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Determination of Insulin Concentration in Camel Milk Using Ultra Violet –Visible Absorption Spectroscopy

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ABSTRACT: Camel milk contains sufficient levels of insulin and does not form the coagulum in the stomach preventing degradation of insulin and act as an effective vehicle to take the milk insulin unchanged to the intestine. In present study direct spectroscopy and standard addition method, well-known methods in analytical chemistry were used for determining insulin concentration in camel milk. The concentration of insulin was determined by UV absorption measurement at wavelength of 276 nm. The linear range for calibration curve in direct spectroscopy was 2 to 8 units/liter insulin solution indicating good agreement with Beer-Lambert law. The camel milk was centrifuged and upper clear solution was separated. According to calibration curve, insulin concentration was determined by direct spectroscopy. In standard addition method, upper clear solution and sufficient amount of distilled water were mixed with insulin standard solution to prepare solutions for Uv-Vis spectroscopy. The insulin levels in upper clear solution were determined; 17.91 ± 0.40 and 18.65 ± 0.38 units/ liter, using standard addition method and direct spectroscopy, respectively. Both methods are capable of being used in determining insulin concentration in camel milk. However, it is suggested to use standard addition method for measuring insulin concentration due to decreasing matrix error during determination.

Keywords: *Camel Milk, Iinsulin Determination, Standard Addition Method, UV- Vis Absorption Spectroscopy.*

Introduction

According to Food and Agriculture Organization report, there are about 19 million camels in the world of which 15 million are in Africa and 4 million in Asia (Farah *et al*., 2007).

Some reports have shown that vitamin C content of camel milk is about three times more than that of cow's milk and there are remarkable levels of other vitamins namely the B group, unsaturated fatty acids and mineral compounds that cause camel milk to be more salty than cow milk (Sawaya *et al*., 1984).

Insulin is a peptide hormone that is secreted from the beta cells in pancreas and its main role in the body is regulating the blood glucose levels (Martini, 2007). Insulin is composed of two peptide chains including 51 amino acids. The chain A consists of 21 amino acids and the chain B consists of 30 amino acids. These chains are linked covalently together by two cysteine disulfide bridges (Schlein *et al*., 2002) (Figure 1).

Camel milk contains sufficient levels of insulin and it does not form the coagulum in the stomach or acidic environment therefore it prevents degradation of insulin in the stomach and this property of camel milk allows it to pass quickly through the

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stomach without changing of insulin structure (Al-Numair *et al*., 2011). It was observed that there was 30-35 % reduction in daily doses of insulin in patients of type 1 diabetes consuming raw camel's milk (Agrawal *et al*., 2003).

Radio immunology kit (RIA) is the method applied to determine insulin levels in camel milk (Zagoriski *et al*., 1998). However using this method has been faced with some problems such as availability of the kits and expensive supplementary equipments. Therefore the main purpose of this study is to find an easier and more available method for determining insulin content in camel milk. Ultra Violet –Visible (UV-Vis) spectroscopy is well-known method for detecting some molecules that have the ability to absorb ultraviolet and visible radiation. The amount of absorbance is proportional to the concentration of absorbent, therefore we can determine the concentration of the sample according to the amount of absorbed light (Skoog *et al*., 2007). Since insulin structure has amino acid sequenecs including double bonds in their structure, it is capable of absobing UV-Vis radiation. In this study, UV-Vis spectroscopy was applied to determine insulin levels in camel milk. Standard addition method (SAM) is particularly useful for analyzing complex samples in which the likelihood of matrix effects is remarkable (Skoog *et al*., 2007). In order to solve the matrix effect problem, we applied SAM for insulin determination in camel milk.

Materials and Methods

- *Materials*

Bovine insulin was purchased from Sigma (St. Louis, MO). Hydrochloric acid (37 %), Sodium Hydroxide and Phosphate buffered saline were provided by Merck (Darmstadt, Germany). Camel milk was prepared from 10 different one-humped camels by hand milking in Dirgachin, Varamin, Iran. These camels were fed fodder,shrubs and herbs in the arid regions and milk sampling was done between 5-6 a.m. in the summer.

- *Standard insulin solution*

Insulin solution was freshly prepared by dissolving the insulin powder in 0.1N HCl and then neutralized with 0.1N NaOH, and diluted with Phosphate buffered saline, pH 7.4 (Pourhosseini *et al*., 2007). Since International unit (IU) of insulin is about 45.5 µg, we prepared standard insulin solutions by solving appropriate amount of insulin powder (Hinwood, 1992).

