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Original Article

Formulation and preparation of oil in water cream sample based on OPH enzyme and evaluate enzyme performance in the cream

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Organophosphorus hydrolase enzyme commonly named OPH is listed in phosphoric triester hydrolases group (EC: 3.1.8). OPH can hydrolyze a variety of organophosphate pesticides and neurotoxins and has important roles in bioremediation. Organophosphate components are the main group of pesticides that diffusion of them into the soil, wastewater, and groundwater is noticeable. In this study, formulation of oil in water cream is optimized based on activity of OPH enzyme and its stability during the time in cream. OPH enzyme was extracted and purified from strain flavobacterium. The oil in water cream was made by different formulations. The best formulation was selected for making cream, on the basis of visual examination of viscosity and physical stability of cream. 5U/90g enzyme was used for each formulation. The stability of OPH in the creams was investigated in 4 and 30℃. In order to measuring the enzyme activity, reverse extraction method was used, and activity of enzyme was determined based on parathion hydrolysis. Formulation of oil in water cream was optimized. The half-life of the enzyme in the cream was determined and it was more than 50 days in 4℃. The results showed that biological activity of OPH enzyme in cream was conserved and OPH enzyme in cream can be stable almost for two months in suitable condition.

Keywords: Organophosphorus hydrolase enzyme (OPH), Organophosphate components, Emulsion oil in water, enzyme stability

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Introduction

Organophosphate compounds due to having major properties regarding their vital impacts create specific toxicology issues in the industry and in consumption [1]. Most of phosphate or organophosphate toxins were discovered by German scientist Gerhard Schrader during World War II [2]. Organophosphate compounds are one of the most common causes of toxicity and their number is very high, some of them are highly toxic and are considered as nerve gases [3]. The impact of organophosphate compounds is because of their impact on esterase enzyme which acetylcholinesterase is the most important of them. The reactivation of the enzyme varies depending on the type of organophosphate compound and this causes differences in the treatment of severe poisoning resulting from different types of nerve agents. These toxins cause dysfunction in peripheral nervous system, brain nerves and weakness in the skeletal, respiratory and eye muscles [4].

Biodegradability of these factors is highly regarded in the world today. In order to eliminate these pollutions some methods such as chemical procedures, burning and burial at sea are used. These toxins act in humans through inhalation and dermal absorption and disrupt cholinesterase

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activity in the synapses of nerve tissue [4-6]. Organophosphorus hydrolase enzyme (OPH) is an enzyme which can hydrolyze many organophosphate compounds [5]. OPH, as phosphotransacetylase (PTE) resulting from Pseudomonas diminuta and Flavobacterium species is the second hydrolytic enzyme to eliminate large-scale pollution [7]. This stable and globular enzyme is highly regarded in detoxification of organophosphate insecticides and nerve agents [5, 8-10]. To avoid skin penetration of these compounds and according to the hydrolase function of OPH enzyme and negating the organophosphate effects of toxic substances, this enzyme can be used in the cream formulation. This study was designed and carried out to investigate the stability of the OPH enzyme in the sample of o/w1 cream.

Materials and Methods

Applied materials

In this study, for the manufacture of the cream of emulsions o / w type based on OPH enzyme, polyethylene glycol 6000, Span 60 and Tween 60, liquid paraffin, petrolatum gel, beeswax, glycerol, lactylate alcohol, methyl paraben and distilled water were used. Parathion substrate was used to determine the performance of the enzyme. All these compounds had high purity and were manufactured by Sigma, Merck and Floka companies. According to previous research OPH enzyme was isolated and purified from wild strains of Flavobacterium and was used in this study.

