International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.10, pp 298-307, 2018

Synthesis and Development of Some Quinolone/Fluoroquinolone- Latentiated Drug Polypeptide Systems and Their Antimicrobial Evaluation

Shailendra K. Saraf[1]*, M. S. M. Rawat[2], Avinash C. Tripathi[1]

[1]Division of Pharmaceutical Chemistry, Faculty of Pharmacy, Babu Banarasi Das Northern India Institute of Technology, Lucknow-226028, U.P., India.
[2]Department of Pharmaceutical Sciences, H. N. B. Garhwal University, Srinagar-246174, Uttarakhand, India.

Abstract : The present study envisages to synthesize and evaluate some Quinolone/Flouroquinolone-polypeptide conjugates and to investigate whether, such drug latentiated systems possess any biological activity (antimicrobial) by themselves and to what extent the physical properties of these conjugates vary with different polypeptides. To accomplish this, four established antibiotics namely Nalidixic acid (NDA), Norfloxacin (NFC), Ciprofloxacin (CFC) and Ofloxacin (OFC) were conjugated with two different polypeptides, polyglutamic acid (PGA) and polyaspartic acids (PAA), respectively and evaluated for their physicochemical properties as well as antimicrobial action. The synthesized derivatives were characterized by various physicochemical and other methods. The partition coefficient of the NFC-PGA derivative was found to be highest amongst others. The rates of hydrolysis in simulated gastric and intestinal fluids showed that latentiated derivatives were resistant to hydrolysis in the gastric fluid but show hydrolysis in the intestinal fluid. Finally, the synthesized drug conjugates were screened for antibacterial activity, at concentrations of 10 and 50 µg/mL concentrations, against *P. morganii* and *S. aureus* bacterial strains using agar diffusion (filter paper disc) method. Almost all the drug-polymer conjugates showed good antibacterial potency but in particular, CFC and OFC conjugates of PGA exhibited maximum zone of inhibition against both the Gram positive as well as Gram negative microorganisms. The enhanced antibacterial potency activities observed for all the latentiated derivatives can be attributed to the synergistic effect of drug and polypeptides. Especially, improved activities of NDA-PGA and NDA-PAA against *S. aureus* was possible only due to conjugation with the polypeptides because NDA is not effective against Gram positive microorganisms.
Keywords : Drug-Polymer conjugates, Drug latentiation, Antibacterial, Quinolones, Fluoroquinolones, *In vitro hydrolysis*, Availability factor.

DOI= http://dx.doi.org/10.20902/IJCTR.2018.111037

Introduction

Any number of inherent disadvantages may preclude the use of parent drug molecule in clinical practice. These may be bitterness or tartness, offensive odour or taste, gastric or intestinal upset, lack of stability in bulk state or dosage form to be used *in vivo* and slow or rapid metabolism [1]. In many cases, the use of drugs with a high therapeutic potential is restricted due to severe side effects. To overcome these problems, the concept of drug latentiation has been promulgated by Harper [2]. It involves a chemical modification of a known biologically active compound to form a new substance, which upon *in vivo* enzymatic attack will liberate the parent compound. Although drug latentiation is not a novel approach, it has gained renewed interest in improving general cytotoxicity reduction, better internal control of active drug, passive drug absorption and duration of action [3]. The behaviour of prodrugs or drug conjugates in the body is determined by physicochemical properties of the prodrugs as well as by the biological environment [4].

Two of the most advanced drug latentiation technologies being evaluated at present are antibody directed enzyme prodrug therapy (ADEPT) [5] and use of polymeric prodrugs [6]. These systems could be designed specifically for body organs exploiting biochemistry and physiology of that organ. Further extension of this technology includes retro-metabolic drug delivery or soft drugs [7]. The latentiated systems can be converted to colloidal systems and studied for sustained and controlled delivery. Additionally, the ability of certain congeners to cross the blood brain barrier makes them ideal for targeted delivery to the brain. The latentiated derivatives can be used in liposomes, niosomes, emulsions, solid lipid nanoparticles or in micelle forming polymeric drug systems.

The chemical conjugation of drugs to macromolecules presents an efficient way of modifying pharmacological and biochemical characteristics of the drugs [8]. Generally, the polymer backbone of drug-polymer conjugates as prodrugs or latentiated derivatives is modified as to get the desired hydrophilicity/ hydrophobicity, site specificity and such physical, pharmacological and biochemical characteristics. The biodegradable polyanionic polypeptides conjugated with different drugs not only alter their untoward effects but also modify their absorption, distribution, metabolism and elimination properties.

