academicJournals

Vol. 12(47), pp. 6661-6671, 20 November, 2013
DOI: 10.5897/AJB2013.12957
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Preliminary spectroscopic characterization of PEGylated mucin, a novel polymeric drug delivery system

Franklin Chimaobi Kenechukwu[1]*, Emmanuel Chinedum Ibezim[1], Anthony Amaechi Attama[1], Mumuni Audu Momoh[1], John Dike Nwabueze Ogbonna[1], Petra Obioma Nnamani[1], Salome Amarachi Chime[2] , Chukwuebuka Emmanuel Umeyor[3] and Emmanuel M. Uronnachi[3]

[1]Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.
[2]Department of Pharmaceutical Technology and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.
[3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Accepted 16 September, 2013

The objective of this study was to evaluate, spectrophotometrically, the compatibility of non-mucinated polyethylene glycol (PEG) 4000 and non-PEGylated mucin in a PEGylated mucin matrices for drug delivery application. Mucin was extracted from the giant African land snails (*Archachatina maginata*) using chilled acetone and characterized in terms of qualitative properties and solubility profile. Polymeric matrices composed of PEG 4000 and mucin in ratios of 2:0 (A), 1:1 (B), 2:1(C) and 3:1 (D) were prepared by co-precipitation using chilled acetone. The matrices were characterized with respect to compatibility using the Fourier transform infrared (FT-IR) spectroscopy. Results of the qualitative tests performed on the snail mucin showed that carbohydrates, proteins and trace amounts of fats were present; the extracted mucin was light-brownish in colour, with a pleasant meaty odour. Snail mucin, when dispersed in water yielded a slightly viscous dispersion, but is not soluble in ethanol, acetone, 0.1 M sodium hydroxide, ammonium hydroxide and sulphuric acid. The presence of different peaks in the FT-IR spectra of the PEGylated mucin matrices compared with the non-PEGylated mucin (2:0) matrix and non-mucinated PEG 4000 (0:2) matrix indicated the formation of new polymers, which could be employed in drug delivery. This study has shown that PEGylation of mucin gives rise to new polymeric system with principal FT-IR peaks quite different from those of non-PEGylated mucin and non-mucinated PEG, and this may be employed in the delivery of drugs.

Key words: PEGylation, drug delivery, mucin, Fourier transform infrared (FT-IR) spectroscopy, *Archachatina maginata*.

INTRODUCTION

Drug delivery can be of importance for both new chemical entities as well as established drugs. The basic goal of any drug delivery system is to achieve steady state of blood concentration or tissue level that is therapeutically effective and non-toxic for an extended period of time (Muller et al., 2000; Gillies and Frechet, 2004). Many biolo-

gically active molecules are limited in their therapeutic values by properties such as poor solubility, limited bioavailability, and rapid elimination (Muller et al., 2000). In addition, while the beneficial effects of many drugs arise from their interaction with specific tissues, their exposure to other cell types frequently leads to undesirable side effects and toxicity (Gillies and Frechet, 2004). In recent decades, there has been increased awareness of the need to develop drug delivery systems to improve the properties of therapeutic compounds, increase their effectiveness, and reduce their harmful side effects. Oral administration of therapeutic agents represents by far the easiest, safest and most convenient route of drug delivery, especially in the case of chronic therapies (Kreuter, 1991). Unfortunately, the oral delivery route is beset with problems such as gastrointestinal (GI) destruction of labile molecules and low levels of macromolecular absorption (Francis et al., 2004). The development of oral forms of many drugs however remains a challenge either on account of their stability or their absorption from the GIT thus leading to sub-therapeutic bioavailability (Kreuter, 1991). To reduce the impact of digestive enzymes and ensure the absorption of bioactive agents in an unaltered form, molecules may be incorporated into polymeric matrices, which could shield the encapsulated drug from the external harsh conditions, and may favour uptake by intestinal cells (Delie and Blanco-Prieto, 2005). Many polymers have been used in the formulation of polymeric matrices (Ravi-Kumar, 2000; Pamujula et al., 2004; Lagarce et al., 2005; Bernkop-Schnurch et al., 2004; Attama and Nwabunze, 2007). The engineering of new polymer biomaterials with special physicochemical properties appears to be an answer to the afore-mentioned problems. To obtain new polymer biomaterials with better physicochemical qualities that will meet the carrier needs of challenging drug molecules, blending of polymers with desirable properties becomes imperative. Thus, mucin and PEG could be blended to improve the physico-chemical properties of the individual polymers.

Mucins are high molecular weight (0.2 – 10 million Dalton) glycoproteins containing both highly glycosylated domains and naked domains. The glycosylated domains are enriched in serine and threonine residues which serve as anchoring points for oligosaccharide chains. These O-linked oligosaccharide side chains are complex both in terms of composition and length. The naked domains are typically found in the N-terminal and C- terminal parts of the protein and are enriched in cysteine residues (Carlstedt et al., 1983). The cysteine residues can form intermolecular bonds, and in the native state mucins are often found as oligomers composed of several end-to-end linked mucin subunits (Perez and Proust, 1987). A common feature of mucins, apart from a high molecular weight and a high carbohydrate content (68 - 81%), is the abundance of negatively charged groups. The negative charges arise mainly from sialic acid residues and in some cases from sulphated sugars. These acidic groups account for the low isoelectric point (2-3) of mucins (Durrer et al., 1995). The basic amino acids of the protein component are serine and threonine. The glycosylated regions of mucins interact favourably with water and force the molecule to an extended random coil conformation, and the high molecular weight enables individual mucin molecules to overlap and entangle at relatively low concentrations (Lee et al., 2005). These features are ideal with respect to the formation of hydrogels. Studies have shown that reconstucted mucous gels from mucins have similar rheological properties as native mucous gels at physiological concentrations (Raynal et al., 2003). The ability of mucin to form the structural backbone of the mucous gel is one of its most important functions (Raynal et al., 2003).