Formulation and method of preparing oil-in-water cream

For making this cream, based on table 1 two oily and aqueous phases were separately prepared and heated to 75℃, and using a mechanical stirrer oily phase was gradually and steadily added to the aqueous phase. When the temperature of cream dropped to 30℃, mechanical stirrer was turned off, and the cream was stored at 4℃. The best formula for making cream in Table 2 with an investigation of stability, viscosity and physical form was selected in accordance with the chemical composition of Table 3 [11, 12]. This method of manufacture was used in order to make the cream containing enzyme. The difference is that at 40 a certain amount of enzyme was added to the cream and stirring operation for uniform distribution of the enzyme was done in all parts of the cream for 1 hour. The type of cream was selected to be oil in water emulsion because of the enzyme being hydrophilic. To maintain the stability of the OPH enzyme in the cream, temperature and acidity of cream was controlled. According to the mentioned method for making the o / w emulsion, different types of formulations with various percentages are mentioned in order to make this cream.

Table 1. Necessary materials for making cream

Oil phase	Water phase
Fluid Paraffin	Glycerol
Beeswax	Methyl paraben
Lactylate alcohol	Polyethylene glycol 6000
Petrolatum jelly	Tween 60
Aspen 60	Water

Table 2. Different formulations of oil in water cream Based on the percentage of materials used

Materials	Formulation 1	Formulation 1	Formulation 2	Formulation 3
Fluid Paraffin	10%	10%	10%	10%
Polyethylene glycol 6000	2%	5%	4%	8%
Beeswax	1%	1%	1%	1%
Lactylate alcohol	2%	2%	2%	2%
Petrolatum jelly	5%	5%	5%	5%
Glycerol	8%	5%	5%	5%
Aspen 60	0.86%	0.86%	0.86%	0.86%
Tween 60	2.13%	2.13%	2.13%	2.13%
Methyl paraben	0.22%	0.22%	0.22%	0.22%
Water	68.8%	68.8%	68.8%	65.8%

Table 3. Optimized Formulation of oil in water cream with OPH

Materials	Percent (%W)
Fluid Paraffin	10
Polyethylene glycol 6000	8
Beeswax	1
Lactylate alcohol	2
Petrolatum jelly	5
Glycerol	5
Aspen 60	0.86
Tween 60	2.13
Methyl paraben	0.22
Water	65.8
Enzyme	5 U/90gr

Method of OPH enzyme activity measurement

An enzyme unit of (U) is defined as the amount of enzyme capable of hydrolyzing a micromole of parathion within one minute under optimum conditions [13]. Since PNP has a maximum absorption at a wavelength of nm410 harm in the alkaline environment; its Stock solution with a concentration of 100 mg⁄ml was prepared in phosphate buffer and using it solution at different concentrations (12.5, 25, 50, 60 and 80) were prepared and absorption of the samples was measured at a wavelength of 410 nm and standard PNP absorption - concentration curve was drawn. Equation 1 was used to measure enzyme activity (7)

(1) $U/mL = (\Delta A_{sample} - \Delta A_{blank})/10.6 * 1/t * V_t/V_s$

Vt = the volume in which activity is measured (mL)

Vs = volume of the sample containing the enzyme used (mL)

t = time when absorption changes are measured (min)

ΔA= *Change in absorbance at the relevant time*

U = enzymatic activity (mol / mLμ).

OPH enzyme extraction method from the cream and measuring its activity

In order to extract OPH enzyme from the cream and measuring its activity, from 3 different points of the cream 1 g was removed and using 1 mL of PBS buffer was uniformed and were equally divided in 3 micro-tubes. Micro-tubes with 13000 rpm centrifuges, Liquid phase of samples was extracted, and they were emptied in 3 other micro-tubes, in 3 new micro-tubes 485 µL extracted samples were mixed with 5 µL cobalt (Co2 +) and incubation was performed for 30 minutes. Then

10 µL of parathion Substrate was added to the incubation solution and the reaction time was recorded using a chronometer and then absorption amount was read at a wavelength of 410 nm. A cream with the same formulation but without the OPH enzyme was used as control

Checking the stability of the enzyme in cream

In the Stability testing, cream samples were maintained and incubated at 4 and 30 ^{o} C. Enzymatic activity of cream was measured at different times based on equation [1] and it was compared to zero time in order to calculate its relative activity using the following formula (1, 7)

$$\text{Relative activity}(\%) = \frac{Activity_{time=t}}{Activity_{time=0}}$$

Results

With the variety of formulations made and results of checking the stability and physical form of creams, optimal formulation was prepared containing the ingredients listed in Table 2.

