A SIMPLE TECHNIQUE FOR DETERMINING CONTROLLED RELEASE KINETICS

by

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ABSTRACT

A SIMPLE TECHNIQUE FOR DETERMINING CONTROLLED RELEASE KINETICS

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Master of Science

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A simple technique is developed which measures the hydrolytic release profiles of drugs from a multicomponent polymeric system. A monomer, 1-(N-2-ethylmethacrylcarbamoyl)-5-fluorouracil (EMCF), was prepared by reacting 5-fluorouracil (5-FU) with isocyanatoethyl methacrylate (IEM). EMCF was further copolymerized with methyl methacrylate (MMA) in the molar ratios of 25/75, 50/50, and 75/25. The hydrolytic rates of these copolymers and EMCF were determined by placing the pulverized and sieved samples in gas dispersion tubes of two different sizes. The tubes were immersed in a flask containing one liter of deionized water which was fitted with a mechanical stirrer. The reaction was kept at a constant temperature of $37 \pm 0.5^{\circ}C$ using a water bath. Aliquots of the solution were removed periodically and assayed for the 5-FU concentration spectrophotometrically at 265 nm.

The copolymers showed zero-order kinetics over extended periods of time. In the earlier studies done in this laboratory, Gebelein and Hartsough[21,22] had used a more complex technique involving a dialysis membrane supported in a wire-mesh cage. The present

technique is much easier to assemble than the dialysis membrane/steel cage method. This new technique can be useful to differentiate between different sized samples and also between the different copolymers. The use of the technique provides high reproducibility and the results correlate with the earlier studies.

ACKNOWLEDGMENTS

I dedicate this thesis to my parents and to my advisor Dr. Charles G. Gebelein, without whose encouragement and support, this work would not have been completed. I thank Dr. T. Dobbelstein and Dr. E. Foldvary for reading and correcting this thesis. Also, I thank all the members of the faculty and staff for extending their cooperation.

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CHAPTER I

GENERAL INTRODUCTION AND HISTORICAL

The Developnent Of Cancer Therapy

Cancer chemotherapy evolved during World War I. It was observed that people gassed with mustard gas, [bis(beta-chlorethyl)sulfide], suffered from damage of bone marrow and lymphoid tissue. Further studies on animals revealed that heavy exposure to different nitrogen mustard gases destroyed lynphoid tissue. This led to the idea of using mustard gases in the treatment of lymphoid cancer. Very soon scientists discovered that exposure to this kind of treatment was causing damage to the patient's healthy bone marrow.[1]

Mustard gases are mainly alkylating agents and depend on the electron releasing capacity of their nitrogen atom for their biological action. In order to make these drugs mild in action, scientists made compounds replacing the methyl group with various electron-withdrawing groups (Mustragen or Mechlorethamine).[1] A very promising drug in this line was Uracil Mustard (Figure 1). It inhibits the incorporation of precursors into nucleic acids and the amino acids into t-RNA. Its trade name is DOPAN and it is used in the treatment of Hodgkin's disease, chronic lynphatic leukemia and carcinoma of breast

and ovary. As side effects it causes nausea, bone marrow depression, vomitting, diarrhea and dermatitis.[2,3,4]

Cyclophosphamide (Figure 2) is another nitrogen mustard derivative still used in treating Hodgkin's disease, breast, lung and ovarian cancer.' The alkylating agents such as nitrogen mustards may produce mutations in at least three ways: 1) addition of methyl or ethyl groups to guanine, causing it to behave as a base analog of adenine and thereby producing a pairing error; 2) loss of alkylated guanine bases (depurinated) producing gaps in the DNA replication or a shortened nucleotide sequence; and 3) crosslinking between the strands of same or different DNA molecules causing loss or excision of nucleotides.[5]

Since 1947, a different approach has developed in the treatment of cancer by chemotherapy. This involves using an antagonist which inhibits the biosynthesis of nucleic acids by competitive mechanism. In 1954, Rutman, Canterov, and Paschkis discovered the enhanced utilization of uracil for DNA synthesis in rat hepatoma contrary to normal cells use of ortic acid.[6]

In 1956, Cohen and Barner reported the phenomenon of "thymine1ess death" in bacteria. They observed that E. Coli die when placed in a nutrient medium lacking thymine. Later they suggested the possible use of such compounds inhibiting synthesis of DNA in cancer chemotherapy.[1,7]

In 1957, Heidelberger, et al did some in vitro studies and observed that the conversion of C^{14} -labeled formate into the methyl group of thymine was inhibited by 5-fluorouracil. Since then

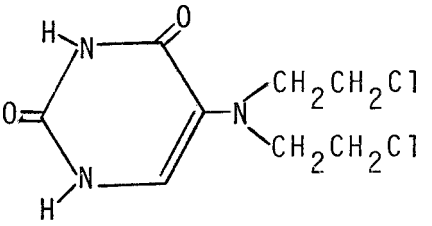


Figure 1. Structure of Uracil Mustard.

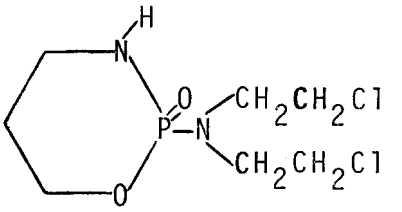


Figure 2. Structure of Cyclophosphamide

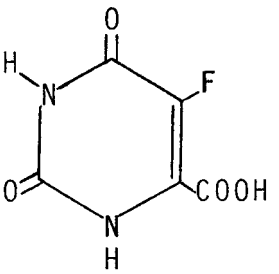


Figure 3. Structure of 5-Fluoroortic acid.

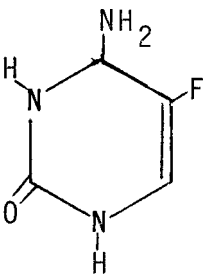


Figure 4. Structure of 5-Fluorocytosine.

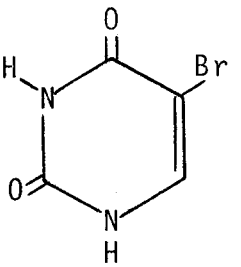


Figure 5. Structure of 5-Bromouracil.

5-fluorouracil has been in use in treating certain cancers. In further studies it was found that the activity of 5-fluorouracil was faster by 25 times, 5 times and 500 times than the activities of 5-fluoroortic acid (Figure 3), 5-fluorocytosine (Figure 4) and 5-bromouracil (Figure 5) respectively.

Heidelberger chose fluorine because it is more similar to the methyl group in thymine in its molecular weight, atomic radii etc. as opposed to bromine or chlorine. In 1957, he also reported the mechanism of action and the results of the first clinical trials on rats of 5-fluorouracil (5-FU) as an anti-cancer agent. The results are summarized below:

1) The LD_{50} for 5-FU in rats was 200 mg/Kg.
2) The maximum tolerated dose over ten days was 25 mg/Kg.
3) 5-FU was effective in treating different kinds of cancer and sarcoma 180.
4) Large dose caused convulsions and resulted in cardiac activity changes.

In order to minimize side effects, Duschinsky, et al synthesized 5-fluorodeoxyuridine and 5-fluorouridine. 5-Fluorouridine showed some promising results when tried on animals.[8,9]

Curreri, et al did the first clinical trials on cancer patients and found 5-FU to be of significant therapeutic value especially for treating breast and gastro-intestinal cancers. 5-FU has also been found to be effective in treating cancers of colon, rectum, skin, thyroid, pancreas, kidney, bladder, uterus, cervix, pharynx, liver, stomach and prostate.[3,8,9,10] It has been found useful in treating

sarcoma 180, adenosarcoma 775 and EO771, Walker carcinosarcoma, ascites tumors and some leukemia strains.[2]

5-FU has been found to be more effective if given by infusion for a few days to a few weeks than if given by mouth.[8,9] Curreri and Ansfield proposed a high initial "loading" dose followed by lower doses until no side effects are seen.[11] It has been observed that doses of 15 mg/Kg 5-FU did not result in side effects for 36 days if given by infusion. Another approach was proposed by Meyers, Young and Chabner in which 5-FU would be administered based on differential intake of 5-FU by cancerous cells as compared to the normal healthy cells. In a study done by Curreri et al increased survival times were noted when 5-FU and 5-FUDR were used in adjunctive therapy.[8] A combination of radiation therapy and 5-FU have been tried by Hall and Good.[5,12]

However, by using the above-mentioned approaches, damage to the normal cells can not be avoided. The damage is caused by the. conversion of 5-FU to 5-fluorouridine which is 30 times more toxic than 5-FU. The use of some sort of controlled release system might solve the problem by restricting the drug to the site of pharmacological action. Over the years two approaches have been taken by scientists in order to increase the TI (the ratio of toxic to minimum effective concentration) of 5-FU and to restrict it to the tumor:

1) by incorporating the drug into a polymer

2) and by making derivatives of 5-FU.

Derivatives of 5-FU

Hillers, Zhuk and Lidkas were the first to prepare a derivative of 5-FU. In 1967 they prepared 1-(2'-furanidyl)-5-fluorouracil. In 1972, Hillers, Zhuk, Lidkas and Zindermane prepared 1-(2'-puranidyl)-5-fluorouracil. These derivatives were found to be less toxic than 5-FU.[9]

Meiren, and Belousova demonstrated that 1-(2'-furanidyl)-5-fluorouracil could be the transport system for 5-FU and its action was due to hydrolytic release from the parent molecule.[13]

Nesnow, Miyazaki, Khwaja, Meyer and Heidelberger, in 1972, prepared some 5-FU related pyridine nucleosides which included 2,4-dimethyl-5-fluoropyridine, 4-hydroxy-5-fluoro-2-pyridone, 4-hydroxy-5-fluoro-1-(2,3,5-tri-o-benzoyl-alpha-D-ribofuranosyl)-2-pyridone, 4-hydroxy-5-fluoro-1-(alpha-D-ribofuranosyl)-2-pyridone, 2-hydroxy-5-fluoro-1-(alpha-D-arabino-furanosyl)-4-pyridone(0--2')-cyclonucleotide and 4-hydroxy-5-fluoro-1-(2-deoxy-alpha-D-erythro-pentofuranosyl)-2-pyridone. In vitro studies showed no inhibitory activity on cells.[14]

In 1947, Okada, Nakayama and Mitsui prepared 1-acetyl-5-FU, 1-benzoyl-5-FU, 1,3-dimethyl-5-FU, allyl-FU, butenyl-5-FU, carboethoxymethyl-5-FU, acetoxyethyl-5-FU and 1-o-toluoyl-5-FU and demonstrated that 1-acetyl-5-FU and 1-benzoyl -5-FU were more effective and less toxic than 5-FU in vitro.[15]

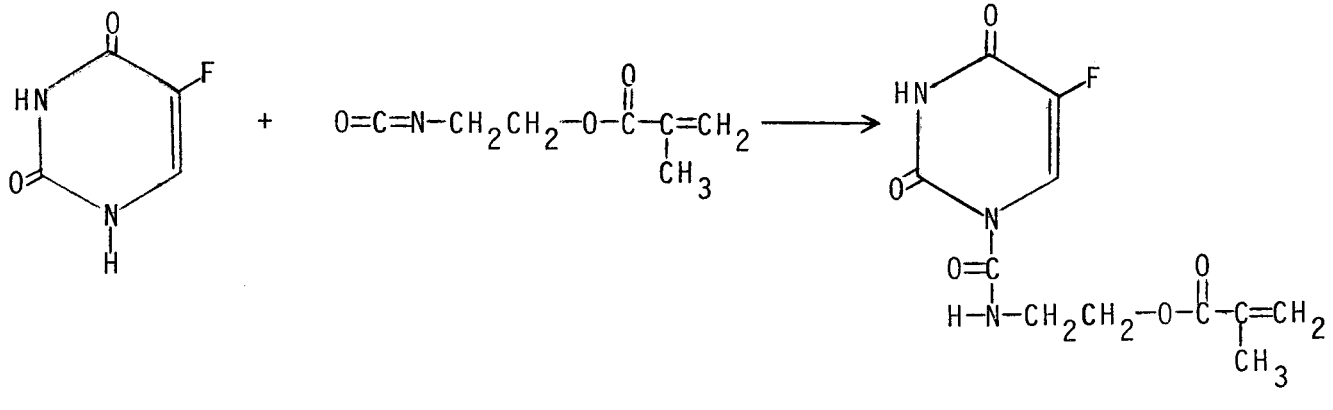
In 1975, Hoshi, Iigo, Yoshida and Kuretani synthesized some 1-carbamoyl derivatives which included methylcarbamoyl-5-FU, isopropylcarbamoyl-5-FU, phenylcarbamoyl-5-FU and cyclohexylcarbamoyl-5-FU. These derivatives were more active and less toxic than 5-FU.[16]

In 1978, Gebelein and Morgan reported the synthesis of 5-fluoro-N-(N^1-ethylcarbamoyl)uracil, 5-fluoro-N-(N^1-allylcarbamoyl)uracil, 5-fluoro-N-(N^1-isopropylidenecarbamoyl)-uracil and 5-fluoro-N-(N^1-vinylcarbamoyluracil). The acryloyl derivative hydrolyzed too rapidly to be of any practical application while the allylic derivative was not polymerizable.[17,18,19]

Umrigar, Ohashi and Butler made 1-(2-carbomethoxyacrylol)-5-FU and found out that the monomer hydrolyzed too rapidly.[20]

In 1984, Gebelein and Hartsough reported the synthesis of 1-(N-2-ethylmethacrylcarbamoyl)-5-FU monomer which could be further copolymerized with various acrylates or methacrylates.[22] (Figure 6)

In May 1986, Burr and Bundgaard reported results of synthesis and hydrolysis studies of seven 1-alkoxycarbamoyl derivatives of 5-FU. The derivatives were more lipophilic than 5-FU. One of the derivatives, 1-(butoxycarbamoyl)-5-fluorouracil when administered through the rectum of rabbits showed 100% absorption while no bio-availability was observed when 5-FU was administered through the same route.[23]



5-Fluorouracil (5-FU) | Isocyanatoethyl methacrylate (IEM) | 1-(N-2-ethylmethacrylcarbamoyl)-5 luorourafci l(EMCF)

Figure 6. Synthesis of EMCF.

