DEVELOPMENT OF CONTROLLED DRUG DELIVERY SYSTEM OF ANTIMICROBIALS FOR THE LOCAL TREATMENT OF PERIODONTAL DISEASE

By
Firas F. Hamudi

Supervisor
Dr. Ena'am Khalil

Co- Supervisor
Dr. Al-Sayed Sallam

This Thesis was submitted in Partial Fulfillment of the Requirements for Master's Degree of Science in Pharmaceutical Sciences

Faculty of Graduate Studies
University of Jordan

December 2003

المنـــارة للاستشارات
www.manaraa.com

This thesis (Development of Controlled Drug Delivery System of Antimicrobials for the Local Treatment of Periodontal Disease) was successfully defended and approved on: ------------------------------------

Examination Committee	**Signature**
Dr. Ena'am Khalil, Chairman Assoc. Prof. of Physical Pharmacy	---------------------------
Dr. Al- Sayed Sallam, Member Prof. of Pharmaceutics	---------------------------
Dr.Mutaz S. Salem, Member Prof. of Biopharmaceuticals	---------------------------
Dr. Khalid Aiedeh, Member Assoc. Prof. of Pharmaceutics	---------------------------
Dr. Bassam Amro, Member Assoc. Prof. of Pharmaceutics	---------------------------

المنارة للاستشارات

www.manaraa.com

DEDICATION

To Iraq

Our beloved

Our painful wound

Our beautiful dream

المنارة للاستشارات

www.manaraa.com

ACKNOWLEDGMENT

I have to thank my supervisor Dr. Ena'am Khalil and Dr. Al-Sayed Sallam for their guidance.

Special thanks are expressed to the examination committee members for their important comments.

My thank is devoted to Dr. Isma'eil, Dr. Emel Al-Bakri, Mr. Muhammad Zuairi and Ms. Tuhfa Nirokh for their valuable help.

المنارة للاستشارات

www.manaraa.com

TABLE OF CONTENTS

Subject	page
Committee Decision………………………………………………..	ii
Dedication………………………………………………………….	iii
Acknowledgement………………………………………………….	iv
List of Contents…………………………………………………….	v
List of Tables……………………………………………………….	vi
List of Figures…………………………………………………….	vii
List of Abbreviation……………………………………………...	xi
Abstract…………………………………………………………….	xii
Introduction……………………………………………………….	1
Materials and Experimental Methods…………………………….	30
Results and Discussion…………………………………………..	40
Conclusions and Future Work ……………………………….….	92
References…………………………………………………………..	94
Abstract in Arabic………………………………………………..	102

المنارة للاستشارات
www.manaraa.com

LIST OF TABLES

No.	Title	Page
1	Summaries of some drug delivery system used in the treatment of periodontal disease	10
2	Percentage of PG and water in GMO/EC/Metronidazole formulations.	35
3	Values of absorbance against different concentration of metronidazole and the relative standard deviation between (B) and within (W) day in distilled water	41
4	Values of absorbance against different concentration of metronidazole and the relative standard deviation between (B) and within (W) day in phosphate buffer pH 7.4	41
5	Values of absorbance against different concentration of metronidazole and the relative standard deviation between (B) and within (W) day in isotonic saline	42
6	Equilibrium solubility of metronidazole in different aqueous solutions at 37C°	43
7	The phase behavior of GMO and Physical State of EC in mixture contain water, GMO, metronidazole, EC and PG	48
8	Similarity factor (f2) for dissolution profiles between different concentration of EC in GMO that contain 20% Metronidazole	50
9	Similarity factor (f2) for the dissolution profiles between formulations having different concentration of PG in GMO containing 7%EC and 20% Metronidazole	52
10	Similarity factor (f2) for the dissolution profiles between formulations having different concentration of PG in GMO that containing 20% Metronidazole	53
11	Similarity factors (f_2) for the dissolution profiles of formula A using dissolution media having various tonicities	64
12	Flow indices of the formulations containing GMO (7% EC), 20% Metronidazole and different percentage of water and PG	68
13	Similarity factors (f2) for the dissolution profiles of Formula A stored at different storage conditions	70
14	The average work and standard deviation of mucoadhesion of unswollen GMO and Formula A	78
15	Percentage of PG and water in GMO 7%EC in different loading of metronidazole formulations	88

المنارة للاستشارات
www.manaraa.com

LIST OF FIGURES

No.	Title	Page
1	Bacteria in subgingival plaque have caused a periodontal pocket to develop inflaming surrounding tissue and causing loss of alveolar bone.	4
2	Structural formula of GMO (for pure material)	15
3	The cubic phase of GMO	17
4	Phase diagram of the glycerol monooleate–water system depicting the reversed micellar phase (L_2), the lamellar phase (Lα), cubic phase (C) and reversed hexagonal phase (H_{II}).	17
5	Structures of the lipid liquid crystalline lamellar L_{α}, inverted hexagonal H_{II} and inverted bicontinuous cubic phases	18
6	Calibration curve of metronidazole in phosphate buffer pH 7.4.	40
7	Calibration curve of metronidazole in normal saline solution.	40
8	Calibration curve of metronidazole in distilled water	41
9	Dissolution profiles from formula containing (20% PG, 20% Metronidazole and 60% GMO containing 7% EC) determined spectrophotometrically using (▲) phosphate buffer as a reference solution and (■) filtered solution of the dissolution medium containing excipients	42
10	Partial triangular phase diagram of water, GMO and Metronidazole	44
11	Partial triangular phase diagram of water, GMO and EC	45
12	Partial triangular phase diagram of water, GMO and PG	46
13	Release profiles of metronidazole in GMO base with different concentration of EC as a function of time	51
14	Release profiles of metronidazole from GMO base containing 7%EC and different concentration of PG as a function of time	52
15	Release profiles of metronidazole from GMO base containing different concentration of PG as a function of time	53
16	Fitting of dissolution profile data of metronidazole in GMO base containing different concentration of EC into Peppas equation	55
17	Fitting of dissolution profile data of metronidazole in GMO base with 7%EC containing different concentrations of PG into Peppas equation	55

المنارة للاستشارات
www.manaraa.com

No.	Title	Page
18	Fitting of dissolution profile data of metronidazole in GMO base contain different concentration of PG into Peppas equation	56
19	Release profiles of metronidazole in GMO base containing different concentrations of EC as a function of square root of time	57
20	Effect of EC concentration on the dissolution rate constant of metronidazole from GMO base	57
21	Release profiles of metronidazole in GMO base containing different concentration of PG as a function of square root of time	58
22	Release profiles of metronidazole in GMO base with 7%EC containing different concentration of PG as a function of square root of time	58
23	Effect of PG concentration on the dissolution rate constant of metronidazole from GMO base contain 7%EC	59
24	Release profiles of metronidazole from Formulas A, B, C and D as a function of time	60
25	Fitting of dissolution profile data of metronidazole from Formulas A, B, C and D into Peppas equation	61
26	Release profiles of metronidazole from Formulas A, B, C and D as a function of square root of time	61
27	Effect of the amount of formula A on metronidazole release as a function of time	62
28	Release profiles of metronidazole from various amounts of Formula (A) fitted into Peppas equation	63
29	Release profiles of metronidazole from various amount of Formula A as a function of square root of time	63
30	Release profiles of metronidazole from Formula A in dissolution media having various tonicities as a function of time	64
31	Fitting of Release profiles of metronidazole from Formula A in dissolution media having various tonicities different ionic strength into Peppas equation	65
32	Release profiles of metronidazole from Formula A in dissolution media having various tonicities as a function of square root of time	65
33	Flow curves represent the influence of PG and/or water on the rheological behavior of GMO containing 7% EC, in addition to metronidazole 20% at 30°C	67

المنارة للاستشارات
www.manaraa.com

No.	Title	Page
34	Flow curves represent the influence of PG and/or water on the rheological behavior of GMO containing 7% EC, in addition to metronidazole 20% at 30C°Flow curve represents the influence of PG and/or water on the rheological behavior of GMO containing 7% EC, in addition to metronidazole 20% at 30°C.	69
35	Release profiles of metronidazole from Formula (A) kept under different storage conditions for 5 weeks as a function of time	71
36	Fitting of dissolution profile data of metronidazole Formula (A) kept under different storage conditions for 5 weeks into Peppas equation	71
37	Release profiles of metronidazole from Formula (A) kept under different storage conditions for 5 weeks as a function of square root of time	72
38	Log-probability plots of the cumulative under and over size percent of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and metronidazole powder	73
39	The geometric means and the geometric standard deviation of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 11 weeks at room temperature, 10 weeks at 4°Cand metronidazole powder	74
40	Histograms represent the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 11 weeks at room temperature, after storage for 10 weeks at 4°C and metronidazole powder	74
41	Log-probability plots of the cumulative under and over size percent of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 11 weeks at room temperature and after storage for 10 weeks at 4°C	75
42	Histograms represent the particle size distribution of suspended metronidazole in freshly prepared sample and after storage for 2 and 4 weeks at room temperature	76
43	The geometric means and the geometric standard deviation of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 2 and 4 weeks at room temperature	76

المنارة للاستشارات
www.manaraa.com

No.	Title	Page
44	Log-probability plots of the cumulative under and over size percent of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 2 and 4 weeks at room temperature	77
45	DSC thermogram of GMO at a heating rate of 2°C/min.	79
46	DSC thermogram of GMO containing 7%EC at a heating rate of 2°C/min.	80
47	DSC thermogram of Metronidazole powder at a heating rate of 10°C/min.	80
48	DSC thermogram of Metronidazole (20%w/w) and GMO at a heating rate of 10°C/min	81
49	DSC thermogram of Formula A at a heating rate of 2°C/min	81
50	DSC and thermogravimetric thermograms of Formula A at a heating rate of 10°C/min.	82
51	DSC and thermogravimetric thermograms of Formula A without metronidazole at a heating rate of 10°C/min.	82
52	DSC and thermogravimetric thermograms of fresh sample of Formula A at a heating rate of 10°C/min, stored at room temperature for 3 months	84
53	DSC and thermogravimetric thermograms of fresh sample of Formula A at a heating rate of 10°C/min., stored at 4°C for 3 months	84
54	DSC thermogram of rerunning sample of Formula A at a heating rate of 10°C/min. stored at room temperature for 3 months	85
55	DSC thermogram of rerunning sample of Formula A at a heating rate of 10°C/min, stored at 4°C for 3 months	85
56	Release profiles of metronidazole from 10% and 20% drug loading Formula A as a function of time	87
57	Fitting of dissolution profile data of metronidazole from 10% and 20% drug loading Formula A into Peppas equation	89
58	Release profiles of metronidazole from 10% and 20% drug loading Formula A as a function of square root of time	89
59	Release profiles of metronidazole from 30% and 40% drug loading Formula A as a function of time	90
60	Fitting of dissolution profile data of metronidazole from 30% and 40% drug loading Formula A into Peppas equation	90
61	Release profiles of metronidazole from 30% and 40% drug loading Formula A as a function of square root of time	91
62	Effect of metronidazole concentration on the dissolution rates constant of metronidazole from Formula A	91

المنارة للاستشارات
www.manaraa.com

List of Abbreviation

Glycerol monooleate	GMO
Ethyl cellulose	EC
Propylene glycol	PG
Thermogravimetry	TGA
Differential scanning calorimeter	DSC

المنارة للاستشارات
www.manaraa.com

DEVELOPMENT OF CONTROLLED DRUG DELIVERY SYSTEM OF ANTIMICROBIALS FOR THE LOCAL TREATMENT OF PERIODONTAL DISEASE

By
Firas F. Hamudi

Supervisor
Dr. Ena'am Khalil

Co- Supervisor
Dr. Al-Sayed Sallam

ABSTRACT

This study describes the development and characterization of mucoadhesive controlled release formulation of metronidazole for use in the treatment of periodontal disease. Metronidazole was formulated as a liquid suspension, which undergoes transformation to a release-controlling semi-solid gel on contact with gingival fluid.

The system was based on the ability of glycerol monooleate to form a viscous liquid crystalline cubic phase when comes in contact with water. Ethyl cellulose was added to the glycerol monooleate to increase the viscosity of the cubic phase and to prolong the sustained release of metronidazole from the cubic phase. The release constant of the metronidazole from the cubic phase decreased significantly ($P < 0.05$) as the concentration of ethyl cellulose in the glycerol monooleate increased.

المنارة للاستشارات

www.manaraa.com

The viscosity of the formulations was controlled by incorporation of a small quantity of water that allowed the formation of the liquid crystalline phases of glycerol monooleate like reversed micellar and lamellar phases that are characterized by having low viscosity and being easily injectable. Furthermore, propylene glycol was added to the formula to decrease the viscosity. The release exponents of the formulations ranged from 0.4 to 0.55; which indicated that the drug release was diffusion controlled.

A formula containing 20% metronidazole, 10% propylene glycol, 5% water and 65% glycerol monooleate that containing 7% ethyl cellulose was found to have the optimum drug release property and to be easily injectable at room temperature. The formula characteristics were assessed by thermal analysis and viscosity measurements. Particle size analysis and the drug release results indicated that the best storage condition for the formula was at 4°C in a dark place. Polarized light microscope was used to observe the phase changes of the glycerol monooleate.

There was no effect of the different additives on the mucoadhesive property of glycerol monooleate. The effect of the tonicity of the dissolution medium on the release of the drug was not significant ($P > 0.05$) but the effect of matrix thickness and the drug loading were significant ($P < 0.05$) on the controlled release property of the drug.

Therefore, it could be concluded that the metronidazole containing suspension formula described in this study could be administered easily by syringe and it forms in the periodontal pocket a controlled release, mucoadhesive gel that enhances the removal of the anaerobic pathogen, thus improving the periodontal health.

المنارة للاستشارات
www.manaraa.com

1.INTRODUCTION

1.1 Periodontal disease

Periodontal disease is a general term, which encompasses several pathological conditions affecting the tooth supporting structures. Periodontal diseases include conditions such as chronic periodontitis, aggressive periodontitis and necrotizing periodontitis (Brown *et al.,* 1996).

A destruction of the periodontal ligament, a resorbtion of the alveolar bone and the migration of the junctional epithelium along the tooth surface characterize these conditions (Figure.1). The clinical signs of periodontitis are changes in the morphology of the gingival tissue, bleeding upon probing as well as periodontal pocket formation. This pocket provides an ideal environment for growth and proliferation of anaerobic pathogenic bacteria; these bacteria colonizing the subgingeval area are the main etiology to the development of the inflammation and tissue destruction (Marsh, 1989).

Bacteria associated with periodontal disease include gram-negative organism such as *Porphyromonas gingvalis* and anaerobes such as *Actinobacillas actinomycetemcomitans* and *Bactericides* species (Wayne *et al.,* 2001). Deposits of gray-yellow bacterial plaque on the teeth in conjunction, erythma, edema, and bleeding of the gingiva are seen in gingivitis. In periodontitis, halitosis and unpleasant tastes may accompany these symptoms and signs. The teeth may become loosened, and destruction of the periodontal ligament proceeds. Spaces may develop between teeth, chewing may be difficult, and abscesses may occur (Dwight, 2000).

المنارة للاستشارات
www.manaraa.com

1.1.1 Pathogenesis

The microbial flora associated with periodontal diseases is comprised of Gram negative bacteria, such as *Porphyromonas gingvalis* and anaerobes such as *Actinobacillas actinomycetemcomitans* that have been identified as potential periodontal pathogens. However, although a Gram-negative infection is a prerequisite to periodontal destruction, the host response plays an important role in determining the outcome of the process (Slots and Jorgensen, 2000).

Early inflammatory lesions of the gingival tissues are associated with a predominately mononuclear cell infiltrate. Once microorganisms have colonized the gingival crevice, lipopolyscharide derived from periodontal pathogens have the ability to penetrate the root cementum and gingival tissues. Lipopolyscharide can stimulate the minor cell population of monocytes and macrophages to secrete cytokines. These cytokines have the ability to stimulate other cell types associated with inflammatory infiltrate, such as lymphocytes and fibroblasts, to secrete additional inflammatory mediators, amplifying inflammatory response. This cascade of events suggests an important role for LPS-inflammatory mediators in the tissue destruction. High level of pro-inflammatory mediators such as tumor necrosis factor-α , intraleukins-1β and prostaglandines-E_2 with the potential to induce bone resorbtion, further more intraleukins-1β and prostaglandines-E_2 have been correlated to the active phases of periodontal breakdown.

Pocket formation starts as an inflammatory change in the connective tissue wall of the gingival sulcus. The cellular and fluid inflammatory exudate causes degeneration of the surrounding connective tissues, including the gingival fibers as shown in figure 1. Just apical to the junctional epithelium, collagen fibers are destroyed and the area become occupied by inflammatory cell and edema (Gray, 2000).

المنارة للاستشارات

www.manaraa.com

Two mechanisms are considered to be associated with collagen loss: (1) collagenases and other enzymes secreted by fibroblasts (Takada and Donath, 1988), polymorphonuclear leukocytes and macrophages (Quirynen *et al.,* 1999) become extracellular and destroy collagen, these enzymes are called *matrix metallprotinases* (Ten, 1994). (2) Fibroblast phagocyte collagen fibers by extending cytoplasmic process to the ligament cementum interface and degrade the inserted collagen fibrils and the fibril of the cementum matrixes (Deporter *et al.,* 1980).

The transformation of a gingival sulcus into a periodontal pocket creates an area where plaque removal becomes impossible, and the following feedback mechanism is established:

Plaque ⟶ Gingival inflammation ⟶ Pocket formation ⟶ More plaque formation.

1.1.2 Systemic effect

In addition to local complication, periodontal disease may have systemic consequences. Bacterial respiratory tract pathogen colonize dental plaque and are believed to contribute to pulmonary infections and exacerbation of chronic obstructive pulmonary disease through aspiration, generation of cytokines, and alteration in the antibacterial propriety of saliva (Scannapieco, 1999).

In patient in the intensive care unit, this colonization has prospectively linked to development of nosocomial pneumonia and bateremia. Recently, much attention has been given to the possible relationship between periodontitis and the development of atherosclerosis. This was postulated after showing that bacterial species such as *Porphyromonas gingvalis* can activate thrombotic pathways and that periodontal infections are associated with release of cytokines and other inflammatory mediators (Loos *et al.,* 1998).

المنارة للاستشارات
www.manaraa.com

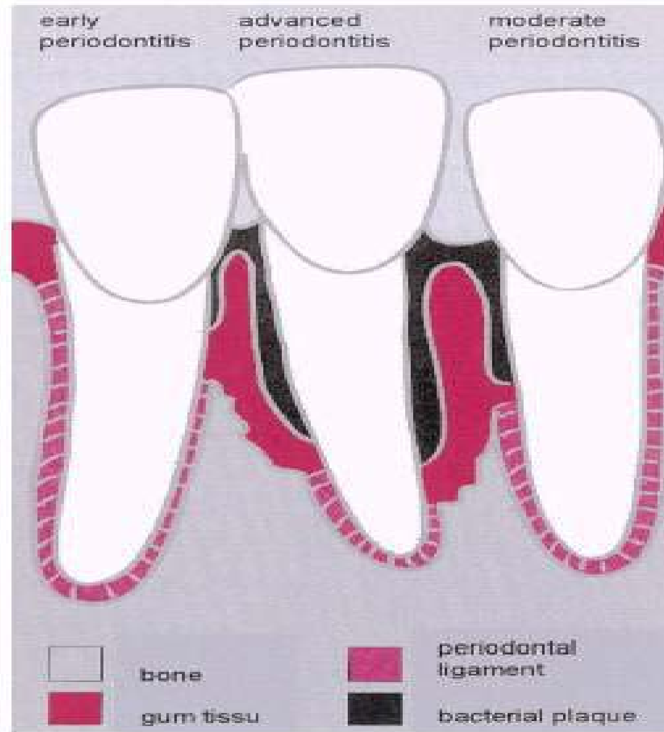


Figure 1. Bacteria in subgingival plaque have caused a periodontal pocket to develop, inflaming surrounding tissue and causing loss of alveolar bone.

1.1.3 The periodontal pocket

The periodontal pocket is lined with epithelium on one side and tooth cementum on the other. Lack of attachment is demonestrated by insertion of periodontal probe and change in attachment level or pocket depth has been monitered to assess disease progression. Pocket depth ranging from 4 to 12 mm is generally observed at disease sites (Haffajee and Socransky, 1986).

They have reviewed the characteristics of the gingival crevicular fluid (Cimasoni *et al.,* 1983), which fills the periodontal pocket. Healthy sites are associated with small volume (0.04µl) and low flowing rates (0.03µl/min) and the protein concentration similar to extracellular fluid (Hattingh and Ho, 1980). In contrast, at diseased site there is increase in fluid production and the protein composition like serum. The volume and fluid flow rate depends on the degree of inflammation. Volumes of about 0.5µl and flow

المنارة للاستشارات
www.manaraa.com

rates 0.33-0.5 µl/min. Goodson calculated the turn over rate of gingival fluid to be 40 times per hour (Goodson, 1989).

1.1.3.1 Pocket contents

Periodontal pocket contain dibers consisting principally of microorganism and their enzymes, endotoxines and metabolic products, gingival fluid, food remnant, salivary mucin, desquamated epithelial cell, and leukocytes. Plaque-covered calculus usually projects from the tooth surface (McMillan *et al.,* 1958).

المنارة للاستشارات

www.manaraa.com

1.2 Antibacterial agents in the treatment of periodontal disease

Antibacterial agents can be adminestered locally, orally, or parenterally in all cases, their purpose is to reduce the number of bacteria present in the infected periodontal pocket (Christersson *et al.,* 1985). An ideal antibiotic for use in the preventation and treatment of periodontal diseases should be specific for periodontal pathogen, nontoxic, substantive, not in general use of treatment of other diseases, and inexpensive. Antibiotics therapy should not be used as a monotherapy; that its must be part of comprehensive periodontal treatment plan. This treatment should have department of root surfaces, optimal oral hygiene, and frequent supportive periodontal therapy at the center of therapy (Jorgensen and Slots, 2000). Local administration of antibacterial agents, directly in the pocket to provide greater concentration to the infected area and reduce possible systemic side effect.

1.2.1 Antimicrobial Choice

A-Tetracyclines

Tetracyclines have been widely used in the treatment of periodontal diseases. Tetracyclines have the ability to concentrate in the periodontal tissues and inhibit the growth of *Actinobacillas actinomycetemcomitans*. In addition, they exert anticollaginase effect that can inhibit tissue destraction and may aid bone regeneration (Solts and Rams, 1990).

B-Metronidazole

Metronidazole is not the drug of choice for treating *A. actinomycetemcomitans* infections but it may be effective at therapeutic levels owing to its hydroxy metabolite (Rams and Solts, 1992). Metronidazole is also effective against anaerobes such as *Porphyromonas gingvalis* and *Prevotella intermedia* and spirochetes (Greenstein, 1993).

المنارة للاستشارات
www.manaraa.com

C-Ciprofloxacin & Ofloxacin

These showed marked antibacterial activity against periodontopathic bacteria including Bacteriod species, Fusobacterium species and *A. actinomycetemcomitans* (Rams *et al.,* 1992). Furthermore, ciprofloxacin and ofloxacin have high chemical stability and because they demonstrate minimal effect on *streptococcus* species, which are associated with periodontal health, therapy may faciliate the reestablishment of microflora (Kimura *et al.,* 1991).

D-Clindamycin

Clindamycin is effective against anaerobic bacteria like *Porphyromonas gingvalis* and *Prevotella intermedia*. It is effective in cases in which the patient is allergic to penicillin (Sauvetre *et al.,* 1993).

E-Macrolides

Spiramycin use for treament of periodontal disease, it is effecive against gram positive and excreted in high concentration in saliva (Mills *et al.,* 1979).

Azithromycin is effective against anaerobes and gram-negative bacilli. The conctration of azithromycin in tissue specimens from periodontal lesions is higher than that of normal gingiva. The Azithromycin is actively trasported to sites of inflammations by phagocytes and then released directly into the sites of inflammations as the phagocyte rupture during phagocytosis (Hoepelman and Schneider, 1995).

F-Sanguinarine

Sanguinarine, a benzophenanthridine alkaliod with a wide-spectrum antiseptic activity in vitro, was obtained from the bloodroot plant (*Sanguinaria canadensis*) (Godowski *et al.,* 1995).

المنارة للاستشارات
www.manaraa.com

G-Chlorhexidine

The use of chlorhexidine as an antifungal and antibacterial agent in dentistry is well known, also it is effective in periodontitis (Swai *et al.,* 1997).

1.2.2 The appropriate route of administration of antimicrobiales

Systemically applied antimicrobials have been advocated for the treatment of severe forms of periodontitis. However, in the early 1970s, concern emerged with respect to systemic antibiotic therapy for chronic infections such as periodontal disease. Indeed, side effects including hypersensitivity, gastrointestinal intolerance and the development of bacterial resistance have been described (Bollen and Quirynen, 1996).

Some studies also reported poor results due to the fact that the active product could not achieve an adequate concentration at the site of action and/or due to the inability of the active product to be retained locally for a sufficient period of time (Vandekerckhove *et al.,* 1997). These drawbacks would be markedly reduced if antimicrobial agents applied locally could be used, although unwanted effects such as gastrointestinal disturbances and development of antibiotic resistance cannot be totally ruled out. Incorporating the active agent into controlled release delivery systems to be placed directly in the periodontal pocket can enhance the local tissue concentration of a drug. Such systems may have applications where systemic drugs are currently used, for instance localized juvenile periodontitis, refractory periodontitis and periodontitis with secondary systemic involvement, e.g. HIV periodontitis. Sustained local delivery systems might also be recommended for sites considered as difficult to instrument because of depth or anatomical complexity (Kornman, 1993).

More controversial would be the replacement of root planing by controlled release devices. However, studies suggest that these systems, used as adjuncts to scaling

المنارة للاستشارات
www.manaraa.com

and root planning, give a slight advantage over mechanical treatment alone, although the clinical difference has often been insignificant. This may be because scaling and root planning alone is usually quite effective in producing clinical and statistically significant improvements (Lie *et al.,* 1998).

On the other hand, few studies have evaluated the effects of local drug delivery systems on sites that responded poorly or showed recurrence after scaling and root planning (Noyan *et al.,* 1997). If improvements were maintained for a long-term period, then such systems would be an interesting tool in the management of localized periodontal lesions. For most studies dealing with local delivery systems, the treatments did not result in any serious adverse effects and were well tolerated by the patients. Conversely, a biodegradable sustained release drug delivery system which can be placed into the periodontal pocket and maintain therapeutic concentrations for prolonged periods of time would be advantageous. Indeed, in addition to improving compliance over systemic antibiotics, biodegradable devices are cost effective, as they will not require a second visit to the periodontist for removal.

To be useful for periodontal therapy, it is desirable to have a bioerodible drug delivery system that can maintain an effective drug release rate in the periodontal pocket while simultaneously eroding throughout the duration of treatment up to several days.

1.2.2.1 The advantages of using local controlled delivery systems for antimicrobial agents

There are two approaches to improve drug action: (1) sustained and controlled drug release to reduce side effects;(2) site-specific drug delivery to minimize systemic effects (Van Wachem *et al.,* 1991).

المنارة للاستشارات
www.manaraa.com

Drug delivery system can be classified according to the mechanism controlling drug release, the distinguished methods are (1) Solvent controlled matrix systems based on macromolecular matrix permeability to drug molecules after matrix swelling into hydrated medium; (2) Reservoir systems controlled by drug diffusion across a polymeric membrane; (3) Chemically controlled systems, where the rate of drug released controlled by the rate and extent of chemical bond degradation and the erosion of polymer matrix(Steinberg *et al.,* 1990).

Many polymer based systems for antibiotic delivery in the periodontal diseases have been studied and evaluted in vitro and/or in vivo (Table 1).

Some of these systems are not resorbable, while most are biodegradable. Nonbiodegradable systems have to be removed after complete drug release, which may cause irritation and inflammation of the treated site. Conversely, a biodegradable system can be placed in the periodontal pocket and maintain therapeutic concentrations for prolonged period of times and will not require a second visit to the periodontist for removal.

Table 1 Summaries of some drug delivery system used in the treatment of periodontal disease

Type of device, product name (company)	Polymer	Drug loading (%)	Degrad-ability	Reference
Microtubules	Diacetylenic phosphatidyl cholines	Tetracycline, 5	No	Price and Patchan, 1991
Monolithic fiber Actisite®(Alza, Corporation)	Ethylene vinyl acetate copolymer	TetracyclineHCl, 25	No	Tonetti et al., 1990
Strip	PHBA	TetracyclineHCl 50	Yes	Collins et al., 1989
	PHBA	Metronidazole, 25	Yes	Deasy et al., 1989
	PHEMA	Doxycycline, 30	No	Larsen, 1990
	PMA + HPC	Ofloxacin, 10	No	Kimura et al., 1991
Film	Cross-linked collagen	Tetracycline, 50	Yes	Minabe et al., 1989

المنارة للاستشارات
www.manaraa.com

	Bycoprotein + glycerol	Chlorhexidine diacetate, 10-15	Yes	Steinberg et al., 1990
	Eudragit	Clindamycin, 5	No	Higashi et al., 1991
	PLGA	Tetracycline HCl, 5/25	Yes	Webber and Mathiowitz, 1997
	CAP+PEO-PPO	Metronidazole, 10	Yes	Gates et al., 1999
	POE	Metronidazole, 5-10	Yes	Vasavada and Junnarkar, 1997
Insert, (Periochip®)	Cross-linked hydrolyzed +Gelatin+ Glycerin	Chlorhexidine gluconate, 33	Yes	Goffrins, 1998
Microspheres	PLGA	Minocycline, 25	Yes	Jones et al., 1994
	PLGA	Histatin peptides, 0.125	Yes	Jeyanthi et al., 1997
	PLGA, PLA	Tetracycline, 20	Yes	Esposito et al.,1997
Mucoadhesive gel	Carbopol	Clindamycin,	No	Sauvetre et al.,1993
	HEC+ PVP	Tetracycline, 5	No	Jones et al.,1996
	HEC+ carbopol+ polycarbophil	Metronidazole, 5	No	Jones et al.,1997
	HPMC	Histatin peptides 0.125	No	Paquette et al.,1997
Lipid-like gel	Glycerol monooleate +sesame oil(Elyzol®)	Metronidazole, 25	Yes	Norling et al.,1992
	PEO-PPO+ glycerol Monooleate	Tetracycline, 5	Yes	Esposito et al.,1996
	HEC+ aminoalkyl Methacrylate copolymer+triacetine+ magnesium chloride glycerin	Minocycline, 2		Nakagawa et al., 1991
Liquid system, Atrigel, (Atridoxe®) Laboratories)	PLA+NMP	Doxycycline hyclate, 10	Yes	Polson et al., 1996
Injectable semi-solid system	POE+ Mg (OH) 2	Tetracycline HCl, 10	Yes	Roskos et al., 1995

Notes:
Abbreviations: PHBA, poly (hydroxybutyric acid); PHEMA, poly (2-hydroxyethyl)-methacrylate; PMA, poly (methacrylic acid); HPC, hydroxypropyl cellulose; PLGA, Poly (lactide-co-glycolide) 50/50; CAP, cellulose acetate phthalate; PEO-PPO, poly (ethyleneoxyde-co-propyleneoxyde) (PluronicL101); EC, ethyl cellulose; POE, poly (ortho ester); PLA, poly (lactide); HEC, hydroxyethyl cellulose; PVP, polyvinylpyrrolidone; HPMC, hydroxypropylmethyl cellulose; NMP, Nmethyl-2-pyrrolidone.
Commercial names in bold.