Since the maximum number of latentiated compounds are biologically inactive, this concept is important for those drugs that are metabolized or excreted too rapidly to provide adequate clinical efficacy. The carboxylic group containing organic compounds are generally converted to ester or amide derivatives. With this, their lipophilic character increases resulting in easy access through the lipophilic barriers and entry to systemic circulation. A strategic group attached to increase the lipophilicity not only protects the otherwise vulnerable group and stabilizes the molecule but also guides the drug to a target tissue or site. Also, the compounds of type R-OH and R-NH_2 can be directly acetylated through Schotten-Boumann method [9].

Materials and methods

Procurement and characterization of drugs and polypeptides

Nalidixic acid (NDA), Norfloxacin (NFC), Ciprofloxacin (CFC) and Ofloxacin (OFC) were procured from Moraceae Labs Ltd. and Ranbaxy Labs Ltd. as gift samples and were subjected to different identification and characterization tests. However, polypeptides such as polyglutamic acid (PGA) and polyaspartic acids (PAA) were synthesized and characterized in our own laboratory [10].

Melting point: NDA: 228°C, NFC: 222°C, CFC: 318°C, OFC: 255°C.

Solubility profile: Solubility studies were conducted in solvents- water, dilute hydrochloric acid, dilute sodium hydroxide, alcohol, chloroform, acetone and ether. Results are given in Table 1.

Table 1: Solubility profile of selected drugs.

Solvent	Solubility			
	NDA	**NFC**	**CFC**	**OFC**
Water	-	+	++	++
Dilute hydrochloric acid	N/A	N/A	+++	+++
Dilute sodium hydroxide	+++	N/A	N/A	N/A
Acetic acid	N/A	+++	+	N/A
Methanol/Ethanol	+	+	+	+
Chloroform/DCM	+++	++	N/A	N/A
Acetone	+	+	-	-
Ether	+	-	N/A	N/A

+++: Completely soluble; ++: Sparingly soluble +: Slightly; -: Insoluble; N/A: Not Applied.

UV-Visible spectral characterization:

UV spectrophotometric method was followed for the determination of NDA (0.0005%) in 0.1M NaOH at an absorbance maximum of 334 nm. Accurately weighed 20 mg of NDA was dissolved in 0.1M NaOH solution and make up volume was made up to 100 mL. From this solution, aliquots of 1mL, 2mL,….were pipetted out into a series of 10 mL volumetric flasks and the volume was made up to get the required concentration range. The absorbance was measured at 334 nm on a Spectronic Genesis 10 spectrophotometer. The standard curve was plotted between concentration range and absorbance and was found to follow Beer-Lambert's Law in the given concentration range. The results of the study are given in Table 2. Similarly, the desired concentrations of NFC were prepared in a minimum quantity of methanol and the volume was made up with 0.1M NaOH solution. The absorbance was measured at 276 nm. The results of the study are given in Table 2.

For CFC, 20 mg of sample was dissolved in 0.1M HCl and the volume was made up to 100 mL. From this solution, aliquots of 0.1mL, 0.2mL,….were pipetted out into a series of 10 mL volumetric flasks, 1 ml of 1% w/v solution of ferric chloride was added to each volumetric flask and the volume was made up with 0.1 M HCl to get the required concentration range. The absorbance was measured at 550 nm on a Spectronic Genesis 10 spectrophotometer. The standard curve was plotted between concentration range and absorbance and was found to follow Beer-Lambert's Law in the given concentration range. The results of the study are given in Table 2. Similar method was followed for OFC as that of CFC, except the absorbance which was measured at 526 nm to obtain the desired result.

Table 2: Standard curve data for estimation of NDA, NFC, CFC and OFC.

S. N.	Concentration (in µg/mL)	Absorbance			
		NDA (at 526nm)	**NFC (at 276nm)**	**CFC (at 550nm)**	**OFC (at 526nm)**
1.	2	0.059	0.075	0.029	0.263
2.	4	0.106	0.151	0.049	0.417
3.	6	0.158	0.220	0.067	0.607
4.	8	0.213	0.296	0.091	0.945
5.	10	0.265	0.372	0.118	0.134
6.	12	0.324	0.440	0.139	0.326

Infra red spectral characterization:

IR spectra were recorded on Shimadzu 8400S and Perkin Elmer RX1 FTIR spectrophotometers (Shimadzu Corporation, Japan) using KBr discs and the values are expressed in cm^{-1}.