Polyneric Systems Containing 5-Fluorouracil

Ballweg, et al, in 1969 prepared a polymer (Figure 7) containing 5-FU for the first time. This polymer contined 5-FU as a part of its backbone and had shown some biological activity.[24,25,26]

Umrigar, Ohashi and Butler, in 1979, copolymerized 2-carbomethoxyacryloyl-5-FU with styrene, divinyl ether and 2-chloroethyl vinyl ether and showed that the copolymer hydrolyzes more slowly than the monomer. (Figures 8,9,10,11 and 12). They also reported that the rate of hydrolysis depended on two important factors: 1) hydrophobicity of the polymer and 2) the strength of the bond attached to the 5-FU. These copolymers have shown some activity against P388 leukemia in mice.[20]

In 1980, Gebelein, et al reported the synthesis of poly[5-fluoro-1-(N-vinylcarbamoyl)uracil] (Figure 13). This polymer showed some activity against P38 leukemia.[17]

Yoshida and Kaetsu, in 1979, studied the release of 5-FU from polymers containing an adsorbent and reported that as the concentration of the adsorbent increased, the release of 5-FU decreased.[27]

Kaetsu, Yoshida and Yamada, in 1980, demonstrated that the release rates could be varied by varying the hydrophobicity and shape of the composite in which 5-FU was entrapped. They studied release of 5-FU entrapped in a matrix of poly(diethylene glycol dimethacrylate) including a small quantity of poly(styrene), poly(vinyl formal), poly(vinyl-acetate) or poly(methyl methacrylate) and poly(ethylene glycol).[28]

Drobnik, Spacek and Wichterele did studies on permeation of

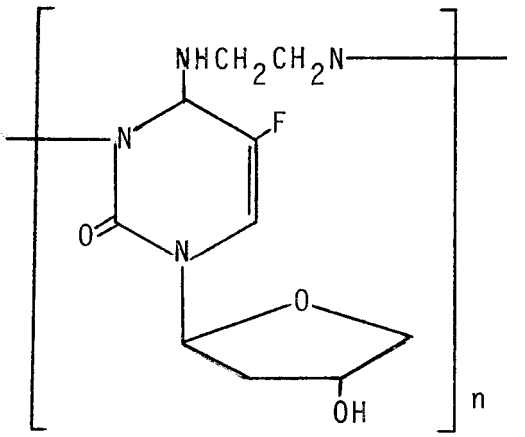


Figure 7. Structure of the polymer made by Ballweg et al.[26]

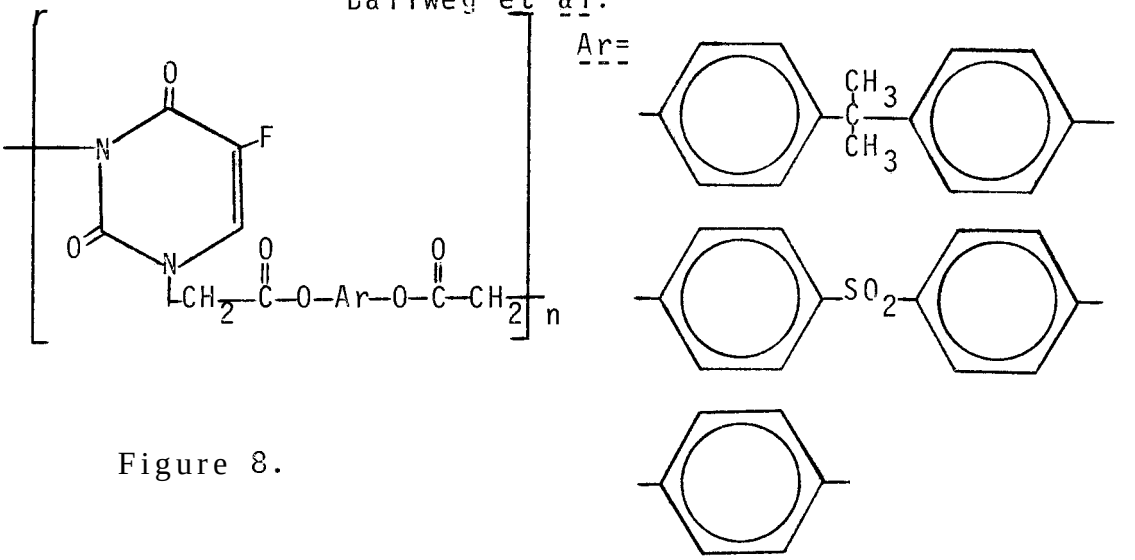


Figure 8.

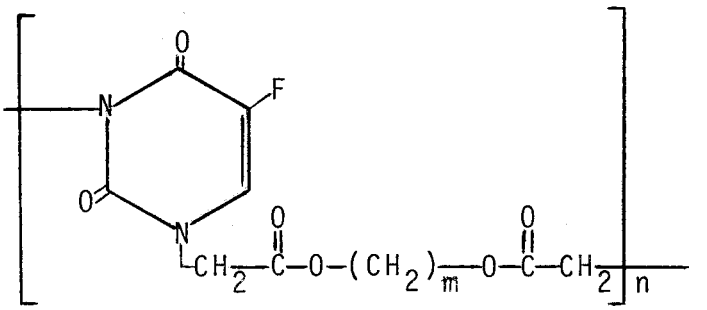


Figure 9.

Figures 8 and 9. Structures of the polymers made by Butler et al.[20]

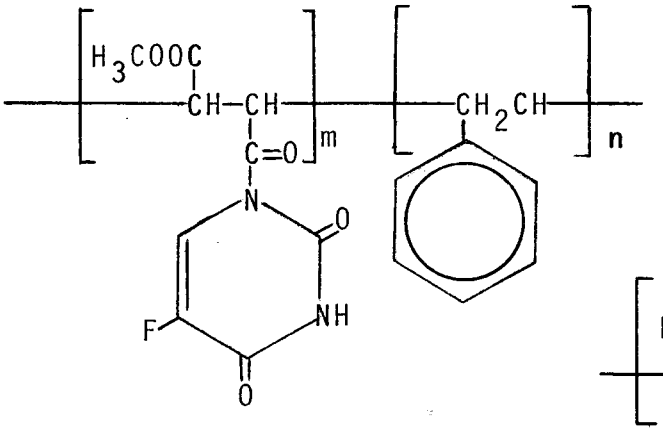


Figure 10.

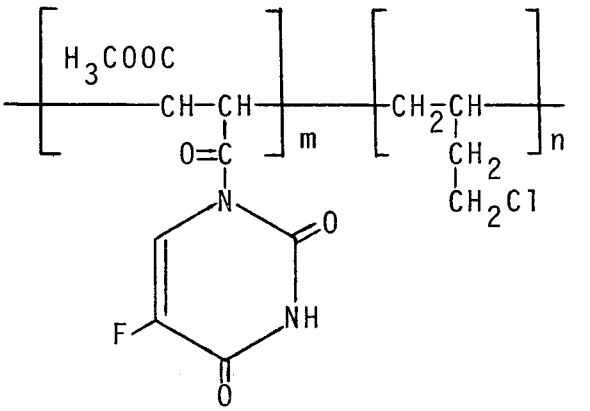


Figure 11.

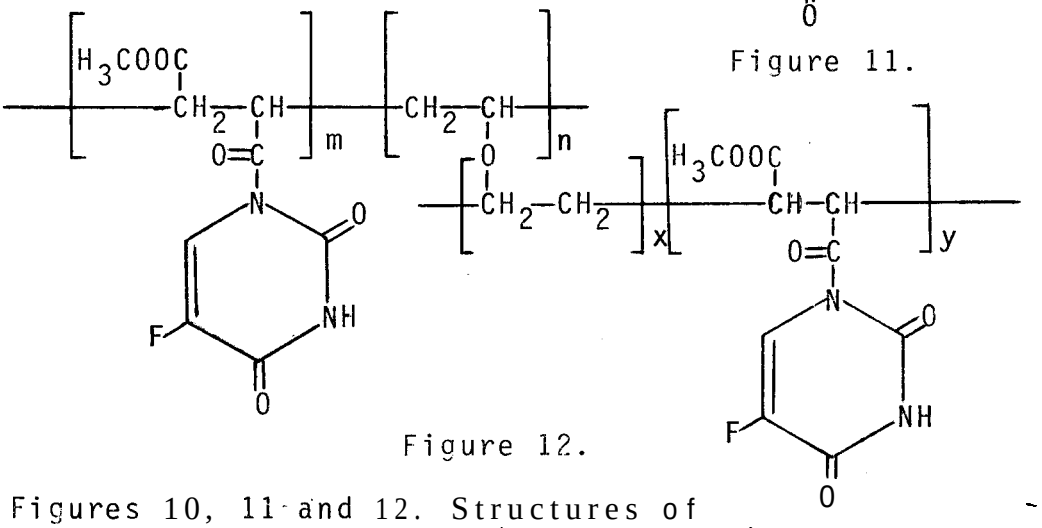


Figure 12.

Figures 10, 11 and 12. Structures of the polymers made by Butler et al.[20]

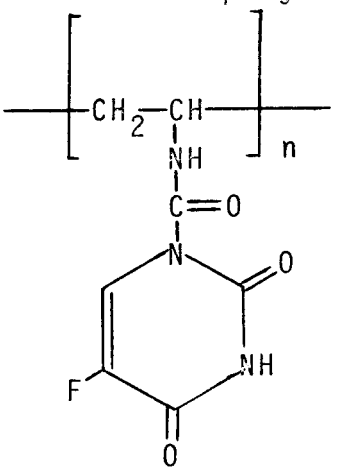


Figure 13. Structure of the polymer made by Gebelein et al.[17,39,42]

5-FU through hydroxyethyl methacrylate and butyl methacrylate. Their work demonstrated that dilution could be accomplished by 1) increasing the hydrophobicity of the membrane, 2) by increasing the thickness of the membrane and 3) by using dilute solutions.[28]

Levowitz, La Guerra, Calem, Gould, Scherrer and Schoenfield impregnated 5-FU into Hydron discs and studied its release by placing these discs in buffer or human plasma.[29]

Gebelein and Hartsough copolymerized a 5-FU derivative [EMCF], [1-(N-2-ethylmethacrylcarbamoy1)-5-fluorouracil], with methyl methacrylate, methyl acrylate, butyl acrylate, butyl methacrylate and stearoyl methacrylate. They prepared a wide range of copolymers by varying the EMCF and comonomer molar ratios as 25:75, 50:50 and 75:25. Hydrolysis studies were done on these polymers by placing a weighed sample in a wire-mesh-supported dialysis membrane cage and by immersing this assembly in a conical flask containing water which was continuously stirred. Samples were taken out periodically and assayed spectrophotometrically to determine the concentration of 5-FU released. The hydrolysis studies showed perfect zero-order release kinetics. The rate of release could be varied by varying the comonomer used or the amount of EMCF in the copolymer.[21,22]

Polymeric Controlled Release Systems

To quote Gebelein, "A controlled release system is simply any polymeric system that regulates or controls the release of some type of bioactive agent. Generally, these systems are developed to restrict the concentration of this agent to some fairly narrow range in order to elicit the desired activity while reducing the other potentially dangerous side effects to acceptable levels".[31]

Polymeric controlled release systems can be classified on the basis of physical and chemical approaches.[32] Gebelein distinguishes these systems into three broad categories:

1) controlled release systems
2) biologically active systems
3) immobolized bioactive materials

A drug may be enclosed in a polymeric material which eventually dissolves or gets destroyed in some part of the body thereby releasing the bioactive agent. "Timed capsules" for headaches and cold problems are good examples of this category. The advantage of such erodable systems is that the polymeric material degrades in the body and is not required to be removed surgically. The most commonly used synthetic polymers in erodable systems are poly(lactic acids). The drug may be placed in the erodable polymer as a depot (reservoir system) or dispersed through the polymer (monolithic system).[33]

In another system, the drug is allowed to diffuse through a non-degradable polymer like poly(dimethyl siloxane) or a hydrogel poly(hydroxyethyl methacrylate). The rate of diffusion depends on the composition of polymer, the chemical structure of the drug and nature

of the system. Diffusion-controlled systems can be either reservoir type or monolithic type. Some medical devices of diffusion-controlled type are available which include release of pilocarpine (for glaucoma treatment) from an ethylene-vinyl acetate copolymer (Ocusert^{R}, Alza Corp.), and the control of fertility by the release of progesterone from the same polymeric system (Progestasert^{R}, Alza Corp.).[34,35,36] Artificial tears, scopolamine, a motion sickness drug delivered through skin by a transdermal system and nitroglycerine for prolonged release are also available.