There is an on-going effort aimed at improving the potential use of mucin in controlled delivery of certain drugs. One of the strategies that have been successfully employed is PEGylation (Momoh et al., 2010; Momoh et al., 2011; Momoh et al., 2010). Previous studies on PEGylation (the process of attaching PEG to any polymer, or the molecular attachment to polyethylene glycols with different molecular weights to active drug molecules or surface treatment of drug-bearing particles with PEGs) have shown that the interaction between PEG and mucin is either molecular interaction in a solvent which resulted in the formation of new bond or attachment of the PEG to a functional group in the protein molecule to form a copolymer (Schnurrer and Lehr, 1996). This technology is one of the most promising and extensively studied strategies for improving the pharmacokinetics of drugs (Abuchowski et al., 1977). Clinically proven PEGylation technology can improve the performance and dosing convenience of peptides, proteins, some water soluble drugs, antibodies, oligonucleotides and many small molecules by optimizing pharmacokinetics, increasing bioavailability and decreasing immunogenicity and dosing frequency. PEGylation has been shown to increase therapeutic efficacy by enabling increased drug concentration, improved biodistribution, and longer dwelling time at the site of action (Kodera et al., 1998). This would result in the achievement of therapeutic drug concentrations with less frequent dosing which would be a significant benefit to patients who are taking injected drugs

*Corresponding author. E-mail: chimafrankduff@yahoo.com; frankline.kenechukwu@unn.edu.ng.
Tel: +234 8038362638. Fax: +234-42-771709.

Abbreviations: APIs, Active pharmaceutical ingredients; **FT-IR,** Fourier transform infrared; **DSC,** differential scanning calorimetry; **GI,** gastrointestinal.

and long time medication for example in diabetes management. Therefore, among the different polymer-based drug delivery systems, PEGylated polymeric bioadhesive microparticles represent a promising delivery vehicle especially intended for short-acting active pharmaceutical ingredients (APIs) employed in chronic therapies in order to improve their therapeutic effects.

Recently, PEGyalted mucin matrices were characterized with respect to thermal properties. The results of the thermal properties of PEGylated mucin which was measured by differential scanning calorimetry (DSC) indicated that an interaction occurred between PEG and mucin (Momoh et al., 2010). This serves as a basis for further characterization of the interaction between PEG and mucin in PEGylated mucin matrices for drug delivery application. Fourier transform infrared (FT-IR) spectroscopic analysis can be used to determine the functional groups of the molecules (Sahoo et al., 2012; Sahoo et al., 2012; Sahoo et al., 2011). Therefore, to obtain more information in detail about chemical interaction between PEG and mucin, the FT-IR analysis was carried out. In this study, admixtures of PEG 4000 and mucin obtained from African land snail (*Archachatina marginata*) were prepared by combining the two at different ratios. Some physicochemical properties as well as compatibility of the PEGylated mucin matrices were assessed. The objective of this study was to evaluate, spectroscopically, the compatibility of non-mucinated polyethylene glycol (PEG) 4000 and non-PEGylated mucin in a PEGylated mucin matrices for drug delivery application.

MATERIALS AND METHODS

The following materials were purchased from their local suppliers and used without further purification: polyethylene glycol-4000 (Ph. Eur. Carl Roth GmbH + Co.KG Karlsruhhe Germany), citric acid, sodium hydroxide (Merck, Germany), methyl red, ethanol, nitric acid, silver nitrate, tetraoxosulphate (vi) acid, sodium chloride, acetone, concentrated hydrochloric acid (BDH, England). The giant African land snails (*A. marginata*) used were procured from a local market in Nsukka, Enugu State. All other reagents were of analytical grade and were used as such.

Extraction of snail mucin (slime)

The method of Adikwu and Nnamani (2005) was employed for the extraction with modifications. The giant African land snails (*A. maginata*) were washed with clean water and the shells were knocked open at the apex and a spirally coiled rod was inserted to remove the fleshy body. The mucus layer (slime mucus) was gently scraped off from the fleshy parts, pooled together in a container and precipitated with chilled acetone. These precipitates were then dried with a flush of cold air (-4°C) to obtain greyish-brown flakes of the snail mucin, which were powdered and used for the study.

Physicochemical properties of snail mucin

Some physicochemical properties of the snail mucin were also assessed using standard procedures (Adikwu and Ikejiuba, 2005).

Test for proteins (amines; oxidation test)

Biuret test

Two drops of water and 1 ml of dilute sodium hydroxide were added to 2 % dispersion of mucin in water. Two drops of 1 % copper sulphate solution were added with the solution shaken thoroughly after each drop and observed. A purple or pink colour shows the presence of proteins.

Xanthoproteic reaction

Two drops of concentrated nitric acid were carefully added to 2% dispersion of mucin in water. A white precipitate was formed, which turned yellow on heating. The contents of the test tube were cooled, three drops of dilute sodium hydroxide solution added and the precipitate observed. A yellow colour which changes to orange indicates the presence of proteins.

Test for fats and oils

A drop of the acetone extract of the mucin was placed on a filter paper. The solvent was allowed to evaporate and the filter paper observed carefully for any transluscence.

Test for sugars

Three drops of freshly prepared Fehling's solutions I and II were added to 1% w/v aqueous dispersion of snail mucin which was then heated in a boiling water bath for 5 min and observed.

Test for carbohydrates

Iodine test

Two drops of 1% iodine solution were added to 1 ml of 1% w/v of mucin and then observed for blue-black colouration.

Molisch's test

Two drops of ɑ-naphthol solution was added to 2 ml of the snail mucin dispersion and the two mixed thoroughly. Then 1 ml of concentrated sulphuric acid was gently poured down the side of the tube and observed.

