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Research Article

EXTRACTION, IDENTIFICATION, FORMULATION AND EVALUATION OF PIPERINE IN ALGINATE BEADS.

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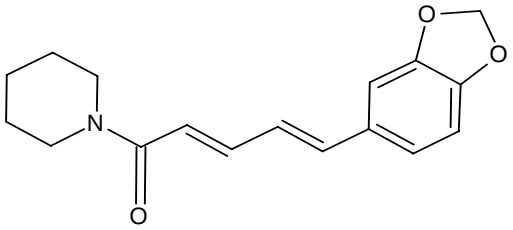
ABSTRACT

Piperine {[1-5-(1, 3)-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]-piperidine},an alkaloid responsible for the pungency of black pepper & long pepper. Systemic pharmacological studies on piperine have revealed that this compound elicited diverse pharmacological activities; analgesic, anti-pyretic, anti-inflammatory, anti-convulsant & CNS-depressant activities. Piperine was isolated from *Piper nigrum Linn.* (Piperaceae) and identified by TLC. Piperine was fabricated into alginate beads using sodium alignate. The main aim of this study was to demonstrate the sustained release of piperine from alginate beads by *in vitro* evaluation. The drug release studies were showed that the alginate beads sustained the release of the drug with % drug released in hours.

Keywords: Piperine, Alginate beads, Sustain release.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. The plants and the phyto constituents in the traditional systems of medicine of many countries continue to play an essential role in healthcare. Black pepper (*Piper nigrum*) is native to India and other southeastern Asian countries. Pepper is the dried unripe fruit of perennial climbing vine *Piper nigrum Linn.* , belonging to the family Piperaceae. Piperine is the principle pungent substance in pepper species. It is a constituent of *piper nigrum* and *piper longum*. Piperine is a piperidine derivative with multiple pharmacological activities. The traditional uses include analgesic, anti-pyretic, CNS depressant, anti-inflammatory, antioxidant, anticonvulsant, anti-bacterial, anti-tumor and hepatoprotective activities[1]. Pepper contains an alkaloid piperine (5-9%), volatile oil (1-2.5%), pungent resin (6.0%), piperidine and starch (about 30%). Piperine content is 3- 9% and 3-5% (on dry weight basis) in piper nigrum and piper longum respectively. The volatile oil (yellowish) contains mainly l-phyllandrene and caryophyllene. It has the specific gravity of 0.898 - 0.900, optical rotation -3 to -5° and refractive index of 1.4539 – 1.4977[2,3].



(E,E)1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine.

Fig. 1: It shows the structure of piperine

Black pepper suffers with certain side effects when taken orally, like it causes gastro intestinal disturbances and stomach upset. The reason for this may the availability of whole amount of the drug at a time. In this regard, a sustained release system for the drug to be in the therapeutic window all the time during the treatment would be a appropriate idea[4].

Recent research is concentrating much on the natural polymer as drug carriers to sustain the action[5]. Here in this study sodium alginate is used as the polymer and micro beads are formed basing on the ionotropic gelation technique. The gelation is caused by forming an egg box junction to associate divalent metal ions of alginate polymer chain [6].

MATERIALS AND METHODS

Black pepper was obtained from a local source. Sodium alginate and calcium chloride are from Fine chemicals. All other reagents used were of analytical grade and magnetic stirrer (Remi Magnetic Stirrer), U.V spectrophotometer (Elico SL159), USP Dissolution Apparatus (Electrolab) were used.

Isolation of piperine from pepper powder

10g of ground pepper powder and 20ml of dichloromethane were placed in a 100ml round bottom flask with a magnetic stirrer. A water condenser was attached to the top of flask and allowed water to run through it to condense the dichloromethane vapors while refluxing the solution for 20min. After cooling the flask, by using vacuum filtration with a Buchner funnel pepper grounds were filtered. The grounds were washed with 10ml dichloromethane.