1.2.3. Considerations in designing a sustained release delivery system.

1-Drugs used in subgingival delivery systems

Garant and Cho (1979) described the limits of actions of subgingival plaque bacteria and the substances they release into the periodontal pocket to a radius of up to 2

المنارة للاستشارات
www.manaraa.com

mm, so antibacterial agents directed on the intra pocket bacteria like chlorhexidine and tetracyclines would preferably be an agents that act locally in the pocket without penetrating the gingival tissues (Rapley *et al.,* 1992). Using chemotherapeutic agents that can penetrate into the tissues was intended on eliminating bacteria that had penetrated into the pocket wall like metronidazole (Stoltze and Stellfeld, 1992) or affecting inflammatory mediator that destroy bone and connective tissues like tetracycline (Rapley *et al.,* 1992).

2-Release profile

As the release of drug from systems is dependant on both diffusion and dissolution mechanisms, the flow rate of gingival crevicular fluid will influence the release rate of the drug. Therefore, the rate of release should be high after intial placement of device, to achieve and maintain therapeutic level of the drug in the pocket (Goodson, 1989). As the gingival crevicular fluid flow decreases due to decrease in gingival inflammations a slower release rate is required to maintain therapeutic level of the drug in the pocket. Also poorly absorbed drug enables drug levels in the periodontal pocket to reach higher concentrations and prolongs their retentions in the pocket.

3-Device morphology

The device should be easily atraumatic insersions into the pocket, minimizing pain and discomfort during insertions and once inserted not interfere with normal hygiene procedures like tooth brushing with little effect on eating habits of patients.

The devices have been used as adjunctive therapy to routine scaling and root planning and as alternative to routine maintance of periodontal pocket after definitive therapy. Evidence for the effecivness of this approch is beginning to become available.

Despite the reported clinical successes, currently available controlled release formulations suffer from several disadvantages including: (i) the requirement for

المنارة للاستشارات
www.manaraa.com

mechanical binding of the drug delivery system to a tooth surface to prevent removal of the system from the periodontal pocket a result of the positive flow of gingival crevicular fluid from the pocket into the oral cavity; indeed, the Atrigel® delivery system has to be maintained in the pocket by the addition of periodontal adhesive (Octyldente) or periodontal dressing (Coe-Pake Periocare®); (ii) the requirement for removal of tooth bound, non-biodegradable drug delivery systems at the termination of treatment, as for Actisite® fibers; (iii) poor retention of oil-based delivery systems within the aqueous environment of the periodontal pocket as in the case of Elyzol® (iv) potential deleterious effects of plasticizers leached from solid polymeric drug delivery systems on the periodontal tissues (Schwach-Abdellaoui *et al.,* 2000).

المنارة للاستشارات

www.manaraa.com

1.3 Glycerol monooleate (GMO or Monoolein)

Monoolien is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. The acyl side chain (oleic acid) is attached to the glycerol backbone by an ester bond (Figure 2). The two remaining carbons of glycerol have active hydroxyl groups, giving polar characteristics to this portion of the molecule (Engstrom *et al.,* 1992).

Monoolein occurs as a waxy yellow paste with characteristic odor. It swells in water, giving rise to several lyotropic liquid crystalline structures (Engstrom *et al.,* 1988). Monoolein is a nontoxic, biodegradable, and biocompatible material. Its biodegradability comes from the fact that monoolein is subject to lipolysis due to different types of esterase activity in different tissues (Appel *et al.,* 1994).

1.3.1 The pharmaceutical uses of glycerol monooleate

In the pharmaceutical field, the applications of monoolien can be classified as follows:

Emulsifier

Due to amphliphlic character, it has been classified as nonionic surfactant (Adriana *et al.,* 2000). Recently, it has been proposed as cosurfactants for the development of an injectable oil-in-water emulsion containing lipophilic antioxidants (Lyons *et al.,* 1996).

Solubilizer

Luner**,** showed that the addition of increasing amount of monoolein to glycocholate solutions resulted in a linear increase in the solubility of gemfibrozil (Luner *et al.,* 1994)

المنارة للاستشارات
www.manaraa.com

Absorption Enhancer

Monoolein–bile salts aqueous solutions have been shown to be capable of promoting the intestinal and nasal absorption of poorly absorbed drug incorporated in oily, liposomal (Muranushi *et al.,* 1980), and emulsion formulations (Karali *et al.,* 1992).

Drug delivery systems

Also monoolein is used for preparation of different drug delivery system like oral (Wyatt and Dorschel, 1992), periodontal (Norling *et al.,* 1992), parentral (Ericsson *et al.,* 1988) and vaginal (Geraghty *et al.,* 1996). This is due to the special swelling characteristics of monoolein, as well as biodegradability, mucoadhesion and biocompatibility which makes this substance an ideal candidate for the design of drug delivery systems (Adriana *et al.,* 2000).

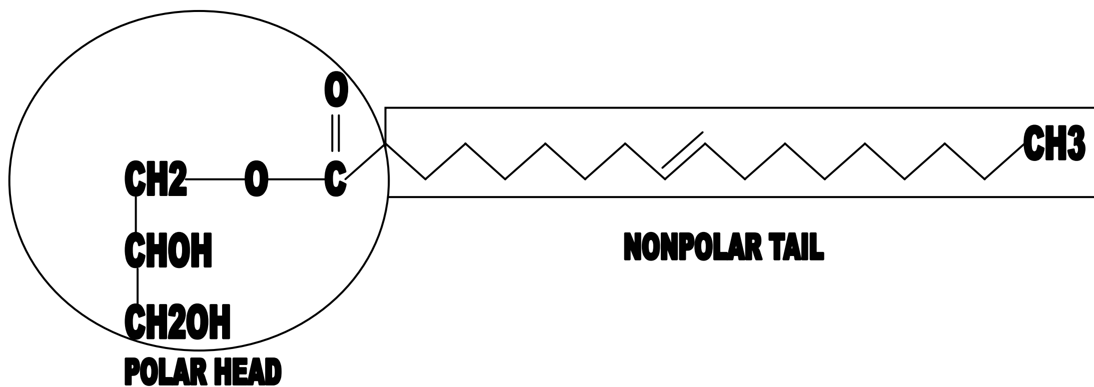


Figure.2: Structural formula of GMO (for pure material)

Storage system for protection of macromolecules susceptible to degradation

Cubic phases are unique in their ability to accommodate proteins (Ericsson *et al.*, 1983). The particular structure of the cubic phase may function as a protector medium for proteins like insulin against enzymatic degradation because of the diffusion of the enzyme in to the cubic phase was negligible due to its large size compared to the water pores in the cubic phase (Sadhle and Shah, 1995). Also the protection against chemical

المنارة للاستشارات
www.manaraa.com

degradation in the cubic phase, due to the apparent reduction of mobility of water due to the highly structured nature of the gel (Wallins *et al*., 1993).

1.3.2 Phase behavior of GMO

Polar amphiphilic lipids such as glyceryl monooleate (GMO) when placed in water reorganize into lipid bilayers forming a reversed micellar phase (L_2) and three types of liquid crystalline phases (lamellar, reversed hexagonal and the cubic phase) depending upon the temperature and water content as shown in the phase diagram presented in figure 4 (Larsson, 1989).

The lamellar (L_α) phase has a long-range order in one a dimension. Its structure consists of a linear arrangement of alternating lipid bilayers and water channels as shown in figure 5. The reversed hexagonal phase (H_{II}) consists of infinite water rods arranged in a two-dimensional lattice and separated by lipid bilayers as shown in figure 5. The cubic phase is usually observed between the lamellar and the reversed hexagonal phases as the water content is increased as shown in figure 3. As seen from the phase diagram of glyceryl monooleate (GMO) / water, with increased hydrocarbon chain disorder, obtained either by heating or by increasing the water content, there is a transition from the (L_α) phase to the cubic a phase (C) and finally into the (H_{II}) phase. A large cubic phase region dominates the phase diagram. The cubic phase have the ability to coexist in equilibrium with excess water (monoolein solubility in water is only about 10^{-6} M) (Wyatt and Dorschel, 1992).

1.4.3 Influence of lamellar to cubic phase transition on drug release

The practical use of cubic phase as a delivery system requires formulating drug in less viscous lamellar phase and expecting it to undergo rapid transformation into the rate-controlling stiff cubic phase in vivo upon absorption of excess water, but the burst of drug release from lamellar phase and during its conversion into cubic phase

المنارة للاستشارات

www.manaraa.com

significantly limits the release kinetics and duration (Engstroem *et al*., 1992, Chang and Bodmeier, 1998). As seen for various drugs, release from lamellar phase is very fast following pseudo first-order kinetics with duration of release dependent on the property of the incorporated drug.

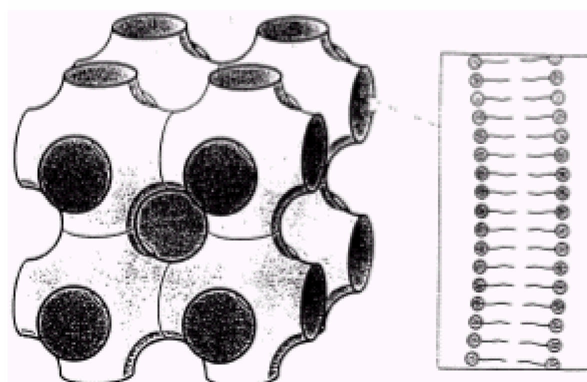


Figure 3. The cubic phase of GMO.

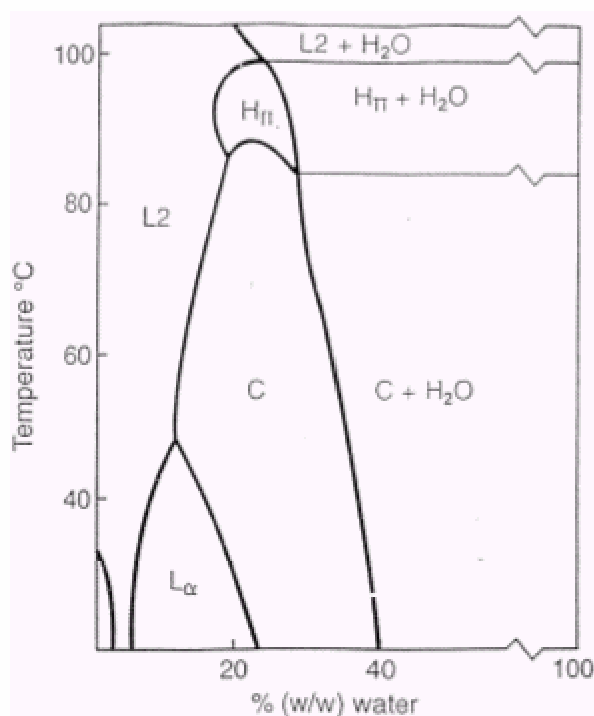
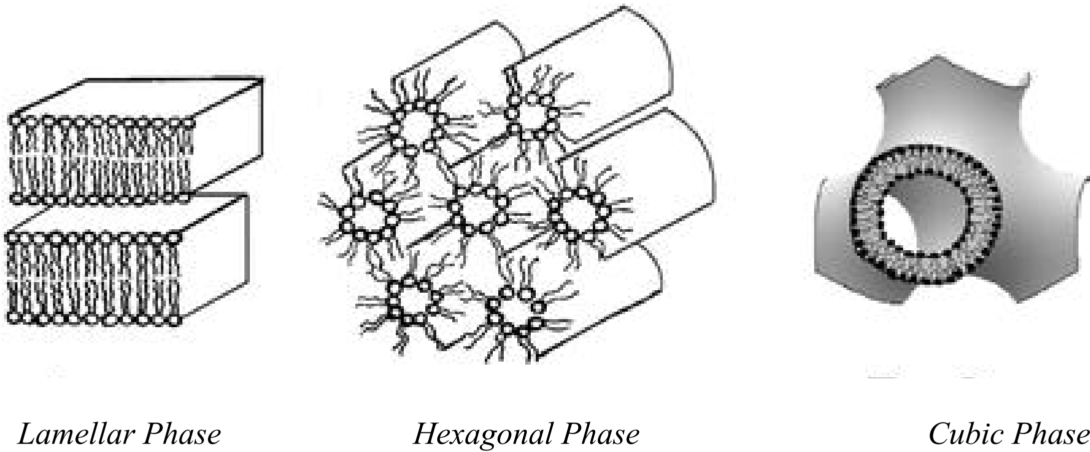


Figure 4 Phase diagram of the glycerylmonoleate–water system depicting the reversed micellar phase (L_2), the lamellar phase (Lα), cubic phase (C) and reversed hexagonal phase (H_{II}).

المنارة للاستشارات
www.manaraa.com



Lamellar Phase *Hexagonal Phase* *Cubic Phase*

Figure 5. Structures of the lipid liquid crystalline lamellar L_{α}, inverted hexagonal H_{II} and inverted bicontinuous cubic phases

Furthermore, in vitro drug release is not a very good predictor of in vivo release from cubic phase systems, due to the biodegradable nature of lipids such as GMO. Relatively small oligopeptides such as desmopressin and medium sized insulin were completely released in less than 9 h in vivo after intramuscular injection (Sadhale and Shah, 1999, Ericsson *et al*., 1991).

Thus, very innovative approaches are required to provide long-term release of small to large molecular weight drugs of varying polar characteristics without compromising the Interesting physical structure of these liquid crystalline systems.

1.3.4 Mucosal drug delivery using cubic phase gel

The high viscosity of the in situ formed cubic phase may make it bioadhesive, which was used by Nielsen *et al.* (1998) to propose cubic phase of glycerol monooleate as bioadhesive mucosal drug delivery system. The bioadhesive nature of cubic phase may be useful in targeting drugs to different parts of oral and vaginal cavity as well as to different sites in the gastrointestinal tract.

المنارة للاستشارات

www.manaraa.com

1.3.4.1 Cubic phase gel for vaginal delivery of drugs

GMO–water system was studied for vaginal delivery of antimuscarinic drugs, propantheline bromide and oxybutynin hydrochloride to treat urinary incontinence. The incorporated drug induced the formation of lamellar phase, which upon water uptake formed cubic phase with equilibrium water content of 40% (w/w). The cubic phase would also be retained in the vaginal cavity due to its bioadhesive characteristics (Geraghty *et al*., 1996).

1.3.4.2 Local delivery of antibiotics and analgesics for post-surgical infections and pain

The cubic phase gel has been used to deliver local anesthetics such as bupivacaine and lidocaine and applied at the wound site to provide sustained release of the drug locally (Engstrom and Engstrom, 1992). The release kinetics of bupivacaine from cubic phase gel with a final objective of designing a long acting local anesthetic for conditions such as herniorhaphy and thoracotomy has been studied. Local antibiotic delivery is very important to prevent post-surgical infections for which current mode is parenteral delivery of safe and effective broad-spectrum antibiotics such as cefazolin. Sadhale and Shah demonstrated the enhanced stability of cefazolin and cefuroxime in GMO cubic phase gel, which is a significant advantage as these antibiotics undergo rapid degradation in solution (Sadhale and Shah, 1998)

1.4.4.3 Periodontal drug delivery using cubic phase gel

Another interesting application of the in situ formed cubic phase gel was for periodontal delivery of antibiotics for the prevention and treatment of infections. The lamellar phase or pure GMO can be injected into the periodontal pocket where it would

المنارة للاستشارات

www.manaraa.com

transform into a stiff, cubic phase gel and release the antibiotic locally preventing infection (Norling *et al*., 1992, Esposito *et al*., 1996)

Monoglycerides and triglycerides were used in suspension form to slowly deliver metronidazole into periodontal pocket following conversion of suspension into viscous cubic and reversed hexagonal phases. The system was characterized for viscosity, thermal behavior and in vitro release; however, cubic phase did not result in desired release characteristics (Esposito *et al*., 1996). In another study, viscous solutions prepared with poloxamer and GMO were delivered by a syringe and needle into a periodontal pocket and were shown to provide prolonged in vivo release and clinical efficacy. Both formulations undergo a transformation to gel upon administration resulting in local drug delivery. While poloxamer undergoes thermoreversible gelling, GMO formed the viscous cubic phase in situ upon absorption of water. In this study, the sol–gel transformation was rheologically characterized before performing the clinical study. The successful results of the above study demonstrate an interesting application of cubic phase gel for local antibiotic delivery for infection (Esposito *et al*., 1996).

1.3.5 Modulation of drug release from cubic GMO phase

Incorporation of a negatively charged phospholipid was shown to slow and hinder the release of positively charged timolol maleate from cubic phase into water, and the amount released was inversely proportional to amount of charged phospholipid in the system (Lindell *et al*., 1998). However, the negatively charged phospholipid had no effect on release of timolol maleate in saline because of the high ionic strength of the release medium preventing the ionic interactions between the positively charged drug and the negatively charged phospholipid. Although the in vitro release of an ionic drug can be sustained to some extent by addition of a charged lipid in the cubic phase; but its

المنارة للاستشارات
www.manaraa.com

practical usefulness is limited due to the isotonic physiological fluids overcoming any ionic interactions (Lindell *et al*., 1998).

1.3.5.1 Effect of drug incorporation on phase behavior

Lidocaine, chlorpheniramine maleate, diltiazem and propranalol HCl and other drugs have been observed for their effect on the phase transformations in GMO–water systems (Chang and Bodmeier, 1997, Engstrom and Engstrom, 1992). Generally, hydrophilic drugs favored transformation into a lamellar phase while lipophilic drugs converted cubic phase into reversed hexagonal phase (Chang and Bodmeier, 1997). The incorporation of lidocaine HCl into cubic phase induced the transformation into lamellar phase in contrast to lidocaine free base, which converted cubic phase into reversed micellar phase (Engstrom and Engstrom, 1992).

1.3.6 Kinetics and mechanism of drug release from cubic phase

The release kinetics could be described well by first order as diffusion controlled for a variety of drugs from low to high drug load (Burrows *et al*., 1994). Overall, the release of a variety of drugs from a constant surface of GMO cubic phase gel has been consistently observed to be following Higuchi's square root of time release kinetics and the duration of release is not very prolonged. This is to be expected considering the fact that drug is dispersed or dissolved in a matrix formed by the cubic phase irrespective of drug's location in the cubic phase. The release occurs by diffusion in aqueous channels of the cubic phase assuming the cubic phase is stable during the duration of release study, and if a drug is lipophilic enough to be incorporated in the lipid bilayer, the rate-limiting step may become partitioning into the aqueous channel.

Depending on the physicochemical properties of the drug, it may reside in the lipid bilayer or the aqueous channels of the gel and the location of the drug in the gel may influence its release. Chilukuri and Shah (1997) studied the release mechanism

المنارة للاستشارات

www.manaraa.com

based on the interaction between the physicochemical characteristics of the drug and the cubic phase gel. Two main variables studied for their influence on the release; the form of drug used, and the release medium pH.

Bupivacaine is a weak base with p*Ka* of 8.17 and is an available in the free base form and the hydrochloride salt form (Shah and Maniar, 1993, Park *et al*., 1998). So the release studies of bupivacaine (both as free base and HCl salt forms) from cubic phase gels, prepared with pH 5 and 9 buffer, were performed from a constant surface area of the gel, in a USP Dissolution apparatus at 37C° and 100 rpm into release media of pH 5 and 9. The release profiles were found to follow the Higuchi's square root of time model and the apparent diffusion coefficient was calculated using the simplified Higuchi's diffusion.

A linear relationship between the amount of bupivacaine released as a function of the square root of time indicated that the mechanism of release was typical for a matrix-type delivery system, dissolution of the drug followed by diffusion out of the gel matrix. However, interestingly, irrespective of the form of bupivacaine in the gel, release was always faster at pH 5 than at pH 9 indicating that release medium pH had a significant role in controlling the release rate of bupivacaine.

The diffusion coefficients obtained from the release study were compared with the intrinsic dissolution rates (IDR) obtained earlier for bupivacaine base and hydrochloride salt at pH 5 and 9 (Shah and Maniar, 1993). While the IDR of bupivacaine at pH 5 was 721 times higher than the IDR at pH 9, diffusion coefficient from gel at pH 5 was only 3 times higher than that at pH 9. This suggest that at pH 9, the low solubility and IDR of bupivacaine results in the aqueous diffusion boundary layer controlling drug release irrespective of form of drug incorporated or the location of drug in the cubic phase gel; aqueous channels or lipid bilayers. In contrast, in spite of

المنارة للاستشارات

www.manaraa.com

high IDR at pH 5, lower diffusion coefficients of bupivacaine from the cubic phase gel at pH 5 suggested that the gel controls the release of drug irrespective of form of drug incorporated or the location of drug in the cubic phase gel; aqueous channels or lipid bilayers. Therefore, when diffusion across aqueous boundary layer was not the release rate limiting, gel controls the release of incorporated drug.

From bupivacaine drug delivery perspective, at physiological pH of 7.4, GMO gel can be used to control the release of bupivacaine since at that pH, bupivacaine with a p*Ka* of 8.17, would behave similarly as at pH 5 and release of bupivacaine would be controlled by cubic phase gel (Park *et al*., 1998).

However, these results suggest that release rate of drug can be controlled to only a limited extent by the cubic phase gel dependent on drug's aqueous solubility and diffusivity in the aqueous diffusion layer. Thus, for a drug with limited aqueous solubility, cubic phase gel may not be able to control its release, and other controlled release technologies such as polymeric systems may have to be used.

Furthermore, the form of drug incorporated, method of incorporation or where it may reside in the cubic phase gel do not influence the release kinetics and duration significantly. This is further supported by other studies reporting only moderate modulation of drug release by factors altering the physicochemical properties of drug in the cubic phase (Shah and Maniar, 1993).

The release of drug from cubic phase minimally affected by the ionic strength of the medium (Chang and Bodmeier, 1997). This may be due to the fact that GMO is non-ionic amphiphilic molecule. Therefore, the ionic strength of the buffer media did not considerably affect the GMO swelling and drug release. Also the effect of storage temperature and the drug loading on the release profile have been studied (Burrows *et al.,* 1994).

المنارة للاستشارات

www.manaraa.com

1.4. Bioadhesion

Although the term bioadhesion can refer to any bond formed between biological surface and synthetic surface or two biological surfaces, but it is specifically be used to describe the adhesion polymer samples, either synthetic or natural, and soft tissue like gastrointestinal mucosa (Mathiowitz, 1999). For instance when the bond involves the mucus coating and the polymer device, many authors used the term mucoadhesion (Leher *et al.,* 1990).

The advantages of mucoadhesive drug delivery system include the following: *(1)* enhanced bioavaliability and effectiveness of drug due to targeted delivery to a specific region, *(2)* maximized absorption rate due to intimate contact with the absorbing membrane and decreased diffusion barrier *(3)* improved drug protection *(4)* longer transit time resulted in extended periods of absorption (Peppas and Buri, 1985).

1.4.1 Mechanisms of mucoadhesion

The Mechanisms involved in the formations of bioadhesive bonds are not completely clear. The process involved in the formation of such bioadhesive bonds has been described in three steps: (1) wetting and swelling of the polymer to permit intimate contact with biological tissues; (2) interpenetration of bioadhesive polymer chains and entanglement of polymer and mucin chains (3) formation of weak chemical bonds. In the case of hydrogels, it has been determined that several polymer characteristics are required to obtain adhesion: (1) sufficient quantities of hydrogen bonding chemical groups (-OH and –COOH), (2) anionic surface charges, (3) high molecular weight, (4) high chain flexibility, (4) surface tensions that will induce spreading into the mucous layer (Peppas and Buri, 1985).

These characteristics favor the formation of a bond that is either mechanical or chemical in nature.

المنارة للاستشارات
www.manaraa.com

1.4.2 The mucoadhesive properties of GMO

Monoolein has some molecular characteristics similar to those seen in polymeric materials used as bioadhesives. It is an amphiphilic molecule with secondary bond forming like hydroxyl and ester groups (Geraghty *et al*., 1997). It is classified as having moderate to excellent bioadhesive properties (Engstrom *et al*., 1995). Another study showed that the unswollen GMO has the greatest mucoadhesions, followed by lamellar and cubic phases (Nielsen *et al*., 1998). The cubic phase is mucoadhesive when formed on wet mucosa. The possible mechanism involves the dehydration of the mucosa. The adhesive nature of the cubic phase appears to be due to secondary chemical bonds, such as van der Waals forces, but is limited by the cohesive strength of the gels. The physicochemical properties of drugs or excipients and their concentrations influenced the mucoadhesive properties of the GMO because the additives change the ability of GMO to form the cubic phase (Nielsen *et al*., 1998).

المنارة للاستشارات

www.manaraa.com

1.5. Ethyl cellulose (EC)

EC is cellulose ether prepared by the reaction of alkali cellulose with ethyl chloride and it is characterized by the degree of ethoxy substitution and the solution viscosity. Commercial grades of EC have a degree of substitution between 2.25 and 2.58 (44 to 50% ethoxyl contents) per anhydroglucose unit (Rekhi and Jambhekar, 1995).

EC is water insoluble but soluble in a variety of organic solvents/solvent mixtures like acetone and ethanol. The desired use of EC will determine the choice of particular grade, for example, lower molecular weight grades are used for coating, and higher molecular weight grades are used for microencapsulation. The polymer is tasteless and odorless, physiologically inert, stable in a pH range between 3 and 11, and because of its nonionic character, compatible with most drug substances (Harris and Ghebre-Sellassie, 1996).

EC has excellent film forming properties and has been used by the pharmaceutical industry for almost 40 years for the coating of solid dosage form, beside the predominant use as controlled release barrier; in matrix system and in microencapsulation (Mathiowitz, 1999).

المنارة للاستشارات
www.manaraa.com

1.5. Metronidazole

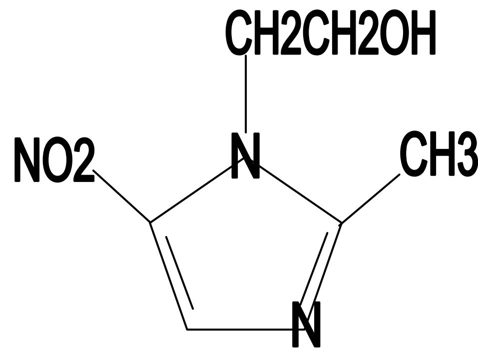
Chemical name: 2-Methyl-5-nitromidazole-1-ethanol.

Empirical Formula: $C_6H_9N_3O_3$.

Molecular Weight: 171.2.

Physical Properties: Metronidazole is a pale crystalline substance that is sparingly soluble in water. It is stable in air, but is light sensitive. Metronidazole is a weak base having the pKa of 2.5 and Log *P* (octanol/pH 7.4), -0.1. Metronidazole melts in the range 159° to 163°.

Structural Formula:



Drug Action: Metronidazole was used for topical treatment of *Trichomonas vaginalis* vaginitis. Also the drug possesses a useful amebicidal activity and used in the treatment of both intestinal and hepatic amebiasis. It is particular effective against gram negative anaerobes, such as Bacteriodes and Fusobacterium species. Because of its bactericidal action Metronidazole has become an important agent in treatment of serious infections (such as septicemia, pneumonia, peritonitis and meningitis) caused by anaerobic bacteria (Jaime and William, 1998).

The liquid phase of Elyzol® formulation consists of a water free mixture of melted glycerol monooleate and metronidazole benzoate to which sesame oil has been added to lower the melting point and improve the flow property of the gel in the

المنارة للاستشارات
www.manaraa.com

syringe, but then on contact with the water of the gingival fluid, it undergoes an in situ transformation to a highly viscous mucoadhesive cubic and reversed hexagonal phase of GMO. The matrix is degraded by neutrophils and bacterial lipase present in the GCF (Norling *et al*., 1992).

Therapeutic levels of metronidazole were reported for a period of 2-3 days in the periodontal pocket after the administration of Elyzol® gel (Stoltze, 1992). The best application regimen of the metronidazole gel was determined in a controlled, randomized, multicenter study and was recommended as two separate applications into each pocket, one-week apart (Klinge *et al*., 1992).

The results of clinical studies comparing this therapeutic approach alone with scaling and root planning, indicate that the Elyzol® results in reduction in probing pocket depth and bleeding on probing which is not significantly different from the results obtained with scaling and root planning (Grossi *et al*., 1995). Controversial results reported in another study do not support the routine use of Elyzol® because of the poor clinical and microbiological efficacy compared to scaling and root planning alone (Palmer *et al*., 1998). This is probably due to the rapid elimination of gel from the periodontal pocket (Stoltze, 1995).

المنارة للاستشارات
www.manaraa.com

1.7. The objective

The objectives of this study are: -

1- Studying the effect of ethyl cellulose, propylene glycol and metronidazole on the phase changes of GMO.

2- Studying the effect of ethyl cellulose and propylene glycol on the release of metronidazole from the cubic phase of GMO.

3- Optimization of the flowability of GMO delivery system.

4- Evaluation of the rheological and thermal behavior of the optimized formula.

5- Studying the effect of drug loading and matrix thickness of the optimized formula and the tonicity of the dissolution medium on the release property of metronidazole.

6- Studying the mucoadhesive property of the optimized formula.

المنارة للاستشارات

www.manaraa.com

2. Materials and Experimental methods

2.1 Materials.

The following materials and chemicals were used:

2.1.1 Ethyl Cellulose (Gift from APM, QC No.01991089).

2.1.2 Metronidazole (Gift from APM, QC No.01990216).

2.1.3 GMO (Croda-England), with the following properties:

Monoglyceride content	≥ 94.0%
C18:1	≥ 75.0%
C18:2+C18:3	≤ 15.0%
C16:0+C18:0+C20:0	≤ 10.0%
Water	≤ 2.0%
Acid value	≤ 3.0%
Saponification value	155-165
Residue on ignition	< 0.1%

2.1.4 Miscellaneous Chemicals

Reagent grade chemicals were used from the following materials: Sodium hydroxide (Lonover, England), 1,2-Propylene glycol (Scharlau, Spain), Potassium-dihydrogen phosphate (Panreac, Spain). All chemicals were used without further purification. Distilled water was used in all experiments.

2.2 Equipments

- Analytical balance, Sartoruis, Germany.
- UV spectrophotometer, Cary-Varian, Germany.
- Dissolution Tester (VK 7000/7010), USA.
- Orbital Incubator, Stuart Scientific, England.

