

**MICROENCAPSULATION AND APPLICATION OF  
*CATHA EDULIS* EXTRACT ON SEXUAL BEHAVIOUR,  
BODY WEIGHT AND BLOOD LIPID PROFILES IN RATS**

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LIPID PROFILES IN RATS**

**By**

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## LIST OF ABBREVIATION & SYMBOLS

SPSS	Statistical procedures for social science
ANOVA	Analysis of variance
Tukey-HSD	Tukey Honestly Significant Difference
CV	Coefficient of variation
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
SD	Standard deviation
UV	Ultra violet
°C	Degree centigrade
%	Percent
μ	Micro
mg	Milligram
kg	Kilogram
mg/mL	Milligram per millilitre
μg	Microgram
μL	Microlitter
μm	Micrometer
cm	Centimetre
Co.	Company
GC	Gas chromatography
GC-MS	Gas chromatography-Mass Spectrometry
L%	Loading percent
EE%	Entrapment efficiency percent
Y%	Yield percent
SEM	Scanning electron microscope
UK	United Kingdom
USA	United State of America
T50%	Time for 50% of drug release
v/v	Volume in volume
w/w	Weight in weight
o/w	Oil in water
h	Hour

CU	Curcuminoid Powder
CUGM	Curcuminoid-gelatin microparticles
CUOGM134	Curcuminoid-ovalbumin-gelatin microcapsules at 1:3:4
UNODC	United Nations Office on Drug and Crime
WHO	World Health Organization
PE	Pseudoephedrine HCl powder
PEGM	Pseudoephedrine-gelatin microcapsules
PEOGM112	Pseudoephedrine-ovalbumin-gelatin microcapsules at 1:1:2
VP	Verapamil HCl powder
VPGM	Verapamil-gelatin microcapsules
VPOGM112	Verapamil-ovalbumin-gelatin microcapsules at 1:1:2
PP	Propranolol HCl powder
PPGM	Propranolol-gelatin microcapsules
PPOGM112	Propranolol-ovalbumin-gelatin microcapsules at 1:1:2
PC	Paracetamol powder
PCGM	Paracetamol-gelatin microcapsules
PCOGM112	Paracetamol-ovalbumin-gelatin microcapsules at 2:3:5
PCPE	Paracetamol-pseudoephedrine powder
PCPEGM	Paracetamol-pseudoephedrine-gelatin microparticles
PCPEOGM235	Paracetamol-pseudoephedrine-Ovalbumin-gelatin microparticles at 2:3:5
KE	Khat extract
KGM	Khat-gelatin microcapsules
KOGM235	Khat-ovalbumin-gelatin microcapsules at 2:3:5
OralKOGM235	Oral Khat-ovalbumin-gelatin microcapsules 2:3:5
SQInjKOGM235	Subcutaneous injection of Khat-ovalbumin-gelatin microcapsules 2:3:5
GE	Garlic extract
KG	Khat-garlic

## LIST OF PUBLICATIONS

### International Journals:

- 1 **Aziz HA**, Peh KK, Tan YT. (2007). Solubility of core materials in aqueous polymeric solution effect on microencapsulation. *Drug Development and Industrial Pharmacy*. **33** (11), 1263-1272.
- 2 **Aziz HA**, Peh KK, Tan YT. (2009). Extraction and microencapsulation of khat: effects on sexual motivation and estradiol level in female rats. *Journal of Sexual Medicine*. **6** (3), 682-695.
- 3 **Aziz HA**, Tan YT, Peh KK, Yam MF (2010). Direct effect of khat and garlic extracts on blood lipids contents: Preliminary *in-vitro* study. *Obesity Research & Clinical Practice*. **4** (4), e247-e252.
- 4 **Aziz HA**, Peh KK, Tan YT (2011). Effects of repeated and continuous administration of encapsulated khat-extracts on body weight of rats. *Obesity Research & Clinical Practice*. doi:10.1016/j.orcp.2011.03.008

### National Journals:

1. **Aziz HA**, Peh KK, Tan YTF (2009). Anti-obesity effect of khat in Sprague Dawley rats. *Malaysian Journal of Pharmacy*, 1(7), S25.
2. **Aziz HA**, Peh KK, Tan YT (2009). Solubility of core materials in aqueous polymeric solution and ovalbumin effect on microencapsulation of khat (*Catha edulis*) extract. Proceeding of symposium of USM Fellowship Holder. IPS, USM.
3. Ang LF, Peh KK, Tan YTF, Mallikarjun CM, **Aziz HA** (2009). Enhancement of solubility of Quercetin and Rutin via micro-emulsion drug delivery system. *Malaysian Journal of Pharmacy*, 1(7), S43.
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- 1 **H.A. Aziz**, K.K. Peh and Y.T.F. Tan. Effect of khat extract on the sexual motivation of SD female rats. Proceeding of GEA-NUS PPRL 10<sup>th</sup> Anniversary Commemorative Pharmaceutical Technology Conference, **Singapore**, Dec, **2007**, 175-182. **Poster Presentation.**
- 2 YTF Tan, K.K. Peh and **H.A. Aziz**, Microencapsulation and evaluation: Effect of core material in coacervating solvent and/or aqueous polymeric solution. Proceeding of GEA-NUS PPRL 10<sup>th</sup> Anniversary Commemorative Pharmaceutical Technology Conference, **Singapore**, Dec, **2007**, 140-142. **Oral Presentation.**

### National Conferences:

- 1 **Aziz HA**, Peh KK, Tan YTF. (2008). The protective effect of ovalbumin in microencapsulation of water soluble drugs using a simple coacervation method. Asian Scientific Conference in Pharmaceutical Technology **2008**. Current Trend in Pharmaceutical Technology. 1-3 June 2008. School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia. **Oral Presentation.**
- 2 **Aziz HA**, Peh KK, Tan YTF. (2009). Anti-obesity effect of khat in Sprague Dawley rats. 4<sup>th</sup> Asian Association of Schools of Pharmacy – 9<sup>th</sup> Malaysian Pharmaceutical Society Pharmacy Scientific Conference 2009 (AASP-MPSPSC 2009). 10-13 June **2009**, Penang, Malaysia. School of Pharmaceutical Sciences, Universiti Sains Malaysia. **Oral Presentation.**
- 3 **Aziz HA**, Peh KK, Tan YTF. (2009). Solubility of core materials in aqueous polymeric solution and ovalbumin effect on microencapsulation of khat (*Catha edulis*) extract. Proceeding of symposium of USM Fellowship Holder. IPS, USM. **Oral Presentation.**

- 4 Ang LF, Peh KK, Tan YTF, Mallikarjun CM, **Aziz HA. (2009).** Enhancement of solubility of Quercetin and Rutin via micro-emulsion drug delivery system. 4<sup>th</sup> Asian association of Schools of Pharmacy – 9<sup>th</sup> Malaysian Pharmaceutical Society Pharmacy Scientific Conference 2009 (AASP-MPSPSC 2009). 10-13 June 2009, Penang, Malaysia. School of Pharmaceutical Sciences, Universiti Sains Malaysia. **Oral Presentation.**

5 **PHARMACEUTICAL TECHNOLOGY SYMPOSIUM**

Oral Presentation on Microencapsulation of Drugs with Different Solubility and Plant Extracts by Hesham Abdul Aziz. One Day Symposium in Pharmaceutical Technology 2009, 24<sup>th</sup> March 2009, Venue: Meeting Room, School of Pharmaceutical Sciences, USM.

