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Development and validation of an infrared spectroscopy-based method for the analysis of moisture content in 5-fluorouracil

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The determination of moisture content in pharmaceuticals is very important as moisture is mainly responsible for the degradation of drugs. Degraded drugs have reduced efficacy and could be hazardous. The objective of the present work is to replace the Karl Fischer (KF) titration method used for moisture analysis with a method that is rapid, involves no toxic materials and is more effective. Diffuse reflectance infrared (IR) spectroscopy, which is explored as a potential alternative to various approaches, is investigated for moisture analysis in 5-fluorouracil, an anticancer drug. A total of 150 samples with varying moisture content were prepared in laboratory by exposing the drug at different relative humidities, for different time intervals. Infrared spectra of these samples were collected with a Fourier transform infrared (FTIR) spectrophotometer using a diffuse reflectance accessory. Reference moisture values were obtained using the Karl Fischer titration method. A number of calibration models were developed using the partial least squares (PLS) regression method. A good correlation was obtained between predicted IR values and reference values in the calibration and validation set. The derived calibration curve was used to predict moisture content in unknown samples. The results show that IR spectroscopy can be used successfully for the determination of moisture content in the pharmaceutical industry.

Keywords: diffuse reflectance; 5-fluorouracil; infrared spectroscopy; moisture content

Introduction

5-Fluorouracil (Figure 1) is one of the oldest antitumour drugs, which has been used for gastrointestinal, pancreatic, colon, liver, stomach and colorectal cancer for several decades.[1–6] It is a pyrimidine analogue, which belongs to the family of drugs called antimetabolites.[7] The drug effectively blocks the replication of DNA viruses.[8,9] Determination of moisture content in pharmaceuticals is an important part of the manufacturing and storage process, as some compounds may degrade rapidly under high or low moisture conditions. In some studies it was reported that moisture can diminish the efficacy of drug compounds and in some cases it is responsible for the formation of degradation products.[10] Degraded drugs not only have reduced efficacy but can be hazardous. Moisture content in pharmaceuticals is conventionally determined by the thermogravimetric method, Karl Fischer (KF) titration[11,12] and gas chromatography.[13] Methods based upon gas chromatographic analysis are most sensitive, however they are invasive. Karl Fischer titration is very precise and is the most common method for determining moisture content, but the method is time consuming, requires hazardous reagents and is destructive. An ideal method for the determination of moisture content for a routine manufacturing schedule should be noninvasive, nondestructve and rapid. Infrared spectroscopy meets these requirements and hence is superior to conventional techniques.

Infrared spectroscopy has been used by several workers for different applications, from identification of raw materials[14,15] to process monitoring.[16] Moisture content has been determined in different pharmaceutics using near-IR spectroscopy.[17–25] We have previously determined moisture content in aspirin formulation by partial-least-squares regression and near-infrared (NIR) diffuse reflectance spectroscopy.[26] Mid-infrared (MIR) spectroscopy has been used for the quantitative determination of various components in pharmaceuticals.[27,28]. Quantitative analysis of a mixture of polymorphs was performed by diffuse reflectance IR spectroscopy.[29] Determination of active components in a pharmaceutical formulation using Fourier transform IR-attenuated total reflectance spectroscopy has been reported.[30]

In the present work we report the feasibility of a diffuse reflectance IR spectroscopic technique for the accurate and fast determination of moisture content in 5-fluorouracil. Determination of moisture content in 5-fluorouracil is important as the drug may become degraded in the presence of moisture and light.[31] Mid-IR spectroscopy was chosen because of the strength of the absorption coefficients for the fundamental vibrations.[32] In this spectral region organic functional groups have characteristic and well delineated absorption bands. Moreover calibration data in the MIR are much more generic than in the NIR. The nondestructive and noninvasive IR spectroscopy-based method may alleviate the need for sample preparation and use of hazardous solvents necessary in the Karl Fischer method that is currently used.

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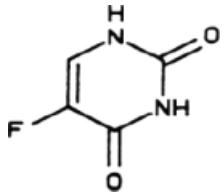

Figure 1. Chemical structure of 5-fluorouracil.

Table 1. Sample prepared at different relative humidity

Relative humidity (RH %)	Number of samples
20–30	25
30–40	30
40–50	25
50–60	35
60–70	35

Experimental

Materials

The 5-fluorouracil used in the study was procured from HiMedia Laboratories Pvt Ltd, Mumbai, India. Specially dried methanol for KF titration was procured from Qualigens Fine Chemicals. Pyridine-free 526 KF reagent obtained from Qualigens fine Chemicals, India, was used for KF titration. Potassium bromide (KBr) used was of spectroscopy grade, procured from BDH Laboratory Suppliers, England.