المنارة للاستشارات

www.manaraa.com

- Magnetic stirrer hotplate, Stuart, England.
- pH meter, Hanna, Italy.
- Differential scanning calorimeter (DSC), DSC 20 and therogravimetric analysis (TG 50 Thermobalance); Mettler TC11, TA processor, TA 4000 System, Mettler, Switzerland.
- Rotovisco 1, ThermoHaake, Germany.
- Sonicator, Julabo, West Germany.
- Versa Test, force measurement device, Mecmesin, England.
- Optical microscope, Optiphot, Nikon, Japan.
- Polarizing optical microscope, BAUSCH&LOMB, USA.

المنارة للاستشارات

www.manaraa.com

2.3 Experimental Methods

2.3.1. Spectrophotometric scanning and Calibration curve of metronidazole

Spectrphotometric scanning of a selected concentration (100μg/ml) of metronidazole in different solvents (distilled water, phosphate buffer pH 7.4 and isotonic solution) was done using Cary IE Spectrophotometer in the range of 200-400nm.

0.1-mg/ml stock solution of metronidazole in these solvents was prepared and a suitable dilution has been done to prepare different concentrations (3,5,7.5,10,15 and 18μg/ml). Samples were analyzed at $\lambda max=319$ nm ,and calibration curves were constructed. The spectrphotometric method was validated.

2.3.2 Equilibrium Solubility Study

Equilibrium solubility was measured for metronidazole in phosphate buffer pH 7.4, distilled water and normal isotonic solution (0.9% NaCl). An excess of metronidazole powder, 1500mg, was introduced into 50 ml of media in 75-ml glass bottles. The bottles were fixed on the sample holder in an incubator at 37°C±0.5 protected from light and mechanically shaken at fixed rate of 150 stroke per minute.

Samples were taken after 24 hours and 48 hours. Aliquots (5 ml) of solutions were drawn using plastic syringe and filtered through 0.45 μm membrane filter and suitable dilutions were done. Drug concentrations were determined by measuring the UV absorbance at 319 nm. Solubility measurements were done in triplicates.

2.3.3.The Incorporation of ethyl cellulose in GMO base

The required quantity of ethyl cellulose was added to preweighed melted GMO base and then mixed well. The mixture remains in an oven at temperature 50ºC for 10 hours with continuous stirring until the solution became clear.

المنارة للاستشارات
www.manaraa.com

2.3.4. Effect of Additives on the phase behavior of GMO

Samples of GMO mixtures that contain metronidazole, propylene glycol (PG) or EC, and water were examined for their phase's changes using the polarized microscope. The changes in viscosity of the different phases were also observed visually at room temperature. The reversed micellar phase (L_2) was identified as being clear liquid and isotropic. Cubic phase was identified as being very viscous gel and isotropic.

Reversed hexagonal (H_{II}) phase was less viscous than cubic and showed anisotropic phase under the polarized microscope. The lamellar phase had lower viscosity than reversed hexagonal and was anisotropic. The fan shape was very clear in reversed hexagonal phase with bright blue and purple color.

Samples for optical examination were prepared by mixing of EC, PG or metronidazole with GMO. The metronidazole was added in a percentage ranged from 10% to 30% (w/w) while EC was added in a percentage ranged from 3 to 7% (w/w) and PG was added in a percentage ranged from 10 to 30% (w/w).

Water preheated to 40°C was added gradually to adjust the final weight of the mixture to 1 gm, then the components were mixed well and the vials were tightly closed and left at room temperature in a dark place for 24 hours to reach equilibrium. The observation was done by placing a small quantity of the mixture on clean glass slide and covered with cover slip and examined at room temperature at 10x-magnification power using polarized microscope.

Also the overall effect of additives (EC, PG, Metronidazole) on the viscosity and phase behavior of GMO was studied by the same procedures as described above. The effect of these additives on the solubility of EC was also examined.

المنارة للاستشارات
www.manaraa.com

2.3.5.Preparation of samples for drug release study

The GMO base (with EC or without) was melted at 50ºC on a water bath, then the required amount of metronidazole, PG or water was added and mixed well until complete dispersion or dissolution of the additive. The hard gelatin capsules were filled immediately and left to acquire room temperature before running the dissolution experiments.

2.3.6.In vitro dissolution studies

The paddle method described in USP (XXIII) apparatus II was used. The medium was 500 ml of phosphate buffer pH 7.4 and the experiment was carried out at 37±0.2 °C. The paddle was operated at 50 rpm. The samples were prepared in hard gelatin capsule each one contained 275 ± 5 mg of the selected formula.

Samples from the medium were withdrawn at different time intervals and replaced with fresh prewarmed phosphate buffer. The water bath of the dissolution apparatus was covered with aluminum foil to protect the drug solution from light.

The absorbance of the properly diluted samples was measured at 319 nm. The dissolution tests were done in hexaplicate.

2.3.7.Effect of addition of PG and/or EC on the release of metronidazole from GMO

The release kinetics of metronidazole (20%w/w) from GMO were investigated and the effect of addition of EC to GMO in different concentrations (5,7,10 %w/w) was studied. Furthermore, the effect of addition of PG to GMO in different concentrations (10 % and 30 %) and to GMO contained 7 % EC (10,20,30,40 %) on the release profile of metronidazole (20 %) was studied.

المنارة للاستشارات

www.manaraa.com

2.3.8.Effect of addition of PG and water on the release of metronidazole (20%) from GMO contain 7%EC.

The effect of addition of PG and water on the release of metronidazole (20 %) from GMO contained 7 % EC have been studied. PG and Water added to GMO led to the formation of different phases that had different rate and capacity of water uptake when the capsule were added to the dissolution medium. Table 2 shows the percentage of each constituent in the formula.

Table 2: Percentage (w/w) of PG and water in GMO/EC/Metronidazole formulations.

Formula	*GMO7%EC*	*Metronidazole*	*PG*	*Water*
A	65 %	20 %	10 %	5 %
B	65 %	20 %	7.5 %	7.5 %
C	60 %	20 %	10 %	10 %
D	50 %	20 %	20 %	10 %

2.3.9.The effect of matrix dimensions on the release profile

The effect of matrix dimensions on drug release was studied by using two different sizes of hard gelatin capsules. The larger contained 275mg (15*5.5 mm) of Formula A and the smaller one contained 150 mg (13.5*4.5 mm). Both were containing 20 % of metronidazole.

2.3.10. Particle size analysis

Particle size analysis was done to observe any crystal growth in the suspension of Formula A during the storage in dark place either at room temperature or at 4C°.

A stage micrometer of 10,000µm was used. Microscopic examination of samples has been undertaken to detect presence of agglomerates and different shapes of the

المنارة للاستشارات
www.manaraa.com

particles. One drop of well-mixed suspension was placed on clean microscope slide, and a cover slip was applied. The magnification of 40 times was selected such that the entire range of particle size can be counted under one-magnification power.

A British Standard Graticule was placed in one of the objective eyepieces. The field was scanned from one side to the other using a mechanical microscopic stage and particles were sized according to the nearest equivalent circle of the graticules. The sized particles were tallied under their appropriate class interval.

The counted particles were not less than 200 particle for each sample. The data were arranged in tables to calculate the geometric mean and geometric standard deviation and then were plotted. Also the cumulative under and over size for each sample have been plotted.

2.3.11. Assessment of the mucoadhesive properties of GMO and Formula A

The mucoadhesive properties of GMO and Formula A were evaluated by using force measurement device. Pieces of the mucous membrane of the chicken pouch have been isolated and cleaned and placed in simulated saliva solution (2.38 g Na_2HPO_4, 0.19 g KH_2 PO_4 and 8.00 g NaCl in 1000 ml of distilled water at pH 6.75) and stored in a freezer and used within 2 weeks of storage (Wong *et al.*, 1999).

Pieces of the mucous membrane of the chicken pouch of 4 cm^2 were fixed by clips to a polystyrene support. The pieces came in contact with thin layer of unswollen GMO or Formula A for 15 minutes at room temperature and the force of contact equal to 0.4 Newton (N). The work required for detachment of these two layers was determined per one cm^2.

The work determined using computerized force measurement device that was attached to the support by metallic hook and moved at constant speed (50mm/min.).

المنارة للاستشارات

www.manaraa.com

The force in N. required for this movement recorded and plotted versus the distance in millimeter. The work in milijoule (mJ) was equal to the area under the curve (AUC) of force N. versus distance (millimeter) as presented in equation 1.

Work (mJ) = AUC of Force (N) versus Distance (mm)……………………(1)

The work per one cm^2 was calculated by dividing the total work in mJ. by the application surface area that was equal to 4 cm^2. The experiment was repeated for six times for GMO and Formula A.

2.3.12. Rheological measurements

The rheological behavior of samples were studied using cone-plate geometry on a Thermo Haake RS 100 rheometer, cone radius and angle were 5cm and 0.056°, respectively. The samples were thermostated at 30°C±0.1 using circulating bath connected to the viscometer. The samples were equilibrated at 30°C prior to each measurement for 5 minutes. Samples were applied to the lower plate using a spatula to ensure that formulations shearing did not occur (Jones *et al*., 1997).

The shear rate was increased from 0 to 200 s^{-1} during one minute. The viscosity was determined from the flow curve obtained at different values of shear rate.

Rheograms were fitted to the Ostwald relationship (Gebhard Schramm, 2000):

$\tau = k\gamma^n$...(2)

Where γ is the shear rate, τ is the shear stress, k is related to the amount of resistance of flow (the consistency index), n is called the flow index and quantifies the degree of non-Newtonian flow behavior. The flow index is equal to one if the flow is Newtonian, a value greater than one if the flow is dilatant and a value smaller than one if the flow is pseudoplastic (Gebhard Schramm, 2000). Indices k and n were determined by

المنارة للاستشارات

www.manaraa.com

regression of power equation and the correlation coefficient was calculated using Sigma Plot version 5.0 software.

2.3.13.The effect of ionic strength of the dissolution medium on the release profile.

The release profile was studied in phosphate buffer (pH=7.4) with different ionic strength values; in hypotonic solution (0.45%NaCl), isotonic solution (0.9%NaCl) and hypertonic solution (1.3%NaCl).

2.3.14. Effect of drug loading on the release profile and phase changes in Formula A

Different drug concentration (10,20,30,40 %w/w) have been incorporated in Formula A without changing the proportion of the base, 65 % w/w (GMO contains 7 % w/w EC), 10% w/w PG and 5% w/w water. The effect of drug loading on the phase of GMO was investigated before and after the release study. The release studies were done by the same procedure described in section 2.3.5.

2.3.15.The effect of the storage conditions on the release profile of Metronidazole from Formula A.

Formula A was subjected to one of the following storage conditions:

(1) Stored in a dark place at 4˚C for 5 weeks.

(2) Stored in a dark place at room temperature for 5 weeks.

Dissolution experiments were done and the release profiles were compared with the release profile of a freshly prepared Formula A. Any phase changes during the storage also was examined.

المنارة للاستشارات

www.manaraa.com

2.3.16. Chemical stability of Formula A.

The chemical stability of metronidazole in formula A which stored for 3 months at 4C° and at room temperature was done using validated HPLC method at APM Laboratories.

2.3.17. Thermal analysis.

DSC thermal analyses of GMO, GMO containing 7% EC and Formula A were performed. The calorimeter was previously calibrated using indium and then operated at heating rate of 2 °C/min. Samples (30-50mg) were placed in pierced crimped aluminum pans and weated over the temperature range of -13 to 100°C.

Furthermore, Formula A, GMO containing 20% metronidazole and placebo of the Formula A were examined at heating rate of 10 °C/min., over temperature range from -13 to 220°C. Both the melting range and the peak temperature were recorded. Formula A which stored for 3 months at 4°C or at room temperature were examined twice. The first one was done on a fresh sample over temperature range from -13 to 220°C. The second one was done over temperature range from -13 to 100°C and then the same samples were rerun over temperature range from -13 to 220°C. Thermal analysis of metronidazole powder was examined over temperature range from 100 to 220°C.

Thermogravimetric analysis was performed for freshly prepared Formula A and placebo and that stored for 3 months under different storage conditions at heating rate of 10 °C/min. Samples (30-50 mg) were placed in aluminum oxide crucibles and weight loss versus temperature was monitored over the temperature range of 37°C to 220°C.

المنارة للاستشارات
www.manaraa.com

3. Results and Discussions

3.1 The spectrophotometric scanning and the calibration curve of metronidazole.

The spectrophotometric scanning of 100µg/ml of metronidazole dissolved in water gave maximum absorbance at the range of 318.5-321nm. The wavelength of 319 nm had been selected to construct the calibration curve.

Plotting of the absorbance of different concentrations of metronidazole in different media at λ=319 nm had resulted in calibration curves illustrated in the figures 6,7 and 8.

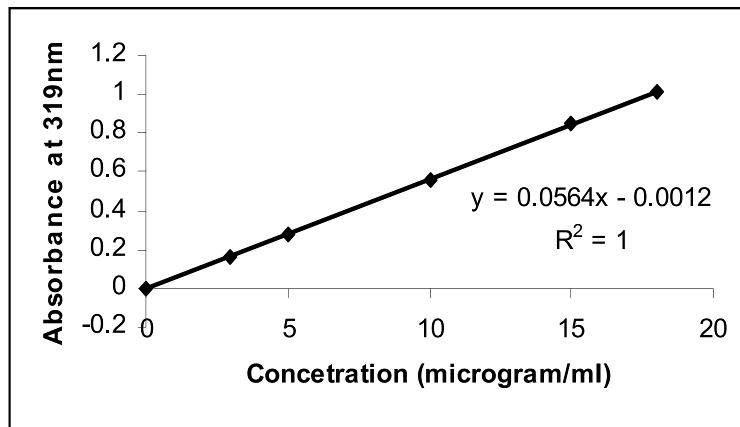


Figure 6. Calibration curve of metronidazole in phosphate buffer pH 7.4.

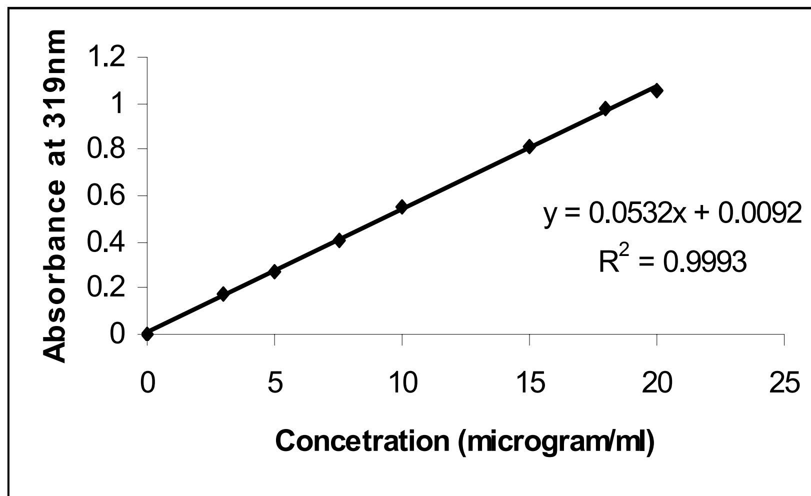


Figure 7. Calibration curve of metronidazole in normal saline solution.

المنارة للاستشارات
www.manaraa.com

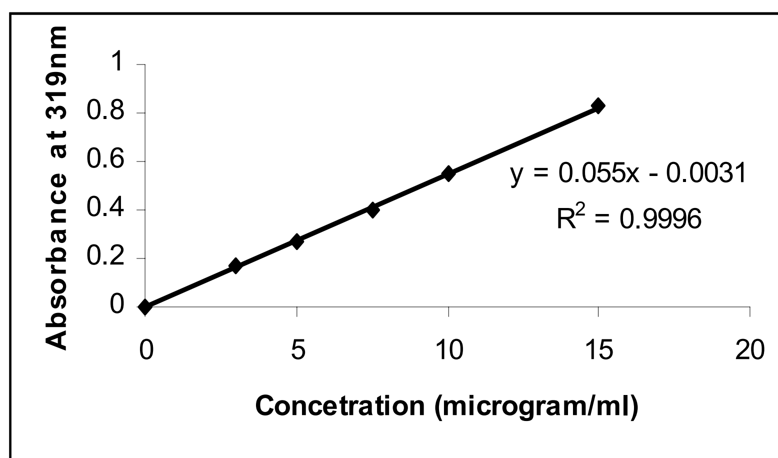


Figure 8. Calibration curve of metronidazole in distilled water.

Intra and interday variation of the calibration curve were carried out. The results are shown in table 3,4 and 5.

Table 3. Values of absorbance against different concentration of metronidazole and the relative standard deviation between (B) and within (W) day in distilled water.

Conc.µg/ml	Absorbance	RSD B Day	RSD W Day
0	0		
3	0.1669	1.02669	0.33185
5	0.26837		
7.5	0.40051		
10	0.54526	1.242	0.389605
15	0.82694	0.7418	0.2802

Table.4 Values of absorbance against different concentration of metronidazole and the relative standard deviation between (B) and within (W) day in phosphate buffer pH 7.4.

Conc. µg /ml	Absorbance	RSD B Day	RSD W Day
0	0		
3	0.16712	1.0088	0.882
5	0.27865		
10	0.562914	1.039	0.55426
15	0.84667		
18	1.01117	1.53	0.19126

المنارة للاستشارات
www.manaraa.com

Table 5. Values of absorbance against different concentration of metronidazole and the relative standard deviation between (B) and within (W) day in isotonic saline.

Conc. Mg/ml	Absorbance	RSD B Day	RSD W Day
0	0		
3	0.173167	0.97564	1.19
5	0.27415		
7.5	0.4043		
10	0.554489	1.547	0.453463
15	0.814038		
18	0.977038	1.0711	0.296173
20	1.05525		

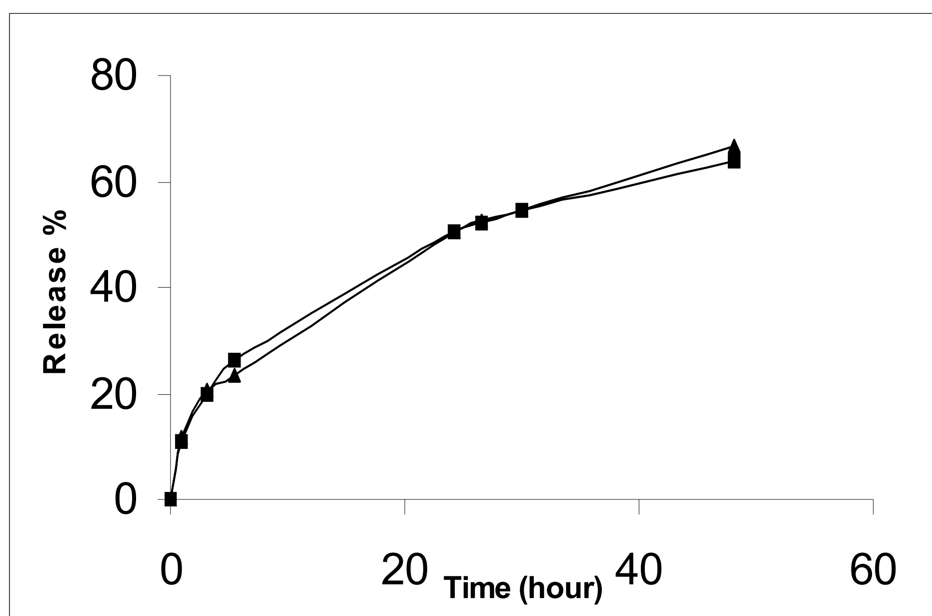


Figure 9. Dissolution profiles from formula containing (20% PG, 20% Metronidazole and 60% GMO containing 7% EC) determined spectrophotometrically using (▲) phosphate buffer as a reference solution and (■) filtered solution of the dissolution medium containing excipients.

The interference due to the presence of PG and other constituents in the formula on the spectrophotometric determination method was examined by choosing formula that contained 20% metronidazole, 20% PG and 60% GMO contained 7% EC. The release profile of metronidazole is shown in figure 9, the absorbance of metronidazole

المنارة للاستشارات
www.manaraa.com

was measured from one vessel two times, in the first the reference was the diluted dissolution media that contained all the constituents of the formula except the drug and in the second the reference was the phosphate buffer pH 7.4. The release profiles were similar in each time as shown in figure 9, and there was no interference due to the presence of PG and other constituents in the formula on the spectrophotometric determination method of metronidazole. From these results the spectrophotometric determination method was assured to be a valid method for the determination of metronidazole in dissolution studies.

3.2 Equilibrium Solubility Study

Equilibrium solubility of metronidazole was measured in different aqueous solutions (Phosphate buffer pH 7.4, Distilled water, Normal isotonic solution 0.9% NaCl) and the results are shown in table 6.

Table 6. Equilibrium solubility of metronidazole in different aqueous solutions at 37°C

Aqueous solution	Solubility(g/100mL)at 37°C	Standard deviation
Distilled water	1.2377	0.0541
Phosphate buffer pH 7.4	1.1478	0.0364
Isotonic solution	1.1954	0.0411

As a weak basic drug (pK_a=2.5)(CODEX, 1994), the solubility of metronidazole did not change drastically by increasing pH. Also Metronidazole solubility did not change by increasing sodium chloride concentration.

المنارة للاستشارات

www.manaraa.com

3.3 Effect of Additives on the phase behavior of GMO.

3.3.1. The effect of metronidazole.

The effect of metroniodazole on the phase behavior of GMO at room temperature is shown in figure 10.

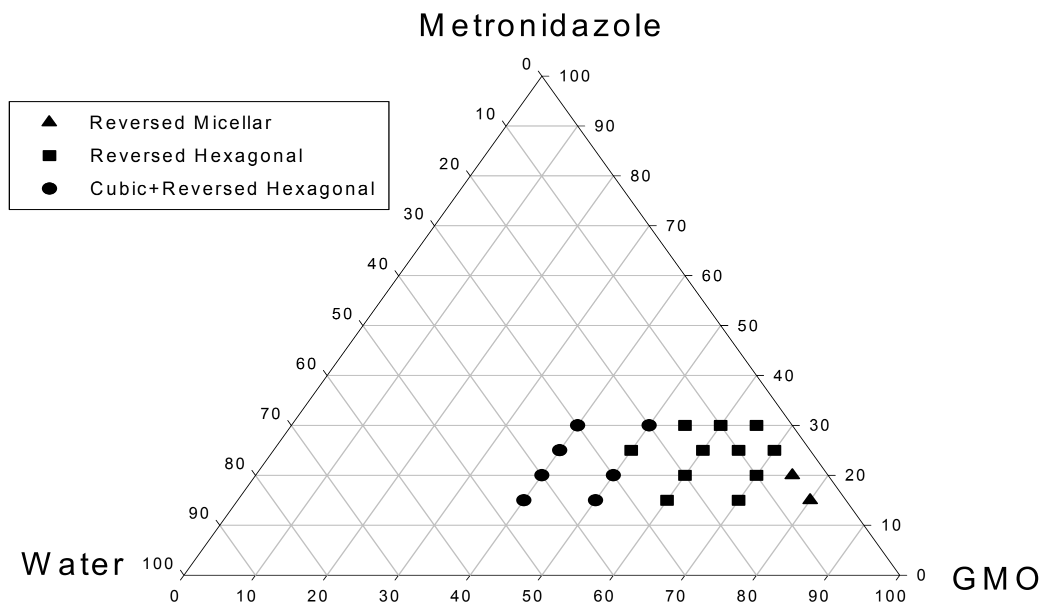


Figure 10. Partial triangular phase diagram of water, GMO and Metronidazole.

In the case of metronidazole, it had limited solubility in GMO base and it was mainly suspended in the base. The soluble part of metroniodazole prevented the cubic phase formation and increased area of reversed hexagonal phase. This effect could be explained by the partial presence of drug in the hydrophobic domain, which affected the phase behavior. The phenomenon was described using Ninham's ratio (Mitchell and Ninham, 1981) as follows:

$$R = \frac{V_h}{a_0 I_c} \qquad (3)$$

المنارة للاستشارات

www.manaraa.com

Where V_h is the volume of hydrocarbon chain, a_0 is the cross-sectional area of the polar group, and I_c is the length of amphiphile. Since I_c is considering constant, V_h and a_0 affected the transformation of the mesophases. The location of the solubilized drug component influences these two parameters. Any increase in the amount of the drug will increase V_h and, therefore, transform the cubic phase where ($V_h > a_0$) into an inverted hexagonal phase or an inverted micellar phase where ($V_h >> a_0$).

3.3.2.The effect of EC

EC started to precipitate as water concentration exceeded 5% in mixtures containing GMO, water and EC as shown in figure 11. Also EC prevented the formation of cubic phase which might be due to precipitation of EC polymer chain as network.

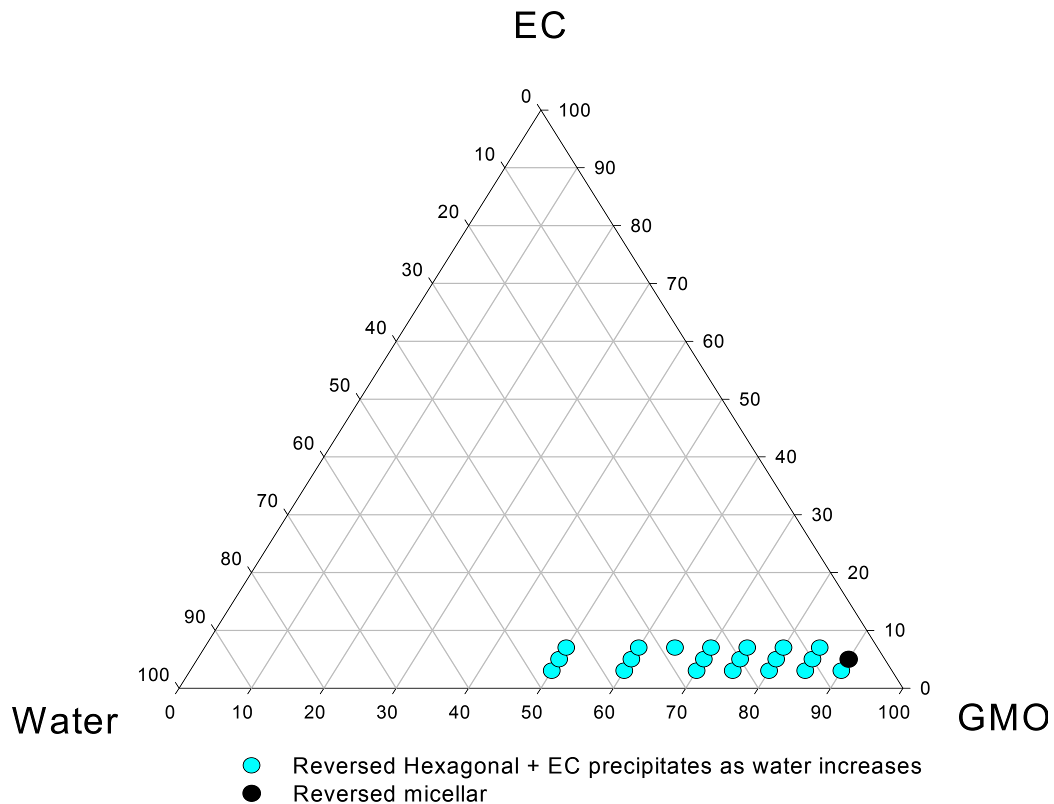


Figure 11. Partial triangular phase diagram of water, GMO and EC.

المنارة للاستشارات
www.manaraa.com

This network has hydrophobic character and prevented water ingress inside GMO base, as a result, the cubic phase appeared at the edge of the sample, while at the center of the sample EC precipitated network had separated or connected islets of reversed hexagonal phase of GMO.

3.3.3.The effect of PG

The effect of PG on the phase behavior of GMO is shown in figure 12. It is clear that PG did not prevent the formation of the cubic and lamellar phases of GMO compared with EC and metronidazole.

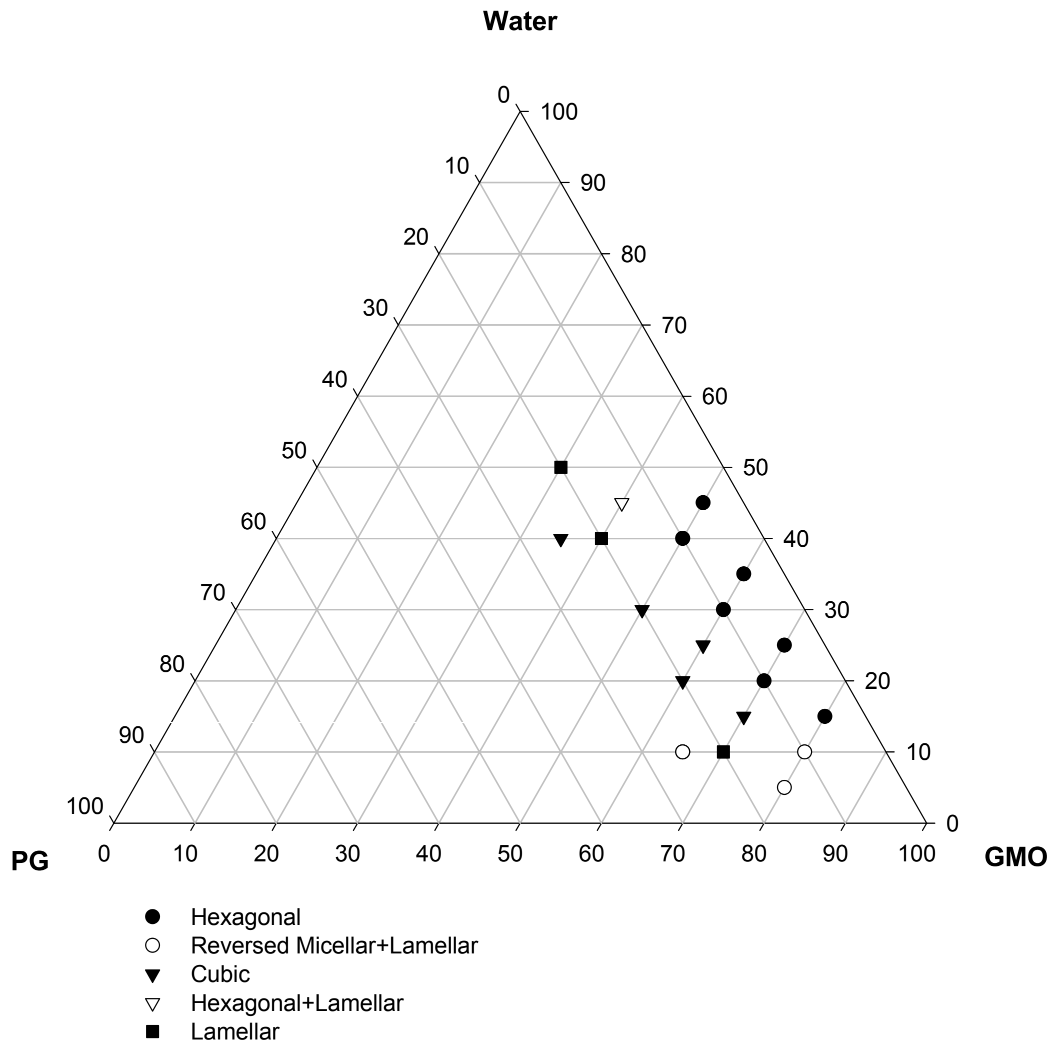


Figure 12. Partial triangular phase diagram of water, GMO and PG.