Isolation and purification

The filtrate was transferred to a 50 ml round bottom flask and using a sand bath the dichloromethane was removed until dark brown oil was left. The oil was cooled in an ice-bath and 6ml of cold ether was added. After stirring for 5min., the solvent was removed again by sand bath. Cool the oil in an ice bath and add 6ml of cold ether again. The flask was kept in an ice bath for 15minutes with occasional stirring to precipitate out piperine. The crystals were washed with cold ether (2 - 4ml) and 5mg of crystals were saved for TLC analysis.

To re-crystallize, the piperine was placed in a test tube and dissolved it in similarly 5ml of hot 3:2 acetone: hexane solution. It was allowed to sit for 15 min at room temperature and then 30 min in an ice bath. The crystals were filtered using a Hirsch funnel and washed with 4ml of cold ether. A second crop of crystals from the mother liquor were obtained to improve yield after dried[7].

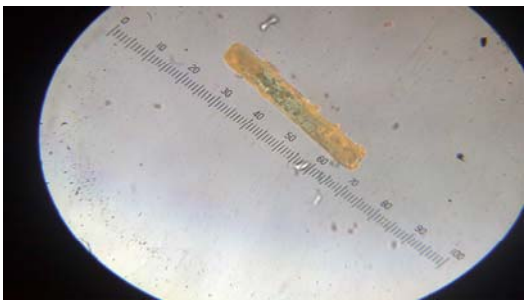


Fig. 2: It shows the microscopic view of the piperine crystal.

Identification test

The piperine (in mcL) was subjected on to the precoated and activated (kept the plates in oven for 1hr at 70^0C) silica gel TLC-plates. The mobile phase is Toluene: Ethyl acetate in 70:3 ratios and the detecting agent is Vanillin- Sulphuric acid reagent. After the TLC run and spraying the detecting agent the yellow spots of piperine were identified visually. Rf value was calculated[8].

Preparation of standard calibration Curve

The calibration curve is obtained by dissolving piperine in distilled water and further dilutions were made using distilled water and absorbance measured spectro photometrically at 343 nm.

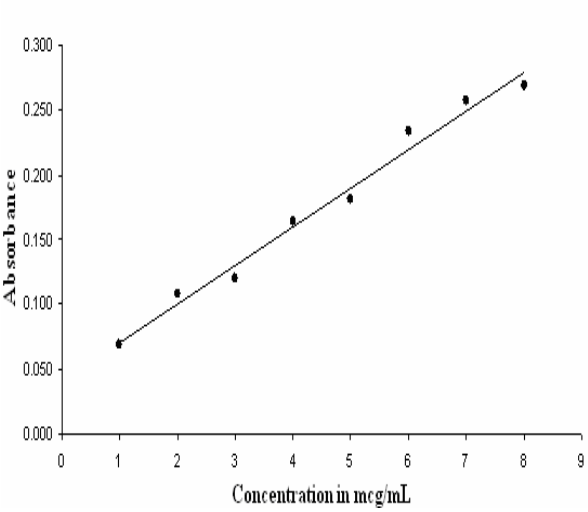


Fig. 3: It shows standard calibration curve of piperine. (r- Value is 0.9902)

FTIR studies

FTIR spctra of selected pure piperine, pure sodium alginate and piperine along with sodium alginate were recorded on a spectrometer using conventional KBr pellet method

Preparation of Alginate Beads

2.5 % w/v solution of sodium alginate was prepared by dispersing sodium alginate in deionized water under continuous stirring. The drug was dispersed (drug: polymer ratio 1:1) in the alginate solution. The above dispersion was added drop wise in to 2 % w/v solution of calcium chloride using a hypodermic syringe with a flat tip needle (20 G). Simultaneously the calcium chloride solution was stirred at a speed of 50 rpm using a magnetic stirrer. The drug loaded pellets were formed immediately on contact with the calcium chloride solution were allowed to cool for 30min. in calcium chloride solution to complete the gelation reaction. Then the so formed micro pallets were filtered and dried using hot air oven at 60^{o}C for 5 hrs[9].