Sample preparation

A total of 150 samples of different moisture content were prepared by exposing the samples to different relative humidity for different timings. Details of sample preparation are given in Table 1. Samples were well homogenized before the experiments. Each sample was divided into two parts. One part was used for the IR spectroscopic measurement and the other part for the Karl Fischer titration experiment. Both the experiments were carried out simultaneously to minimize the experimental error.

Infrared spectroscopic measurements

Infrared spectra of the samples were collected in diffuse reflectance mode with a Bio Rad 175 C Fourier transform IR spectrophotometer. The sample mixtures were prepared by homogenous mixing of the drug powder with spectroscopy grade potassium bromide (KBr). Samples were then placed in a small sample cup and kept in the sample holder. Spectra with a resolution of 4 cm^{-1} were obtained by averaging 64 scans for each spectrum. Background spectra were obtained with KBr powder for each sample. Unscrambler 9.1 software was used for data processing and developing a calibration model.

Karl Fischer titration measurements

Reference moisture analysis was carried out by Karl Fischer titration using LABINDIA automatic KF titrator, equipped with magnetic stirrer and double pin platinum electrode. For the quantification of moisture content by Karl Fischer titration, about 20 ml of dry methanol was kept in the titration vessel and neutralized with KF reagent until the end point was displayed on the screen of KF titrator. An accurately weighed amount of 5-fluorouracil (about 300 mg) was added to the neutralized methanol and titrated with KF reagent till the end point was reached. The end point was determined by polarized electrode. The percentage (w/w) moisture content of the sample was automatically displayed by the KF titrator. Different dissolution times were tried during KF titration measurements in order to optimize the results. Finally a stirring time of 4 minutes was selected to dissolve entrapped water present in the sample in the titrator. Solvent was replaced before every experiment. Random analysis was repeated for the same sample to cross-check the results.

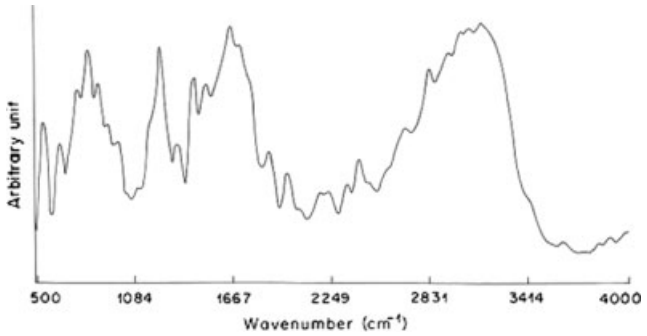

Figure 2. Infrared spectrum of 5-fluorouracil in the region 500–4000 cm^{-1}.

Results and Discussion

Figure 2 shows the diffuse reflectance IR spectra of 5-fluorouracil in the region of 500–4000 cm^{-1}. The medium-intensity absorption band in the region 3100–3000 cm^{-1} may be attributed to =C–H stretching. The medium intensity bands in the region 3450–3300 cm^{-1} may be due to the –OH stretching mode of the water molecules. The band at about 1650 cm^{-1} may be assigned to the bending vibration of water present in the sample.[33] The absorption bands at about 2938 and 2831 cm^{-1} correspond to $-CH_2$. Absorptions observed in the region 1580–1600 cm^{-1} may be due to C=N and C=C ring stretching vibrations. The absorption band at about 1724 cm^{-1} is due to the stretching frequency of C=O group. The bands at about 1450 and 1350 cm^{-1} are vibrations of the substituted pyrimidine compounds.[33] The absorption band at 1180 and 1251 cm^{-1} may be assigned to the vibration of C–O and C–N respectively. The absorption band at about 1230 cm^{-1} may be due to the fluorine atom on the ring. The absorption bands are also observed in the 820–550 cm^{-1} region, which may be attributed to the C–F deformations.[33] The characteristic absorption bands of 5-fluorouracil are also shown in Table 2. The overlaid IR spectra of 5-fluorouracil with different moisture content are shown in Figure 3. There are significant changes in the spectra of the samples. The absorbance in the region 3450–3300 cm^{-1} (bands due to –OH stretching) and at 1650 cm^{-1} (bending vibration) increases as moisture content increases, which shows that there is a quantitative relationship between absorbance and moisture level.