Tollen's reagent test

Tollen's reagent prepared as 1 ml of 5% silver nitrate solution was treated with a few drops of 5 % sodium hydroxide solution. A volume of aqueous ammonia just enough to redissolve the precipitate was added to 3 drops of the snail mucin dispersion and the mixture warmed in a boiling water bath for few minutes. The colour of the precipitate formed was observed.

Solubility profile of snail mucin

The solubility of snail mucin in several solvents was determined by dispersing 100 mg of the snail mucin in definite volumes of each solvent- acetone, ethanol, water, sodium hydroxide, hydrochloric acid and ammonium hydroxide respectively at different temperatures (25, 30, 40°C).

Table 1. Physicochemical properties of snail mucin.

Test	Observation	Inference
Carbohydrate	+++	Present
Protein	++	Present
Fats	+	Present

+, Present in trace amount; ++, moderately present; +++, copiously present.

Table 2. Solubility profile of snail mucin.

Temperature (°C)	Acetone	Ethanol	0.1 M NaOH	0.1 M H_2SO_4	0.1 M NH_4OH	H_2O
25	-	-	-	-	-	-
30	-	-	-	-	-	+
40	-	-	-	-	-	++

-, Not soluble; +, less soluble; ++, more soluble.

Preparation of PEGylated-mucin matrices

Polymer hybrids (matrices) were generated from mucin and poly(ethylene glycol) (PEG M_w 4000) by controlled coacervation in aqueous medium (Momoh et al., 2010; Momoh et al., 2011; Momoh et al., 2010). The matrices were prepared in the following molecular ratios (M:P): 1:1, 2:1, 3:1, 2:0, 0:2. Briefly, known quantities of mucin and PEG 4000 were weighed into separate 100 ml beakers and 20 ml of distilled water was added into the samples and allowed to stand for 72 h for interaction of the solvent and the polymers. The contents of the two beakers were then mixed and allowed to stand for another 72 h for molecular interaction. The hybrids were precipitated with chilled acetone, dried, pulverized and kept in an airtight container until used.

Spectroscopic characterization of the matrices

FTIR spectroscopy study was conducted using a Shimadzu FTIR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1} with threshold of 1.303, sensivity of 50 and resolution of 2 cm^{-1} range. The procedure consisted of dispersing a 5 mg sample in KBr and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum obtained (Sahoo et al., 2011, 2012, 2012; Builders et al., 2008).

RESULTS AND DISCUSSION

Physicochemical properties of snail mucin

Results of the physicochemical tests performed on the snail mucin (Table 1) showed that carbohydrates, proteins and trace amounts of fats were present. This is consistent with previous reports (Carlstedt et al., 1983; Perez and Proust, 1987; Durrrer et al., 1995; Lee et al., 2005; Raynal et al., 2003). In both wet and dry states, the extracted mucin was light-brownish in colour, with a pleasant meaty odour. Snail mucin when dispersed in water yielded a slightly viscous dispersion (Table 2). The snail mucin is not soluble in ethanol, acetone, 0.1 M sodium hydroxide, ammonium hydroxide and sulphuric acid (Table 2), in agreement with previous reported studies ((Momoh et al., 2010, 2010, 2011; Adikwu and Nnamani, 2005; Adikwu and Ikejiuba, 2005).

Fourier transform infrared (FT-IR) spectroscopy

The IR spectrum of a given compound is always unique and characteristic. Thus, IR spectroscopy is a quick and relatively cheap technique for identifying compounds (Sahoo et al., 2012; Sahoo et al., 2012; Sahoo et al., 2011; Builders et al., 2008). FT-IR spectra of the matrices (2:0, 0:2, 1:1, 2:1 and 3:1) are shown in Figures 1 – 5 . FT-IR spectra of non-PEGylated mucin (2:0) matrix, non-mucinated PEG (0:2) matrix and PEGylated mucin (1:1, 2:1 and 3:1) matrices were carried out as finger prints to identify PEGylated mucin relative to non-PEGylated mucin and non-mucinated PEG matrices. FT-IR spectrum of non-PEGylated mucin matrix (that is, mucin alone 2:0) (Figure 1) shows that principal peaks were observed at wave numbers of 1074.39, 1240.27, 1528.64, 1641.48, 2946.36, 3275.24 and 3421.83 cm^{-1} corresponding to C-N vibrations, -C-O stretching, N-H bending vibration for secondary amines, C=C stretching of α,β–unsaturated ring, C-H stretching, O-H stretching and N-H amide bending, respectively.

FT-IR spectrum of non-mucinated PEG matrix (that is, PEG 4000 alone 0:2) (Figure 2) shows characteristic peaks at 840.99, 950.94, 1114.89, 1273.06, 1355.04, 1466.91, 2882.71 and 3429.55 cm^{-1} corresponding to aromatic C-H bending (2 adjacent free H's), aromatic C-H bending (1 FT-IR spectra of PEGylated mucin matrices (that is, 1:1, 2:1 and 3:1) (Figures 3 - 5) were different from those of non-PEGylated mucin matrix (2:0) and non-mucinated PEG matrix (0:2). Spectrum of 1:1 matrix (Figure 3)