Drugs may be released from microcapsules by the process of diffusion. The process itself consists of three steps: 1) water diffuses into the coating, 2) an aqueous solution of the drug forms inside the coating and 3) the drug solution diffuses out of the coating.[37]

Polymeric Drugs

In another approach, the drug unit is directly attached to the polymer by covalent bonding to form the polymeric drug. According to Gebelein, "A polymeric drug is a macromolecule that contains a drug unit attached to or within the backbone chain of a polymer that exhibits medical activity without having such unit".[32] A good example of a polymeric drug without a known therapeutic unit is the divinyl ether/maleic anhydride cyclocopolymer (DIVEMA or 'Pyran' copolymer).[38]

There are three types of polymeric drugs:

1) Insoluble polymeric drugs: These are surgically transplanted or injected at the site of pharmacological action for extended periods of time. However, these drugs are good for only easily accessible

sites and not desirable for treating internal organs for fear of causing damage to the delicate organs.

2) Soluble polymeric systems: These have advantage over the insoluble polymeric drugs as they require no surgery and can be injected or ingested orally. Moreover, they have a lot of versatility, which means that the solubility or the specificity of the polymer can be varied by varying the nature and amount of copolymer used. Their drawback is lack of complete specificity to the target cells.

3) Directed soluble polymeric drugs: These are multicomponent polymeric drugs, generally composed of three parts: a) the therapeutic agent, b) a solubilizing unit, and c) a directing unit. The directing unit may be another monomer or a biological unit, such as an enzyme, protein, etc.[39,40,41]

Numerous references are available in the literature describing the synthesis of a monomer containing a bioactive agent which could then be. attached to another monomer or polymeric matrix or encapsulated by a polymeric membrane.[42]

The cancer drugs which have been attached to polymer chains include 5-FU, cyclophosphamides, and 5-methyl-2-phenyl-l,3-dioxy-boriman-5-yl methyl methacrylate.[43] References are cited in the literature describing attachment of phenyl ethyl amine, dl-amphetamine, 1-ephedrine and tyramine to poly(methacrylic acid) and starch.[44]

The copolymer of 1-vinyluracil has been found to be active against murine lukemia virus.[45-49] Poly(9-vinyl-adenine) has been found to be antiviral and has shown inhibition of E. Coli RNA polymerase. Statalon is a naturally occurring polysaccharide and has been found to

been found to be active against mouse leukemia. Actinomycin is another example of a natural polymer used in treating Hodgkin's disease. 9-Alpha-fluorocorticoids are used the in treatment of some neoplasms of lymphoid tissue. They inhibit incorporation of precursors of purines in nucleic acid synthesis, and the incorporation of formate into DNA.[44]

Poly(sodium acrylate) has been found to be active against intramuscular Walker carcinoma 256 in rats and has also shown anti-tumor activity against solid tumor cells. Certain polymers are capable of inducing production of interferon, which is known to be a potential neoplastic agent.[40] The alternating 1:2 divinyl ether:maleic anhydride copolymer (Pyran) has been found useful in treating Ehlrich and Lewis lung cancer in mice.[20]

Ascoli, Casini, Ferappi and Tubaro have reported many polymers containing anti-bacterial agents showing antibacterial action.[50] By varying the structure of some synthetic hormones and enzymes a considerably higher activity than their natural counterparts could be achieved.[51] Poly(2-vinyl pyridine)-1-oxide has shown antisilicosis activity.[52] Copolymers of dirnethylolurea-sulphamid or dimethylolureasulphone have been reported as antimalarial drugs.[53] Choulis and Papadoupolis prepared timed-release tablets containing quinine sulfate.[54] To mask its unpleasant taste and odor, Clofibrate (hypocholestrolemic agent) was encapsulated.[55]

Mason, Theis and Cicero did in vitro and in vivo studies on the release of cyclazocine (a narcotic agent) from dl-poly(lactic acid) microcapsules and demonstrated that the larger capsules showed slower

Newton and Razzo studied the release of nitrofuranotin, nitrofurazon, oxytetracycline and tetracycline from gelatin capsules containing diluent with or without a lubricant and wetting agent.[56] They reported that the type of drug, type of diluent, level of diluent and lubricant each affect drug release, but the wetting agent does not. Gentamycin in poly(methyl methacrylate), when used as a bone cement, exhibited localized bacteriocidal action in dogs. *In vitro* studies on this system have shown sustained release for over 70 weeks.[57,58] The release rate was dependent on the gentamycin content of the system, the amount of elution liquid, shape and size of the system and period of contact.

Abrams and Ronel have reported a Hydron device for zero order release rate of cyclazocin.[59] They demonstrated that the release rates could be controlled by varying the copolymer composition and crosslink density.

Drug Elution Systems

In the *in vitro* studies, the proper design of a suitable device to measure the controlled release kinetics of a drug is a very vital task. The variables which have to be considered in designing such a controlled release drug delivery device include analytical sensitivity, maintenance of sink conditions, hydrodynamic characteristics of solution diffusion, reproducibility of results and accurate maintenance of temperature. Reports of many such designs can be found in the literature.[61-65] Chien classifies these designs into two main groups:

1) Continuous flow apparatus and 2) Constant rotation apparatus.[60]

Continuous flow apparatus:

This kind of apparatus was used by Kalkwarf[61] and by Roseman and Higuchi.[62] The prototype of the drug delivery device is fixed in an elution column maintained at 37°C and the solution to be eluted into is passed through it. The flow rate of the elution solution is controlled by using a peristalic pump. Samples of the eluent solution are collected periodically and the concentration of the released drug is determined.[61,63] Using this system, D. R. Kalkwarf studied the in vitro release of progesterone from a matrix-type polyethylene drug delivery device. As the flow rate increased, the rate of drug release also increased till it reached a plateau beyond which any further increase in flow rate did not affect the rate of drug release. A high flow rate (300 mL/minute) of elution solution was used as the drug has low aqueous solubility. The release profile of the drug could be determined either by measuring the concentration of the elution solution periodically or by assaying the residual drug content in the device. This principle was also used by Roseman and Higuchi[62] and Roseman[64] to investigate the controlled release of progesterone and its derivatives from matrix type medicated vaginal rings made from silicone rubber. The drug release profiles depended on the polymer solubility and polymer diffusibility.[62,64] Continuous flow apparatus is also used to determine controlled drug release profiles from capsule and sandwich type drug delivery systems.[60]

Constant Rotation Apparatus:

This system developed by Chien et al[65] is simpler in design than the continuous flow apparatus. The drug delivery device was mounted in a circular Plexiglas[R] holder and rotated in the elution medium at a constant speed using a spin bar. A constant angular rotation speed was required in order to maintain constant solution hydrodynamics. The release of deoxycorticosterone acetate from matrix type and sandwich type silicone formulations was studied and the results compared. The daily dose of the drug released from the matrix type drug delivery system was much higher than the sandwich type and gradually decreased with time. Sink conditions required for the study of the release profiles using the constant device can be maintained by the use of a water-miscible compatible co-solvent. In this way the aqueous solubility of low solubility drugs such as the steroids can be appreciably enhanced. Another advantage of using a co-solvent system. is that only a small volume of elution solution is required. By changing the solubility of the drug in the elution medium, the rate of-release can be varied.[60]

Using medroxyprogesterone acetate as a model drug, experiments were carried out to study its release from a matrix-type drug delivery system using the continuous flow apparatus and the constant rotation apparatus. The results obtained using these devices showed the same drug release profiles. The results were also comparable to the theoretical calculations.[65]

Drug Delivery Device Used by Gebelein et al in Earlier Studies

Gebelein et al developed a device in order to study the release profiles of 5-FU from EMCF, poly[EMCF] and EMCF copolymers. The apparatus was basically comprised of a dialysis membrane supported by a wire mesh cage (Figure 14,15). A cellulose dialysis membrane, 6 cm in length and 2.5 cm in diameter, was placed into 7-8 ml molten wax in a beaker. When the wax hardened, the membrane became embedded in the wax. This wax-embedded membrane was retrieved from the beaker and was fixed to the bottom of a stainless steel cage, 4.0 cm high and 2.75 cm in diameter, using molten wax. The cage had a stainless steel rod soldered to it. The dialysis membrane was trimmed to make it even with the bottom of the cage. The bottom was further reinforced by fixing a circular wire mesh piece to it using molten wax. A tubular dialysis membrane, 4.0 cm high and 2.75 cm in diameter, was introduced into the cage and fixed to the the bottom by pouring molten wax from a pasteur pipette. The top of the cylindrical cage was also covered by employing the dialysis membrane/molten wax technique. A square port was cut into it for the purpose of introducing the sample. The top was reinforced with a same-sized, circular piece of wire-mesh, again using wax;

At the start of the experiment, a small hole was made in the top of the wire mesh/dialysis membrane assembly and water was introduced through it to expel air. Once this was done, the hole was sealed with wax. This apparatus was slowly placed in a conical flask containing 1000 mL of water. The contents in the flask were equilibrated at $37 \pm 0.5^{o}C$ using a water bath and were stirred using a

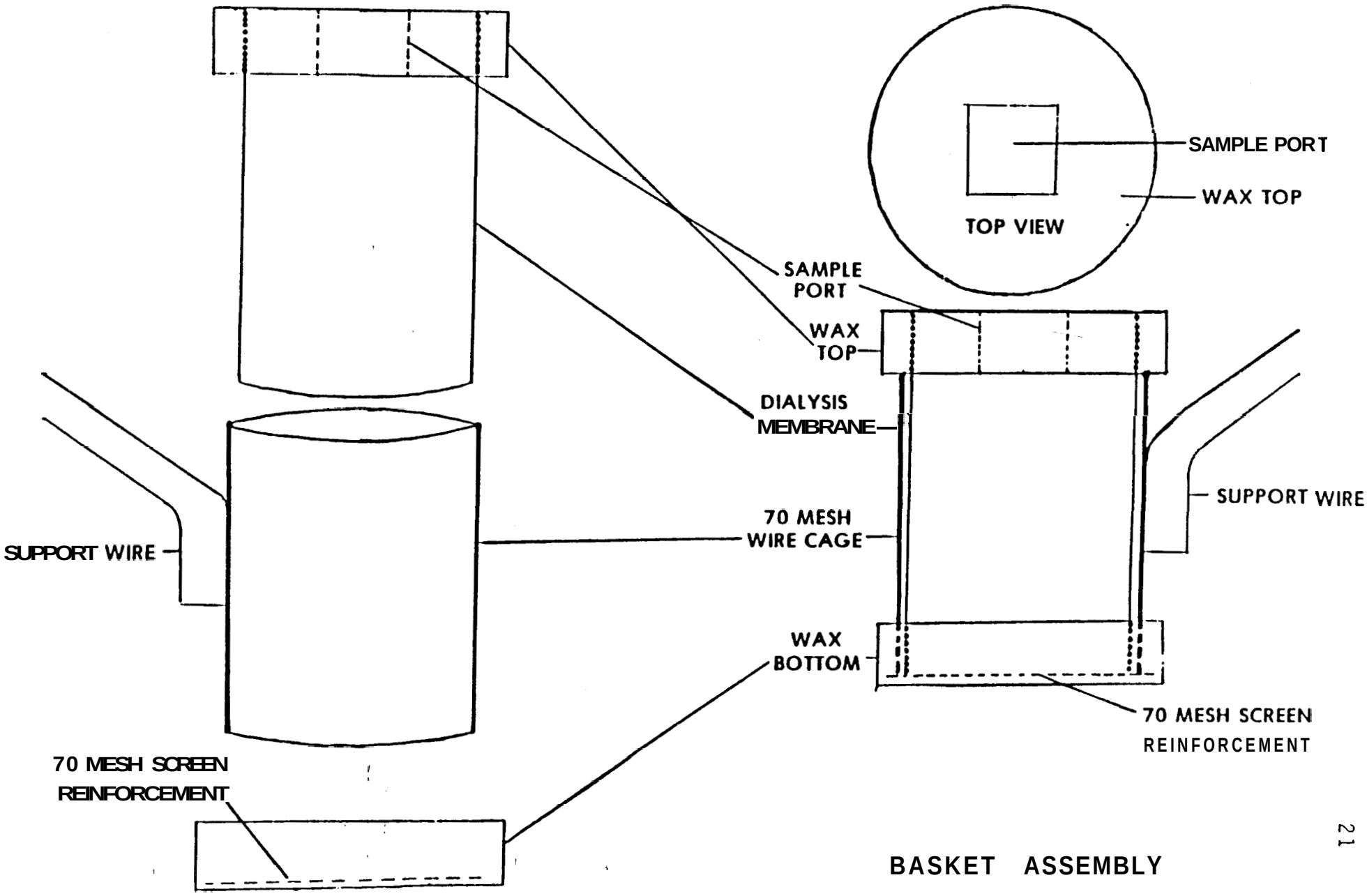


Figure 14. Dialysis membrane/wire mesh anparatus used by Gebelein and Hartsough. Diagram taken from

mechanical stirrer. Aliquots were removed periodically and assayed spectrophotometrically for 5-FU.

CHAPTER II

STATEMENT OF THE PROBLM

The major problem associated with any kind of medication is obtaining an effective concentration level in the body. A high concentration level results in side effects and at low concentration the drug becomes ineffective. Keeping this in mind, dosage level is designed in such a way that it falls between these upper and lower limits. The ratio of toxic to the minimum effective concentration is known as the therapeutic index, commonly denoted as TI. A drug with a higher TI value is considered to be safer than the one with a low TI.

The treatment of cancer or leukemia using therapeutic agents is difficult because the medications usually cause undesirable side effects such as loss of apettite, nausea, loss of hair, etc. In addition, such drugs have low TI values. As a result the patient is required to take medication more often, aggravating the already-existing problem of side effects.

