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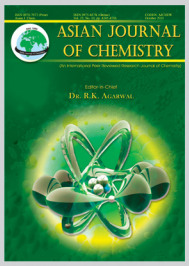

Preparation and Evaluation of Diclofenac Sodium Polyelectrolyte Microparticles for Controlled Drug Delivery

L. SRINIVAS

GITAM Institute of Pharmacy, GITAM University, Rushikonda, Visakhapatnam-530 045, India

Corresponding author: Tel: +91 891 2840551; E-mail: lsrinivas2001@yahoo.com

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The electrostatic interactions between oppositely charged polyelectrolytes lead to formation of insoluble polyelectrolyte complexes in aqueous medium. The polyelectrolyte complexes formed between a poly acid and poly base are little affected by the pH variation of the dissolution medium. In the present study attempts were made to prepare polyelectrolyte complexes of polyvinyl pyrrolidone (poly base) and carbopol (poly acid) into which diclofenac sodium is incorporated and studied for its controlled release. The polyelectrolyte complexation was evaluated by pH, conductivity, Fourier transform infrared spectroscopy, X-ray diffractometry and scanning electron microscopy studies. The dried polyelectrolyte complexes were also evaluated for micromeritic properties and drug release kinetics. Promising results were obtained suggesting the application of these polyelectrolyte microparticles of diclofenac sodium in the design of controlled release systems.

Key Words: Polyelectrolyte complexes, Diclofenac sodium, Carbopol, Polyvinyl pyrrolidone, Poly ions.

INTRODUCTION

Polyelectrolyte complexes (PECs) are the association complexes formed between two oppositely charged particles (*e.g.* polymer-polymer, polymer-drug, polymer-drug-polymer). These are formed due to electrostatic interaction between oppositely charged polyions. This avoids the use of chemical crosslinking agents, thereby reducing the possible toxicity and other undesirable effects of the reagents. The polyelectrolyte complexes formed between a poly acid and poly base are little affected by the pH variation of the dissolution medium. This concept of complexation, between DNA and Chitosan[1], has extensively been studied in the development of delivery vehicle for gene therapy and oral vaccination.

The occurrences of charge-charge interactions between ionic polymers and drugs were considered to be a negative event when the ionic polymers are used as excipients in pharmaceutical formulations. In these systems release of drugs may be strongly affected by the occurrence of charge-charge interactions. However, in recent years these negative events of polymer - drug and polymer-polymer interactions have been exploited positively for controlled drug release[2,3].

Many researchers extensively studied on the properties of the polyelectrolytes[4] and on the formation of polylectrolyte complexes[5-7]. The formation process of polyelectrolyte complexes may be divided into three main classes (a) primary complex formation (b) formation process within intracomplexes (c) intercomplex aggregation process[8]. The inter molecular forces responsible for formation of complexes are either covalent or coordinated bonds or van der Waals forces of dispersion or ion-dipole or dipole-dipole interactions. Several factors like ion site, charge density, polyelectrolyte concentration, pH, ionic strength, solvents and temperature affect the formation of polyelectrolyte complexes.

Polyelectrolyte complexes have gained much attention in the past few years because of their potential applications. These can be used as membranes[9-11], for coating on films and fibers[12], for isolation and fractionation of proteins[13,14], for isolation of nucleic acid[15-17], for binding pharmaceutical products[18], as supports for catalyst[19] and for preparation of microcapsules for drug delivery[20,21].

Multiple charged macromolecular compounds with ions of opposite charge precipitate from aqueous solutions depending on the charge distribution and the molecular weight of the final product. In this case, the higher molecular weight compound displaces low molecular weight ions of the same charge. The polyelectrolyte complexes are composed of a macromolecular multiple charged component of one polarity and many low molecular weight ions of the other polarity or two macromolecular partners with different polarity.

The active substance can be incorporated in to the polyelectrolyte complexes by four ways[22]. In the first case the active

substance will be entrapped from the solution during precipitation of the complex. The active substance will absorb from the solution and gets incorporated into the already formed complex on contact in the second case. In the third case the active substance may chemically bound to at least one complex partner and precipitates during complexation. In the last case the active compound itself may act as poly ion and form polyelectrolyte complex. The active substance from these PECs will be released either by solution equilibration or by ion exchange mechanism or by charge interaction and slow decomplexation as well as breakdown and dissolution of the complex.

The present study is planned to investigate the interpolymer complexation of Carbopol 934P (CP) and poly(vinyl pyrrolidone) (PVP) and also to prepare and evaluate polylectrolyte microparticles of diclofenac sodium (sparingly soluble drug) and these polylectrolyte blends. Carbopol (polyacid) and poly(vinyl pyrrolidone) (poly amide), which are having the tendency for interpolymer complexation, are chosen for the preparation of polyelctrolyte complexes.

EXPERIMENTAL

Diclofenac sodium I.P, was a gift sample obtained from M/s. Aurobindo Pharma Ltd., Carbopol 934P (polyacrylic acid cross-linked with allyl sucrose) obtained from M/s. Ajantha Pharma Ltd., poly(vinyl pyrrolidone) (PVP K90) purchased from Loba Chem., All other reagents and chemicals used were of analytical grade or pharmacopoeial grade and used as obtained.