6 **PRE-VIVA PRESENTATION OF THESIS**

Microencapsulation and application of *Catha edulis* extract on sexual behaviour, body weight and blood lipid profiles in rats by Hesham Hasan Hussein Abdul Aziz. 17<sup>th</sup> April 2011. Venue: Meeting Room, School of Pharmaceutical Sciences, USM.

**MIKROENKAPSULASI DAN PENGGUNAAN EKSTRAK *CATHA EDULIS*  
TERHADAP TINGKAHLAKU SEKSUAL, BERAT BADAN DAN PROFIL  
LIPID DARAH DALAM TIKUS**

**ABSTRAK**

*Catha edulis* (CE) yang biasa dikenali sebagai khat telah dilaporkan dapat meningkatkan motivasi seksual dan mempunyai kesan anti-obesiti. Khat secara tradisional diambil melalui pengunyahan daunnya diikuti dengan penelanan perlahan-lahan jus untuk kesan yang dikehendaki. Kajian ini bertujuan untuk meniru pelepasan bertahan kandungan khat melalui penyediaan mikrokapsul pelepasan bertahan ekstrak khat. Kesan ekstrak khat dan mikrokapsul pelepasan bertahan terhadap motivasi seksual, rembesan vagina, paras estradiol, pengambilan makanan, berat badan, paras kolesterol dan trigliserida pada tikus telah diperiksa. Gelatin digunakan untuk menyediakan mikrokapsul tetapi ia tidak dapat enkapsulat sebatian teras yang larut air, seperti pseudoefedrin HCl, verapamil HCl, propranolol HCl dan parasetamol serta campuran parasetamol dan pseudoefedrin HCl. Dengan itu, ovalbumin dimasukkan untuk sebatian teras yang larut air. Ovalbumin dapat bertindak sebagai lapisan pelindung di antara teras sebatian dan salutan gelatin, menghalang partisi teras sebatian larut air keluar dari mikrokapsul. Di samping itu, salutan ovalbumin-gelatin dapat melambatkan pelepasan drug. Ovalbumin-gelatin seterusnya digunakan untuk menyediakan mikrokapsul ekstrak khat. Mikrokapsul khat-ovalbumin-gelatin meningkatkan motivasi seksual, estradiol, dan rembesan vagina tikus betina. Pengambilan oral ekstrak khat didapati mengurangkan berat badan, pengambilan makanan, paras kolesterol dan trigliserida darah tikus, dan



kesannya dapat bertahan selama satu minggu. Apabila ekstrak khat diformulasi sebagai mikrokapsul, kesan dapat dipanjangkan ke tiga minggu. Kesan itu berlarutan sehingga 8 minggu tanpa pemerhatian “rebound”, apabila mikrokapsul diberikan sebagai suntikan subkutaneus. Memperlambatkan pelepasan ekstrak khat berkorelasi secara langsung dengan pengurangan berat badan, paras kolesterol dan trigliserida darah. Suntikan subkutaneus lebih berkesan daripada pemberian oral untuk ekstrak khat. Kesan ekstrak khat dalam mengurangkan paras kolesterol dan trigliserida darah, ditingkatkan lagi apabila digabungkan dengan ekstrak bawang putih. Kesan ini mungkin disebabkan oleh sifat pengemulsian ekstrak garlic dan aktiviti lipolisis ekstrak khat. Secara kesimpulan, pelepasan bertahan mikroenkapsulasi ekstrak khat mungkin kaedah yang berkesan untuk merawat keinginan seksual hipoaktif dalam perempuan dan mengurangkan berat badan dan profil lipid manusia.

**MICROENCAPSULATION AND APPLICATION OF *CATHA EDULIS*  
EXTRACT ON SEXUAL BEHAVIOUR, BODY WEIGHT AND BLOOD  
LIPID PROFILES IN RATS**

**ABSTRACT**

*Catha edulis* (CE) which is commonly known as khat has been reported to improve sexual motivation and has anti-obesity property. Khat is traditionally consumed via chewing of the leaf followed by slowly swallowing the juices for the desired effect. This study aimed to simulate the sustained release of khat content through the preparation of sustained release microcapsules of khat extract. The effect of khat extract and the sustained release microcapsules on the sexual motivation, vaginal secretions, estradiol levels, food intake, body weight, blood cholesterol and triglycerides levels was examined in rats. Gelatin was used to prepare the microcapsules but it was not able to encapsulate water soluble core compounds, such as pseudoephedrine HCl, verapamil HCl, propranolol HCl and paracetamol as well as a mixture of paracetamol and pseudoephedrine HCl. In this regard, ovalbumin was incorporated for soluble core compounds. Ovalbumin could act as a protective layer between the core material and the gelatin coating, preventing the partitioning of the soluble core compound out of the microcapsules. Moreover, the ovalbumin-gelatin coat could sustain the release of drug. The ovalbumin-gelatin was subsequently used to prepare khat extract microcapsules. The khat-ovalbumin-gelatin microcapsules enhanced sexual motivation, up-regulated estradiol, and increased vaginal secretions in female rats. Oral administration of khat extract was found to reduce the body weight, food intake, blood cholesterol and triglyceride levels of rats, and the effect

could last for one week. When khat extract was formulated as microcapsules, the effect was extended to three weeks. The effect was extended further to 8 weeks without observation of rebound, when the microcapsules were given as subcutaneous injection. Sustaining the release of khat extract correlated directly with the reduction in body weight, blood cholesterol and triglyceride levels. The subcutaneous injection was more effective than the oral route for khat extract administration. The effect of khat extract in reducing blood cholesterol and triglyceride levels was further enhanced when combined with garlic extract. The mechanism could be attributed to the emulsifying property of garlic extract and the lipolysis activity of khat extract. In conclusion, control release of the microencapsulated khat extract might be an effective means for treating hypoactive sexual desire of females and reducing the body weight and lipid profiles in human.

## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### 1.1 BACKGROUND

Sexual health is important to overall good health and well-being (Mulhall et al., 2008). Recently, female sexual dysfunction has attracted much interest in the field of medical sciences (Clayton, 2007; Aslan et al., 2008; Both et al., 2008a; Both et al., 2008b; Derogatis and Burnett, 2008; Garcia et al., 2008; Harte and Meston, 2008; Hayes et al., 2008; Laan et al., 2008). Female sexual dysfunction, which consists of multiple disorders, are categorized as hypoactive sexual desire disorder, orgasmic disorder, sexual arousal disorder, and sexual pain disorder (Berman et al., 1999; Goldstein et al., 2000; Traish et al., 2004; Clayton, 2007; Aslan et al., 2008; Beharry et al., 2008; Derogatis and Burnett, 2008). Female sexual arousal disorder refers to a lack of responsiveness to sexual stimulation in women (Wincze and Carey, 2001).

Obesity and overweight are the most important risk factor contributing to the overall burden of disease worldwide (Flint et al., 2010; Khan et al., 2010; Sato et al., 2010). The identification of the biochemical basis for hunger and meal initiation, and of the signals controlling these neurobiological processes have been the subject of extensive research and debate for some decades regarding the control of food intake (Chaput and Tremblay, 2009).

Many natural herbs are purported either to enhance libido or to treat obesity. One of such plants, commonly known as khat (*Catha edulis*) is thought to affect libido and/or anorexia. Its positive effects occur when khat is chewed.

## **1.2 KHAT (*CATHA EDULIS*)**

Khat, *Catha edulis* Forsk, an evergreen shrub or tree, belongs to the suborder Rosidae and family Celastraceae. The cultivation areas of khat are in Ethiopia, Yemen, Kenya, South Africa, Uganda, Tanzania, Rwanda, Zimbabwe, Turkistan, Afghanistan and northern Hejaz. It is cultivated at an altitude of 1670–2590 meters and can live up to 75–100 years if taken care properly (Al-Hebshi and Skaug, 2005). Other khat names reported were tchat, qat, qaad/jaad, miraa, mairungi, muhulo, cat, catha, gat, tohai, muraa, Abyssinian tea or Arabian tea (Feyissa and Kelly, 2008).

### **1.2.1 ACTIVE CONSTITUENTS OF KHAT**

The chemical constituents of khat leaf include alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals (Feyissa and Kelly, 2008; Pennings et al., 2008). The major alkaloids in fresh khat leave of various origins are cathinone, cathine and norephedrine at 0.95, 1.98, and 0.54 mg/g of khat leaf, respectively (Geisshüsler and Brenneisen, 1987; Kalix, 1990; Widler et al., 1994; Dimba et al., 2004; Feyissa and Kelly, 2008). The major contents of adult or dry khat leaves are cathine and norephedrine at an approximate ratio of 4:1 (Schorno and Steinegger, 1979; Kalix, 1990; Sporkert et al., 2003; Feyissa and Kelly, 2008). Cathinone is unstable and undergoes decomposition to relatively inactive compounds after harvesting and during drying or extraction of the plant material (Pennings et al., 2008).

### **1.2.2 TRADITIONAL MODE OF KHAT ADMINISTRATION**

Several million people are habitual khat users and khat chewing is predominantly a male habit. However, female's habit is less and smaller quantities of khat are chewed

for shorter periods (Al-Motarreb et al., 2002b; Al-Hebshi and Skaug, 2005). Chewing of khat leaves is a common practice during social gatherings in the local populations that often last for several hours (Kassie et al., 2001; Al-Motarreb et al., 2002b). After the first few hours of consumption, users of khat demonstrate increased alertness, better self-esteem, augmented feeling of elation, enhanced imaginative ability, and improved capacity to relate ideas (Cox and Rampes, 2003). However, towards the end of the khat session, some users experience a depressive stage often manifested as irritability, anorexia, hyperthermia, insomnia, mydriasis, and endocrine disturbances (Al-Motarreb et al., 2002b; Hassan et al., 2002).

The mastication of khat leaves is the exclusive method of khat use. However, khat (dry leaves) is rarely used as tea (namely Abyssinian or Arabian tea) in close connection with the use of coffee. The khat chewing habit has a deep rooted social and cultural tradition. Khat is usually chewed at special social gatherings, but is also used frequently during work to keep alert and reduce physical fatigue. The way of khat use is described by chewing the 100–200 g of khat leaves one by one for duration of 2-4 h. During mastication, the juice is swallowed while the residue is stored in cheek (Al-Hebshi and Skaug, 2005; Feyissa and Kelly, 2008; Pennings et al., 2008).

Chewing khat leaves in traditional manner results in enhancement of some activities as well as diminished side effects (Cox and Rampes, 2003; Toennes et al., 2003; Feyissa and Kelly, 2008). A habitual user masticates the leaves slowly one by one, liberating alkaloids consistently and slowly swallowing the juice during the khat session period of 3-4 hours. Hence, special emphasis on the sustained release of

alkaloids consistently from khat leaves could be obtained from the traditional way of khat consumption by chewing.

### **1.2.3 HABITUAL OR ADDICTIVE PROPERTY OF KHAT**

Khat was described by several studies as a habitual substance (Al-Motarreb et al., 2002b; Numan, 2004; Al-Hebshi and Skaug, 2005; Feyissa and Kelly, 2008; Pennings et al., 2008). A recent review by Pennings et al. (2008) on the physical and psychological dependence liability of khat reported that pure cathinone had an addictive potential due to the fast onset of action when administered parenterally. However, khat leave was not classified as drug dependence due to the self-limiting way of administration by chewing. It was concluded that khat has low abuse potential in human and khat dependence is mild. Furthermore, Al-Motarreb et al. (2002b) reported that mild craving and tolerance to the effect of khat do exist but there is no definite withdrawal syndrome. Withdrawal symptoms after prolonged use may consist of lethargy, mild depression, slight trembling and recurrent bad dreams (Kalix, 1988), but these symptoms are mild and resolve in a short time (Kalix, 1990). Another recent review by Feyissa and Kelly (2008) reported that the habitual use of khat is in many instances compulsive, as indicated by the tendency of khat chewers to secure their daily supply of the leaves at the expense of vital needs (Kalix and Braenden, 1985; Nencini et al., 1988). This is described as a psychological dependence by many authors (Kalix, 1990; Gosnell et al., 1996; Connor et al., 2002). In eastern African countries, the prevalence of khat dependence is estimated to be 5–15% of the population (Nielen et al., 2004). The review concluded that generally it is believed that there is no physical withdrawal symptoms occur in khat users, as experienced with alcohol, morphine or barbiturates (Luqman and Danowski, 1975;

Kalix, 1984; Al-Motarreb et al., 2002b; Sulzer et al., 2005). Abandoning the khat chewing habit however is followed by symptoms including lassitude, nightmares, slight trembling, and depression (Luqman and Danowski, 1975; Elmi, 1983; Alem et al., 1999; Cox and Rampes, 2003). Indeed, habitual users report that they have no serious difficulties when moving to an area where khat cannot be obtained (Kalix, 1990).

