Cumulative release of 10 wt.% of 5-FU through poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing different amounts of crosslinking agent at 37°C: symbols are representative of different amounts (weight %) of crosslinking agent used viz., *N,N'*-methylene bisacrylamide: (■) 1 %, (•) 2% and (▲) 3%.

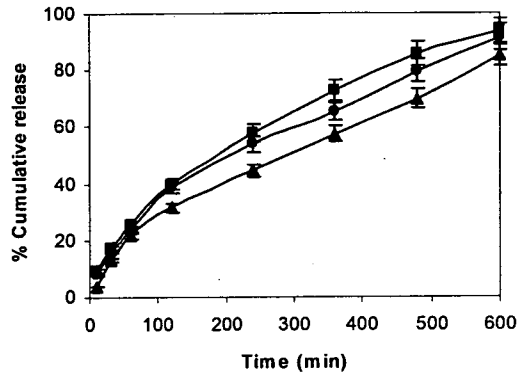

Figure 7: shows the % Cumulative release of 10 wt.% of 5-FU from poly(*N*-vinyl caprolactam-*co*-acrylamide) copolymeric microparticles containing different weight % of 5-FU with respect to weight of the copolymer at 37 °C: symbols for different weight % of drug loaded in the matrix system: (■) 5 %, (•) 10 % and (▲) 15 %.