Research Article

Received: 20 March 2014 Revised: 30 April 2014 Accepted article published: 7 May 2014 Published online in Wiley Online Library: 17 June 2014

(wileyonlinelibrary.com) DOI 10.1002/jctb.4420

UV resonance Raman spectroscopy: a process analytical tool for host cell DNA and RNA dynamics in mammalian cell lines

Lorna Ashton,[a*] Catherine E.M. Hogwood,[b] Andrew S. Tait,[c] Julia Kuligowski,[d§] C. Mark Smales,[b] Daniel G. Bracewell,[c] Alan J. Dickson[e] and Royston Goodacre[a]

Abstract

BACKGROUND: Recent advances in Raman spectroscopy have resulted in the development of rapid, *in situ* Raman probes that can identify and allow the assessment of the quality of complex constituents in mammalian cell culture. One specific Raman technique, UV resonance Raman (UVRR) spectroscopy, has potential as a probe for residual cellular DNA and RNA in mammalian cell culture medium.

RESULTS: Variations in DNA and RNA UVRR spectral profiles of medium-cellular footprint samples were identified and related to time of harvest and increased cell lysis that is associated with a loss in cell viability. Increased DNA and RNA were also observed in the cell culture supernatant in response to sodium butyrate treatment. Variation in DNA and RNA profiles as a result of both primary and secondary clarification methods during downstream bioprocessing could also be determined with UVRR spectroscopy.

CONCLUSIONS: This study has demonstrated the utility of UVRR spectroscopy as a tool to monitor variations in residual DNA and RNA that may contaminate cell culture medium. Application of this technique has the potential for both improvement of recovery techniques and assurance of reliable clearance of DNA/RNA to acceptable safety levels.

Keywords: UV resonance Raman; mammalian cell culture; cellular DNA; sodium butyrate; clarification

INTRODUCTION

It is widely accepted that to meet the ever-increasing demand for biopharmaceuticals produced from cell expression systems, it will be essential to improve product yield and quality control. This will require the development of reliable and cost-effective process analytical techniques that can be used to determine and monitor critical quality attributes of such molecules during their manufacture.[1–3] Non-destructive techniques would be ideal, allowing application in real time, and it would be valuable if the techniques developed provided information on multiple aspects of process performance. Raman spectroscopy fits these criteria. This approaches utilises the interaction of light with molecules to measure functional group vibrations to provide both quantitative and qualitative information. The chemical and structural sensitivity of Raman spectra enables multiple analytes to be measured simultaneously and, therefore, a wide range of components in cell cultures can be detected and monitored. Further, advances in Raman instrumentation have resulted in the development of portable Raman probes that meet the criteria of non-destructive, rapid, *in situ* techniques and such instruments have successfully been used to identify and quality assess complex constituents in mammalian cells.[4,5]

We have previously shown how one specific Raman technique, UV resonance Raman (UVRR) spectroscopy, can be used to monitor multiple components in mammalian cell cultures, including recombinant protein titre.[6] Measuring Raman spectra with UV lasers (excitation wavelength 180–260 nm) has the advantage of no fluorescent background and creating a resonance Raman effect, enhancing the signal by a factor of 10^3–10^5. In resonance Raman spectroscopy, if the energy of the incident laser is

* *Correspondence to: Lorna Ashton, School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, Manchester M1 7DN, UK. E-mail: Lorna.Ashton@manchester.ac.uk*

§ *Current Address:* Neonatal Research Group, Health Research Institute Hospital La Fe, Bulevar Sur s/n, 46026 Valencia, Spain

a *School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, Manchester M1 7DN, UK*

b *Center for Molecular Processing and School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK*

c *Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK*

d *Department of Analytical Chemistry, University of Valencia, Edificio Jerónimo Munoz, Dr. Moliner 50, 46100 Burjassot, Spain*

e *Faculty of Life Sciences, Michael Smith Building, University of Manchester, Manchester M13 9PT, UK*

within the molecular absorption bands of chromophores or aromatics in the molecules then the signal from these molecules is enhanced. This effect gives UVRR the advantage of selectivity; by choosing an appropriate UV wavelength individual species in complex samples can be selectively enhanced. Although not yet available as a Raman probe, using a 244 nm excitation source, UVRR has the advantage of being extremely sensitive for detection of aromatic amino acids and nucleic acid components without the interference from background fluorescence which can often mask important spectral features. One exciting consequence of this specific sensitivity is the potential of UVRR spectroscopy to monitor residual DNA and RNA directly during biopharmaceutical manufacture.

The removal of product and process related impurities from the target molecule and to define the reproducible clearance of these (chief among which are host cell proteins (HCPs) and nucleic acids) is a major goal of downstream processes. The measurement of HCPs during the production of therapeutic recombinant proteins is a complex issue due to the number of protein species present.[7,8] The standard approach is to use ELISA technology to monitor HCPs while more recently proteomic-based approaches have provided information on proteins present in the cell culture supernatants and lysates.[7,8] A more recent study of HCP dynamics in monoclonal antibody-producing Chinese hamster ovary (CHO) cell lines, using 2D-PAGE and LC-MS, demonstrated that the majority of HCPs in the medium arose from cell lysis or breakage associated with loss of viability.[9,10] Virtually nothing has been reported on the presence of DNA and RNA across a whole process in medium during culture. However, the presence of both HCPs and DNA can produce an impurity burden with the potential to induce adverse short term and long term clinical effects.[11,12] Acceptable amounts are reported as <1–100 ppm for HCPs and <10 ng per dose for DNA and vast resources have been invested in processes for clearance from culture harvests and for robust confirmation of less than acceptable amounts in the final product/drug substance.[11–13]