Investigation of association between polyions: Polyelectrolyte complex formation or phase separation of polyelectrolytes from the aqueous solution is the basic principle for the preparation of polyelectrolyte complexes. Hence in the present study the association character of the polyelectrolytes was studied by taking the 1 % w/v aqueous solution of one of the polyelectrolytes and titrating it with an oppositely charged polyelectrolyte solution (1 % w/v). The formation of associated complexes was studied by the pH and conductivity studies.

pH and conductivity study: The change in the pH and conductivity with respect to the addition of poly ions at various weight ratios of the polymers was determined by using pH meter (Systronics, Model: 361) and conductivity meter (Systronics, Model: 360) with cell constant 1.0 cm^{-1} at 25 ºC respectively. To 100 mL of 1 % w/v carbopol 943P solution, 1 % w/v PVP solution was added at increments of 10 mL and the respective changes in the pH and conductivity were measured until the attainment of constant readings.

Preparation of polyelectrolyte complex: The degree of complexation between carbopol and PVP was studied by considering their weight ratios. This study showed maximum yield of complex between carbopol and PVP at the weight ratio of 1:1 and hence further studies on the incorporation of diclofenac sodium in these complexes was done at 1:1 weight ratios of carbopol and PVP. The PECs were prepared at drug, carbopol and PVP ratio of 80:10:10 (DCP-80), 60:20:20 (DCP-60), 50:25:25 (DCP-50) and 40:30:30 (DCP-40). In the preparation of PECs, 0.5 % w/v aqueous stock dispersions of carbopol 934P and PVP K90 were first prepared. Appropriate quantity of carbopol solution was kept for stirring at 300 rpm. A remi medium duty stirrer with speed meter (model RQT-125) was used for stirring. Appropriate quantity of PVP solution was taken and diclofenac sodium was dissolved in it. This solution was added slowly to carbopol solution under stirring. The stirring continued for 1 h. This solution was centrifuged and the settled complex was collected, washed thoroughly with water and dried at 80 ºC for 6 h. The dried mass obtained was crushed and shifted through mesh # 20. These shifted particles were further screened for size distribution.

Micromeritics of polyelectrolyte complexes: The particle size analysis of all the ratios of prepared PECs was done by sieve analysis using a set of standard sieves of sieve numbers #20, #30, #40, #60, #80, #100 and the amount retained on each sieve was determined. The static angle of repose (θ) was measured according to the fixed funnel and free standing cone method[23]. A funnel with the end of the stem cut perpendicular to its axis of symmetry is secured with its tip 2 cm above a graph paper placed on a flat horizontal surface. Powder is carefully poured through the funnel until the apex of the cone thus formed just reaches the tip of the funnel. The mean diameter of the base of the powder cone is determined and the tangent of the angle of repose is obtained.

The bulk densities of pure drug (diclofenac sodium) and for the prepared PECs of diclofenac sodium were determined by the three-tap method[24]. Compressibility on tamping[25] was measured with a sample of 25 g placed in a 100 mL graduated cylinder and the occupied volume (V_i) was determined. After 500 vibrations, occupied volumes were determined as V_f. With this data we obtained the compressibility index (CI).

$$CI = \frac{V_f - V_i}{V_f} \times 100$$

FTIR spectroscopy: The pure drug diclofenac sodium, carbopol 934P, PVP K90 and the polyelectrolyte complex of diclofenac sodium (DCP-50) samples were analyzed for the determination of complex formation by Fourier transformed infrared spectroscopic (FTIR, make Perkin Elmer) studies. The IR spectra were done against the KBr background.

X-Ray diffraction: The powder X-ray diffraction patterns of pure diclofenac sodium, carbopol 934P, PVP K90 and the polyelectrolyte complex of diclofenac sodium (DCP 50) were recorded by using an automated Siemens D/5000. The samples were irradiated with monochromatized Cu K_α radiation and analyzed between 2 angles of 30-300. The voltages, current and time per step used were 30 KV, 20 mA and 0.5 s, respectively.

Scanning electron microscopy (SEM): The SEM photographs of diclofenac sodium, carbopol 943P, PVP and DCP50 were obtained by scanning electron microscope (Jeol, JSM-840 A, Japan) with 20 kV accelerating voltage.

Drug content: Accurately weighed 100 mg of the drug loaded PEC microparticles and transferred into a 100 mL volumetric flask. 5 mL of 5N sodium hydroxide solution was added and sonicated for 15 min. 50 mL of phosphate buffer of pH 7.4 was added to this solution and vigorously shaken for 15 min and made up to volume with buffer. The resulted solution was filtered through 0.45 μm filter paper and suitably diluted and the drug content was estimated spectrophotometrically

by measuring the absorbance at 275 nm. The drug loading efficiency of the prepared PECs was calculated by using the following formula.