Particle size measurement

Conventional light microscopy is used to determine the shape and outer structure of the beads. **Capture Efficiency**

The micro beads equivalent to 100mg of piperine micro pellets were weighed and dispersed in distilled water. The resulting mixture was agitated on mechanical shaker for 24 hours. The solution was then filtered and drug content was estimated by UV spectrophotometer.

Release Studies and kinetic model fitting

Drug release studies were conducted in distilled water for 12 hours using basket type dissolution apparatus under sink conditions. Accurately weighed samples of the Alginate beads were added to dissolution medium kept at 37oC ±0.5oC. At preset time intervals aliquots were withdrawn and replaced by an equal volume of dissolution medium to maintain constant volume. After suitable dilution, the samples were analyzed by UV spectrophotometer at 343nm[10, 11, 12 and 13].

RESULTS AND DISCUSSION

The piperine was isolated from the black pepper and the percentage yield of piperine from pepper powder was found to be 6.7%. After isolation it is identified by TLC. The standard Rf- value of piperine from the literature was 0.25. The Rf- value of purified piperine from TLC was found to be 0.24. So it was confirmed that the product obtained from the black pepper powder contains piperine (Fig 4).

From the FTIR studies, it was confirmed that the drug and polymer were compatible with each other. Data was given in Fig 5.

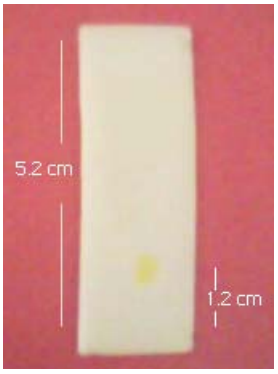


Fig. 4: It shows RF Value of isolated piperine 0.24

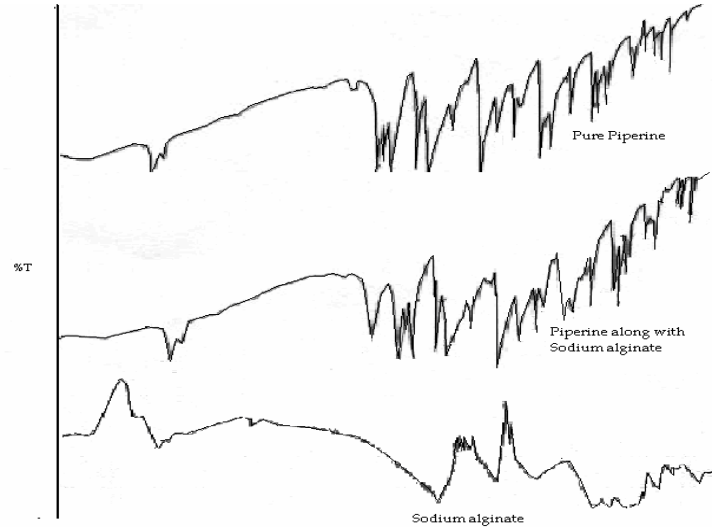


Fig. 5: It shows FTIR spectra of Piperine, Sodium alginate and Piperine along with Sodium alginate.

Particle size was determined by optical microscopy. The prepared spheres were almost spherical in shape and these are having the average particle size was found to be 53± 0.652 µm (Fig 6).

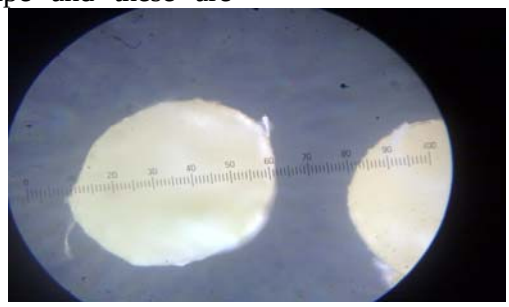


Fig. 6: It shows microscopic picture of piperine loaded alginate bead.