Synthesis of drug-polymer conjugates or latentiated systems

Synthesis of NDA/NFC/CFC/OFC chloride

NDA (23 g, 0.1 M)/NFC, (32 g, 0.1 M)/CFC (39 g, 0.1 M)/OFC (36 g, 0.1 M) each were added to 100 mL of chloroform and then the suspension was added to 17.9 g (11 mL, 0.15 M) of thionyl chloride. The contents were refluxed at 25-30 °C under reduced pressure for four hours. The solvent and excess of thionyl chloride was distilled off under reduced pressure to get NDA/NFC/CFC/OFC chloride, respectively.

Synthesis of NDA-PGA/PAA conjugate

Potassium carbonate solution (10%, 50 mL) was cooled on an ice bath at 10 °C. PGA, 6.6 g (0.0001 M) was added in small portions with continuous stirring. Then, 0.025 g (0.0001 M) of NDA chloride was added to the alkaline PGA in portions, with constant stirring for 2 hours at 10°C. The separated compound (NDA-PGA conjugate) was washed with 0.5% cold sodium hydroxide solution followed by ether and then dried. The above procedure was repeated using PAA, 4.8 g (0.0001 M) instead of PGA to obtain NDA-PAA conjugate.

Synthesis of NFC-PGA/PAA conjugate

Potassium carbonate solution (10%, 50 mL) was cooled on an ice bath at 10 °C. PGA, 6.6 g (0.0001 M) was added in small portions with continuous stirring. Then, 0.034 g (0.0001 M) of NFC chloride was added to the alkaline PGA in portions, with constant stirring for 2 hours at 10°C. The separated compound (NFC-PGA conjugate) was washed with 0.5% cold sodium hydroxide solution followed by ether and then dried. The above procedure was repeated using PAA, 4.8 g (0.0001 M) instead of PGA to obtain NFC-PAA conjugate.

Synthesis of CFC-PGA/PAA conjugate

Potassium carbonate solution (10%, 50 mL) was cooled on an ice bath at 10 °C. PGA, 6.6 g (0.0001 M) was added in small portions with continuous stirring. Then, 0.04 g (0.0001 M) of CFC chloride was added to the alkaline PGA in portions, with constant stirring for 2 hours at 10°C. The separated compound (CFC-PGA conjugate) was washed with 0.5% cold sodium hydroxide solution followed by ether and then dried. The above procedure was repeated using PAA, 4.8 g (0.0001 M) instead of PGA to obtain CFC-PAA conjugate.

Synthesis of OFC-PGA/PAA conjugate

Potassium carbonate solution (10%, 50 mL) was cooled on an ice bath at 10 °C. PGA, 6.6 g (0.0001 M) was added in small portions with continuous stirring. Then, 0.038 g (0.0001 M) of OFC chloride was added to the alkaline PGA in portions, with constant stirring for 2 hours at 10°C. The separated compound (OFC-PGA conjugate) was washed with 0.5% cold sodium hydroxide solution followed by ether and then dried. The above procedure was repeated using PAA, 4.8 g (0.0001 M) instead of PGA, to obtain OFC-PAA conjugate.

Physicochemical characterization of synthesized drug-polymer conjugates

Physical characterization and solubility studies

The physicochemical characters such as color, odor and solubility studies of the latentiated derivatives are reported in Table 3.

Table 3: Physicochemical characterization data of the synthesized drug latentiated systems.