Here we have utilized the sensitivity of UVRR spectroscopy to nucleic acids to assess the presence of DNA and RNA in mammalian cell culture medium. In order to establish the robustness of this technique we have compared medium samples of three CHO cell lines harvested throughout batch culture, including cell lines where cell proliferation was inhibited by addition of sodium butyrate. Sodium butyrate has been reported to increase specific recombinant protein production in a variety of mammalian cell lines but has a simultaneous detrimental effect on cell growth and viability[14–16] which will affect the levels of DNA and RNA within the medium. To demonstrate further the potential of UVRR spectroscopy as a process analytical technique for both upstream and downstream processes we have also compared medium samples after the application of various primary and secondary clarification techniques.

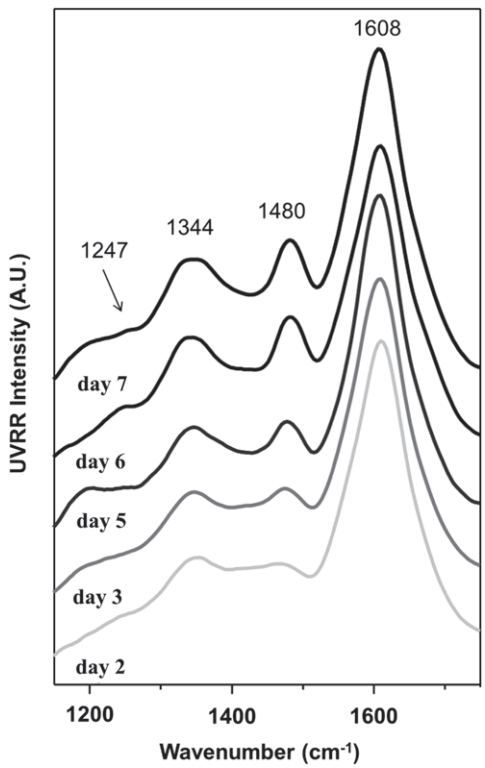

Figure 1. Averaged UVRR spectra from monitoring IgG4 fermentation in LB01 cell line. Medium samples were harvested on day 2, day 3, day 5, day 6 and day 7. Six spectra were acquired for each day and averaged, with the exception of day 5 spectrum which is the average of three repeat spectra.

MATERIALS AND METHODS

Cell lines

Experiments were carried out using Chinese hamster ovary (CHO) cell lines stably expressing an IgG4 monoclonal antibody (LB01 and CY01) and a CHO cell line transfected with a blank glutamine

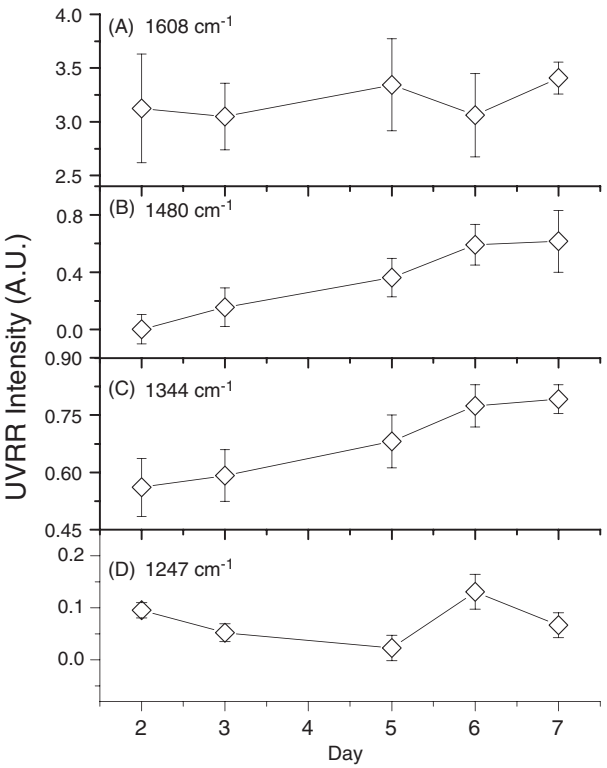

Figure 2. UVRR spectral intensity profiles of IgG4 fermentation in LB01 cell line as a function of days during cultivation. The difference in maximum peak height between day 2 and days 3–7, at (A) 1608 cm^{-1} measured from 1655 to 1608 cm^{-1}, (B) 1480 cm^{-1}, measured from 1520 to 1480 cm^{-1}, (C) 1344 cm^{-1}, intensity measured from 1262 to 1344 cm^{-1} and (D) 1247 cm^{-1}, measured from 1228 to 1247 cm^{-1}. Points are the average of six measurements (except Day 5 which is the average of three measurements) and error bars represent standard deviations.

synthetase plasmid (Null8), generously provided by Lonza Biologics (Slough, UK). All cells were grown in shake flasks using chemically defined medium (CD CHO medium, Invitrogen, Paisley, UK) and supplemented with 25 μmol L^{-1} methionine sulfoximine (Sigma-Aldrich, Gillingham, UK).

