Journal of Research in Pharmacy

Research Article

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Development of Polyelectrolyte Complex Beads Containing Vancomycin Hydrochloride for Colon-targeted Drug Delivery

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Received: 20 June 2022 / Revised: 14 September 2022 / Accepted: 19 September 2022

ABSTRACT: Vancomycin Hydrochloride is a glycopeptide antibiotic used for the treatment of Pseudomembranous colitis. This drug is susceptible to proteolytic degradation in the gastric environment and it is associated with nephrotoxicity. As the therapeutic action of vancomycin hydrochloride is intended in the intestine, colon-targeted drug delivery could help the drug achieve sufficient concentration in the target site. A polyelectrolyte complex using chitosan and hupu gum is used to prepare the beads that control the drug release and minimize the adverse effects. Eudragit S100 is used as an enteric coating material to bypass the gastric environment. The beads thus formed by polyelectrolyte complex were filled into capsules and coated with Eudragit S100. The formulation (CHP3C8) containing chitosan and hupu gum with polyethylene glycol 400 and 8% Eudragit S100 coating has shown a controlled drug release of up to 24 hours with a predetermined lag time. The *ex-vivo* studies have shown higher drug release in rat cecal content which can be attributed to the degradation of polyelectrolyte complex by intestinal bacteria. The *in-vivo* studies are carried out using white New Zealand rabbits where the capsules (CHP3C8) and solution of pure drug of vancomycin hydrochloride are administered via the oral route. The peak plasma concentration (C_{max}) of Vancomycin Hydrochloride from CHP3C8 and the oral solution was found to be 809.53 µg/ml and 402 µg/ml respectively. All the results have shown the superiority of Vancomycin Hydrochloride polyelectrolyte beads (CHP3C8) over the pure drug indicating its suitability for colon drug delivery.

KEYWORDS: Polyelectrolyte complex; Pseudomembranous colitis; Colon targeted drug delivery; Hupu gum; Chitosan.

1. INTRODUCTION

Multiple unit dosage forms like beads are beneficial over single unit dosage forms as they enable controlled, temporal drug delivery with fewer adverse effects and comparatively low doses [1]. In this study, beads of Vancomycin Hydrochloride are prepared by using the polyelectrolyte complex technique. The absorption of the drugs in the colon region is favored by its almost neutral pH (6.8), longer transit time, and comparatively lesser proteolytic activity. The polyelectrolyte complex method is a novel technique for the delivery of drugs showing therapeutic action in the colon as it protects the drug from the gastric environment as well as curtails the drug release in a controlled manner [2]. A literature survey revealed that a polyelectrolyte complex using chitosan-pectin can be successfully employed for colon drug delivery, however, the research is limited to in vitro studies and poor drug release performance [3]. Hence, this study is aimed to increase the drug release performance in the colon with a desirable lag time and to perform in vivo and ex vivo studies that depict a complete pharmacokinetic picture of the developed beads. The main objective of this work is to develop vancomycin hydrochloride beads using the polyelectrolyte complex technique which are then loaded into capsules with enteric coating. Chitosan and Hupu gum are used to form the polyelectrolyte complex while polyethylene glycol 400 (PEG 400) and Eudragit S100 are used for the rigidization of the beads formed [4,5]. Once the beads are loaded into the capsules, Eudragit S100 is again used over the capsules as an enteric coating to prevent gastric degradation of the drug as well as release the drug in a controlled manner to avoid nephrotoxicity associated with vancomycin hydrochloride.

How to cite this article: Kudupudi V, Kakarparthy R, Sarella P, Kolapalli V. Development of polyelectrolyte complex beads containing vancomycin hydrochloride for colon targeted drug delivery. J Res Pharm. 2023; 27(4): 1658-1672.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.450

2. RESULTS

2.1. Drug excipient incompatibility

Drug excipient incompatibility studies by Fourier Transform Infrared Spectroscopy (FTIR) revealed that the characteristic peaks representing the parent functional groups in the pure drug vancomycin hydrochloride remained intact.

2.2. Determination of polyelectrolyte mixing ratio on % dry yield

The concentration of hupu gum and chitosan are taken at various concentrations (1:0.5, 1:1, 1:1.5, 1:2) to determine the % dry yield and favorable pH that gives optimum results. The results of these preliminary studies are shown in Table 1.

Table 1. Effect of polyelectrolyte mixing ratio on the % dry yield of Hupu gum-Chitosan polyelectrolyte complex

Ratio of HG-CH	Concentration		% dry yield PEC	pH	Conductivity
	HG (%w/w)	CH (%w/w)			
1:0.5	66.66	33.33	71.08±1.32	7.86	0.98
1:1	50.00	50.00	88.33±1.44	7.82	1.02
1:1.5	40.00	60.00	86.75±1.53	7.92	1.06
1:2	33.33	66.66	78.33±1.28	7.95	1.00

2.3. Evaluation of beads

The prepared beads are evaluated for color, shape, surface, % yield, particle size, % drug loading, % drug entrapment, and swelling index. The results of the evaluation tests are shown in Table 2.

Table 2. Evaluation of vancomycin hydrochloride beads

Formulation code	% Yield	Particle size[a] (mm)	% Drug loading[b]	% Drug entrapment efficiency[b]	% Swelling index[b]		Weight of beads equivalent to 125 mg of drug	Capsule size
					0.1N HCl	pH 7.4 phosphate buffer		
CHP1	89.62	0.102±0.07	50.55±0.22	80.88±1.05	19.03±1.71	89.70±0.67	247.28	2
CHP2	76.62	0.093±0.00	35.38±0.17	84.92±0.82	19.67±1.59	90.89±0.56	353.26	1
CHP3	73.23	0.104±0.02	25.71±0.25	92.57±1.33	20.72±1.36	89.58±1.08	486.11	0
CHE4	93.26	0.105±0.03	54.51±0.23	87.21±1.56	19.77±1.20	83.32±1.22	229.33	2
CHE5	88.10	0.102±0.07	37.36±0.18	89.67±0.60	19.89±1.24	84.87±0.31	334.56	1
CHE6	80.21	0.105±0.00	27.25±0.16	98.11±0.73	20.39±1.02	88.42±1.60	458.67	0
CHPE7	90.62	0.107±0.08	52.09±0.21	83.34±0.78	21.43±1.02	86.76±0.60	239.98	2
CHPE8	83.41	0.107±0.04	36.92±0.16	88.62±1.25	21.90±0.58	88.79±0.56	338.54	1
CHPE9	78.12	0.108±0.03	26.37±0.13	94.95±1.33	22.91±1.60	88.74±0.65	473.96	0

a: mean ± S.D., n=100;
b: mean± S.D, n=3

2.4. Evaluation of capsules

The beads were loaded into the capsules by taking an equivalent weight of 125 mg of vancomycin hydrochloride. 6%, 8%, and 10% Eudragit S100 coating is applied to the capsules and the coated and uncoated capsules were evaluated as per Indian Pharmacopeia (I.P). The results are shown in Table 3.