#### **1.2.4 SEXUAL BEHAVIOR EFFECT OF KHAT**

The early reports on the sexual activity of khat were reported in 1959 by Dr. Kervingant and Dr. Trelu, United Nations Office on Drug and Crime (UNODC) (Kervingant, 1959; Trelu, 1959). Kervingant (1959) reported that khat is an aphrodisiac. Trelu (1959) reported that khat acts like coffee, which is one of the most potent aphrodisiacs.

The report of Halbach (1972) documented that khat was an aphrodisiac. Initially, it increased libido but spermatorrhoea and subsequently impotence might occur with chronic use of khat. Luqman and Danowski (1975) reported that khat is an aphrodisiac which enhances sexual activity in the depressed person. Khat chewing delays the ejaculation phase, which treats premature ejaculation. However, a spontaneous secretion of spermatic fluid may occur in many khat chewers, when khat exacerbates or accentuates anxiety states. On the other hand, the advisory group of WHO reported that the chronic administration of khat is believed to have spermatorrhoea effect in males (Chanoit et al., 1980).



Elmi (1983) conducted an epidemiological research on khat to estimate its prevalence, social characteristics of consumers, patterns of use and effects during and after consumption. Consumers and non-consumers (7485 people) were randomly interviewed. The results showed that about 60% of the male population reported increase of libido which was not sustained by an equal increase of sexual potency and 18.78% reported improvement of sexual performance, while 61% denounced its impairment. The situation in the female population was very different; the increase of sexual desire (71.72%) was in fact followed by improvement of performance ability (78.26%). Sixty one percent of the male population reported either spermathorrea or precocious ejaculation.

Islam et al. (1990) investigated the reproductive toxicity of a chemically synthesized (-)-cathinone on male rats. Three dose levels of cathinone were administered intraperitoneally as 5, 10 and 30 mg/kg body weight of rats to assess their reproductive toxicity. The results showed that cathinone produced a dose-dependent decrease in sperm count and increased the number of abnormal sperms. Furthermore, the plasma testosterone was also decreased.

Taha et al. (1995) studied the effect of (-)-cathinone, caffeine and their combinations on the sexual behaviour of male rats. Male sexual activities were assessed by recording the erectile responses (grooming of genitalis, stretching and homosexual mounting), in the absence of females. The copulatory behaviour was observed by caging males with receptive females brought into oestrus with subcutaneous injection of oestradiol benzoate and progesterone. The copulatory pattern of male rats (mounting, intromissions, ejaculations and refractory period) was recorded. The

results showed that the oral treatment of the combination of cathinone and caffeine (5 and 50 mg/kg/day) for 15 days increased sexual arousal (motivation) in male rats (increased mounting performance and anogenital investigatory behaviour with no stimulatory effect on erectile and ejaculatory responses).

Adeoya-Osiguwa and Fraser (2005) investigated the effects of cathine and norephedrine on the function of mammalian uncapacitated sperm suspensions using mouse and human spermatozoa. The result showed that cathine and norephedrine significantly accelerated capacitation and the treated sperm suspensions were significantly more fertile than controls. The study concluded that cathine and norephedrine can directly affect mammalian sperm function, accelerating capacitation and inhibiting spontaneous acrosome reactions. It was suggested that cathine and norephedrine, at appropriate doses, might enhance fertility.

Mwenda et al. (2006) determined the effects of oral administration of crude khat juice extract on the circulating hormones of male Olive Baboon (*Papio anubis*, Cercopithecidae) for 1 and 2 months. The results showed that khat administration (fresh juice made of khat leaves and peeled bark) to male baboon (250g/50 mL/baboon) caused a significant increase in the mean testosterone levels, while prolactin and cortisol levels were reduced. These effects were also evident 1 month post treatment, indicating that khat may exert a transient effect on male fertility by interfering with the hormonal profiles.

Abdulwaheb et al. (2007) evaluated the effect of oral administration of khat extract on sexual behavior in male rats. Adult albino Wistar male rats were administered

khat extracts (100, 200, 400 mg/kg), amphetamine (1 mg/kg), sildenafil (1 mg/kg), ethanol (2 mL/kg of 2% and 10%), and combination of khat and ethanol (2% + 10%) for 15 days. The results showed that rats treated with 400 mg/kg of khat demonstrated a statistically significant increase in all sexual parameters except in mounting frequency, intercopulatory interval and copulatory efficiency. Whereas, rats treated with 200 mg/kg showed a statistically significant increase only in ejaculation latency. In contrast, low dose of khat extract at 100 mg/kg was found to significantly reduce both intromission latency and mount latency, thereby enhancing sexual motivation/arousal in male rats. Similar results were obtained when khat extract (200 mg/kg) and ethanol (10%) were administered concomitantly despite the inhibitory effect observed in male sexual behavior when administered alone. The study concluded that higher doses of the extract inhibited sexual behavior in male rats. In contrast, low dose of the extract as well as the concurrent administration of the extract followed by ethanol was found to enhance male rat sexual motivation/arousal.

Nyongesa et al. (2007) investigated the *in-vitro* effect of khat extract on mouse interstitial cells. The isolated mouse interstitial cells were incubated with different concentration of khat extract (0.06, 0.6, 6.0, 30.0 and 60 mg/mL). Testosterone level was measured at 30-min intervals over 3 h of incubation period. The results showed that khat extract at high concentration (30 and 60 mg/mL) inhibited testosterone production, while low concentration (0.06, 0.6 and 6 mg/mL) stimulated testosterone production. The study assumed that khat extract at high concentration might cause impairment of reproductive function but at low concentration might enhance reproductive functions.

Nyongesa et al. (2008) investigated the effect of fresh khat extract on luteinizing hormone in male rabbits. The rabbits were divided into five groups. One group served as control fed with normal saline, and four groups fed with khat extract (1.5, 4.5, 13.5 and 40.5 g/kg) twice a week for 5 weeks. The results showed that all doses of khat extract lowered plasma luteinizing hormone and plasma testosterone levels. However, plasma cortisol levels were elevated in a dose-dependent manner. The study assumed that khat may impair reproductive function in male rabbits by interfering with sex hormone profiles.

Bentur et al. (2008) conducted a prospective observational study on the side effects of cathinone capsules (200 mg, marketed in Israel as a natural stimulant and aphrodisiac). The data of 34 patients aged between 16-54 years were analyzed. The results showed that the main clinical symptoms were hypertension, headache, vomiting, nausea, tachycardia, dyspnoea, myalgia and chest pain. The main complications were pulmonary edema, intracerebral haemorrhage and myocardial ischemia, all in young subjects. The study concluded that exposure to synthesized cathinone was associated with serious cardiovascular and neurological toxicity.