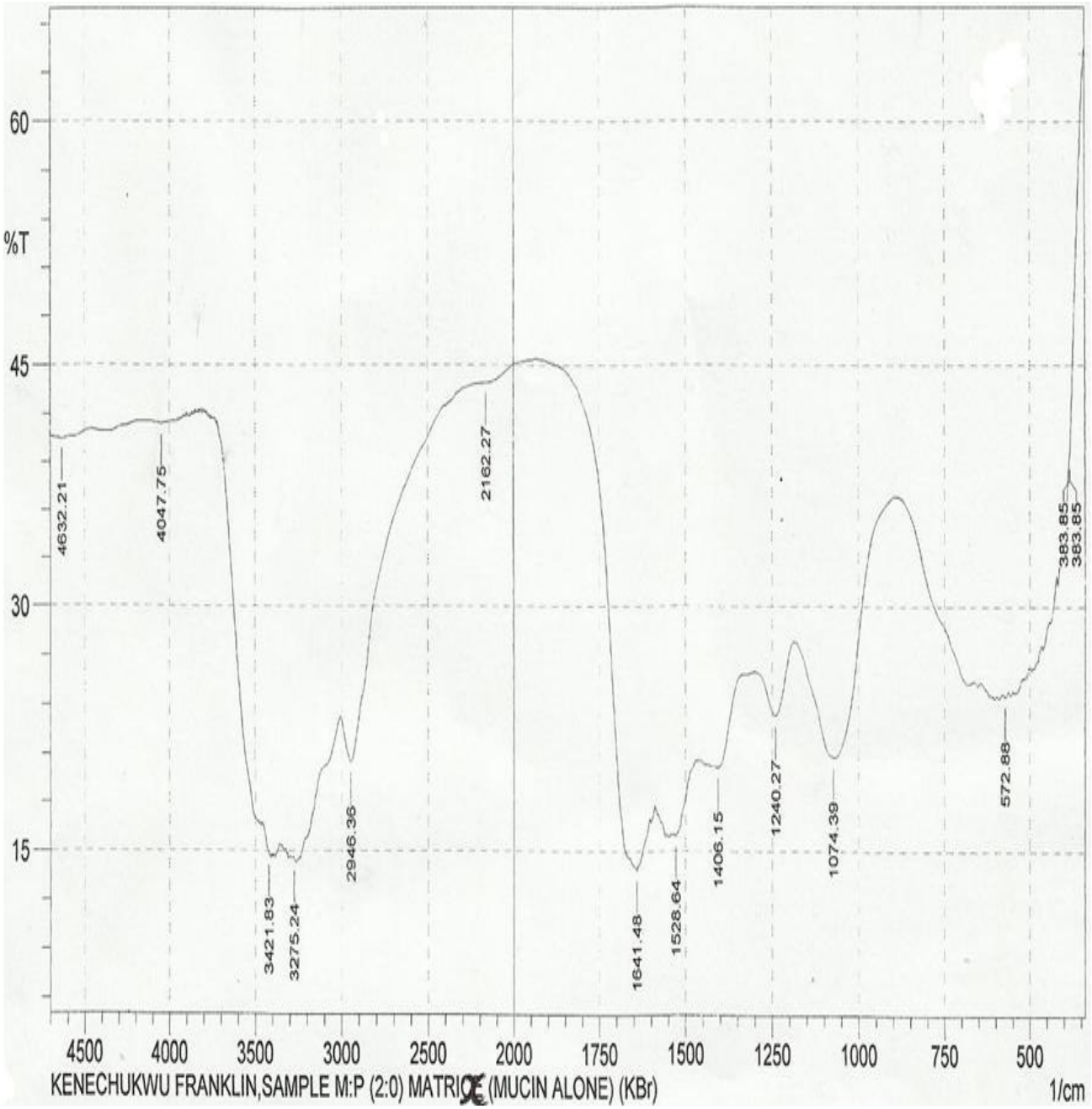

Figure 1. FT-IR spectrum of polymer matrix (2:0) (mucin).

shows strong peaks at 838.10, 956.72, 1116.82, 1264.38, 1362.75, 1536.35, 1665.59, 2885.60 and 3298.38 cm^{-1} corresponding to aromatic C-H deformation (2 adjacent free H's), C-N vibrations, C-O stretching (strong), C-O stretching, C-H deformation (CH_3), N-H bending vibration for secondary amines, C=C stretching of α,β–unsaturated ring, O=C-H stretching and N-H amide bending, respectively; in 2:1 matrix, the characteristic peaks were found at 625.92, 1113.93, 1251.84, 1404.22, 1679.09, 2888.50 and 3444.02 cm (Figure 4) due to aromatic C-H deformation, C-O stretching, C-O stretching, C-H deformation (CH_3), C=C stretching of α,β–unsaturated ring, C-H stretching and N-H amide bending, respectively; while the FT-IR spectrum of 3:1 matrix (Figure 5) shows that principal peaks were observed at wave numbers of 595.06, 1079.21, 1244.13, 1450.52, 1597.11, 2889.46