Table 3. Evaluation of vancomycin hydrochloride capsules

Uncoated Capsules			Coated Capsules					
Formulation code	**Uniformity of weight[a] (mg)**	**Drug content[b] (%)**	**Formulation code**	**Uniformity of weight[a] (mg)**	**Drug content[b] (%)**	**Formulation code**	**Uniformity of weight[a] (mg)**	**Drug content[b] (%)**
CHP1	248.86±0.032	99.67±0.01	**CHP1C6**	262.98±0.012	99.56±0.02	**CHE4C8**	248.90±0.012	98.81±0.01
CHP2	354.74±0.033	99.35±0.01	**CHP1C8**	267.58±0.028	99.20±0.01	**CHE4C10**	253.62±0.038	98.86±0.02
CHP3	487.35±0.045	98.92±0.03	**CHP1C10**	272.86±0.042	99.83±0.04	**CHE5C8**	362.84±0.022	99.08±0.04
CHE4	230.95±0.044	98.92±0.01	**CHP2C6**	375.73±0.032	99.30±0.03	**CHE5C10**	369.56±0.028	99.10±0.02
CHE5	336.12±0.045	99.12±0.04	**CHP2C8**	382.84±0.016	99.37±0.02	**CHE6C8**	496.64±0.015	98.79±0.03
CHE6	460.15±0.048	98.92±0.02	**CHP2C10**	389.16±0.024	99.28±0.01	**CHE6C10**	506.01±0.032	98.92±0.01
CHPE7	242.51±0.048	99.62±0.02	**CHP3C6**	516.64±0.032	98.76±0.01	**CHPE7C8**	260.46±0.018	99.60±0.01
CHPE8	339.99±0.032	98.63±0.01	**CHP3C8**	526.25±0.028	98.82±0.02	**CHPE7C10**	265.25±0.026	99.48±0.04
CHPE9	475.41±0.038	99.25±0.02	**CHP3C10**	536.06±0.015	98.82±0.03	**CHPE8C8**	366.91±0.021	98.61±0.02
						CHPE8C10	373.64±0.030	98.79±0.01
						CHPE9C8	513.07±0.028	99.18±0.03
						CHPE9C10	522.77±0.042	99.22±0.01

a: mean ± S.D., n=100;
b: mean±S.D., n=3

2.5. Disintegration test for coated capsules

The main objective of the Eudragit S100 coating is to prevent the drug release in the stomach and achieve a sufficient lag time of 4-6 hours before the drug release. Hence, the coated capsules of vancomycin hydrochloride are subjected to a disintegration test in 0.1 N HCl and pH 6.8 phosphate buffer adopting the procedure given in I.P.

2.6. *In vitro* dissolution test for uncoated capsules

All the uncoated vancomycin hydrochloride capsules containing a 1:1 drug-polyelectrolyte complex have shown a cumulative drug release of 53.51 to 87.48% at the end of 2 hours in 0.1 N HCl. The uncoated capsules containing beads made up of a 1:2 drug-polyelectrolyte complex have shown drug release for up to 16 hours. The results are shown in Table 4.

2.7. *In vitro* dissolution test for coated capsules

A sequential buffer change method (with 1.2 pH HCl buffer, 6.8 pH, and 7.4 pH phosphate buffer) is employed to carry out the dissolution test for coated capsules. The % cumulative drug release of the capsules is shown in Figure 4.

Table 4. Cumulative % drug release from vancomycin hydrochloride uncoated capsules

Dissolution medium	Time (hours)	% Cumulative drug release (mean ± S.D, n=3)								
		CHP1	CHP2	CHP3	CHE4	CHE5	CHE6	CHPE7	CHPE8	CHPE9
0.1N HCl	1	76.99±0.22	72.85±0.18	68.10±0.28	54.51±0.15	49.87±0.21	44.45±0.31	64.36±0.18	59.66±0.15	53.51±0.17
	2	87.48±0.31	83.60±0.31	78.24±0.21	60.53±0.23	56.51±0.19	50.68±0.13	70.79±0.22	64.61±0.19	58.48±0.24
	3	90.40±0.15	86.33±0.23	82.57±0.19	65.90±0.27	61.60±0.18	56.35±0.32	76.53±0.32	70.18±0.29	64.66±0.10
pH 7.4	4	97.09±0.21	92.79±0.22	89.97±0.31	72.13±0.17	66.77±0.23	62.82±0.15	80.54±0.21	76.76±0.17	69.19±0.21
	5	100.16±0.16	95.63±0.15	92.97±0.27	79.87±0.12	72.67±0.29	65.51±0.25	86.34±0.26	82.39±0.15	76.44±0.34
	6		100.08±0.13	96.48±0.18	84.54±0.13	78.83±0.32	71.46±0.31	94.43±0.34	87.28±0.23	81.36±0.15
pH 6.8	8			100.06±0.21	89.82±0.31	84.26±0.21	77.18±0.15	100.21±0.16	95.34±0.19	88.54±0.19
	10				94.73±0.24	89.49±0.23	82.69±0.24		100.12±016	96.36±0.25
	12				100.08±0.15	95.12±0.31	86.23±0.27			100.06±0.12
	14					100.03±0.12	94.42±0.17			
	16						100.12±0.14			