For OPH enzyme to remain stable, this enzyme was added to the cream at a low temperature (40 ° C). According to the formulations made and the results of investigation of physical form of cream and the addition of OPH enzyme at different concentrations and temperatures, 0.37 mL enzyme was added to the optimal formulation. Using equation [1], consumed enzyme unit per 1 mL stoke was calculated and enzyme activity was obtained. In Figure 1 cream stability was evaluated at 4 and 30° C. Applying the mentioned extraction method for o/w cream containing the enzyme is measurable. The results of investigating the stability of cream at 4 and 30 ° C show that stability of the enzyme is reduced while increasing temperature. The greatest resistance was for 4° C which has been stable after 1 month and no significant reduction has been observed in enzyme activity

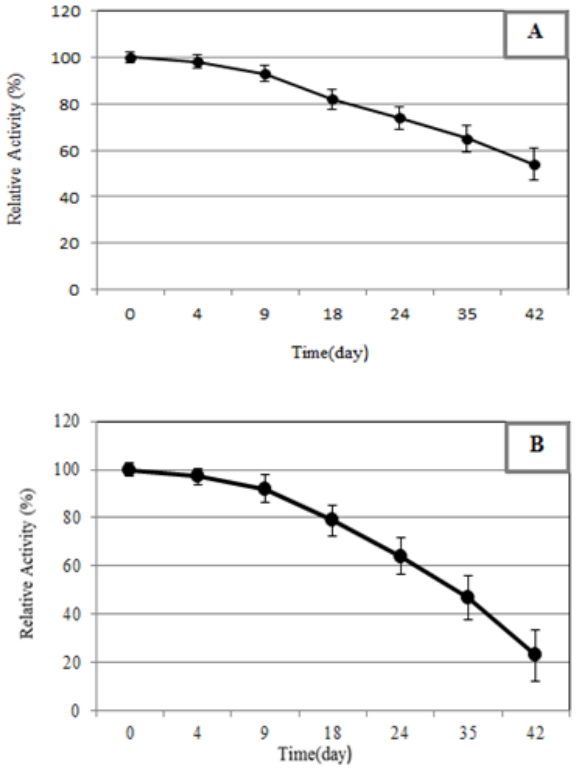

Figure 1. The measurement of thermal stability
A. Stability at 4 ° C.
B. Stability at 30 ° C.

Discussion

In the studies of cream preparation, using HLB system and calculations based on RHLB fatty substances and surfactants, the amount of ingredients in the formulation was determined by

testing and checking the physical appearance, viscosity and stability of the cream. And optimal formulation of o/w cream containing the enzyme was determined in accordance with Table 2. The stability of the o / w cream was studied for 2 months and stability graph was plotted in figure 1. The results of cream stability in two temperatures of 4 and 30 ° C show that with increasing temperature the stability of the enzyme is reduced, and the enzyme is more stable at 4 ° C compared to 30 ° C. The results of the stability studies of the o/w cream showed that it takes 45 days in 4 ° C for enzyme activity in the o/w cream to reach to its 50% of its initial amount but it takes 35 days for it in 30° C.

Conclusion

Overall, this study shows that there is a possibility to prepare cream containing OPH enzyme and if the cream is kept at the right temperature, it can remain stable for at least two months. It is suggested that encapsulated enzyme be used in the cream structure for better stability of it. The lack of easy access to OPH enzyme can be cited as a limitation of this study.

Author's contribution

Mariye Rajai, and Mortaza Robatjazi Contributed in study design and experimental procedures. Hamid Akbari contributed in experimental works and data analysis. Amir Hossein Goudarzian also contributed in significant revising the manuscript. All the authors proved the final version of it.

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Conflicts of interest

The authors have no conflict of interests to declare.

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