Drug loading efficiency

$$= \left(\frac{\text{Practical amount of drug loaded}}{\text{Theoretical amount of drug loaded}} \right) \times 100$$

***In vitro* diffusion study:** *In vitro* diffusion studies were performed by dialysis technique using dialysis membrane - 60 (Avg. flat width - 25.27 mm, Avg. diameter - 15.9 mm, capacity approx. - 1.99 mL/cm, molecular weight cut off 12000-14000 KD). The dialyzing medium was phosphate buffer pH 7.4. One end of pretreated dialysis tubing (7 cm in length) was tied with thread and then sample equivalent to 25 mg of diclofenac sodium was placed in it along with 0.5 mL of dialyzing medium. The other end of the tubing was also secured with thread and was allowed to rotate freely in 300 mL of dialyzing medium and stirred continuously with magnetic bead on magnetic plate at 37 °C. Aliquots of 1 mL were removed at different time intervals and diluted further. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for diclofenac sodium dialyzed across the membrane at corresponding time by using UV-visible spectrophotometer at 275 nm. All the diffusion experiments were conducted in triplicate and the mean values were reported.

The diffusion studies were carried for the pure diclofenac sodium and for all the ratios of DCP microparticle. The drug release data was further subjected for calculation of diffusion coefficient of drug based on modified Higuchi equation by using the following formula.

$$D = \frac{a^2 \pi}{(2C_0)^2 A^2}$$

where, D is diffusion coefficient, C_0 initial concentration of drug, α is the slope of curve between cumulative amount released *versus* square root time and A is the area of the diffusion cell membrane. The data was also subjected for analysis by calculating mean dissolution time (MDT)[26] to characterize the release rate of drug in different experimental conditions. The mean time for the drug to dissolve under *in vitro* conditions was calculated from dissolution data using statistical moment analysis using the following equation.

$$\text{Mean dissolution time } (MDT) = \frac{\int_0^\infty M_\infty - M_t \, dt}{M_\infty}$$

where, M_∞ is initial amount of dose and M_t is amount of the drug released at time t.

***In vitro* dissolution:** *In vitro* dissolution studies were carried out in 900 mL of alkaline phosphate buffer of pH 7.4 using USP XXIV type-II (Paddle) dissolution rate test apparatus (model DISSO 2000, M/S Labindia). Sample equivalent to 100 mg of diclofenac sodium, a speed of 50 rpm and a temperature of 37 ± 1 ºC were used in each test. A 5 mL aliquot was withdrawn at different time intervals, filtered and replaced with 5 mL of fresh dissolution medium. The filtered samples were suitably diluted whenever necessary and assayed for diclofenac sodium by measuring absorbance at 275 nm. All the dissolution experiments were conducted in triplicate and the mean values were reported. The dissolution studies were carried for the pure diclofenac sodium and the prepared PECs of #30/40 mesh size.

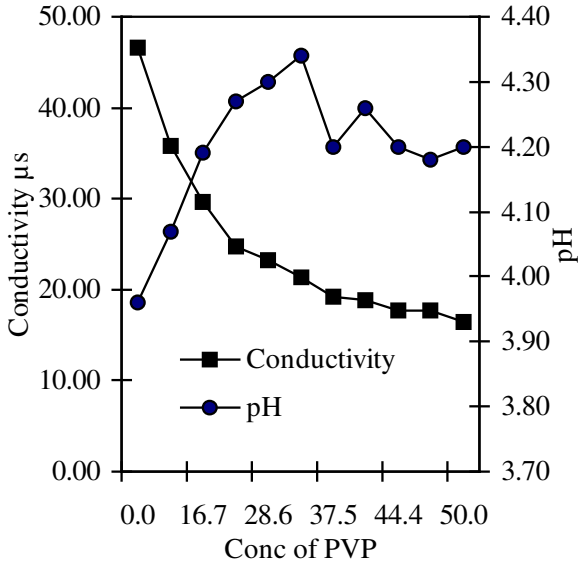
RESULTS AND DISCUSSION

pH and conductivity study: Polyions posses certain charge when they are present in the aqueous solution. Oppositely charged polyions form an insoluble polyelectrolyte complex in the aqueous medium. There is a possible change in the charge that may occur when the polyelectrolytes interact and form complexes. These interactions and the formation of PECs can be well judged by pH and conductivity studies. In the present study the polyelectrolytes in solution exhibited various pH and conductivity profiles (Fig. 1) when they interact with each other. In the case of carbopol and PVP, when PVP solution was added to carbopol solution the conductivity decreased gradually (form 46.6-16.5 μs) and sharp fall in conductivity (29.6-24.8 μs) was observed during complex formation. Though there was a change in conductivity very little change was observed in the case of pH (3.96-4.20). When carbopol solution was added to the solution of diclofenac sodium and PVP (1:1) the conductivity decreased from 108-43.0 μs and sudden change in conductivity (from 91.0-77.3 μs) was observed during complexation. The pH was little affected. The pH changed from 6.29-5.48. Thus the pH and conductivity studies clearly indicated the formation of polyelectrolyte complex between carbopol, PVP K90 and diclofenac sodium.