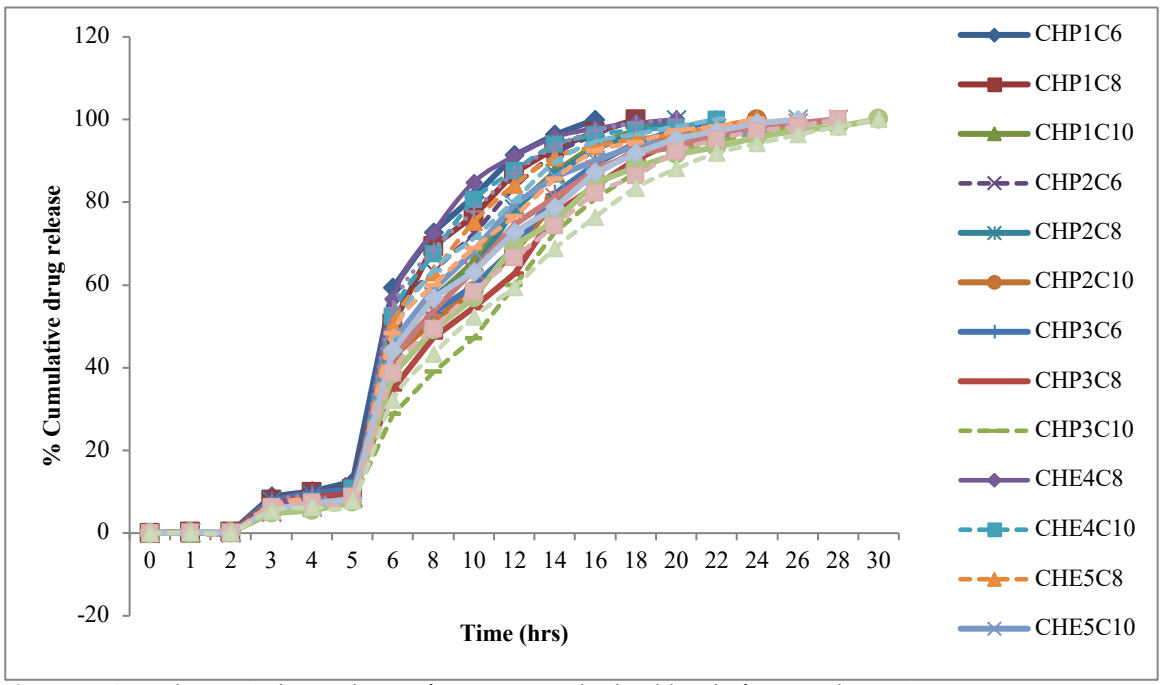

Figure 4. Cumulative % drug release of vancomycin hydrochloride from Eudragit S100 coated capsules

2.8. Drug release kinetics

The *in vitro* drug release data is fitted into several mathematical models to know the mechanism of drug release and the best fit model to describe the drug release. The results of the drug release kinetics are shown in Table 5.

Table 5. Drug release kinetics of the *in vitro* drug release data

Formulation	Zero order		First order		R^2			Peppas exponent 'n'
	R^2	k_0	R^2	k_1	Higuchi	Erosion	Peppas	
CHP1C6	0.8859	4.737	0.9882	0.448	0.9912	0.938	0.991	0.223
CHP1C8	0.8541	4.341	0.9877	0.369	0.9862	0.932	0.969	0.265
CHP1C10	0.9092	4.642	0.9798	0.324	0.988	0.927	0.992	0.314
CHP2C6	0.9174	4.653	0.9716	0.368	0.9908	0.932	0.994	0.262
CHP2C8	0.9248	4.383	0.9692	0.314	0.9831	0.928	0.982	0.300
CHP2C10	0.9206	3.583	0.9781	0.250	0.9803	0.926	0.982	0.315
CHP3C6	0.938	4.053	0.9663	0.276	0.9806	0.928	0.978	0.314
CHP3C8	0.9261	3.949	0.9655	0.245	0.9863	0.926	0.986	0.373
CHP3C10	0.882	3.462	0.9896	0.245	0.9752	0.922	0.985	0.426
CHE4C8	0.9031	4.026	0.9943	0.301	0.9875	0.924	0.969	0.234
CHE4C10	0.8938	3.668	0.9932	0.247	0.9803	0.921	0.976	0.248
CHE5C8	0.8849	3.246	0.9925	0.198	0.9696	0.922	0.983	0.250
CHE5C10	0.8968	3.070	0.9945	0.172	0.9717	0.920	0.987	0.268
CHE6C8	0.9181	3.058	0.9753	0.187	0.9743	0.918	0.994	0.295
CHE6C10	0.9158	2.941	0.9891	0.156	0.9669	0.916	0.990	0.320
CHPE7C8	0.9635	4.264	0.9671	0.275	0.9934	0.924	0.992	0.247
CHPE7C10	0.9244	3.573	0.9795	0.245	0.9851	0.922	0.985	0.279
CHPE8C8	0.9757	3.928	0.9721	0.192	0.9912	0.920	0.993	0.269
CHPE8C10	0.9461	3.043	0.9658	0.187	0.9925	0.918	0.990	0.281
CHPE9C8	0.9862	3.797	0.9706	0.141	0.9926	0.916	0.994	0.323
CHPE9C10	0.9598	3.254	0.9756	0.145	0.9909	0.914	0.992	0.372

2.9. *Ex vivo* drug release studies in rat cecal contents

The formulation containing chitosan-hupu gum-PEG 400 with 10% coating (CHP3C8) fulfilled the objectives of the study maintaining 5 hours of lag time and controlling drug release for 24 hours. Thus, it is selected as the best formulation and used for *ex vivo* drug release studies using the rat cecal content medium. The results of the ex vivo drug release studies are shown in Figure 5.

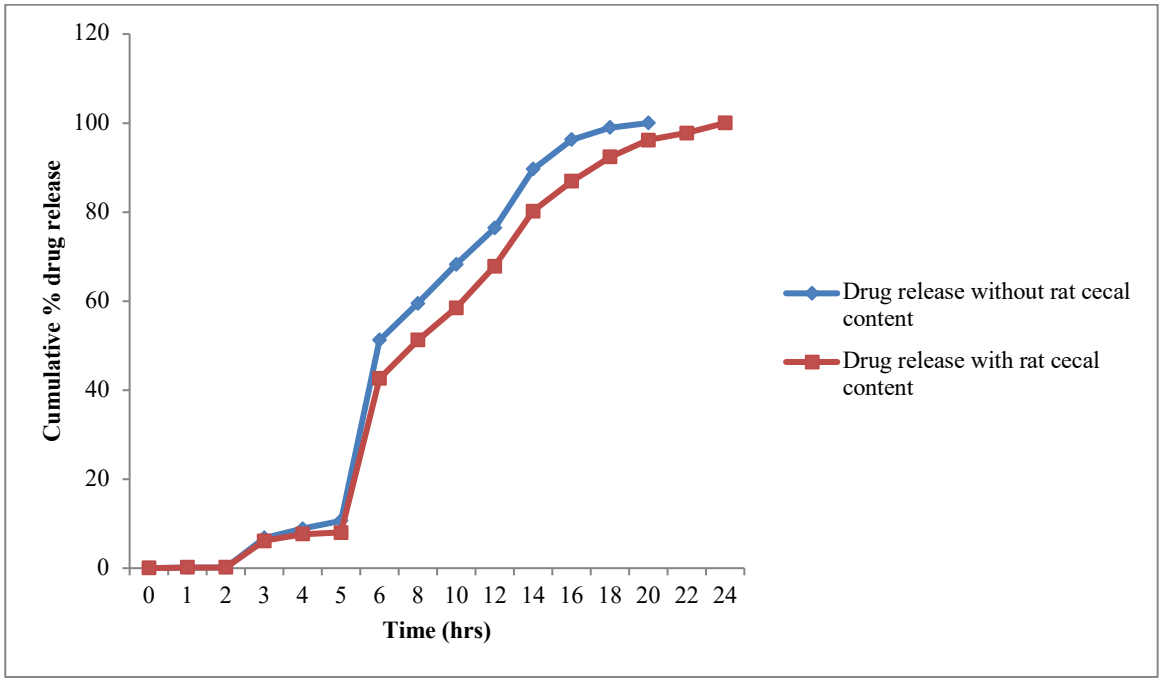

Figure 5. Comparative dissolution profile of CHP3C8 formulation with and without rat cecal contents

2.10. In vivo drug release studies for the optimized formulation

The results of plasma concentrations of vancomycin hydrochloride and various pharmacokinetic parameters after administration to New Zealand rabbits are shown in Table 6 and Figure 6. The results are compared with the administration of a pure solution of vancomycin hydrochloride. The peak plasma concentration of vancomycin HCl from the formulation CHP3C8 (809.53 µg/ml) is found to be greater than that of the pure drug solution (402.12 µg/ml).