Calibration was carried out by the partial least square (PLS) regression method. This is a full-spectrum method that extracts principal components from whole wavelength regions and correlates the spectral data in these regions with the concentration

Table 2. Characteristic absorption bands of 5-fluorouracil

Absorption Bands (cm^{-1})	Assignments
3100-3000	=CH Stretching
3450-3300	water content (-OH stretching mode)
2938-2831	$-CH_2$
1724	C=O stretching
1650	In plane bending mode of water
1580-1600	C=N and C=C ring stretching vibrations in pyrimidines
1180 and 1251	Vibrations of C-O and C-N respectively
820-550	C-F Deformations
1450-1350	Vibrations of substituted pyrimidine compounds

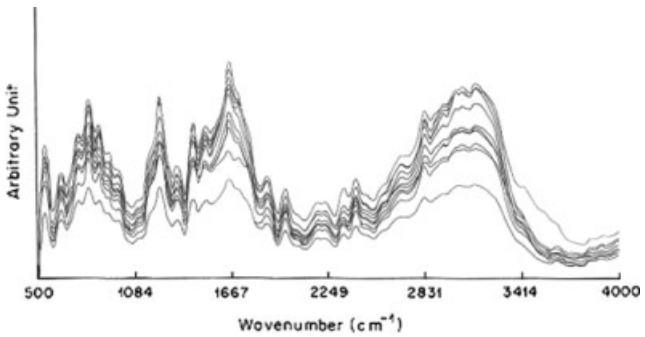

Figure 3. Overlaid infrared spectra of 5-fluorouracil with different moisture content in the region 500–4000 cm^{-1}.

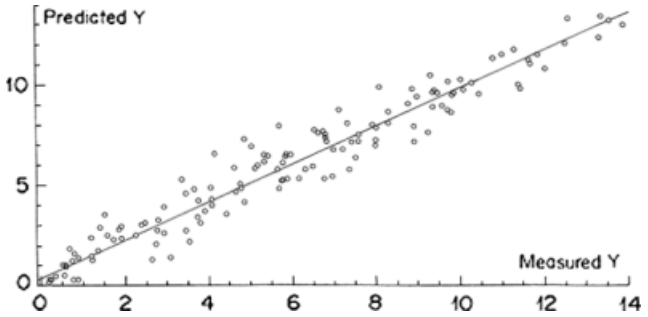

Figure 4. Actual (KF) versus predicted (IR) values of moisture content in calibration set.

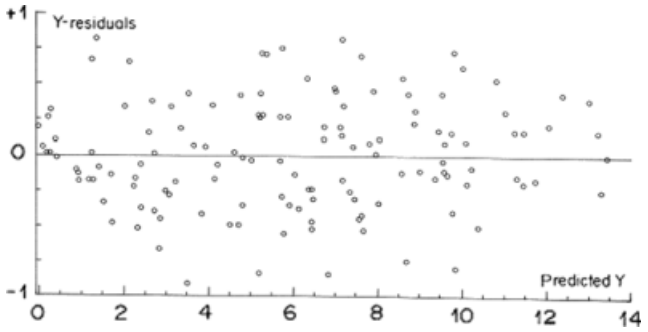

Figure 5. Concentration residual plot for moisture content.

of the constituent of interest. It is a factor-based analysis during which the variables of the original data matrix are reconstructed with new variables. Partial least square models give more accurate predictions due to their ability to resolve interfering and overlapping peaks of complex matrices.[34] Several calibration models were developed in different wavelength regions to determine the wavelength region in which spectral data are best correlated with moisture contents of the samples. The PLS factors were determined for each combination of parameters. The calibration models were developed using KF values as references for all samples. The optimum number of PLS factors is calculated on the basis of the minimum root mean square error of prediction (RMSEP). To improve the precision and accuracy of the calibration model, outliers, if found, were discarded and the calibration was repeated. Outliers may be attributed to errors in reference and spectra analysis or due to sample contamination. The statistics of different calibrations is shown in Table 3. The best calibration model was selected on the basis of maximum R^2 and minimum RMSEC. It is evident from Table 3 that the best calibration model is obtained in the wavelength region 1550–1780 cm^{-1}. The model has Rc^2 0.9999, Rp^2 0.9998 and RMSEC 0.0111 with no outliers.