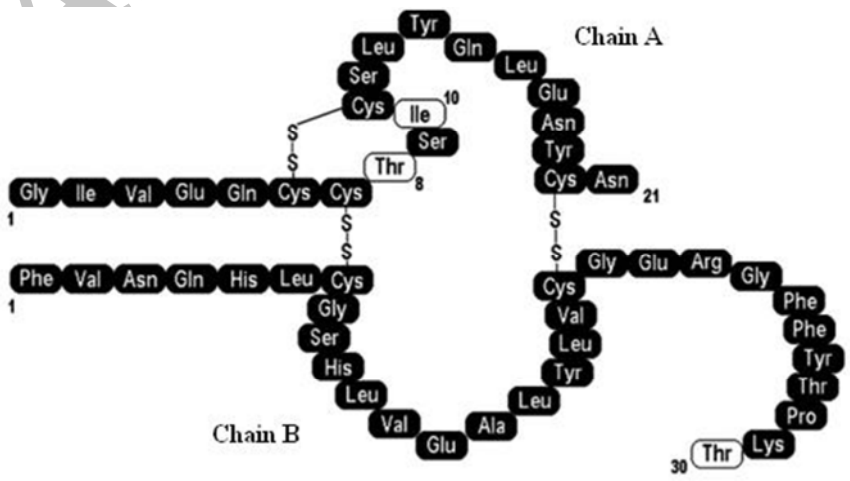

Fig. 1. Structure and sequence of human insulin including chain A and B (Ortiz *et al*., 2004)

- Determination of insulin concentration

In order to determine insulin concentration, UV-Vis spectrophotometer (RayLightw ,UV-1600, China) was used. To detremine absorption maximum wavelenght (λ_{max}) for insulin, its spectrum was obtained in the wavelength ranging from 260 to 350 nm.

- Direct spectroscopy

25 ml of milk sample was centrifuged at 7800 rpm for 60 min at 25 ^{o}C . The upper clear solution (5 ml) was separated and diluted up to the mark in 25-mL volumetric flask with distilled water. The concentration of insulin in stock solutions was determined by UV absorption measurement at 276 nm.

- Standard addition method

In this method, the absorbance of five mixtures was measured at 276 nm with a total volume of 25 ml, containing 5 ml of centrifuged milk sample and 20, 15, 12, 8, and 4 ml of distilled water and mixed with 0, 5, 8, 12 and 16 ml of standard insulin solution (5 units/liter).

Results and Discussion

- UV-visible spectroscopy

In this method, electromagnetic waves have the wavelength range between 190 to 780 nm. According to Beer-Lambert law, the amount of absorbance is proportional to the concentration of absorbent (Eq. 1):

$$A=\varepsilon bc \qquad (1)$$

Where A, ε, b and c are absorbed radiation, molar absorptivity, the length of cell and analyte concentration respectively.

The wavelength, in which the absorbent has absorption maximum is known as λ_{max}. Deviation from the Beer-Lambert law in λ_{max} is lower than other wavelengths. λ_{max} has been applied for spectroscopic determination of the samples' concentration and the law is not obeyed at high concentrations, therefore linear range of calibration curve is used to determine the analyte concentration (Skoog *et al*., 2007). In order to determine λ_{max}, the absorption spectrum of insulin solution (2 units/liter) was taken at wavelength 260 – 350 nm (Figure. 2). λ_{max} was obtained at about 276 nm that is consistent with another report (Pourhosseini *et al*., 2007).