Physicochemical parameters		**NDA-PGA**	**NFC-PGA**	**CFC-PGA**	**OFC-PGA**	**NDA-PAA**	**NFC-PAA**	**CFC-PAA**	**OFC-PAA**
Colour		Reddish brown	Brownish yellow	Greyish brown	Light brown	Yellowish brown	Brownish yellow	Greyish brown	Light brown
Odour		Slight	Odourless	Odourless	Slight	Odourless	Odourless	Odourless	Slight
Melting Range (**°C**)		182-185	171-174	207-210	200-203	179-181	167-169	197-201	193-195
Solubility	Water	+	+	-	+	+	+	-	+

	Methanol	+	+	-	+	+	+	-	+
	Acetone	+	+	+	+	+	+	+	+
	Ethylene glycol	+	+	-	+	+	+	-	+
	Ether	-	-	-	-	-	-	-	-
	Benzene	-	-	+	-	-	-	+	-
	Chloroform	-	+	+++	+	-	+	+++	+
	Pyridine	+	+	+++	+	+	+	+++	
	DMF	+	+++	+++	+++	+++	+++	+++	+++

+++: Completely soluble; +: Sparingly soluble; -: Insoluble.

IR spectra

IR spectra were recorded on Shimadzu 8400S and Perkin Elmer RX1 FTIR spectrophotometer (Shimadzu Corporation, Japan) using KBr discs and the values are expressed in cm^{-1}.

Partition coefficients

The ratio of the drug distribution in the organic phase and aqueous phase is the partition coefficient (P). 1-Octanol was used as the organic phase and double distilled water as aqueous phase.

Availability factors (AF)

The availability of a relatively insoluble compound for oral absorption depends on both the aqueous solubility, which is directly related to the rate at which the compound dissolves in the gut lumen and the lipid solubility, which is directly related to the rate at which the compound partitions into the lipid-gut barrier.

The availability factor is the product of the molar aqueous solubility and a factor related to the lipid/water distribution characteristic of the compound. Since partition coefficient can be extremely low or extensively high, thus offsetting significant changes in the aqueous solubility, the lipid-water distribution characteristic of the compound has been expressed in the terms of fractional distribution (f.d.).

Table 4: Partition coefficient, fractional distribution, and availability factor data of latentiated derivatives.

Latentiated derivatives	**Solubility**		**Partition coefficient (Octanol/Water at 37 °C)**	**Fractional distribution (f.d.)**	**Availability factor**
	Water at 37 °C (mg/ml)	**Octanol at 37 °C (mg/ml)**			
NDA-PGA	0.29	0.71	2.44	0.71	0.0438
NFC-PGA	0.24	0.76	3.17	0.76	0.0362
CFC-PGA	0.41	0.59	1.44	0.59	0.0618
OFC-PGA	0.32	0.68	2.12	0.68	0.0482
NDA-PAA	0.33	0.67	2.03	0.67	0.0684
NFC-PAA	0.27	0.73	2.70	0.73	0.0559
CFC-PAA	0.45	0.55	1.22	0.50	0.0930
OFC-PAA	0.35	0.65	1.86	0.65	0.0724

The following empirical equation was used to calculate the values-

$$f.d. = \frac{\text{Octanol solubility}}{\text{Octanol solubility} + \text{Aqueous solubility}}$$

Fractional distribution varies only from 0 to 1 in comparison to 0 to infinite variation in partition coefficient.

Availability factor = f. d. x Molar aqueous solubility x 10^4

In-vitro hydrolysis profile

In vitro hydrolysis rate of the compounds correlates drug release from the derivative in the body. These studies are carried out in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) truly mimic the *in vivo* milieu outside the body providing an artificial environment to drugs administered through oral route. Koshy proposed both alkaline and acidic hydrolysis mechanisms [11].

Latentiated derivatives, equivalent to 5 mg of the drugs NDA, NFC, CFC, and OFC, were separately and accurately weighed. Each was dissolved in a minimum quantity of ethanol and transferred to flasks, containing 100 mL of SGF, which were kept in mechanical shaker at 37°C. Samples of 2 mL were removed at half an hour intervals from each flask, diluted to 10 mL in volumetric flask, and the absorbance was measured at 334 nm, 276 nm, 550 nm and 526 nm, respectively, against a SGF blank using Thermospectronic Genesys 10 spectrophotometer. The volume withdrawn was replaced with SGF. The same procedure was followed for the hydrolysis of the derivatives in simulated intestinal fluid (SIF). The values are reported in Table 5.

Table 5: *In vitro* hydrolysis data of latentiated derivatives in SIF.