Cell culture: LB01

LB01 cell lines were sub-cultured every 3–4 d with a seeding density of 0.2×10^6 cells mL^{-1}. The cells were grown in 250 mL Erlenmeyer flasks in a volume of 50 mL medium. All cultures were grown at 37°C with 100 rpm shaking. When present, sodium butyrate was added to cells on day 3, (at a final concentration of 1 or 2 mmol L^{-1}) and controls were incubated simultaneously without sodium butyrate. Growth was assessed by light microscopy using an improved Neubauer haemocytometer at 24 h intervals. Samples were appropriately diluted and mixed 1:1 with 0.5% Trypan Blue in PBS. Samples of medium were collected for UVRR spectroscopy. Cells were removed by centrifugation and the supernatant was kept at −20°C until required for UVRR spectroscopy.

Cell culture: CY01 and Null8

CY01 and Null8 cell lines were cultured in parallel in two bioreactors with working volumes of 3.5 L and set points of pH: 7.1, 37°C, and DOT 30% of air. The glucose concentration was monitored throughout and the culture fed to maintain a glucose concentration of 2 g L^{-1} using 10 times concentrated CD CHO medium with glucose added to give a final concentration of 150 g L^{-1}. On days 10, 12, and 14 (CY01 and Null8) of culture equal volumes of medium were taken from each bioreactor and combined for UVRR spectrometry. Cells were removed by centrifugation and the supernatant was kept at −20°C until required for UVRR spectroscopy.

Pilot-scale downstream processing of bioreactor samples

Centrifugation

Centrifugation was carried out using a CSA-1 discstack centrifuge (Westfalia Separator GmbH, Oelde, Germany) operated with a soft feedzone and bowl speed of 9800 rpm. The combined cell culture suspension from two 3.5 L fermenters was centrifuged at a flow rate of 100 L h^{-1} for 10 bowl volumes to ensure steady state clarification was achieved. The feed material was agitated throughout to ensure a homogeneous suspension was delivered to the centrifuge. Supernatant was collected from the centrifuge for UVRR spectroscopy and subsequent pilot processing. The sigma value for this centrifuge was calculated to be 1640 m^2, giving a $Q/c.\Sigma$ value of 4.2×10^{-8} m s^{-1} (assuming a correction factor of 0.4). The supernatant was kept at −20°C until required for UVRR spectroscopy.

Depth filtration

Depth filtration was carried out at room temperature using disposable BioCap® capsules (Cuno Incorporated, CT, USA) that contain the Zeta Plus SP depth filters. An Amicon 8010 system (unstirred cell) (Millipore Corporation, MA, USA), modified to accommodate an additional 500 mL feed reservoir, was used to regulate pressure onto the depth filter capsules attached to the reservoir outlet. Nitrogen gas was supplied to pressurize the whole system (controlled by a regulator valve) in order to generate a positive gauge pressure for filtration. The operating pressure was kept constant at 70 kPa throughout the duration of each study and permeate mass was logged on a computer to determine the cumulative filtrate volumes. For comparison of primary recovery unit operations (i.e. centrifugation and depth filtration), cell culture broth was filtered using 05SP (nominal removal 0.5–5 μm) grade BioCap® capsules, while clarified broth taken from the CSA-1 centrifuge was filtered using 30SP (nominal removal 0.2–2 μm) and 90SP (nominal removal 0.05–0.2 μm) grade BioCap® capsules. Filtrate samples were kept at −20°C for subsequent analysis with UVRR spectroscopy.

UVRR spectroscopy

UVRR spectra were acquired using a Raman microscope (Renishaw, Wotton-under-edge, Gloucestershire, UK). All medium samples were thawed at room temperature immediately prior to the measurement of UVRR spectra and 40 μL of sample was pipetted into a small well on the lid of a polystyrene microplate.

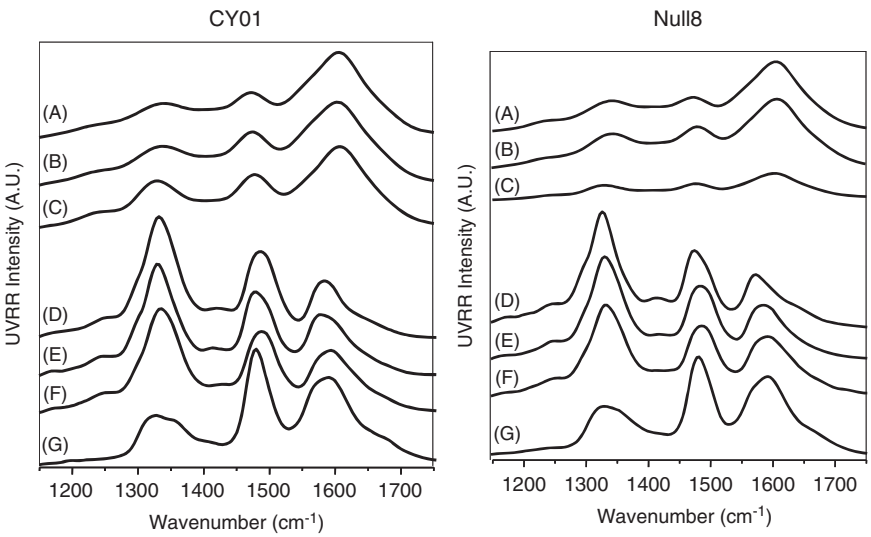

Figure 3. UVRR spectra of CY01 and Null8 medium harvested on day14 spiked with adenosine and guanine containing analytes. (A) Supernatant only, (B) plus 0.1 mg mL^{-1} plasmid DNA (pcDNA3.1 v5H is supplied by Invitrogen), (C) plus 0.25 mg mL^{-1} adenosine, (D) plus 10 mg mL^{-1} NAD, (E) plus 10 mg mL^{-1} AMP, (F) plus 10 mg mL^{-1} ATP and (G) plus 10 mg mL^{-1} GTP. Three spectra were acquired for each sample and averaged.