The solution to these problems in medication might be accomplished by using a controlled release system. If implanted at the site of desired pharmacological action, it would release the drug molecules at a controlled rate in the near vicinity. This way the action of the drug would be localized rather than systemic thus minimizing its exposure to the non-target areas.

Some polymeric systems utilizing 5-fluorouracil have been reported in the literature. However, no implantable polymers have been developed. One such system was developed by Gebelein and Morgan,[17] but it released 5-FU at a faster rate than desired.[18,69,70]

In another system, the reaction product of 5-FU and fumaroyl chloride was utilized. It released 5-FU at a rapid rate and would not be of any practical use.[20] In a recent article, Gebelein and Hartsough,[21] have reported the controlled release of 5-FU from acrylate copolymers of 1-(N-2-ethylmethacrylcarbamoyl)-5-fluorouracil[EMCF] monomer. The controlled release studies demonstrated slow release of 5-fluorouracil over extended periods of time and exhibited zero-order kinetics. The amount of 5-FU could be varied by varying the comonomer ratio and/or the comonomer.

This study will be an extension of the Hartsough and Gebelein work emphasizing mostly the controlled release studies using a more simplified technique. A carbamoyl derivative of 5-FU, [EMCF], will be prepared and copolymerized with methyl methacrylate in 25/75, 50/50, and 75/25 molar ratios. The solvent system for these reactions will be 1,4-dioxane. In order to avoid the possibility of photopolymerization, the synthesis of EMCF will be carried out in a round bottom flask covered with aluminum foil. The reactants, isocyanatoethyl methacrylate and 5-FU will be reacted in the presence of a catalyst at room temperature.

The copolymers of EMCF and MMA will be made at 60°C using AIBN as the initiator. Earlier, Gebelein and Hartsough[21,22] reported that copolymers of EMCF and MMA prepared at 70-80°C did not dissolve in any solvents. The insolubility of the copolymers may be due to crosslinking of the polymeric chains at higher temperatures or to the release of 5-FU from the polymer followed by crosslinking (deblocking). In the present study a lower reaction temperature (60°C) will be

maintained with the expectation that the resulting copolymer would dissolve in some solvent.

The hydrolytic release rates on the EMCF monomer and the following copolymers will be carried out using a much simpler technique instead of the more complex one used previously which involved a dialysis membrane supported by a steel mesh basket (Figure 14).

EMCF:MMA	EMCF:MA
25:75	25:75
50:50	50:50
75:25	75:25

In the present new technique, gas dispersion tubes (Filter-sticks) of two different sizes will be used. Carefully-weighed, sieved (60-100 mesh), powdered samples of EMCF monomer or the EMCF copolymers will be placed in the filter-sticks (Figure 15) immersed in a one liter flask fitted with a mechanical stirrer, in a constant-temperature bath maintained at $37 \pm 0.5^{o}C$. One liter of water will be poured in the flask and stirred. Studies will be done using different sample sizes and tubes. Aliquots will be removed from the flask periodically and the concentration of the 5-FU released will be determined spectrophotometrically at 265 nm. Beckman DU-7 and/or Beckman-26 spectrophotometers will be used for the assays. The results of these studies will be compared with the results of Hartsough and Gebelein.

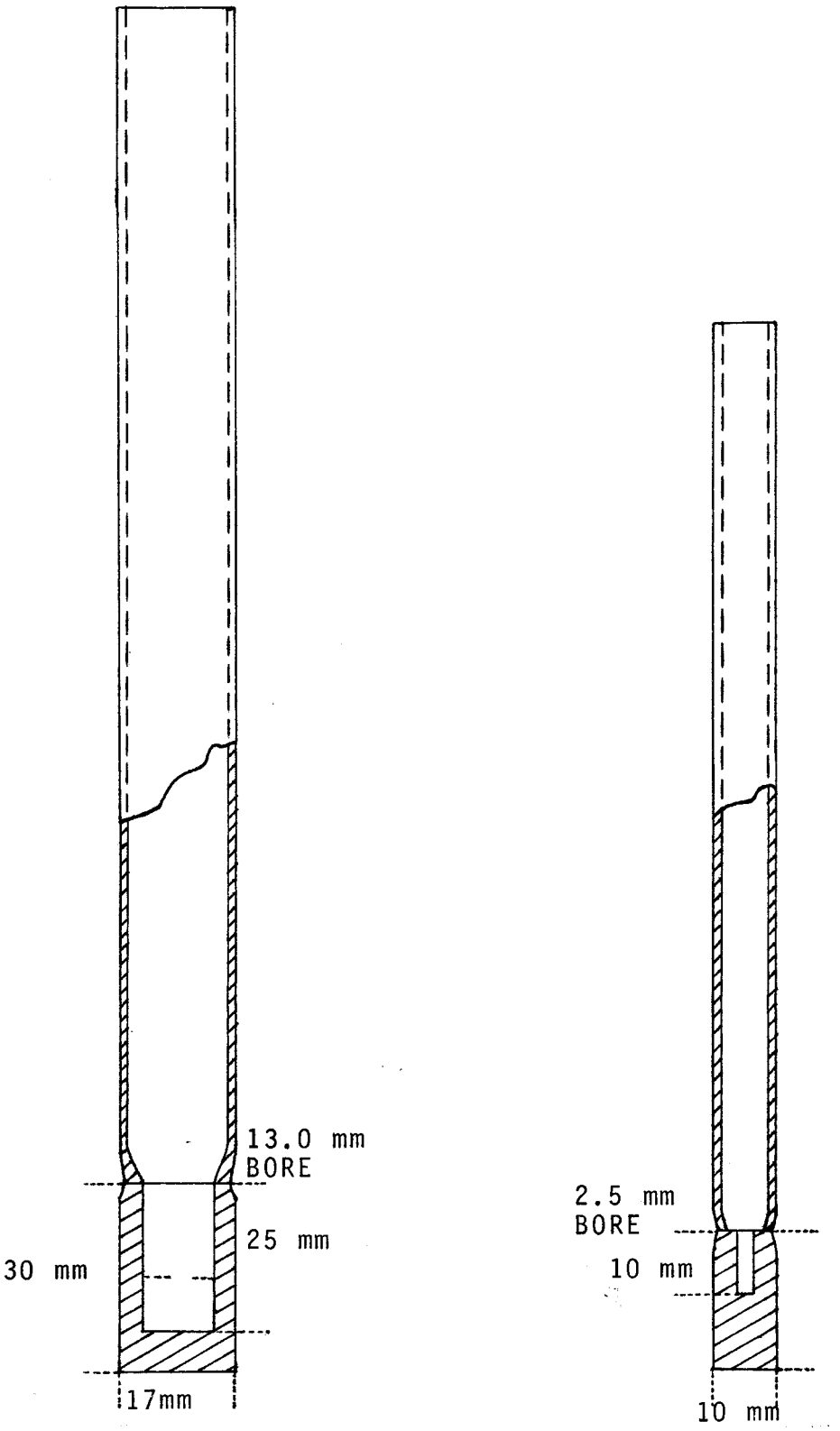


Figure 15. Gas dispersion tubes.

CHAPTER III

EXPERIMENTAL

Reagents

The reagents used in the study were of practical or analytical grade. In order to remove the peroxide, 1,4-dioxane was passed through alumina. 5-FU was dried in an oven at 110°C for 24 hours before use. Methyl methacrylate was washed several times with 5% sodium hydroxide to remove the phenolic inhibitor and then dried over calcium chloride. A list of reagents used, their grades and the names of suppliers is presented below:

Table 1. Reagents used

Material	Formula	Grade	Supplier
2,2'-azobisisobutyronitrile	$C_6H_{12}N_6$	Laboratory	Aldrich
1,4-Dioxane	$C_4H_8O_2$	Reagent	Aldrich
5-Fluorouracil	$C_4H_3N_2O_2F$	Practical	P.C.R.
Hydroquinone	$C_6H_8O_2$	Purified	Fisher
Isocyanatoethyl Methacrylate	$C_7H_9NO_3$	Experimental	Dow
Methyl Methacrylate	$C_5H_8O_2$	Laboratory	Rohm & Haas

EQUIPMENT

All of the glassware used had 24/40 standard tapered joints. In order to avoid the possibility of the monomer or the polymer undergoing premature hydrolysis, it was necessary to dry the apparatus before use. All of the apparatus was washed with soap, rinsed with deionized water and then dried in the oven at 110°C.

A Buchler flash evaporator was used to evaporate 1,4-dioxane from EMCF monomer, a Mettler P1000 balance was used to determine the weights of the chemicals, a Beckman Acculab 4 Infrared spectrophotometer was used for obtaining the infrared spectra of the monomer and the polymer, Beckman DU-7 spectrophotometer and/or a Beckman 26 spectrophotometers were used for the hydrolysis studies, a Sargent-Welch control was used to maintain a constant bath temperature of 37±0.5°C during hydrolysis studies and sintered glass tuber (porosity C) of two different sizes ordered from ACE Glass Company were used.

PROCEDURES

Preparation of 1-(N-2-ethylmethacrylcarbamoyl)-5-fluorouracil

The procedure for the following synthesis was taken from Robert R. Hartsough's Master's thesis.[66] Into a three-necked round bottom flask, wrapped in an aluminum foil and fitted with a mechanical stirrer, was poured 450 mL 1,4-dioxane, 0.5000 g hydroquinone, 0.5000 g triethylamine and 20.0000 g(O.1358 moles) 5-fluorouracil. Nitrogen was

purged through the flask for one hour at the end of which 25.0000 g (0.1611 moles) isocyanatoethyl methacrylate was added slowly. The contents of the flask were continuously stirred. The reaction (Figure 6) was allowed to proceed for five days. The solution was filtered and transferred into a preweighed one-liter round-bottom flask. The dioxane was flash evaporated at 40 torr and 45°C. The solid crude EMCF monomer was yellowish-white in color. It was dried in a vacuum at 25 torr and room temperature. The average yield of four syntheses was 36.2g (83% of the theoretical yield). The melting point was found to be 151-152°C.

Crude monomer (30 g) was dissolved in 250 mL dioxane and passed through a silica-gel column. Fractions were collected between 50 to 250 mL and flash evaporated under 40-torr vacuum and at 45°C. The purified monomer was then dried under 25-torr vacuum and the average yield was 28.3 g. The pure product had a sharp melting point of 154°C. The infrared analysis further confirmed the product and was identical to that reported by Hartsough.[66]

The monomer was found to be soluble in 1,4-dioxane, acetone, methylene chloride, chloroform, dimethyl sulfoxide, and cellusolve acetate. It swelled in n-butyl acetate, ethyl acetate, methyl cellusolve, methyl ethyl ketone, gamma-butyrolactone, and tetrahydrofuran. The monomer was insoluble in n-butyl alcohol, carbon tetrachloride, toluene, n-heptane, ethyl ether, and benzene. When heated to 80°C, it dissolved in gamna-butyrolactone, methyl iso-butyl

ketone, methyl ethyl ketone, acetonitrile, toluene, ethyl acetate and n-butyl alcohol.

Preparation of Copolymers of Methyl Methacrylate and EMCF

To a three-necked flask, fitted with a mechanical stirrer and calcium chloride drying tube, were added the appropriate amounts of comonomer, EMCF and 100.00 mL of 1,4-dioxane. Before adding the initiator (AIBN), the flask and its contents were purged for one hour with nitrogen. The flask was then submerged in an oil bath maintained at 60°C. The reaction was allowed to be continued for twenty-four hours while the contents in the flask were stirred. At the end of the reaction, the copolymer was recovered by precipitating in 600 mL of methanol. The copolymer was allowed to dry in a vacuum oven at 25 torr at room temperature. Weight of AIBN and comonomers and yields are given in tables (2 & 3).

The IR spectra of the copolymers confirmed the presence of respective peaks expected due to the comonomers. The magnitude of the peaks also varied according to the monomer concentration. It is worth noting that the copolymerization reactions were carried out at 60°C instead of 70-80°C, as reported by Hartsough,[66] with the hope of synthesizing soluble copolymers. The copolymers were insoluble in all the solvents tried; the results are tabulated in Table 4.

Table 2. Synthesis data

Comonomer charge	Amount of comonomer (g)	(moles)	Amount of EMCF (g)	(moles)
50% MMA	5.1300	0.5120	4.8726	0.0171
50% MMA	2.5981	0.0260	7.4019	0.0260
25% MMA	1.0474	0.0105	8.9526	0.0314

Table 3. Synthesis data

Comonomer charge	Amount of AIBN (g)	(moles)	Yields (g) This study	Earlier study[66]
75% MMA	0.1125	0.0688	4.0200	9.9310
50% MMA	0.0860	0.0524	5.7500	9.8723
25% MMA	0.0695	0.0432	6.3000	8.4539

Table 4. Solubilities of EMCF:MMA Copolymers

Solvent	25% MMA	50% MMA	75% MMA
Acetone	i	i	sw
Acetonitrile	i	i	sw
Benzene	i	i	i
Butyl alcohol	i	i	i
Gamma-butyrolactone	i	i	i
Carbon tetrachloride	i,f	i,f	sw
Chloroform	i.f	i,f	sw
Cellusolve acetate	i	i	sw
1,4-Dioxane	i	i	i
DMSO	i	i	i
Ethyl ether	i	i	i
n-Heptane	i	i	i
MEK	i	i	i
Methylene chloride	i	i	i
THF	i	i	i
Toluene	i	i	i

i = insoluble, f = floats, sw = swells

Hydrolysis studies

The release of 5-FU from EMCF and the copolymers of EMCF was studied using gas dispersion tubes of two different sizes (Figure 15). A typical gas dispersion tube is basically a glass tube, one end of which is open and at the other end is a hollow, porous bulb made out of sintered glass. The smaller gas dispersion tube had a cavity of 2.5 mm diameter and a height of 10.0 mm. while the cavity of the larger tube had a diameter of 13.0 mm and a height of 25 mm. The cavity volume of larger tubes was 67 times bigger than that of smaller tubes and could accomnodate larger sample sizes. The porosity was "C" in both sizes.