FTIR spectroscopy: The FTIR spectra (Fig. 2) of polyelectrolyte complex of diclofenac sodium (DCP-60) showed all the characteristic peaks of diclofenac sodium. The sodium salt of carboxylic acid stretch (COONa) at 1580 cm^{-1}, secondary acyl amine (-NH-) stretch at 1550 cm^{-1} and chloride (Cl) stretch at 750 cm^{-1} were present in both the spectra with minor shifts. The DCP-60 spectra also showed the presence of carboxylic acid (COOH) stretch of carbopol at 1750 cm^{-1} and amide stretch of PVP at 1720 cm^{-1}. This data clearly indicated the absence of any chemical interaction between diclofenac sodium, carbopol and PVP in the formation of polyelectrolyte complex. Thus it indirectly confirmed the electrostatic interactions between the polyelectrolytes, which are responsible for the formation of these polyelectrolyte complexes.

X-Ray diffraction: The X-ray diffractograms are shown in Fig. 3. The spectra of diclofenac sodium showed the sharp peaks at 6.63, 8.5, 15.2 and 21.7 angle (2θ) indicating the crystallinity of the drug. The spectra of carbopol and PVP did not have any sharp peaks indicating their amorphous nature. The spectra of DCP-60 showed the disappearance of the peaks at 6.63 and 8.5 and showed new peaks at 13.5, 20.5 and 24.4 angle (2θ). This may be due to fine dispersion and entrapment of the drug in the polyelectrolyte complex.

SEM: The scanning electron microphotographs (Fig. 4) of the pure diclofenac sodium showed crystalline structure. The powders of carbopol 943P and PVP were irregular in shape. The surface of DCP microparticles was porous and they were irregular in shape.



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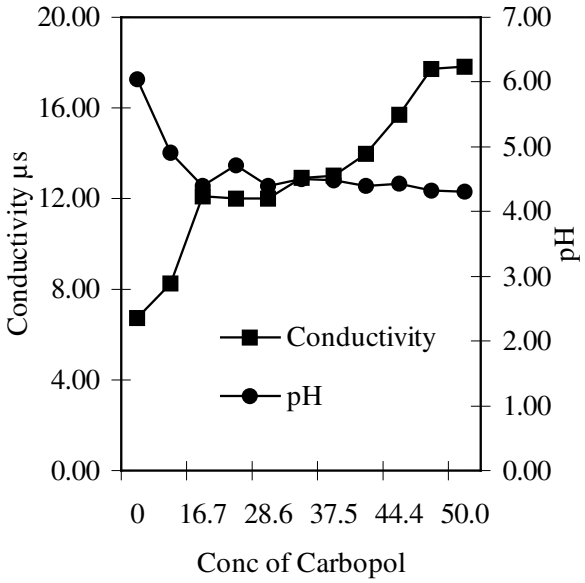

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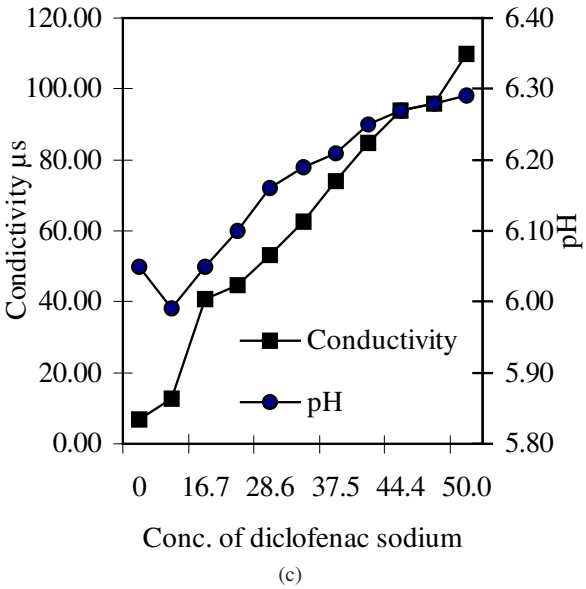

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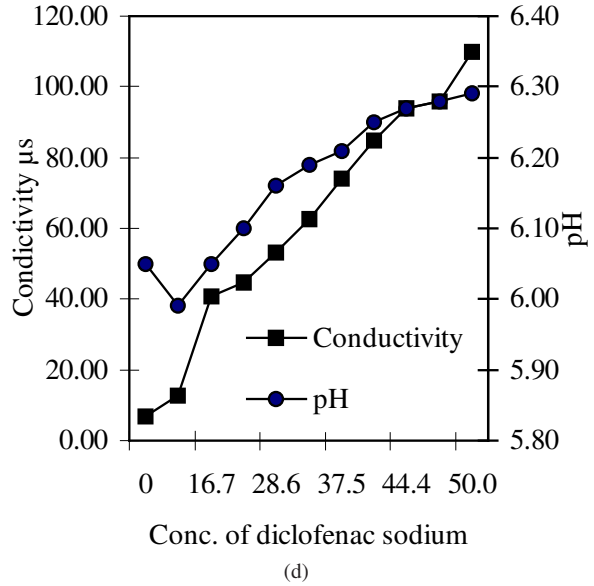