In light of the above studies, it seems that the sexual behavior is influenced by the source of alkaloid used, dose level and mode of administration. When pure, chemically synthesized alkaloids particularly cathinone was given parenterally or orally, severe side effects are observed. However, moderate side effects are associated with ingestion of khat leaves. Low doses of khat-extract enhanced the sexual motivation of male rats more than sexual performance, while high doses produced opposite effects on both sexual motivation and performance. Also, the

positive effect of khat on sexual desire is more frequently observed in females than in males. It seems that khat is more effective on the sexual motivation/arousal or libido, particularly in females. Studies on the effect of khat on sexual behavior were summarized in Table (1.1).

Table 1.1: Summary of studies on the effect of khat on sexual behavior.

Reference	Effects	Compound	Dose	Mode of administration	Duration	Subject	Side effects																
(Kervingant, 1959)	Aphrodisiac	khat	-	-	-	-	-																
(Trellu, 1959)	Aphrodisiac	khat	-				↓ desire																
(Halbach, 1972)	Aphrodisiac ↑ libido initially	-	-	-	-	-	spermatorrhoea and subsequent impotence may occur in a chronic use of khat.																
(Luqman and Danowski, 1975)	Aphrodisiac ↑ sexual activity in depressed person ↓ ejaculation, treats premature ejaculation	Khat	-	Chewing	-	-	a spontaneous secretion of spermatic fluid may occur in many khat chewers, when khat exacerbate or accentuate anxiety states																
(Chanoit et al., 1980)	-	-	-	-	-	-	Chronic administration of khat is believed to have a spermatorrhea in males.																
(Elmi, 1983)	↑ female sexual desire 60% ↑ male sexual desire 40%	Khat	-	Chewing	-	Humans	inability to sustain male erection																
(Islam et al., 1990)	<table border="1"> <thead> <tr> <th colspan="4">Level change of Testosterone (%)</th> </tr> <tr> <th>(mg/kg)</th> <th>5</th> <th>10</th> <th>30</th> </tr> </thead> <tbody> <tr> <td>(-)-Cathinone</td> <td>↑ 5</td> <td>↓28</td> <td>↓43</td> </tr> <tr> <td>(+)-Cathinone</td> <td>0</td> <td>0</td> <td>↓32</td> </tr> </tbody> </table>		Level change of Testosterone (%)				(mg/kg)	5	10	30	(-)-Cathinone	↑ 5	↓28	↓43	(+)-Cathinone	0	0	↓32	5, 10 & 30 mg/kg	Intraperitoneal injection	15 days	24 male Wistar rats	(-)-Cathinone in rats showed a decrease in sperm count, an increase in number of abnormal sperms and a decrease in plasma testosterone.
Level change of Testosterone (%)																							
(mg/kg)	5	10	30																				
(-)-Cathinone	↑ 5	↓28	↓43																				
(+)-Cathinone	0	0	↓32																				
(Kalix, 1990)	↑ libido	Khat	-	Chewing	-	-	Spermatorrhoea																

(Tariq et al., 1990)	% of change in	(-)-Cathinone (mg/kg)			5, 10 & 30 mg/kg	Intraperitoneal injection	15 days	rats	↓ Food and water intake
		5	10	30					
	Sperm count	↑ 5	↓ -21	↓ -28					
	Sperm motility	↓ -25	↓ -38	↓ -40					
	Abnormal sperm	↑ 49	↑ 106	↑ 154					
(Giannini et al., 1992)	Aphrodisiac	Khat			-	-	-	-	-
(Taha et al., 1995)	↑ sexual motivation ↑ mounting performance	Cathinone + caffeine			5 + 50 mg/kg/day	Oral	15 days	male rats	anogenital investigatory behavior with no stimulatory effect on erectile and ejaculatory responses.
(Adeoya-Osiguwa and Fraser, 2005)	↑ Capacitation ↑ Fertility	Cathine-HCl Norephedrine-HCl			0.01–10 mmol/L	Incubation	1 h	uncapacitated sperm suspensions of mouse and human spermatozoa	
(Mwenda et al., 2006)	↑ Testosterone ↓ Prolactin and cortisol	250g khat leaves blended with water → juice			juice (50mL/baboon)	Oral	month	Baboons	-
(Abdulwaheb et al., 2007)	Low dose ↑ sexual motivation Low dose + ethanol ↑ sexual motivation	khat extract			Low dose 200mg/kg/day High dose 400mg/kg/day	Orally by gavage	-	male rats	<u>Low dose</u> ↓ mounting latency ↓ intromission latency <u>High dose</u> ↓ sexual motivation
(Nyongesa et al., 2007)	Low dose, ↑ testosterone No effect on interstitial cell viability	khat extract			Low dose 0.06-6 mg/mL High dose 30-60 mg/mL	Cells incubated in khat extract	-	Isolated mouse interstitial cells	High dose ↓ testosterone ↓ viability of interstitial cell
(Nyongesa et al., 2008)	↓ Luteinizing ↓ Testosterone ↑ Cortisol in a dose-dependent manner	khat extract			1.5, 4.5, 13.5, 40.5 g/kg	fed	5 weeks	Rabbits	Impair reproductive function in male rabbits by interfering with sex hormone profiles.
(Bentur et al., 2008)	Aphrodisiac	cathinone			200mg	Oral capsules	-	-	Serious neurological and cardiovascular complications,

### 1.2.5 ANOREXIC ACTIVITY OF KHAT

Anorexia, a characteristic effect of khat has been used for centuries to alleviate the sensation of hunger (Feyissa and Kelly, 2008). However, the anorectic effect of khat was only studied in the last 30 years (Table 1.2). Due to the relatively complex extraction method from the khat plant, chemically synthesized alkaloid was investigated (Halbach, 1972; Kalix, 1990).

The synthesized alkaloid was reported to possess anorexic properties and body weight reducing activity in animals. These alkaloid include (-)-cathinone and (+)-cathine (Knoll, 1979), phenylpropanolamine and alpha-aminopropiophenone (Zelger and Carlini, 1980), dl-cathinone (Foltin and Schuster, 1983; Woolverton and Johanson, 1984), dl-cathinone oxalate (Goudie and Newton, 1985), d-norpseudoephedrine-HCl and dl-norephedrine-HCl (Eisenberg et al., 1987), cathinone-HCl (Nencini et al., 1988), (-)-cathinone and (+)-cathinone (Islam et al., 1990), norpseudoephedrine (Nencini et al., 1996) or dl-cathinone-HCl (Wolgin and Munoz, 2006).

In those studies, the alkaloids were mainly given parenterally, for example intraventricularly (Knoll, 1979), intraperitoneally (Zelger and Carlini, 1980; Foltin and Schuster, 1983; Woolverton and Johanson, 1984; Goudie and Newton, 1985; Eisenberg et al., 1987; Nencini et al., 1988; Islam et al., 1990; Nencini et al., 1996; Wolgin and Munoz, 2006) or intravenously (Schuster and Johanson, 1979; Yanagita, 1979).



