

ARTÍCULO ORIGINAL

Effect of surfactant on the release of ciprofloxacin from gelatin microspheres

Kesavan S. and Swarnambal Alamelu Bai*

Department of Biochemistry

Asan Memorial College of Arts & Science. Jalladampet campus, Chennai- 600 100, India.

Email id: dr_vidyraj@yahoo.co.in

Fax no: 91- 044- 22460138

ABSTRACTS

Gelatin microspheres with high entrapment efficiency were prepared by emulsification (using different surfactants) and crosslinking by glutaraldehyde with a view to improve stability of the microspheres. The microspheres were characterized for entrapment efficiency of ciprofloxacin, swelling, FT-IR, scanning electron microscopy (SEM) and leakage properties. From the results obtained we inferred that the gelatin microspheres entrapped with ciprofloxacin prepared by using three different surfactants increased the shelf life of gelatin microspheres.

KEYWORDS: Microspheres, Surfactants, Ciprofloxacin, Glutaraldehyde

INTRODUCCIÓN

*To whom correspondence should be made

Microspheres gained a lot of attention among drug delivery systems. Colloidal carriers in the form of microspheres and nanoparticles are being investigated as potential drug delivery systems^{1,2,3}. These systems involve

microspheres in diameters ranging from below 1 μ m to over 100 μ m. Considerable interest has been shown in the formation of biodegradable nanoparticles^{4,5,6}. As they have the advantage of obviating the need to surgically remove the drug-depleted device^{7,8,9}.

Gelatin microspheres are generally prepared by solvent evaporation method^{10,11,12} and crosslinking method¹³⁻¹⁸.

In this article we have prepared gelatin microspheres by emulsification and crosslinking method.

Gelatin microspheres were prepared with two different percentage of glutaraldehyde as crosslinkers.

Cationic, anionic and neutral surfactants were used for emulsification as a external adduct in the preparation of gelatin microspheres and their effect on leakage of the drug were determined. Ciprofloxacin, an antibacterial drug^{19,20} was encapsulated in the microspheres. These microspheres were characterized for entrapment efficiency, %swelling, SEM, FT-IR and leakage properties.

An attempt was made to improve the shelf life of gelatin microspheres using two different percentages of glutaraldehyde and three different surfactants as external adduct.

MATERIALS AND METHODS

Materials

Gelatin (Bacteriological grade), glutaraldehyde (25%w/v aqueous solution), Toluene rectified (LR), Acetone, Span-80(sorbitan monooleate), Cetyl trimethyl ammonium bromide, Sodium dodecyl sulphate and Oil. All chemicals were purchased from S.d fine chemicals limited Mumbai, The drug Ciprofloxacin (ciplox-500) was purchased from pharmacy.

Preparation of gelatin microspheres using three different surfactants:

Gelatin microspheres were prepared by emulsification and cross linking method. Gelatin microspheres were prepared using an overhead stirrer with a five-blade paddle¹⁴. Five ml of gelatin solution (20%, *m/V*, in water) was preheated to 80 °C and added drop-wise to 70 ml of mustard oil containing 1% (*m/m*) of surfactants (Span 80 or cetyl trimethyl ammonium bromide or sodium dodecyl sulphate) (with respect to the mass of the oil phase). The biphasic system was stirred under turbulent flow conditions using an overhead stirrer to form a w/o emulsion. A known amount of drug were dispersed in gelatin solution and stirred to obtain a uniform dispersion.

Glutaraldehyde - Saturated toluene was prepared by mixing equal volumes of glutaraldehyde and toluene in a decantation funnel. After shaking for 15 minutes, the mixture was allowed to separate. The upper toluene layer saturated with glutaraldehyde was separated and added to the w/o emulsion to stabilize the particles²¹. Stirring was continued for 3 hrs.

Microspheres formed were separated by centrifugation at 1500 rpm for 10 minutes. The microspheres were then washed and dehydrated with 20ml of acetone. Finally microspheres were allowed to dry at room temperature (25° C). Upon drying a yellow to yellowish orange coloured free flowing, fine powder was obtained.

The formation of gelatin microspheres using three different surfactants was observed by scanning electron microscopy. (Figure 1-3)

Figure 1 SE Micrograph of glutaraldehyde crosslinked gelatin microspheres 1:1 [Gel: Glu] using span-80 as surfactant Magnification (1000)