Table 6. Pharmacokinetic parameters of vancomycin hydrochloride from CHP3C8 and oral solution

Parameters	CHP3C8[a]	Oral solution[a]
C_{max} (µg/ mL)	1326.02±3.19	402.12±5.12
T_{max} (hours)	6.10±0.44	2.0±0.45
K_a (hours^{-1})	0.011	0.008
K_{el}(hours^{-1})	0.035±0.26	0.055±0.001
$t_{1/2}$ (hours)	1.33±0.39	0.383±0.007
AUC_{0-24} (µg.hours/mL)	117.31±7.31	7.84±5.24
$AUMC_{0-24}$ (µg.hours^2/mL)	29625.64±44.85	594.36±10.85
MRT_{0-24} (hours)	7.37±7.38	8.62±5.87
V_d(litres)	0.147±0.010	4.7±2.96
Cl (mL.hour^{-1})	0.084±0.016	1.05±0.75

a. Mean ± SD, n=3

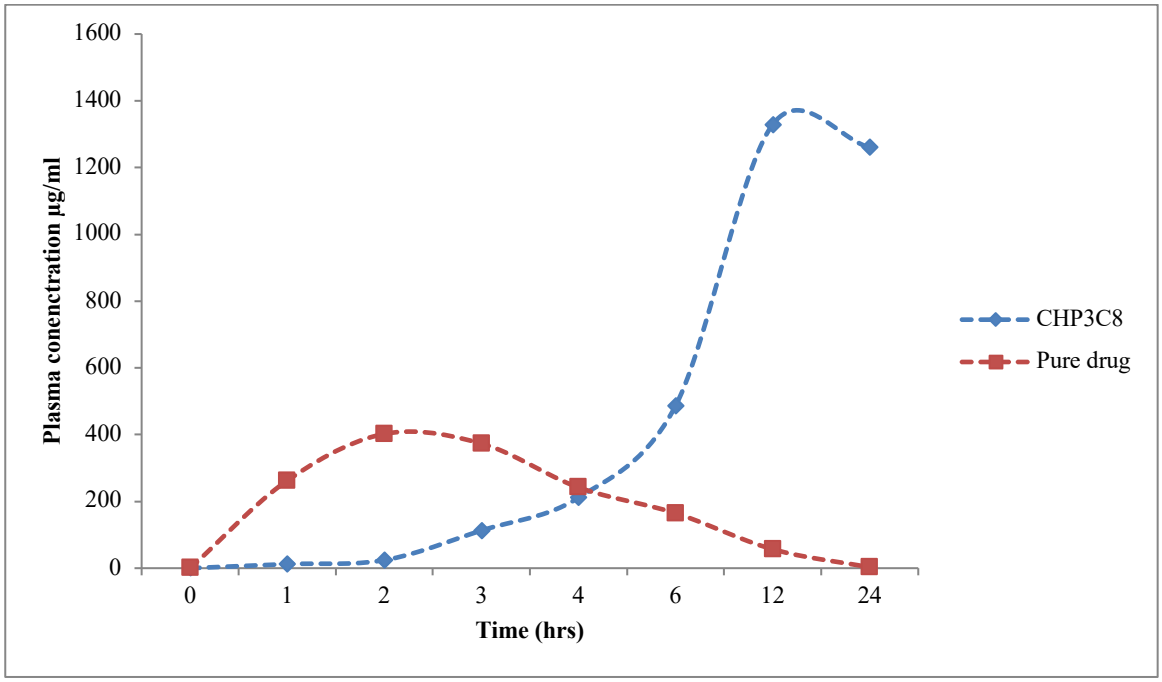

Figure 6. Comparative mean plasma drug concentration of vancomycin hydrochloride from CHP3C8 formulation and oral solution

3. DISCUSSION

3.1. Drug excipient incompatibility

The characteristic peaks of the fingerprint region such as OH stretching (3422.8cm^{-1} to 3454.3 cm^{-1}), C=O stretching (2046.8 cm^{-1} to 2114.6 cm^{-1}), C=C stretching (1400.9 cm^{-1} to 1475.2 cm^{-1}), and C-O stretching denoting the primary alcohol (1022.1 cm^{-1} to 1060.1 cm^{-1}) remained intact as shown in Figure 1 for all the pure drug-excipient mixtures indicating no signs of drug-excipient incompatibility.

3.2. Determination of polyelectrolyte mixing ratio on % dry yield

The maximum % dry yield (88.33%) of the polyelectrolyte complex is observed at a ratio of hupu gum-chitosan ratio of 1:1. The optimum pH is found to be 7.82. As the ratio is further increased, the % dry yield is decreased indicating that the optimum concentration is 1:1. The pH and conductivity values gradually increased as this ratio is increased which denotes the degree of dissociation associated with polyelectrolytes. However, the beads formed at a 1:1 ratio lack desirable rigidity, to increase the rigidity, Eudragit S100 and PEG400 are added during bead formation. The total polyanion ratio is maintained at 1:1 by fixing the chitosan concentration and varying the ratio of polyanions (Hupu gum, PEG400, and Eudragit S100) such that the total parts of polyanion are always equal to that of chitosan concentration.

3.3. Evaluation of beads

The prepared beads are white with a round shape and smooth surface without cracks. A representative photograph of the wet beads is shown in Figure 2. The percentage yield of the beads is in the range of 73.23 to 93.26%, the average particle size of the beads is in the range of 0.093 to 0.108 mm, the % drug loading is in the range of 25.71% to 54.51%, and the % drug entrapment efficiency is in the range of 80.88 to 94.95%. The swelling index is more pronounced in pH 7.4 phosphate buffer (83.32 to 90.89%) while it is lower in the 0.1 N HCl (19.03 to 22.91%) indicating the suitability for colon drug delivery. The poor swelling index in 0.1 HCl can be attributed to the protonation of amino groups which then interact with the carboxylic groups of hupu gum leading to a stronger polyelectrolyte complex [6]. On the other hand, at pH 7.4 deprotonation of chitosan weakens the ionic crosslinking resulting in a higher degree of swelling [7]. The scanning electron micrograph of a representative bead denoting the smooth surface is shown in Figure 3.