Validation was performed by a cross-validation process that leaves one sample of the calibration set at a time for prediction and predicts its value on the basis of a model of all other samples. Figure 4 shows the actual (KF) versus the predicted (IR) values of the calibration set. The effectiveness of the calibration may be observed from the closeness of the data points to linear fit. Actual (IR) values of moisture content are very close to the predicted (KF) values in the calibration set. It is also evident from the calibration statistics given in Table 3. The concentration residual moisture values are shown in Figure 5. Concentration residual gives a clear picture of the calibration. It explains the range of variation that the predicted concentration values exhibit for those samples present in calibration set. It is evident from Figure 5 that most of the moisture residual values lie in the range of −1 to +1.

Table 3. Calibration statistics for moisture in 5-fluorouracil

Wavelength range (cm^{-1})	No. of spectra	Factor	R_C^2	R_p^2	RMSEC	RMSEP	No. of outliers
500–4000	150	2	0.9379	0.8028	0.4877	0.5081	5
	145	2	0.9420	0.8287	0.3849	0.4981	–
1550–1780	150	2	0.9881	0.9721	0.1463	0.1952	2
	148	1	0.9999	0.9998	0.0111	0.1434	–
2550–3450	150	3	0.9815	0.9713	0.1312	0.2113	3
	147	3	0.9997	0.9301	0.0888	0.1625	–

Table 4. Actual (KF) versus predicted (IR) values of percentage of moisture in prediction set

Sample name	KF values	IR values	Bias
Sample1	0.227	0.241	0.014
Sample2	2.561	3.027	0.466
Sample3	3.138	3.078	−0.060
Sample4	3.684	3.396	−0.288
Sample5	3.167	2.764	−0.403
Sample6	5.954	6.132	0.178
Sample 7	6.157	6.211	0.054
Sample 8	7.224	7.294	0.070

A separate set of eight samples was employed for prediction. Spectral measurements were made on these samples and their moisture values were determined using the developed calibration model. Karl Fischer titration was also performed for the same sample set to determine the moisture present. The predicted IR values were compared with the KF titration values of these samples. Table 4 shows the comparison between IR predicted values and KF titration values. A good correlation was obtained between the two values. The IR spectroscopic and KF titration results were very close, which indicates the accuracy of spectroscopic techniques.

Conclusions

It is evident from the results that IR spectroscopy can be used as a noninvasive, nondestructive and rapid technique for the determination of moisture content in pharmaceuticals. The work reported in the paper determined moisture content in 5-fluorouracil using diffuse reflectance mid-IR spectroscopy. A successful calibration model was developed with 150 samples of 5-fluorouracil with different moisture content using the partial least squares (PLS) regression method. The calibration was carried out in different wavelength regions. The model developed in the region 1550–1780 cm^{-1} was found to be the best with the correlation coefficient Rc^2 0.9999 and RMSEC 0.0111. Eight samples of unknown moisture values were analysed using IR analysis to predict the moisture content. The results of IR spectroscopy and KF titration method are quite comparable. A similar approach may also be employed for other drug substances. The calibration developed may be transferred to another instrument of same make for the same compound; however, different calibration has to be developed for different compounds for better accuracy as there may be slight shifting in the water absorption bands due to the chemical environment and complex matrix. It is advantageous towards real-time process monitoring to apply IR spectroscopic technique for the determination of moisture content in pharmaceutical industries. However, the technique has a few limitations. The main disadvantage is that the IR technique is indirect and is based on reference values that may be subject to errors. Moreover new calibration has to be constructed for each pharmaceutical formulation.