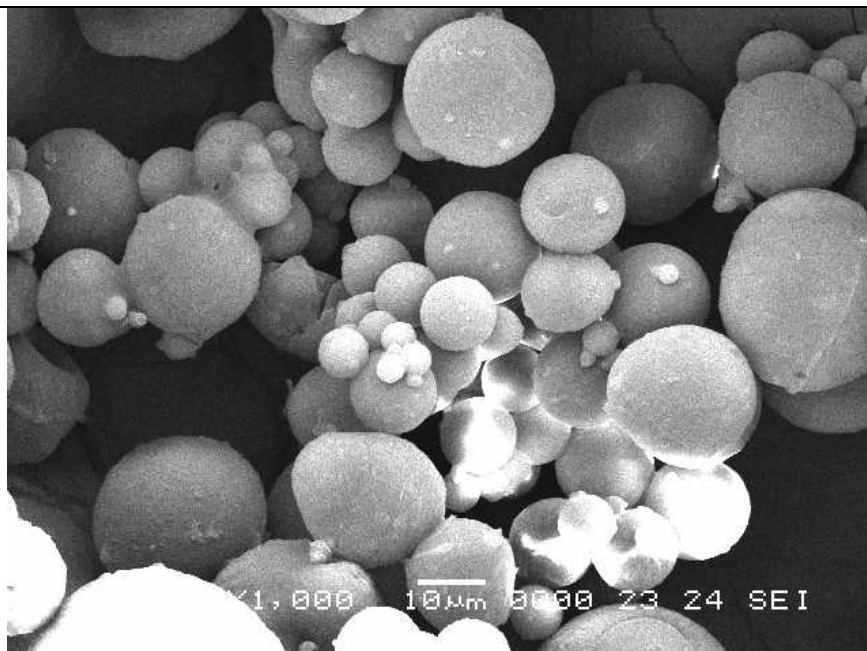


Figure 2 SE Micrograph of glutaraldehyde crosslinked gelatin microspheres 2:1 [Gel: Glu] using CTAB as surfactant Magnification (1000)

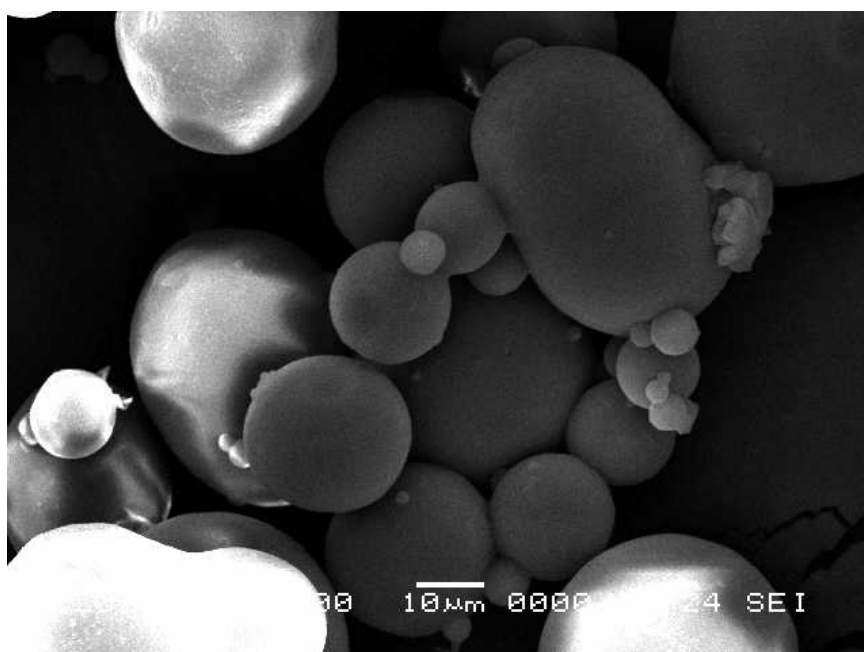
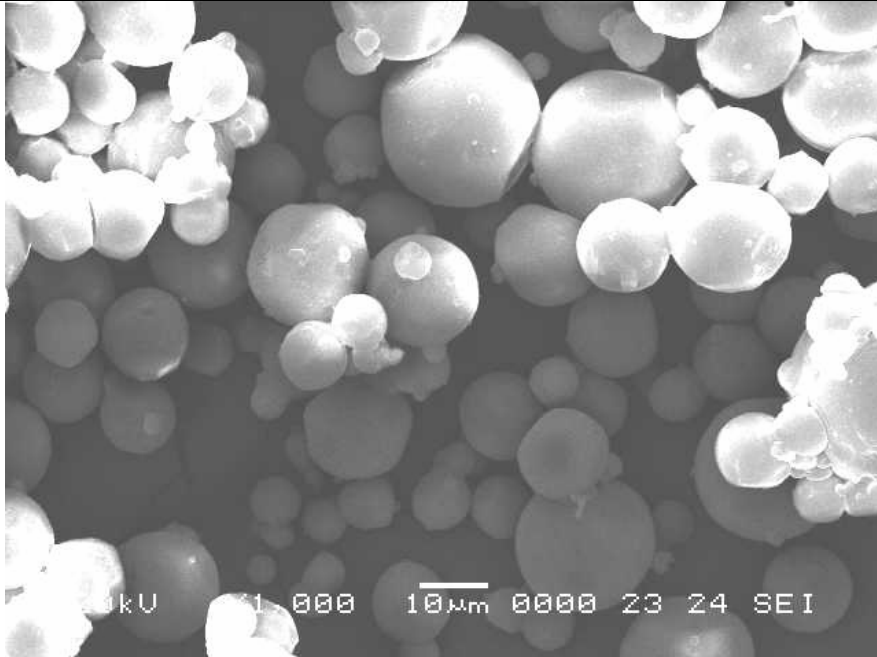