Pulverized samples, sieved to be in the 60-100 mesh particle size, were used. Exactly 1000 mL of deionized water was pipetted into a three-necked round-bottom flask fitted with a stirrer. The flask was then immersed in a water bath maintained at $37 \pm 0.5^{o}C$ and held in place with a clamp for 24 hours before starting the experiment so the flask and contents would equilibrate to temperature. The pulverized and sieved sample was placed in a gas dispersion tube and then immersed in the round-bottom flask. The contents of the flask were continuously stirred using a mechanical stirrer. Aliquots were removed at regular intervals from the flask using a pipette. These samples were then assayed for 5-FU using a Beckman DU-7 spectrophotometer at 265 nm. Test samples were returned to the reaction vessel after the assay was done.

In order to establish the optimum sample size for the gas dispersion tubes, hydrolysis studies were done using 0.1000-g, 0.2000-g and 0.5000-g EMCF samples. With the smaller-sized gas dispersion tubes, 0.1000 g proved to be the maximum amount of sample that could be used; above this the cavity was overfilled and the release rates tended to be identical with 0.1000-g data. Hence, in the subsequent studies on the copolymer samples 0.1000-g samples were used. With the larger-sized gas dispersion tubes, samples of 0.5000 g or larger could be used without overloading the cavity. The larger tubes could also accommodate pellets of the samples.

In order to establish the reproducibility of the technique, duplicate studies were done on EMCF samples at different times. To investigate if the amount of 5-FU released depended on the sample size, studies were carried out on 0.1000-g, 0.2000-g and 0.5000-g samples. Finally, to establish whether the technique could be used to differentiate between copolymers varying only in molar ratio, hydrolysis studies were done on the following copolymers

EMCF:MMA	EMCF:MA
75:25	75:25
50:50	50:50
25:75	25:75

Comparisons will be done between the following results:

a) the different-sized samples (Figure 17)

b) the different copolymers of MMA/EMCF (Figure 19)

c) the different copolymers of MA/EMCF (Figure 20)

d) gas dispersion tubes vs dialysis membrane (Figure 21) and

e) small tubes vs larger tubes (Figures 22 and 24)

CHAPTER IV

RESULTS AND DISCUSSION

As mentioned earlier in the introduction, there are very few examples available in the literature in which 5-FU has actually been attached to the main polymeric backbone or as a part of the backbone. Gebelein, *et al*[21,22] have done the most promising work in this field by synthesizing a polymerizable monomer (EMCF), from 5-FU and isocyanatoethyl methacrylate (IEM). The EMCF monomer was further copolymerized with different acrylates and the hydrolysis release profiles of the resulting copolymers were determined using a complicated wire mesh/dialysis membrane device. The copolymers were insoluble in any solvent and showed zero-order release kinetics.

The purpose of the present study was to synthesize the copolymers using different reaction conditions with the hope that the copolymers would dissolve in some solvent. In other words, this study was aimed at making soluble copolymers of EMCF and at the same time simplifying the technique for measuring the release profile of a drug from a multicomponent polymeric system by using gas dispersion tubes.

The reaction involving synthesis of EMCF proceeded well and gave a high yield (about 83% of theoretical). The monomer was purified by passing it through a chromatography column packed with 50-200 mesh silica gel. The yields before and after purification were 93.3% and 77% of the theoretical yield. The infrared analysis showed the spectral peaks characteristic of a methacrylate monomer. The monomer had a sharp melting point of 154°C.

EMCF was copolymerized with MMA in 25/75, 50/50, and 75/25 mole

% ratio. Gebelein and Hartsough[21,22] carried out the copolymerization reactions at 70-80°C and obtained insoluble copolymers. In this study the reaction temperature was maintained at 60°C, hoping that the crosslinking would be minimized at lower temperatures and that the copolymer would dissolve in some solvent. But this attempt proved to be unsuccessful as the copolymers were insoluble in any of the solvents tried (Table 4). Soluble copolymers were desired in order to study the effects of crosslinking, crystallinity, molecular weights etc. on the release rates of the therapeutic agents.

Crosslinking is one of the important factors that affect release rates. In a study by Chien et al, polymer diffusivity of progestrins in a hydrogel matrix was observed to decrease in response to the addition of a crosslinking agent.[67,68] Polymer diffusivity is a linear function of the reciprocal of the extent of crosslinking. The addition of a crosslinking agent results in the crosslinking of some polymer chains. Crosslinking reduces the mobility of the polymer chains and decreases the porosity for the diffusion of drug molecules in the polymer matrix.[67]

Crystallinity also affects release rates in a similar fashion to crosslinking. A study done by A. S. Michaels and H. J. Bixler[71] demonstrated that crystallinity introduced regions of very low diffusion, relative to the diffusion in the surrounding amorphous structure, leading to a significant reduction in the gross polymer diffusivity in polyethylene.

In order to maintain uniformity in the results, it was necessary to use the same range of particle size in all of the

hydrolysis studies. The powdered sample was sieved and only the particles of 60-100 mesh size were used. The smaller tubes could accommodate a maximum of 0.1000-g sample, above which the cavity overfilled and the data tended to become similar to that of a 0.1000-g sample (Figure 17). It is worth noting that only a 0.1000-g sample was sufficient for obtaining a clear release profile of a drug. Pellets and sample sizes of 0.5000-g and larger could be used in the larger tubes.

Release profiles of the EMCF monomer and of the copolymers showed that constant amounts of 5-FU were released over a period of more than ten days. Therefore, the profiles indicated that the EMCF monomer followed almost zero-order kinetics and that the copolymers exactly followed zero-order kinetics (Figures 17 and 18).

Figure 16 shows release profiles of two EMCF samples. The curve shows that 5-FU was released at a faster rate in the initial stages. The release profiles of the two EMCF samples are very similar to each other. Since the experiments were run at different times, the close replication suggests that the technique is reproducible.

As expected, the hydrolysis studies of 0.0500-g, 0.1000-g and 0.2000-g samples demonstrated that the smaller-sized samples released lesser amounts of 5-FU. The data is plotted in figure 17, and clearly highlights that the technique can differentiate between different-sized samples.

The profile data of the different EMCF:MMA and EMCF:MA (methyl methacrylate) copolymers is presented in Figure 18. The profiles indicate that the amount of 5-FU released from the copolymers increased

as the amount of EMCF increased. The data also indicates that the rate of release was higher in MMA copolymers than the MA copolymers (also see figures 19) and 20). Gebelein and Hartsough[21,22] reported that BA copolymers released 5-FU slower than MA copolymers. From these findings, it can be inferred that the hydrophobicity of the comonomer affects the rate of release. Increase in the hydrophobicity of comonomer inhibits the diffusion of water into the polymeric matrix and the subsequent diffusion of the drug solution out of the matrix. Similar observations were made by some other researchers.[28,29] Further, it can be noted that the polymer releases 5-FU more slowly than the monomer and all of the copolymers show zero-order kinetics. Umrigar, Ohashi, and Butler made similar observations with their monomer and homopolymer, but both hydrolyzed 5-FU at a rapid rate and would not have any practical application.[20]

The data obtained from the new technique when normalized could be correlated with that of the wire basket apparatus (Figure 21). In the wire basket apparatus, Gebelein and Hartsough[22,22] had used 0.50-g samples. The results obtained from small gas dispersion tubes, with 0.1000 g sample, when multiplied by a factor of 5.0 fitted on the same line as the release data of the same copolymer in the wire basket apparatus. This demonstrates that the techniques are measuring the same thing and that the technique tself does not cause the observed release rates for the drug. Similarly the data obtained from hydrolysis of 0.2000-g, or 0.5000-g samples using larger tubes, can be coplotted with the data obtained from 0.1000-g EMCF samples using smaller tubes, multiplied by a factor of 2.0 or 5.0 respectively

(Figure 22).

Figure 23, shows the release profiles of 0.1000-g, 0.2000-g and 0.5000-g samples obtained by using larger tubes. The profiles show that a proportionately higher amount of 5-FU was released from 0.5000-g and 0.2000-g samples when compared to that released from 0.1000-g sample.

The release data obtained from small and large tubes using 0.1000-g EMCF samples are plotted in Figure 24. The data fall on the same curve, further confirming that the release rates are not affected by the technique used.

The release of a drug from a monolithic polymeric matrix normally demonstrates the popular "Higuchi" kinetic pattern, which is a linear relationship between the amount of drug released and the square root of time. But the release of 5-FU from the copolymers of EMCF shows zero-order kinetics, which means that the amount of the drug released is directly proportional to the time elapsed. In order to understand this behavior of 5-FU, one has to look into the process of hydrolysis which occurs in three steps: a) water diffuses into the polymer matrix, b) hydrolysis of the drug from the polymer occurs, and c) the drug solution diffuses out of the matrix. According to Gebelein,[21] this process is further complicated by the fact that the chemical nature of the EMCF portion of the polymer (Figure 25) changes with the extent of the release of 5-FU, which would change diffusion rates and possibly the rate of release.

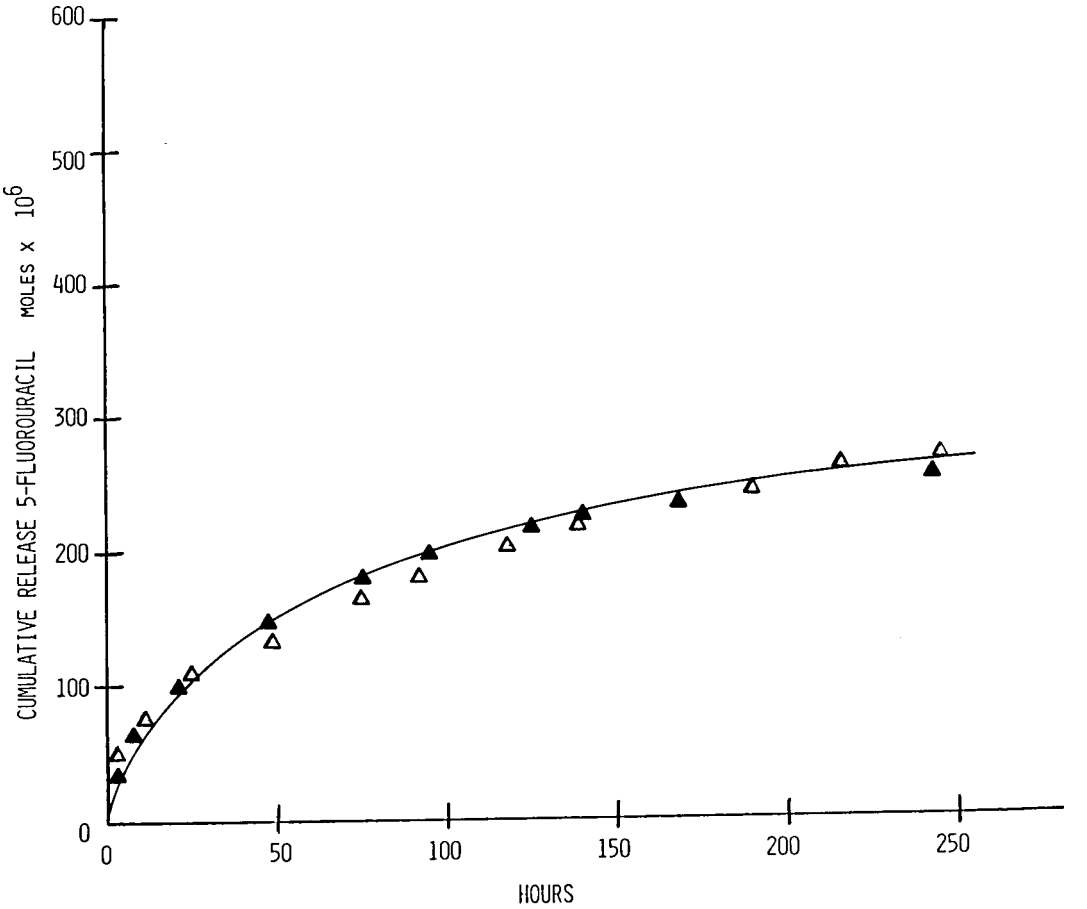


Figure 16. Release of 5-FU from two different (△,▲) 0.1000-g EMCF samples using small tubes.

