characterization of encapsulated berberine in yeast cells of saccharomyces cerevisiae

roshanak salari, omid rajabi, zahra khashyarmanesh, mohsen fathi najafi and biibi sedigheh fazly bazzaz

department of drug and food control, school of pharmacy, mashhad university of medical sciences.

targetted drug delivery research centre, school of pharmacy, mashhad university of medical sciences, mashhad, iran.
department of veterinary research and biotechnology, razi vaccine and serum research institute, mashhad, iran.

abstract

berberine was loaded in yeast cells of saccharomyces cerevisiae as a novel pharmaceutical carrier to improve the treatment of many diseases. the yeast-encapsulated active materials showed high stability and bioavailability due to the enhanced solubility and sustained releasing. in this study, different characteristics of prepared berberine loaded yeast cells (loading capacity, release kinetic order, mic and stability) were evaluated by different analytical methods (fluorescence spectroscopy, hplc and sem). the loading capacity was about 78% ± 0.6%. berberine release patterns of microcapsules happened in two different stages and followed by zero and first-order kinetic, respectively. about 99% of all active material released during 34 hours. mic was improved by berberine loaded microcapsules in comparison with berberine powder. the microcapsules were completely stable. berberine loaded sac. cerevisiae could be considered as a favorite sustained release drug delivery system. the yeast would be applied as an efficient carrier to improve various properties of different active materials.

keywords: berberine; kinetic; mic; microencapsulation; yeast cells.

introduction

in every encapsulation process, physical characteristics of the product for example encapsulation efficiency, the release kinetic of active material, etc should be determined and optimized for better drug encapsulation (1). kinetics is the study of rates of different reactions. it provides information about the reaction’s mechanism by mentioning mathematical models which describe the characteristics of a chemical reaction. kinetics means how various conditions can affect the speed of a chemical reaction. kinetic studies for pharmaceutical dosage forms in medicine is very important because the final results help us to know that how many times a drug should be prescribed a day. patients are more familiar with such medicines which are prescribed once a day (2).

microencapsulation is a process that is applied a lot in pharmaceuticals, cosmetic and food industries due to its extension of shelf-life, protection against oxidation and control release of active component (3). microencapsulation nowadays developed in many fields of sciences.
and different drug and food industries. It is known as a process in which small active compounds are surrounded by another material, which is called coat or carrier. Encapsulation causes stabilization to the active materials since the wall material acts as a physical barrier to protect the active compound. Besides, the material can be released in a controlled way in product applications (4).

The structure of yeast cell wall made it an excellent encapsulating material and its natural properties caused many advantages over other microencapsulation carriers (5). Yeast-cell-based microencapsulation process has been applied in the encapsulation of different materials such as essential oils and flavors (6). Baker’s yeast (Saccharomyces cerevisiae) has been proved as an appropriate host for the development of a new kind of drug delivery system (7).

Sac. cerevisiae yeast cells can be regarded as food-grade and low cost food materials (8). Their phospholipid membranes could behave as liposomes and have been used for the encapsulation of different molecules with different lipophilicity (9). The yeast cell wall composed of beta glucan network and a small amount of chitin with a mannanprotein layer which made it more beneficial comparing to other carriers. The wall allows molecules to diffuse conveniently (10).

Berberine has many pharmacological properties. Several pharmacological studies have indicated the cardiovascular effects of berberine against ischemia induced by ventricular tachyarrhythmia. It is used to treat cardiac contractility and decreasing blood pressure (11, 12). Berberine could improve the damaged heart function of CHF rats (13), has high capacity in antiplatelet aggregation in patients (14) and it shows immunosuppressive influences (15). Recent report showed berberine could be a good candidate for further studies as a new anticancer drug in the treatment of human breast cancer (16).

Many studies have been shown antiinflammatory activity for Berberis vulgaris and its most alkaloid, berberine, but the exact mechanism is unknown (17, 18). B. vulgaris fruit (barberry) was administered for its sedative effect in traditional medicine (19). It is reported that the intake of 3 g/d B. vulgaris fruit extract for 3 months may have beneficial effects on different factors in type 2 diabetic patients, such as lipoproteins, apoproteins, glycemic control and TAC (20). Berberine has usually been used as an antihyperglycemic medicine in China (21). Berberine can be used as an expectorant according to its ability to increase mucin release (22). Berberine has been known to cure diarrhea in different population (23). Berberine low solubility and its instability to environmental conditions are the major problems that prevent its consumption in various industries (24, 25).