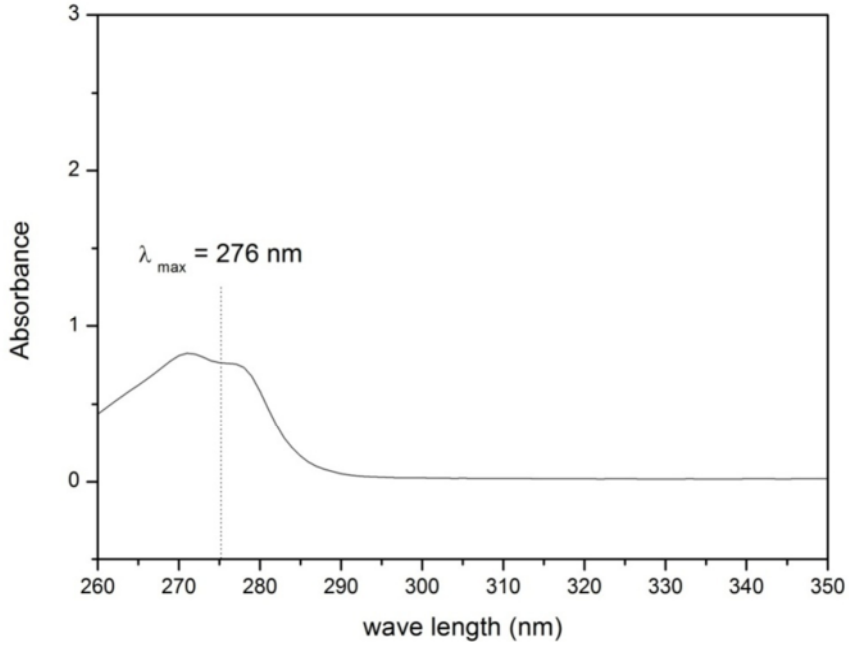

Fig. 2. Spectrum of insulin solution with concentration 2 units/liter

- *Direct Spectroscopy*

A calibration curve is prepared and used to determine the analyte concentration in an unknown solution of insulin. This curve is obtained from absorbance of some standard solutions of insulin from 2 to 8 units/liter to provide linear range that follows Beer-Lambert law. Correlation coefficient square (r^2) of standard curve was 0.99, indicating good agreement with Beer-Lambert law and also linear regression (Figure 3).

According to fitting data with linear regression, the calibration equation follows as:

$$A = 0.216 + 0.285C \quad (2)$$

Where A is absorbance of insulin solution at wavelength of 276 nm and C is the concentration of insulin standard solution in units/liter. Table.1 shows the absorption of insulin solutions.

Direct spectroscopy is also used to detect a specific compound based on spectrum taken from the sample. UV-Vis spectroscopy spectrum of camel milk was obtained by scanning absorbed radiation at wavelengths of 250 – 350 nm. λ_{max} at 276 nm indicates the existence of insulin in camel milk (Figure 4).

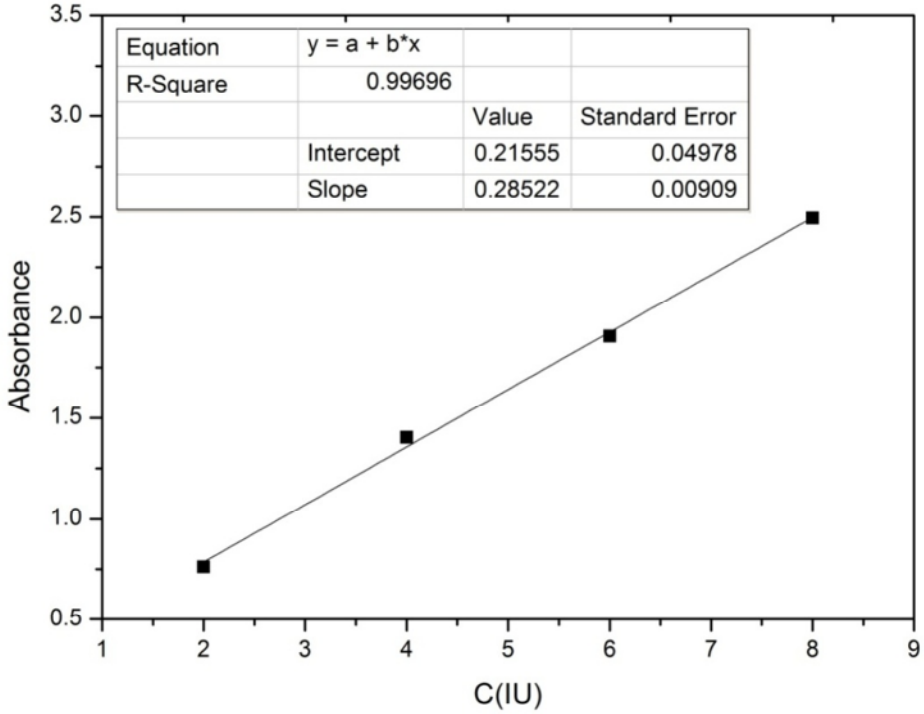

Fig. 3. Calibration curve of standard solutions of insulin (2 to 8 units / liter)