Schuster and Johanson (1979) investigated the behavioral effects of dl-cathinone and l-cathinone in rhesus monkeys and rats. They concluded that dl-cathinone served as a positive food reinforce when given intravenously in drug self-administration experiments. When connected to surgical indwelling venous catheters, caused a decrease in milk intake and produced tolerance to the anorexic effects.

Foltin and Schuster (1982) examined the effects of dl-cathinone (0.25-48 mg/kg) on milk intake in rats. The study was conducted before, during and after a period of repeated daily dose given intraperitoneally. The results indicated a transient reduction in water intake followed by tolerance and supersensitivity.

Goudie and Newton (1985) compared the potency between dl-cathinone-oxalate and d-amphetamine sulphate on the conditioned taste aversion (reduced food intake) and adipsia (reduced water intake) in rats. The results showed that the potency ratio of dl-cathinone to d-amphetamine for inducing conditioned taste aversion was 1:17 and adipsia was 1: 4.

Wolgin and Munoz (2006) investigated the effects of dl-cathinone-HCl (2, 4, 8, 16 & 32 mg/kg) given intraperitoneally on milk intake and motor activity in bottle- and cannula-fed rats. The study suggested that cathinone suppresses intake by inducing locomotion and stereotypy, which interfere with the appetitive phase of feeding, and tolerance to drug-induced hypophagia (reduced food intake) involves learning to suppress such movements (Wolgin and Munoz, 2006).

Methanolic khat extract administered orally by gavage to rats during days 6 to 15 of gestation at doses of 0, 125, 250 and 500 mg/kg/day reduced the food consumption and maternal weight gain and also lowered the food efficiency index, as compared to control mothers. The study revealed that khat retarded fetal growth, possessed both dose-related embryotoxic and teratogenic properties (Islam et al., 1994).

Al-Habori and Al-Mamary (2004) investigated the long term (6 months) effects of khat leaves on the plasma concentration of triglycerides and cholesterol of animal rabbits. The results showed that there were significant decreases in plasma cholesterol and triglycerides levels.

Administering fresh juice made of khat leaves, peeled bark and water to male baboon (250g/50 mL/baboon) decreased their mean body weight by as much as 10% (Mwenda et al., 2006).

Hundred human male volunteers involved in a study, where 50 was control, 25 chewed 200 g of khat leaves and 25 chewed 400 g of khat leaves. The result showed that the plasma leptin and non-esterified fatty acids were significantly increased in the group receiving 400 g of khat leaves, while plasma triglycerides were significantly decreased in both groups receiving khat leaves (Al-Dubai et al., 2006).

Murray et al. (2008) showed that chewing of khat for a period of 3 h by six habitual khat chewers caused a significant decrease in the subjective feelings of hunger and increase in the fullness but had no effect on orexigenic ghrelin and anorexigenic peptide PYY. Normally, the orexigenic ghrelin is present in the stomach, its level

increases in time of hunger to increase appetite and decreases immediately post-meal to decrease appetite. On the other hand, the anorexigenic peptide YY is present in the intestine, its level increases following a meal to decrease appetite (Gale et al., 2004).

### **1.2.6 SIDE EFFECTS OF KHAT**

The associated side effects were investigated by numerous studies. A medical survey carried out with 751 users and non-user of khat reported that the common adverse effects of khat use are gastritis, insomnia, anorexia, constipation, headache, and respiratory difficulties (Kennedy et al., 1983).

The effect of tube feeding of khat leaves to eight awake, chronically catheterized, late-pregnant guinea pigs suggested a reduction in placental blood flow and impairment in fetal growth (Jansson et al., 1988).

The effects of repeated oral administration of khat or the commercial S-(-)-cathinone enhanced aggression in rats, presumably by decreasing the serotonin and its corresponding metabolite levels. These effects were produced by dose levels of S-(-)-cathinone-HCl at 1.5 mg/kg and fresh-khat-extract at 200 mg/kg (Banjaw et al., 2006).

Al-Motarreb et al. (2002a) reported that chewing of fresh khat leaves resulted in acute myocardial infarction. Kuczkowski (2005) documented two cases of chest pain, tachycardia and hypertension in two pregnant patients who used fresh khat in familial gatherings. Mujlli et al. (2005) reported that khat is a risk to blood pressure in acute cerebral infarction patients. However, all the above studies did not consider

the adjustment of the confounding effects of smoking (since 80% of khat chewers are smokers). As such, their findings might be due to either khat chewing or both khat chewing and smoking habits (Al-Hebshi and Skaug, 2005).

Pennings et al. (2008) stated that the level of abuse and threat to public health of the green vegetables' khat leaves are not significantly enough to warrant international control. In addition, epidemiological studies over the last 50 years (1945–2006) reviewed by Warfa *et al.* (2007) did not support a causal relationship between khat-chewing and mental illness.

In light of all the above studies, it seems that the chemically synthesized alkaloids possess anorexic, body weight reducing properties and many other associated side effects. The severity of these side effects is influenced by the source of alkaloid used, dose level and mode of administration. When pure alkaloid particularly cathinone was given parenterally (intraperitoneally, intraventricularly or intravenously), severe side effects are observed. However, moderate side effects are associated with ingestion of khat leaves.

Table 1.2: Comparison between studies on the effect of khat on body weight, loss of appetite (anorexia), triglycerides, cholesterol or lipolysis

Reference	Effects	Compound used	Mode of administration	Dose	Treatment period	Experimental subject	Side effects
(Knoll, 1979)	↓F.I	(-) Cathinone (+) cathine	Intraventricular	-	-	rats	-
(Schuster and Johanson, 1979)	Positive food reinforce ↓ milk intake in rats	dl- Cathinone, l-Cathinone & d-amphetamine	Intravenous Injection	-	-	3 Rhesus monkeys & Rats	Tolerance to its anorexic effects
(Yanagita, 1979)	-	l-cathinone, dl-cathinone, dl-ephedrine, d-amphetamine d-norpseudoephedrine	Intravenous Injection	- 1 mg/kg - 10-5g/ml - 0.06-0.25 mg/kg /injection	- 1 h - 1 h - Month	- anesthetized rats - isolated guinea pig atria - 2 Rhesus monkeys	- ↑ Blood pressure, ↑ heart rate - Inotropic - Drug self administration
(Zelger and Carlini, 1980)	↓ B.W Anorexia	Phenylpropanolamine α-aminopropiophenone	Intraperitoneal injection	-	-	rats	-
(Foltin and Schuster, 1982)	↓F.I 25%	dl-Cathinone	Intraperitoneal injection	4 mg/kg	Month	20 SD male Rats	transient nature of the tolerance and supersensitivity after 10 days
(Kennedy et al., 1983)	Anorexia	Khat leaves	Chewing	-	-	Humans	constipation, insomnia, headaches, respiratory difficulties
(Goudie and Newton, 1985)	Anorexia	dl-Cathinone oxalate	Intraperitoneal injection	1, 4 & 16 mg/kg	Month	rats	- Taste aversion 1:17 (dl-cathinone : d- amphetamine) - Adipsia (1: 4)
(Eisenberg et al., 1987)	AD <sub>50</sub> (50% ↓ F.I) NPE = 22 mg/kg NE = 18 mg/kg	phenylpropanolamine d-Norpseudoephedrine- HCl dl-Norephedrine-HCl	Intraperitoneal injection	10-50 mg/kg	1 day	24 SD male Rats	NPE increase locomotor activity.
(Nencini et al.,	Acute: - ↓F.I	Cathinone-HCl	Intraperitoneal	2, 4 or 8	Month	48 SD male	- Tolerance after 9 days