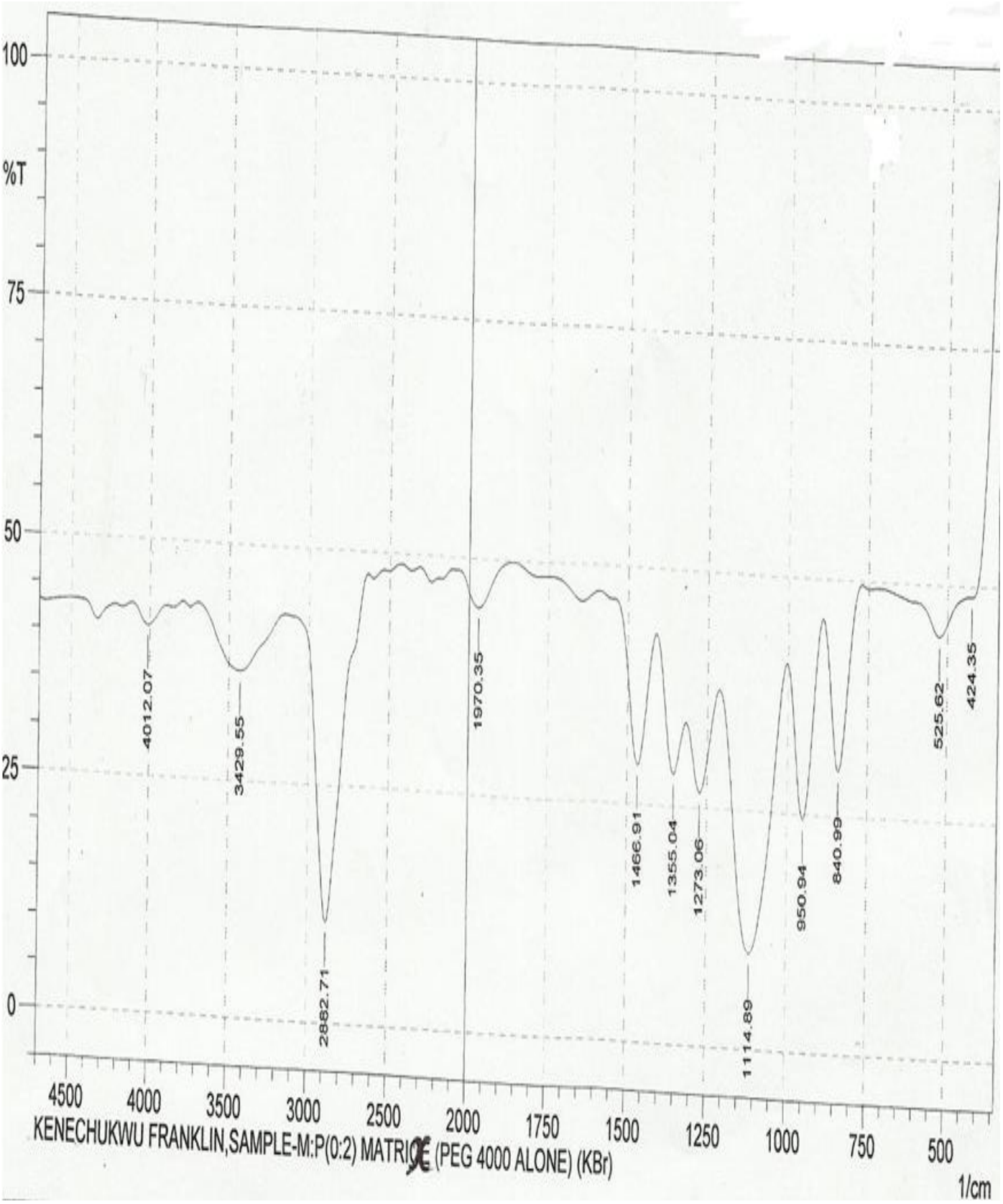

Figure 2. FT-IR spectrum of polymer matrix (0:2) (PEG-4000).

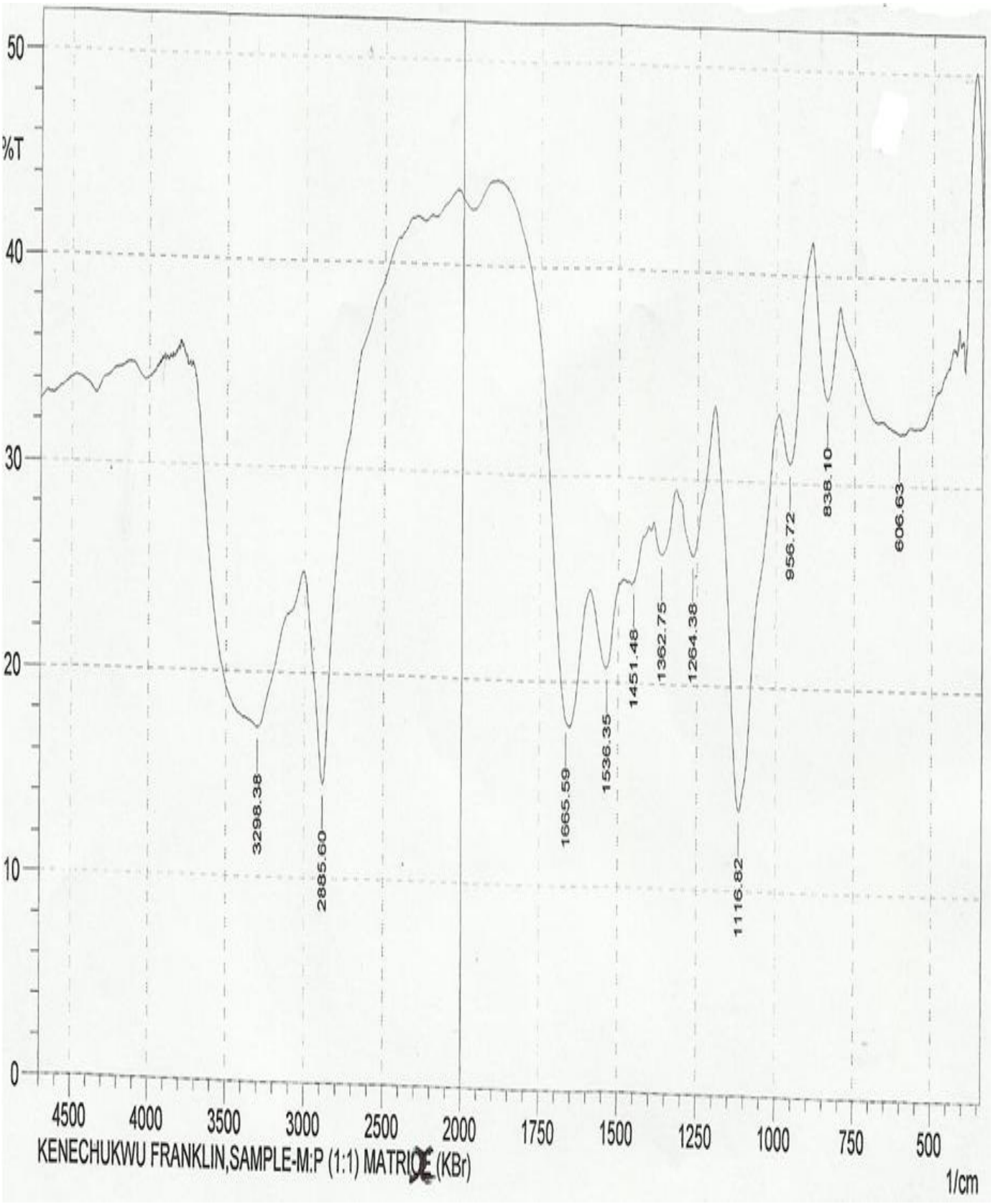

Figure 3. FT-IR spectrum of polymer matrix (1:1).