Latentiated derivatives		**Time (in Hours)**							
		0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**
NDA-PGA	Absorbance	0.32	0.37	0.40	0.43	0.48	0.53	0.59	0.64
	Conc. (µg/mL)	12	14	15	16	18	20	22	24
	% Release	24	28	30	32	36	40	44	48
NFC-PGA	Absorbance	0.51	0.55	0.58	0.66	0.73	0.80	0.88	0.95
	Conc. (µg/mL)	14	15	16	18	20	22	24	26
	% Release	28	30	32	36	40	44	48	52
CFC-PGA	Absorbance	0.08	0.09	0.12	0.15	0.17	0.18	0.23	0.24
	Conc. (µg/mL)	07	08	11	13	15	16	20	21
	% Release	22	26	30	32	40	44	48	52
OFC-PGA	Absorbance	1.1	1.2	1.3	1.5	1.7	1.9	2.4	2.4
	Conc. (µg/mL)	10	11	12	14	16	17	22	22
	% Release	20	22	24	28	32	34	44	44
NDA-PAA	Absorbance	0.26	0.38	0.40	0.48	0.54	0.59	0.61	0.64
	Conc. (µg/mL)	10	14	15	18	20	22	23	24
	% Release	20	28	30	36	40	44	46	48
NFC-PAA	Absorbance	0.47	0.55	0.58	0.66	0.73	0.80	0.88	0.99
	Conc. (µg/mL)	13	15	16	18	20	22	24	27
	% Release	22	26	30	32	36	40	44	48
CFC-PAA	Absorbance	0.15	0.17	0.18	0.19	0.21	0.24	0.25	0.29
	Conc. (µg/mL)	13	15	16	17	19	21	22	23
	% Release	26	30	32	34	38	42	44	46
OFC-PAA	Absorbance	1.3	1.4	1.9	2.2	2.3	2.4	2.5	2.6
	Conc. (µg/mL)	12	13	17	20	21	22	23	24
	% Release	24	26	34	40	42	44	46	48

***In-vitro* screening of latentiated systems for antibacterial activity**

The antibacterial activity [12-17] study of the drug-polypeptide conjugates was performed in order to ensure: The potency of the drug-latentiated systems and; the sensitivity of selected microbes to the known concentration of the synthesized derivatives. Different methods [18-21] are in practice, each with its own limitations and advantages. These include agar diffusion (cup, disc and cylinder) method, serial dilution method, turbidimetric method etc. with the first two being used most often. In the present study, agar diffusion with disc method was followed, selected on the grounds of convenience, sensitivity and practice.

Preparation of culture media

A 2% w/v agar media was prepared by using the following formula: Beef extract (1g), yeast extract (2g), peptone (5g), sodium chloride (5g), agar (20g), distilled water up to (1000 mL). Accurately weighed quantities of all the ingredients, except agar, were dissolved in a small quantity of distilled water by heating on a water bath. The solution was filtered through a muslin cloth. The pH of the solution was adjusted to 7.2 to 7.4 by adding 0.1 N sodium hydroxide solution. Then, weighed quantity of agar was added and the volume made up with distilled water. The media was then sterilized by autoclaving at121°C, 15 pounds psi pressure for 2 hours [22-25].

Proper diffusion of the compound through the media is a necessity in determining the antibacterial activity. Thus, water solubility of compounds is desirable [26]. However, since the synthesized compounds were sparingly soluble in water, 10% dimethyl formamide (DMF) was used. This concentration of DMF was considered to possess no antibacterial activity by itself [27]. The selection of micro-organism were such as to include both gram-negative and gram-positive bacteria. The strains selected were *Proteus morganii* and *Staphylococcus aureus.*

Preparation of test/standard samples

Latentiated derivatives (10 mg each) were dissolved in DMF and aliquots with 0.1 mL and 0.5 mL were withdrawn and diluted to 10 mL with sterilized distilled water to get solutions of 10 µg/mL and 50 µg/mL concentration of each polypeptide. Similarly 10 µg/mL and 50 µg/mL concentration of NDA, NFC, CFC and OFC were also prepared as stated above.

Preparation of inoculums

The inoculums were prepared by transferring a loopeful of the corresponding micro-organism from the stock culture into the sterile broth and incubating. A two days old young culture of the concentration of $3x10^{-6}$/µL was used.

