Comparative Study of Antimicrobial Activity of lactosylated form amides bulk solution with its nanoparticles

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Abstract:
By observing biological application of Nanoparticles and desulphurized compounds of carbohydrates in industrial and medicinal research, it was found intresting to carry out the Antimicrobial activity of newly synthesized series of1-Hepta –O-benzoyl –β-D-lactopyranosyl-3H/aryl formamides nanoparticlesand compare it with its bulk solution.

Key words: LactosylatedFormamides, Nanoparticles and Antimicrobial activity.

Introduction:

Nanostructure materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications [1,2]. Recent studies have demonstrated that especially formulated nanoparticles have good antibacterial activity [3,4].

Similarly In view of this application[5] of lactosyl compounds and Nanoparticles in this we have synthesis to investigate the chemistry of this new compound with reference to their application.

Desulfurization is the removal of sulfur or sulfur compounds (as from coal or flue gas), mostly from fuels. The most commonly required desulfurization process is natural gas, but it is also required for flue gas, coal and oil.Sulfur in crude oil, natural gas, process gas and natural gas liquids (LNG) may take many forms, including hydrogen sulfide (H_2S), carbonyl sulfide (COS), sulfur oxide(Sox) and the whole family of marcaptans.

Experimental:

UV-visible Spectra is measured using UVSpectrophotometer by using model Single Beam UV-Visible Spectrophotometer with software(BI/CI/SP/SB-S-03)of Bio Era make.. IRspectra were recorded on Perkin-Elmer spectrum RXI FTIR spectrophotometer (4000-450 cm^{-1}). ^1H NMR was recorded in $CDCl_3$ on Bruker DRX-300 spectrometer operating at 300 MHz.

a) Synthesis of hepta-O-benzoyl-α-D-lactosyl bromide:

The finally powdered lactose octabenzoate(0.03M, 21.0g) was added gradually to the brominating agent. After the addition the flask was kept for 2hr at room temperature. Then the reaction mixture with chloroform (130ml) then the mixture was shaken vigorously for about 15 min. The resultant mixture was poured into ice cold water. The chloroform layer was then separated. It was washed several with aqueous sodium bicarbonate to remove excess of acetic acid followed by aqueous sodium metabisulphite to remove excess of bromine and finally 2-3 times with water. To the chloroform addition of petroleum

ether afforded a solid (16.5 gm). This solid was expected hepta-O-benzoyl-α-D-lactosyl bromide (yield 77%). It was purified by dissolving it in minimum quantity of chloroform and reprecipitating it with petroleum ether,m.p.168^{0}C.

b) Preparation of lead thiocyanate :

Lead thiocyanate was prepared by mixing aqueous solution of lead nitrate and ammonium thiocyanate. The white granular lead thiocyanate was filtered washed with distilled water and dried at 50^0 C.

c) Preparation of hepta-O-benzoyl-β-D-lactosyl isothiocyanate[6] :(1)

To a suspension of hepta-O-benzoyl-α-D-lactosyl bromide (21 gm, 0.03M) in sodium dried xylene (80ml) was added lead thiocyanate (6gm, 0.03M). The reaction mixture was then treated for microwave synthesis for about 3 min. This solution was then cooled and liberated lead bromide was removed by filtration. The xylene filtrate was then treated with petroleum ether (60-80^{0}C) with stirring, a white solid mass obtained (13gm). This solid was expected hepta-O-benzoyl- β-D-lactosyl isothiocyanate.

It was purified by dissolving it in minimum quantity of chloroform and reprecipitating it with petroleum ether, m.p. 118-120^{0}C. [Found; C, 67.07, H; 4.46, N; 1.22, S; 2.9; $C_{62}H_{49}O_{17}NS$ requires; C; 66.96, H; 4.41, N; 1.26, S; 2.88%].

Preparation of 1-hepta O-benzyl –β-D –lactosyl 5 phenyl 2,4,Dithiobluerts:(3a)

A suspension of 4 gm of Hepta O-benzyl-β-D lactosyl isothiocyante with 20 ml of benzene and 1 gm of aniline thiourea(2a) was treated for microwave synthesis for about 3 min. This solution was then cooled and the benzene filtrate was then treated with petroleum ether (60-80^{0}C) with stirring, a white solid mass obtained (13gm). This solid was expected 1 –hepta-O–β–D lactosyl 5-phenyl 2, 4 dithiobiurets.

It was purified by dissolving it in minimum quantity of chloroform and reprecipitating it with petroleum ether, m.p. 145-146^{0}C.

Desulphurization of Hepta-O-benzoyl-β-D-lactosyl-5-phenyl-2,4dithiobiuret

1. Preparation o Raney Nickel:

The required Raney nickel was prepared by earlier method [29] by action of sodium hydroxide solution on powdered NI-Al alloy.

