ENCAPSULATION OF BLACK SEED OIL IN ALGINATE BEADS: FORMULATION, CHARACTERIZATION, AND STABILITY EVALUATION

BY

HAMZEH ALKHATIB

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Kulliyyah of Pharmacy
International Islamic University Malaysia

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ABSTRACT

Taste of medicines has a high number of reports as an administration problem for paediatric medicines. Black seed oil (BSO) is known widely for its medical applications, but children are facing difficulties to take it orally because of its bitter taste. Alginate as a polymer is widely used to encapsulate medications for different purposes including taste masking. Thus, the objectives of this study were to characterize BSO in terms of thymoquinone (TQ) content as the major bioactive compound, to formulate and characterize the alginate beads containing BSO, to study the stability of the beads and to evaluate the taste masking efficacy in human. Stability-indicating HPLC method for TQ analysis in BSO was validated. For encapsulation of BSO in alginate beads, BSO was emulsified with 2% w/v alginate solution by the aid of Tween 80 as a stabilizer. The concentration of BSO and Tween 80 was selected as 15% v/v and 5% w/v respectively to obtain the most stable emulsion. The optimized emulsion was dropped wisely into 1% w/v calcium chloride bath to fabricate BSO-alginate beads. Electrospray technique was used to apply controlling on size of the produced beads. Influence of changing flow rate of BSO-alginate emulsion and applying various values of high voltage on each of encapsulation efficiency, shape and size of the beads were studied. It was found that BSO was completely encapsulated within alginate beads without any significant difference during changing both the flow rate and values of high voltage. All the fabricated beads were in spherical shape. Size of the beads decreased significantly as the value of the applied high voltage increased. On the other hands, changing the flow rate did not have influence on the beads size without applying high voltage. While the beads size decreased significantly as the flow rate increased during applying high voltage. Based on the factorial design analysis and to make producing of BSO-alginate beads is more practical, flow rate of BSO-alginate emulsion was selected at 0.8 mL/min. Moreover, the applied value of high voltage was selected at 4000 v to produce beads within average size of 1.1 mm. The *in vitro* release study showed that all amount of BSO was released at 110 min in simulated intestine medium. Attenuated total reflection-Fourier Transform infrared spectroscopy (ATR-FTIR) and differential scanning calorimetry (DSC) analysis showed that BSO was encapsulated in alginate beads without chemical or physical interactions with the excipients. Stability study was carried out under real time and accelerated conditions. TQ in encapsulated BSO was more stable than TQ in aqueous solution and in original BSO. Moreover, visual inspection and ATR-FTIR confirmed stability of BSO-alginate beads against oil leaking. Evaluation of BSO-alginate beads palatability was done based on an open-label, single-dose study in adults. Encapsulation of BSO in alginate beads significantly improved the oil palatability, making it more accepted to be administered. This study suggests that encapsulation of BSO in alginate beads can improve the oil palatability especially the taste without reducing TQ stability, making BSO more suitable as pediatric formulation.