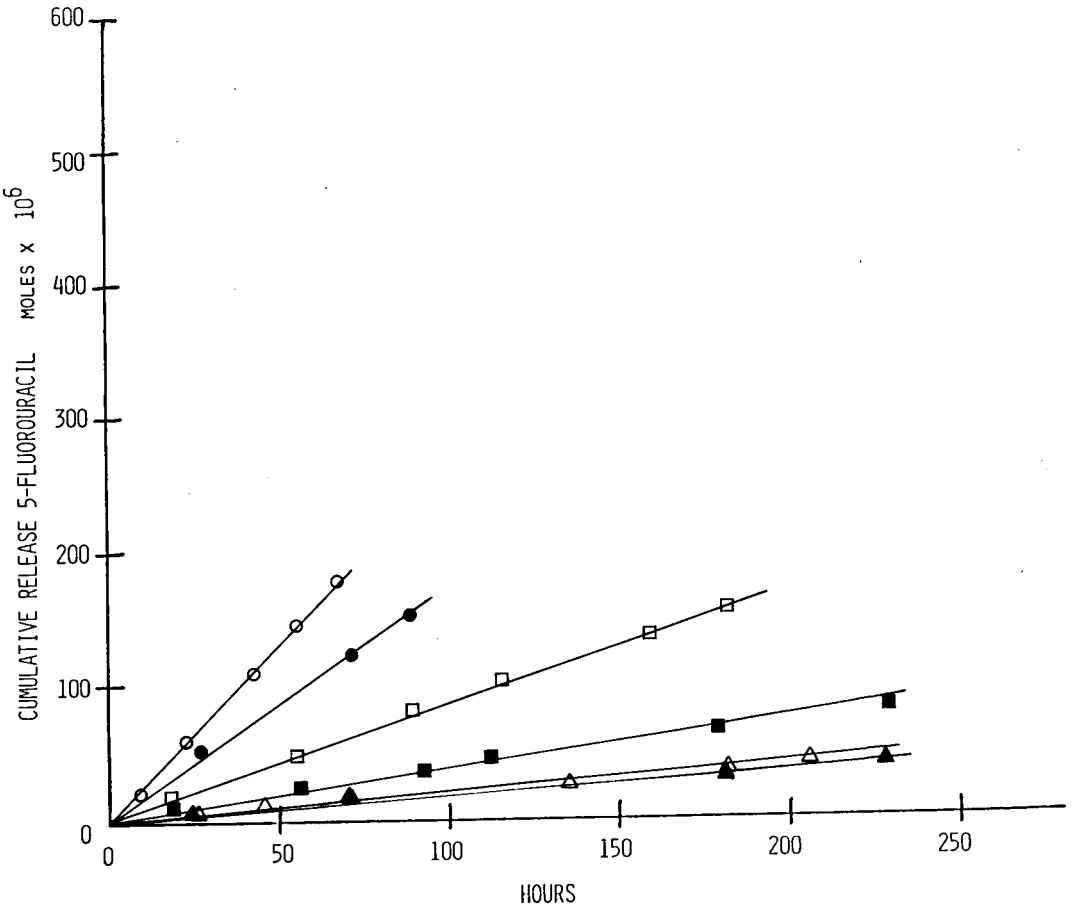


Figure 18. Release of 5-FU from 25% (A), 50% (■), 75% (●) MA and 25% (A), 50% (□), 75% (○) MMA copolymers using 0.1000–g samples in small tubes.

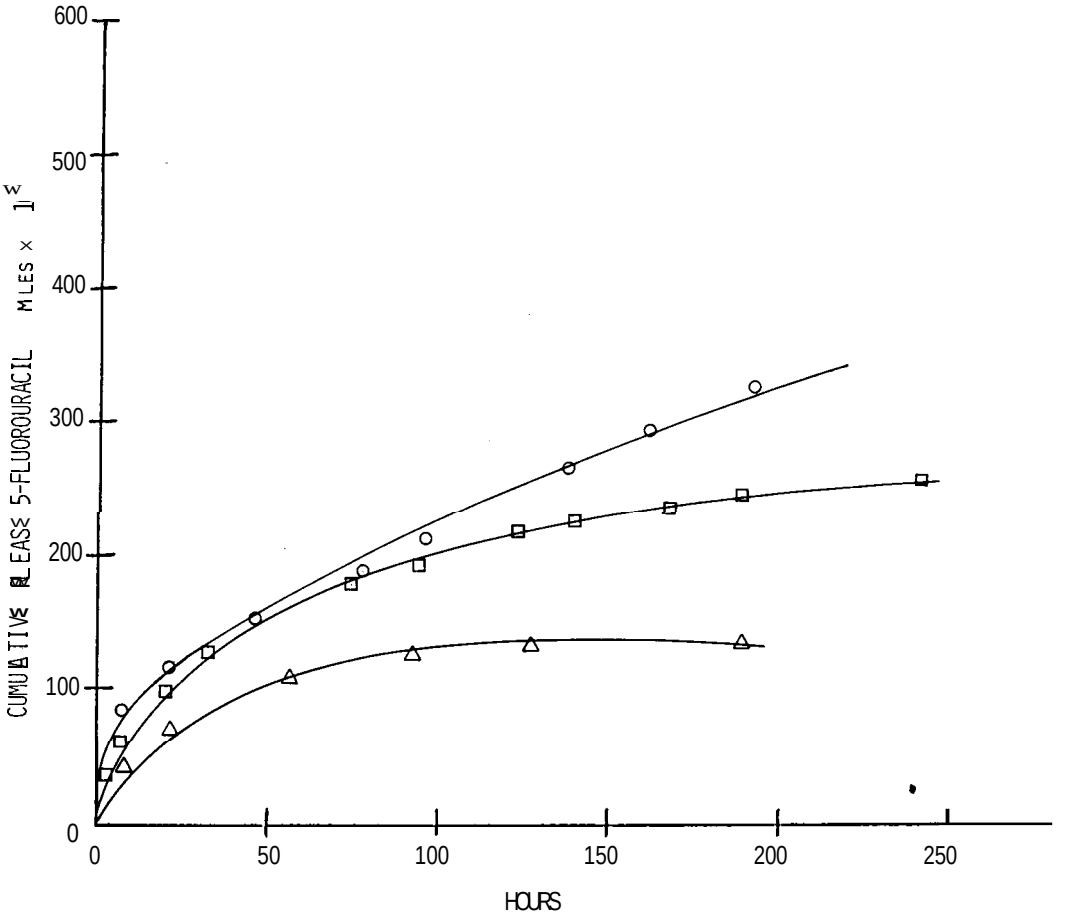


Figure 17. Release of 5-FU from 0.0500-g, 0.1000-g and 0.2000-g (△,□,○)EMCF samples using small tubes.

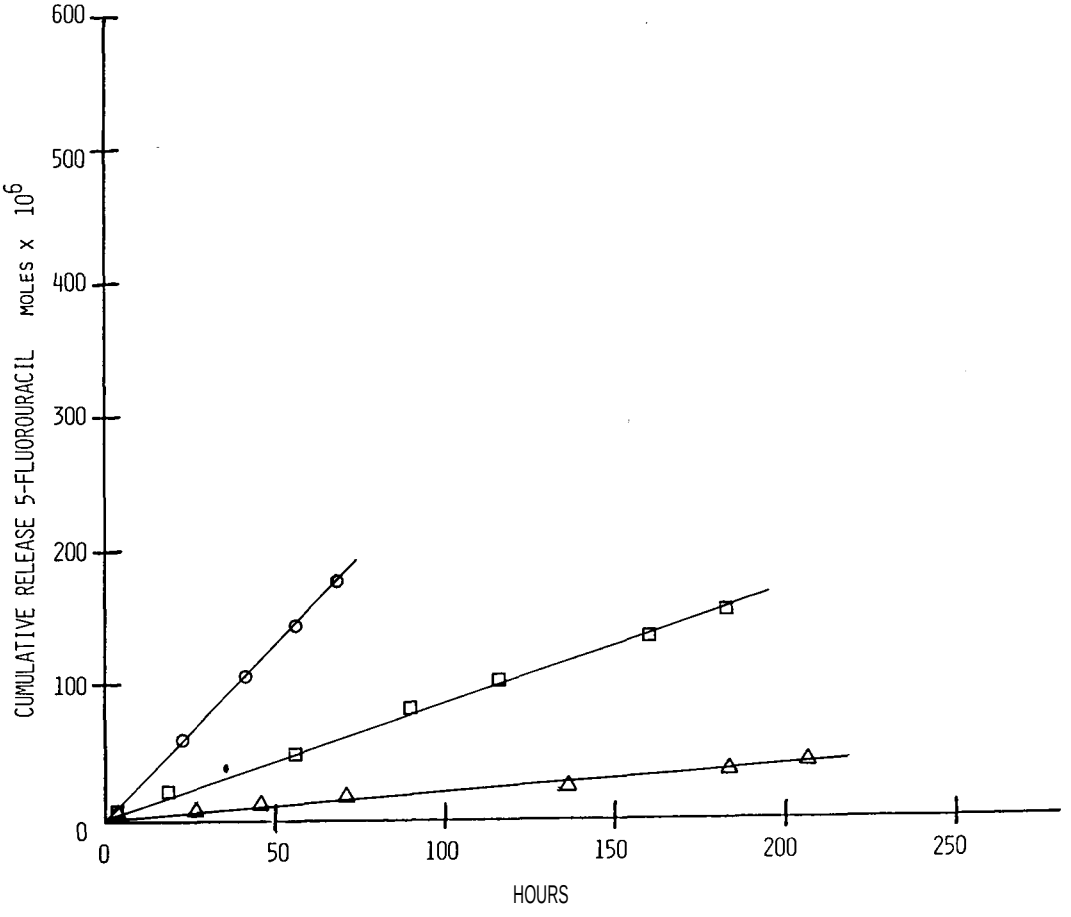


Figure 19. Release of 5-FU from 25% (A), 50% (□), 75% (o) MMA copolymers using 0.1000-g samples in small tubes.

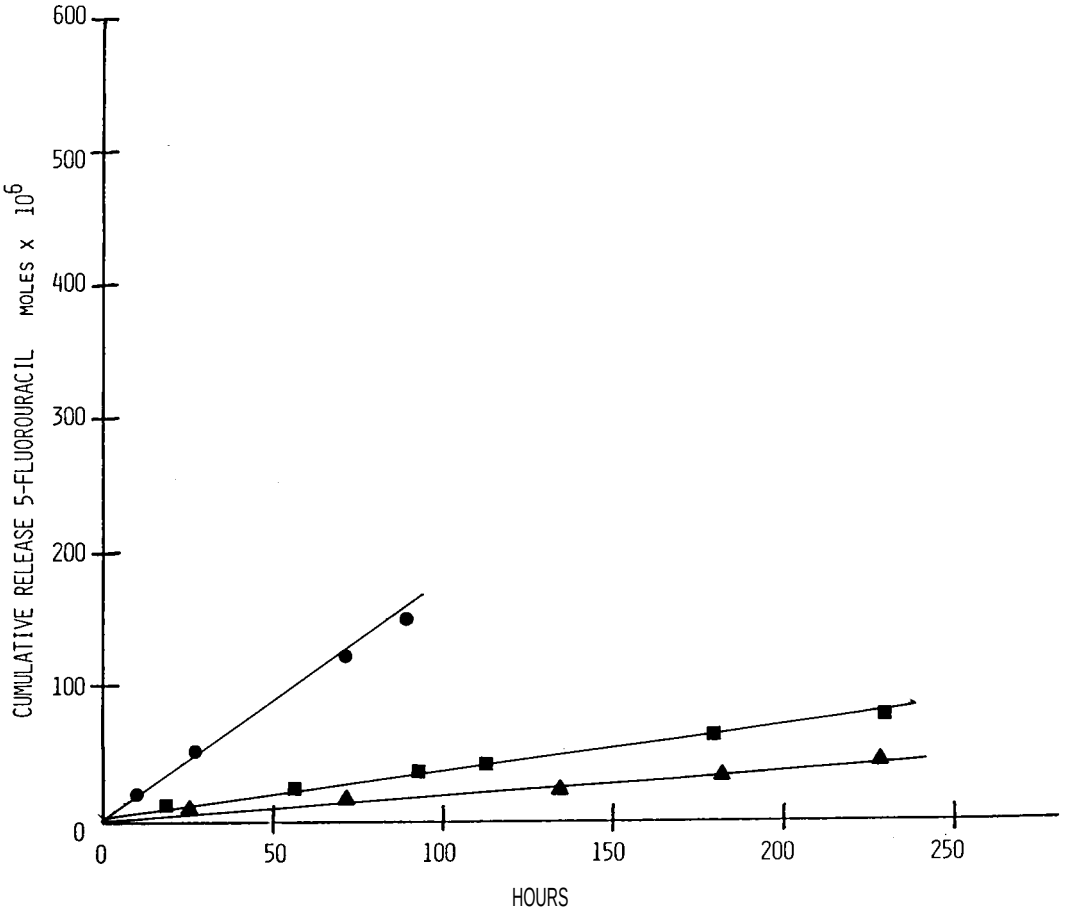


Figure 20. Release of 5-FU from 25% (▲), 50% (■), 75% (●) MA copolymers using 0.1000-g samples in small tubes.

