Formulation and Physicochemical Evaluation of Azathioprine Loaded Ethyl cellulose Microspheres using Solvent Evaporation Technique

A. Kokilavani [1]*, P. Jeyaprabha [2], V. Ganesan[3], R. Senthamarai [4]

[1]*Department of Pharmacology, Periyar College of Pharmaceutical sciences, Tiruchirappalli-620021, Tamilnadu.*

kokilavanigpat@gmail.com

[2]*Department of Pharmaceutics, The Erode college of Pharmacy, Erode-638112, Tamilnadu.*

jayaprava@gmail.com

[3]*Department of Pharmaceutics, The Erode college of Pharmacy, Erode-638112, Tamilnadu*

[4]*Department of Pharmacognosy, Periyar College of Pharmaceutical science, Tiruchirappalli-620021, Tamilnadu.*

Abstract

The present study is associated with Formulation and physicochemical evaluation of azathioprine loaded Ethyl cellulose microspheres. Azathioprine is an immunosuppressant drug which used in the treatment of Rheumatoid arthritis. In this study Azathioprine microspheres were prepared by solvent evaporation technique by using Ethyl cellulose as polymer. Prepared microspheres were evaluated by particle size analysis, UV spectroscopy, SEM Analysis, FT-IR spectroscopy and encapsulation efficiency. Particle size of prepared microspheres was ranges from 10 to 50 micrometer. SEM pictures of microspheres conforms the perfect spherical particles and uniform smooth surface. FT-IR spectroscopy reveals there is no interaction between drug and polymer while formulating the microspheres. The percentage yield of microspheres was 91.42%. Encapsulation efficiency was found to be 48%. The formulation plays an important role in controlled release as well as prolonged release in the treatment of inflammation.

Key words: *Azathioprine, Ethyl cellulose, Microspheres, Rheumatoid Arthritis.*

Introduction

Microspheres are defined as monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion or particles. Particle size of microspheres 1-100 micrometer.

Azathioprine is an immunosuppressant drug which used in many diseases such as, Rheumatoid Arthritis, organ transplantation, crohn's disease and chronic hepatitis. Other than these effects it also has some adverse reactions such as hepatotoxicity, teratogenic effect and suppression of haemopoietic system.

Azathioprine is having shorter biological half-life; it ranges from 12 to 15 minutes. So it is necessary to take the drug frequently to maintain effective plasma concentration.
The applications of Azathioprine microspheres are it has prolonged release, reduces the frequent administration of drug, and it reduces the adverse effect produced by the drug.
In the present study, Azathioprine was encapsulated in ethyl cellulose microspheres, so it has longer plasma half-life when compared to other formulations like tablets.

Materials and methods

Materials required:

Azathioprine was obtained as a gift sample from ANAMAYEE Pharma chem private Ltd., Boisar. Ethyl cellulose obtained from LOBA CHEMIE private Ltd., Mumbai. Span 60 was obtained from CENTRAL DRUG HOUSE private Ltd., Mumbai. Light liquid paraffin was procured from MERCK LIFE SCIENCE private Ltd., Bangalore.

Preparation of azathioprine microspheres:

Microspheres of azathioprine were prepared by solvent evaporation technique using ethyl cellulose as polymer. Briefly, ethyl cellulose was dissolved in a mixture of acetone and ethanol in the ratio of 1:0.5. Azathioprine was added to the above polymeric solution under stirring. The drug-to-polymer (azathioprine: ethyl cellulose) ratio was kept constant at 1:3. The resultant mixture was extruded through a syringe (# 20) into light liquid paraffin containing 0.5% v/v of Span 60 and stirring was carried out using a propeller stirrer at 1000 rpm for 3 hours. Prepared microspheres were collected by filtration and washed several times with petroleum ether to remove liquid paraffin. The microspheres were then dried at room temperature for 24 hours.

Results and discussion

1. **Particle size analysis**

The particle size of microspheres was measured by optical microscopy. The microspheres were dispersed in light liquid paraffin, and a smear was made on a glass slide and the size of 200 particles was measured by using a micrometer attached with a microscope. The average particle size was calculated.