خلاصة البحث

لقد نال طعم الأدوية النصيب الأكبر من التقارير على أنه مشكلة استساغة لدى الأطفال. لقد عرف زيت الحبة السوداء (BSO) بتطبيقاته العلاجية الواسعة، لكن الأطفال يواجهون صعوبات بتناوله عن طرق الفم بسبب طعمه اللاذع. بالإضافة الى ذلك لقد عرف الألجينات كبوليمر بتطبيقاته القوية في تغليف الأدوية لأغراض اخفاء الطعم. وبالتالي تهدف هذه الدراسة لتوصيف BSO من ناحية محتواه من الثايموكينون (TQ) باعتبارها المادة الفعالة حيويا في BSO، والى صياغة وتوصيف حبيبات ألجينات تحتوي على BSO، والى دراسة استقرار الحبيبات ومن ثم الى تقييم فعالية اخفاء الطعم من قبل الانسان. لقد تم التحقق من طريقة تحليل والاشارة الى استقرار الثايموكينون باستخدام HPLC. من أجل تغليف BSO في حبيبات الألجينات تم استحلاب BSO مع محلول الألجينات بمساعدة توين 80 كمثبت. تم اخيار تركيز كل من BSO وتوين 80, 15% v/v و5% w/v على التوالي من أجل اختيار المستحلب الأكثر استقرارا. ثم تم تنقيط المستحلب الأمثل الى حمام يحتوي على شوارد الكالسيوم لصناعة حبيبات الألجينات، استمرت المعالجة في محلول شوارد الكالسيوم لمدة 20 دقيقة. استخدمت تقنية الرذاذ الكهربائي للتحكم بحجم الحبيبات المنتجة. تم دراسة تأثير تغير تدفق المستحلب وتطبيق قيم مختلفة للتوتر العالي على كل من كفاءة التغليف وشكل وحجم الحبيبات. لقد وجد أن زيت BSO تم تغليفه بشكل كامل ولم يكن هناك اختلاف ملحوظ اثناء تغير تدفق المستحلب وقيم التوتر العالي. تمت دراسة كفاءة التغليف عن طريق قياس العكارة. ولقد كانت كل الحبيبات المنتجة كروية الشكل. نقص حجم الحبيبات بشكل ملحوظ بازدياد قيمه التوتر العالي المطبق. من ناحية أخرى، لم يكن هناك تأثير ملحوظ بحجم الحبيبات عند تغير تدفق المستحلب دون تطبيق التوتر العالي. بينما ازداد حجم الحبيبات بشكل ملحوظ بازدياد التدفق أثناء تطبيق التوتر العالي. وأظهرت دراسة التحرير أن كامل الزيت المغلف تم تحريره عند الدقيقة 110 في بيئة الأمعاء الدقيقة. أظهرت تحاليل ATR-FTIR وDSC أن BSO تم تغليفه ضمن الحبيبات دون أي تداخل كيميائي او فيزيائي بين الزيت والمكونات الأخرى. لقد أنجزت دراسة الاستقرار تحت ظروف الوقت الحقيقي وتحت الظروف المسرعة. وأظهرت الدراسة أن TQ في الزيت المغلف كان مستقر أكثر من TQ في الزيت الأصلي وفي الطور المائي. كما أكد ATR-FTIR عدم تسرب الزيت من الحبيبات بالإضافة الى استقراره كيميائيا.

دراسة تقييم الاستساغة تضمنت 6 متطوعين (3 ذكور و3 اناث). ان تغليف BSO حسن بشكل ملحوظ استساغة الزيت الأصلي جاعلا منه أكثر قابلية للتناول.

APROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

…………………………………..
Abd Almonem Doolaanea
Supervisor

…………………………………..
Farahidah Binti Mohamed
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Science (Pharmaceutical Technology).

…………………………………..
Bappaditya Chatterjee
Internal Examiner

…………………………………..
Haliza Katas
External Examiner

This thesis was submitted to the Department of Pharmaceutical Technology and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

…………………………………..
Muhammad Taher Bin Bakhtiar
Head, Department of
Pharmaceutical Technology

This thesis was submitted to the Kulliyyah of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

…………………………………..
Juliana Bt. Md. Jaffriti
Dean, Kulliyyah of Pharmacy

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

BSO	Black seed oil
TQ	Thymoquinone
HPLC	High performance liquid chromatography
ATR-FTIR	Attenuated total reflection-Fourier Transform infrared spectroscopy
SF	Sphericity Factor
EDTA	Ethylenediaminetetraacetic acid disodium salt dehydrate
DSC	Differential scanning calorimetry
ICH	International Conference on Harmonisation
LOD	Limit of detection
LOQ	Limit of quantification
SD	Standard deviation
RSD	Relative standard deviations

LIST OF SYMBOLS

°	degree
p	P value
μ	micro
±	plus or minus
R^2	linear correlation coefficient

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Black seed is one of the Prophet Mohamed (s.a.w) medicines (At'tib Annabawi). The Prophet stated that black seed (habatussawda) had healing for everything except death (Ali et al., 2003). Black seed has several beneficial influences related to humans especially children. New studies went to support that black seed as a potential candidate against neurodegeneration related diseases, and it has a positive effect on learning and memory (Sahak et al., 2016). In addition to the above, there is enough information to confirm that black seed oil (BSO) is useful in allergy and inflammation (Gholamnezhad et al., 2015) and in the prevention of diseases (Rahmani et al., 2015). It is very important to say that its benefits are confirmed for children suffering some widely spread diseases like epilepsy (Bhandari, 2014). Several studies showed that BSO could be worth further studies as a product with new several healthy uses (Cheikh-Rouhou et al., 2007).