المنارة للاستشارات
www.manaraa.com

Also the addition of PG led to the formation of a mixture of lamellar and reversed micellar phases of GMO, at low percentage of water (5-10%) w/w and at 10% to 25%w/w of PG. Incorporation of PG also produced low viscosity, easily injectable formula.

The swelling was accomplished by PG, which had some general properties: (1) it is completely miscible with water and freely miscible with GMO, (2) it seems to have a tendency to partition into the aqueous domain as revealed by the octanol-water partition (Leo, et al, 1971) and (3) it will almost probably accumulate at the interface. This implies that it is possible to fine-tune the interfacial curvature with water since when water was added; the solvent was extracted from the lipid domain to the interface, thereby changing the curvature of the latter.

3.3.4.The overall effects of EC, metronidazole, water and PG.

The overall effects of (EC, metronidazole, water and PG) on GMO phase behavior were summarized in table 7. From this table it was clear that as water increased and PG decreased, the system transfered from reversed micellar to hexagonal phase.

Also as water increased the EC started to precipitate in the mixtures as polymeric amorphous network. The viscosity of these mixtures was also observed and found that hexagonal phase had higher viscosity than lamellar and reversed micellar phases as previously mentioned (Chang and Bodmier, 1998).

The lamellar and reversed micellar phase had low viscosity and they were easily injectable. The overall effects of additives that mentioned above led to the formation of GMO phases having low viscosity like the lamellar and reversed micellar phases, these were further investigated for the release of metronidazole.

المنارة للاستشارات
www.manaraa.com

Table 7. The phase behavior of GMO and Physical State of EC in mixtures was containing water, GMO, metronidazole, EC and PG.

Type	GMO	Propylene Glycol	Metronidazole	Water	Phase	ECppt	Drug crystals
A	65	5	20	10	Hexagonal	Yes	Yes
B	65	7.5	20	7.5	Reversed micellar+lamellar	No	Yes
C	65	10	20	5	Reversed micellar+lamellar	No	Yes
D	65	15	20	0	Isotropic solution	No	Yes
E	60	5	20	15	Hexagonal	Yes	Yes
F	60	10	20	10	Reversed micellar	Yes	Yes
G	60	15	20	5	Lamellar	No	Yes
H	60	20	20	0	Isotropic solution	No	Yes
I	55	20	20	5	Lamellar	No	Yes
K	50	5	20	25	Hexagonal	Yes	Yes
L	50	10	20	20	Hexagonal	Yes	Yes
M	50	15	20	15	Reversed micellar+lamellar	Yes	Yes
N	50	20	20	10	Hexagonal	Yes	Yes
O	50	25	20	5	Reversed micellar+lamellar	No	Yes
Q	50	30	20	0	Isotropic solution	No	Yes

The effect of PG was due to the modulation of the polarity of the mixtures that might affect EC and metronidazole solubility in these mixtures. Also might affect the rate and capacity of water uptake by the GMO. For example the presence of EC or metronidazole prevented the formation of the lamellar phase of GMO but the presence of PG led to the formation of the lamellar phase of GMO in spite of presence of EC and metronidazole. In all these mixtures, metronidazole was present as suspended particles in the liquid phase of the GMO.

المنارة للاستشارات
www.manaraa.com

3.4. In vitro Metronidazole release kinetics.

Figure 13, shows the release profiles of metronidazole form GMO gels containing different concentrations of EC. Metronidazole release was slower as the concentrations of EC increased. The similarity factor (f_2 –value) was chosen to compare the dissolution profile of different formulations.

The similarity factor was calculated according to equation 4 (Shah *et al.,* 1998)

$$f_2 = 50 \bullet \log\left\{\left(1 + 1/P\sum_{i=1}^{p}(\mu_{ti} - \mu_{ri})^2\right)^{-1/2} \bullet 100\right\} \qquad (4)$$

Where, f_2 is a population measure for assessing the similarity between two dissolution profiles, P is the number of sample points, μ_{ti} and μ_{ri} represent the dissolution measurements at P time points on the test profile (μ_{ti}) and the reference profile (μ_{ri}) respectivly. If (f_2) value lies in the range between 50 and 100, the two dissolution profiles can be considered equivalent. If (f_2) value is below 50, the dissolution profiles may not be considered similar (FDA, 1997). Results of (f_2) similarity factor are shown in table 8. The results show that the dissolution profiles of metronidazole from GMO base containing 0% and 5% EC were similar, and GMO containing 7% and 10% of EC were also similar. However, the dissolution profiles for metronidazole from GMO base that contained 5% EC differed from that contained 7% EC. By using one-way ANOVA test for the dissolution rate constant of metronidazole at different percentage of EC, the rates were significantly different at P=0.05, however no significant difference was observed between the release rate constant at 7% and 10% EC. The EC in GMO base started to precipitate at room temperature when the concentration reached 10%. The release of metronidazole through water channel in the cubic phase decreased due to precipitation of EC network that increased tortuosity of these channels.

المنارة للاستشارات
www.manaraa.com

Table 8. Similarity factor (f2) for dissolution profiles between different concentration of EC in GMO that contain 20% Metronidazole.

Reference formula	Test formula	f_2-value
0%EC in GMO	5%EC in GMO	50.9365
0%EC in GMO	7%EC in GMO	35.885
10%EC in GMO	7%EC in GMO	69.5102
5%EC in GMO	7%EC in GMO	48.8993
10%EC in GMO	5%EC in GMO	42.5237

Also the increase in EC concentration led to increase the viscosity of the cubic phase of the GMO, which might delay the release of metronidazole. The porosity of the system due to the dissolution of the suspended particles of the drug was not changed because of almost constant ratio of metronidazole in the formulations. The effects of porosity and tortuosity were further discussed upon explaining the effect of drug loading on the release profile of metronidazole in section 3.13. From figure 13, it is clear that incorporation of EC in GMO base can decrease the dissolution rate constant of metronidazole from GMO base. This effect can be explained if the GMO cubic phase considered as granular matrix in which the release of a solid drug involves the simultaneous penetration of the surrounding liquid, dissolution of drug and leaching out of the drug through interstitial channels. The EC network when formed in the cubic phase of the GMO decreased the simultaneous penetration of the surrounding liquid along with entrapment of drug particles.

So the formula that contains 20% metronidazole and 7% EC has been chosen for further development of the controlled release formulation.

To decrease the viscosity of the formula PG was added to the formula that contained 20% metronidazole and 7% EC in different percentages from 10% to 40%. The effect of PG on the sustained release performance of the formula was studied as shown in figure 14.

المنارة للاستشارات

www.manaraa.com

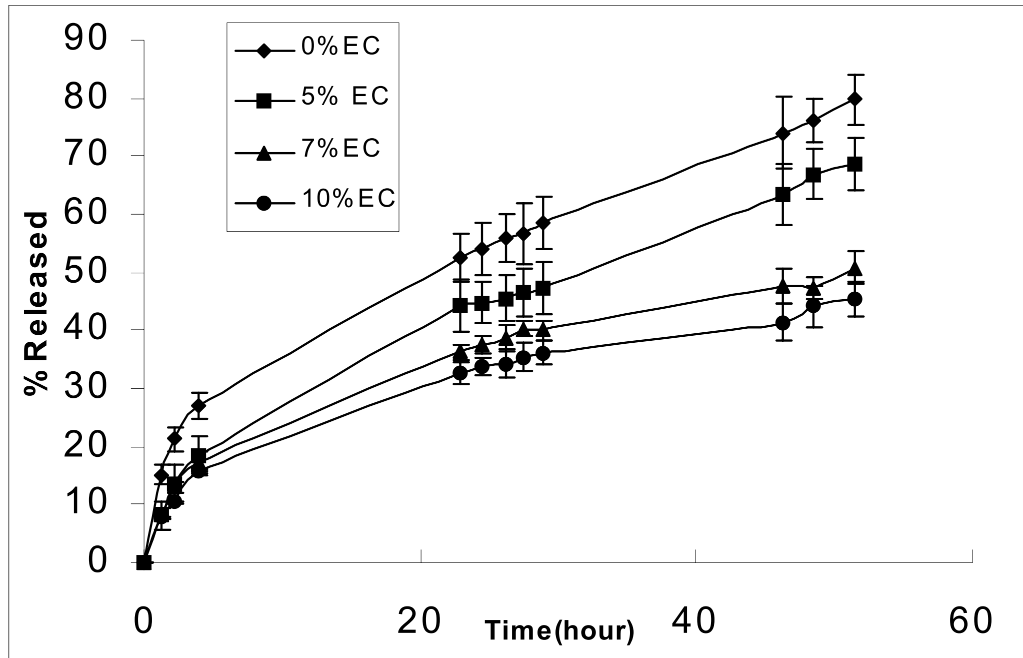


Figure 13. Release profiles of metronidazole in GMO base with different concentrations of EC as a function of time.

Also similarity factor f_2 -value was calculated and presented in table 9. From the f_2 values, it is clear that there was no difference between formulas containing 0% and 10% PG, while there was a difference between formulas containing 20,30 and 40% PG (Table 9). Also one-way ANOVA test was applied on the dissolution rate constants of formula GMO-7%EC containing different percentages of PG. It appeared that, there was no significant difference observed between the formulas that contained 0% and 10% PG ($P>0.05$). But there were significance differences observed between the formulas that contained 10% and 20% PG and that contained 0% and 20%PG ($P<0.05$).

المنارة للاستشارات

www.manaraa.com

Table 9. Similarity factor (f2) for the dissolution profiles between formulations having different concentration of PG in GMO containing 7%EC and 20% Metronidazole.

Reference formula	Test formula	f_2-value
10%PG in GMO	0%PG in GMO	70.4054
10%PG in GMO	20%PG in GMO	54.2500
20%PG in GMO	0%PG in GMO	47.0061
40%PG in GMO	30%PG in GMO	29.1211
30%PG in GMO	20%PG in GMO	54.4237

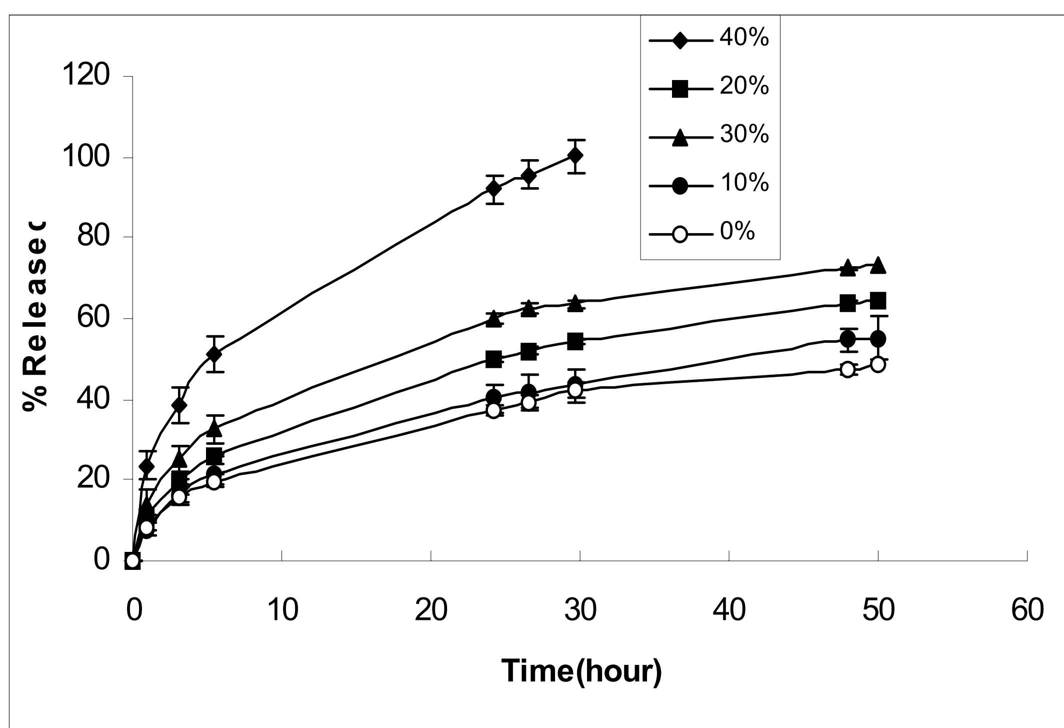


Figure 14. Release profiles of metronidazole from GMO base containing 7%EC and different concentration of PG as a function of time.

The effect of PG was further investigated when used in formulations containing no EC as shown in figure 15.

المنارة للاستشارات
www.manaraa.com

From table 10, it was clear that PG when added to GMO alone did not affect the release profile of metronidazole. Also by using one way ANOVA test, there were no significant differences in the rate constant of metronidazole release. The conclusion from the above results that the addition of PG to the GMO did not increase the solubility of metronidazole in the formula.

Table 10. Similarity factor (f2) for the dissolution profiles between formulations having different concentration of PG in GMO that containing 20% Metronidazole.

Reference formula	Test formula	f_2-value
10%PG in GMO	0%PG in GMO	66.8394
10%PG in GMO	30%PG in GMO	73.8053
0%PG in GMO	30%PG in GMO	77.7168

.

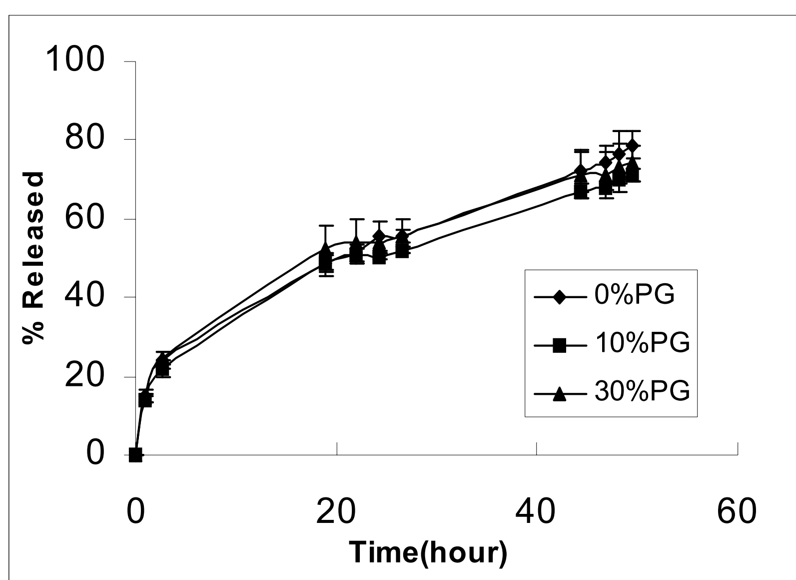


Figure 15. Release profiles of metronidazole from GMO base containing different concentrations of PG as a function of time.

PG being hydrophilic component might increase the permeability of the network formed by EC when the GMO started to swell. This might be one of the possible

المنارة للاستشارات

www.manaraa.com

explanations of the PG effect on the release profile. Furthermore, it was the decrease in the EC percentage in the formula as the percentage of PG increased, so the percentage of the hydrophobic barrier decreased and consequently the release rate of metronidazole from the formula increased.

In all the formulations, which contained PG, GMO formed the cubic phase except when the concentration of PG was 40%. When EC was present as 2.8 % in the formula (40%PG), there was no EC precipitated network observed under the microscope. These observations might explain the high release rate of metronidazole from formula containing 40% PG as shown in figure 14.

In order to investigate the mechanism of drug release from controlled release formulations, the values of the kinetic parameter *n* from equation 5, were determined (Ritgar and Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n \quad (5)$$

Where M_t is the cumulative amount of drug release at time t, M_∞ is the total amount of drug incorporated. *K* is the proportionality constant (the value of which depends on the structural and geometrical properties of the matrix), and *n* is the release exponent (its value depends on the mechanism of drug release).

Based on release exponent n values are classified as Fickian diffusion ($n \leq 0.5$), Case II transport ($n = 1$), non- Fickian or anomalous transport ($0.5 < n < 1$) and Super Case II transport ($n > 1$)(Ritgar and Peppas, 1987)

المنارة للاستشارات

www.manaraa.com

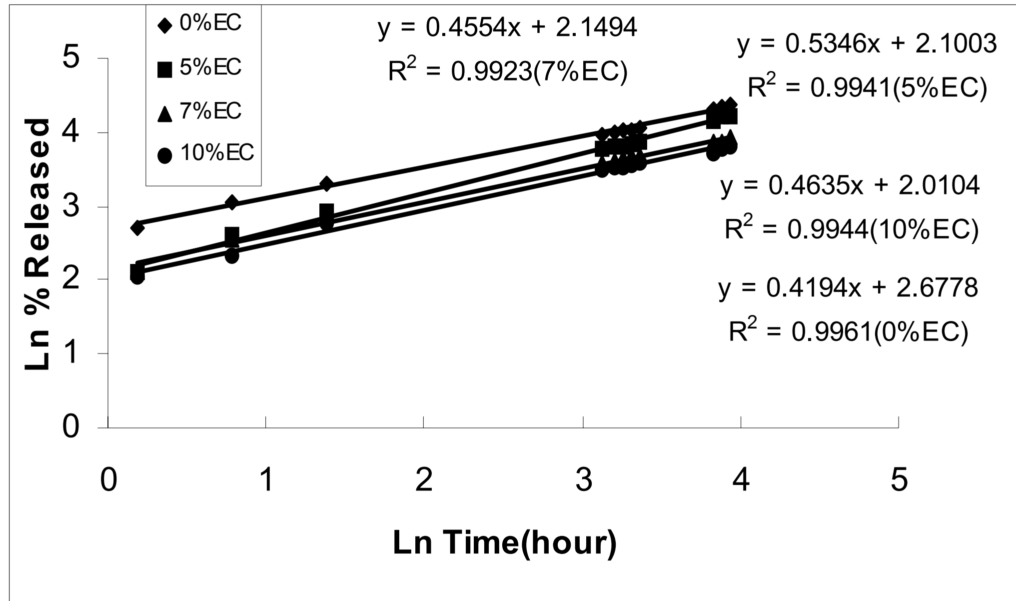


Figure 16. Fitting of dissolution profile data of metronidazole in GMO base containing different concentrations of EC into Peppas equation.

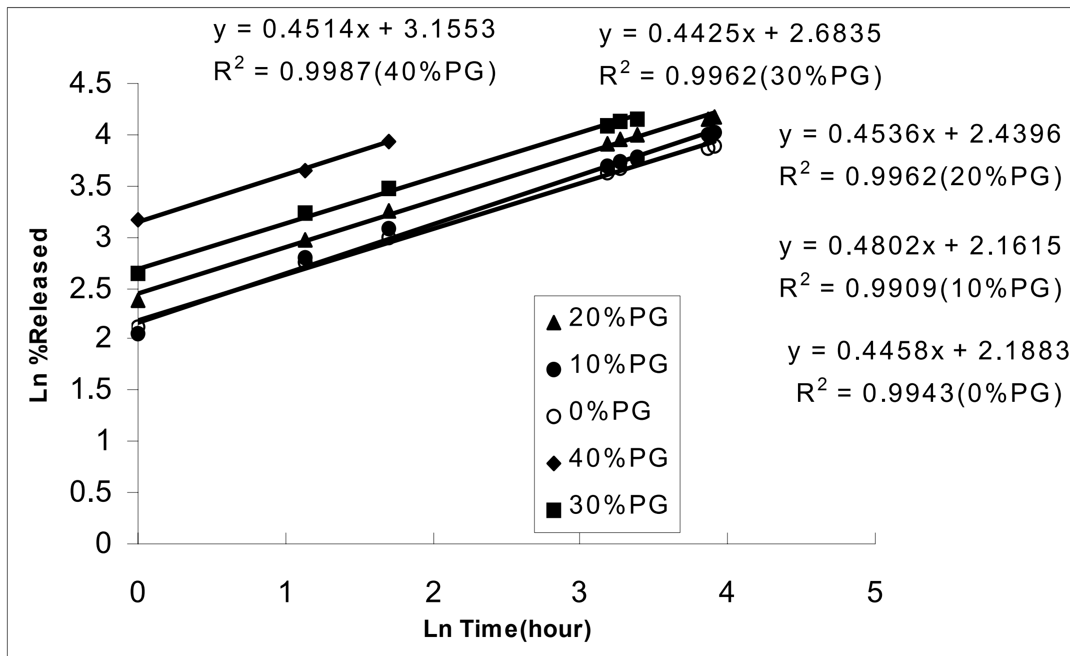


Figure 17. Fitting of dissolution profile data of metronidazole in GMO base with 7%EC containing different concentrations of PG into Peppas equation.

المنارة للاستشارات

www.manaraa.com

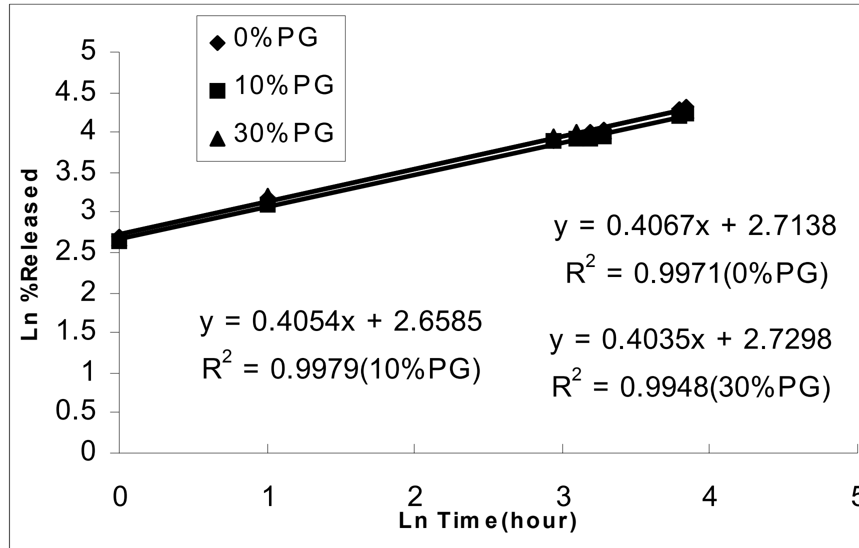


Figure 18.Fitting of dissolution profile data of metronidazole in GMO base contain different concentration of PG into Peppas equation

From figures 16,17 and 18, the results of this analysis show that *n* values ranged from (0.4035-0.53) and these values indicated a situation where drug release occurred by means of diffusions partially through the swollen matrix and partially through the water filled channels. The release pattern did not change on increasing or decreasing the percentage of EC or PG in the formula, it was a diffusion mechanism.

In the diffusion-controlled release, the cumulative amounts of drug released (Q) per unit surface area of the system is proportional to the square root of time, *t* as shown in equation 6:

$$Q = kt^{\frac{1}{2}} \qquad (6)$$

Where *k* is the release rate constant. The equation 6 describes the release from a system where the solid drug is dispersed in an insoluble matrix and the rate of drug release is controlled by the rate of drug diffusions. In the case of metronidazole in GMO, the drug was in the dispersed or suspended form in the GMO base. In figures 19,21 and 22, where the percent of drug released was plotted versus the square root of time, for all

المنارة للاستشارات
www.manaraa.com

different formulations, the relations were linear with R^2 values above (0.99), suggesting that the release behavior followed Highushi diffusion model (Martin, 1993)

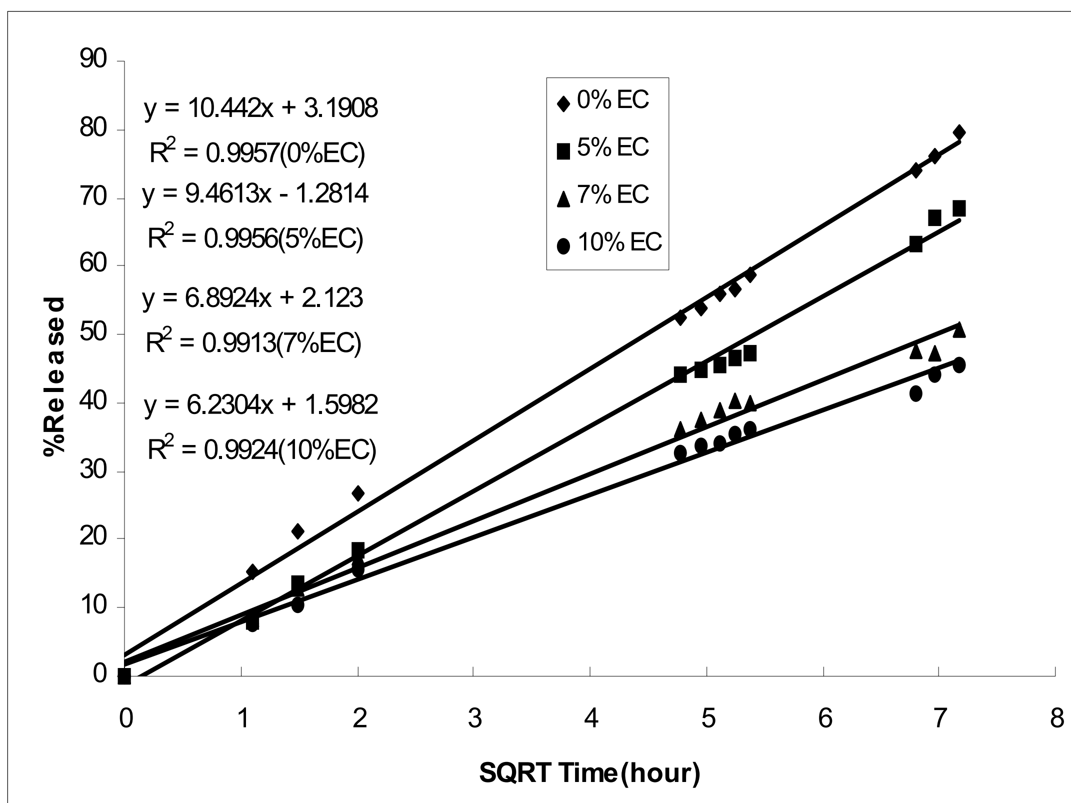


Figure 19. Release profiles of metronidazole in GMO base containing different concentrations of EC as a function of square root of time.

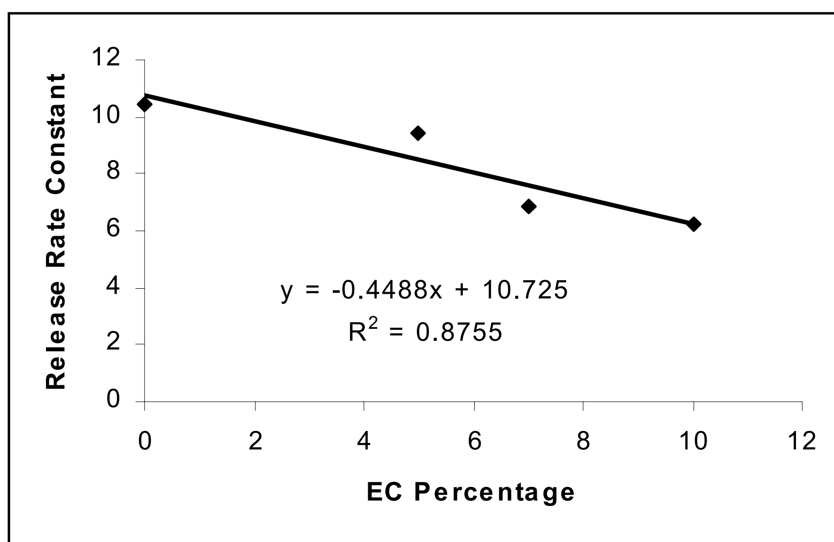


Figure 20. Effect of EC concentration on the dissolution rate constant of metronidazole from GMO base.

المنارة للاستشارات
www.manaraa.com

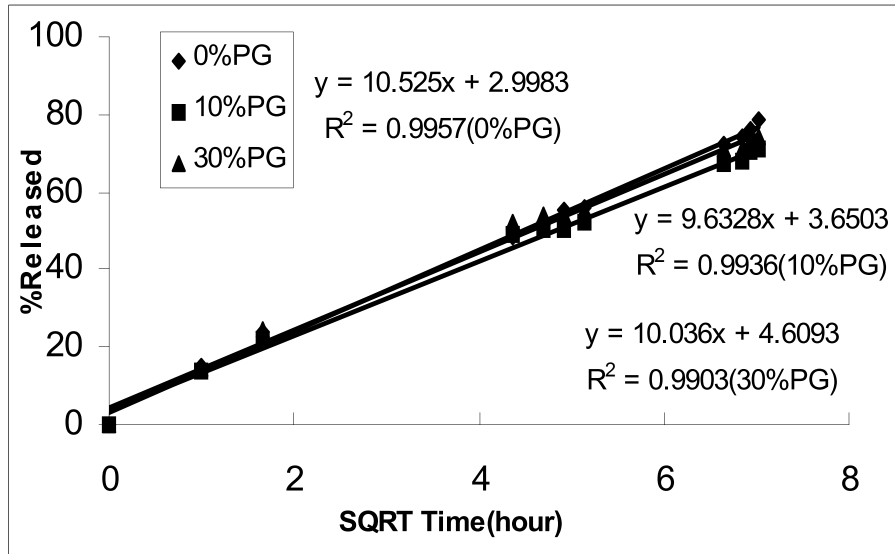


Figure 21. Release profiles of metronidazole in GMO base containing different concentration of PG as a function of square root of time.

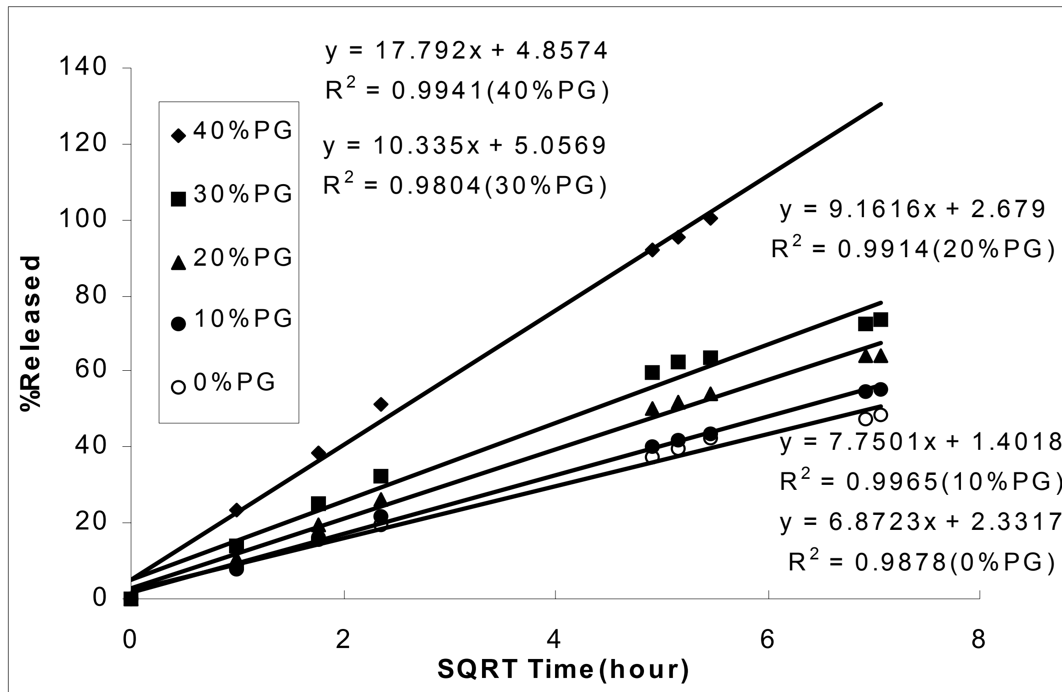


Figure 22. Release profiles of metronidazole in GMO base with 7%EC containing different concentration of PG as a function of square root of time.