Table 1. Spectroscopic absorption data at 276 nm for insulin solutions

Standard solution (units/liter)	Absorbance
2	0.76
4	1.40
6	1.91
8	2.49

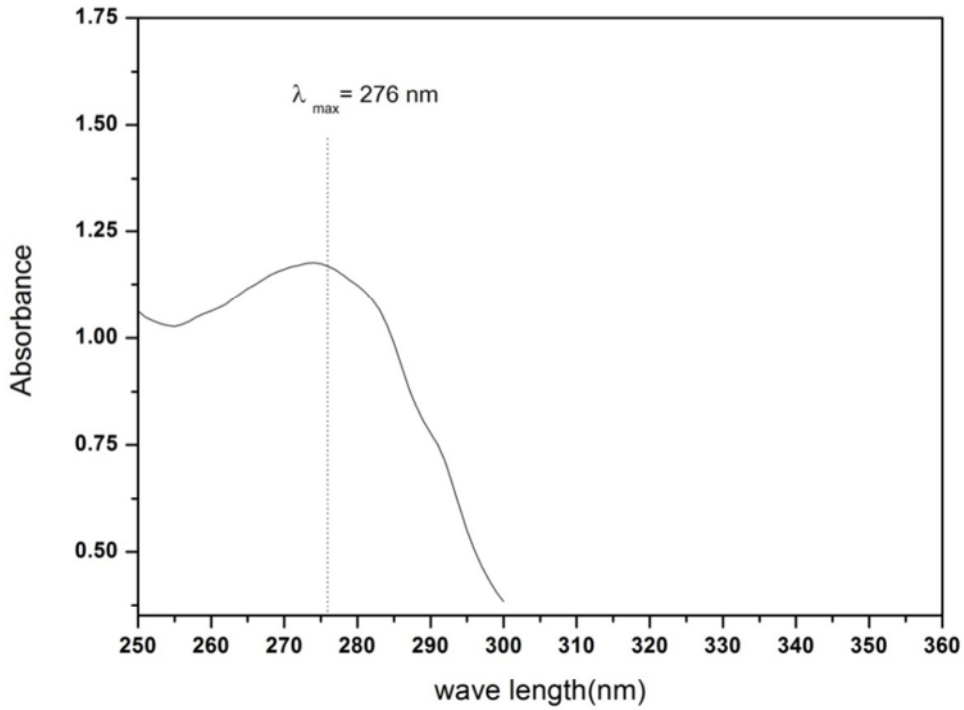

Fig. 4. Camel milk UV-Vis spectrum

Table 2. Volume of solution components for SAM and their absorbance

	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5
Camel milk (ml)	5	5	5	5	5
Distilled water (ml)	20	15	12	8	4
Standard solution (ml)	0	5	8	12	16
Total volume (ml)	25	25	25	25	25
Absorbance	1.21	1.4	1.6	1.91	2.18

- *Standard addition method (SAM)*

In this method, sample solutions were prepared by mixing various volumes of standard insulin solution (5 units/liter) with camel milk according to Table 2, and their absorbance at wavelength of 276 nm were measured (Table 2).

Standard curve was prepared using absorbance data against volume of added standard solution (Figure. 5). The slope and intercept (m and b) of the calibration plot of the absorbance against volume of the standard was used to calculate the concentration of the sample (Eq. 3):

$$C_x = bC_s/mV_x \quad (3)$$

In this equation, C_x and C_s are the concentrations of insulin in the sample and standard solutions, respectively. V_x is the volume of sample in the mixtures that was 5 ml in this study (Skoog *et al*., 2007).

Correlation coefficient square (r^2) of standard curve was 0.98, indicating good agreement with the linear regression (Figure 5). In order to reduce measurement errors in all the experiments, the UV absorption intensity of each solution sample was measured five times and the concentration was calculated according to the standard calibration curve in direct spectroscopy.