Approximately 0.2 mW of power were delivered to the sampling point using a Lexel Model 95 Ion Laser emitting at 244 nm. The UV laser was focused into the solution with care being taken not to focus on the lid itself.[6] The polystyrene microplate has a distinct UVRR spectrum (not shown) with unique peaks not observed in the medium samples and therefore it could be easily determined if the sampling point was incorrect and only spectra with absolutely no signal from the plate were analysed for this study. The well was continuously rotated under the laser to avoid photodegradation.

For the majority of samples six repeat spectra from two different wells were collected for 60 s each. The exceptions to this were day 5 for LB01 without sodium butyrate and day 3 for LB01 with 2 mmol L^{-1} sodium butyrate where the small amount of available sample resulted in only three reliable spectra being acquired from one well. Furthermore, for both day 4 of LB01 without sodium butyrate and day 3 for LB01 with 1 mmol L^{-1} sodium butyrate insufficient sample volume was available to collect reliable spectra without interference from the sample plate.

Data preprocessing and chemometric analysis

Before chemometric processing wavenumbers were calibrated using a diamond peak at ~1332 cm^{-1} and cosmic spikes were electronically removed from spectra using GRAMS WiRE (Galactic Industries Corp., Salem, NH). All further data processing and analysis was applied using MATLAB software version 2011a (The Math Works, MA, USA). In order to compare spectra directly the data were normalized by standard normal variate (SNV) so that each spectrum has a 0 mean and standard deviation of 1 and smoothed using a triangular sliding average.[17]

RESULTS AND DISCUSSION

UVRR spectra

Figure 1 shows the UVRR spectra of medium samples from the control LB01 cell line (without any addition of sodium butyrate) collected between days 2 and 7 of batch culture. As we have previously shown,[6] UVRR spectra of supernatant samples have three distinctive peaks measured at ~1344, 1480 and 1608 cm^{-1} and spectral changes in these peaks can be observed in Fig. 1. What is particularly relevant for the detection of DNA and RNA is the large increase in intensity with day of culture in the adenosine (A) and guanine (G) assigned peaks at ~1344 and 1480 cm^{-1} as well as a much weaker peak assigned to A, G and U molecules.[18,19] Peaks in the region of ~1480 cm^{-1} have been categorically assigned to both A and G molecules, arising from C–N and C=N stretching in G molecules and NH_2 bending in A molecules.[20] However, the peak at ~1344 cm^{-1} while frequently assigned to C–N and C=N stretching in both A and G molecules[20] can also be assigned to tryptophan residues[18,21] that are also present in the medium samples as a result of both medium amino acid composition and