In this work, the physical characteristics of berberine loaded microcapsules were studied.

Experimental

Materials

Saccharomyces cerevisiae lyophilized powder was purchased from Industrial Research Center (PTCC No. 5269), Tehran, Iran. Berberine hydrochloride was obtained from China (XI ANRongsheng Biotechnology CO., LTD). Double distilled water was prepared to carry out the experiments. Trytonte soya broth (TSB) culture medium was purchased from Himedia, India. Merck brand of tetrazolium salt was used in the experiments.

Preparation of microcapsules

Microcapsulation was performed with some modification according to the method by Paramera, et al (8). The freeze dried yeast cells (7 mg) were suspended in a flasks containing 10 ml berberine in water solution (275 µl/ml) at 45°C. The flasks were stirred at 200 rpm for 72 h and then centrifuged (7000 rpm, 15 min). The precipitants were washed three times to remove the free and excess berberine. Then the microcapsules which loaded with berberine were freeze dried.

Preparation of standard solutions to plot the standard curve

Berberine standard solutions were prepared by dissolving different amounts of berberin in double distilled water to obtain five standard concentrations. The concentrations were 500, 400, 300, 250, 125, 62.5 and 31.25 microgram (µg). The
fluorescence emissions of these concentrations were measured and the standard curve was plotted.

**Determination of loading capacity**

To define the encapsulation efficiency, 7 mg berberine loaded microcapsules was dissolved in 10 ml double distilled water. This suspension was stirred (200rpm) for 48 h. The final supernatant was centrifuged and the fluorescence emission (Shimadzu spectrophotometer RF-540) was determined. Based on the standard curve, the concentration of loaded berberine was determined.

**Release kinetic studies**

Microcapsules (7 mg) (equal to 215 µg/ml pure berberine) were suspended in 10ml of water in a flask (kinetic flask). The flask was put on a stirrer (100 rpm) for 48 h. The samples (100 µl) were centrifuged at predetermined time points and the supernatant was separated. The precipitated microcapsules were suspended in 100 µl distilled water and returned back into the flask(26). The supernatants were then analyzed by fluorescence method.

In fluorescence spectrophotometry method, measurements were carried out in 520 nm (Ex: 375 nm). According to the standard curve, the concentration of free berberine in each sample was determined. Due to higher sensitivity of fluorescence method, mathematical calculations to estimate berberine release order carried out based on fluorescence data. Matlab mathematical software was used to define the equations of all curves.

Effect of pH on berberine release process was studied by introducing 0.5 ml 0.01 M HCl to the kinetic flask. The experiment was carried out in the way mentioned above.

**Antimicrobial activity measurement**

Minimum inhibitory concentration (MIC) was evaluated for berberine as an active material, berberine loaded microcapsules and physical mixture of berberine and yeast cells. Three organisms (Staphylococcus aureus(PTCC 1337), Pseudomonas aeruginosa(PTCC 1707) and Esherichia coli(PTCC 1330)) were used to estimate the MICs. 20 µl of 10^6 suspensions of each microorganism was added to 200 µl of three antimicrobial agents (berberine, berberine loaded microcapsules and physical mixture of berberine and yeast cells) in different pure berberine concentrations (500, 400, 300, 200, 100, 75, 50, 37.5 µg/ml) in separate wells of 96 well microplate. The plate was incubated for 24 h. After incubation, 50 µl of tetrazolium salt (C_19H_15ClN_4) solution (5mg/ml) was added to each well. Then incubation was done for 45 min. The well before the one that the color of its content became red, showed us MIC.

To define the Minimum bactericidal concentrations (MBC), 20 µl of all concentrations higher than MIC which showed no microorganisms growth, were added to 200 µl TSB culture medium in separate wells. The microplate was incubated for 24 hours. Tetrazolium salt solution was used to distinguish the live microorganisms that they were not killed, and in fact only their growth was inhibited by the active material. The well before the one that its red color was appeared, indicated MBC concentration. These experiments were repeated for three times.