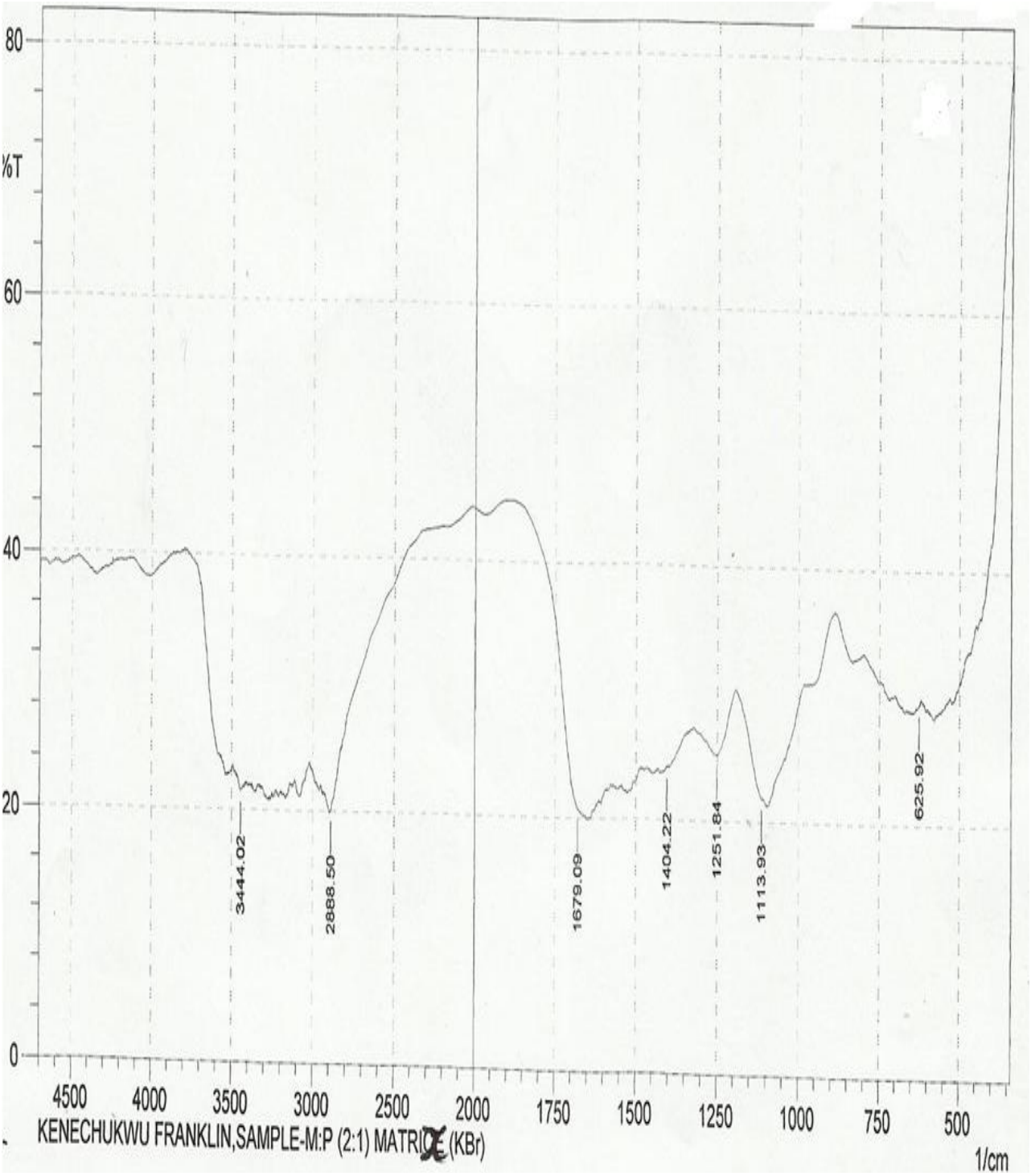

Figure 4. FT-IR spectrum of polymer matrix (2:1).

and 3197.12 cm^{-1} corresponding to aromatic C-H deformation, C-N vibrations, C-O stretching, C=H deformation (CH_3, CH_2), aromatic C=C (conjugated ring), C-H stretching and N-H amide bending, respectively. FT-IR spectra of 2:1 and 3:1 matrices (Figures 4 and 5) showed decrease in the number of peaks due to overlapping of peaks corresponding to 2:0 (mucin) and 0:2 (PEG 4000) matrices. The characteristic differences between the spectra of PEGylated mucin matrices (1:1, 2:1 and 3:1) on one hand and between the spectra of PEGylated