It is known that children usually have problems in swallowing capsules (Kluk et al., 2015) so, a method to give BSO to children is still lacking. Nowadays, BSO is available commercially as capsule (hard or soft) or in its original oil form with unpleasant taste. To facilitate the administration of BSO to children, this study would develop a new taste masking formulation relying on encapsulation technologies. Sodium alginate is a biomaterial that is widely used for encapsulation purpose and other applications in biotechnical engineering due to its practical applications (K. Y. Lee et al., 2012).

Patel et al. (2013) illustrated that, alginates encapsulation provides physical barrier between drug or supplement and the taste buds, resulting in the masking of the unpleasant taste. From a study done by Radhika et al. (2012), they proved the taste masking ability of alginates without causing any chemical modifications during the beads preparation. In addition, the encapsulation by alginates may not only mask the taste, but also improve the solubility of BSO, thus enhancing its bioavailability. Mallappa et al. (2015) claimed that alginates encapsulation could improve solubility, absorption, and bioavailability of poorly water soluble aceclofenac sodium, that is in agreement with this research because BSO also is poorly water soluble. In summary, alginate encapsulation offers taste masking opportunity as well as improves solubility and bioavailability of BSO.

1.2 PROBLEM STATEMENT

There are many pharmaceutical dosage forms of BSO, but not all of them are suitable for children. Materials that tastes bitter and/or irritates the mouth and throat are aversive to children. Bad taste of drugs and supplements like bitterness can be avoided by many methods like formulation of drug in pill or tablet form for adults, but for children this can be problematic, as they can not swallow solid dosage forms. BSO as a good supplement for children is not available in suitable dosage form. Currently, it is available as an oil or capsule. Therefore, this research would try to find a suitable method to mask the taste of BSO by the encapsulation in alginate beads that can be taken by children.

1.3 RESEARCH HYPOTHESIS

The encapsulated BSO in alginate beads will significantly reduce the unpleasant taste of the original oil. Thus, the beads successfully mask the taste of the oil.

1.4 RESEARCH OBJECTIVES

This study aimed to mask BSO taste, and this is the general objective of this research, hence, the study embarks the following specific objectives:

- To characterize BSO in terms of thymoquinone content using HPLC method.
- To formulate and characterize the alginate beads containing BSO.
- To determine the stability of the beads.
- To evaluate the taste masking efficacy in human subjects.

1.5 STUDY DESIGN

Following the introduction and the literature review chapters, this project is divided into four main steps. Each step was written in a form of complete manuscript included with its related methods, results and discussion. First step is a validation of stability-indicating method for thymoquinone analysis in BSO, represented here in Chapter 3. The second step is formulation and characterization of BSO-alginate beads, represented in Chapter 4. To assess stability of the fabricated BSO-alginate beads after encapsulation and stability of thymoquinone in encapsulated BSO, the beads were subjected to stability study. Thus, the third step is stability study of BSO loaded in alginate beads, represented here in Chapter 5. The final step is a human evaluation of palatability and taste masking of BSO-alginate beads, represented as Chapter 6.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Uses of herbal medicines are increasing day by day, where the herbal extracts are used for many therapeutic purposes such as antidepressants, immune system fortifiers, anti cancer, and in wide range of other diseases (Mohammadi et al., 2017). The interested researches in field of herbal medicines have shown that 60% of people with cancer use herbal medicine in addition to conventional cancer treatments (Mohammadi et al., 2017). According to the World Health Organization, 80% of people in developing countries depend on the alternative medicine to reach or supplement the basic health needs (Bonifácio et al., 2014).