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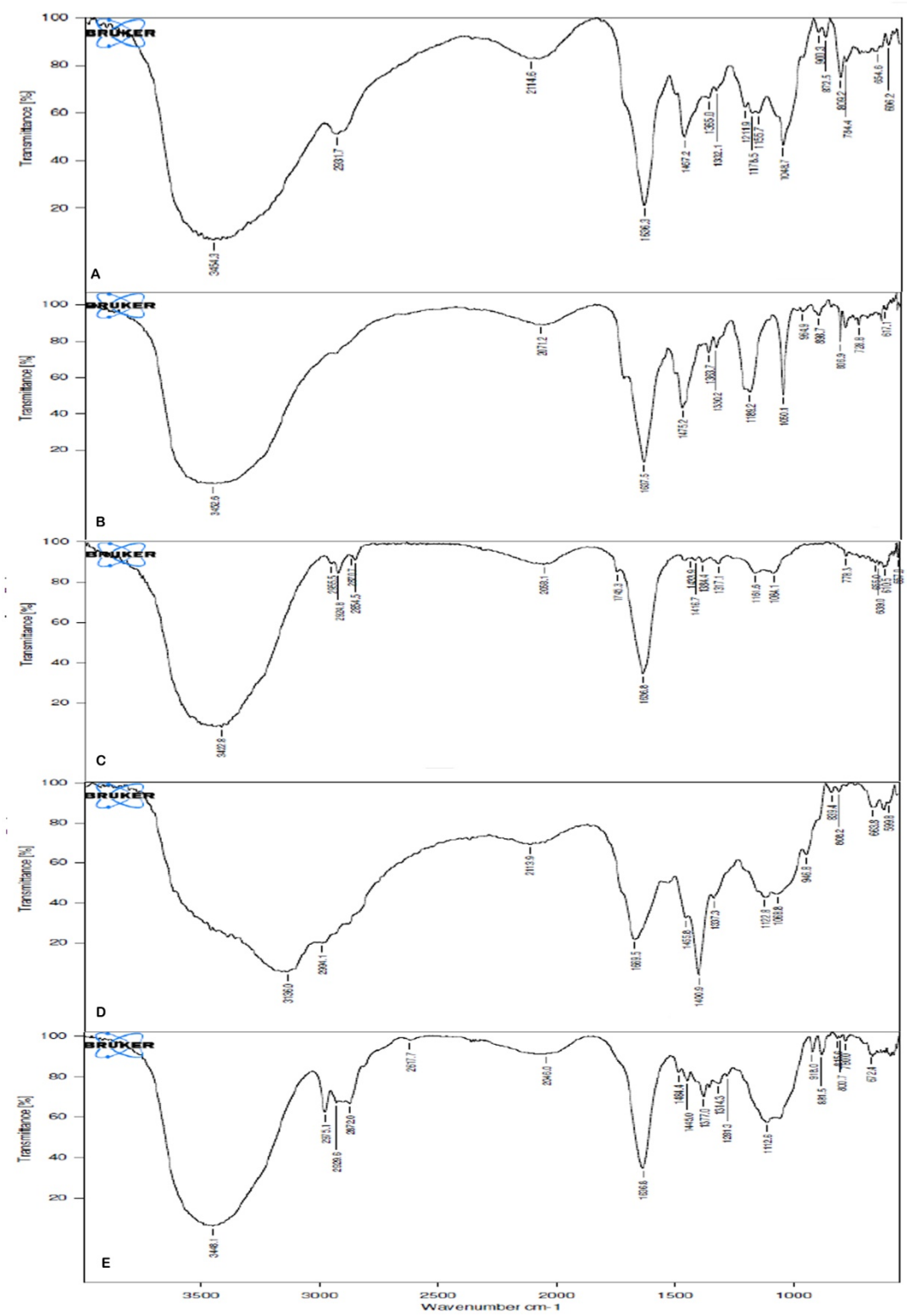

Figure 1. FTIR spectrum A. pure drug B. pure drug: chitosan (1:1 mixture) C. pure drug: hupu gum (1:1 mixture) D. pure drug: PEG 400 (1:1 mixture) E. pure drug: Eudragit S100 (1:1 mixture).

http://dx.doi.org/10.29228/jrp.450
J Res Pharm 2023; 27(4): 1658-1672

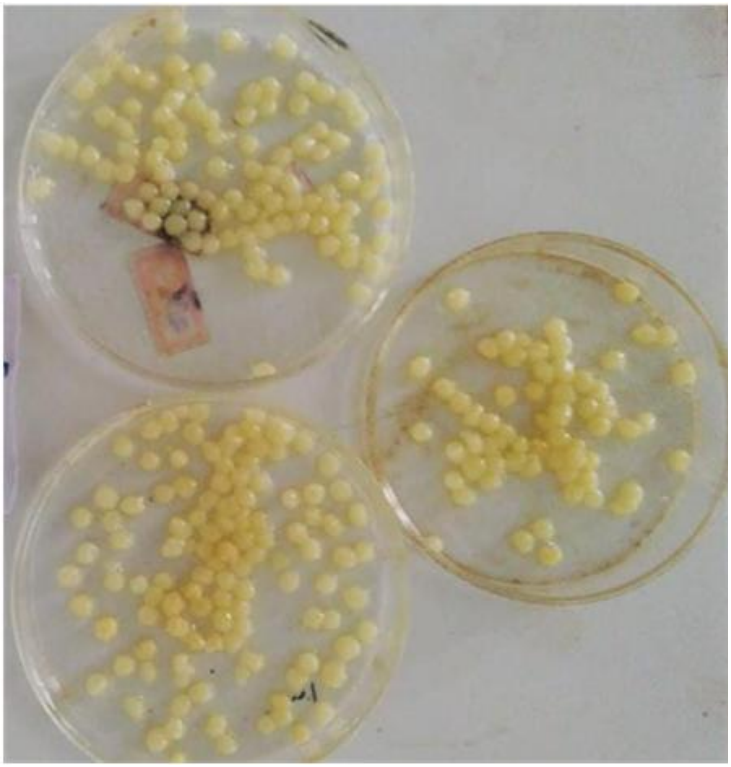

Figure 2. Representative figure showing the beads of vancomycin hydrochloride before drying. The size of the beads is reduced after drying

3.4. Evaluation of capsules

The uncoated and coated capsules passed the evaluation tests for uniformity of weight. The capsules weighing less than 300 mg have shown the weight variation within the official limits of ± 10% while the capsules weighing more than 300 mg are within the limits of ± 7.5%. The drug content in the capsules is found to be in the range of 98.61 to 99.83%.

3.5. Disintegration test for coated capsules

The uncoated capsules disintegrated in 0.1 N HCl within 5.23 minutes indicating the need for enteric coating. The Eudragit S100 coated capsules showed resistance in 0.1N HCl with no signs of rupture denoting gastric resistance and the capsules are completely disintegrated in the 7.4 pH phosphate buffer in 8.11 minutes showing the efficiency of the coating.