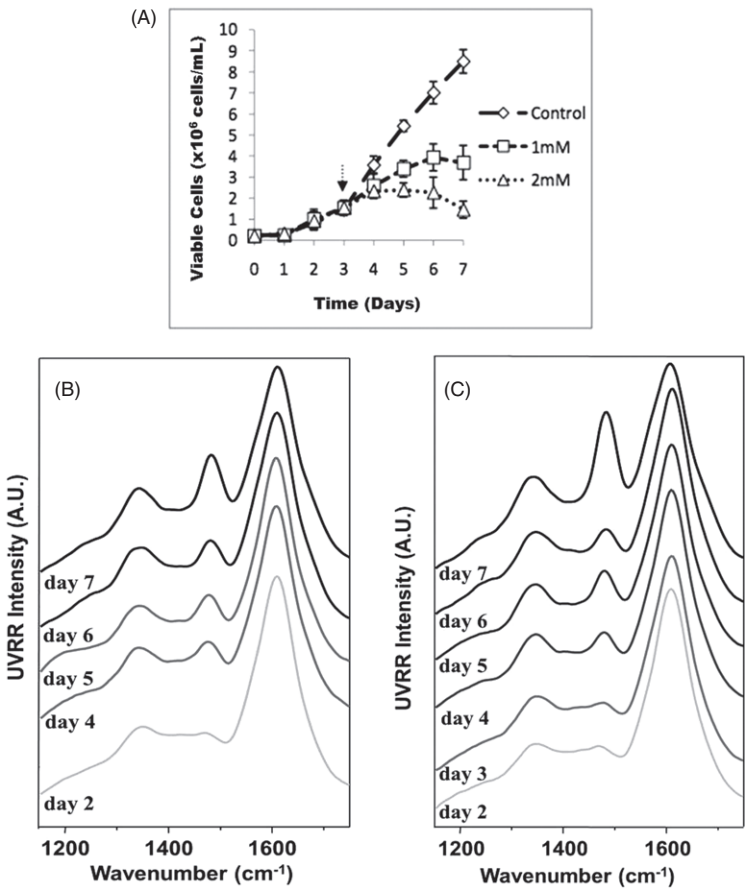

Figure 4. IgG4 fermentation in LB01 cell line spiked with sodium butyrate. (A) The effect on the number of viable cells in the cell culture in response to the addition of sodium butyrate on day 3 to LB01 cells in batch culture; in this plot points are the average of six measurements (with the exception of day 3 LB01 with 2 mmol L^{-1} sodium butyrate) and error bars represent the standard deviations. Averaged UVRR spectra of medium samples harvested on days 2 to 7 with the addition of (B) 1 mmol L^{-1} and (C) 2 mmol L^{-1} of sodium butyrate on day 3. Averaged spectra were calculated from six repeat spectra, with the exception of the Day 3 with 2 mmol L^{-1} of sodium butyrate spectrum, which is the average of three repeat spectra.

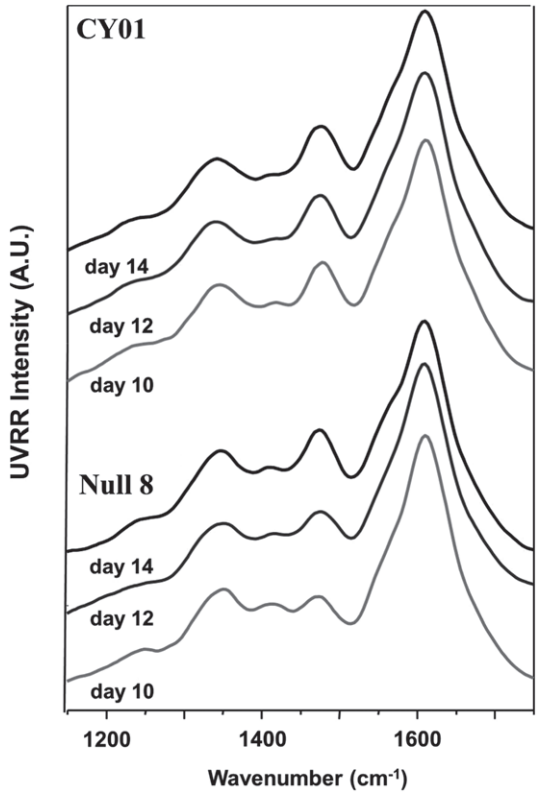

Figure 5. Averaged UVRR spectra monitoring IgG4 fermentation in CY01 and Null8 cell lines. Medium samples were harvested on day 10, day 12 and day 14, six spectra were acquired for each day and averaged.

protein secretion from the cells. In order to determine the origin of the spectral variations observed at ~1344 cm^{-1} we compared the changing intensity profiles of the peak at ~1344 cm^{-1} with both a nucleic acid assigned peak (~1480 cm^{-1}) and a tryptophan assigned peak (1608 cm^{-1}). The peak at ~1608 cm^{-1} has been extensively assigned to tryptophan and tyrosine molecular vibrations.[18,22] If the maximum peak intensity is plotted as a function of time (rather than against wavenumber frequency as in Fig. 1) changing intensity profiles can be observed as cell culture progressed from day 2 to day 7 (Fig. 2). In Fig. 2(C), for the peak measured at ~1344 cm^{-1} a steady increase in intensity is observed from day 2 to day 3 to day 4 before a larger intensity increase from day 5 to 6 with a small increase from day 6 to 7. This changing intensity profile is very similar to intensity changes observed for the peak at ~1480 cm^{-1} (Fig. 2(B)) and different from the changing profile for the peak at ~1608 cm^{-1} (Fig.2(A)) where most noticeably there is a decrease in intensity from day 5 to 6. Consequently, the intensity profiles (or trends in behaviour of the maximum peak intensity) indicate that the peak measured at ~1344 cm^{-1} should, for this investigation, be assigned to A and G molecular vibrations. The peak measured at ~1247 cm^{-1} has a slightly different intensity profile (Fig. 2(D)) to the peaks at 1480 and 1344 cm^{-1}. The peak at 1247 cm^{-1} is assigned not only to A and G but also U molecules and it is most likely that changes in residual RNA content as well as residual DNA content contribute to intensity changes in this peak.

Although we assigned the peaks as described above, there is the potential that the increase in intensity observed at ~1344 and 1608 cm^{-1} (Fig. 1) may be due to an increase in the presence of A and G monomers or an increase in other components including ATP, AMP, GTP or NAD, rather than DNA and RNA. In order to help establish the molecules that correspond with the spectral intensity increases, UVRR spectra were recorded of CY01 and Null8 medium samples harvested on day 14 and subjected to CSA-1 primary clarification followed by 90SP depth filtration (see clarification methods for further details) with various A and G analytes (Fig. 3). As can be seen in Fig. 3, for both CY01 and Null8 medium samples spiked with either ATP, AMP, GTP or NAD (Fig. 3(D)–(E)) the acquired UVRR spectra have significantly different profiles compared with the medium sample spiked with DNA (Fig. 3(B)). A shift in wavenumber of the maximum peak intensity is observed in all the spectra that are not observed in the UVRR spectra when monitoring throughout cell culture (Fig. 1). Peak positions of maximum intensity in the supernatant spiked with plasmid DNA are the same as those observed in the supernatant samples suggesting the observed increases in intensity in these bands (Fig. 1) are most likely due to an increase in host DNA.