Among various herbal medicines, black seed (*Nigella sativa*) is going up to appear as a miracle herb through a lot of pharmacological studies (Ahmad et al., 2013). In addition to that, black seed oil (BSO) has been widely used for therapeutic purposes throughout the world and through centuries (Ahmad et al., 2013). However, BSO has pungent and bitter taste (Padhye et al., 2008), that will make it difficult to be administrated orally to children. Presenting medications to children requires real challenges in terms of taste, smell, and texture feeling in mouth (Venables et al., 2015). The challenge is to find formulation that is able to achieve all these conditions without to be as capsule or tablet because it is difficult for children with age less than 6 years to take these forms orally (Kluk et al., 2015). Generally, alginate is one of polymers which used to encapsulate drugs through producing cross-linked beads in addition to its ability to mask their taste (Liu et al., 2017).

2.2 BLACK SEED AND BLACK SEED OIL

Black seed or *Nigella sativa Linn* comes from Ranunculaceae family. It is an annual plant that has been generally used as a part of Indian culture, Arabian countries and the countries bordering the Mediterranean Sea for therapeutic purposes as a treatment for a considerable number of ailments including asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and flu (Ali et al., 2003). Based on Islamic culture, black seed is able to heal all illnesses except death (Ali et al., 2003). Moreover, it is important to mention that thymoquinone (TQ) is an active component extracted from black seed and has been investigated for its anti-oxidant, anti-inflammatory and anticancer activities in both *in vitro* and in vivo models (Woo et al., 2012). BSO has very important therapeutic uses especially for paediatric and geriatric such as its positive effect on the learning and memory reinforcing (Sahak et al., 2016).

Black seed is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves. Color of the soft flowers is white, yellow, pink, pale blue or pale purple. Petals of the flowers are ranged from 5 to 10. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds (Figure 2.1) (Ahmad et al., 2013; Goreja, 2003).

Figure 2.1 Plant black seed (*Nigella sativa*), flower and seeds
(Sourced from Ahmad et al. (2013))

2.2.1 Black Seed and Black Seed oil as Complementary Medicine

The use of *Nigella sativa* or more commonly known as black seed plant, as a complementary medicine has dated back to the earlier centuries where its historical uses have been recorded and mentioned in numerous religious and ethnics books such as the Holy Bible and Hippocrates (Saad et al., 2011). It is considered as an important drug in the Indian traditional medicinal systems such as Ayurveda and Unani, as well as other medicinal systems, the medicinal values of black seed have been widely acknowledged throughout the world as a miracle herb with many healing properties. This emergence of black seed as a miracle herb with a rich historical and religious background has led to many researches revealing its wide spectrum of pharmacological potential which in turn allows for extensive research studies to be carried out (Ahmad et al., 2013).

However, more studies are still needed to fully understand its mechanisms. The medicinal properties of black seed are known to be attributed to the chemical compositions that are mainly concentrated in its seeds and oils (S. Desai et al., 2015). Native to the Southern Europe, South Africa and Southwest Asia, black seed is cultivated all around the world where for thousands of years, the seeds and oil of

Nigella sativa have been used to treat various ailments such as headaches, toothaches and nasal congestions as practiced during the ancient Egyptian and Greek (Hajhashemi et al., 2004). Also recorded, the ancient Egyptian and Greek physicians also prescribe BSO for intestinal worm as well as a diuretic to promote menstruation and milk production in women. Meanwhile, in Ayurvedic medicine, the seeds are commonly administered with butter milk to obstinate hiccups and treat loss of appetite and vomiting. They are also used in large doses as an abortifacient or in different combinations to treat obesity and dyspnea (Tembhurne et al., 2014). As for the Arabic medical system, black seeds are mainly regarded as a valuable remedy for hepatic and digestive disorders. Through these systems, Avicenna, or Ibn Sina, also wrote in his most famous volumes called The Canon of Medicine, where he referred to Nigella as the seed that stimulates the body's energy and helps recovery of fatigue and dispiritedness (Ahmad et al., 2013) . Traditionally, in folk medicine, it is claimed to be used as a diuretics, anti-helminthic agents, antihypertensives and immunity enhancers to fight infections as well as for the treatment of a variety of disorders, diseases and conditions related to the respiratory system, digestive tract, kidney and liver function (Sharma et al., 2000).