Figure 3 SE Micrograph of glutaraldehyde crosslinked gelatin microspheres 2:1 [Gel: Glu] using SDS as surfactant Magnification (1000)



Entrapment efficiency

A known amount of drug was loaded in microspheres during preparation. It was centrifuged and the supernatant was diluted suitably with distilled water and the absorbance of the resulting solution was measured at 274.2nm on a SL-164 UV-VIS spectrophotometer to determine the amount of Ciprofloxacin (CF) present in the supernatant.

Drug entrapment was calculated using the formula

$$\text{Entrapment efficiency} = \frac{\text{(Total amount of drug added to the system (initial)} - \text{Drug present in the solution outside Microspheres)}}{\text{Total amount of drug added to the system.}} \times 100$$

Scanning electron microscopy (SEM)

Scanning electron microscopy of the gelatin microspheres was carried out to

examine the microsphere structure and surface morphology. The microspheres were mounted on metal stubs and coated with a 150 Å layer of gold.

Photographs were taken using Joel Scanning Electron Microscope [Joel- JSM-5610LV SEM]. The samples were given for analysis in Central Instrumentation Lab, Annamalai University, Chidambaram, India.

Fourier transform infra-red {FT-IR} measurements

FTIR spectral measurements were performed using a Perkin Elmer FTIR spectrometer. Microspheres were ground with KBr and FTIR spectra were taken in the range 4000 – 400 cm^{-1} . The samples were given for analysis in IIT, Madras- 36, India. (Figure 4- 6)

Figure 4 FT- IR spectrum of (a) 1:1 [Gel: Glu], (b) 2:1[Gel: Glu]

(Drugs Using Span 80 as Surfactant)

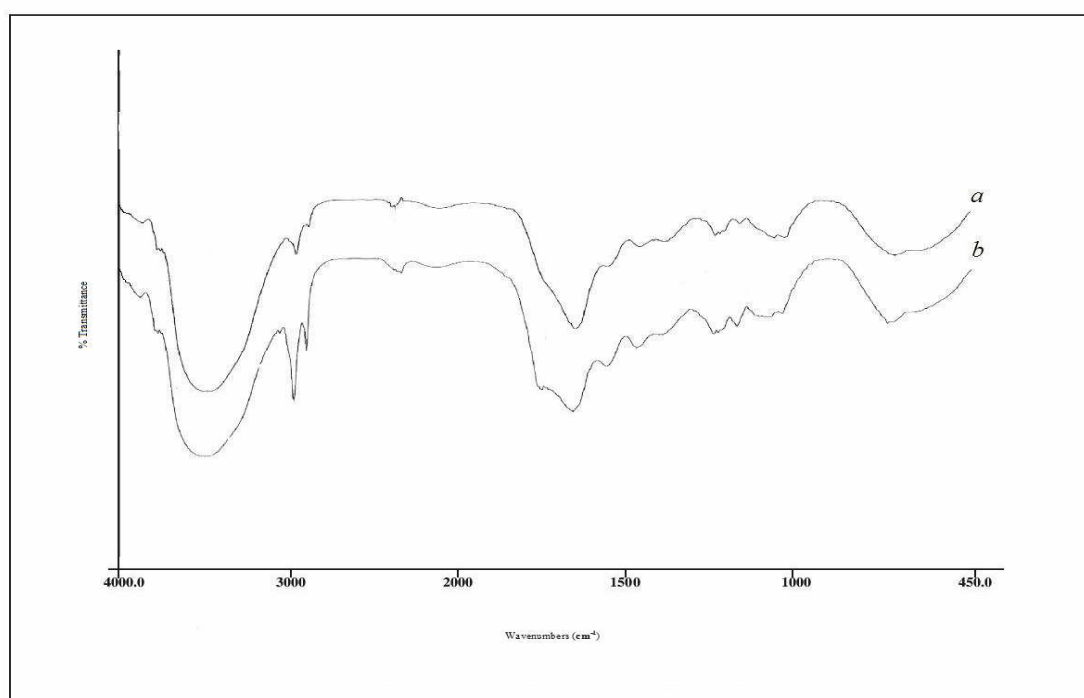


Figure 5 FT- IR spectrum of (a) 1:1 [Gel: Glu], (b) 2:1[Gel: Glu]

(Drugs Using CTAB as Surfactant)