Effect of addition of sodium butyrate on spectra

Sodium butyrate, a histone deacetylase inhibitor, can increase productivity of recombinant proteins in mammalian cells but can also have a negative effect on cell viability.[14–16] Figure 4(A) shows the effect on the number of viable cells in the culture in response to the addition of sodium butyrate to LB01 cells in batch culture. In the presence of 2 mmol L^{-1} sodium butyrate a decline in viable cells was observed after day 4 with a slower, less-pronounced effect in response to the addition at 1 mmol L^{-1} concentrations. In the absence of sodium butyrate, a continual increase in cell viability was observed through culture to day 7.

Figure 4(B) and 4(C) show the averaged UVRR spectra of the medium samples from the two cell cultures with the addition of 1 mmol L^{-1} and 2 mmol L^{-1} sodium butyrate, respectively, harvested on days 3–7. For the peak at ~1480 cm^{-1} a large increase in intensity is observed from day 6 to day 7, with a much greater increase occurring with the addition of 2 mmol L^{-1} of sodium butyrate compared with 1 mmol L^{-1}. A similar increase in peak intensity, although to a lesser extent, was also observed for the peak at ~1344 cm^{-1} and the weak peak occurring at ~1247 cm^{-1} after day 6. Comparing these spectral intensity changes with that of the control LB01 cell culture (Fig. 1) it can be concluded that the addition of sodium butyrate results in a much greater amount of DNA and RNA being present in the medium, presumably due to cell lysis.

Comparisons of antibody-producing and non-producing cell lines

Previous research on two of the CHO cell lines used in this current study (antibody-producing CY01 and non-producing Null8) suggested that the majority of HCPs (assessed by ELISA, 2D-PAGE and LC-MS/MS) in the supernatant arose as a result of cell lysis or breakage of cells associated with a loss in viability.[9] To investigate the ability of UVRR spectroscopy to monitor constituents in the host cell culture further, we also assessed UVRR spectra of the same samples that had been harvested for HCP analysis on days 10, 12 and 14 of culture.[9] Again, three strong bands were observed (~1344, 1480 and 1608 cm^{-1}) in all UVRR spectra (Fig. 5). Intense peaks at ~1344 and 1480 cm^{-1} and the weaker peak at 1247 cm^{-1} occur in the spectra from both the Null8 and CY01 medium samples harvested on day 10 indicating that DNA and RNA are present in the sample. In the Null8 spectra increases in intensity can be observed from day 10 to day 14 which were not present in the CY01 spectra, specifically the band at ~1480 cm^{-1}. Tait *et al.*[9] reported that the Null8 cell line declined