(d)

Fig. 1. Conductivity and pH profiles of polyelectrolyte complexation. (a) PVP added to carbopol, (b) carbopol added to PVP, (c) diclofenac sodium added to PVP and (d) carbopol added to mixture of PVP and diclofenac sodium

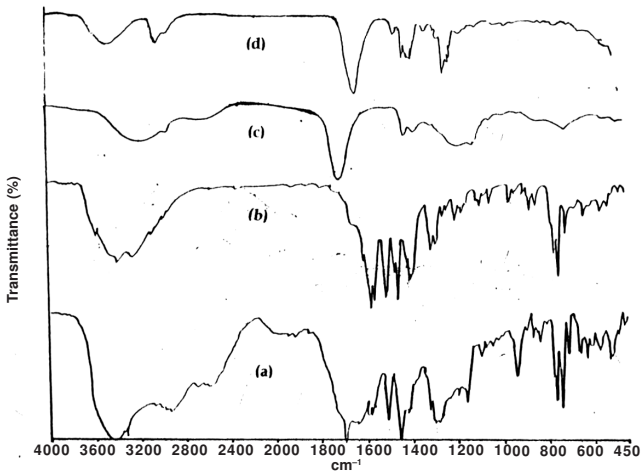


Fig. 2. FTIR spectra of (a) DCP-50, (b) diclofenac sodium, (c) carbopol 934P and (d) PVP K90

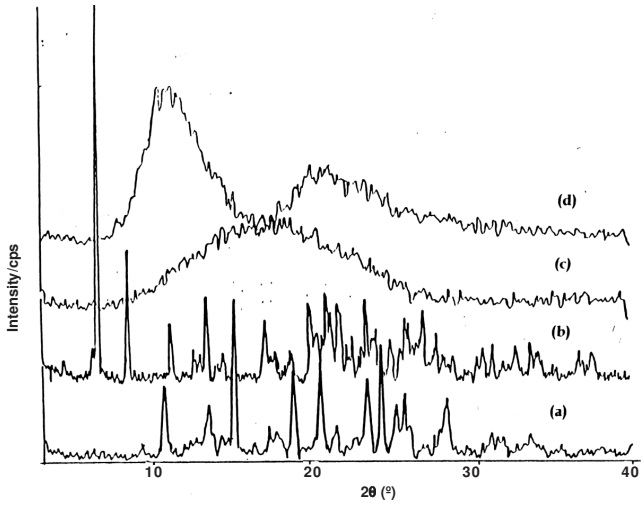
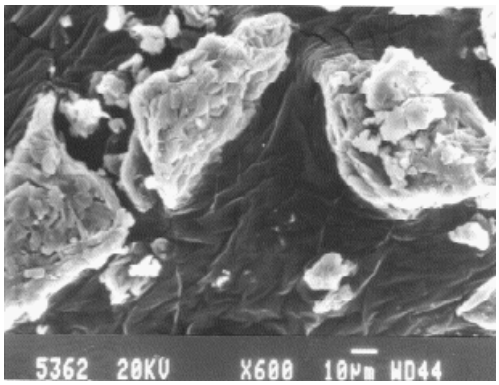


Fig. 3. X-Ray diffractograms of (a) DCP-50, (b) diclofenac sodium, (c) carbopol 934 P and (d) PVP K90



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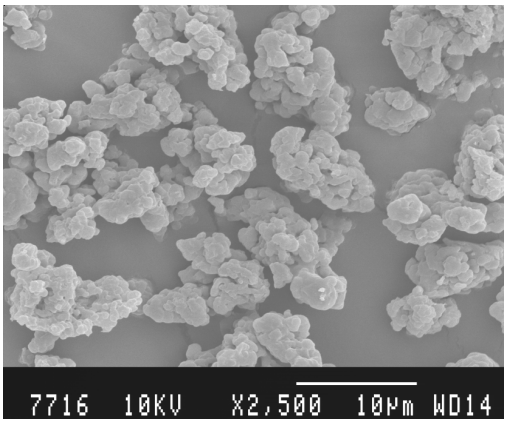

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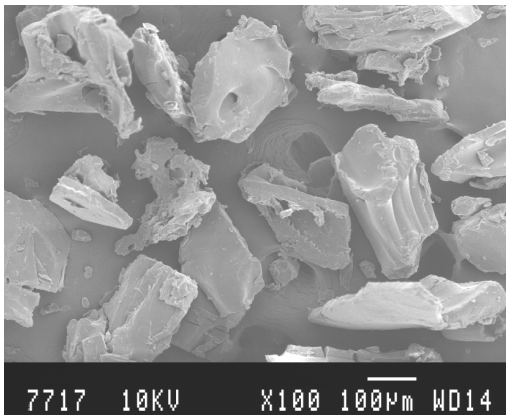