Black seeds are also commonly given orally either with food or mix with honey. This may be because black seed has a slightly aromatic smell but bitter taste when consumed in which many may not be tolerable towards it. The bitter taste of black seed is thought to be due to the presence of active compound nigellin in its structure (An, 2014). However, there are limited studies in relation to the bitter taste of BSO, its mechanism and the best masking methods for its bitter taste. Therefore, efforts to mask the bitter taste of BSO have been made in the current study.

2.2.2 Composition and Physicochemical Properties of BSO

Black seed contains many active components, such as thymoquinone (TQ), alkaloids (nigellicines and nigelledine), saponins (alpha-hederin), flavonoids, proteins, fatty acids, and many others, that have positive effects in the treatment of patients with different diseases. TQ is the most abundant constituent in the volatile oil of black seeds, and most of the herb's properties are attributed to it (refer). Black seed contains both fixed oil and volatile oil. Fixed oil constitutes about 30% and volatile oil as 0.4-0.45% of the seeds. However, TQ constitutes about 18.4-24% of the volatile oil (Aboutabl et al., 1986). Cheikh-Rouhou et al. (2007) studied the properties of two black seed varieties, from Tunisian and Iranian origins. Physical and chemical analyses were done on crude oils extracted by cold method from seeds. The resulting amount of extraction oil (on a dry-weight basis) for Tunisian and Iranian sorts showed: 28.48% and 40.35%, respectively. The main unsaturated fatty acids were linoleic acid (49.2 to 50.3%), followed by oleic acid (25.0 to 23.7%), while the main saturated fatty acid was palmitic acid (17.2 to 18.4%). Myristic, myristoleic, palmitoleic, margaric, margaroleic, stearic, linolenic, arachidic, eicosenoic, behenic and lignoceric acids were also detected.

Physiochemical properties of the oils for Tunisian and Iranian varieties, exists: saponification number 211 and 217, peroxide value 5.65 and 4.35, iodine value 120 and 101, and an acid value of 22.7% and 18.6% , respectively (Cheikh-Rouhou et al., 2007). Proximate analysis of Saudi black seeds showed a composition of 20.85% protein, 38.20% fat, 4.64% moisture, 4.37% ash, 7.94% crude fibre and 31.94% total carbohydrates. Potassium, phosphorus, sodium and iron were the predominant elements present. Zinc, calcium, magnesium, manganese and copper were found at lower levels. However, lead, cadmium and arsenic were not detected in the seeds.

Linoleic and oleic acids were the major unsaturated fatty acids while palmitic acid was the main saturated one. Glutamic acid, arginine and aspartic acid were the main amino acids present while cystine and methionine were the minor amino acids. These results indicate the high nutritional potential of black seeds especially as a source of protein and fat. The total aerobic bacterial count was 7 × 107 cfu/g and the yeast and mould counts were 4 × 102 cfu/g. The low numbers observed for *Staphylococcus aureus* and *Bacillus cereus* make black cumin seeds acceptable, without any associated health hazard (Al-Jassir, 1992).

2.3 ENCAPSULATION OF ACTIVE PHARMACEUTICAL INGREDIENTS

Encapsulation or in specific term microencapsulation has been established since 1950s (Fang et al., 2010). It can be defined as a means to envelope or surrounds the solids, liquids or gaseous materials in sealed capsules that have ability to release their contents at controlled rates with specific conditions (K. G. H. Desai et al., 2005). Microencapsulation also can be considered as a method of exerting a thin coating to small particles solids or droplets of liquids. The packaged materials which can be called as coated material or the internal phase can be either pure material or a mixture. On the contrary, the wall material or packaging material can be built by synthetic polymers, natural and modified polysaccharides as well as lipids, proteins, sugars and gums (Mozafari et al., 2008).

In general, there are three steps involved in encapsulation technique. Firstly, the development and generation of the wall around the material which need to be encapsulated. Then, undesired leakage must be avoid from occur. Lastly, to avoid unwanted materials from the bioactive agents (Mozafari et al., 2008). Basically, encapsulation can be done by several techniques such as spray drying, extrusion,