A) Particle size analysis of azathioprine pure drug:

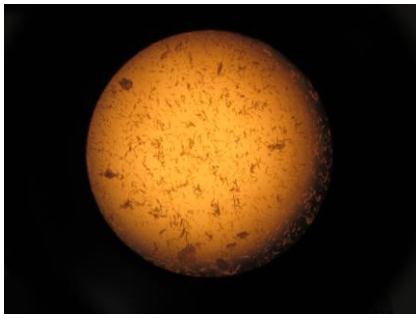

Figure 1. Optical microscopic photograph of Azathioprine pure drug.

The average particle size of azathioprine pure drug was measured by using optical microscope and average particle size was found to be 100 micrometers.

B) Particle size determination of azathioprine microspheres

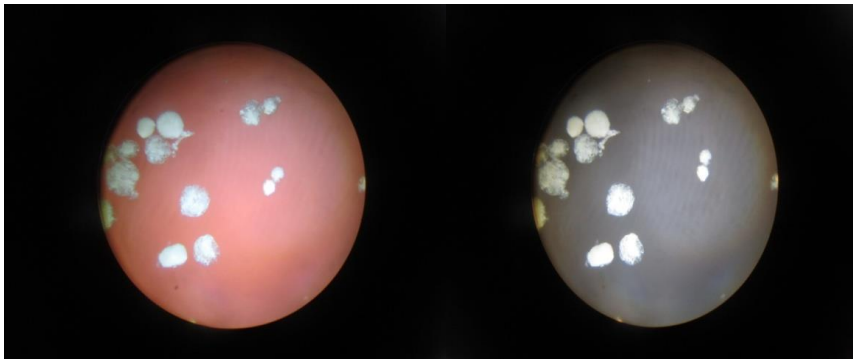

Figure 2 Figure 3

Figure. 2 and 3 optical microscopic photographs of azathioprine microspheres

The average particle size of prepared azathioprine microspheres were determined by using optical microscope was found to be 10 - 50 micrometer.

2. Scanning electron microscopy (SEM)

The sample for the SEM analysis was prepared by sprinkling the microspheres on to one side of double adhesive stub. The stub was then coated with gold using JOEL JFC 1100 sputter coater. The SEM analysis of the microspheres was carried out by JOEL JFC 1100, Japan. The microspheres were viewed at an accelerating voltage of 15-20kV.

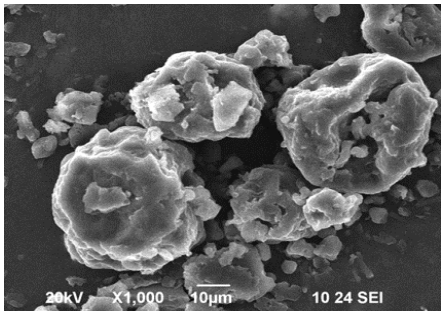

Figure 4

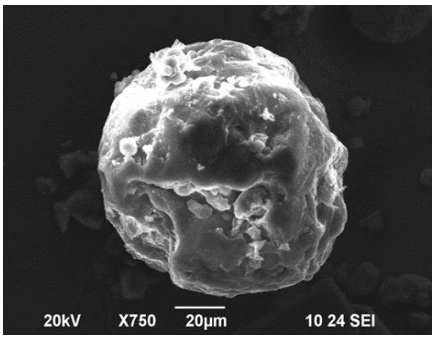

Figure 5

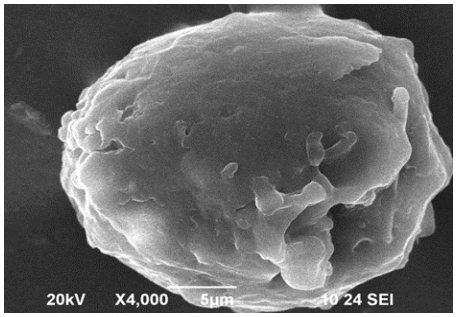

Figure 6

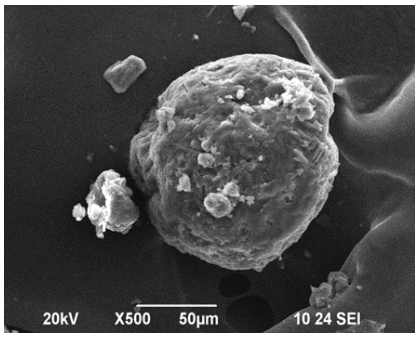

Figure 7

Figure. 4,5,6,7 shows Scanning electron microscopic photograph of azathioprine microspheres.