**Stability of microcapsules**

The stability of microcapsule powder was studied according to ICH time points (0, third month, sixth month and a year) by HPLC (Waters, 600 controllers) analytical method. The analysis was performed on a C_18 column with phosphate buffer 0.02 mol/L (pH 5)-acetonitrile (75:25) as mobile phase at a flow-rate of 1.0 ml/min, with UV/Visible detection at 340 nm. The pure berberine concentrations in the samples were 300µg/ml. The suspensions were centrifuged several times then supernatants (berberine solutions) were collected.

**SEM images**

Scanning electronic microscopy (Oxford Company, S-360) was used to study the cell wall morphological differences between the yeast cells and berberine loaded yeast cells. The yeast cells and microcapsules were embedded in paraffin, sectioned, de-paraffin, and sputter-coated with gold.
Results and Discussion

In the previous study, we proved the entry of berberine inside the yeast cells by different analytic techniques\(^{(27)}\). In this study only the physical characterization of this drug dosage form was determined.

In order to evaluate the loading capacity and the release mechanism, fluorescence spectrophotometry was applied. The standard curve was drawn based on the standard concentrations of berberine (Table 1) (Figure 1).

**Loading capacity**

The fluorescence emission of berberine solutions which obtained by degradation of microcapsules was 31.9% (concentration = 215 \(\mu\)g/ml). According to standard curve, the loading capacity (Equation 1) is about 78% \(\pm\) 0.6% (215/275 \(\mu\)g/ml \(\times\) 100).

\[
\text{%Loading capacity} = \frac{C}{C_0} \times 100 \quad (\text{Equation 1})
\]

In equation 1, \(C\) refers to the concentration of released berberine by degradation of microcapsules and \(C_0\) is the concentration of the berberine solution used for encapsulation process. Due to yeast cells availability, low cost production process and the high loading capacity, the yeast cells could be applied as beneficial natural carriers to load active materials. Their unique structural properties like the beta glucan network cause the active material to release in a controlled way and it could protect the materials against any harmful environmental factors. In comparison with other studies, 78% loading capacity is high and completely acceptable\(^{(8)}\).

**Release kinetic studies**

The release profile of berberine was studied and the curves equations were calculated by Matlab software. The equations which were obtained by Matlab software and showed below each curve, describe the release profile of berberine in mathematical formula.

Berberine released in two stages (Figure 2). In the first stage (0-4 h), a sharp slope was observed. This sharp slope at the beginning of berberine release curve was represent of zero-order kinetic pattern which is independent of berberine concentration \(^{(28)}\) (Figure 3) (Equation 2).

Zero order equation \(\quad (\text{Equation 2})\):

\[
y = 0.1467x + 0.3466 \quad R^2 = 0.9995
\]

---

**Table 1.** Berberine standard concentrations data.

| Concentration (\(\mu\)g/ml) | 0     | 31.25 | 62.5 | 125 | 200 | 250 | 300 | 400 | 500 |
|-----------------------------|-------|-------|------|-----|-----|-----|-----|-----|-----|
| % Flourescence              | 0     | 4.6   | 10.5 | 19  | 29.7| 36  | 44.3| 59.2| 74  |
| SD                          | 0.21  | 0.33  | 0.2  | 1   | 0.41| 0.37| 1   |     |     |
Figure 2. Release profile of encapsulated berberine (First equation under the curve is based on berberine%Flourescence and the second one is based on berberine concentration (µg/ml)).

Figure 3. Diagram of released berberine concentrations in the first 4 hours (SD = ± 1%).
In Equation 2, $Q_t$ is the amount of drug dissolved in time $t$, $Q_0$ is the initial amount of drug in the solution (most times, $Q_0 = 0$) and $K_0$ is the zero order release constant expressed in units of concentration/time.

Then the rate of drug release was slowed down but continued until 99% of the active material (Figure 2) released in 34 hours. This pattern of release followed first-order kinetic which is dependent on berberine concentrations (28). The diagram of Log concentration against time is linear about 12 hours after releasing which confirms that the second stage obey first-order kinetic (Figure 4) (Equation 3).