The drug entrapment efficiency was found to be 77.87%. From the dissolution studies it was found that, the release was sustained up to 12 hours by using the 1:1 ratio of the drug polymer ratio.

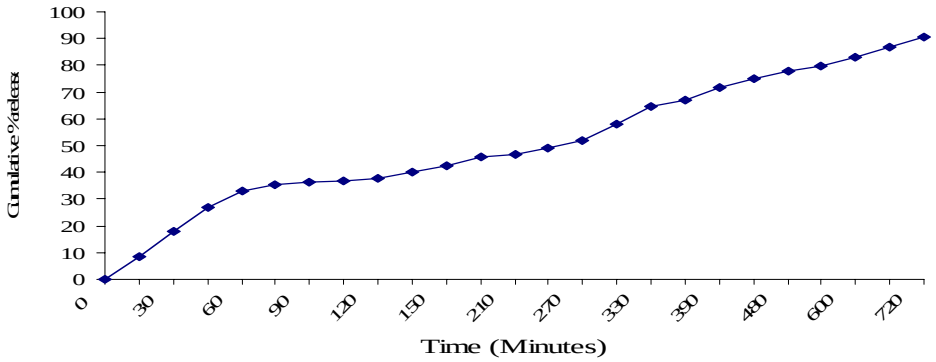


Fig. 7: It shows dissolution graph of prepared alginate beads

Table 1: It shows dissolution data for piperine loaded alginate beads

Sr.No.	Time (minutes)	Cumulative %Release± SD	SE-mean	RSD
1	0	0.000 ± 0.00	0.00	0.00
2	15	8.709 ± 0.21	0.15	2.36
3	30	17.930 ± 0.08	0.06	0.45
4	45	26.939 ± 0.49	0.35	1.84
5	60	32.912 ± 0.50	0.35	1.51
6	75	35.510 ± 0.12	0.08	0.33
7	90	36.112 ± 0.20	0.14	0.55
8	105	36.716 ± 0.13	0.09	0.35
9	120	37.847 ± 0.46	0.32	1.21
10	135	39.915 ± 0.30	0.21	0.74
11	150	42.430 ± 0.26	0.18	0.60
12	180	45.657 ± 0.13	0.09	0.29
13	210	46.658 ± 0.96	0.68	2.05
14	240	48.886 ± 0.14	0.10	0.29
15	270	52.057 ± 0.35	0.25	0.68
16	300	58.069 ± 0.51	0.36	0.88
17	330	64.637 ± 0.02	0.02	0.04
18	360	67.076 ± 0.55	0.39	0.82
19	390	71.681 ± 0.06	0.04	0.09
20	420	74.795 ± 0.76	0.54	1.02
21	480	78.041 ± 0.35	0.25	0.45
22	540	79.906 ± 0.14	0.10	0.17
23	600	83.089 ± 0.34	0.24	0.41
24	660	86.956 ± 0.51	0.36	0.59
25	720	90.436 ± 0.31	0.22	0.34

After fitting in to various kinetic models, as the R- value (0.9989) is more for Peppas model, the best fit model for the dissolution of Piperine beads was found to be the “Peppas Model”with n value of 0.9896 and k value of 0.1168.

Table 2: It shows R and K Values for Pharmacokinetic models from 20-60% of drug release.

Model	R	K
Zero order(T- test)	0.9985	0.1305
First order	0.9714	0.0029
Matrix	0.9912	4.9467
Peppas	0.9989	0.1168

CONCLUSION

Piperine was successfully extracted and purified from black pepper. It was identified by running TLC. The extracted piperine was formulated into alginate beads and evaluated for all the possible in-vitro studies. The results of identification and in vitro evaluation were encouraging.

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