SEM pictures of azathioprine microspheres confirms the discrete spherical structure. The surface of drug loaded azathioprine microspheres with non-uniform surface was obtained.

3. Compatibility studies

i) UV SPECTROSCOPY

Accurately weighed 10 mg of Azathioprine pure drug was transferred to 100 ml volumetric flask, dissolved in 20 ml 0.1N NaOH by shaking manually for 10 min. The volume was adjusted up to the mark with distilled water to give final strength i.e. 100 µg/ml. Different aliquots of Azathioprine pure drug in range 1-5 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with distilled water to get concentrations 10,20,30,40 and 50µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was recorded at 281 nm. The calibration plot was constructed as Absorbance vs concentration (Fig. 8)

Table 1. Absorbance of Azathioprine pure drug.

CONCE NTRATION (µg/ml)	**ABSORBANCE**
10	0.315
20	0.744
30	1.216
40	1.667
50	2.138

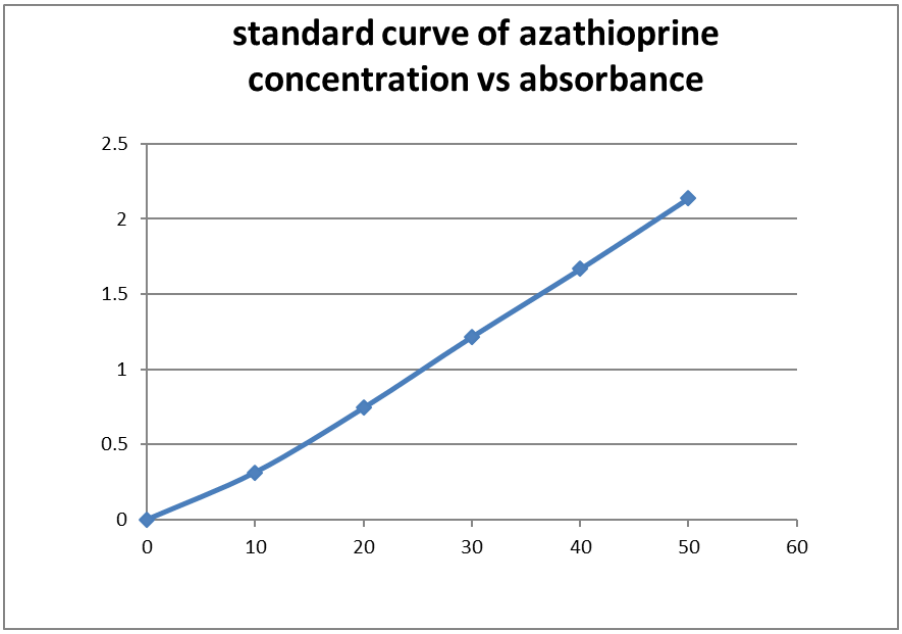

Figure.8 Standard curve of azathioprine pure drug

ii) Thin layer chromatography

0.2 gm of microspheres was dissolved in dilute ammonia solution to produce 10 ml.

Stationary phase: silica gel

Mobile phase: Butanol saturated with dilute ammonia solution.

Reference standard:

a) 0.02%w/v solution of chloro methyl nitro imidazole RS in dilute ammonia solution.

b) 0.02%w/v solution of mercaptopurine in dilute ammonia solution.

Apply to the plate 5mcl of each solution. After development dry the plate and examine in ultraviolet light.

Table 2. Rf value of Azathioprine microspheres

S.NO	**CONTENTS**		**R_f VALUE**
1.	Standard	Mercaptopurine	0.55
2.	Test	Azathioprine microspheres	0.57

The R_f value of standard and test was compared and it was found that there is no significant change in the R_f values, thereby confirms the compatibility between the drug and polymer.

iii) Fourier transform infrared spectroscopy

Infrared spectra of azathioprine and formulated microspheres were taken by using KBr pellet technique and were recorded on BOMEM MB-II FT-IR spectrometer.