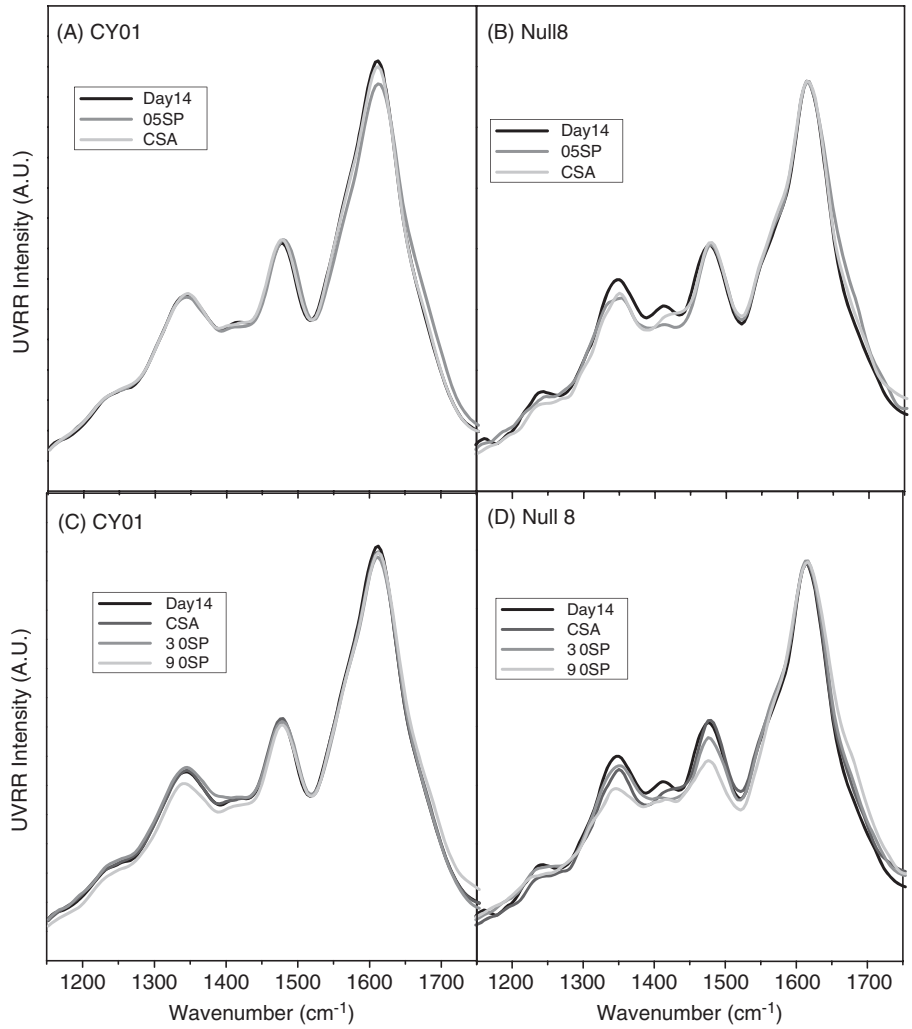

Figure 6. UVRR spectra of CY01 and Null8 medium harvested on day 14 and after various clarification methods. (A) CY01 and (B) Null day 14 bench-top centrifuged only compared with two primary clarification methods, 05SP depth filtration (05SP). (C) CY01 and (D) Null8 day 14 compared with the pilot-scale disc stack centrifuge (CSA-1) and followed by further secondary clarification through either 30SP or 90SP depth filter (30SP and 90SP).

more rapidly than the CY01 cell line with a lower viability when harvested on day 14 as indicated by only 35% of cells remaining viable compared with 67% in the producer cell line suggesting that these UVRR spectra (Fig. 5) are potentially monitoring increasing levels of DNA and RNA due to a loss of cell viability.

Monitoring and comparing clarification methods using Raman spectroscopy

The sensitivity of UVRR spectra to the increasing amounts of DNA and RNA has potential as a technique to monitor and track these species during downstream purification. Hogwood and co-workers[10] applied 2D-PAGE analysis to compare HCP profiles after different primary and secondary clarification techniques demonstrating that clarification methods can have significant impact upon the HCP profile post-clarification.[10] However, they did not monitor nucleic acids. We have therefore acquired UVRR spectra of the same samples used in the previous study of Hogwood *et al.* to assess the potential of UVRR spectroscopy to determine differences in DNA and RNA amounts after different clarification approaches.

UVRR spectra were recorded of CY01 and Null8 supernatant samples (harvested on day 14) after the application of different clarification methods. Figure 6(A) and 6(B) compare the CY01 and Null8 UVRR spectra, respectively, of day 14 supernatant samples (after centrifugation only) with samples after primary clarification through 05SP depth filtration (05SP) and through a pilot-scale disc stack centrifuge (CSA-1). As previously stated, during cell culture a greater loss of cell viability was observed in the Null8 cell line compared with CY01 and this may explain why very little spectral variation is observed for the CY01 samples while a change in intensity can be observed for the Null8 samples. Comparing the Null8 day14 UVRR spectrum with 05SP and CSA spectra a loss of intensity was observed at ~1344 and 1247 cm^{-1} indicating a lower concentration or changes in DNA and RNA content, demonstrating the potential of UVRR spectroscopy to monitor different DNA/RNA profiles as a result of primary clarification techniques.

Figure 6(C) and 6(D) compare UVRR spectra of supernatant samples from day 14 of culture (after bench-top centrifugation only) to the pilot-scale disc stack centrifuge (CSA) and followed by further secondary clarification through either 30SP or 90SP depth filters (30SP and 90SP). Again, different UVRR spectral profiles can be observed for the different depth filters used. In particular, a large decrease in intensity can be observed in the Null8 and the CY01 spectra after 90SP depth filtration compared with day 14 and

other primary and secondary clarification methods, suggesting this method removes more of the DNA and RNA from the supernatant samples. The ability to monitor the removal of such nucleic acids contaminants rapidly during downstream bioprocessing offers the ability not only to follow the fate of such species and confirm their removal, but such approaches can also be applied to the development and assessment of downstream bioprocesses.

CONCLUSIONS

In this study we have demonstrated the potential of UVRR spectroscopy as a tool to monitor variations in residual DNA and RNA that may contaminate cell culture medium, before and after purification of valuable recombinant proteins from the same medium. UVRR has the distinct advantage of being extremely sensitive for the detection of nucleic acids without interference from background fluorescence, which is often a major problem with conventional Raman spectroscopy. This offers the advantage over traditional PCR based approaches in that it could be used on-line in real time to monitor nucleic acids throughout a bioprocess. In UVRR spectra of mammalian cell culture medium, collected at an excitation wavelength of 244 nm, two distinct peaks are observed at ~1344 and 1480 cm^{-1} assigned to A and G residues. Specific variations in intensity and shape of these peaks as a result of increasing residual cellular DNA have been determined through the comparison of medium samples spiked with plasmid DNA and alternative nucleic acid components including ATP, AMP, GTP and NAD. Similar spectral variations as a result of increasing DNA levels were observed in medium cell culture samples harvested at different days of cell culture for both antibody and non-antibody producing cell lines, as a result of increasing cell lysis associated with a loss in cell viability. Variations in UVRR spectral profiles could also be observed in medium samples after sodium butyrate treatments, with a greater increase in peak intensity observed with the addition on day 3 of 2 mmol L^{-1} concentration of sodium butyrate compared with 1 mmol L^{-1}, most likely as a result of cell lysis and changing gene expression characteristics. Furthermore, comparison of UVRR spectra of cell culture medium after the application of various clarification techniques shows that UVRR spectroscopy is an excellent tool to assess different DNA and RNA profiles during primary and secondary clarification. The ability of this spectroscopic technique to quantify the presence of residual DNA and RNA has significant potential for both improvement of recovery techniques and assurance of the reliable clearance of this impurity to achieve acceptable safety levels.