3.6. *In vitro* dissolution test for uncoated capsules

The partial degradation of polyelectrolyte complex in the acidic environment and the release of beads in the gastric medium could be attributed to the faster drug release. To release vancomycin hydrochloride in the colon, the capsules are coated with an enteric polymer like Eudragit S100. This will prevent the dissolution of the capsule shell in the stomach and achieve a lag time of 4 to 6 hours.

3.7. *In vitro* dissolution test for coated capsules

The initial drug release from all the coated capsules in 0.1 N HCl is less than 0.25% indicating the effectiveness of the coating for preventing the drug release in the stomach. The drug release in 7.4 pH phosphate buffer is also not more than 13% thus fulfilling the objective of 5 hours lag time before drug release in the colon. The drug release from chitosan-hupu gum-PEG400 (CHP) extended the drug release to an extent of 16 to 28 hours. The increased degree of swelling of PEG400 in the simulated colon medium was due to the formation of hydrogen bonds between hydroxyl groups of PEG400 and amine groups of chitosan. 8% and 10% Eudragit coating prevented the initial burst release and extended the drug release.

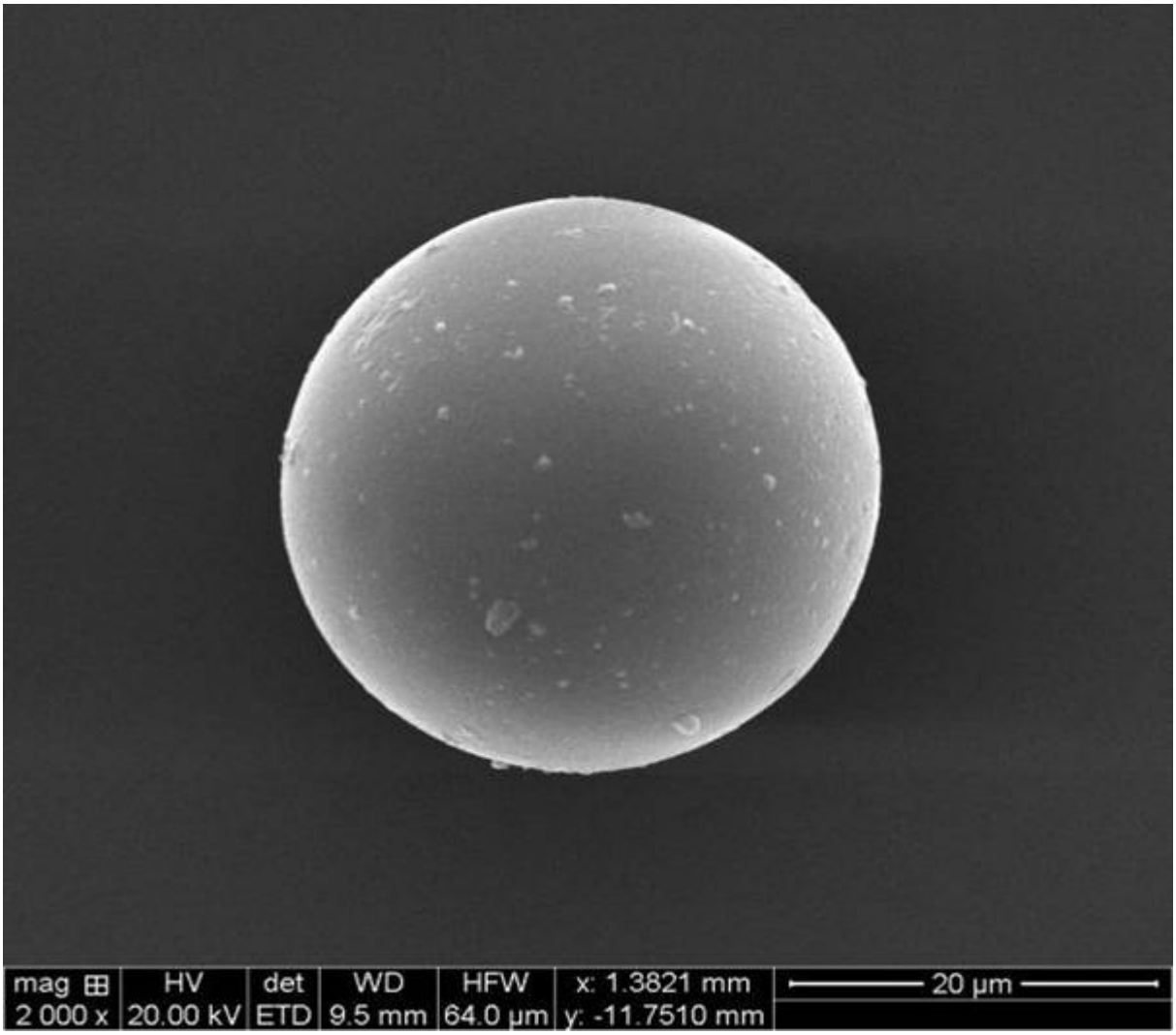

Figure 3. Scanning electron micrograph of vancomycin hydrochloride beads depicting smooth morphology

The drug release from chitosan-hupu gum-Eudragit S100 extended the drug release to a period of 20 to 30 hours. The extended drug release in beads containing Eudragit S100 could be due to the methacrylic acid groups. Once, the polyelectrolyte complex is swollen completely, it served as a barrier to drug release, increasing the diffusion path length and controlling the drug release for a prolonged period [8].

3.8. Drug release kinetics

The drug release kinetics are calculated from the *in vitro* drug release data where the first 5 hours of lag time are not taken into consideration. The drug release from the 6th hour follows linearity in the Higuchi model as evident from the higher r^2 values (0.9669 to 0.9912) indicating that the drug release followed diffusion rather than erosion. The drug release data fitted into the Korsmeyer-Peppas model also implies the same and the n value ranged from 0.223 to 0.426 concluding that the mechanism of drug release is Fickian diffusion [9, 10].

3.9. *Ex vivo* drug release studies in rat cecal contents

The extent of drug release was observed for the first 5 hours using 0.1 N HCl and pH 7.4 phosphate buffer. After 5 hours the medium is replaced by pH 6.8 phosphate buffer with 3% rat cecal content. The drug release in this medium is compared with normal pH 6.8 buffer without rat cecal content. It is observed that the drug release is higher in the rat cecal content medium which could be attributed to the cleavage of polymeric complexes by the bacteria present in the rat cecum [11]. The drug release in the rat cecal medium is shortened to 20 hours as compared to 24 hours in the medium without cecal contents.

3.10. *In vivo* drug release studies for the optimized formulation

The peak plasma concentration of vancomycin hydrochloride from CHP3C8 at the end of 24 hours is higher than the peak plasma concentration of pure drug solution indicating the slow and consistent release of the drug from the capsules. The time to maximum concentration for the optimized formulation is 6 hours while it is 2 hours for pure drug solution. The apparent volume of distribution is found to be 0.55 and 4.7 liters for optimized formulation and pure drug solution respectively. The total body clearance values are lower for the optimized formulation when compared to pure drug indicating slower elimination.