Determination of anti-bacterial activity

The sterilized nutrient agar was poured into the petri dishes aseptically. A swab of absorbent cotton was soaked in the inoculums and gently applied on the surface of the cooled agar plates to get a uniform distribution of the cultures. Sterile filter paper discs (Whatman No. 1) of 8 mm diameter were soaked in the test solution. Each plate was labeled according to the micro-organism and test solution used. A filter paper disc from each solution was carefully kept on the surface of the respective agar plate. The plates were incubated at 37°C for 72 hours. The diameter of the zones of inhibition was then recorded.

Results and discussion

The latentiated drug-polypeptide systems were synthesized by modified Schotten-Baumann reaction. The physicochemical and other parameters would be a guide to their physiological behavior and formulation development.

The organoleptic properties of the latentiated systems were quite a contrast from the parent drugs, and so were the melting point and solubility profiles (Table 3). The changes in melting range further consolidated their formation. The congeners had varying hydrophobicity, as was reflected in the partition coefficient (Octanol/water) studies and was in the order of: NFC-PGA>NFC-PAA>NDA-PGA>OFC-PGA>NDA-PAA>OFC-PAA>CFC-PGA>CFC-PAA. The availability of the compounds is of vital considerations in the assessment of physiological importance. It was in the order of: CFC-PAA>OFC-PAA>NDA-PAA>CFC-PGA>NFC-PAA>NDA-PGA>NFC-PGA. The acidic and alkaline hydrolysis of the latentiated systems revealed more about their nature. They resisted acidic hydrolysis but were rapidly hydrolysable in the alkaline

medium. The rate of availability of drugs by hydrolysis of the compounds was in the order of: NFC-PAA>NFC-PGA>NDA-PGA=NDA-PAA=OFC-PAA>OFC-PGA>CFC-PGA. The data for the same has been given in Table 5.

The results of the antimicrobial clearly reflect the antibacterial nature of the latentiated drug-polypeptide systems against different microorganisms at different concentrations. It is significant to note the increase in activity with increasing concentration of the compounds. The results show varying activity of the compounds against different microorganisms. However, on an overall estimate, the antibacterial activity of the compounds against *P. morganii* was in the order of: CFC-PGA>OFC-PGA>CFC-PAA>OFC-PAA=NDA-PGA=NDA-PAA>NFC-PGA>NFC-PAA. Whereas, the activity in the case of *Staphylococcus aureus* was in the order of: CFC-PAA=OFC-PGA>CFC-PGA>OFC-PAA>NFC-PGA>NFC-PAA>NDA-PGA>NDA-PAA. The detailed results of antibacterial activity of the synthesized latentiated derivatives were recorded and are represented in Table 6 and Figure 1.

On the basis of the above results it can be deduced that the physicochemical properties of the latentiated systems show marked change from those of the drugs. The completely water insoluble drugs became sparingly soluble in latentiated systems. This can be attributed to the polypeptides. The availability assessments also show an increase due to altered partition values. The resistance of the derivatives towards acidic hydrolysis led to the assumption that they would cause almost no gastric irritation and ulcerogenicity since they were not hydrolyzed in the simulated gastric fluid. This is a manifestation of the solubility profile of polyacids, which is strongly pH dependent. At low pH, the carboxylic groups of polyacids are protonated, i.e. not ionized, and upon an increase in the pH, of the solution, they release hydrogen and become ionized.

Table 6: Data showing antibacterial activity of synthesized latentiated systems.

S. N.	Drug-polypeptide latentiated systems	Concentration (µg/mL)	Diameter of zone of inhibition (mm)	
			Proteus morganii	***Staphylococcus aureus***
1.	NDA	10	24	---
		50	29	0
2.	NFC	10	23	10
		50	27	16
3.	CFC	10	25	21
		50	32	25
4.	OFC	10	25	20
		50	33	22
5.	NDA-PGA	10	31	10
		50	34	11
6.	NFC-PGA	10	25	14
		50	31	17
7.	CFC-PGA	10	30	25
		50	37	28
8.	OFC-PGA	10	26	25
		50	36	29
9.	NDA-PAA	10	32	10
		50	34	11
10	NFC-PAA	10	24	12
		50	26	15
11	CFC-PAA	10	29	22
		50	35	29
12	OFC-PAA	10	26	23
		50	34	26
13	Control (Distilled water)	---	00	00