1988)	Chronic: ↑ W.I		injection	mg/kg		Rats	- diuresis
(Jansson et al., 1988)	↓F.I	Khat leaves	Feeding	2.2 g/kg	10 days	Pregnant guinea pigs	↓ Birth weight (7%)
(Islam et al., 1990)	5, 10 & 30 mg/kg (-)-Cathinone ↓F.I: 27,44 & 72% ↓W.I: 3,19 & 25% ↓B.W: 15,28 & 40% (+)-Cathinone ↓F.I: 6,11 & 50% ↓W.I: 0,19 & 26% ↓B.W: 28,36 & 42%	(-)-Cathinone (+)-Cathinone	Intraperitoneal injection	5, 10 & 30 mg/kg	15 days	24 male Wistar rats	(-)-Cathinone: 5, 10 & 30 mg/kg Testosterone: ↑ 5, ↓ 28 & ↓ 43%  (+)-Cathinone: 5, 10 & 30 mg/kg Testosterone: 0, 0 & ↓ 32%
(Brenneisen et al., 1990)	-	Cathinone-HCl	Oral capsules	0.5 mg/kg	One day	6 humans	↑ Blood pressure, ↑ heart rate
(Islam et al., 1994)	↓F.I ↓ B.W ↓ Food efficiency	Methanol khat extract	Orally by gavage	125, 250 & 500 mg/kg	6-15 days	rats	- ↑ resorptions & fetal wastage. - Intrauterine growth retardation. - embryotoxic & teratogenic.
(Nencini et al., 1996)	↓F.I	Norpseudoephedrine	Intraperitoneal injection	17, 32 & 56 mg/kg	6 days	SD Rats	Antidipsic ↓ water intake
(Griffiths et al., 1997)	Anorexia ↓ B.W	Khat	Chewing	-	-	Humans	- Trouble sleeping - Feeling depressed - Mood swings - Feeling anxious
(Al-Habori and Al-Mamary, 2004)	↓Cholesterol ↓Triglycerides	Dried khat leaves	added to the diets	20 & 30% of food/rabbit	6 months	Rabbits	-
(Wolgin and Munoz, 2006)	↓F.I	dl-Cathinone-HCl	Intraperitoneal injection	2, 4, 8, 16, & 32 mg/kg	2 weeks	SD Rats	- Tolerance (less than amphetamine). - Cathinone induced sensitization of hypophagia in saline-treated controls (not

							found with amphetamine).
(Mwenda et al., 2006)	↓ B.W (10%)	250g khat; leaves & shoots removed and bark peeled was blended with water → juice	Oral	Juice (50mL/ baboon)	month	baboons	-
(Al-Dubai et al., 2006)	Anorexia ↓ B.W ↓ Triglycerides	Khat leaves	Chewing	200-400g leaves/person	one day	humans	-
(Warfa et al., 2007)	Anorexia	-	-	-	-	-	- Minor psychosis due to cathinone
(Hassan et al., 2007)	Anorexia	-	-	-	-	-	- Psychosis due to cathinone - ↑ Blood pressure - Hypertension - Constipation
(Murray et al., 2008)	Anorexia	Fresh Khat leaves	leaves chewed but not swallowed	200-400 g of leaves / person	3 h	6 men habitual khat chewer	-
(Feyissa and Kelly, 2008)	↓ F.I ↓ B.W	-	-	-	-	-	- Minor psychosis due to cathinone - Aggression due to cathinone - Food-reinforced
(Pennings et al., 2008)	↓ Cholesterol ↓ Triglycerides ↓ F.I ↓ B.W ↓ Food efficiency	-	-	-	-	-	- Acute myocardial infarction - Tachycardia & Hypertension - ↑ Blood pressure

**F.I** (Food Intake), **B.W** (Body Weight), **W.I** (Water Intake)

### **1.3 CONTROLLED RELEASE DRUG DELIVERY SYSTEM**

The controlled-release drug delivery is defined as dosage form that allows reduction in dosing frequency or significant increase in patient compliance or therapeutic performance when compared with that presented as a conventional dosage form. The rationale for development of controlled-release formulation of a drug is to enhance its therapeutic benefits, minimizing its side effects while improving the management of the diseased condition (Tiwari and Rajabi-Siahboomi, 2008).

#### **1.3.1 MICROCAPSULES**

Microcapsules are small particles (3-800  $\mu\text{m}$ ) that contain an active agent or core material (10-90 wt%) surrounded by a coating or shell (Thies, 2006). The core material can be consisting of single or multiple compounds with hydrophilic and/or hydrophobic properties and the coat can be matrix, single layer or multilayer, and can be hydrophilic and/or hydrophobic (Benoit et al., 2006). The choice of coating materials depends on the physicochemical properties of the core materials (Magdassi and Vinetsky, 2006). If a water-insoluble core material is dispersed in the system and the coacervate wets this core material, each droplet or particle of dispersed core material is spontaneously coated (Thies, 2006). A complex system of multiple emulsions could be used in microencapsulation of single or multiple aqueous compartments with hydrophilic/hydrophobic variation (Garti and Aserin, 2006). However, the composition ratio entrapped into microcapsules could be negatively affected by the core partition problem.