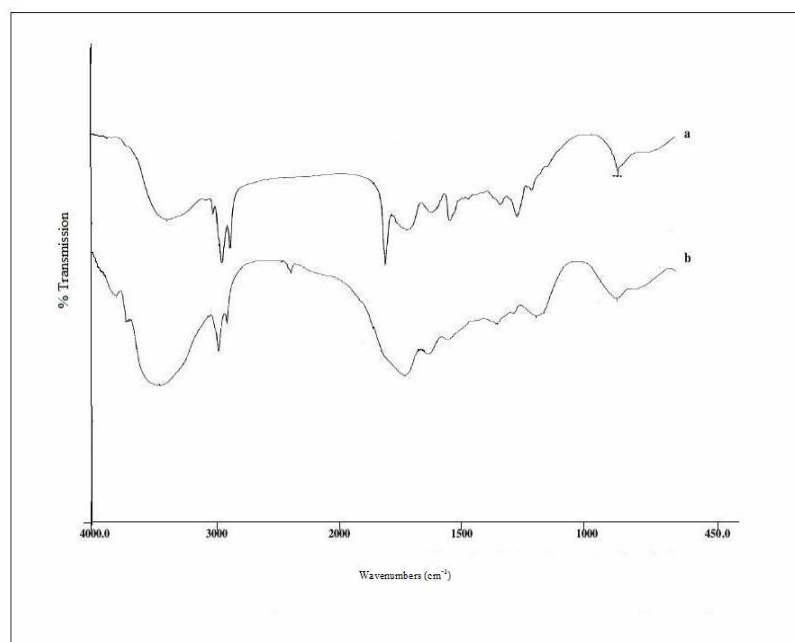
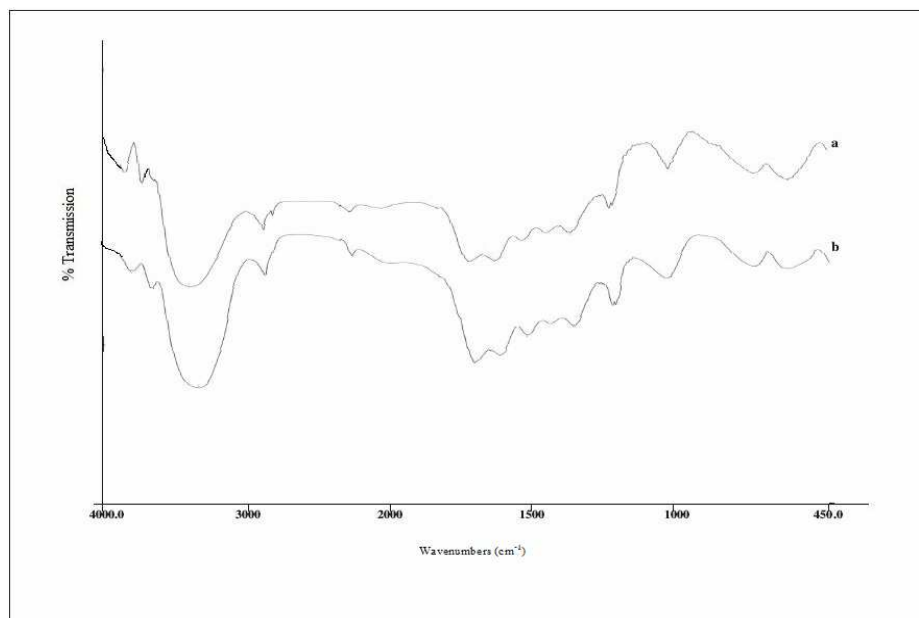


Figure 6 FT – IR spectrum of (a) 1:1 [Gel: Glu], (b) 2:1 [Gel: Glu]

(Drugs Using SDS as Surfactant)



Swelling tests

Gelatin microspheres were placed in water at 25°C for 24 hr to swell. The swelling ratio²² “q” was calculated according to the Equation [1] by measuring the diameter of gelatin microsphere, assuming a spherical geometry for particles.

$$q = V_s/V_d \quad - 1$$

Where, V_s and V_d are the volume of the swollen microspheres and dried microspheres respectively.

Drug release

Drug release from the gelatin microspheres prepared with three different surfactants was determined using three different buffers, Acidic buffer (citrate buffer, 0.1M, pH-5.8), alkaline buffer (phosphate buffer, 0.2M, pH-9.2), Neutral buffer (phosphate buffer, 0.1M, pH-6.8). Drug ciprofloxacin was scanned UV - VIS Spectrophotometrically and it was found to absorb light at 274.2nm. 10mg of buffered sample were immersed in 10ml of respective buffer kept for one week at room temperature. Periodically buffer samples were analyzed for the presence of released drug in UV – VIS Spectrophotometer.

RESULTS AND DISCUSSION

Entrapment efficiency

All the types of microspheres were found to have an entrapment efficiency ranging between 96% – 98%. **The results are shown in Table 1.**

Table 1 Total entrapment efficiency of crosslinked gelatin microspheres

| Total entrapment % | | | |
|--------------------|---------------------|--------------------|--------------------|
| Sample No | Cationic surfactant | Anionic surfactant | Neutral surfactant |
| 1:1(Gel:Glu) | 97.23% | 97.4% | 97.97% |
| 2:1(Gel:Glu) | 96.82% | 97.94% | 97.97% |

Swelling tests

The microspheres were characterized by equilibrium swelling experiments and percentage of swelling is calculated by the formula.

$$q = V_s/V_d$$

Where,

V_s – Volume of the swollen microspheres

V_d – Volume of dried microspheres

The results are shown in the Table 2.