المنارة للاستشارات
www.manaraa.com

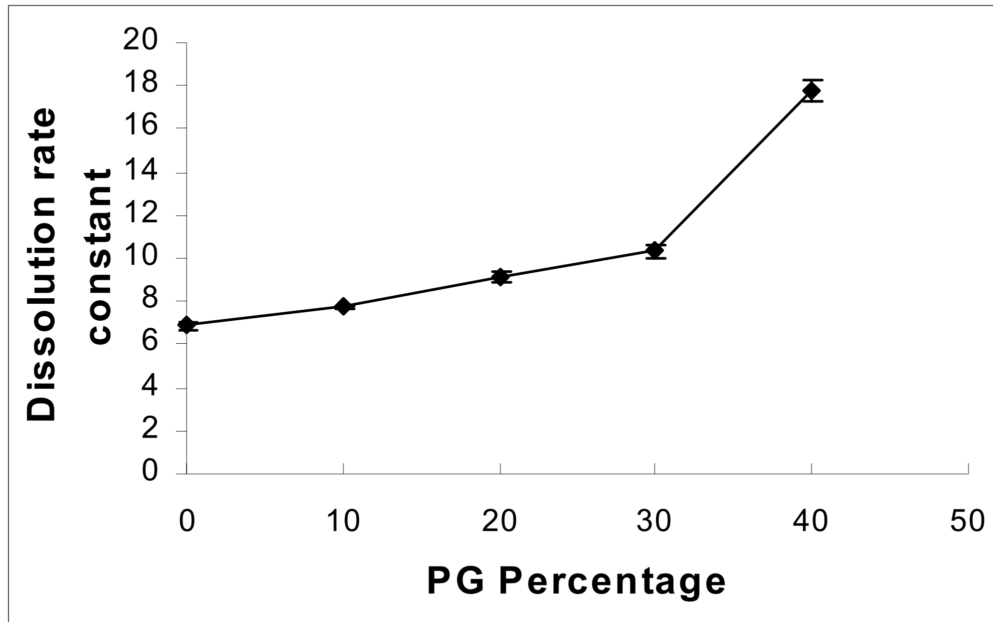


Figure 23. Effect of PG concentration on the dissolution rate constant of metronidazole from GMO base contain 7%EC.

المنارة للاستشارات
www.manaraa.com

3.5.Effect of addition of PG and Water on the release of metronidazole (20%) from GMO containing 7%EC.

Figures 24 and 26, show that the release rate from Formula A was lower than others. There was a significant difference between the release rate constant of metronidazole from Formulas A, B, C and D by using one way ANOVA test ($P < 0.5$). Formula A has a significantly lower release rate constant than Formulas B, C and D. The phase of the GMO in the Formula A was a mixture of reversed micellar and lamellar. The less physically stable lamellar phase enables rapid water uptake (Farakas, E., et al 2000). As a result of the rapid water uptake the swollen liquid crystalline system inhibited further drug release and increased the rate of formation of EC network. The release from the highly ordered system hexagonal phase in Formula D was the faster one because of slower water uptake. Also there was an effect on the solubility of metronidazole that increased by increasing the portion of co-solvent (PG+water) in the formula. Formula A has been chosen for further investigation.

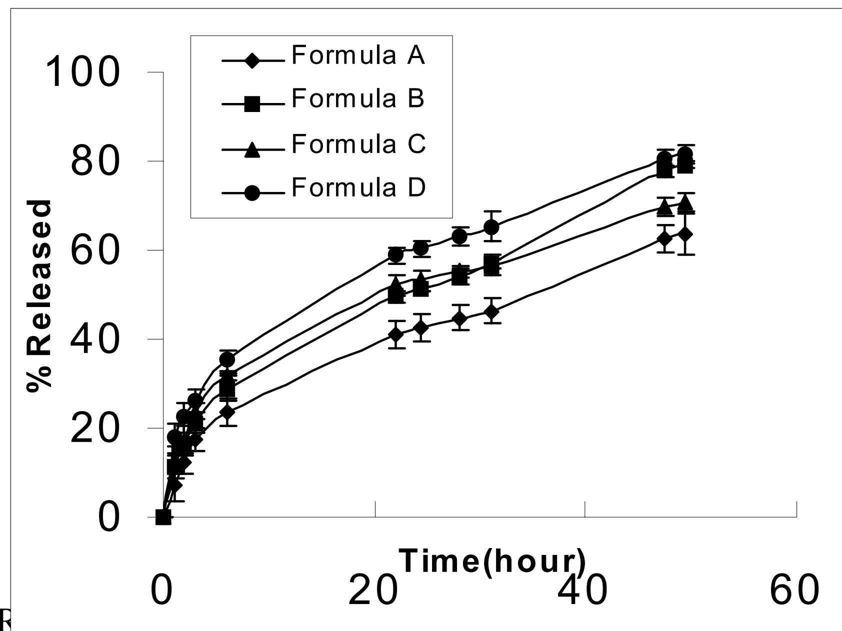
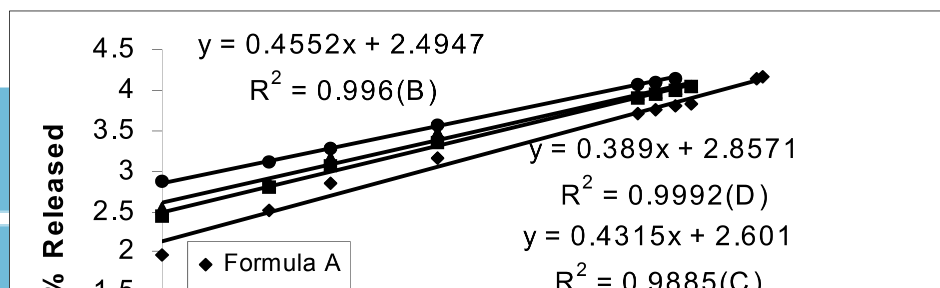


Figure 24. R [illegible] and D as a function of time.



للاستشارات
anaraa.com

Figure 25.Fitting of dissolution profile data of metronidazole from Formulas A, B, C and D into Peppas equation.

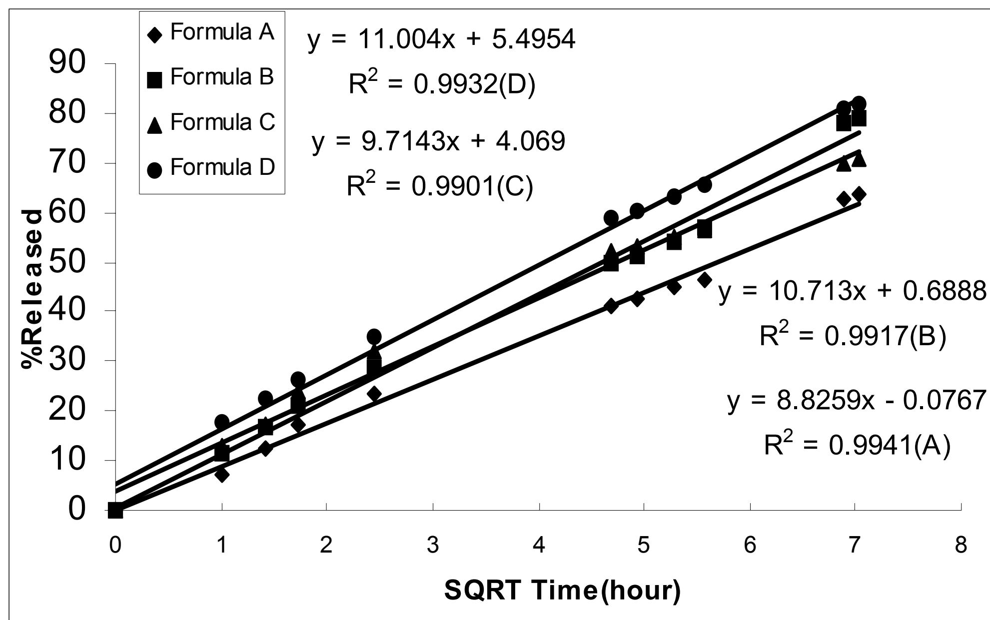


Figure 26. Release profiles of metronidazole from Formulas A, B, C and D as a function of square root of time.

From figure 25, the results of this analysis show that *n* values ranged from (0.389-0.5165) and these values indicated a situation where drug release occurred by means of diffusions partially through the swollen matrix and partially through the water filled channels.

المنارة للاستشارات
www.manaraa.com

3.6. The effect of matrix thickness on the release profile.

The rate of drug release increased as the capsule weight decreased as shown in figures 27 and 29. The difference was significant between the rate constant of drug release by using student-t test ($P < 0.05$). Furthermore, the similarity factor between the two release profile was lower than 50% ($f_2$value = 44.41%).

These results could be explained either by the decrease in the rate of drug release and the increase in the duration of drug release with increasing the matrix thickness, or by the lower surface to volume ratio (Norling, et al 1992, Chang and Bodmeier, 1997). The surface area available for drug release was proportionally larger at smaller matrix thickness.

Also the effect could be explained when one considered the diffusion pathlength of the water uptake as it increased with increasing matrix thickness. Therefore, the quantity of the injected formula has a significant effect on the drug release profile. So if the diameter of the gel matrix is not small enough, the enzymatic degradation of the gel matrix is necessary in order to attain 100% drug release.

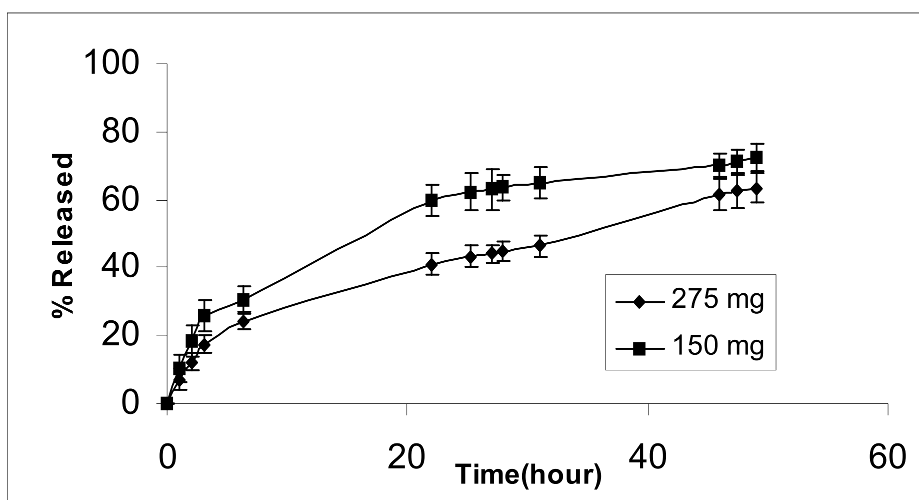


Figure 27. Effect of the amount of formula A on metronidazole release as a function of time.

المنارة للاستشارات
www.manaraa.com

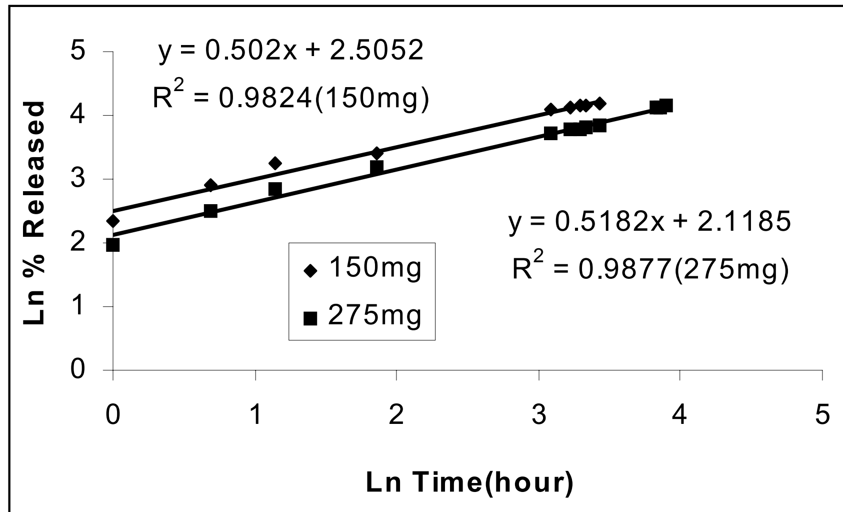


Figure 28. Release profiles of metronidazole from various amounts of Formula (A) fitted into Peppas equation.

From figure 28, the results of this analysis show that *n* values indicated a situation where drug release occurred by means of diffusions partially through the swollen matrix and partially through the water filled channels.

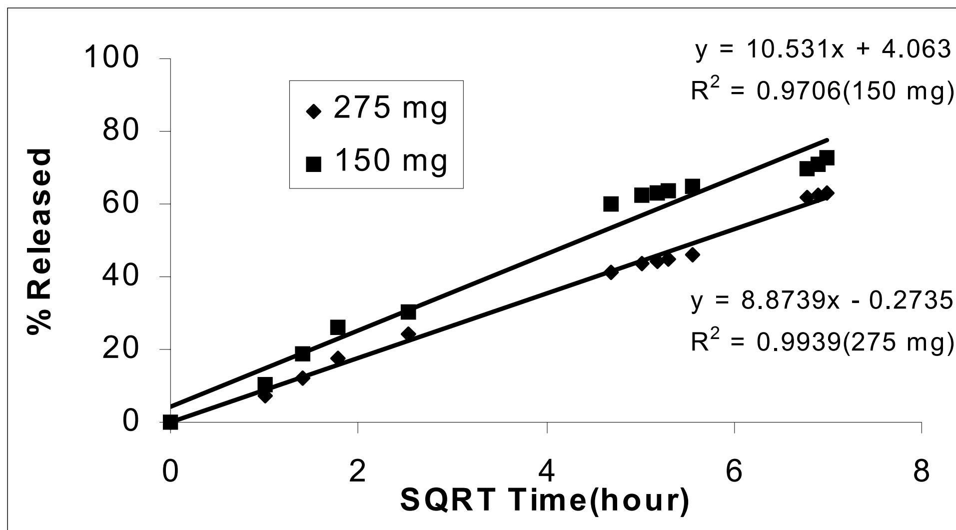


Figure 29.Release profiles of metronidazole from various amount of Formula A as a function of square root of time**.**

المنارة للاستشارات
www.manaraa.com

3.8.The effect of tonicity of the dissolution media on the release profile.

The effect of ionic strength on the release profile of metronidazole is shown in figures 30 ,31 and 32. There were no significant differences among the dissolution rate constant of metronidazole release in different ionic strengths of the dissolution media by using one way ANOVA (P > 0.05). Also the similarity factors (f_2-value) have been used to compare the release profile of different tonicities and the results are shown in table 11. The influence on the water uptake by the GMO, the swollen mesophases were identified by the polarized microscope. The difference in the ionic content did not prevent formation of the cubic phase of the GMO. Also the ionic content did not change the mechanism of drug release from the cubic phase.

Table 11. Similarity factors (f_2) for the dissolution profiles of formula A using dissolution media having various tonicities.

Reference tonicity	Test tonicity	f_2-value
Isotonic (0.9%NaCl)	Hypertonic (1.3%NaCl)	69.4
Hypotonic (0.45%NaCl)	Hypertonic (1.3%NaCl)	68.6
Hypotonic (0.45%NaCl)	Isotonic (0.9%NaCl)	86.2

.

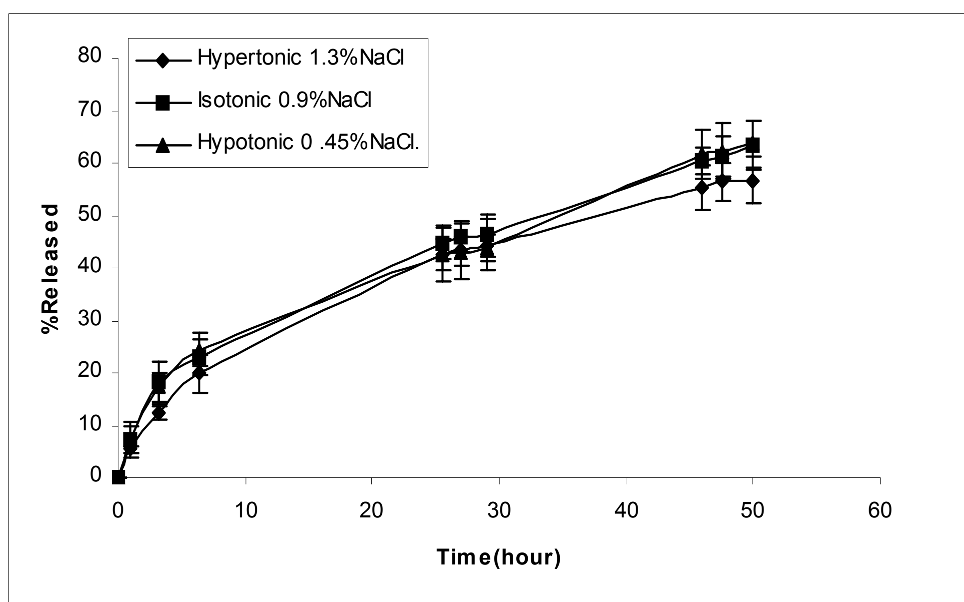


Figure 30. Release profiles of metronidazole from Formula A in dissolution media having various tonicities as a function of time.

المنارة للاستشارات
www.manaraa.com

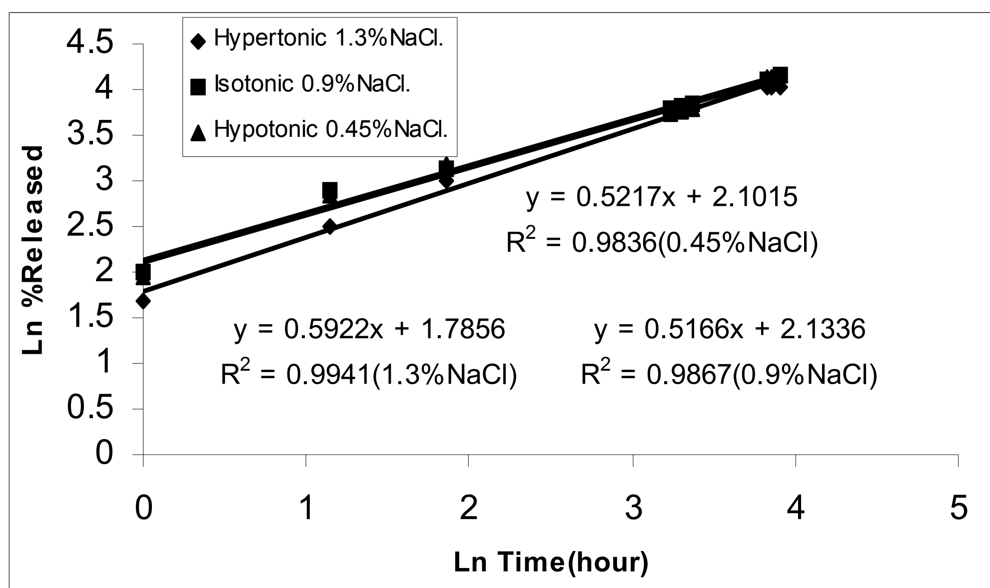


Figure 31. Fitting of Release profiles of metronidazole from Formula A in dissolution media having various tonicities different ionic strength into Peppas equation.

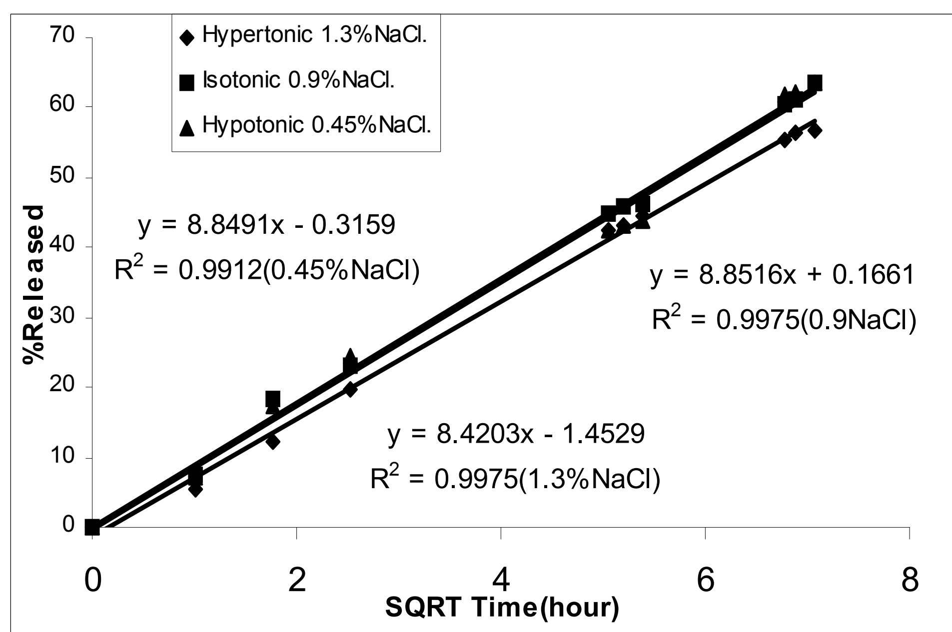


Figure 32. Release profiles of metronidazole from Formula A in dissolution media having various tonicities as a function of square root of time

Monoglycerides like GMO are non-ionic amphliphilic molecules; therefore, the ionic content did not considerably affect the GMO swelling and drug release.

المنارة للاستشارات
www.manaraa.com

3.7.Rheological measurements

Figures 33and 34 show that the formulations have pseudoplastic flow behavior and the apparent viscosity decreases as the amount of PG increases. The viscosity was determined from the flow curve obtained at different values of shear rate and rheograms were fitted to the Ostwald relationship.

The results obtained after fitting the rheological data to Ostwald equation confirmed the visually observed pseudoplastic flow behavior of these formulations. The calculated values of n were less than 1, also the K value decreased as the amount of PG increased from 10 to 40%. Calculated values of n are listed in table 9. The presence of a higher PG concentration improved the injectability of the formula through syringe gauge 21 but adversely affected the sustained release of metronidazole as previously mentioned (section 3.4).

The aim of having low viscosity formula without affecting the sustained release property was achieved by the addition of water in 5% to the formula containing 10% PG and 20% metronidazole in GMO containing 7% EC. However, this composition led to the formation of formula had reversed micellar and lamellar phase of GMO.

This effect was obvious when the K value of formulas containing 10% PG with 5% water was compared with the formulas containing 10% PG without 5 % water.

The K value of the formulas containing 10% PG without 5 % water was 5 times higher than that of formula containing 10% PG with 5% water as shown in table 12. Also from figure 34 the apparent viscosity in presence of water reached to lower value at low rate of shear. Furthermore, it was easily injectable through syringe gauge 21 when compared with the formula that did not contain water.

المنارة للاستشارات
www.manaraa.com

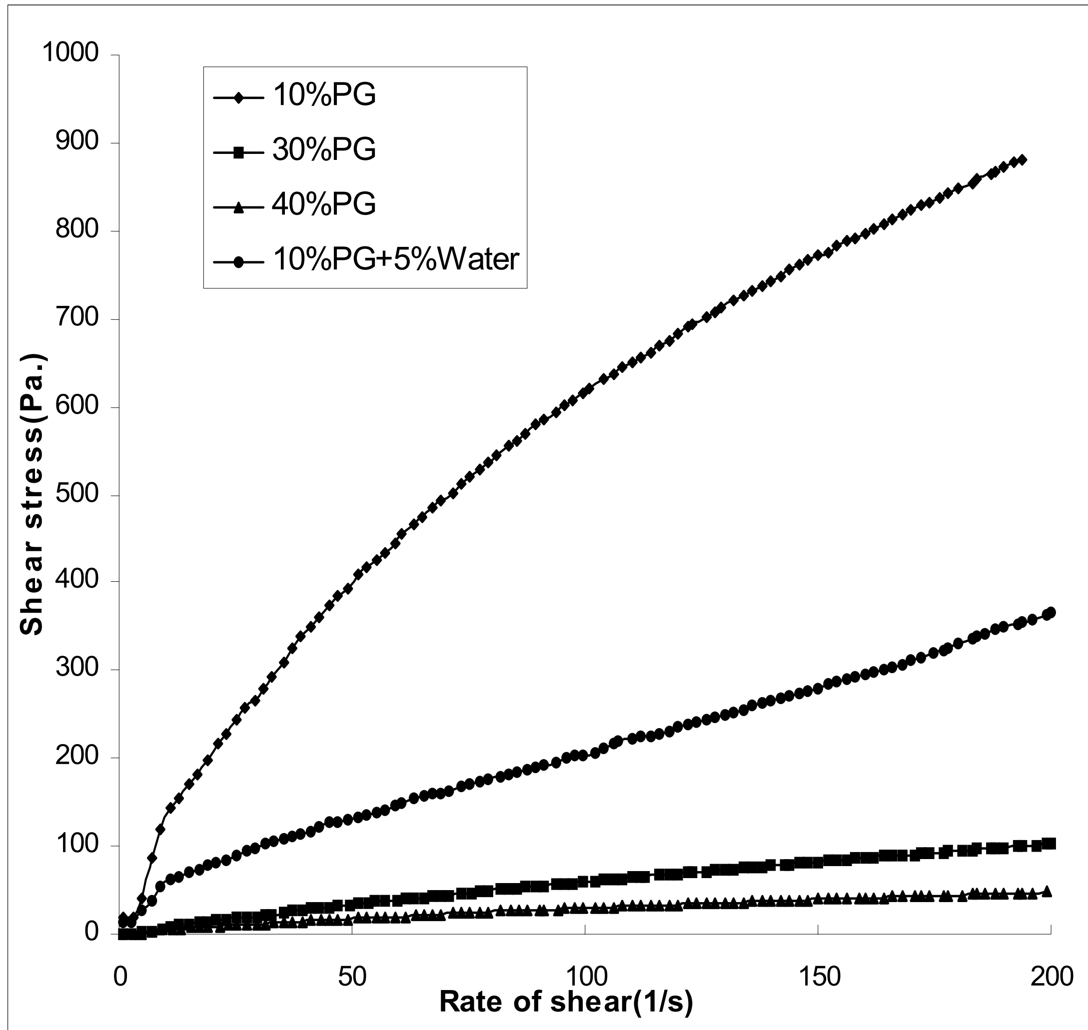


Figure 33. Flow curves represent the influence of PG and/or water on the rheological behavior of GMO containing 7% EC, in addition to metronidazole 20% at 30°C.

To explain the rheological behavior of metronidazole suspensions, the concept of pseudoplastic behavior of such suspensions and the effect of shape of drug particles are discussed in the following paragraphs.

It is worthwhile to indicate that some reasons for the shear thinning effect of pseudoplastic materials are orientation, stretching, deformation and disaggregation. Many liquid products that seem homogeneous are in fact composed of several ingredients: particles of irregular shape of one liquid are dispersed in another liquid. On the other hand there are polymer solutions like EC with long entangled and looping

المنارة للاستشارات
www.manaraa.com

molecular chains. At rest, all of these materials will maintain an irregular internal order and correspondingly a sizable internal resistance against flow, i.e. a high viscosity, characterizes them. The microscopical examination of metronidazole in GMO contained 7% EC showed oblong particles. This shape is compatible with shear thinning behavior of the suspension since this shape is more conductive to orientation in the direction of flow.

With increasing shear rates, oblong-like particles suspended in the liquid will be turned lengthwise in the direction of the flow. Chain-type molecules in a melt or in a solution like EC can disentangle, stretch and orient themselves parallel to the driving force.

Particle or molecular alignments allow particles and molecules to slip past each other more easily. The rheological literature points out one other possible reason for the shear-thinning of a material: in addition, some of the solvent associated with molecules may be released, resulting in an effective lowering of the concentration and the size of dispersed molecules. This, too, will effect a lowering of the apparent viscosity

Table 12.Flow indices of the formulations containing GMO (7% EC), 20% Metronidazole and different percentage of water and PG.

	n	*K*	R^2
40%PG	0.7315	1.0059	0.99
30%PG	0.8210	1.3386	0.99
10%PG	0.6071	36.8356	0.99
10%PG+5%water	0.7133	7.9667	0.99

المنارة للاستشارات
www.manaraa.com

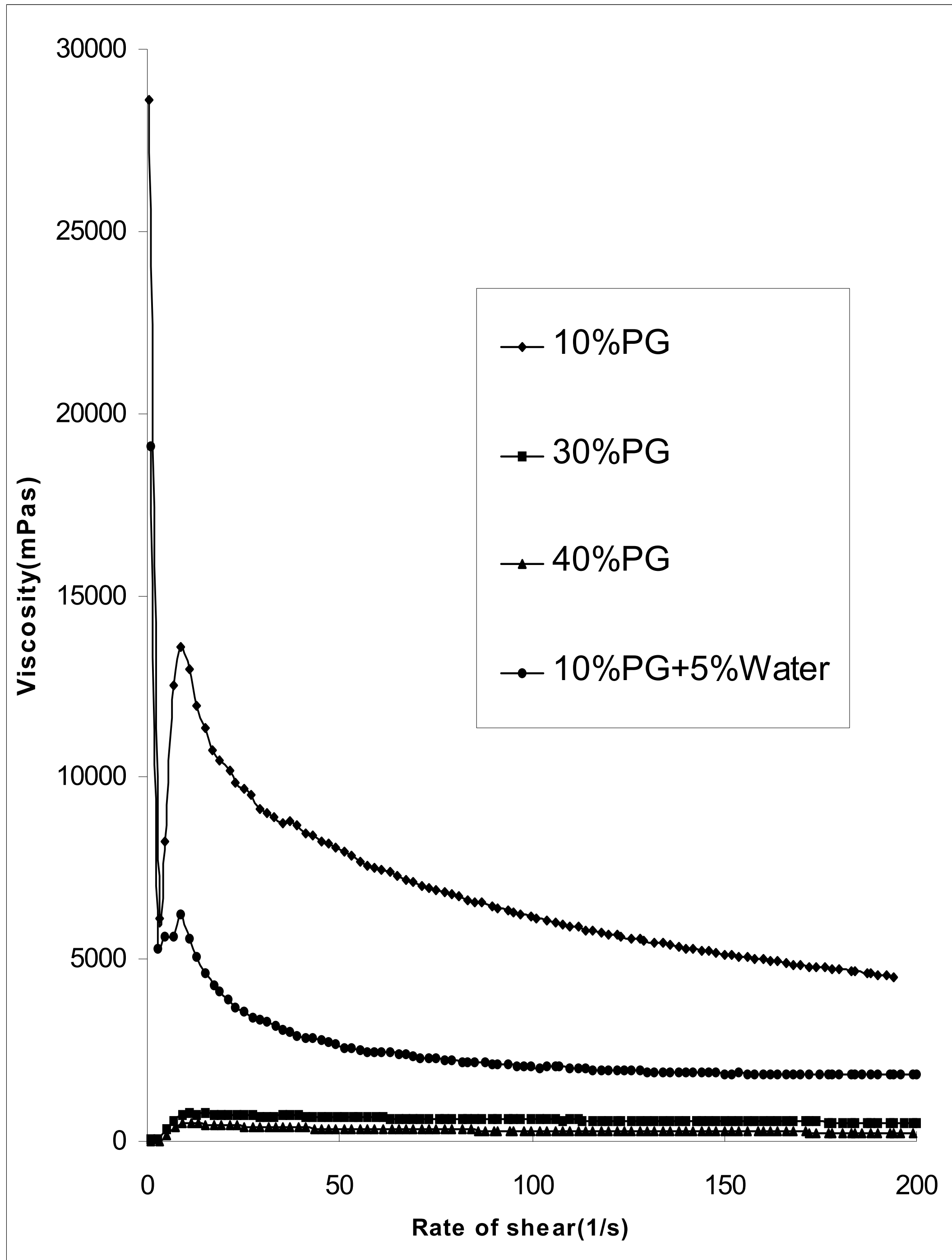


Figure 34. Flow curves represent the influence of PG and/or water on the rheological behavior of GMO containing 7% EC, in addition to metronidazole 20% at 30C°.