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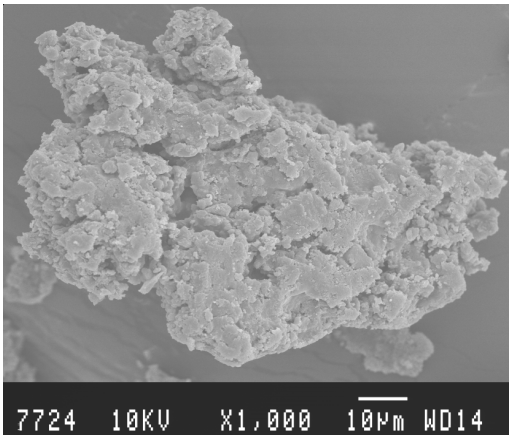

(d)

Fig. 4. Scanning electron micrographs of (a) diclofenac sodium, (b) carbopol 934P, (c) PVP K90 and (d) DCP50

Micromeritics: The micromeritic properties of PEC microparticles showed very good results (Table-1). These PECs possess very good flowability (angle of repose θ = 26-29°) and compressibility (compressibility index = 10-14).

Drug content: The drug content (mg) of the PEC microparticles was 65.20 ± 1.45, 49.20 ± 0.92, 46.30 ± 0.86 and 37.6 ± 1.77 per 100 mg of DCP-80, DCP-60, DCP-50 and DCP-40 respectively (Table-1). Low standard deviation values in drug content show reproducibility from batch to batch. The drug loading efficiency of DCP-80, DCP-60, DCP-50 and DCP-40 was 81.5, 82.0, 92.6 and 94.0 %, respectively. The results show that there was no loss of drug during the process of complexation. The drug loading efficiency increases as the polymer concentration increases.

***In vitro* diffusion:** The drug release from PEC microparticles was studied using *in vitro* drug diffusion studies. The results (Table-2) indicated that diclofenac sodium release was gradual and complete. In case of pure drug 100 % of release was obtained in 2 h. Diclofenac sodium release from PEC microparticles extended for a period of 12 h. The diffusion coefficient of pure diclofenac sodium was 32.810×10^{-5} cm^2/s, whereas for DCP microparticles the diffusion coefficient decreased from 9.552×10^{-5}-0.471×10^{-5} cm^2/s as the drug to polymer ratio increased. The mean dissolution time of pure drug was 25 min and for DCP microparticles it was in the range 134-356 min. As the drug to polymer ratio increased the mean dissolution time also increased.

***In vitro* dissolution:** The drug release from the PEC microparticles was (Fig. 5) uniform and extended for longer duration of time. Within 10 min, 100 % of the drug is dissolved in the case of pure diclofenac sodium, whereas the release from the PEC was extended for a period of 2 h. The T_{90} (time taken for 90 % drug to dissolve) value for pure diclofenac sodium was 4 min, whereas for DCP microparticles it was in the range of 34-56 min. The release of drug from the PECs decreased as the polymer concentration increased.

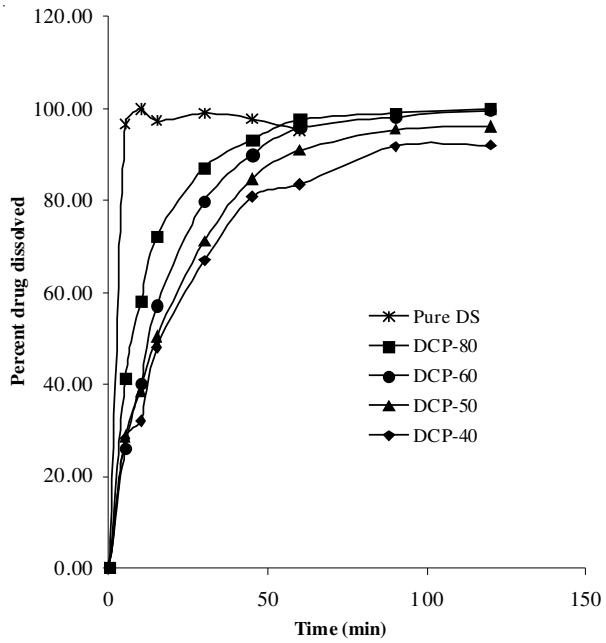

Fig. 5. Comparative dissolution profiles (n = 3) of diclofenac sodium from polyelectrolyte complex microparticles

TABLE-1
COMPARATIVE PHYSICAL PROPERTIES OF POLYELECTROLYTE COMPLEX MICROPARTICLES

Name	Bulk density (g/cc)	Tapped density (g/cc)	Compressibility index (CI) (%)	Angle of repose (θ) (º)	Drug content (mg) ± s.d.	Encapsulation efficiency (%)
Diclofenac sodium	0.445	0.523	22.22	34.38	–	–
DCP80	0.528	0.638	14.35	26.87	65.2 ± 1.45	81.50
DCP60	0.584	0.642	11.25	27.48	49.2 ± 0.92	82.00
DCP50	0.562	0.598	10.14	27.25	46.3 ± 0.86	92.60
DCP40	0.582	0.632	10.54	28.64	37.6 ± 1.77	94.00