Table 2 Swelling properties of crosslinked gelatin microspheres.

| Sample No | Cationic surfactant | Anionic surfactant | Neutral surfactant |
|--------------|---------------------|--------------------|--------------------|
| 1:1(Gel:Glu) | 80% | 300% | 300% |
| 2:1(Gel:Glu) | 400% | 300% | 300% |

Microspheres prepared in anionic surfactant

1:1(Gel: Glu) and 2:1(Gel: Glu) gave a swelling percentage of 300. This meant crosslinking concentration did not affect the percentage swelling of the microspheres in the case of anionic surfactant.

Microspheres prepared in cationic surfactant

Expect 1:1(Gel: Glu) microspheres which gave a value of 80% swelling other microspheres gave a value of 400% swelling. This low value of 1:1 (Gel: Glu) is may be due to the presence of higher concentration of glutaraldehyde which shows an increased number of crosslinks and a reduced solvent absorption capacity of the microspheres.

Microspheres prepared in neutral surfactant

Microspheres prepared with neutral surfactant, showed a maximum percentage swelling of 300.

Scanning electron microscopy (SEM)

The formation of microspheres and their particle size were analyzed by scanning electron microscopy. The Figure 1-3 shows the scanning electron micrographs of various microspheres. The range of particle size of various microspheres is tabulated Table 3.

Table 3 Particle size distribution of crosslinked gelatin microspheres as per SEM studies.

| Figure no | Sample | Magnification | Particle size(μm) |
|-----------|--------------|---------------|--------------------------------|
| 1 | 1:1(Gel:Glu) | 1000 | 21 |
| 2 | 2:1 CTAB | 1000 | 25 |
| 3 | 2:1 SDS | 1000 | 5 |

- The surface of microspheres of sample 2:1(Gel: Glu) microspheres prepared in anionic and cationic surfactant were found to be smooth and well defined.
- Microspheres prepared in anionic surfactant showed more or less uniform size distribution, while cetyl trimethyl ammonium bromide showed a larger size range.
- Microspheres prepared in span-80 showed a less smooth surface with larger distribution of particle size.

FT-IR spectroscopy

Glutaraldehyde is one of the most popular crosslinking agent, especially for proteins¹³. Because it reacts easily at room temperature with obvious color change characteristic of Schiff base linkages, it is well established that the aldehyde group of the lysine residues of the protein chain to form a Schiff base²³. Crosslinking was observed as the color changed from the pale yellow to deep orange within a few minutes on treatment with glutaraldehyde.

The color change is due to the establishment of aldimine linkages ($\text{CH}=\text{N}$), between the free amino groups of gelatin and glutaraldehyde.

The FT-IR spectrum of gelatin microspheres is shown in figure 4-6. It have two distinct absorption band the $\text{C}=\text{O}$ stretching at 1646 cm^{-1} and the $\text{N}-\text{H}$ stretching at 3435 cm^{-1} . The characteristic absorptions of the back bone occurring at 1540 and 1646 cm^{-1} are the only distinguishing features of the gelatin^{24,25 and 26}

The crosslinked gelatin microspheres show, in addition to the previously mentioned peaks, the aldimine absorption peak at 1450 cm^{-1} .

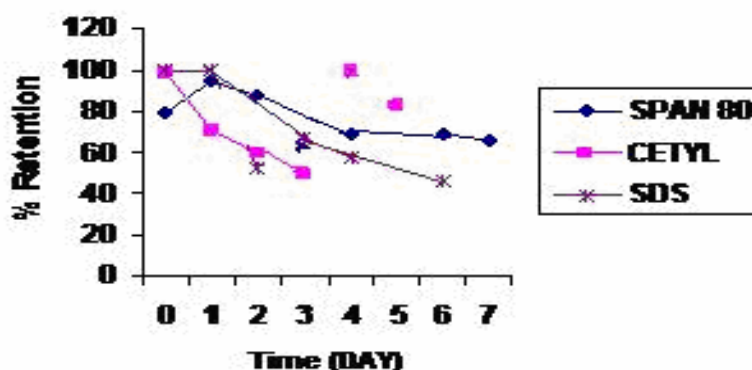
Leakage properties of gelatin microspheres encapsulated with ciprofloxacin