المنارة للاستشارات

www.manaraa.com

3.9.The effect of the storage conditions on the release profile of Metronidazole from Formula A.

Phase changes and crystal growth were observed on the formula stored at room temperature, the reversed micellar and lamellar phase transferred into reversed micellar after storage at room temperature. The formula that stored at 4°C for 5 weeks had the reversed micellar and lamellar phase unchanged and had not shown any significant crystal growth. There were significant differences between the release rate constant of the freshly prepared formula and that stored at room temperature for 5 weeks ($P < 0.05$) as shown in figures 35,36 and 37.

But there was no significant difference between the release rate constant of the freshly prepared formula and that stored at 4°C for 5 weeks by using one way ANOVA test ($P > 0.05$). Also the similarity factors f_2 (Table 13) indicated that formula A which was stored at room temperature had changed significantly.

Table 13. Similarity factors (f2) for the dissolution profiles of Formula A stored at different storage conditions.

Reference	Test	f_2-value
Freshly prepared	Stored at 4C°	72.5
Freshly prepared	Stored at RT	34.67
Stored at 4C°	Stored at RT	35.84

This result could be explained by the modification of the important parameters in Higushi equation, the porosity and the tortuosity due to the crystal growth and the increase in the particle size. Crystal growth led to increase in the pore size that formed in the EC network after the dissolution of the solid particles which resulted in an increase in the porosity and decrease in the tortuosity of the depleted layer in the matrix.

المنارة للاستشارات
www.manaraa.com

This effect led to increase in the diffusion of the drug through such layer and increased the rate of water ingress inside the matrix to dissolve the solid particles of the drug.

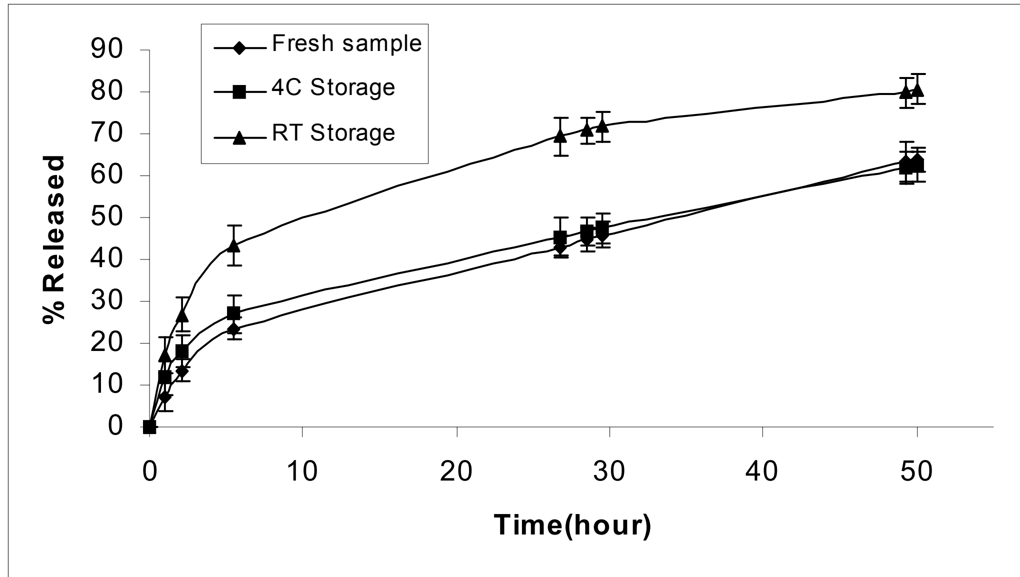


Figure 35. Release profiles of metronidazole from Formula (A) kept under different storage conditions for 5 weeks as a function of time.

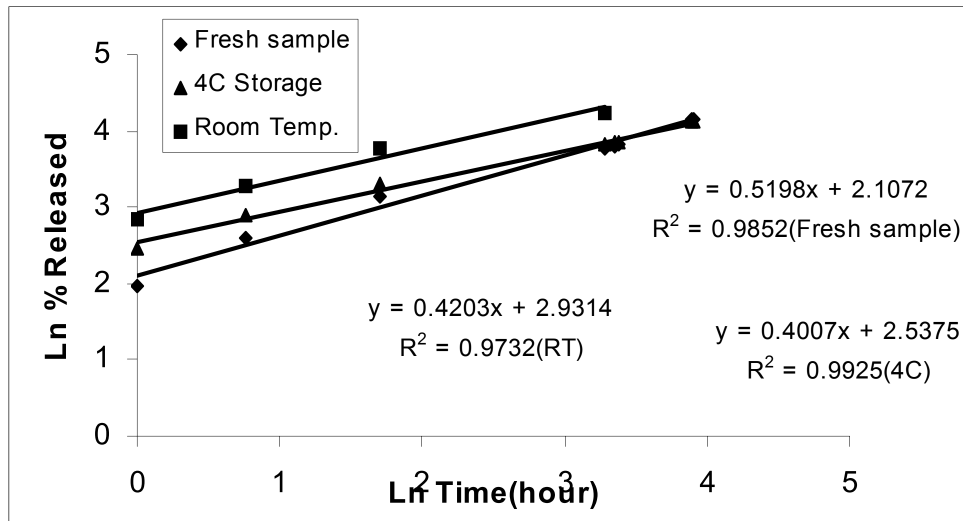


Figure 36.Fitting of dissolution profile data of metronidazole Formula (A) kept under different storage conditions for 5 weeks into Peppas equation.

المنارة للاستشارات
www.manaraa.com

The storage of Formula A at room temperature might lead to the degradation of GMO. The product of degradation like oleic acid might delay the formation of the cubic phase by decreasing the rate of water uptake of GMO and delaying the formation of EC network. This led to increasing the rate of metronidazole release from formula A which stored at room temperature.

In addition to the above possible reasons, the solubility of metronidazole might increase when GMO phase changed from reversed micellar and lamellar into reversed micellar phase during the storage at room temperature. The solubility of the drug in the base play an important role in the initial drug release from the system before the total conversion into the cubic phase and formation of EC network and when the solubility increases the release rate increases.

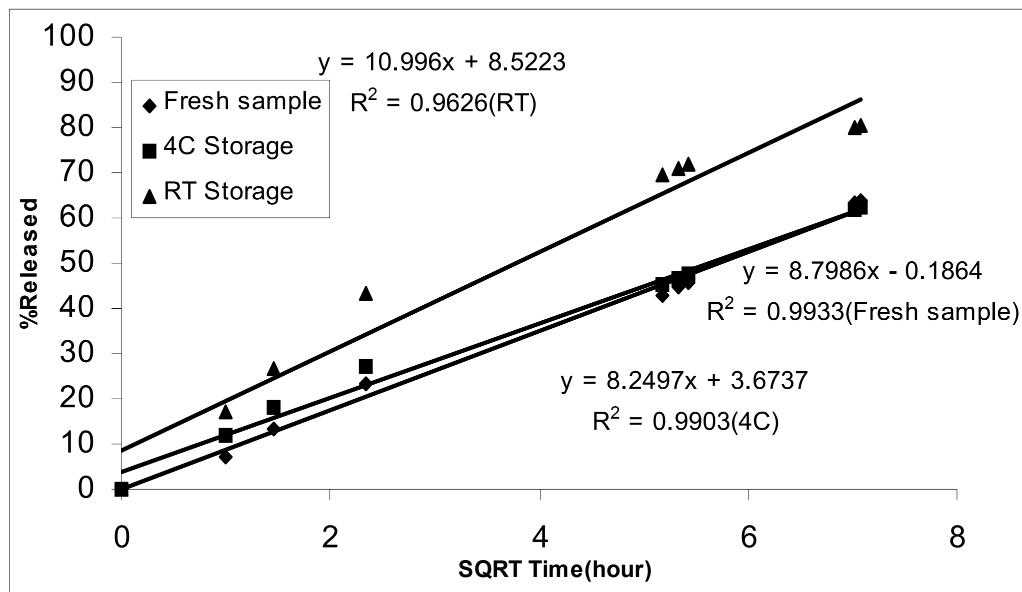


Figure 37. Release profiles of metronidazole from Formula (A) kept under different storage conditions for 5 weeks as a function of square root of time.

المنارة للاستشارات
www.manaraa.com

3.10.Particle size analysis

The results of particle size analysis of metronidazole powder, freshly prepared Formula A and that stored at 4°C for 10 weeks indicated that there was no significant crystal growth observed in stored samples as shown in figures 38,39,40 and 41.

Figures 42,43 and 44 show the significant particle size growth in Formula A when stored at room temperature in dark place. The geometric mean of particles was 25μm in the freshly prepared sample, increased in samples stored for 4 weeks at room temperature to 44μm as shown in figure 43.

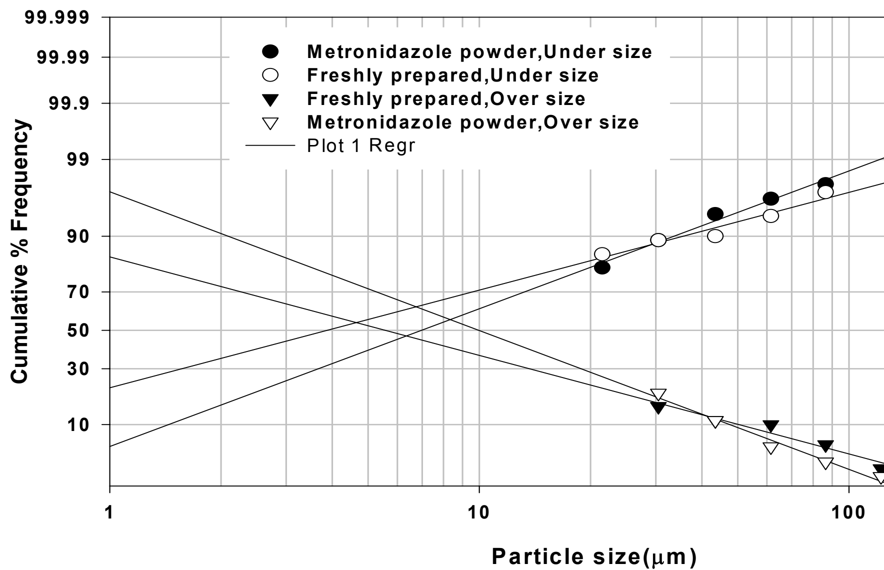


Figure 38. Log-probability plots of the cumulative under and over size percent of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and metronidazole powder.

المنارة للاستشارات
www.manaraa.com

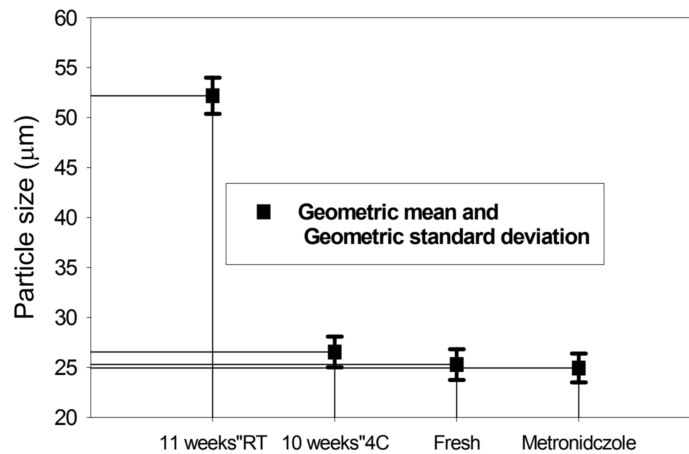


Figure 39. The geometric means and the geometric standard deviation of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 11 weeks at room temperature, 10 weeks at 4°C and metronidazole powder.

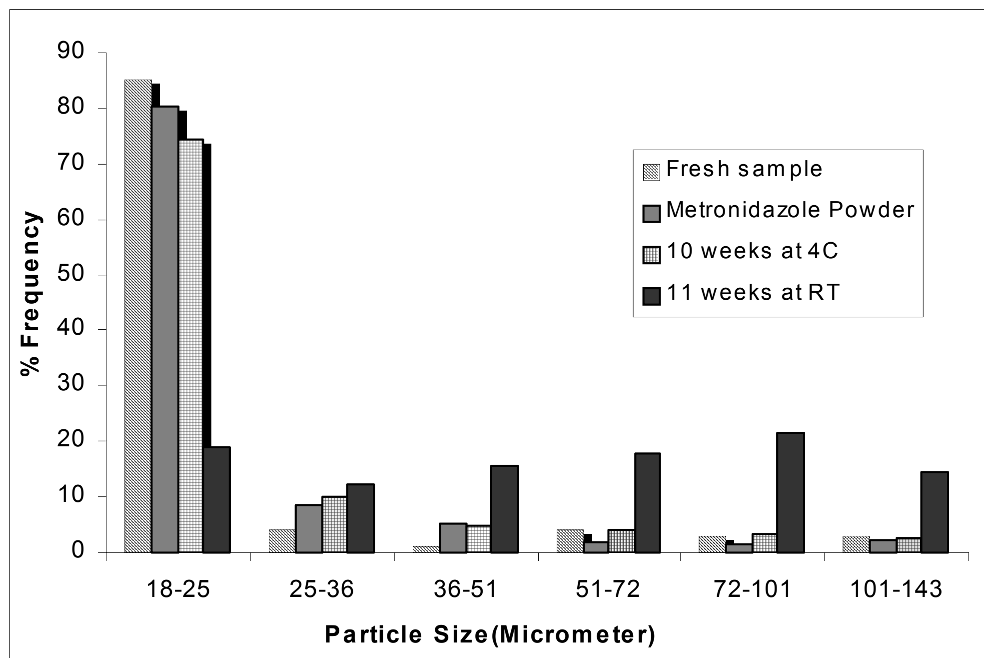


Figure 40. Histograms represent the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 11 weeks at room temperature, after storage for 10 weeks at 4°C and metronidazole powder.

المنارة للاستشارات
www.manaraa.com

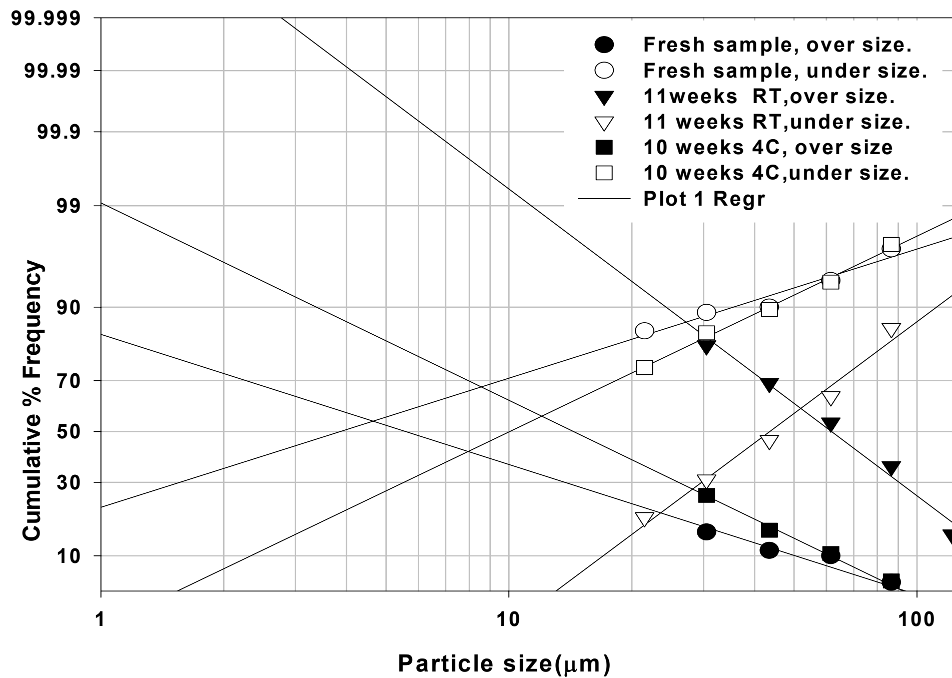


Figure 41. Log-probability plots of the cumulative under and over size percent of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 11 weeks at room temperature and after storage for 10 weeks at 4°C.

From figures 40 and 42, the histogram illustrated the positively skewed distribution of the particle size and more than 80% of the particles had size less than 25μm. The skewness decreased in sample stored for 2 weeks at room temperature and more than 80% of the particles had size less than 50μm. The distribution of the particle size in sample stored for 4 weeks at room temperature could be considered as normal distribution as shown in figure 42.

The formula stored at 4°C did not show crystal growth because there was no fluctuation in the temperature of the environment like in case of storage at room temperature, and the distribution was still positively skewed as shown in figure 40.

المنارة للاستشارات
www.manaraa.com

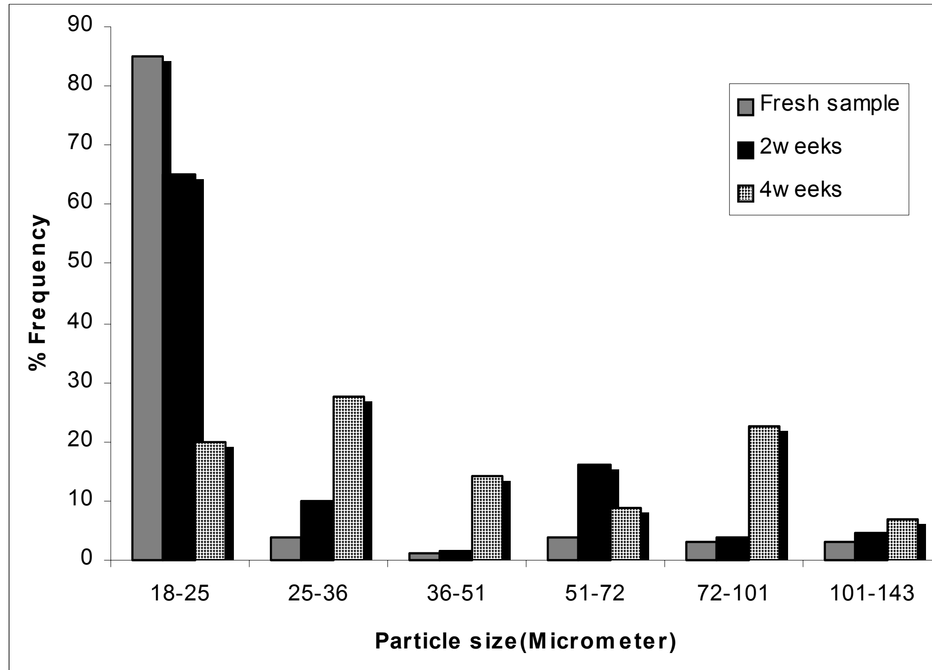


Figure 42. Histograms represent the particle size distribution of suspended metronidazole in freshly prepared sample and after storage for 2 and 4 weeks at room temperature.

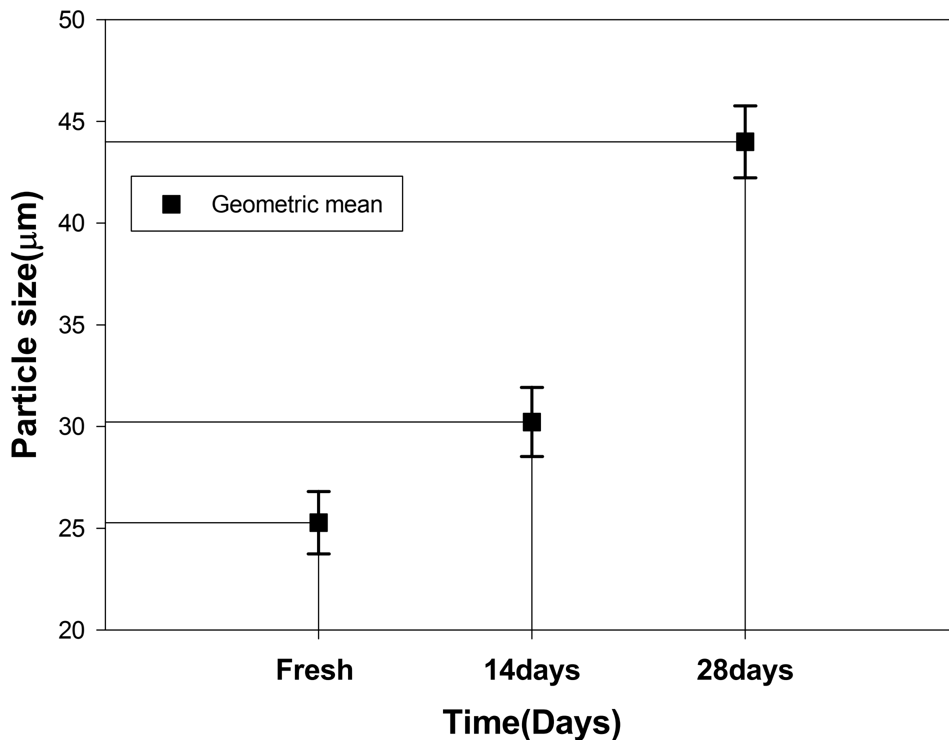


Figure 43. The geometric means and the geometric standard deviation of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 2 and 4 weeks at room temperature.

المنارة للاستشارات
www.manaraa.com

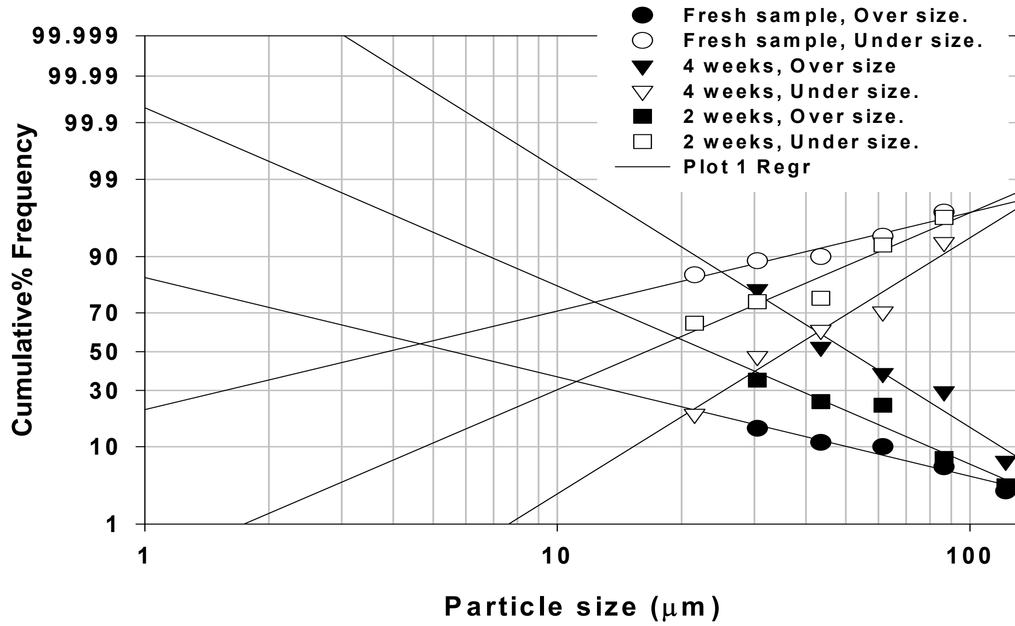


Figure 44. Log-probability plots of the cumulative under and over size percent of the particle size distribution of suspended metronidazole in freshly prepared sample of Formula A and after storage for 2 and 4 weeks at room temperature.

المنارة للاستشارات

www.manaraa.com

3.11. Assessment of the mucoadhesive properties of GMO and Formula A

From table 14, there was no significant differences in the mucoadhesive properties of GMO and Formula A by using Student-t test ($P>0.05$). The mechanism of mucoadhesion of the GMO is still unknown and unspecific (Neilsen et al 1998). The water addition to the GMO affected the mucoadhesive properties, as the addition of water led to decrease the mucoadhesive properties of the GMO. From the above, the dehydration of the mucous membrane may be the possible mechanism of mucoadhesion of GMO (Neilsen *et al*., 1998). The addition of PG in 10% in Formula A might increase the dehydrating activity of the reversed micellar and the lamellar phase of the GMO, that resulted in increase the mucoadhesive properties of Formula A and decrease the effect of the presence of 5% water in Formula A.

Table 14. The average work and standard deviation of mucoadhesion of unswollen GMO and Formula A.

Sample	Average Work(mJ/cm^2)	Standard Deviation
Formula A	0.041924	0.013777
Unswollen GMO	0.033691	0.01011

المنارة للاستشارات
www.manaraa.com

3.12. Thermal analysis (Differential scanning calorimeter DSC)

Figure 45 shows the DSC endothermic peaks related to the melting of GMO. The endothermic peaks due to melting of GMO appeared broad with three peaks at 14,28 and 35°C. GMO is not considered as a pure compound and other constituents that have lower melting point might present. The presence of 7% EC has no significant effect on the thermal behavior of the GMO as shown in figure 46. Figure 47 shows the endothermic peak at 161°C, which was related to the melting of pure metronidazole. Figure 48, describes the thermal behavior of a physical mixture of GMO 80% and metronidazole 20%. In this figure there are two endothermic peaks, the first one at 35°C was due to melting of GMO and the second one at 152°C was due to melting of metronidazole. The decrease in the melting of metronidazole from 161°C in figure 47 to 152°C in figure 48 was due to partial solubility of metronidazole in the melted GMO.

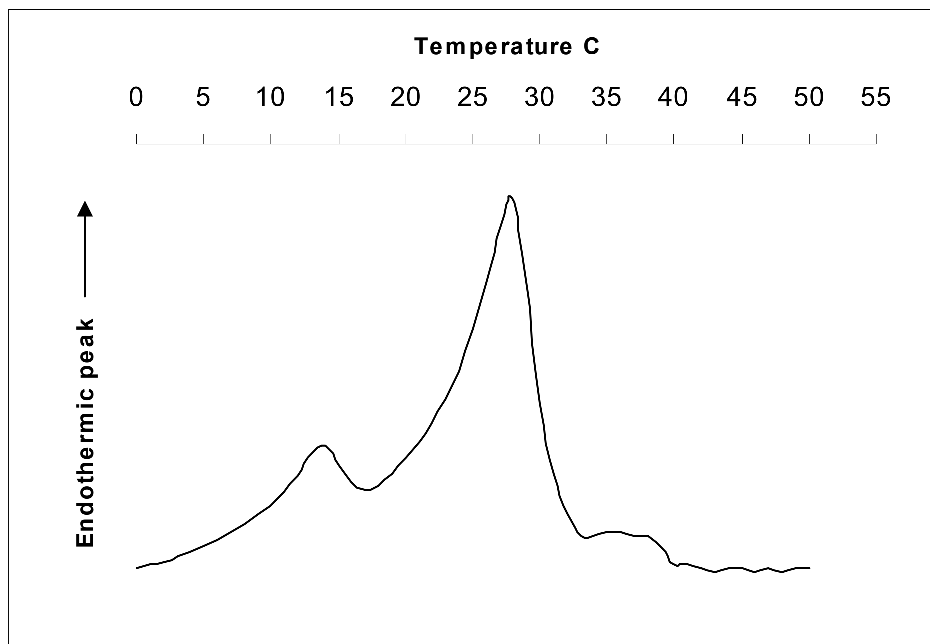


Figure 45. DSC thermogram of GMO at a heating rate of 2°C/min.

www.manaraa.com

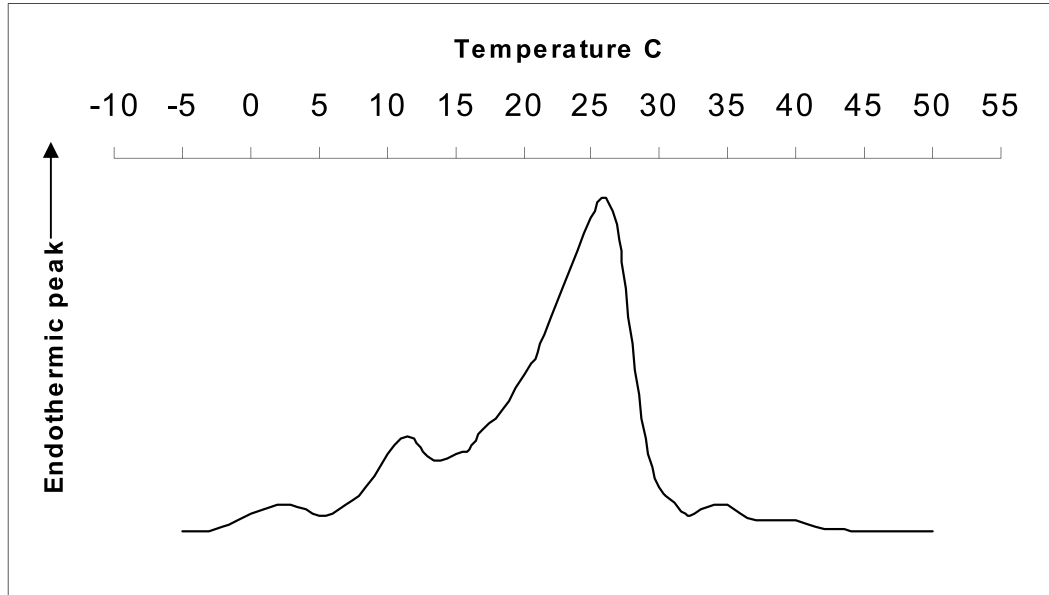


Figure 46. DSC thermogram of GMO containing 7%EC at a heating rate of 2°C/min.