TABLE-2
DIFFERENT RELEASE PARAMETERS OF DICLOFENAC SODIUM (n = 3) FROM POLYELECTROLYTE COMPLEX MICROPARTICLES

Product	Diffusion coefficient ($D \times 10^{-5}$) (cm^2/s)	MDT (min)	T_{90} (min)	Zero order		First order		Peppas equation		Erosion	Higuchi Diffusion
				K_0 ($mg.h^{-1}$)	r	K_1 (h^{-1})	r	r	n	r	r
Pure drug	32.81	25	4	599.880	0.8804	9.6369	0.9931	–	–	–	–
DCP-80	9.552	134	34	79.098	0.8473	0.0595	0.9889	0.9827	0.4432	0.9794	0.9847
DCP-60	5.401	144	45	43.273	0.8255	0.0457	0.9843	0.9907	0.5902	0.9906	0.9980
DCP-50	0.574	172	52	42.071	0.8500	0.0342	0.9882	0.9961	0.5776	0.9948	0.9969
DCP-40	0.471	355	56	40.723	0.8596	0.0275	0.9953	0.9948	0.6137	0.9937	0.9962

The release profile of drug from these PEC microparticles was subjected for zero order[27], first order[28], Higuchi diffusion[29], erosion[26] and Peppas-Korsemeyer equation[30] and the correlation coefficient (r) values are given in Table-2. The results showed that diclofenac sodium release form PEC microparticles followed first order release kinetics as indicated by the correlation coefficient 'r' values (r = 0.9843-0.9953) for DCP microparticles. The release rate (k_1 value shown in Table-2) decreased as the drug to polymer ratio increased.

The plots of log mean per cent of drug released *versus* log time of all the DCP microparticles were found to be linear. The correlation coefficient (r) values were in the range 0.9827-0.9961, respectively for DCP microparticles further indicating the suitability of Peppas-Korsemeyer equation for explaining the release kinetics. It was found that diffusional exponent (n) values for DCP microparticles 0.44-0.61, indicating that the release mechanism followed non-fickian diffusion. The results of the study indicated that the release of drug from DCP microparticles followed first order kinetics *via* anomalous (non-Fickian) diffusion.

The mechanism of drug release was established by constructing plots of cumulative per cent drug released *versus* square root time (Higuchi diffusion) and cube root of fraction of drug remained to be released *versus* time (erosion). The linear correlation coefficients of the slopes for the plots are shown in Table-2. The correlation coefficients for both diffusion as well as erosion are near to 1 indicating good correlation for both the mechanisms of drug release. The causes for diffusion may be due to the swollen insoluble PEC matrix, which entrapped the drug. The difussional path length increased with increase in the size of the swollen matrix there by leading to good correlation for diffusion. The cause for good correlation for erosion may be due to the presence of the surface drug and due to the break down of the swollen matrix to smaller sizes.Hence for the PEC both the mechanisms of drug release were possible.

Effect of the dissolution medium pH on drug release: The effect of dissolution medium pH on the dissolution rate of diclofenac sodium from PEC microparticles was studied using DCP60. The dissolution studies were done in different dissolution media of pH 1.2, 4.5, 6.8 and 7.4 (Fig. 6). In pH 1.2 the release of drug was very low (only 3 % released in 2 h) whereas in the remaining three media release was uniform and extended for 2 h from the microparticles. Similar dissolution profiles were obtained in pH 4.5, 6.8 and 7.4 dissolution media, with 100 % drug release in 2 h DCP60. However the poor release of drug in pH 1.2 medium may be due to the insolubility of the drug (solubility < 1 mg/mL). The results confirmed that the dissolution of PEC microparticles was not affected by the pH of the dissolution medium.

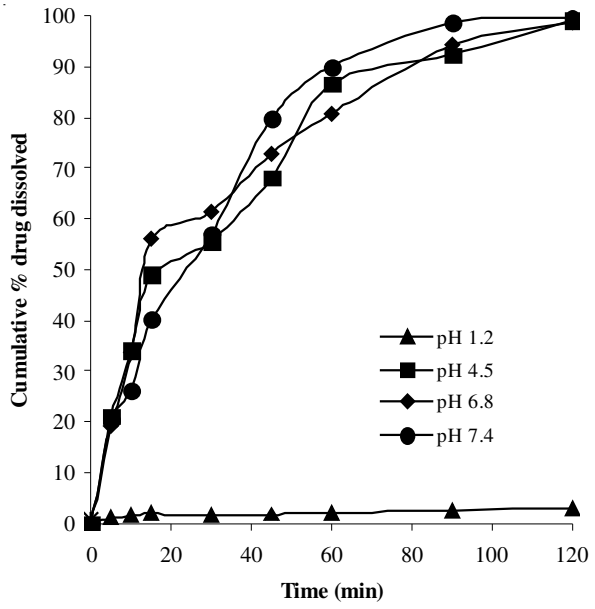

Fig. 6. Comparative dissolution profiles of diclofenac sodium from DCP60 microparticles in different dissolution media of varying pH

Conclusion

Polyelectrolyte complexes are the association complexes formed by the electrostatic interaction between oppositely

charged particles. Polyelectrolyte complexes have gained much attention in drug delivery. In the present study it was proved that the electrostatic interactions between the polyelectrolytes (carbopol, poly(vinyl pyrrolidone) PVP and diclofenac sodium) are responsible for the formation of insoluble polyelectrolyte complex. Diclofenac sodium release from these polyelectrolyte complex microparticles was uniform and prolonged. The results of the study clearly indicated the applicability of polyelectrolyte complexes in the design of controlled release microparticles.