At alkaline pH

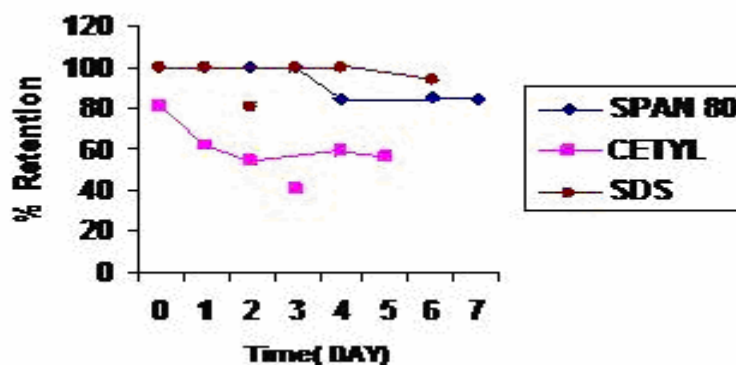
Retention ratio between 1:1(Gel: Glu) and 2:1(Gel: Glu) gelatin microspheres crosslinked with glutaraldehyde and prepared using three different surfactants is shown in the figure 7 and 8.

Figure 7 Release studies of drug encapsulated in gelatin microspheres

At Alkaline pH {1:1 [Gel: Glu]}

**Figure 8** Release studies of drug encapsulated in gelatin microspheres

At Alkaline pH {2:1 [Gel: Glu]}



From the result it was found that microspheres prepared with span-80 showed a boost release of about 20% drug CF after the 2nd day. Till 5th day there is a release of about 30% and after five days the release of CF is constant upto 7th day.

Microspheres prepared by using cationic and anionic surfactant

CTAB and SDS though showed no release initially from 1st day onwards there is a gradual increase in CF release. When compared to the span-80 the release rate by CTAB and SDS was more. This may be due to the interaction between gelatin microspheres and the existing ionic forces of surfactants.

The increase in concentration of the crosslinking agent showed a better retention time as shown in the Figure 7 and 8

From the results obtained it was found that with span-80 and SDS; the

microspheres showed increased stability, showing no release upto 4 days. After 4 days there is a 20% release of the drug by SDS and after that a constant release upto 7 days. However microspheres prepared in span-80 showed no release, offering better retention time. When compared to span-80, SDS and CTAB showed less stability with respect to alkaline medium.

At acidic pH:

Retention rates between 1:1(Gel: Glu) and 2:1(Gel: Glu) gelatin microspheres crosslinked with glutaraldehyde and prepared using three different surfactants is shown in the figure 9 and 10.

Figure 9 Release studies of drug encapsulated in gelatin microspheres

At Acidic pH {1:1 [Gel: Glu]}

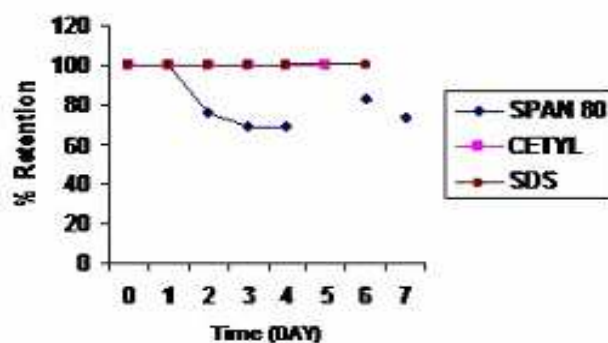
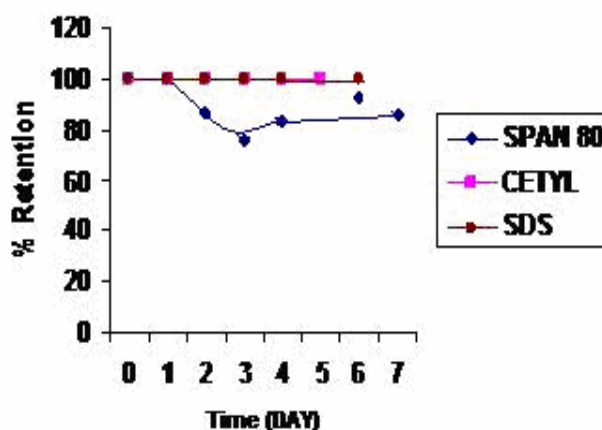


Figure 10 Release studies of drug encapsulated in gelatin microspheres

At Acidic pH {2:1 [Gel: Glu]}



From the results obtained CTAB and SDS microspheres retained the drug upto 7 days while there is a gradual increase in drug release after 2 days from span-80. After 3rd day upto 7 days there was a constant release of the drug. Drug ciprofloxacin has a functional –COOH group which may interact with the acidic medium and this may be the reason for more stability of microspheres of CTAB and SDS.