Preparation of 1-Hepta-O-Benzoyl –D-lactopyranosyl-3-H/phenyl formamides: (4a)

To a benzene solution of Raney nickel (W2:15g in 100ml)(In a 250ml round bottom flask, fitted with a mechanical stirrer, areflux condenser and a dropping funnel). The benzene solution of 1-hepta-O-benzoyl- β-D-lactosyl-5-phenyl-2,4dithiobiuret(7g in 5ml, 0.01M) 3a was taken in dropping funnel and added to Raney nickel in round bottom flask with constant stirring. The reaction mixture was heated gently over heating mental for 150 min with TLC monitoring. After the completion of reaction the reaction mixture was filtered hot to remove excess of Raney nickel. The solvent was distilled off to

afford a semi solid which on triturating with petroleum ether 60-80^0 C for several times afforded faint yellow solid (4a). It was purified with ethanol.

Preparation of Nanoparticles of 1-Hepta-O-Benzoyl –D-lactopyranosyl-3-H/aryl formadimides:(5a)

Take about 1 gm of 1-Hepta-O-benzyl –β-**D-lactopyranosyl-3-H/aryl Formadimides**(4a-4f)and dissolveit completelyin the 50ml of solvent in 250 ml beaker.Now put this beaker in sonicator. The highly penetrating acoustic waves are passedthrough mixture, which create high pressure bubbles in the beaker due to which breakdown ofthe bulk material is takes place and desired sized nanoparticles are formed. The size determination of nanoparticles is done by the X-ray diffraction studies.

IR spectrum of1-Hepta- O-benzyl –β-D-lactopyranosyl-3-H/aryl formaides[7]

Absorption Observed (Cm^{-1})	Assignment	Absorption Expected (Cm^{-1})
3068	C-H Ar-stretching	3040-3010
1728	C=O stretching	1750-1735
1176	C-O stretching	1210-1153
1026,909	Characteristic of lactose	1100-1000 and 910-900
710	Monosubstituted benzene	770-680

NMR SPECTRAL STUDIES[8,9]:

The NMR Spectrum of compound distinctly displayed signals due to N-H Proton at δ 9.05 and d 6.57 ppm, Aromatic Protons at δ7.47-7.15 ppm, lactosyl protons at d 5.77-3.76 ppm.

Characterization of Nanoparticles:

1. **Characterization using UV – Visible Spectrophotometer:** Characterization of nanoparticles was done using visible Spectrophotometer by using model Single Beam UV-Visible Spectrophotometer with software (BI/CI/SP/SB-S-03)of Bio Era make. The UV-Visible Spectroscopy reveals the formation of nanoparticles by showing different absorption those from bulk material.
2. **Size determination of Lactosyl formamidesNanoparticles by X-Ray Diffraction Studies:** From the X-Ray diffraction it comes to know that size of nano octabenzoate is 66nm-90nm.

Antimicrobial Activity:

The bulk Lactosyl formamides and the Nanopartices of Lactosyl formamides have been screened for antibacterial activity using cup plate agar diffusion methodby measuring the inhibition zone in mm. The compounds were taken at a concentration of 1 mg/ ml using dimethyl sulphoxide as solvent. Amikacin (100ug/ml) was used as a standard for antibacterial activity. The compounds were screened for antibacterial activity against *Escherichia coli, Staphylococcus aureus,* in nutrient agar medium.

Antimicrobials	Zone of inhibition in mm					
	Bulk (4a)	Nanoparticles (5a)	Bulk (4b)	Nanoparticles (5b)	Bulk (4c)	Nanoparticles (4c)
E. coli	10	15	12	15	11	16
S. aureus	11	16	11	17	12	18
S. typhi	12	16	12	16	10	17
P. vulgaris	10	15	12	15	10	13
Amikacin	11	20	10	17	12	17
Clandamycine	12	15	11	14	10	14
DMSO						

*including the well diameter of 8mm. ** zone of inhibition in mm (14or less) resistance, (16- 20mm) moderate and (more than 20mm) sensitive

Conclusion: Lactosyl formamides Nanoparticles show good antimicrobial activity as compare to the bulk solution of lactosyl formamides due to their large surface area to volume ratio, which is coming up current interest in the researchers.

ACKNOWLEDGEMENT:

Author is thankful to RSIC, CDRI Lucknow for providing the spectra and also to Dr. S.P.Deshmukh, Shri Shivaji College, Akola, Dr. V.D.Nanoty, Principal Shri R.L.T. College of Science, Akola for providing Guidance and necessary facilities.

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