4. CONCLUSION

Colon-targeted capsules of vancomycin hydrochloride are prepared using chitosan, and hupu gum in a ratio of 1:1. The formulation CHP3C8 with 8% Eudragit S100 coating has shown optimum drug release over 24 hours and fulfilled the study objective of 5 hours of lag time. The in vitro, ex vivo, and in vivo studies have shown the superiority of the formulation when compared to the pure drug solution. It can be concluded from this study that polyelectrolyte complex containing chitosan and hupu gum can be successfully used to target the drug to the colon, reducing its gastric degradation, and can be efficiently used for the management of Pseudomembranous colitis.

5. MATERIALS AND METHODS

5.1. Materials

Vancomycin hydrochloride is obtained as a gift sample from M/s. Concord Biotech Limited, Dholka Gujarat. Chitosan is obtained from M/s. Kemphasol, Mumbai. Hupu gum is sourced from Girijan Co-operative Corporation Limited, Rajahmundry. Eudragit –S100 and Polyethylene glycol 400 was obtained from M/s. Sisco Research Laboratories Pvt. Ltd. Mumbai. Sodium Tripolyphosphate and glacial acetic acid were obtained from SD Finar Chemicals Limited, Ahmedabad, Gujarat. period.

5.2. Drug-excipient incompatibility study

IR spectra of the physical mixture of vancomycin HCl and various excipients are obtained to find out any possible drug-excipients interaction by the KBr pellet method [12] using a Perkin-Elmer FTIR series spectrophotometer between 4000 and 400 cm^{-1}.

5.3. Construction of standard calibration curve

The stock solution of vancomycin hydrochloride is diluted with the respective media to obtain concentrations of 40, 60, 80, 100, and 120µg/ml in a 10ml volumetric flask. The absorbance of these solutions is then measured at 277nm using LAB INDIA double beam UV-Visible spectrophotometer against the respective blank solution i.e. 0.1N HCl/pH 6.8 phosphate buffer/pH 7.4 phosphate buffer [13]. All estimations are done in triplicate and average values were reported with standard deviation. The standard curve is obtained by plotting the absorbance against the concentration of Vancomycin HCl. The equations for the best-fit straight line are obtained to determine the slope and intercept which were later used for the estimation of Vancomycin HCl in the respective media. The values of the coefficient of regression (r^2) are obtained to determine the linearity of the best-fit line

5.4 Preparation of vancomycin hydrochloride beads

The drug and other ingredients required for the preparation of beads are taken according to the formulae mentioned in Table 7. The required quantity of chitosan for the preparation of each batch is dissolved in 300 mL of 2% (v/v) acetic acid and stirred at 250 rpm for 2 hours at 40°C. The remaining ingredients are dissolved separately in 200 mL of distilled water and stirred at 250 rpm for 2 hours at 40°C. Chitosan solution (300 mL) is then added to the drug solution containing the other ingredients with continuous stirring at 250 rpm for the formation of the polyelectrolyte complex. The formed polyelectrolyte complex solution is added drop-wise (using a disposable syringe with a 21 gauge needle) to 500 mL of 1% w/v sodium tri polyphosphate solution (cross-linking agent) adjusted to pH 6.5 with stirring at 250 rpm for 2 hours at room temperature [14]. The obtained beads are then allowed for curing for 20 minutes in the solution. The beads, thus formed are separated by filtration, washed with water, and dried at 40°C for 24 hours in a hot air oven.

Table 7. Composition of vancomycin hydrochloride beads

Ingredients (g)	CHP1	CHP2	CHP3	CHE4	CHE5	CHE6	CHPE7	CHPE8	CHPE9
VancomycinHCl	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Chitosan	3.125	6.25	12.5	3.125	6.25	12.5	3.125	6.25	12.5
Hupu gum	2.325	4.65	9.30	2.325	4.65	9.30	2.325	4.65	9.30
PEG400	0.80	1.60	3.20		-	-	0.40	0.80	1.60
Eudragit S100	-	-	-	0.80	1.60	3.20	0.40	0.80	1.60
Total weight (g)	18.75	25.0	37.50	18.75	25.0	37.50	18.75	25.0	37.50
PEC ratio	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Drug-polymer ratio	1:0.5	1:1	1:2	1:0.5	1:1	1:2	1:0.5	1:1	1:2

5.5 Evaluation of vancomycin hydrochloride beads

5.5.1. Drug entrapment efficiency and drug loading

100 mg of the prepared beads are powdered and soaked in 50 mL of pH 6.8 phosphate buffer for 60 minutes. The dispersion is filtered through a 0.45 µm Millipore nylon filter disc and the residue is washed thoroughly to completely extract the remaining drug with pH 6.8 phosphate buffer and filtered and the washings are collected and the volume is made up to 100 mL. The solution is suitably diluted and assayed for vancomycin hydrochloride content by measuring the absorbance at 277 nm in a UV spectrophotometer [15]. The % drug entrapment efficiency was determined using Eq. 1. The % drug loading for the formulations is calculated using Eq. 2.

(Eq. 1) $$\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{The initial amount of drug-loaded}} * 100$$

(Eq. 2) $$\% \text{ Drug loading} = \frac{\text{Actual drug content}}{\text{Weight of beads}} * 100$$

5.5.2. Swelling index

The swelling index is studied by measuring the percentage of water uptake by the beads. 100 mg of the prepared beads from each batch is taken in a 100 mL volumetric flask and made up to volume with either 0.1 N HCl or pH 6.8 phosphate buffer. After 8 hours the beads are filtered through filter paper grade 591 (particle retention: 12 µm, typical thickness: 180 µm, and basic weight: 161 g/m 2) and the surface is gently dried using filter paper and weighed [16]. The swelling ratio was calculated using Eq. 3.

(Eq. 3) $$\% \text{ Swelling index} = \frac{\text{The final weight of the beads} - \text{Initial weight of the beads}}{\text{The final weight of the beads}} * 100$$

5.6. Preparation of vancomycin hydrochloride capsules

Vancomycin HCl beads equivalent to 125 mg of dose of the drug are filled into capsules of suitable sizes using a hand-operated capsule filling machine [17].

5.7. Enteric coating of vancomycin hydrochloride capsules containing beads

5.7.1. Preparation of enteric coating solution

18 g of enteric coating polymer Eudragit S100 is dissolved in 300 mL of isopropyl alcohol, transferred to a bath sonicator, and homogenized for one hour. After sonication, 6 mL of polyethylene glycol 400 (PEG 400) is added as a plasticizer and 6 g of an anti-sticking agent, talc is added slowly to the solution and mixed thoroughly on a magnetic stirrer for 1 hour at 1000 rpm and left undisturbed to allow for the escape of air bubble [18]. Thus, the obtained solution is used for coating capsules.