First order equation (Equation 3):

$$\log C = \log C_0 + \frac{Kt}{2.303}$$

In equation 3, $C_0$ is the initial concentration of drug, $K$ is the first order rate constant, and $t$ is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of $K/2.303$.

In the first stage, berberine release pattern obeys passive diffusion mechanism and follows zero-order kinetic. Besides, the second stage follows first-order kinetic which is dependent on berberine concentration and happens by the same passive mechanism. These observations show that this kind of drug delivery system could be applied as a sustained release one (29). These microcapsules could be used as kind of natural preservative to protect foods against deterioration process outside the refrigerator for about 34 hours. Effect of other parameters such as pH was evaluated too. Some foods are sour because of their acidic nature. Acidity affects the structure of yeast cells membranes and cell walls. As a result, the acids facilitated berberine release pattern and the sustained release property of yeast cells would be affected. Diagram of berberine release in acidic environment was compared with the one in neutral environment (Figure 5).

Acids could extract cell wall beta glucan network so they would cause damages to natural structure of yeast cell walls. By introducing microcapsules to acidic environment, the time period that the whole berberine released would decrease to 20 hours. It means one of the most significant properties of this carrier as the sustained release one would be damaged. But the release pattern obeys the order in neutral solution. In acidic release profile, in the first stage, zero-order release kinetic happened.
Following that, releasing is dependent on first order kinetic but it was completed up to 20 hours. The mathematical equations below each curve showed us the parameters that the release order is dependent on them.

**Antimicrobial activity measurement**

Berberine loaded microcapsules improve minimum inhibitory concentrations of berberine against *Staphylococcus aureus* and *Escherichia coli* from 200 to 100 µg/ml and 100 to 75 µg/ml respectively in comparison with other antimicrobial samples. But no difference in MIC was seen for *Pseudomonas aeruginosa* (300 µg/ml). Physical mixture of berberine and yeasts cells showed the same MIC as berberine alone. The red color in wells of microplate is represented of the oxidation reaction between the live organism and tetrazolium salt. Encapsulation of berberine in yeast cells improves berberine penetration to the structure of microorganisms. The results show that *P. aeruginosa* might be the most resistant microorganism against berberine and its derivatives. Minimum bactericidal concentration was the same in all samples. MBCs were evaluated 300 µg/ml for *Staph. aureus* and *E. coli* and 500 µg/ml for *P. aeruginosa*. Berberine kills microorganisms by different mechanisms such as its destructive effects on DNA and RNA structures or proteins biosynthesis (30).
Microcapsules stability studies

HPLC chromatogram of loaded berberine in microcapsules is shown in figure 6.

Berberine retention time is about 16 min. The chromatograms (Figure 6) and the data (Table 2) showed that all berberine peaks are approximately the same and the microcapsules were stable after one year of production. The small first peak is due to the impurities of berberine. All characteristics of berberine chromatograms released from microcapsules were the same as pure berberine solution chromatogram. The stability of berberine against light, oxidation and other environmental parameters confirmed the efficiency of yeast cells as carriers.

SEM images

The SEM images of yeast cells (a) and berberine loaded yeast cells (b) are shown in figure 7.

SEM technique was applied to study the effect of encapsulation on the surface structure of yeast cells. The SEM images (Figure 7) showed no significant differences in cell wall morphological properties of the yeast cells and berberine loaded yeast cells. In fact, microencapsulation process did not affect the cell wall organization.

Berberine is the most important alkaloid of Barberry. Its pharmacological effects play an important role in treatment of many diseases. The unfavorable properties of berberine powder are its poor water solubility and susceptibility to different environmental conditions (31). It seems reasonable to find a new carrier to improve these unfavorable properties.

Baker’s yeast (Sac. cerevisiae) could be introduced as a novel kind of drug delivery system. This yeast cell developed a low cost microencapsulation process. The yeast cells as carriers improve the active material shelf-life and protection against oxidation and provide control release drug dosage form. The structure of yeast cell wall and its natural properties made it a unique one over other microencapsulation carriers(32).