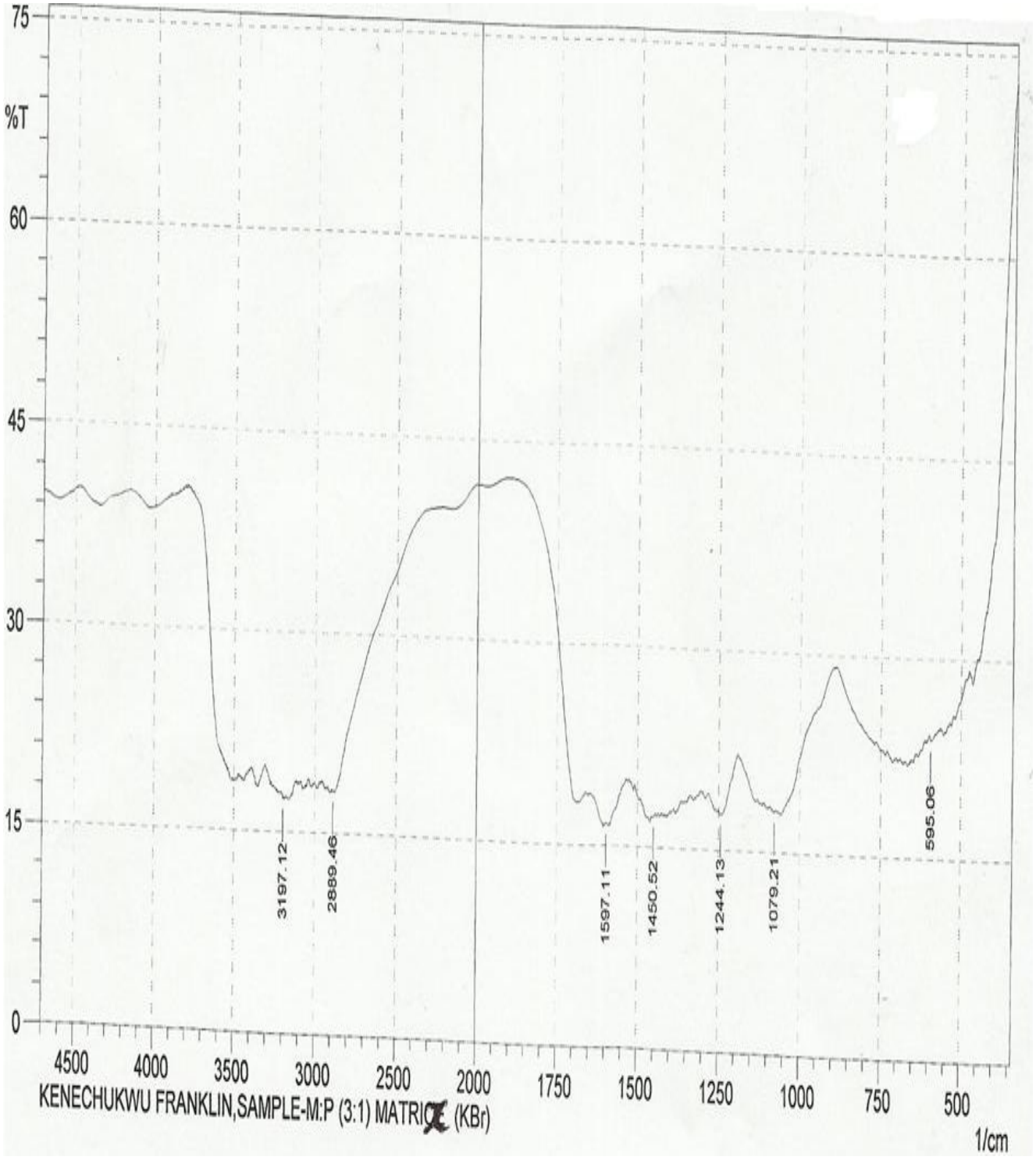

Figure 5. FT-IR spectrum of polymer matrix (3:1).

mucin matrices and those of non-PEGylated mucin (2:0) and non-mucinated PEG (0:2) matrices on the other hand further indicate that new polymeric materials were formed, consistent with a similar report on interaction studies of mucinated cellulose based on FT-IR by Builders et al. (2008).

Although PEGyalted mucin matrices (prepared with PEG 2000 and snail mucin) (Momoh et al., 2010; Momoh et al.,

2011; Momoh et al., 2010) had been characterized with respect to thermal properties by differential scanning calorimetry (DSC) (Momoh et al., 2010), this serves as a basis for further characterization of the interaction between PEG and mucin in PEGylated mucin matrices for drug delivery application. FT-IR spectroscopic analysis can be used to determine the functional groups of the molecules (Sahoo et al., 2012; Sahoo et al., 2012; Sahoo et al., 2011). Therefore, to obtain more information in detail about chemical interaction between PEG and mucin, the FT-IR analysis was carried out on PEGylated mucin matrices prepared with PEG 4000 and snail mucin. The presence of different peaks in the FT-IR spectra of the PEGylated mucin matrices compared with the non-PEGylated mucin (2:0) matrix and non-mucinated PEG 4000 (0:2) matrix indicated the formation of new polymers, which could be employed in drug delivery. Results obtained in this study is in agreement with earlier reports on the drug delivery potential of PEGylated mucin (Momoh et al., 2010). The novelty embodied in this research is that this is the first spectroscopic study to be carried out on PEGylated mucin using PEG 4000 and snail mucin.

Conclusion

The engineering of new polymer biomaterials could be employed to address some problems associated with delivery of some APIs and biomolecules. To obtain new polymer biomaterials that will meet the carrier needs of challenging drug molecules, blending of polymers with desirable properties becomes imperative. In this study, admixtures of PEG 4000 and mucin obtained from African land snail (*A. marginata*) were prepared by combining the two at different ratios. Preliminary spectroscopic characterization was performed on the PEGylated mucin matrices using fourier transform infra-red spectroscopic characterization. Results obtained indicated that a new polymeric carrier was made from admixtures of mucin and PEG 4000 by PEGylation technology. Further solid state spectroscopic charcterization (WAXD and SAXD) studies on PEGylated mucin are currently ongoing in our laboratory.

ACKNOWLEDGEMENTS

This work is a product of research project for the award of M. Pharm. by the Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria. We wish to thank the National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State, Nigeria, for assistance in FT-IR analyses, as well as Ph. Eur. Carl Roth GmbH + Co.KG Karlsruhhe Germany for provision of PEG 4000 used in the study.