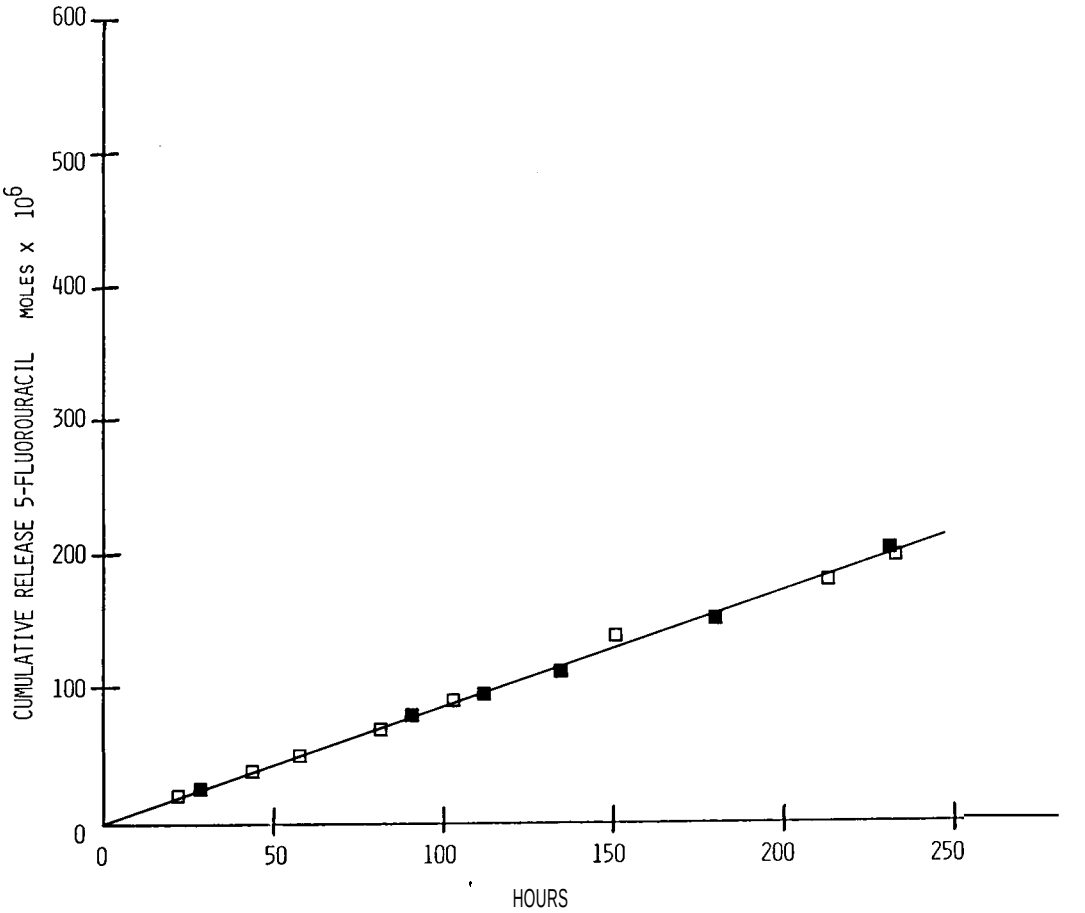


Figure 21. Release of 5-FU from 0.5000-g, 50% MA copolymer in dialysis membrane/wire mesh cage apparatus (□) and 0.1000-g, 50% MA copolymer in small gas dispersion tube multiplied by a factor of 5.0 (■).

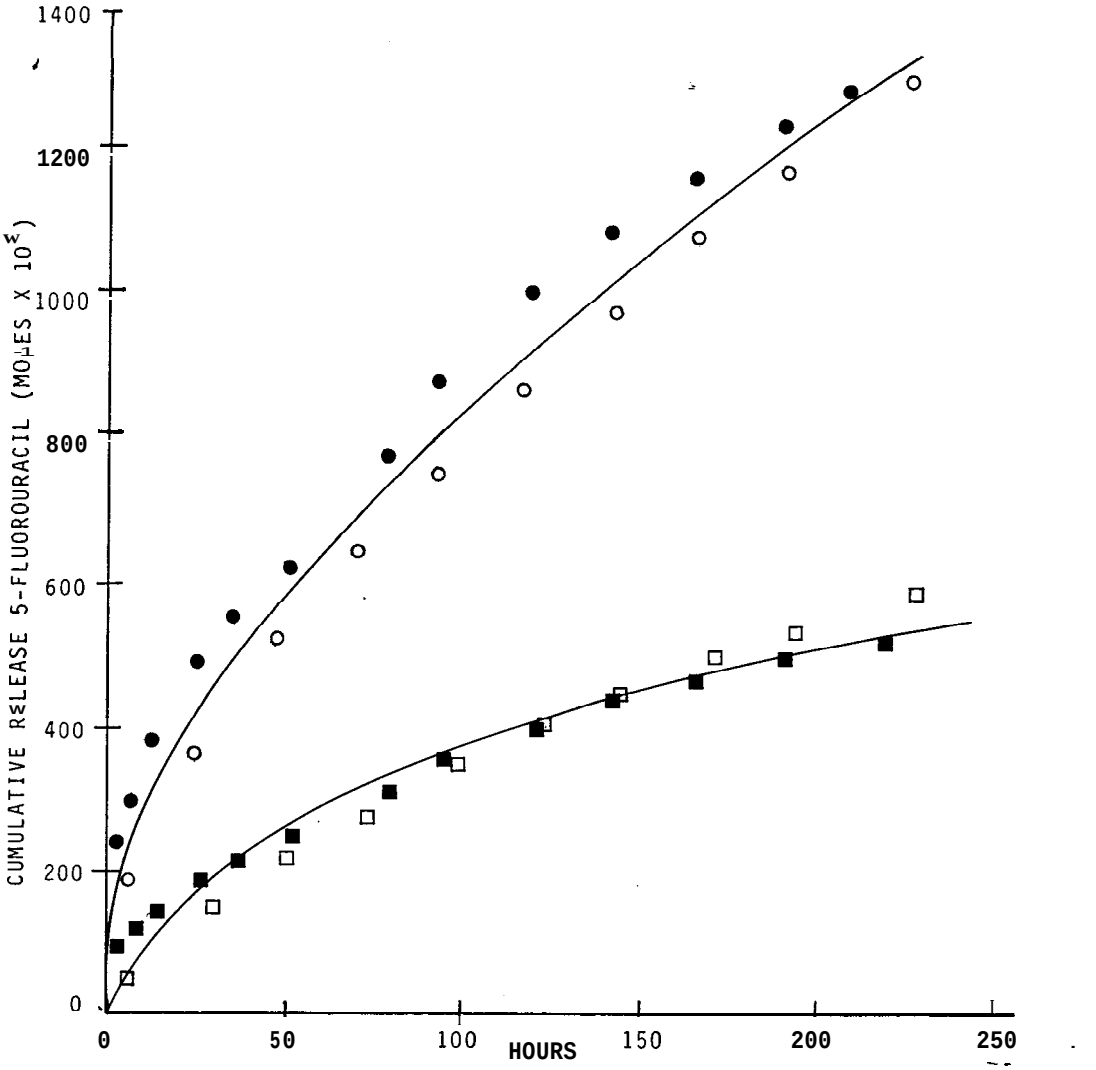


Figure 22. Release of 5-FU from EMCF samples, (●) 0.1003 g x 5.0 using small tubes, 0.1000 g x 2.0 using small tube (■), 0.2300 g using large tube (□), and 0.5000 g using large tube (○).

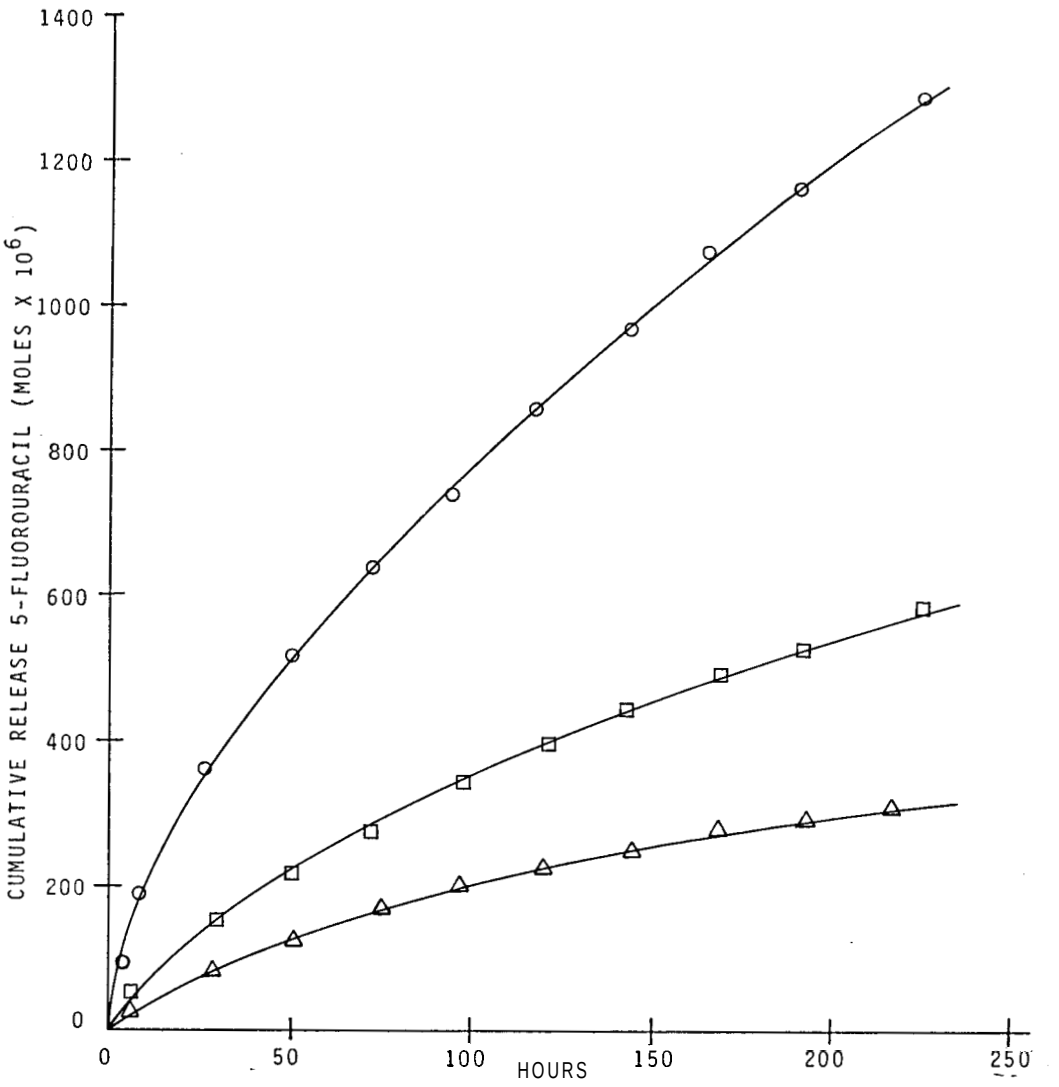


Figure 23. Release of 5-FU from 0.1000-g, 0.2000-g and 0.5000-g EMCF samples in larae tubes.(△),(○),(□).

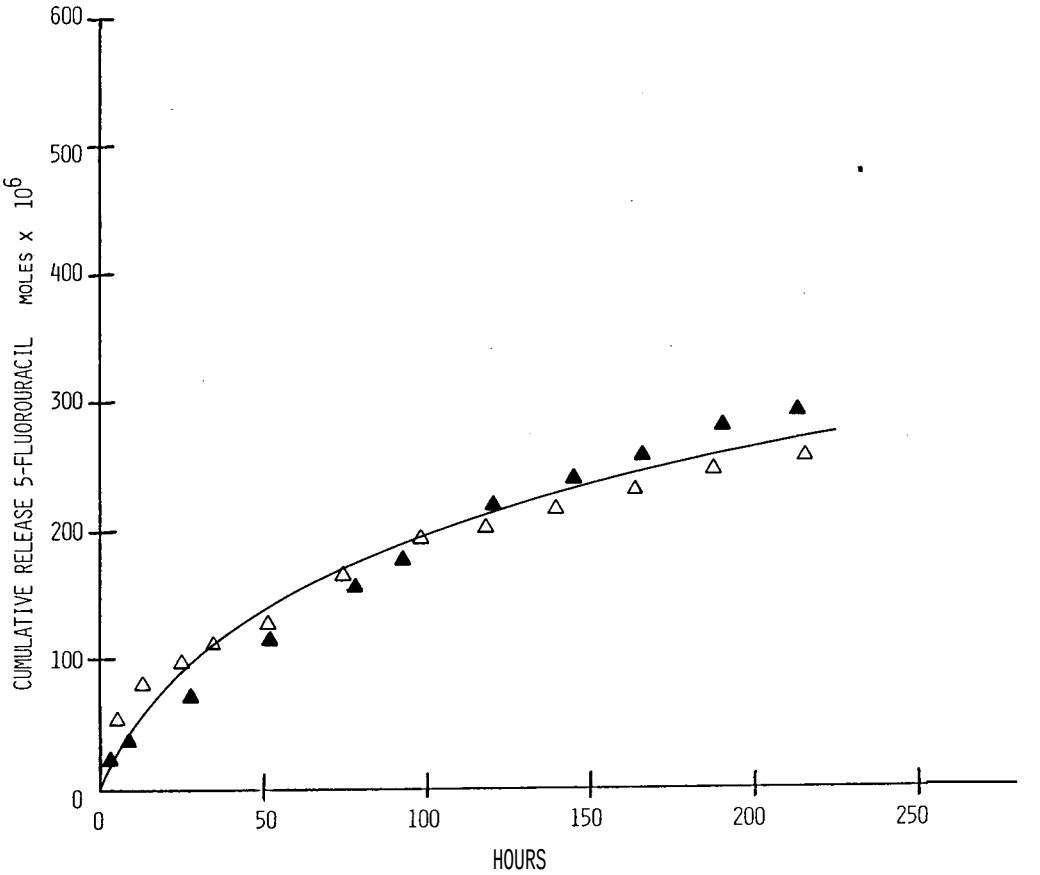


Figure 24. Release of 5-FU from 0.1000-g EMCF samples using small tubes (A) and using large tubes (A).