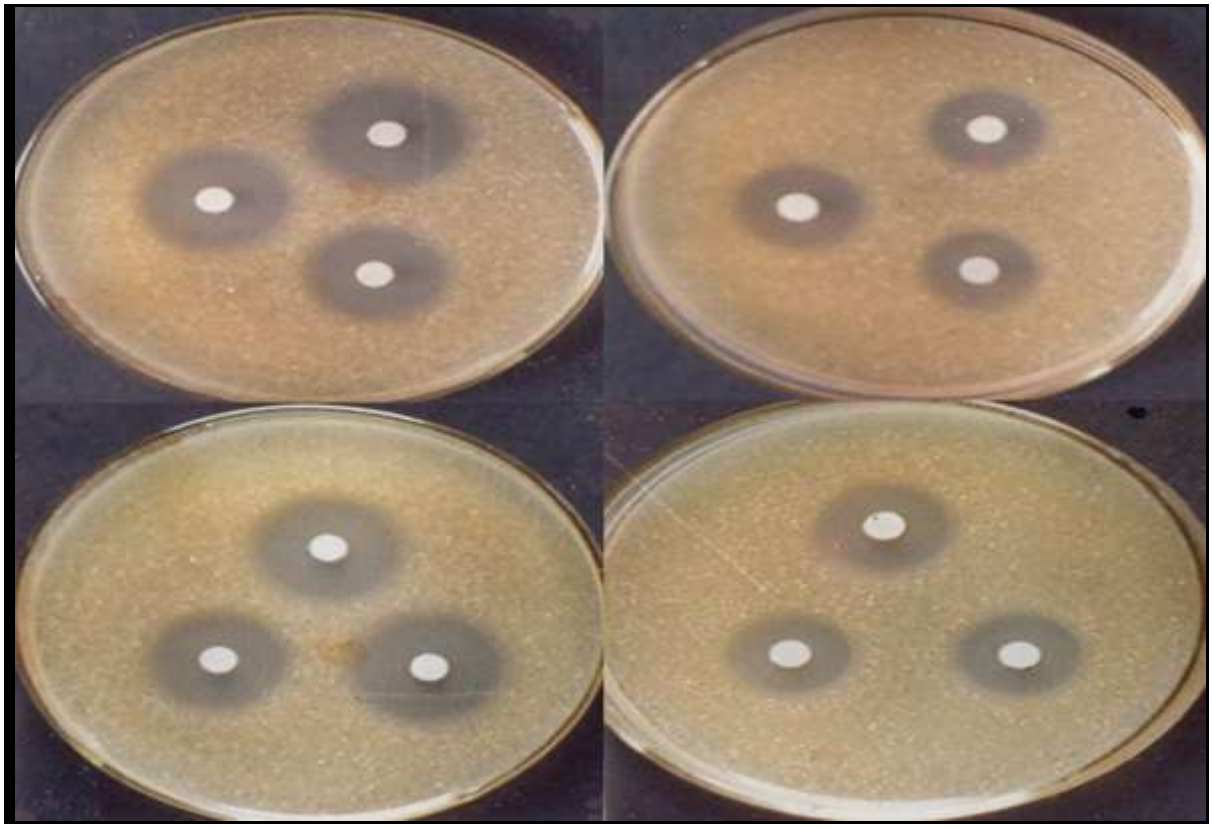

Figure 1: Photograph showing zone of inhibition for antibacterial activity of some active drug latentiated systems against (4a) *Proteus morganii*; (4b) *Staphylococcus aureus*.

The slowly hydrolysable reversible derivatives of the drugs will alter the rate of excretion of the drugs and subsequently enhance the blood levels of the drugs. Also, the latentiated derivatives would decrease the plasma protein binding of the parent drugs, thereby raising their blood levels which results in better activity. The hydrolysis in simulated intestinal fluid is indicative that the hydrolysis of the congeners shall occur by membrane bound enzymes that line the small intestine. The high partition coefficient values for the compounds shall be presumable for their absorption through the lipoidal cell membranes. The enhanced antibacterial activities of NDA-PGA and NDA-PAA against *S. aureus* are possible only due to conjugation with polypeptides because NDA is not effective against Gram negative microorganisms. However, this is not expected of single units of amino acids, thereby justifying the use of macromolecules for conjugation.

Acknowledgments

The authors are thankful to Dr. Hemant Singh, Scientist F (Psychoneuropharmacology), CDRI, Lucknow for his guidance and valuable suggestions in conducting part of this work at CDRI, Luckunow.

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