REFERENCES

Abuchowski D, McCoy S, Palczuk C, van Es A, Davis O (1977). Effect of covalent attachment of polyethylene glycol on immunogenicity and circulation life of bovine liver catalase. J. Biol. Chem. 252(11): 3582-3586.

Adikwu MU, Ikejiuba CC (2005). Some physiological and wound healing properties of snail mucin. Boll. Chim. Farm. 144: 1-8.

Adikwu MU, Nnamani PO (2005). Some physiological and toxicological properties of snail mucin. Bioresearch 5: 1-6.

Attama AA, Nwabunze OJ (2007). Mucuna gum microspheres for oral delivery of glibenclamide: *In vitro* evaluation. Acta Pharma 57: 161 - 171.

Bernkop-Schnurch A, Hornof M, Guggi D (2004). Thiolated chitosans. Eur. J. Pharm. Biopharm. 57: 9-17.

Builders PF, Kunle OO, Adikwu MU (2008). Preparation and characterization of mucinated agarose: a mucin-agarose physical crosslink. Int. J. Pharm. 356: 174–180.

Carlstedt I, Lindgren H, Sheehan JK (1983). The macromolecular structure of human cervical-mucus glycoproteins: Studies on fragments obtained after reduction of disulphide bridges and after subsequent trypsin digestion. Biochem. J. 213 (2): 472-435.

Delie F, Blanco-Prieto MJ (2005). Polymeric particulates to improve oral bioavailability of peptide drugs. Molecules 10: 65-80.

Durrer C, Irache JM, Duchene D, Ponchel G (1995). Mucin interactions with functionalized polystyrene latexes. J. Colloid Interf. Sci. 170 (2): 555-561.

Francis MF, Cristea M, Winnik FM (2004). Polymeric micelles for oral drug delivery: Why and how? Pure Appl. Chem. 76(7-8): 1321-1335.

Gillies ER, Frechet JMJ (2004). Development of acid-sensitive copolymer micelles for drug delivery. Pure Appl. Chem. 76(7-8): 1295-1307.

Kodera R, Matsushima S, Hiroto E, Nishimura D, Inada P (1998). PEGylation of proteins and bioactive substances for medical and technical applications. Progress Polym. Sci. 23(7): 1233-1271.

Kreuter J (1991). Peroral administration of nanoparticles. Adv. Drug Deliv. Rev. 7: 71- 86.

Lagarce F, Renaud P, Faisant N, Nicolas G, Cailleux A, Richard J, Menei P, Benoit J-P (2005). Baclofen-loaded microspheres: preparation and efficacy testing in a new rabbit model. Eur. J. Pharm. Biopharm. 59: 449-459.

Lee S, Muller M, Rezman K, Spencer ND (2005). Porcine gastric mucin (PGM) at the water/poly (dimethylsiloxane) (PDMS) interface: Influence of pH and ionic strength on its conformation, adsorption, and aqueous lubrication properties. Langmuir 21(18): 8344-8353.

Momoh MA (2010). Formulation and evaluation of metformin and aqueous leaf extract of *Vernonia amygdalina* loaded polyethylene glycol-mucin particulate drug delivery systems: Unpublished Ph.D Thesis, Department of Pharmaceutics, University of Nigeria, Nsukka.

Momoh MA, Adikwu MU, Ibezim CE, Ofokansi KC, Attama AA (2010). Thermal characterization of PEGylated mucin. Asian Pacif. J. Trop. Med. 458-460.

Momoh MA, Adikwu MU, Ibezim EC (2010). Formulation and evaluation of the bioadhesive drug delivery system based on PEGylated mucin matrices. Asian Pacif. J. Trop. Med. 461 – 464.

Momoh MA, Adikwu MU, Ibezim EC, Attama AA (2011). Effect of metformin and *Vernonia amygdalina* leaf extract loaded PEGylated-mucin formulation on haematological, kidney and liver indices of healthy and Diabetes rats. J. Pharm. Res. 4(10): 3455-3459.

Muller RH, Mader K, Gohla S (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art. Eur. J. Pharm. Biopharm. 50: 161-177.

Pamujula S, Graves RA, Kishore V, Mandal TK (2004). Preparation and *in vitro* characterization of amifostine biodegradable microcapsules. Eur. J. Pharm. Biopharm. 57: 213-218.

Perez E, Proust JE (1987). Forces between Mica surfaces covered with adsorbed mucin across aqueous solution. J. Colloid Interf. Sci. 118: 182-191.

Ravi-Kumar MNV (2000). Nano and microparticles as controlled drug delivery devices. J. Pharm. Pharm. Sci. 3: 234-258.

Raynal BDE, Hardingham TE, Sheehan JK, Thornton DJ (2003). Calcium-dependent protein interactions in MUC5B provide reversible cross-links in salivary mucus. J. Biol. Chem. 278 (31): 28703-28710.

Sahoo S, Chakraborti CK, Behera PK (2012). FTIR and Raman spectroscopic investigations of a controlled release Ciprofloxacin / Carbopol940 mucoadhesive suspension. Asian J. Pharm. Clin. Res. 5(1): 125-130.

Sahoo S, Chakraborti CK, Behera PK (2012). FTIR and Raman spectroscopic investigations of Ofloxacin / Carbopol940 mucoadhesive suspension. Int. J. PharmTech. Res. 4(1):382-391.

Sahoo S, Chakraborti CK, Mishra SC, Naik S (2011). Analytical characterization of a gelling biodegradable polymer. Drug Invention Today 3(6): 78- 82.

Schnurrer J, Lehr CM (1996). Mucoadhesive properties of the mussel adhesive protein. Int. J. Pharm. 141: 251-256.