Conclusion

The loading capacity, release kinetic pattern, MIC and stability studies showed that yeast cell of

| Table 2. Berberine release HPLC chromatograms data. |
|---------------------------------|---|---|---|---|
| Time (month) | RT. | Area | %Area | Height | SD |
| 0 | 17.13 | 457.13 | 98.73 | 1.52 | ± 2% |
| 3 | 16.46 | 442.27 | 98.68 | 1.61 | ± 1.9% |
| 6 | 16.36 | 430.87 | 98.87 | 1.63 | ± 2.2% |
| 12 | 15.6 | 425.23 | 98.62 | 1.58 | ±1.8% |
| Pure berberine | 17.07 | 461.05 | 98.75 | 1.55 | ±1.9% |
*Sac.cerevisiae* as a carrier could modify unwanted properties of berberine powder and caused a sustain release and stable drug delivery system.

**Acknowledgement**

This work was supported by a grant from Mashhad University of Medical Sciences Research Council, Mashhad, Iran. The authors wish to thank the authorities in University, Biotechnology Research Center and School of Pharmacy for their support. This project was part of Roshanak Salari (PhD Student) thesis. The authors have no conflicts of interest.

**References**

(1) Shariat Sh, Badiee A, Jaafar MR, Mortazavi SA. Optimization of a method to prepare liposomes containing HER2/Neu- derived peptide as a vaccine delivery system for breast cancer. *Iran J Pharm Res.* (2014), 13 (supplement): 15-25.

(2) Levy G. Kinetics of drug action: an overview. *J. Allergy Clin. Immunol.* (1986) 78: 754-61.

(3) Schrooyen PMM, van der Meer R and De Kruijf CG. Microencapsulation: its application in nutrition. *Proc. Nutr. Soc.* (2001) 60: 475-479.

(4) Madene A, Jaquot M, Scher J and Desobry S. Flavour encapsulation and controlled release: A review. *Int. J. Food Sci. Tech.* (2006) 41: 1-21.

(5) Nelson G. Application of microencapsulation in textiles. *Int. J. Pharm.* (2002) 242: 55-62.

(6) Pannell NA. Microencapsulation in microorganisms. *Eur. Patent* (1990) EP 0242135, B1.

(7) Blanquet S, Marol-Bonnin S, Beyssac E, Pompon E, Denaud M and Alric M. The biodrug concept: an innovative approach to therapy. *Trends Biotechnol.* (2001) 19: 393-400.

(8) Paramera EI, Konteles SJ and Karathanos VT. Microencapsulation of curcumin in cells of *Saccharomyces cerevisiae*. *Food Chem.* (2010) 125: 892-902.

(9) Normand V, Dardelle G, Bouquerand P, Nicolas L and Johnston DV. Flavor encapsulation in yeasts: Limonene used as a model system for characterization of the release model. *J. Agric. Food Chem.* (2005) 53: 7532-7543.

(10) De Nobel JG, Klis FM, Munnik T, Priem J and Van Den Ende H. An assay of the relative wall porosity of *Saccharomyces cerevisiae, Kluyveromyces lactis* and *Schizosaccharomyces pombe*. Yeast (1990) 6: 483-490.

(11) Chun YT, Yip TT, Lau KL, Kong YC and Sankawa U. A biochemical study on the hypotensive effect of berberine in rats. *Gen. Pharmacol.* (1979) 10: 177-182.

(12) Marin-Neto JA, Maciel BC, Secches AL and Gallo L. Cardiovascular effects of berberine in patients with severe congestive heart failure. *Clin. Cardiol.* (1988) 11: 253-260.

(13) Huang CG, Chu ZL, Wei SJ, Jiang H and Jiao BH. Effects of berberine on arachidonic acid metabolism in rabbit platelets and endothelial cells. *Thromb. Res.* (2002) 106: 223-227.

(14) Feng CL, Liu SX, Feng QY, Yin JJ and Zhao L. A comparative study of antiplatelet aggregation by berberine hydrochloride with low dose aspirin. *Shandong. Med.* (1996) 36: 11-12.

(15) Marinova EK, Nikolova DB, Popova DN, Gallacher GB and Ivanovska ND. Suppression of experimental autoimmune tubulointerstitial nephritis in BALB/c mice by berberine. *Immunopharmacology.* (2000)48: 9-16.