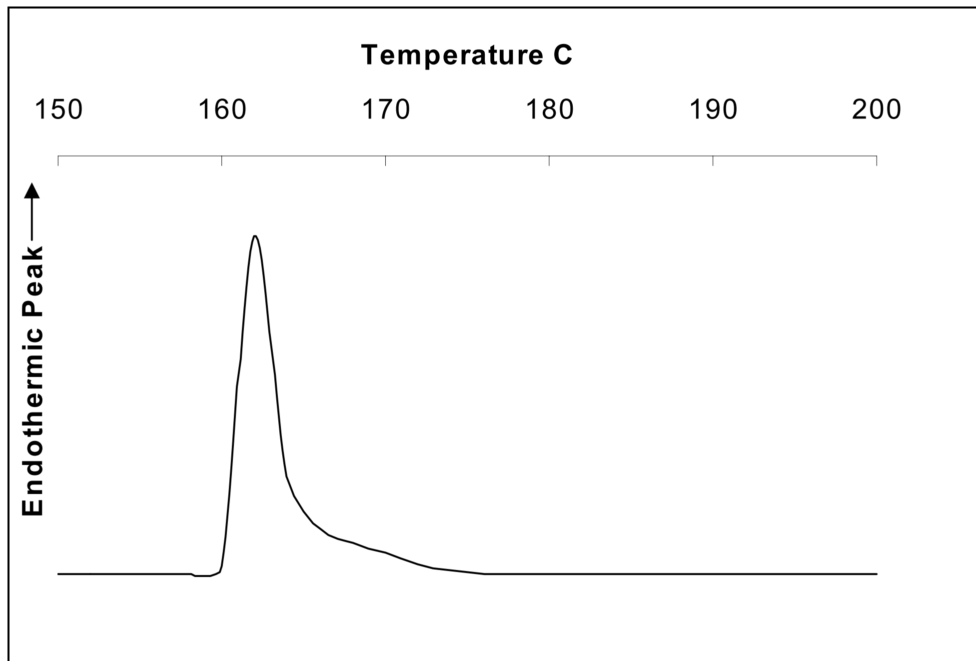


Figure 47. DSC thermogram of Metronidazole powder at a heating rate of 10°C/min

المنارة للاستشارات
www.manaraa.com

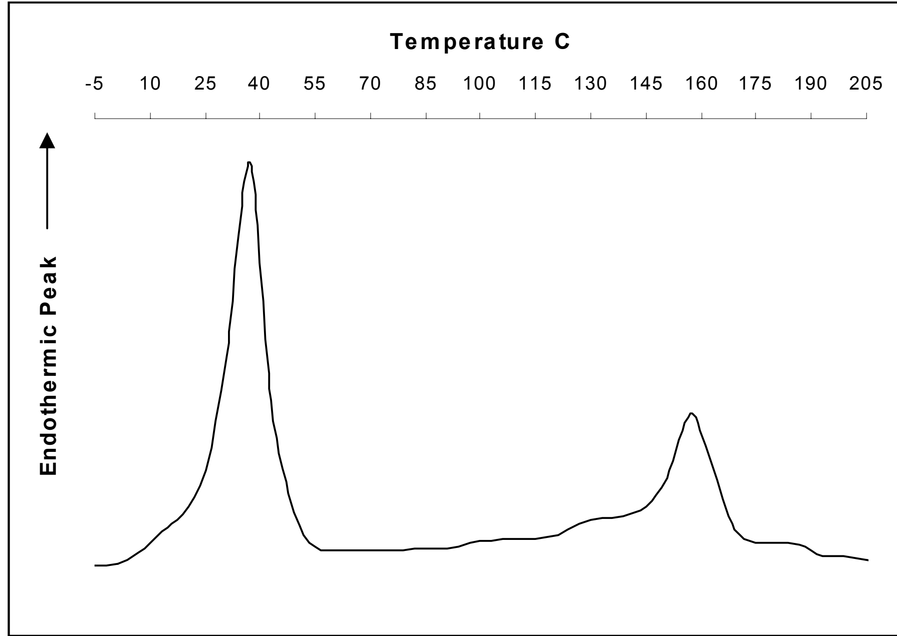


Figure 48. DSC thermogram of Metronidazole (20%w/w) and GMO at a heating rate of 10°C/min

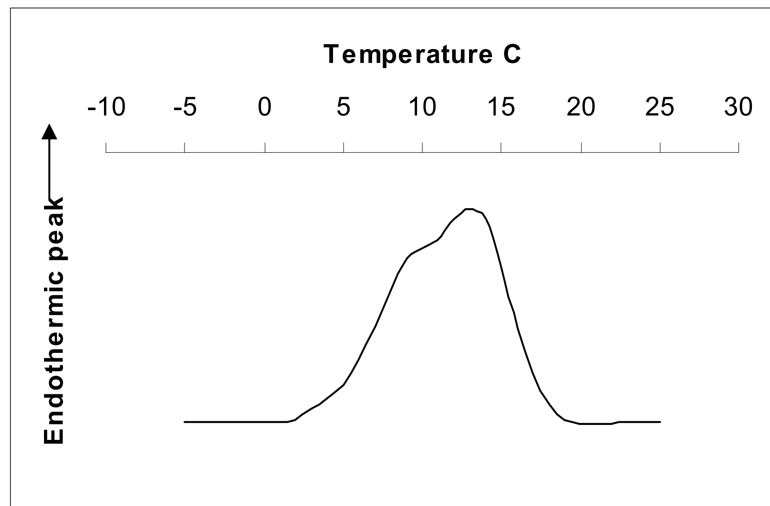


Figure 49. DSC thermogram of Formula A at a heating rate of 2°C/min

The melting point of the GMO decreased (14°C) in Formula A due to the presence of 5%w/w of water and 10%w/w of PG as shown in figure 49. This effect could be explained as the GMO formed the reversed micellar and lamellar phase as indicated by the examination under polarized microscope. This made Formula A had

المنارة للاستشارات
www.manaraa.com

low viscosity at room temperature. No peaks appeared above these temperatures up to 50C°, which indicated that no phase changes took place.

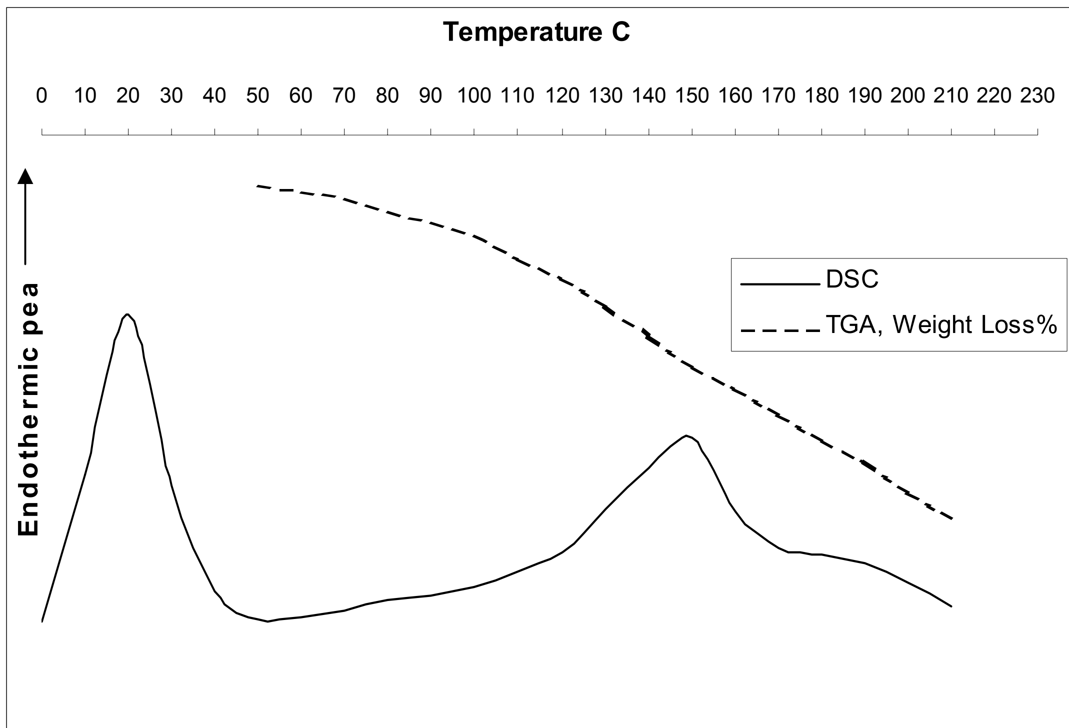


Figure 50. DSC and thermogravimetric thermograms of Formula A at a heating rate of 10°C/min.

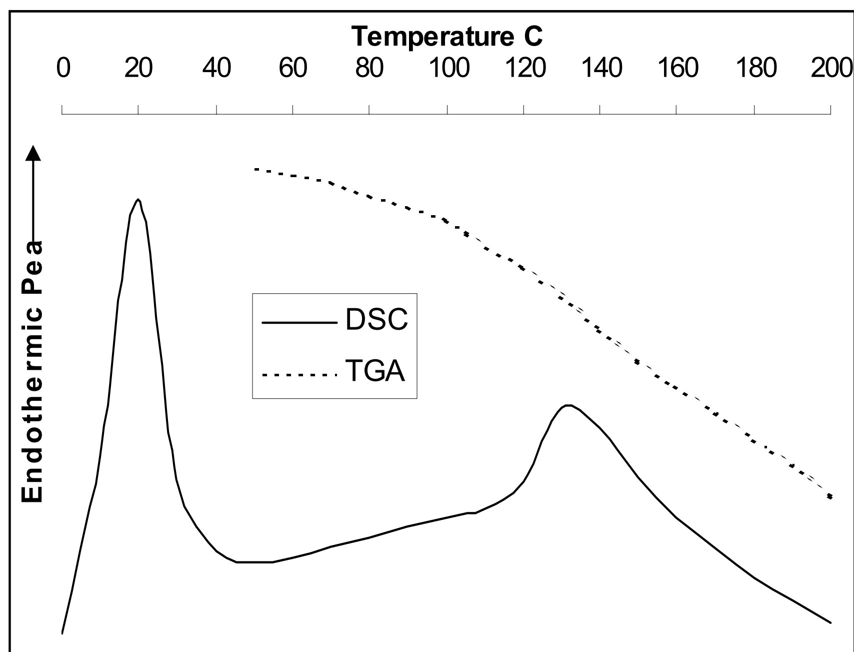


Figure 51. DSC and thermogravimetric thermograms of Formula A without metronidazole at a heating rate of 10°C/min.

المنارة للاستشارات
www.manaraa.com

From figure 50, the endothermic peak at 150°C in DSC thermogram of Formula A was due to melting of the metronidazole, which was also decreased due to effect of PG and water. The tailing in this peak was due to evaporation of water and PG as shown by the DSC and TGA therograms of Formula A placebo in figure 51.

In figure 51, another endothermic peak appeared at 130°C due to evaporation of PG and water, the tailing in this peak was due to evaporation of water which started at 50°C. This was indicated by the weight loss (TGA therogram) which began gradually above 50°C and up to 220°C.

The delay in the evaporation of water occurred because the water activity decreased due to presence of PG and involvement of water in the structure of reversed micellar and lamellar phase of GMO. The thermogravimetric analysis in figures 50.51,52 and 53 indicated the evaporation process of PG and water where the total weight loss from the samples at 210°C was ranged from 12% to 15%, that was related to the percentage of PG and water in the formula. The heating process did not dissolve metronidazole completely in the melted base and the drug was still suspended even at high temperature.

The effect of storage conditions on the thermal behavior of Formula A was shown in figures 52 and 53. The decrease in the melting point of metronidazole to about 120°C in fresh samples as shown in figures 52 and 53,might be related to the transformation of metronidazole crystals from the stable polymorphic form to the unstable one or due to solvate formation during storage at 4°C or at room temperature for three months.

The melting point of metronidazole reappeared in the range of 150-155°C as shown in figures54 and 55,when the samples firstly running from –13 to 100°C and then

المنارة للاستشارات
www.manaraa.com

rerunning from –13 to 210°C. These results confirmed the idea of formation of an unstable polymorph or solvate of metronidazole during the storage.

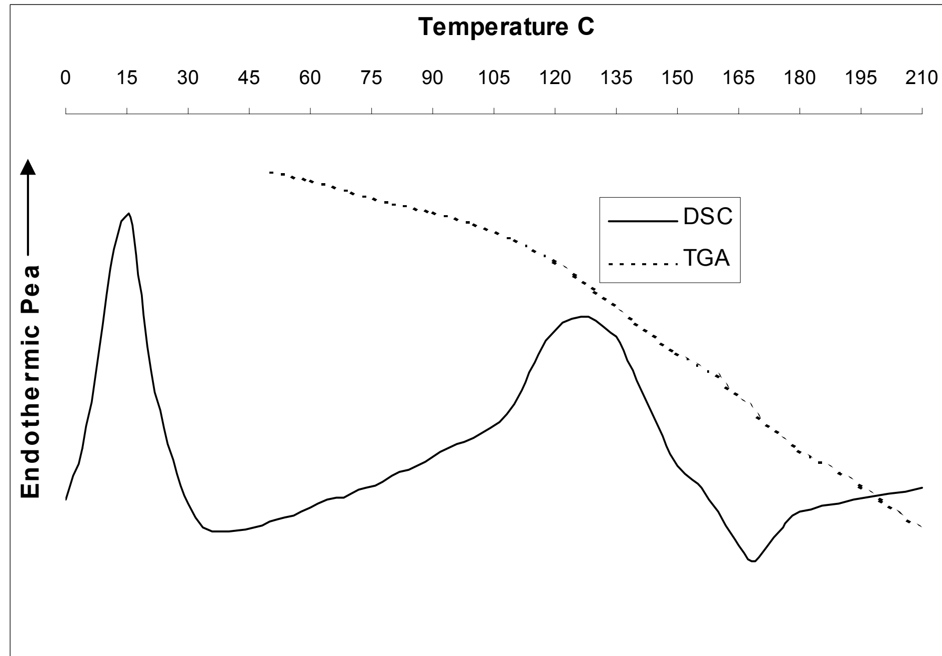


Figure 52. DSC and thermogravimetric thermograms of fresh sample of Formula A at heating rate of 10°C/min, stored at room temperature for 3 months.

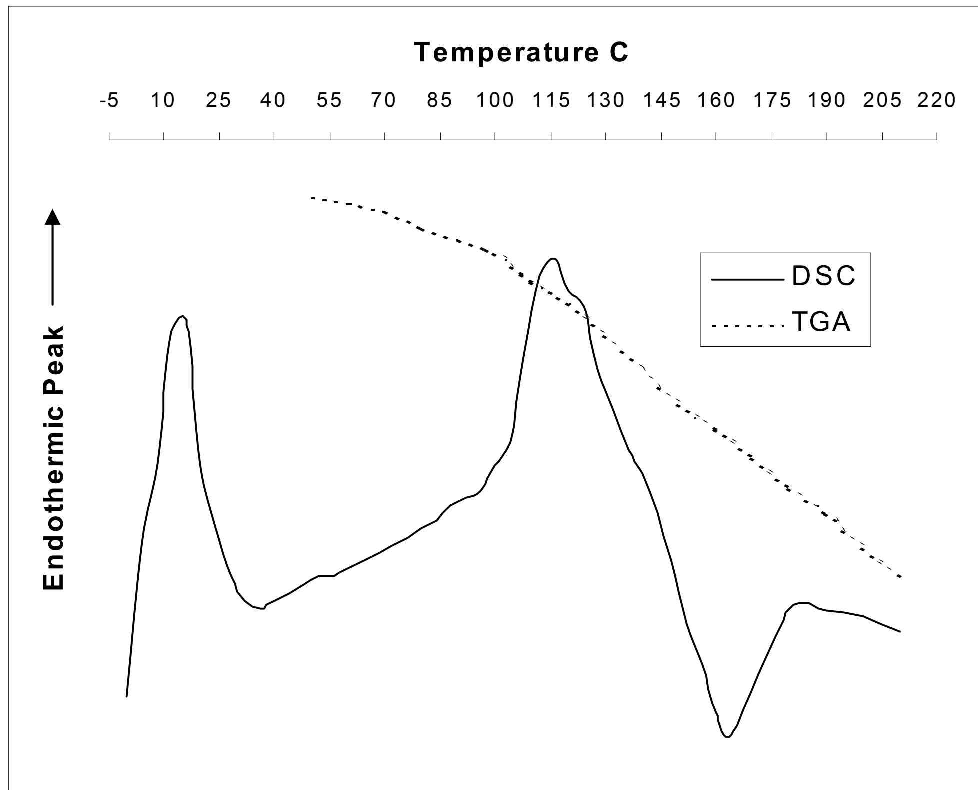


Figure 53. DSC and thermogravimetric therograms of fresh sample of Formula A at heating rate of 10°C/min., stored at 4°C for 3 months.

المنارة للاستشارات
www.manaraa.com

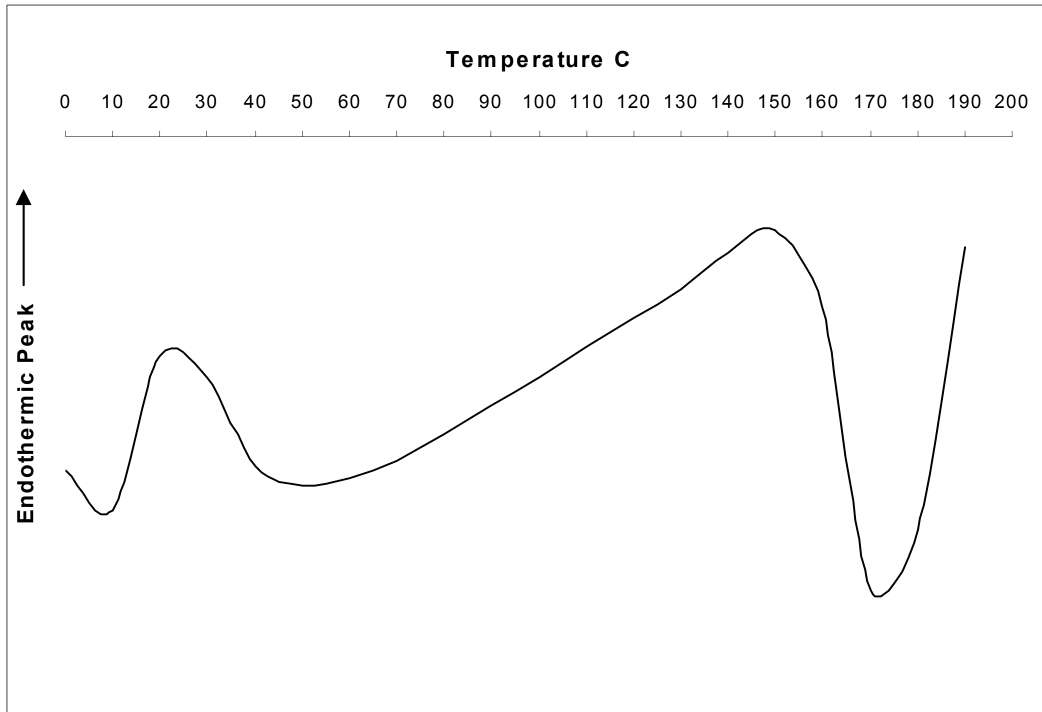


Figure 54. DSC thermogram of rerunning sample of Formula A at heating rate of 10°C/min. stored at room temperature for 3 months.

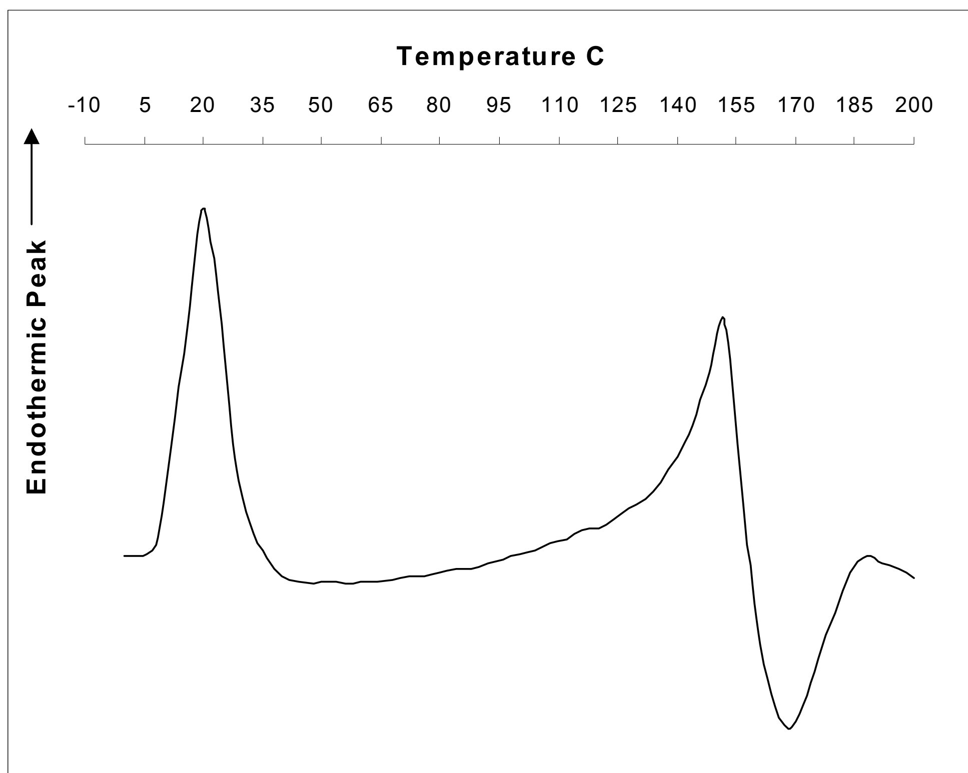


Figure 55. DSC thermogram of rerunning sample of Formula A at heating rate of 10°C/min. stored at 4°C for 3 months**.**

المنارة للاستشارات
www.manaraa.com

Also the tailing of the endothermic peak starting from 50°C was due to evaporation of water and then PG at higher temperature. The thermogravimetric analysis also confirmed the idea of evaporation process due to weight loss as previously discussed. The physical stability of metronidazole in formula A during storage needs further investigation in the future.

3.13. Chemical stability of Formula A .

The result of HPLC analysis of metronidazole in formula A which was stored in dark place at 4°C or room temperature confirmed that the metronidazole was chemically stable under such storage conditions for 3 months. The analysis was done at APM Co. Laboratories and full stability studies are required in future to assess the chemical stability of the formula.

المنارة للاستشارات

www.manaraa.com

3.14. The effect of drug loading on the phase change and release profile of metronidazole from Formula A.

The reversed micellar and lamellar phase of GMO was the same at 0%, 10% and 20% drug loading of metronidazole in Formula A. At 30% and 40% metronidazole there was no definite phase that can be recognized by the polarized microscope because of high solid contents in the formula.

The release rate constant at 10% drug loading was much higher than 20% drug loading at the beginning as shown in figure 56 and 58. The higher rate of release for 10% loading is explained by the increase in the soluble part of the drug in the formula due to increase in the ratio of the co-solvent (water and PG) relative to the drug 17:10, as shown in table 15.

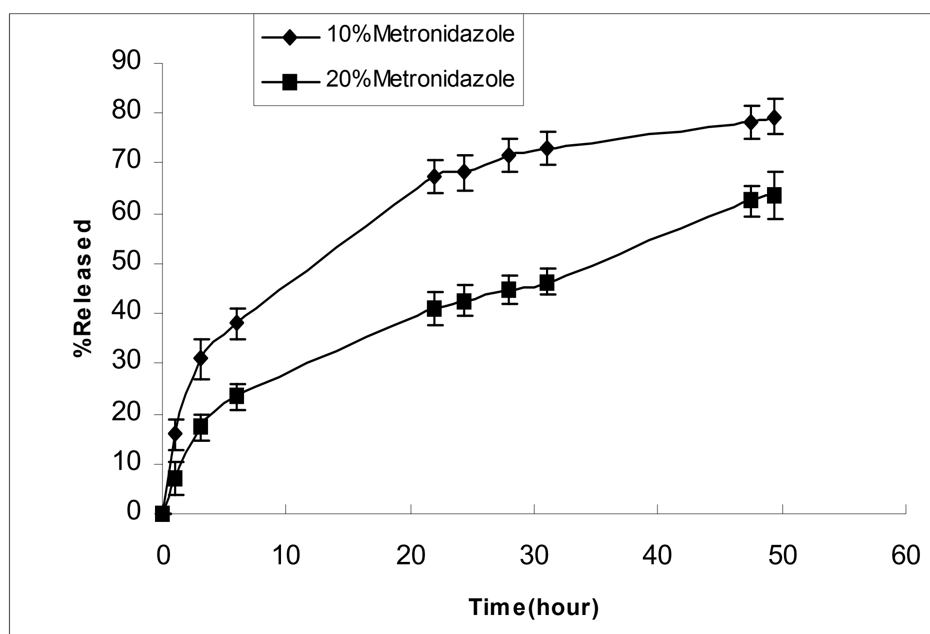


Figure 56. Release profiles of metronidazole from 10% and 20% drug loading Formula A as a function of time.

المنارة للاستشارات
www.manaraa.com

After the formation of the depleted zone that contains EC precipitated network in the cubic phase of GMO, the rate of drug release at 10% drug loading decreased and at 20% drug loading increased. The release of a suspended drug from such granular matrix involved the simultaneous penetration of surrounding liquid, dissolution of drug and diffusion of the drug through water channels of the cubic phase or pores that formed in the EC precipitated network. So the release of drug is believed to be through porous matrix rather than homogeneous matrix. The volume and length of the opening in the matrix have been accounted in the second form of the Higushi equation:

$$Q = \left[\frac{D\varepsilon}{\tau} (2A - \varepsilon C_s) C_s t \right]^{\frac{1}{2}} \text{.......... ....}(7)$$

In which ε is the porosity of the matrix and τ is the tortuosity of the capillary system.

The porosity of the depleted zone in case of 20% drug loading was larger than when the drug loading was 10%. Also the tortuosity of the system increased as the concentration of EC increased. The depleted zone in case of 10% has tortuosity higher than that of 20% drug loading. The f_2-value between 10 and 20% release profile was (34.5). Using Student T test at (P= 0.05) the rate constant of the release from 10% drug loading was found significantly higher than the rate constant of the release from 20% drug loading.

Table 15. Percentage(w/w) of PG and water in GMO 7%EC in different loading of metronidazole formulations.

Metronidazole (%)	PG (%)	Water (%)	GMO 7% EC (%)
10%	11.250%	5.625%	73.125%
20%	10.000%	5.000%	65.000%
30%	8.750%	4.375%	56.875%
40%	7.500%	3.750%	48.750%

المنارة للاستشارات

www.manaraa.com

In case of 30% and 40% drug loading as shown in figure 59, the high solid contents delayed the cubic phase formation until the suspended particles dissolved and released. Also the system contained low percent of EC, that made the depleted zone in these cases of higher porosity and lower tortuosity when compared with that formed in case of 20% drug loading and led to higher release rate of metronidazole from such systems.

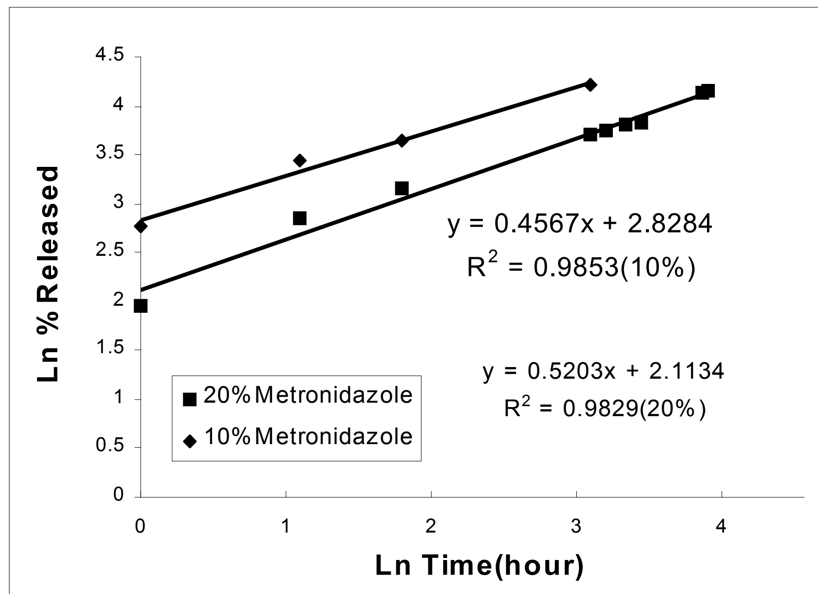


Figure 57. Fitting of dissolution profile data of metronidazole from 10% and 20% drug loading Formula A into Peppas equation.

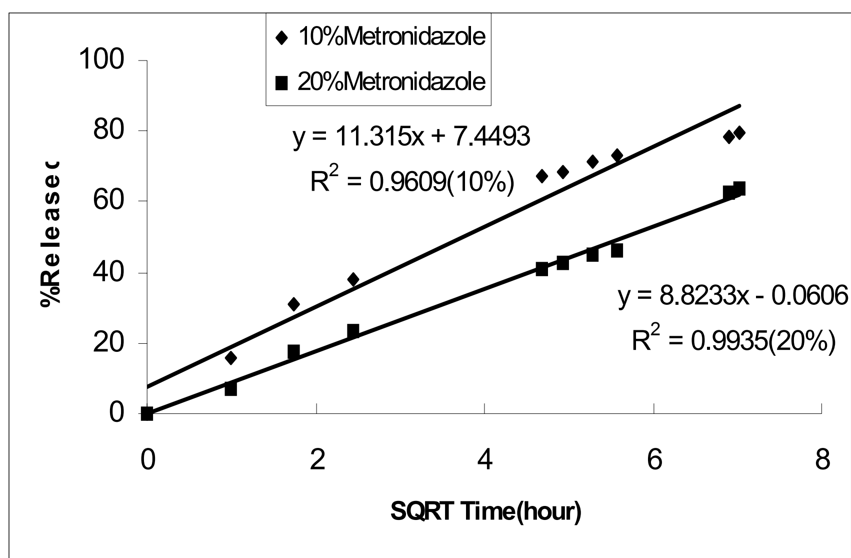


Figure 58. Release profiles of metronidazole from 10% and 20% drug loading Formula A as a function of square root of time.