At neutral pH

Retention rates between 1:1(Gel: Glu) and 2:1(Gel: Glu) gelatin microspheres crosslinked with glutaraldehyde and prepared using three different surfactants is shown in the figure 11 and 12

Figure 11 Release studies of drug encapsulated in gelatin microspheres

At Neutral pH {1:1 [Gel: Glu]}

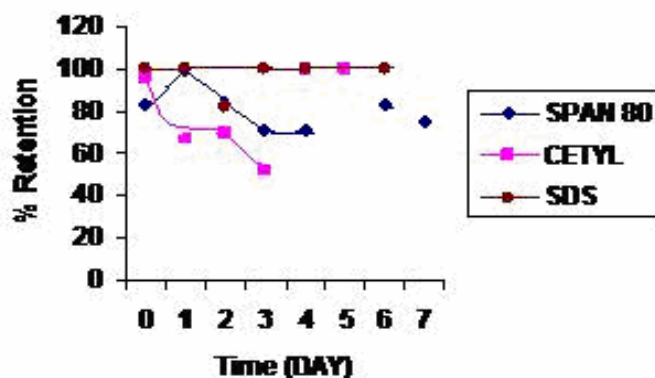
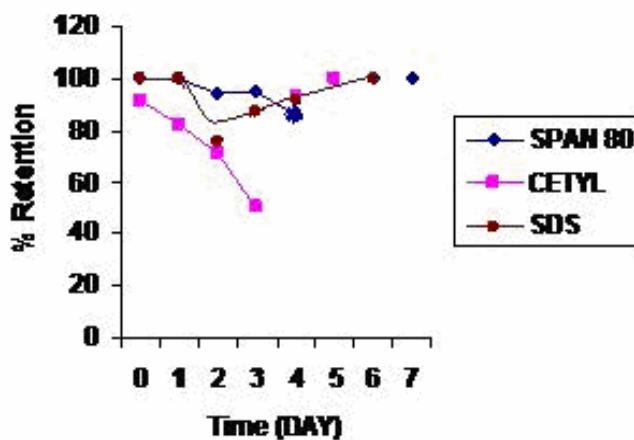


Figure 12 Release studies of drug encapsulated in gelatin microspheres

At Neutral pH {2:1 [Gel: Glu]}



All the three types of microspheres showed same type of release rate (i.e.) CTAB and span-80 showed increased rate of release of CF compared to SDS. SDS showed no release (i.e.) the microspheres prepared out of SDS showed stability upto 7 days irrespective of the medium used.

SUMMARY

Gelatin microspheres were prepared by using emulsification and cross linking method. These microspheres were characterized for % swelling, efficiency of entrapment, FT-IR, SEM studies & leakage property.

1:1(Gel: Glu) & 2:1(Gel: Glu) gelatin microspheres gave a maximum swelling percentage of 300%-600%. This means that the crosslinking didn't affect the percentage swelling of the microspheres.

But in the case of cationic surfactant 1:1 gelatin microspheres increased concentration of glutaraldehyde than 2: 1 Gel: Glu showed a low value of percentage swelling inferring increased number of crosslinks & reduced solvent absorption capacity of microspheres.

All the types of microspheres were found to have fairly good entrapment efficiency ranging between 96%-98% as shown by (Table 1).

The appearance of characteristic absorption bands of carbonyl, amine, aldimine & ester groups confirms the formation of the microspheres & the effect of crosslinking.

The formation of microspheres & their particle size were analyzed by scanning electron microscopy. From the micrograph it is inferred that

- Microspheres with anionic surfactant show more or less uniform size.
- Cationic surfactant microspheres showed a larger size range
- Neutral surfactant showed a less smooth surface with larger distribution.

The leakage property studies showed that in case of ionic surfactants, the rate of release of the drug ciprofloxacin was more, which may be due to the presence of existing ionic forces between gelatin microspheres and presence of -COOH group in drug ciprofloxacin.

In case of neutral surfactant, there was a constant release rate upto 7 days.

From the results obtained it was found that gelatin microspheres entrapped with CIPRO prepared with ionic surfactants showed improved shelf life.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Siva kumar, Central Instrumentation Lab, Annamalai University who helped us in doing SEM studies.

I would also like to express my gratitude to Sridevi K. R project assistant , Indian Institute of Technology (IIT) ,Madras for her help in FT- IR studies.

BIBLIOGRAPHY:

1. Ilum. L and Davis. S.S. The targeting of drugs parenterally by use of microspheres. J.Parenter Sci Technol 1982; 36: 242-251.
2. Majeti. N. V. Ravi kumar. Nano and Microparticles as Controlled Drug Delivery Devices. J. Pharm. Sci 2000; 3 (2): 234-258.
3. Choy YB, Cheng F, Choi H, and Kim K. K. Monodisperse gelatin microsphere as a drug delivery *vehicle*: release profile and effect of crosslinking density. Macromol. Biosci. 2008;8(8):758-65.
4. Gurny. R, Peppas. N.A., Horrington.D.D., and Banker. G.S. Development of biodegradable and injectable latices for controlled release of potent drugs. Drug Dev.Ind.Pharm 1981; 7: 1-25.
5. Okada. H., Yamamoto. M., Heya. T., Inoue. Y., Kamei.S Ogawa. and Y.,Toguchi. H. Drug delivery using biodegradable microspheres. J. Contr. Release 1994;28: 121–129.
6. Zarana S.Patela, Masaya Yamamotob, Hiroki Uedab, Yasuhiko Tabatab and Antonios G. Mikosa Biodegradable gelatin microparticles as delivery systems for the controlled release of bone morphogenetic protein-2. Acta Biomaterialia 2008;4(5): 1126-1138.
7. Langer.R. Invited review polymeric delivery systems for controlled drug release. Chemical Engineering Communication 1980.
8. Langer.R. and Peppas.N.A. Present and future applications of biomaterials in controlled drug delivery systems. Biomaterials 1981; 2(4): 201-214.
9. Pitt.C.G. and Schindler.A. Review biodegradability of synthetic polymers used for medical and pharmaceutical. J.Bioact.Compat.Pol 1986; 1: 467-497.
10. Grandfils.C., Flandroy.P., Nihant.N.,Barbette.S., Jerome. R.,Teyssie. P. and Thibaut.