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References

[1] R. Labianca, M. A. Pessi, G. Zamparelli, *Drugs* **1997**, *53*, 593.
[2] L. J. Schaaf, B. R. Dobbs, I. R. Edwards, D. G. Perrier, *Eur. J. Clin. Pharmacol.* **1987**, *32*, 411.
[3] W. D. Ensminger, A. Rosowsky, V. Raso, D. C. Levin, M. Glode, S. Come, G. Steele, E. Frie, III, *Cancer Res.* **1978**, *38*, 3784.
[4] J. L. Grem, D. F. Hoth, J. M. Hamilton, S. A. King, J. B. Leyland, *Cancer Treat. Rep.* **1987**, *71*, 1249.
[5] H. J. Schmoll, T. Buchele, A. Grothey, W. Dempke, *Semin. Oncol.* **1999**, *26*, 589.
[6] J. L. Abbruzzese, B. Levin, *Hematol. Oncol. Clin. North America* **1989**, *3*, 135.
[7] N. Wolmark, B. Fischer, H. Rockette, C. Redmond, D. L. Wickerham, E. R. Fisher, J. Jones, A. Glass, H. Lerner, W. Lawrence, D. Preger, M. Wexler, J. Evans, A. Cruz, N. Dimitrov, P. Jochimsen, *J. Natl. Cancer Inst.* **1988**, *80*, 30.
[8] B. G. Katsung (Ed.), *Basic and Clinical Pharmacology,* 4th edn, Appleton & Lange: Lebanon, **1989**.
[9] S. A. Schroeder, M. A. Krupp, L. M. Tierney Jr., in *Current Medical Diagnosis and Treatment* (Ed.: S. J. Mc Phee), Appleton & Lange: London, **1990**, 22, 92.
[10] E. W. Ciurczak, *Pharm. Tech.* **1998**, 92.
[11] M. S. Kamat, R. Osawa, R. J. De Angelis, Y. Koyama, P. P. DeLuca, *Pharm. Res.* **1988**, *5*, 426.
[12] E. S. Theimer, J. J. Pavelek, *J. Pharm. Sci.* **1968**, *57*, 887.
[13] R. C. Huber, A. O. Niedermayer, A. L. Weiss, *J. Pharm. Sci.* **1978**, *67*, 1239.
[14] Y. Roggo, C. Roeseler, M. Ulmschneider, *J. Pharm. Biomed. Anal.* **2004**, *36*, 777.
[15] W. L. Yoon, R. D. Jee, A. C. Moffat, *Analyst* **2000**, *125*, 1817.
[16] O. R. Dumitrescu, D. C. Baker, G. M. Foster and K. E. Evans, *Polymer Testing* **2005**, *24*, 367.
[17] R. A. Mattes, R. Schroeder, V. Dhopeshwarker, R. Kowaland, W. Randolph, *Pharm. Tech. Europe* **2005**, February, 41.
[18] A. Dunko, A. Dovletoglou, *J. Pharm. Biomed. Anal.* **2002,**, *28*, 145.
[19] G. X. Zhou, Zhihong Ge, J. Dorwart, B. Izzo, J. Kukara, G. Bicker, J. Wyvratt, *J. Pharm. Sci.* **1997**, *92*, 1058.
[20] P. Frake, D. Greenhalgh, S. M. Grierson, J. M. Hempenstall, D. R. Rudd, *Int. J. Pharm.* **1997**, *151*, 75.
[21] R. A. Mattes, D. E. Root, A. P. Birkmire, *The Role of Spectroscopy in Process Analytical Technologies* **2005**, January, 14.
[22] A. Dunko, A., Dovletoglou, *J. Pharm. Biomed. Anal.* **2002**, *28*, 145.
[23] M. S. Kamat, R. A. Lodder, Patrick P. De Luca, *Pharm. Res.* **1989**, *6*, 961.
[24] X. Zhou, P. Hines, M. W. Borer, *J. Pharm. Biomed. Anal.* **1998**, *17*, 219.
[25] R. M. Leasure, M. K. Gangwer, *Am. Pharm. Rev.* **2003**, *5*, 103.
[26] P. Singh, R. Mehrotra, H. C. Kandpal, *Int. J. Appl. Chem.* **2006**, *2*, 137.
[27] S. M. Patrick, S. A. Arnold, B. McCarthy, L. M. Harvey, B. McNeil, *Biotech. Lett.* **2003**, *25*, 257.
[28] K. Kargosha, M. Khanmohammadi, M. Sarokhani, F. Ansari, M. Ghadiri, *J. Pharm. Biomed. Anal.* **2003**, *31*, 571.
[29] K. J. Hartauer, E. S. Miller, J. K. Guillory, *Int. J. Pharm.* **1992**, *85*, 163.
[30] K. J. Hartauer, J. K. Guillory, *Pharm. Res.* **1989**, *6*, 608.
[31] M. Allwood, A. Stanley, P. Wright, *The Cytotoxic Handbook,* Radcliffe Publishing: Abingdon, **1993**.
[32] E. W. Ciurczak, *Spectroscopy* **2004**, *19*(2), 60.
[33] G. Socrates, *Infrared Characteristics Group Frequencies,* 2nd edn, John Wiley & Sons, Ltd: Chichester, **1994**.
[34] K. R. Beebe, B. R. Kowalski, *Anal. Chem.* **1987**, *59*, 1007 A.