المنارة للاستشارات
www.manaraa.com

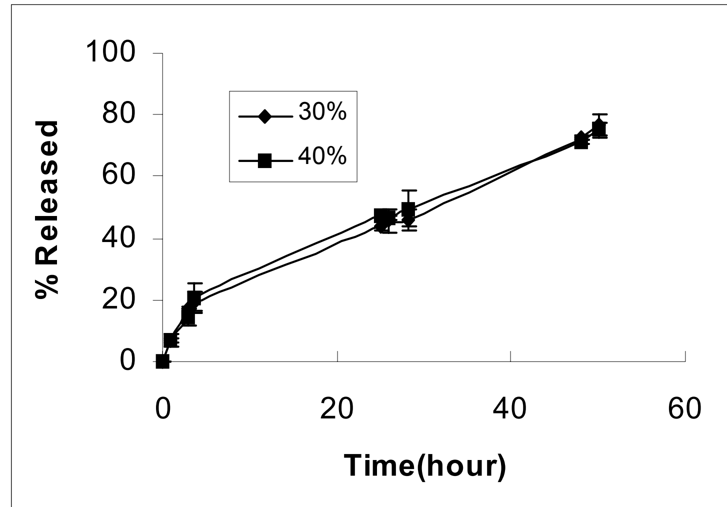


Figure 59. Release profiles of metronidazole from 30% and 40% drug loading Formula A as a function of time.

Using one-way ANOVA at (P=0.05), the rate constant at 30% and 40% was significantly different from that of 10% and 20% drug loading. However, no significant difference was observed between 30% and 40% drug loading and the f_2-value between 30 and 40% release profiles was 81.8%. From *n* values in figures 58 and 60, the value increased from 0.45 to 0.57 with increasing drug loading due to the zero order release system that formed when the drug loading ranged from 20% to 40%.

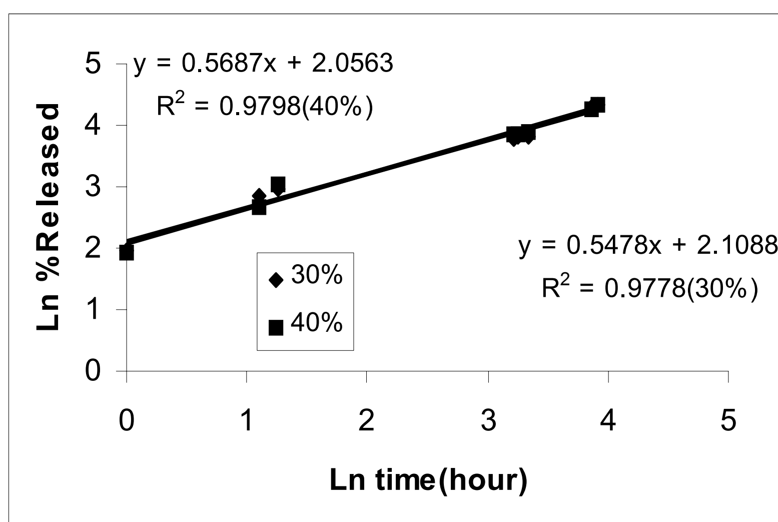


Figure 60. Fitting of dissolution profile data of metronidazole from 30% and 40% drug loading Formula A into Peppas equation.

المنارة للاستشارات
www.manaraa.com

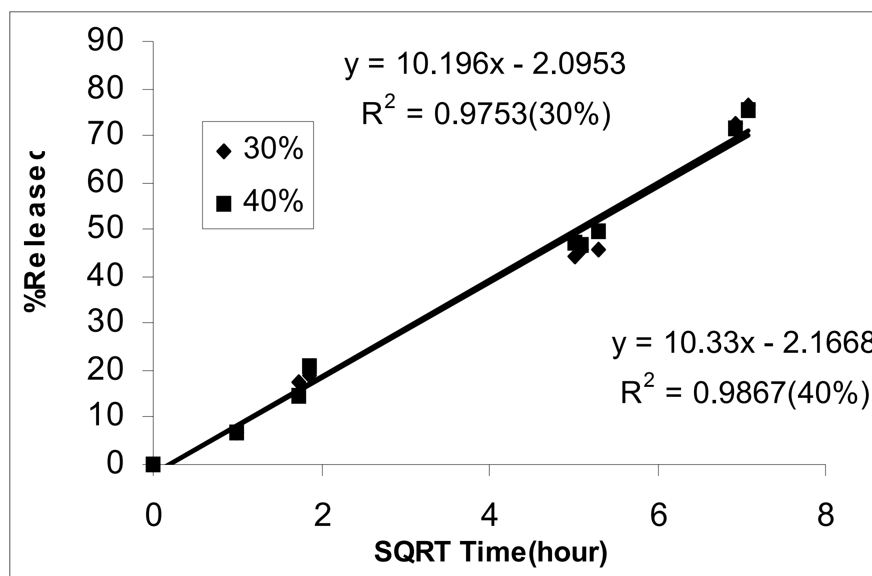


Figure 61. Release profiles of metronidazole from 30% and 40% drug loading Formula A as a function of square root of time.

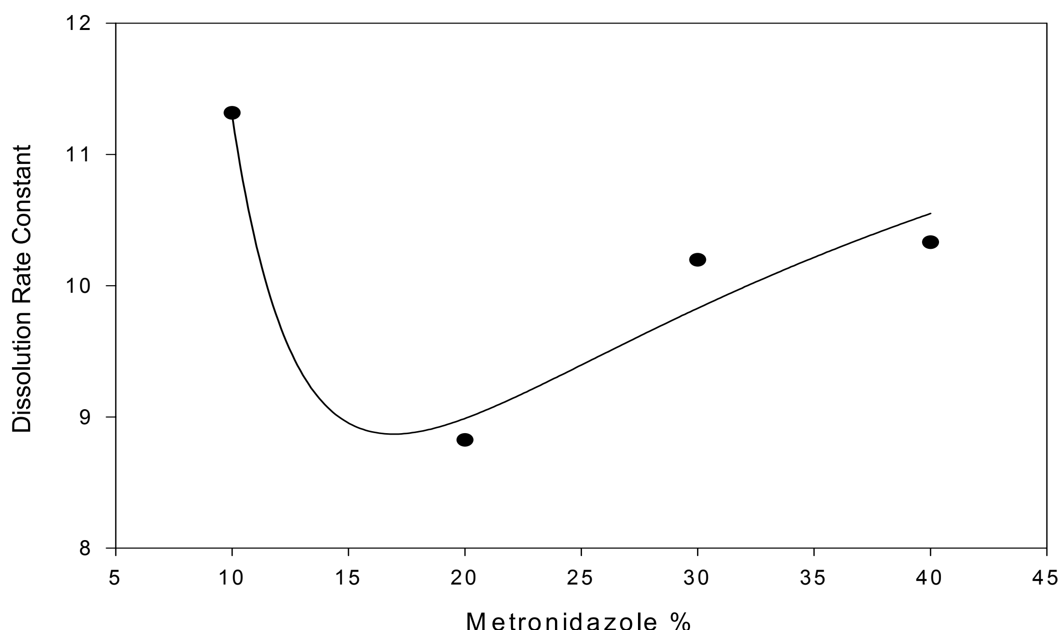


Figure 62. Effect of metronidazole concentration on the dissolution rates constant of metronidazole from Formula A.

The effect of drug loading on the dissolution rate constant of metronidazole from Formula A was represented in figure 62. From this figure, it was clear that the 20% drug loading had the lower releasing rate of metronidazole from Formula A, this result might be due to the optimum effect of co-solvent on the solubility of metronidazole and the optimum percentage of GMO and EC in the formula.

المنارة للاستشارات
www.manaraa.com

4. Conclusions and Future Work:

4.1 Conclusion:

It can be concluded from this study that a controlled drug delivery system was developed to deliver metronidazole in the periodontal pocket directly by utilizing the mucoadhesive and sustained property of the cubic phase of the GMO after addition of 7% EC to decrease the rate of release of metronidazole and addition of PG and water to form a low viscosity phase of GMO.

A formula contains 20% metronidazole, 10% propylene glycol, 5% water and 65% glycerol monooleate that contains 7% ethyl cellulose was found to have the optimum drug release property and to be easily injectable at room temperature. The formula assessment using differential scanning calorimetry indicated that it was completely melted at room temperature. The rheological behavior of the formula was pseudoplastic and the viscosity decreased as the rate of shear increased. The release rate of metronidazole decreased significantly, as the matrix thickness of the gel formed increased.

On studying the particle size analysis and the controlled release property of the formula during three months, it was concluded that the best storage condition of the formula was at 4°C. The formula was mucoadhesive when compared with GMO alone that means that the mucoadhesive property of the GMO was not affected by the incorporation of these additives and metronidazole.

There was no significant effect of the tonicity of the dissolution medium on the controlled release property of the formula. The decrease of the drug loading to 10% led to a significant increase in the rate of release of metronidazole. The rate constant of the drug release was in the following order of drug loading (10%>30 and 40%>20%).

المنارة للاستشارات
www.manaraa.com

4.2 The Future work

1- The aim of this study is the development of controlled drug delivery system for the treatment of the periodontal disease, so the future work can be focused on using the same system to deliver other drugs like antibiotics, anti-inflammatory drugs and bone morphogenic proteins that have different physicochemical properties.

2- Studying the effect of incorporation of another type of polymer having hydro- or lipophilic character on the release kinetics of metronidazole.

3- Studying the effect of EC and other polymer on the rate of biodegradation of GMO in vivo.

4-Preliminary clinical study using the developed formula on the patients and determination of the improvement of the clinical signs like pocket depth, the level of attachment and the distribution of the microbial populations of the periodontal pocket.

المنارة للاستشارات
www.manaraa.com

5. Reference

Adriana Ganem-Quintanar, David Quintanar-Guerrero and Pierre Buri (2000). Monoolein: A Review of pharmaceutical applications. **Drug Development and Industrial Pharmacy,** 26(8), 809-820.

Appel, L., Engle, K., Jensen, J., Rajewski, L. and Zentner, G. (1994). An in vitro model to mimic in vivo subcutaneous monoolein degradation. **Pharmaceutical Research**, 11,S-217.

Bollen, C. M. and Quirynen, M. (1996). Microbiological response to mechanical treatment in combination with adjunctive therapy. A review of the literature. **Journal of Periodontology**, 67, 1143-1158.

Brown, L.J., Brunelle, J. A. and Kingman, A. (1996). Periodontal status in the United States, 1988-1991:prevalence, extent and demographic variation. **Journal of Dental Research**, (75)(special issue), 672-677.

Burrows, R., Collett, J. H. and Attwood, D. (1994). Release of drugs from monoglyceride-water liquid crystalline phases**. International Journal of Pharmaceutics**, 111, 283–293.

Chang, C. M. and Bodmeier, R. (1997). Swelling of and drug release from monoglyceride-based drug delivery systems. **Journals of Pharmaceutical Science,** 86 (6), 747–752.

Chang, C. M. and Bodmeier, R. (1998). Low viscosity monoglyceride based drug delivery systems transforming into a highly viscous cubic phase. **International Journal of Pharmaceutics**, 173 (1–2), 51–60.

Chilukuri, D. M. and Shah, J. (1997). Mechanism of drug release from glyceryl monooleate cubic phase gel using bupivacaine as a model drug. **Pharmaceutical Research**, 14 (11): 532S.

Christersson, L. A., Solts, J. and Rosling, B.G. (1985). Microbiological and clinical effects of surgical treatment of the localized juvenile periodontitis**. Journal of Clinical Periodontology**, 12: 465-471.

Cimasoni, G. (1983). Crevicular fluid updated. **Monographs in Oral Science**, 12,145.

Collins, A. E. M., Deasy, P. B., Maccarthy, D. J. and Shanley, D. B. (1989). Evaluation of a controlled-release compact containing tetracycline hydrochloride bonded to tooth for the treatment of periodontal disease. **International Journal of Pharmaceutics**, 51, 103-114.

Deasy, P. B., Collins, A. E. M., Maccarthy, D. and Russell, R. J. (1989). Use of strips containing tetracycline hydrochloride or metronidazole for the treatment of advanced periodontal disease. **Journal of Pharmaceutical Pharmacology**, 41, 694-699.

المنارة للاستشارات

www.manaraa.com

Deporter, D. A., and Ten Cate A. R. (1980). Collagen resorption by periodontal ligament fibroblasts at the hard tissue-ligament interface of the mouse molar periodontium. **Journal of Periodontology**, (51), 429-435.

Dwight, E. M. (2000). A practical Approach to the diagnosis and treatment of periodontal disease. **Journal of American Dental Association (JADA).** 131, 483-491.

Engstroem, S., Lindahl, L., Wallin, R. and Engblom, J. (1992). A study of polar lipid drug carrier systems undergoing a thermoreversible lamellar-to-cubic phase transition. **International Journal of Pharmaceutics**, 86 (2–3), 137–145.

Engstrom, S. and Engstrom, L. (1992). Phase behavior of the lidocaine-monoolein-water system. **International Journal of Pharmaceutics**, 79, 113–122.

Engstrom, S., Larsson, K. and Lindman, B. (1988). Liquid Crystalline phases as delivery systems for drugs. I. Basic principles. **Proceedings of International Symptom Controlled Release Bioactive Material**, 15,105-106.

Engstrom, S., Lindahl, L., Wallins, R. and Engblom, J. (1992). A study of polar lipid drug carrier systems undergoing a thermoreversible lamellar-to-cubic phase transition. **International Journal of Pharmaceutics**, 86, 137-145.

Ericsson, B., Eriksson, P. O., Loefroth, J. E., Engstroem, S., Ferring, A. B. and Malmoe, S. (1991). Cubic phases as delivery systems for peptide drugs. (**American Chemical Society) Symptom Series. 469 (Polymeric Drugs Delivery Systems)**: 251–265.

Ericsson, B., Larsson, K. and Fontell, K. (1983). A cubic protein-monoolein-water phase. **Biochim. Biophys. Acta**, 729 (1), 23–27.

Ericsson, B., Leander, S. and Ohlin, M. (1988). Liquid crystalline phases as drug delivery systems for drugs. III: In vivo. **Proceedings of International Symptom Controlled Release Bioactive Material**, 15, 382-383.

Esposito,E., Carotta, V., Scabbia, A., Trombelli, L., D'Antona, P., Menegatti, E. and Nastruzzi, C. (1996). Comparative analysis of tetracycline-containing dental gels: poloxamer- and monoglyceride-based formulations. **International Journal of Pharmaceutics**, 142 (1), 9–23.

Garant, P. R. and Cho, M. I. (1979). Histopathogenesis of spontaneous periodontal disease in conventional rats. **Journal of Periodontal Research**, 14, 297-307.

Gates, K. A., Grad, H., Birek, P., and Lee, P. I. (1994). A new bioerodible polymer insert for the controlled release of metronidazole. **Pharmaceutical Research**, 11, 1605-1609.

Gebhard Schramm(2000).**A Practical Approach to Rheology and Rheometry**.(2nded). Gebrueder HAAKE GmbH, Karlsruhe, Federal Republic of Germany.

المنارة للاستشارات

www.manaraa.com

Geraghty, P. B., Attwood, D., Collett, J. H. and Dandiker, Y. (1996). In vitro release of some antimuscarinic drugs from monoolein/water lyotropic liquid crystalline gels. **Pharmaceutical Research**, 13, 1265–1271.

Godowski, K. C., Wolff, E. D., Thompson, D. M., Housley, A. M., Dunn, R. L., Stoller, N. H. and Southard, G. L. (1995). Whole mouth microbiota effects following subgingival delivery of sanguinarium. **Journal of Periodontology**, 66:870-877.

Goodson, J. M. (1989). Pharmacokintic principles controlling efficacy of oral therapy **Journal of Dental Research**, 68, 1625-1632.

Gray, G. (2000). Nonsurgical periodontal therapy in 2000:A literature review. **Journal of American Dental Association, (**131), 1580-1592.

Greenstien, G. (1993). The role of metronidazole in the treatment of periodontal diseases. **Journal of Periodontology**, 63, 1-7.

Grossi, S., Dunford, R.,Genco, R. J., Pihlstrom, B., Walker, C., Howell, H. and Thoroe, U.(1995). Local application of metronidazole dental gel. **Journal of Dental Research**, 74, 468-473.

Haffajee, A. D. and Socransky, S. S. (1986). Attachment level changes in destructive periodontal diseases. **Journal of Clinical Periodontology**, 13:461-472.

Harris, M. R. and Ghebre-Sellassie, I.,(1996). **Aqueous Polymeric coatings for Pharmaceutical Dosage Forms**. (2nd ed). New York: Dekker.

Hattingh, J. and Ho, E. (1980). The concentration of protein in human gingival creviecular fluid. **Journal of Periodontal Research**, 15, 90-95.

Higashi, K., Matsushita, M., Morisaki, K., Hayashi, S. I. and Mayumi, T. (1991). Local drug delivery systems for the treatment of periodontal disease. **Journal of Pharmacobiological Dynamics**, 14, 72-81.

Hoepelman, I. M. and Schneider, M. M. E. (1995). Azithromycin: The first of tissue selective azalides. **International Journal of Antimicrobial Agents**, 5:45-53.

Jaime, N. D. and Willium, A. R.,(1998). **Textbook of Organic Medicinal and Pharmaceutical Chemistry.** (10th ed). New York: Lippincott-Raven.

Jones, D. S., Woolfson, A D., Brown, A. F. and O'Neill, M. J. (1997). Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: in vitro release kinetics, syringeability, mechanical and mucoadhesive properties. **Journal of Controlled Release**, 49, 71-79.

Jones, D. S., Woolfson, A. D., Djokic, J. and Coulter, W. A. (1996). Development and mechanical characterization of bioadhesive semi-solid, polymeric systems containing tetracycline for the treatment of periodontal diseases. **Pharmaceutical Research**, 13: 1734-1738.

المنارة للاستشارات

www.manaraa.com

Jorgensen, M. G. and Solts, J. (2000). Practical antimicrobial periodontal therapy. **Compendium of Continuous Education in Dentistry**, 21, 111-118.

Kararli, T. T., Needham, T. E., Griffin, M., Schoenhard, L., Ferro, L. J. and Alcorn, L. (1992). Oral delivery of a renin inhibitor compound using emulsion formulations, **Pharmaceutical Research**, 9, 888-893.

Kimura, S., Toda, H., Shimabukuro, Y., Kitamura, M., Fujimoto, N., Miki, Y. and Okada, H. (1991). Topical chemotherapy in human periodontitis using a new controlled release insert containing ofloxacin I. Microbiological observation. **Journal of Periodontal Research**, 26:33-41.

Kornman, K. S. (1993). Controlled releases local delivery antimicrobials in periodontics. Prospects for the future. **Journal of Periodontology**, 64, 782-791.

Larsen, T. (1990). In vitro release of doxycycline from bioabsorbable materials and acrylic strips. **Journal of Periodontololgy**, 61:30-34.

Larsson, K. (1989). Cubic lipid-water phases: structures and biomembrane aspects, **Journal of Physical Chemistry**, 93(21), 7301–7314.

Lie, T., Bruun, G. and Boos, O.E. (1998). Effects of topical metronidazole and tetracycline in treatment of adult periodontitis. **Journal of Periodontology**, 69,819-827.

Lindell, K., Engblom, J., Jonstroemer, M., Carlsson, A., and Engstroem, S. (1998). Influence of a charged phospholipid on the release pattern of timolol maleate from cubic liquid crystalline phases. **Progress in Colloidal and Polymeric Science** , 108, 111–118.

Loesche, W. J., Giordano, J., Soehren, S., Hutchinson, R., Rau, C. F., Walsh, L., Schork, A., Arbor, A. and Mich, D. (1996). Nonsurgical treatment of patients with periodontal disease. **Oral Surgery & Oral Medicine Oral Pathology & Endodontices**, 81, 533-543.

Loos, B., Hutter, J. and Varoufaki, H. (1998). Levels of C-reactive protein in periodontitis patients and healthy controls. **Journal of Dental Research**, (77), 666-672.

Luner, P. E., Buba, S. R. and Radebaugh, G. W. (1994). The effects of bile salts and lipids on the physicochemical behavior of gemfibrozil. **Pharmaceutical Research**, 11, 1755-1760.

Lyons, R. T. (1996). Formulation development of an injectable oil-in-water emulsion containing the lipophilic antioxidant (alpha)-tocopherol and β-carotene, **American Association of Pharmaceutical Scientist. Annual Meeting**, 226.

Marsh, P. D. (1989). Host defences and microbial homeostasis: role of microbial interactions. **Journal of Dental Research**, (68), 1567-1575.

Martin, A.(1993) **Physical Pharmacy**(4th ed).Philadelphia: Lea and Febiger.

المنارة للاستشارات

www.manaraa.com

Mathiowitz, E. (1999). **Encyclopedia of Controlled Drug Delivery**, (1st ed). John Wiley and Sons,Inc.

McMillan, L., Burrill, D. Y. and Fosdick, L.S. (1958). An electron microscope study of particulates in periodontal exudate. **Journal of Dental Research**, 37, 51-58.

Mills, W. H., Thompson, G. W. and Beagrie, G. S. (1979). Clinical evaluation of spiramycin and erythromycin in control of periodontal disease. **Journal of Clinical Periodontology**. 6,308-312.

Minabe, M., Uematsu, A., Nishijima, K., Tomomatsu, E., Tamura, T., Hori, T., Umemoto, T. and Hino, T. (1989). Application of a local drug delivery system to periodontal therapy. I. Development of collagen preparations with immobilized tetracycline**. Journal of Periodontololgy**, 60:113-117.

Muranushi, N., Nakajima, Y., Kinugawa, M., Muranishi, S. and Sezaki, H. (1980). Mechanism for inducement of intestinal absorption of poorly absorbed drug by mixed micelles.II.Effecct of the incorporation of various lipids on the permeability of liposomal membranes. **International Journal of Pharmaceutics**, 4, 281-290. .

Nakagawa, T., Yamada, S., Oosuka, Y., Saito, A., Hosaka, Y., Ishikawa, T. and Okuda, K. (1991). Clinical and microbiological study of local minocycline delivery (PERIOCLINER) following scaling and root planing in recurrent periodontal pockets. **Bulletin of Tokyo Dental Collections**, 32, 63-70.

Nielsen, L. S., Schubert, L. and Hansen, J. (1998). Bioadhesive drug delivery systems characterization of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinoleate. **European Journal of Pharmaceutical Science**, 6 (3), 231–239.

Norling, T., Landing, P., Engstrom, S., Larsson, K. Krog, N. and Nissen, S. S. (1992). Formulation of a drug delivery system based on a mixture of monoglycerides and triglycerides for use in the treatment of periodontal disease. **Journal of Clinical Periodontology**, 19, 687–692.

Noyan, U., Yilmaz, S. Kuru, B., Kadir, T., Acar, O. and Buget, E. (1997). A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult periodontitis patients**. Journal of Clinical Periodontology**, 24:158-165.

Palmer, R.M., Matthews, J. P. and Wilson, R. F.(1998). Adjunctive systemic and locally delivered metronidazole in the treatment of periontitis. **British Dental Journal**. 184, 548-552.

Paquette, D. W., Waters, G. S., Stefanidou, V. L., Lawrence, H. P., Friden, P. M., O'Connor, S. M., Sperati, J. D., Oppenheim, F. G., Hutchens, L. H. and Williams, R.C. (1997). Inhibition of experimental gingivitis in beagle dogs with topical salivary histatins. **Journal of Clinical Periodontology**, 24, 216-222.

Park, E. S., Maniar, M. and Shah, J. (1998). Biodegradable polyanhydride devices of cefazolin sodium, bupivacaine, and taxol for site-specific drug delivery: preparation,

المنارة للاستشارات

www.manaraa.com

and kinetics and mechanism of in vitro release**. Journal of Controlled Release**, 52 (1,2), 179–189.

Peppas, N. A. and Buri, P. A. (1985). Surface, interfacial and molecular aspects of polymer bioadhesion of soft tissues. **Journal of Controlled Release**, 2, 257-275.

Polson, A. M., Garrett, S., Stoller, N. H., Bandt, C. L., Hanes, P. J., Killoy, W. J., Southard, G. L,. Duke, S. P., Bogle, G. C., Drisko, C. H. and Friesen, L. R. (1997). Multi-center comparative evaluation of subgingivally delivered sanguinarine and doxycycline in the treatment of periodontitis, II. Clinical results. **Journal of Periodontololgy**, 68, 119-126.

Polson, A. M., Southard, G. L., Dunn, R. L., Yewey, G. L., Godowski, K. C., Polson, A. P., Fulfs, J. C. and Laster, L. (1996). Periodontal pocket treatment in beagle dogs using subgingival doxycycline from a biodegradable system. I. Initial clinical responses, **Journal of Periodontololgy**, 67:1176-1184.

Price, R. and Patchan, M. (1991). Controlled release from cylindrical microstructures. **Journal of Microencapsulation**, 8, 301-306.

Quirynen, M., Gazani, S. and Mongradini, C. (1999). The effect of periodontal therapy on the number of cariogenic bacteria in different intreoral niches**. Journal of Clinical Periodontology**, (26), 322-327.

Rams, T. E. and Solts, J. (1992). Antibiotics in periodontal therapy: An update. **Compendial Continuous Education in Dentistry**, 13:113-140.

Rams, T. E., Solts, J. and Feik, D. (1992). Ciprofloxacin/metronidazole treatment of recurrent adult periodontitis. **Journal of Dental Research**, 71:319-329.

Rapley J. W., Cobb C. M., Killoy W. J. and Williams D. R. (1992). Serum levels of tetracyclines during treatment with tetracyclines containing fibers. **Journal of Periodontology**, 63, 817-822.

Rekhi, G. S. and Jambhekar, S. S. (1995). A review of ethyle cellulose. **Drug Development and Industrial Pharmacy**, 21(1), 61-77.

Ritger, P. L. and Peppas, N. A. (1987). A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the forms of slabs, spheres, cylinders or discs. **Journal of Controlled Release**, 5, 23-26.

Roskos, K. V., Fritzinger, B. K., Rao, S. S., Armitage, G. C. and Heller, J. (1995). Development of a drug delivery system for the treatment of periodontal disease based on bioerodible poly (ortho esters). **Biomaterials**, 16, 313-317.

Sadhale, Y. and Shah, J. C. (1998). Glyceryl monooleate cubic phase gel as chemical stability enhancer of cefazolin and cefuroxime. **Pharmaceutical Development and Technology**, 3 (4), 549–556.

المنارة للاستشارات

www.manaraa.com

Sadhale, Y. and Shah, J. C. (1999). Biological activity of insulin in GMO gels and the effect of agitation. **International Journal of Pharmaceutics**, 191 (1), 65–74.

Sadhale, Y. D. and Shah, J. (1995). Glyceryl monooleate (GMO)-water cubic phase gel as a physical stability enhancer of insulin. **American Association of Pharmaceutical Scientist, Annual Meeting, 234**.

Sauvetre, E., Glupczynsky, Y., Yourassowsky, E. and Pourtois, M. (1993). The effect of clindamycin gel insert in periodontal pockets, as observed on smears and cultures. **Infection**, 21:245-247.

Scannapieco, F.A. (1999). Role of oral bacteria in respiratory infection. **Journal of Periodontology**, (70), 793-802.

Schwach-Abdellaouia, K., Vivien-Castionib, N. and Gurnya, R. (2000). Local delivery of antimicrobial agents for the treatment of periodontal diseases. **European Journal of Pharmaceutics and Biopharmaceutics.** 50, 83-99.

Shah, J. and Maniar, M. (1993). pH dependent solubility and dissolution of bupivacaine and its relevance to the formulation of a controlled release system. **Journal of Controlled Release**, 23, 261–270.

Slots, J. and Jorgensen, M.J. (2000). Efficient antimicrobial treatment in periodontal maintenance care. **Journal of American Dental Association (JADA)**. (131), 1293-1304.

Solts, J. and Rams, T.E. (1990). Antibiotics and periodontal therapy: Advantages and disadvantages. **Journal of Clinical Periodontology**, 17 , 479-485.

Steinberg, D., Friedman, M., Soskolne, A. and Sela, M. N. (1990). A new degradable controlled release device for treatment of periodontal disease. In vitro release study. **Journal of Periodontololgy**, 61, 393-398.

Stoltze, K. (1995). Elimination of Elyzol 25% dental gel matrix from periodontal pockets. **Journal of Clinical Periodontology.** 22, 185-188.

Stoltze, K. and Stellfeld, M. (1992). Systemic absorption of metronidazole after application of a metronidazole 25% dental gel. **Journal of Clinical Periodontology**, 19, 693-701.

Stoltze, K.(1992). Concentration of metronidazole in periodontal pockets after application of a metronidazole 25% dental gel. **Journal of Clinical Periodontology.** 19, 698-701.

Swai, H. S., Patel, M. P. and Braden, M. (1997). Release of chlorhexidine from methacrylate polymers. **Proceedings of International Symptom Controlled Release Bioactive Material**, 24:1025-1026.

المنارة للاستشارات

www.manaraa.com

Takada, T. and Donth, K. (1988). The mechanism of pocket formation. A light microscope study of undecalcified human material. **Journal of Periodontology**, (95), 215-220.

Ten Cate, A. R. Jr. (1994): **Fibroblast and their products In Oral Histology-Development, Structure and Function**, (4th ed.). St Louis: Mosby.

The Pharmaceutical CODEX (1994): Principles and Practice of Pharmaceutics. (12thed). New York:The Pharmaceutical Press.
Tonetti, M., Cugini, M. A. and Goodson, J. M. (1990). Zero-order delivery with periodontal placement of tetracycline-loaded ethylene vinyl acetate fibers. **Journal of Periodontology Research**, 25, 243-249.

Van Wachem, P. B., Van Luyn, M. J. A., Nieuwenhuis, P., Koerten, H. K., Olde Damink, L., Ten Hoopen, H. and Feijen, J. (1991). In vivo degradation of processed dermal sheep collagen evaluated with transmission electron microscopy. **Biomaterials**, 12, 215-223.

Vandekerckhove, B. N. A., Quirynen, M. and VanSteenberghe, D. (1997). The use of tetracycline containing controlled release fibers in the treatment of refractory periodontitis. **Journal of Periodontololgy**, 68:353-361.

Vasavada, R. C. and Junnarkar, G. H. (1997). Release of metronidazole from poly (ortho ester) matrices**. Proceedings of International Symptom Controlled Release Bioactive Material**.,24, 499-500.

Wallins, R., Engstrom, S. and Mandineus C. F. (1993). Stabilization of glucose oxidase by entrapment in a cubic liquid crystalline phase. **Biocatalysis**, 8, 73-80.

Wayne, D. B., Trajtenberg, C. P. and Hyman D. (2001). Tooth and Periodontal Disease: A Review for the primary care physician. **Southern Medical Journal**, 94 (9), 925-932.

Webber, W. L., Mathiowitz, E. (1997). Modulating tetracycline release from PLA/PG films. **Proceedings of International Symptom Controlled Release Bioactive Material**. , 24, 575-576.

Wong, C. F.,Yuen, K. H. and Peh, K. K. (1999) An in-vitro method for buccal adhesion studies: importance of instrument variables. **International Journal of Pharmaceutics**, 180,47–57.

Wyatt, D. and Dorschel, D. (1992). A cubic-phase delivery system composed of glyceryl monooleate and water for sustained release of water-soluble drugs. **Pharmaceutical Technology**, 16 (10), 116–130.

المنارة للاستشارات
www.manaraa.com

المنارة للاستشارات
www.manaraa.com

المنارة للاستشارات
www.manaraa.com