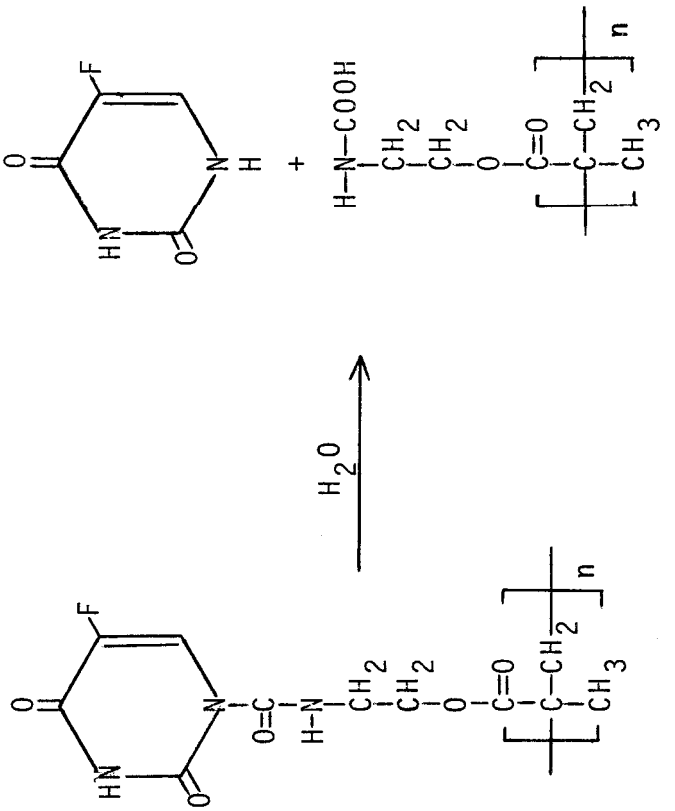


Figure 25. Hydrolysis of EMCF.

CHAPTER V

CONCLUSION

The present technique uses gas dispersion tubes which are much easier to assemble and use than the dialysis membrane/wire-mesh basket assembly of the earlier technique. Another advantage of using this new method is only 0.1000-g of the sample is enough to get a clear and satisfactory release profile of a drug. The technique can also be used to differentiate between copolymers with different release rates and is highly reproducible. This new technique can be used to perfectly demonstrate zero-order kinetics profiles as also reported by Gebelein & Hartsough[21,22] from the data obtained by using the more complex method.

It can be concluded that the gas dispersion tube technique is reproducible, easy to assemble and use, can be used to distinguish between polymers with different release rates, and the results can be correlated with the earlier studies, performed by Gebelein and Hartsough in this laboratory using the more complex dialysis membrane/wire-mesh apparatus.

APPENDIX A

Data From Hydrolysis Studies

DATA FROM HYDROLYSIS STUDIES

TRIAL 1 - 0.1 g/L EMCF

Time (hours)	Dilution	Absorbance	5-FU released ($M \times 10^6$)
0.50	None	0.1920	27.36
1.75	None	0.3294	46.94
2.00	None	0.3387	48.26
3.00	None	0.3782	53.90
6.00	None	0.4194	59.76
7.00	None	0.4468	63.67
12.25	None	0.5425	77.31
25.25	None	0.6904	98.38
35.50	None	0.7801	111.16
52.00	None	0.8841	125.98
80.00	None	1.1211	159.76
95.50	None	1.2632	180.00
121.00	None	1.4382	204.94
143.50	None	1.5610	222.44
168.00	None	1.6765	238.90
193.50	None	1.7678	251.77
222.00	None	1.8507	263.72
252.00	None	1.9364	275.37

Trial 2 - 0.1 g /L EMCF

Time (hours)	Dilution	Absorbance	5-FU released ($M \times 10^6$)
0.25	None	0.1311	17.28
0.50	None	0.2135	30.42
1.00	None	0.2880	41.04
1.50	None	0.3057	43.56
2.00	None	0.3261	46.47
3.00	None	0.3695	52.65
7.00	None	0.4595	65.48
8.00	None	0.4951	70.55
21.00	None	0.7314	104.22
24.00	None	0.7784	110.92
26.00	None	0.7951	113.30
27.00	None	0.8101	115.44
46.00	None	1.0285	146.56
49.00	None	1.0570	150.62
55.00	None	1.1124	158.40
71.00	None	1.2460	177.56
76.50	None	1.2994	185.11
97.25	None	1.4502	206.62
103.75	None	1.4691	209.33
128.00	None	1.6094	229.35
141.00	None	1.6407	233.80
144.00	None	1.6497	235.08
150.00	None	1.6801	239.41
174.25	None	1.7152	244.42
199.00	None	1.7577	250.47
203.00	None	1.7852	254.39
224.00	None	1.8007	256.60
249.00	None	1.8295	260.70
271.00	None	1.8718	266.73

Trial 3 - EMCF 0.1 g/L

Time (hours)	Absorbance	5-FU released ($M \times 10^6$)
0.25	0.1348	25.29
0.50	0.1825	26.01
0.75	0.2132	30.76
1.00	0.2362	33.66
2.00	0.2965	42.25
7.00	0.4314	61.47
8.00	0.4721	67.26
21.00	0.7095	101.10
24.00	0.7511	107.03
26.00	0.7728	110.12
46.00	1.0248	146.03
49.00	1.0560	150.48
55.00	1.1118	158.46
71.00	1.2481	177.81
97.00	1.4157	201.65
103.00	1.4237	202.38
128.00	1.5545	221.52
140.00	1.5860	226.01
149.00	1.6370	233.37
172.00	1.6820	239.85
190.00	1.7295	246.45
215.00	1.7892	254.96
248.00	1.8238	259.90

Trial 4 - 0.2 g/L EMCF

Time (hours)	Absorbance	5-FU released (M X 10^6)
0.25	0.0851	12.11
0.50	0.1517	21.62
1.00	0.2460	35.05
5.00	0.5640	80.37
7.50	0.6118	87.18
12.50	0.6845	97.67
24.50	0.8318	118.53
34.50	0.9020	128.54
5100	1.0577	150.72
81.00	1.3674	194.85
100.00	1.5510	221.02
124.00	1.7698	252.20
142.50	1.9262	274.48
167.00	2.1172	301.70
198.00	2.3344	332.61
225.00	2.4956	355.65
255.00	2.7951	398.50

Trial 5 - 0.05 g/L EMCF

Time (hours)	Absorbance	5-FU released (M X 10^6)
0.50	0.1620	23.09
1.00	0.1727	24.61
1.50	0.1908	27.19
2.00	0.2067	31.47
3.50	0.2570	36.62
7.00	0.3154	44.94
22.75	0.5081	72.46
59.00	0.7780	110.87
95.00	0.8991	128.12
102.00	0.9104	129.73
121.00	0.9417	134.19
167.00	0.9585	135.16
190.00	0.9730	138.65
270.00	0.9771	139.24

Trial 6 - 0.1 g/L 25% MMA

Time (hours)	Absorbance	5-FU released (M X 10^6)
0.50	0.0777	11.07
1.00	0.1242	17.70
2.50	0.2039	29.04
3.00	0.2214	31.55
5.00	0.2694	38.39
21.50	0.6484	92.34
25.00	0.7170	102.17
58.00	1.2492	178.01
94.00	1.4564	207.54
101.00	1.4685	209.26
128.00	1.4820	211.85
166.00	1.4625	208.40
189.00	1.4925	212.68

Trial 7 - 0.1 g/L 25% MMA

Time (hours)	Absorbance	5-FU released (M X 106)
2.00	0.0634	9.03
24.50	0.5652	81.97
49.50	0.8552	123.60
72.50	1.0964	156.25
93.50	1.3762	196.30
114.50	1.4247	203.02
195.00	1.5187	216.41
213.50	1.5224	216.94
237.00	1.5427	219.83
260.00	1.5405	219.52

Trial 8 - 0.1 g/L 50% MMA

Time (hours)	Absorbance	5-FU released (M X 10^6)
0.50	0.0262	3.73
1.00	0.0374	5.33
2.50	0.0617	8.79
3.00	0.0670	9.58
5.00	0.0895	12.75
21.50	0.1741	24.81
25.00	0.1824	25.99
58.00	0.3514	50.07
94.00	0.6144	87.55
101.00	0.6654	94.79
120.00	0.7765	110.59
128.00	0.8434	120.18
166.00	0.9410	134.19
189.00	1.0948	156.01
194.00	1.1062	157.61
223.00	1.1607	171.50
259.00	1.2035	172.85

Trial 9 - 0.1 g/L 50% MMA

Time (hours)	Absorbance	5-FU released (M X 10^6)
2.50	0.0372	15.30
9.25	0.1207	17.10
27.50	0.2167	30.88
94.50	0.6630	90.20
123.50	0.8302	118.30
142.00	0.9341	133.11
160.00	1.0387	148.02
177.25	1.1060	157.61
255.50	1.2767	181.93

Trial 10 - 0.1 g/L 50% MMA

Time (hours)	Absorbance	5-FU released (M X 10^6)
2.00	0.0442	6.28
24.50	0.1834	26.13
49.00	0.2918	41.58
72.50	0.4162	59.31
93.50	0.5867	83.52
114.50	0.7075	100.82
195.00	0.9622	137.11
213.50	0.9734	138.71
237.00	0.9888	140.79
260.00	0.9890	140.93

Trial 11 - 0.1 g/L 75% MMA

Time (hours)	Absorbance	5-FU released (M X 10^6)
0.50	0.0275	3.92
1.00	0.0608	8.66
3.50	0.1075	11.88
5.00	0.1201	17.11
24.00	0.1431	20.39
48.00	0.1591	22.69
53.00	0.1650	23.50
73.50	0.1865	26.58
142.00	0.2511	35.78
165.00	0.2867	40.85
189.50	0.3201	45.61
213.00	0.3482	49.62
257.00	0.3787	53.96

Trial 12 - 0.1 g/L 75% MMA

Time (hours)	Absorbance	5-FU released (M X 10^6)
2.00	0.0500	7.84
24.50	0.1255	17.88
49.00	0.1398	19.92
72.50	0.1574	22.43
93.50	0.1782	25.39
114.50	0.1967	28.03
195.00	0.2710	38.61
213.50	0.2981	42.47
237.00	0.3145	44.82
260.00	0.3605	51.37

Trial 13 - 0.1g/L 25% MA

Time (hours)	Absorbance	5-FU released (M X 10^6)
0.50	0.0001	0.01
2.25	0.0311	4.43
4.00	0.0421	5.99
16.50	0.0788	11.23
75.00	0.1091	15.55
139.00	0.1607	22.90
186.00	0.2048	29.18
237.50	0.2715	38.69

Trial 14 - 0.1g/L 25% MA

Time (hours)	Absorbance	5-FU released (M X 10^6)
3.50	0.1881	26.80
9.00	0.2772	39.50
27.50	0.3067	43.62
94.50	0.4394	62.61
123.50	0.5254	74.87
142.00	0.5605	79.87
177.25	0.5881	83.80
255.50	0.7468	106.42
281.00	0.8292	118.19

Trial 15 - 0.1g/L 50% MA

Time (hours)	Absorbance	5-FU released (M X 10^6)
4.00	0.1951	21.81
29.00	0.2887	41.13
93.75	0.3781	53.88
115.00	0.4004	57.06
139.00	0.4238	60.40
186.00	0.4832	68.86
237.50	0.5674	80.85
264.00	0.6211	88.49

Trial 16 - 0.1g/L 75% MA

Time (hours)	Absorbance	5-FU released (M X 10^6)
1.00	0.0931	13.40
3.00	0.1285	18.31
16.50	0.3647	51.97
75.00	0.9500	135.38
115.00	1.2302	175.28
139.00	1.2787	182.21
162.00	1.2934	184.31
214.00	1.3520	192.66
237.50	1.3704	195.28
264.00	1.3718	195.48

Trial 17 - 0.1g/L 75% MA

Time (hours)	Absorbance	5-FU released (M X 10^6)
3.50	0.1671	23,89
27.50	0.2677	38.15
94.50	0.4592	65.43
123.50	0.5312	75.70
160.00	0.6197	88.31
177.25	0.6515	92.84
255.50	0.8141	116.01
281.00	0.9014	128.44

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Trial 18 - 0.1g/L EMCF

Time (hours)	Absorbance	5-FU released ($M \times 10^6$)
1.00	0.048	3.84
3.00	0.126	17.96
18.00	0.237	33.37
30.00	0.562	77.09
51.50	0.822	117.14
101.00	1.405	202.21
121.00	1.547	220.45
147.00	1.741	248.09
170.00	1.901	270.90
195.00	2.057	293.12
271.00	2.217	315.15

Trial 19 - 0.2g/L EMCF

Time (hours)	Dilution	Absorbance	5-FU Rlegsed ($M \times 10^6$)
1.00	-	0.102	14.55
3.00	-	0.190	27.08
8.00		0.322	45.89
26.75		0.710	102.60
30.00	-	0.747	106.45
51.50	-	1.488	211.21
76.00	-	1.176	167.81
101.00		2.112	300.96
121.00	-	2.339	333.31
170.00	10X	0.310	441.75
195.00	10X	0.367	522.98
219.00	10X	0.413	588.53
271.00	10X	0.472	672.21

Trial 20 - 0.5g/L EMCF

Time (hours)	Dilution	Absorbance	5-FU Released ($M \times 10^6$)
1.00	-	0.310	44.18
3.00	-	0.378	75.10
8.00	-	1.287	183.39
30.00	-	1.733	246.95
51.50	10X	0.359	511.86
76.00	10X	0.455	648.38
98.00	10X	0.550	783.75
121.00	10X	0.612	872.10
170.00	10X	0.766	1091.55
214.00	10X	0.921	1312.42
271.00	10X	1.001	1460.65

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