(16) Barzegar E, Fouladdel Sh, Komelii Movahhed T, Atashpour Sh, Ghahrehmani MH, Ostad SN, Azziz E. Effects of berberine on proliferation, cell cycle distribution and apoptosis of human breast cancer T47D and MCF7 cell lines. *Iran J. Basic Med. Sci.* 2015; 18:334-342.

(17) Yesilada E and Küpeli E. Berberiscrataegina DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *J. Ethnopharmacol.* (2002) 79: 237-248.

(18) Küpeli E, Kosar M, Yesilada E, Hüsni K and Baser C. A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish Berberis species. *Life Sci.* (2002) 72: 645-657.

(19) Fatehi-Hassanabad Z, Jafarzadeh M, Tarhini A and Fatehi M. The antihypertensive and vasodilator effects of aqueous extract from Berberis vulgaris fruit on hypertensive rats. *Phytother. Res.* (2005) 19: 222-225.

(20) Shidfar F, SeyyediEbrahimshia H, HosseiniSh, Heydari I, ShidfardSh, Hajhassanid G. The effects of *Berberis vulgaris* fruit extract on serum lipoproteins, apoB, apoA-I, homocysteine, glycemic control and total antioxidant capacity in type 2 diabetic patients. *Iran J Pharm Res* (2012), 11 (2): 643-652.

(21) Yin J, Hu R, Chen M, Tang J, Li F, Yang Y, Chen J. Effects of berberine on glucose metabolism in-vitro. *Metabolism* (2002) 51: 1439-1443.

(22) Lee CJ, Lee JH, Seok JH, Hur GM, Park YC, Seol IC, Kim YH. Effects of baicalin, berberine, curcumin and hesperidin on mucin release from airway goblet cells. *Planta Med.* (2003) 69: 523-526.

(23) Lin SS, Chung JG, Lin JP, Chuang JY, Chang WC, Wu JY, Tyan YS. Berberine inhibits arylamineN-acetyltransferase activity and gene expression in mouse leukemia L 1210 cells. *Phytomedicine* (2005) 12: 351-358.

(24) Gibbs BF, Kermasha S, Alli I and Mulligan N. Encapsulation in the food industry: A review. *Int. J. Food Sci. Nutr.* (1999) 50: 213-224.

(25) Sadeghi F, Torab M, Khattab M, Homayouni A and
Afrasiabi Garekani H. Improvement of physico-mechanical properties of partially amorphous acetaminophen developed from hydroalcoholic solution using spray drying technique. *Iran. J. Basic Med. Sci.* (2013) 16: 1100-1108.

(26) Shi G, Rao L, Yu H, Xiang H, Yang H and Ji R. Stabilization of photosensitive resveratrol within yeast cell. *Int. J. Pharm.* (2008) 349: 83-93.

(27) Salari R, Fazly Bazzaz BS, Rajabi O and Khashayarmanesh Z. New aspects of Saccharomyces cerevisiae as a novel carrier for berberine. *DARU J. Pharma. Sci.* (2013) 21: 73.

(28) Yadav RC, Kumar GS, Bhadra K, Giri P, Sinha R, Pal S and Maiti M. Berberine, a strong polyriboadenylic acid binding plant alkaloid: spectroscopic, viscometric, and thermodynamic study. *Bioorg. Med. Chem.* (2005) 13: 165-174.

(29) Chang CH, Huang WY, Lai CH, Hsu YM, Yao YH, Ting-Yu Chen TY, Wu JY, Peng SF and Yu-Hsin Lin YH. Development of novel nanoparticles shelled with heparin for berberine delivery to treat *Helicobacter pylori*. *Acta Biomaterialia* (2011) 7: 593-603.

(30) Jian-ling J, Guo-qiang H, Zhen M and Pei-ji G. Antibacterial mechanisms of berberine and reasons for little resistance of bacteria. *Chin. Herb Med.* (2010) 3: 27-35.

(31) Imanshahidi M and Hosseinzadeh H. Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. *Phytother. Res.* (2008) 22: 999-1012.

(32) Omara WAM, Rash BM, Hayes A, Wickham M, Oliver SG and Stateva LI. Conditional cell-wall mutants of Saccharomyces cerevisiae as delivery vehicles for therapeutic agents *in-vivo* to the GI tract. *J. Biotechnol.* (2010) 147: 136-143.

This article is available online at http://www.ijpr.ir