-
- A. Preparation of Poly (D, L) lactide microspheres by emulsion-solvent evaporation and their clinical applications as a embolic material. *J. Biomed. Mater. Res* 1992;26: 467–479.
11. Viswanathan. N. B., Thomas. P. A., Pandit. J. K., Kulkarni. M.G. and Mashelkar. R. A. Preparation of non-porous microspheres with high entrapment efficiency of proteins by a (water-in-oil)-in oil emulsion technique. *J. Controlled Release* 1999; 58: 9–20.
12. Youan. B. B. C., Benoit. M. A., Baras. B. and Gillard. J. Protein-loaded poly (epsilon-caprolactone) microparticles. I. Optimization of the preparation by (water-in-oil)-in water emulsion solvent evaporation. *J. Microencapsulation* 1999; 16(5): 587-599.
13. Akin. H. and Hasirci. N. Preparation and characterization of crosslinked gelatin microspheres. *J. Appl. Polym. Sci.* 1995; 58(1):95-100.
14. Dinarvand.R., Mahmoodi.S., Farboud.E., Salehi. M. and Atyabi. F. Preparation of gelatin microspheres containing lactic acid –Effect of crosslinking on drug release. *Act Pharma* 2005;55: 57-67.
15. Conway. B. R. and Alpar. H. O. Double emulsion microencapsulation of proteins as model antigens using polymers: effect of emulsifier on the microsphere characteristics and release kinetics. *Eur. J. Pharm. And Biopharm.* 1996;42: 42–48.
16. Cascone. M. G., Lazzeri. L., Carmignani. C. and Zhu. Z. Gelatin nanoparticles produced by a simple W/O emulsion as delivery system for methotrexate. *J. Mater. Sci.* 2002;13: 523-526.
17. Vandelli. M. A., Rivasi. P., Guerra. F., Forni and Arletti. R. Gelatin microspheres cross-linked with D, L-glyceraldehyde as a potential drug delivery system: Preparation, characterization, *in vitro* and *in vivo* studies, *Int. J. Pharm* 2001; 215: 175–184.
18. Ozeki. M. and Tabata. Y., *In vivo* degradability of hydrogels prepared from different gelatins by various cross-linking methods. *J. Biomater Sci Polymer Ed* 2005;16: 549-561.
19. Faton. J. H., and Reeves. D. S., Fluoroquinolone antibiotics microbiology, pharmacokinetics and clinical use. *Drugs* 1998; 36:193-228.
20. Deborah. M. Campoli., Richards., Jon. P. Monk., Allan Prince., Paul Benfield., Peter. A. Todd and Alan Ward. Ciprofloxacin- A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* 1988;35:373-447.
21. Thakkar. H. and Murthy. R.S.R. Polyethylene glycol as a de-aggregating agent in the

-
- preparation of celecoxib loaded gelatin microspheres. *Ars Pharm* 2005; 46(1):19-34.
22. Elisabetta Eposito, Rita Coresti and Claudio Nastruzzi Gelatin microspheres: influence of preparation parameters and thermal treatment on chemico-physical and biopharmaceutical properties. *Biomaterials* 1996; 17(20):2009-2020.
23. Chatterji.P.R. Gelatin with hydrophilic/hydrophobic grafts glutaraldehyde crosslinks. *J. Appl Polymer Sci* 1989;37(8):2203-2212.
24. Narayani. R. and Panduranga Rao. K. Sustained release of propranolol hydrochloride from biodegradable gelatin microspheres. *Trends Biomater Artif Organs* 1990; 5(1):22-28.
25. Narayani.R. and Panduranga Rao. K. Preparation, Characterization and In-Vitro Stability of Hydrophilic Gelatin microspheres using a Gelatin-Methotrexate Conjugate. *Int. J. Pharm.* 1993;95(1-3):85-91.
26. Harland. R.S. and Peppas. N. A. Solute diffusion in swollen membranes VII. Diffusion in semi crystalline networks. *Colloid.Polym.Sci* 1989; 267:218.