FT-IR of Azathioprine pure drug

Table 3: FT-IR of azathioprine pure drug

SPECTRUM	**WAVE NUMBER** (cm^{-1})
N-H stretching	3429.20
N-H stretching	3448.49
N-H stretching	3504.42
N-H bending	1498.59
N-H bending	1529.45
N-H bending(Purine ring)	**1577.66**
C-N vibration	1002.92
Alkane	2806.23
C-S stretching	1070.42

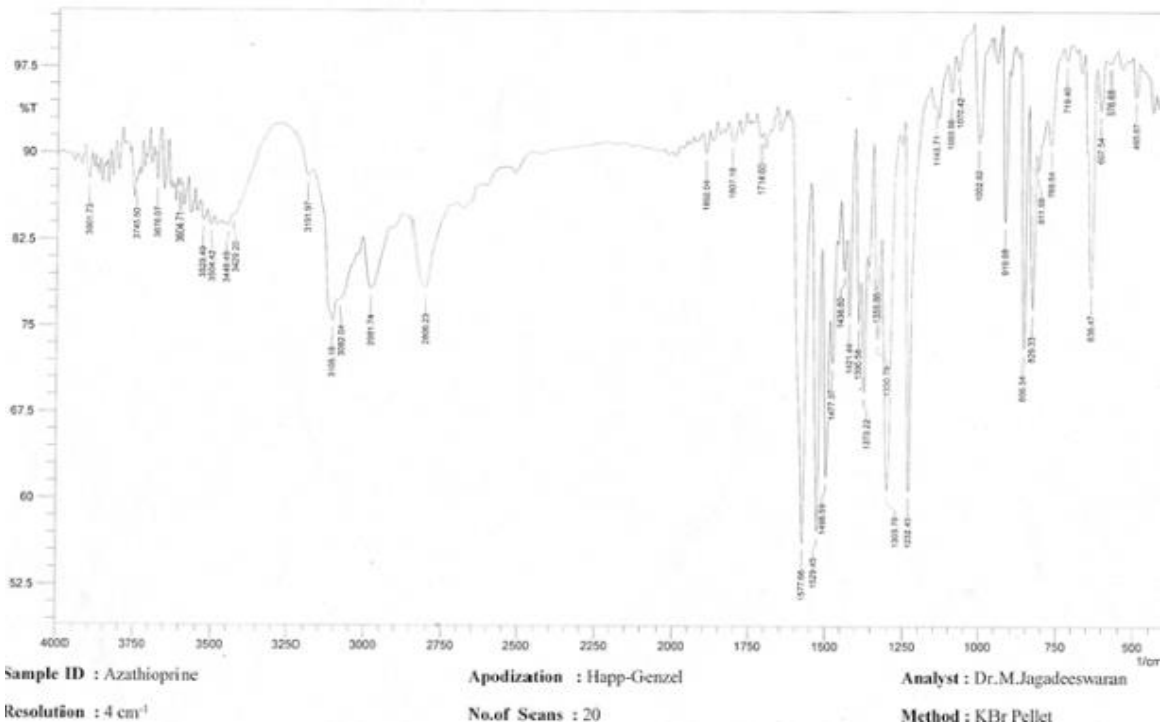

Figure. 9 FT-IR spectrum of Azathioprine pure drug

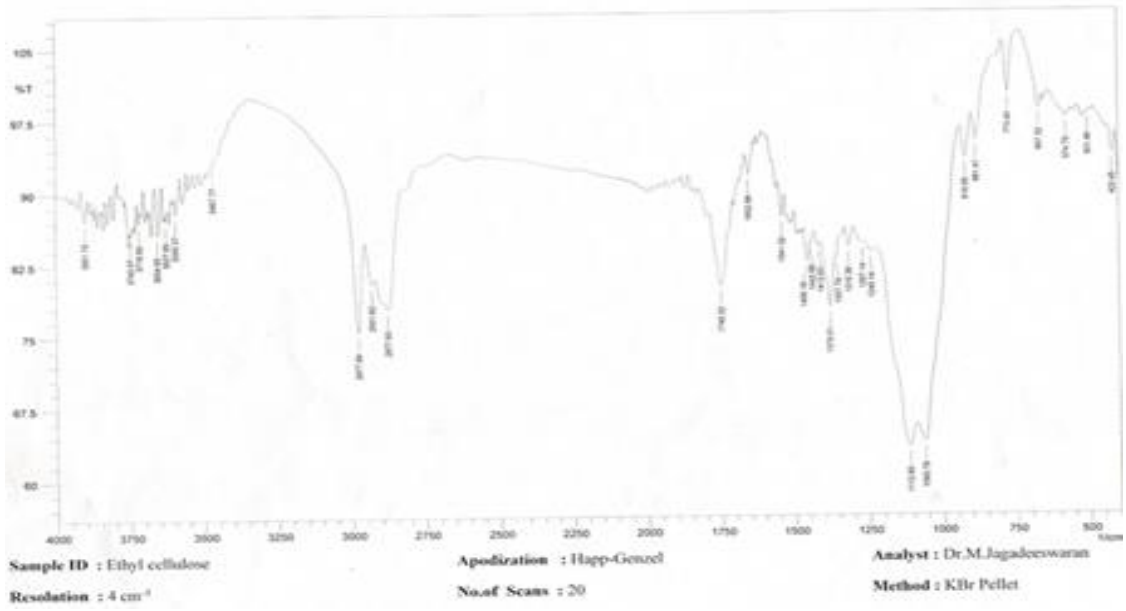

Figure.10 FT-IR Spectrum of Ethyl cellulose

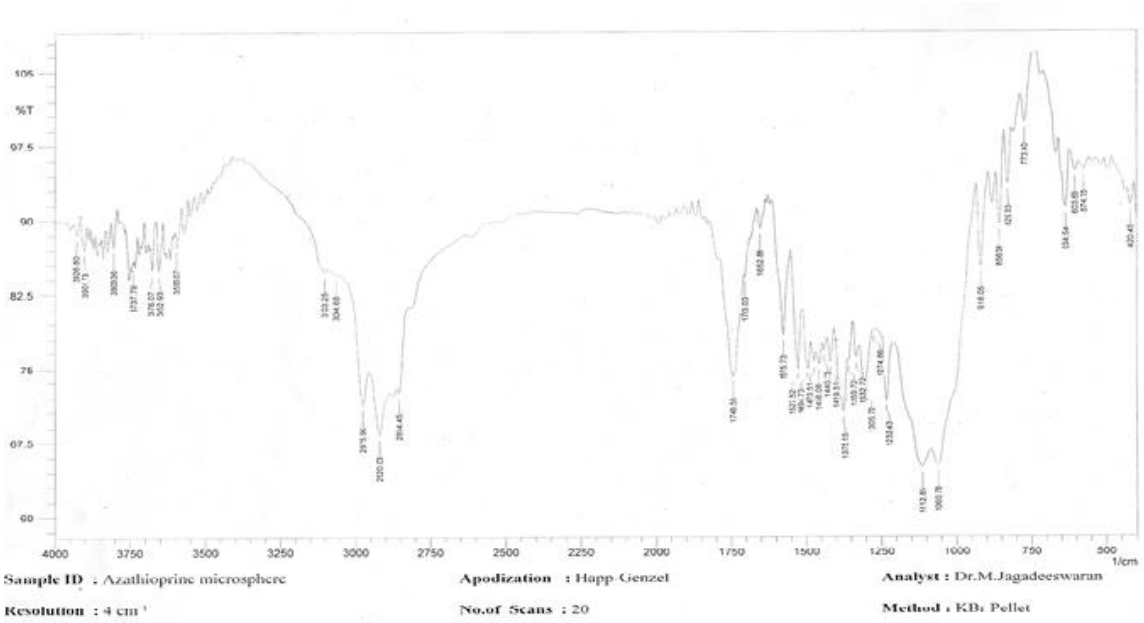

Figure.11 FT-IR of azathioprine microspheres

Table 4: FT-IR of azathioprine microspheres

SPECTRUM	WAVE NUMBER (cm^{-1})
N-H stretching	3103.25
N-H stretching	3595.07
N-H stretching	3652.93
N-H stretching	3676.07
N-H bending (purine ring)	**1575.73**
N-H bending	1527.52
C-N vibration	1112.65
Alkane	2960
C=S stretching	1060.78

FT-IR spectrum of azathioprine and its microsphere were compared with reference spectrum given in British Pharmacopoeia and found to be identical. Most of the peaks also appeared in the drug loaded microspheres (fig.11) which indicates no drug- polymer interaction particularly, a peak at 1575 cm^{-1} represents purine ring was found to be present in drug loaded microspheres, which confirms the stable nature of the drug.