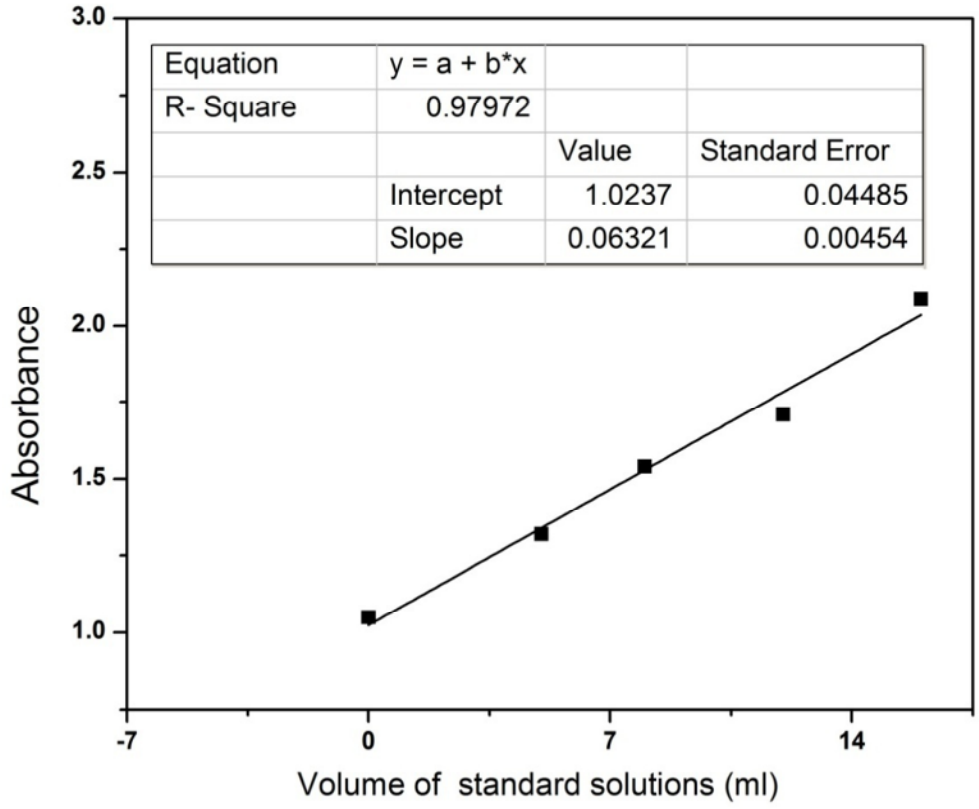

Fig. 5. Calibration plot for SAM (first time)

Table 3. Concentration of insulin in camel milk using both Direct Spectroscopy and SAM.

Methods	1th time (IU /l)	2nd time (IU /l)	3rd time (IU /l)	4th time (IU/l)	5th time (IU/l)	Average concentration(IU/l)
Direct Spectroscopy	18.78	18.4	18.41	18.35	19.30	18.65 ± 0.38
Standard Addition method	18.39	17.98	17.33	18.20	17.65	17.91 ± 0.40

SAM was also repeated five times and the results are listed in Table 3.

The insulin level in camel milk was determined 17.91± 0.40 and 18.65 ± 0.38 units/ liter using SAM and direct spectroscopy, respectively. In other reports, the insulin content in camel milk has been reported in the range of 45 – 125 units per liter using RIA kit (Zagoriski *et al*., 1998). The difference may not be due to the applied method because we used standard insulin solution in direct spectroscopy and this method was also used for the determination of insulin in aqueous solution by Pourhosseini *et al*. (Pourhosseini *et al*., 2007). In addition, we used SAM to reduce the matrix error in camel milk. However this difference between our results and other reports might be due to diverse condition of feeding and living environment for animals.

Both direct spectroscopy and SAM can be used to determine the insulin concentration in camel milk, however SAM is the method employed to reduce the error produced by sample matrix. Therefore standard addition method is a reliable method to determine the insulin concentration in camel milk.

Conclusion

UV-Vis Spectroscopy, based on Beer-Lambert law is a simple method for rapid determination of insulin. λ_{max} at 276 nm was used to determine insulin concentration in camel milk due to reducing deviation from Beer-Lambert law. The λ_{max} at about 276 nm indicated the existance of insulin in camel milk.

In the present study two methods based on absorption in UV-Vis spectroscopy including direct spectroscopy and SAM were used to determine insulin concentration in camel milk. There were not significant differences between the results obtained from these two methods.

In conclusion, the direct spectroscopy and SAM are simple, fast, inexpensive and reliable methods to determine the insulin concentration in camel milk, but SAM might be regarded more reliable due to the decreasing matrix error during insulin measurement. Therefore standard addition method (SAM) might be recommended to be used for measuring the concentration of insulin in camel milk.

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