### **1.3.2 CORE PARTITION PROBLEM IN MICROENCAPSULATION PROCESS**

The core partition effect is the major problem encountered in microencapsulation of water soluble drugs. Several studies highlighted the importance in resolving the core partition effect. One of the solutions reported was dispersion of drug (with or without polymer) into non-aqueous dispersion medium, e.g. oil or non-aqueous solvents (to prevent drug loss) and emulsification as a w/o emulsion with or without the polymer (aqueous or non-aqueous) (Chang and Roland, 1996; Sriwongjanya and Bodmeier, 1997; Kedzierewicz et al., 1999; Nokhodchi and Farid, 2002; Wieland-Berghausen et al., 2002). However, non-aqueous medium is often expensive, requires recycling and difficult to eliminate (Benoit et al., 1996). In addition, emulsification of the drug into an emulsion (o/w) produces irregular and rough surfaces (Cruaud et al., 1999). This might be attributed to drug partitioned into the external phase (aqueous) and drug crystals deposited on the outer layer of the microsphere. In another study, drug was encapsulated by wax using spray congealing (Passerini et al., 2003). As a result of the wetting difficulty between the highly hydrophilic drug crystals and the highly lipophilic molten wax, a limited loading was obtained even after the addition of surfactants. Encapsulation of water-soluble drugs was further improved by using w/o/w double emulsion (Freytag et al., 2000; Hombreiro Pérez et al., 2000). The amount of oil phase decreased, but lower encapsulation efficiency was reported due to the higher solubility of drug in the external aqueous phase. The comparatively higher volume compared to that of the internal aqueous phase caused leakage of drug into the continuous phase (Hombreiro Pérez et al., 2000) and the partition of drug into the external phase during the encapsulation process (Freytag et al., 2000). Some advances in decreasing the drug partition effect have been achieved by decreasing

the drug solubility with initial emulsification of drug in an insoluble biodegradable polymer prior to emulsification into the double emulsion w/o/w (Meng et al., 2003; Ubrich et al., 2004), or by covering a single hydrophilic core with a thin biodegradable polymer membrane using the ink-jet-interfacial phase separation method (Yeo et al., 2003), or layering a drug (22%) into a binder solution [80% (HPMC, PEG, ethanol, and water)] prior to coating with biodegradable polymer film (Lecomte et al., 2004). Alternatively, the initial drug-containing water phase was separated from the dispersion medium by the lecithin-containing oil phase which was advantageous in maintaining encapsulation efficiency of the water-soluble drug (Nii and Ishii, 2005). Recent studies were conducted to separate drug from the aqueous phase and/or from the oily continuous phase prior to preparing the double emulsion either by decreasing the drug's water solubility or by solidifying the drug in the phase to prevent drug migration from one phase to another (Hasan et al., 2007; Puri et al., 2008; Cohen-Sela et al., 2009). Such studies prevented the rapid partitioning of drug into the external phase, but the excess material used with a low drug percentage and total loading capacity could be limited. Capacity of loading was usually increased through supercritical anti-solvent or electrospinning process methods using biodegradable polymers (Lee et al., 2006; Qi et al., 2008), culturing of drug into yeast cells (Shi et al., 2007), gelatin complex coacervation method (Jin and Kim, 2008), or using hydrogen peroxide to make porous microspheres with a thin layer to cover the surface (Bae et al., 2009). In short, it can be concluded that the water soluble cores can be microencapsulated by complicated procedures with various limitations (Tables 1.3 and 1.4).

Table 1.3: Studies conducted for resolving the core partition effect in microencapsulation of water soluble drugs

Reference	Core	Coat	Method	Outcome	Disadvantages
(Chang and Roland, 1996)	- Propranolol HCl	- Ethylcellulose pseudolatexes - Eudragit RS 30D	w/o emulsion	- Microspheres prepared from water-insoluble polymers without use of organic solvents. - Drug-polymer was dispersed into external oil phase, emulsified, microspheres collected by filtration, washed with hexane to remove excess oil from the microsphere surface, and dried under vacuum in a desiccator overnight.	- Difficulty of collecting and drying of microspheres from the oil phase (cottonseed oil). - Washing microspheres with hexane.
(Sriwongjanya and Bodmeier, 1997)	- Chlorpheniramine maleate - Pseudoephedrine HCl - Propranolol HCl	- Ethylcellulose - Poly (methyl methacrylate) - Eudragit RS 100	Aqueous solvent evaporation	Drug was bound to resin, dispersed in an organic polymer solution and followed by emulsification into an external aqueous phase.	- Complicated, long procedures and excess materials were used. - The optimum microparticles selected was produced at ratios of (drug) to (resin) to (polymer) to (surfactant) of (0.5-1) : (1) : (4) : (0.1-1) respectively, which the theoretical drug loading % could be less than 15%.
(Cruaud et al., 1999)	- Baclofen	- Poly(lactic-co-glycolic acid)	o/w emulsion extraction	Microspheres exhibiting smooth surfaces at low drug payload (12.8% w:w), irregular and rough surface at high drug content (33.9% w:w)	- The irregular and rough surface produced at high drug content was due to the using of o/w emulsion, drug partitioned to the external phase (aqueous) and after drying the drug crystallized on microsphere surface. - Methylene chloride used to dissolve PLGA - Lower encapsulation efficiency
(Kedzierewicz et al., 1999)	- Propranolol HCl	- Gellan gum	Ionotropic gelation	Beads were prepared by solubilising the drug in a dispersion of gellan gum and then dropping the dispersion into calcium chloride solution.	- Entrapment efficiencies (92%) and slow release were pH dependent - Lower drug loading; drug (12.5-100 mg) to polymer 1g
(Nokhodchi	- Paracetamol	- Cellulose acetate	Emulsion solvent	Paracetamol was dispersed into liquid paraffin	- Acetone was used for dissolving

and Farid, 2002)		phthalate	evaporation Modified emulsion solvent evaporation Emulsion non-solvent addition	or liquid paraffin hexane mixture, emulsify, liquid paraffin was decanted, and microcapsules were collected, washed twice with 50 mL of chloroform to remove any oil remaining and dried under reduced pressure for at least 12 h.	of polymer - Chloroform and hexane used as non-solvent - Liquid paraffin as dispersion medium. - Difficulty of collecting and drying of microcapsules from the oil phase (liquid paraffin). - Washing microcapsules with chloroform.
(Wieland-Berghausen et al., 2002)	- Nitenpyram Clomipramine HCl	- Ethylcellulose - Eudragit EPO - Eudragit E	- Coacervation - Solvent evaporation (w/o) - Film-coating		- Oppanol B50 was used as coacervating agent and cyclohexane was used as dispersion medium - Acetone was used to dissolve Eudragit E - Acetone was used to dissolve Eudragit EPO
(Meng et al., 2003)	- Blank microparticles for loaded of lysozyme	PELA	- w/o/w double emulsion solvent diffusion		- Using ethyl acetate
(Passerini et al., 2003)	- Verapamil HCl	Wax	- spray congealing	Low encapsulation efficiency was explained by difficult wetting of the highly hydrophilic drug crystals by the highly lipophilic molten wax. But addition of a surfactant (Stearyl alcohol 90) was improved drug wettability, enhances the release and increased the encapsulation efficiency.	The % of drug loaded was 10% which is low.
(Yeo et al., 2003)	- Sodium alginate	Poly(lactic-co-glycolic acid)	- Ink-jet - Interfacial phase separation	Thin biodegradable polymer membrane covering a single hydrophilic core	Ethyl acetate
(Lecomte et al., 2004)	- Propranolol HCl	Ethyl cellulose Eudragit L PEG	- Film coating	Formulation of propranolol-HCl into sustained release pellets	- Complicated, long procedures and - Excess materials (polymers, plasticizers) were used.