5.7.2. Coating of capsules

The dip-coating method is used for coating the capsule and the coating are continued until the capsules achieve a weight gain of 6, 8, and 10%. The enteric coating solution is added to a flat petri dish (12.5 cm diameter) for obtaining a depth of 1 cm (approximately 120 mL). 50 core capsules are weighed initially and placed in the petri dish containing the coating solution and kept for 30 minutes. The coating solution is carefully decanted from the petri dish and kept in a hot air oven at 40°C for one hour for drying [19]. The capsules are weighed and the drying is continued to obtain a constant weight.

5.8. Evaluation of capsules

The prepared vancomycin hydrochloride capsules are evaluated for general appearance, uniformity of weight, and drug content [20].

5.9. Disintegration test for coated capsules

The disintegration test for coated capsules is carried out according to I.P. by disintegration test apparatus without discs in 0.1 N HCl (pH 1.2) maintained at 37±2°C for 2 hours. After 2 hours 0.1 N HCl is replaced with phosphate buffer pH 6.8. A disc is added to each tube and the apparatus is operated for further 60 minutes. The disintegration time of each capsule is recorded [20].

5.10. *In vitro* drug release studies of coated and uncoated capsules

Drug release from capsules was studied using USP dissolution rate test apparatus I (basket method) (M/s. LAB INDIA, Model: Disso 8000). During the first 2 hours, 900 mL of 0.1N HCl (pH 1.2) is used as a dissolution medium and it is replaced with pH 7.4 phosphate buffer at the end of the 2nd hour. The temperature of the dissolution medium is maintained at 37±0.5°C and the basket was stirred at a speed of 100 rpm. 5 mL aliquots are withdrawn with a syringe fitted with a pre-filter (0.45 µm) at appropriate time intervals and immediately replaced with 5 mL of fresh medium maintained at 37±0.5°C. The filtered samples are suitably diluted with the respective medium i.e., 0.1 N HCl or pH 7.4 phosphate buffer wherever necessary and absorbance of the samples was measured at 277 nm. All the dissolution experiments are done in triplicate. The average cumulative percent of vancomycin hydrochloride released at different time intervals was calculated and reported [21].

5.11. Drug release kinetics

The *in vitro* drug release data is then fitted into different mathematical models such as zero order, first order, Higuchi, and Korsmeyer-Peppas to determine the best-fit model that describes the drug release kinetics. The r^2 values are used to determine the linearity while the slope is used to calculate the rate order constants. The release exponent 'n' from the Korsmeyer-Peppas plot is used to determine the mechanism of drug release [22-25].

5.12. *Ex vivo* drug release studies in rat caecal content medium

Healthy Wistar rats of either sex weighing 120-150g are selected for the study. The approved protocol for the use of the animals is compliant with the protocol number (1269/PO/E/S/08/CPCSEA) as per the regulation of the Institutional Animal Ethical Committee of Aditya College of Pharmacy, Surampalem, Andhra Pradesh, India. 2 ml of a 1% w/v aqueous dispersion of Eudragit S100 is given to rats daily for 7 days per oral to induce the degradation of Eudragit S100 in colon region of rats. This ensures the enzymatic environment that simulates the same physiological conditions in the *ex vivo* medium. On the eighth day thirty minutes before the fifth hour of the dissolution study, rats are anesthetized and killed. The rats are dissected and the cecum is located and ligated at both ends. The caecal contents were individually weighed and pooled together and added and the final dilution is made with pH 6.8 phosphate buffer previously bubbled with carbon dioxide to obtain a 3% w/v concentration of caecal contents and kept under continuous bubbling with carbon dioxide. The *Ex vivo* drug release studies were carried out for up to 5 hours i.e. in 0.1N HCl for the first two hours followed by 3 hours in pH 6.8 phosphate buffer. 100 mL of pH 6.8 phosphate buffer containing 3% w/v rat caecal content is taken in a 250 mL beaker and dissolution was continued further for up to 5 hours. The experiment is carried out with a continuous supply of carbon dioxide into dissolution media. 2 mL samples were withdrawn periodically and replaced with fresh buffer bubbled with carbon dioxide [5, 26]. The samples were filtered using a 0.22 µm membrane filter and analyzed at 277 nm as per the procedure described earlier.

5.13. In vivo evaluation of the optimized capsules

The optimized formulation of capsules (CHP3C8) prepared with chitosan and hupu gum could extend the release of the drug over 20 hours as considered in the objectives and these formulations were subjected to further pharmacokinetic evaluations using New Zealand rabbits and compared against pure vancomycin HCl oral solution. The research protocol of animal experimentation for pharmacokinetic studies was approved by an independent institutional Animal Ethical Committee, Aditya College of Pharmacy, Kakinada, Andhra Pradesh, India. vide No. 1269/PO/E/S/08/CPCSEA. With reference to the U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research (CDER), July 2005 Pharmacology and Toxicology for "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" [27], a dose of 40 mg/kg of vancomycin hydrochloride is

recommended for pharmacokinetic study. A dose conversion factor of 0.32 is used to convert the human dose to the animal dose. For an average rabbit weighing 2.5 kg, the dose of vancomycin hydrochloride is found to be 100 mg. The contents (beads) of the capsules are administered to the rabbits through gastric cannula (diameter 4mm). The mouth of the rabbit was opened and a wooden rod is inserted between the jaws such that rabbit does not close the jaws while administration. The beads are then administered along with adequate water through cannula such that the drug enters into the stomach directly.

5.14. In vivo estimation of vancomycin HCl in rabbit plasma using UV-spectroscopy method

The reported *in vivo* methods for quantitative analysis of vancomycin HCl in plasma are HPLC [28], RP-HPLC [29], LC-MS [30], and UV-Spectroscopy [29]. In the present investigation, the UV-Spectroscopy method was used for the in vivo estimation of vancomycin HCl.

Acknowledgments: The authors would like to thank the management of Aditya College of Pharmacy, Surampalem for providing equipment and materials. The corresponding author would like to thank Dr. K. Ravishankar and Dr. K. V. Ramana Murthy for their valuable time and cooperation.

Author contributions: Concept – V.K., R.K., P.N.K.S., V.R.K.; Design – V.K., V.R.K.; Supervision – V.R.K., R.K.; Resources – R.K., P.N.K.S.; Materials – R.K., V.R.K., V.K.; Data Collection and/or Processing – V.K., P.N.K.S., V.R.K.; Analysis and/or Interpretation – V.K., P.N.K.S., V.R.K.; Literature Search – V.K., R.K., P.N.K.S., V.R.K.; Writing – V.K., P.N.K.S., V.R.K.; Critical Reviews – V.K., R.K., P.N.K.S., V.R.K.

Conflict of interest statement: The authors declared no conflict of interest

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