4. PERCENTAGE YIELD OF AZATHIOPRINE MICROSPHERES

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below,

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}}$$

Percentage yield of azathioprine microspheres was found to be ***91.42%***

5. ENCAPSULATION EFFICIENCY

Drug loaded microspheres (100 mg) were digested with 10 ml of 1N sodium hydroxide at room temperature for 12 h. After filtration and suitable dilution, azathioprine present in the solution was determined at 280 nm using a UV visible spectrophotometer. Drug loading in the microspheres was estimated by using the formula,

L= Qm/Wm x 100

Where, L is the percentage loading of microspheres. Qm is the quantity of the azathioprine present in Wm g of microspheres

Drug encapsulation efficiency of prepared microspheres was found to be ***48.0%***

Conclusion

Azathioprine loaded ethyl cellulose microspheres were prepared by adapting solvent evaporation technique, which does not require complex apparatus and special precautions. The prepared microspheres were evaluated for particle size analysis, scanning electron microscopy, compatibility studies, percentage yield and drug entrapment efficiency.

As this study involves only preliminary evaluations, we need further investigations in future for better outcome of the research work.

References

1. *Pinaparolu Devarajulu ArunKumar and Muniyand Saravanan, "Formulation, Physicochemical character and In-vitro release studies of magnetic and compartmental targeting of Azathioprine microspheres using gelatin polymer" Journal of Applied Pharmaceutical Sciences, 2013; 3(10): 017-022.*
2. *Ram Prasad Sekar, Elango Kulanthaivel, Devi Damayanthi and J S Saranya, "Formulation and Evaluation of Azathioprine loaded silver nano particles for treatment of Rheumatoid arthritis" Asian Journal of Biomedical and Pharmaceutical sciences, 2013; 3(23): 28-32.*
3. *Abishek.S. B, Prathiban S, Senthil kumar G P and Tamizh mani T, "Study of Enteric coated sodium alginate Microsphere of Azathioprine for colon targeting" World Journal of Pharmaceutical research, 2018; 7(9): 1767-1781.*
4. *Nidhi, Muzamil Rashid, Veerpal Kaur, Supandeep Singh Hallan, Saurabh Sharma and Neeraj Mishra, "Micro particles as controlled drug delivery carrier for the treatment of Ulcerative colitis: A brief review" Saudi Pharm J. 2016; 24(4): 458-72.*
5. *Nirav R. Patel, Dhagash A Patel, Praful Bharadia, Vikram Pandya "Microspheres as novel drug delivery" International Journal of Pharmacy and Life sciences, 2011; 2(8): 992-997.*
6. *Hussain Mohammad Asif, R.A. Kumar, Rama Rao Tadikonda, M. Anjum, "Preparation and Evaluation of Ethyl cellulose microspheres prepared by Solvent evaporation technique." International Journal of Pharmacy and Pharmaceutical sciences, 2014; 6(7): 264-266.*
7. *Eswar Kumar A, Sharada Nalla and Bhogini Thirupathaiah, "Formulation Development and In-vitro evaluation of Azathioprine tablets for colon drug delivery system." World Journal of Pharmacy and Pharmaceutical sciences, 2016; 5(8): 1067- 1090.*

8. *Patitapabana Parida, Subash Chandra Mishra, Subhashree Sahoo, Ajit Behera, Bibhukalyan Prasad Nayak, "Development and Characterization of Ethyl cellulose based Microspheres for sustained release of Nifedipine" J Pharm Anal, 2016; 6(5): 341-344.*
9. *V.V. Prasanth, Akashmoy Chakraborty, Sam T Mathew, Rinku Mathappan and V. Kamalakkannan, "Formulation and Evaluation of Salbutamol sulphate microspheres by Solvent evaporation method" Journal of Applied and Pharmaceutical Sciences, 2011; 1(05): 133-137.*
10. *Mohd sajid Ali, Vinay Pandit, Mahendra Jin and Kanhiya Lal Dhar, "Mucoadhesive micro particulate drug delivery system of curcumin against Helicobacter pylori infection: Design, development and optimization." Journal of Advanced Pharmaceutical Technology and Research, 2014: 5(1): 48-52*