ACKNOWLEDGEMENTS

We would like to thank the EPSRC, BBSRC and the industrial members of the Bioprocessing Research Industry Club (BRIC) for funding (BB/G010250/1 and BB/G010307/1). We are also extremely grateful to Lonza Biologics (Slough, UK) for providing the cell lines utilized in this study.

REFERENCES

1 Rosa PAJ, Ferreira IF, Azevedo AM and Aire-Barros MR, Aqueous two-phase systems: a viable platform in the manufacturing of biopharmaceuticals. *J Chromatogr A* **1217**:2296–2305 (2010).
2 Undey C, Ertunc S, Misstretta T and Looze B, Applied advanced process analytics in biopharmaceutical manufacturing: challenges and prospects in real-time monitoring and control. *J Process Control* **20**:1009–1018 (2010).
3 Junker BH and Wang HY, Bioprocess monitoring and computer control: key roots of the current PAT initiative. *Biotechnol Bioeng* **95**:226–261 (2006).
4 Li B, Ryan PW, Ray BH, Leister KJ, Sirimuthu NMS and Ryder AG, Rapid characterization and quality control of complex cell culture media solutions using Raman spectroscopy and chemometrics. *Biotechnol Bioeng* **107**:290–301 (2010).
5 Abu-Absi NR, Kenty BM, Cuellar ME, Borys MC, Sakhamuri S, Strachan DJ, Hausladen MC and Li ZJ, Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in line Raman spectroscopy probe. *Biotechnol Bioeng* **108**:1215–1221 (2010).
6 Ashton L, Xu Y, Brewster VL, Cowcher DP, Sellick CA, Dickson AJ, Stephens GM and Goodacre R, The challenge of applying Raman spectroscopy to monitor recombinant antibody production. *Analyst* **138**:6977–6985 (2013).
7 Grzeskowiak JK, Tscheliessnig A, Toh PC, Chusainow J, Lee YY, Wong N and Jungbauer A, 2-D DIGE to expedite downstream process development for human monoclonal antibody purification. *Protein Expr Purif* **66**:58–65 (2009).
8 Jin M, Szapiel N, Zhang J, Hickey J and Ghose S, Profiling of host cell proteins by two-dimensional difference gel electrophoresis (2D-DIGE): implications for downstream process development. *Biotechnol Bioeng* **105**:306–316 (2010).
9 Tait AS, Hogwood CEM, Smales CM and Bracewell DG, Host cell protein dynamics in the supernatant of a mAB producing cell line. *Biotechnol Bioeng* **109**:971–982 (2012).
10 Hogwood CEM, Tait AS, Koloteva-Levine N, Bracewell DG and Smales CM, The dynamics of the CHO host cell protein profile during clarification and protein A capture in a platform antibody purification process. *Biotechnol Bioeng* **110**:240–251 (2013).
11 Wang X, Hunter AK and Mozier NM, Host cell proteins in biologics development: identification, quantitation and risk assessment. *Biotechnol Bioeng* **103**:446–458 (2009).
12 Wang X, Morgan DM, Wang G and Mozier NM, Residual DNA analysis in biologics development: review of measurement and quantitation technologies and future directions. *Biotechnol Bioeng* **109**:307–317 (2011).
13 Chon JH and Zarbis-Papastoitsis G, Advances in the production and downstream processing of antibodies. *New Biotechnol* **28**:458–463 (2011).
14 Gatti MdL, Wlaschin KF, Nissom PM, Yap M and Hu W-S, Comparative transcriptionl analysis of mouse hybridoma and recombinant chinese hamster ovary cells undergoing butyrate reatment. *J Biosci Bioeng* **103**:82–91 (2007).
15 Jiang Z and Sharfstein ST, Sodium butyrate stimulates monoclonal antibody over-expression in CHO cells by improving gene accessibility. *Biotechnol Bioeng* **100**:189–194 (2007).
16 Yee JC, Gatti MdL, Philp RJ, Yap M and Hu W-S, Genomic and proteomic exploration of CHO and Hybridoma cells under sodium butyrate treatment. *Biotechnol Bioeng* **99**:1186–1204 (2007).
17 Bereton RG, *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*. Wiley, Chichester (2005).
18 Chi Z, Chen XG, Holtz JSW and Asher SA, UV resonance Raman-selective vibrational enhancement: quantitative methodology for determining protein secondary structure. *Biochemistry* **37**:2854–2864 (1998).
19 López-Díez EC and Goodacre R, Characterization of microorganisms using UV resonance Raman spectroscopy and chemometrics. *Anal Chem* **76**:585–591 (2004).
20 Hobro AJ, Standley DM, Ahmad S and Smith NI, Deconstructing RNA: optical measurement of composition and structure. *Phys Chem Chem Phys* **15**:13199–13208 (2013).
21 Wen ZQ and Thomas GJ, UV resonance Raman spectroscopy of DNA and protein constituents of viruses: assignments and cross sections for excitations at 257, 244, 238 and 229 nm. *Biopolymers* **45**:247–256 (1997).
22 Wu Q, Hamilton T, Nelson WH, Elliot S, Sperry JF and Wu M, UV Raman spectral intensities of *E. coli* and other bacteria excited at 228.9, 244.0, and 248.2 nm. *Anal Chem* **73**:3432–3440 (2001).