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REFERENCES

1. R. Krishnendu, M. Hai-Quan, H. Shau-Ku and W.L. Kam, *Nature Med*., **5**, 387 (1999).
2. I. Genta, P. Perugini, T. Modena, F. Pavanetto, F. Castelli, R.A.A. Muzzarelli, C. Muzzarelli and B. Conti, *Carbohydr. Polym*., **52**, 11 (2003).
3. S. Graeme Macleod, H. John Collett and T. John Fell, *J. Control. Rel*., **58**, 303 (1999).
4. R. Chang and Y. Arun, *J. Chem. Phys*., **116**, 5284 (2002).
5. G. Petzold, A. Nebel, H.M. Buchhammer and K.Lunkwitz, *Colloid Polym. Sci*., **276**, 125 (1998).
6. Lee Webster and B.H. Malcolm, *Eur. Polym. J*., **33**, 1173 (1997).
7. D.J. Burgess, *J. Colloid Interf. Sci*., **140**, 227 (1990).
8. E. Tsuchida, *J. Macromol. Sci. Pure Appl. Chem*., **31A**, 1 (1994).
9. M. Senuma, S. Kuwabara, K. Kaeriyama, F. Hase and Y. Shimura, *J. Appl. Polym. Sci*., **31**, 1687 (1986).
10. H. Sato, M. Maeda and A. Nakajima, *J. Appl. Polym. Sci*., **23**, 1759 (1979).
11. L.V. Harris, In eds.: E.L.V. Harris and S. Angal, Protein Purification Methods, Oxford University Press, New York (1993).
12. L. Janne and T. Lindström, *Sci. Technol*., **1**, 40 (2001).
13. Y. Hirouki and K. Takeshi, *Chem. Eng. (Japan)*, **41**, B11 (1989).
14. P.L. Dubin, J. Gao and K. Mattison, *Sep. Purif. Methods*, **23**, 1 (1994).
15. R.M. Cordes, W.B. Sima and C.E. Glatz, *Biotechnol. Prog*., **6**, 283 (1990).
16. A. Atkinson and G.W. Jack, *Biochem. Biophys. Acta*, **308**, 41 (1973).
17. J. Jendrisak, In eds.: R. Burgerss and Alan R. Liss, Protein Purification: Micro to Macro, Inc., New York, p. 75 (1987).
18. J. Chen, S. Jo and K. Park, *Carbohydr. Polym*., **28**, 69 (1995).
19. H. Dautzenberg, J. Kotz, K.J. Linow, B. Philipp and G. Rother, In eds.: ed. P. Dubin, J. Bock, R. Davis, D.N. Schulz and C. Thies, Macromolecular Complexes in Chemistry and Biology, Springer-Verlag, Berlin., p. 119 (1994).
20. B. Artur and H. David, *Colloids Surf. B: Biointerfaces*, **21**, 285 (2001).
21. R. Murakami and R. Takashima, *Food Hydrocolloids*, **17**, 885 (2003).
22. V. Krone, M. Magerstadt, A. Walch, A. Groner and D. Hoffmann, United States Patent, 5,700,459 (1997).
23. G.S. Banker and N.R. Anderson, In eds.: L. Lachman, H.A. Lieberman and J.L. Kanig, The Theory and Practice of Industrial Pharmacy, 4th Indian Reprint, Verghese Publishing House, Bombay, edn. 3, p. 317 (1991).
24. A. Martin, P. Bustamante and A.H.C. Chun, Physical Pharmacy, 4th Ed., Indian Reprint, B.I. Waverly Pvt. Ltd., New Delhi, p. 444 (1994).
25. K. Marshall, In eds.: L. Lachman, H.A. Lieberman and J.L. Kanig, The Theory and Practice of Industrial Pharmacy, 4th Indian Reprint, Verghese Publishing House, Bombay, edn. 3, p. 67 (1991).
26. C. Paulo and M.S.L. Jose, *Eur. J. Pharm. Sci*., **13**, 123 (2001).
27. M. Bamba, F. Puisieux, J.P. Marty and J.T. Carstensen, *Int. J. Pharm*., **2**, 307 (1979).
28. J.G. Wagner, *J. Pharm. Sci*., **58**, 1253 (1969).
29. T. Higuchi, *J. Pharm. Sci*., **52**, 1145 (1963).
30. R.W. Korsmeyer, R. Gurny, E.M. Doelker, P. Buri and N.A. Peppas, *Int. J. Pharm*., **15**, 25 (1983).