Significant time savings with simplified cell culturing using ReadyToProcess WAVE 25

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CY13610-20May20-AN

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With today's strict time frames, the demands for automation and ease of use in bioprocessing are rapidly increasing. The ReadyToProcess WAVE 25 bioreactor system was developed with the goal of keeping the simplicity of single-use rocking bioreactor systems, while enhancing system features with time-saving automation technologies (Fig 1). This application note describes how ReadyToProcess WAVE 25 can help reduce hands-on time as much as 25% in fed-batch culturing, while delivering excellent culture performance. For an optimized perfusion application, the use of intelligent control software enabled accurate regulation of process parameters. Over 100 × 10^6 viable cells/mL was easily achieved in seven days.

Fig 1. ReadyToProcess WAVE 25 bioreactor system comprises a rocker, a gas mixer, and a pump unit, all operated by the UNICORN™ software installed on a client computer. The rocker has multiple functions, including heating, culture mixing, and weight measurement. The gas mixer delivers gas of a defined composition to the culture and is used together with optical sensors in the Cellbag™ bioreactor for online control of culture pH and DO. Liquid is delivered to the culture by the pump unit, which supports a range of flow rates to cover multiple applications, from addition of feeds or base to perfusion culturing. The system can also be controlled by an external control system such as the Emerson DeltaV™ using the UNICORN open platform communication (OPC) server.

Introduction

Single-use WAVE Bioreactor™ systems are used in a number of applications, such as culturing of Chinese hamster ovary (CHO) cells for monoclonal antibody (MAb) production, perfusion culturing of T-cells for cell therapy, and culturing of Vero cells on microcarriers for vaccine production. When introduced on the market in the late 1990s, the system was an immediate success because of its simplicity and ease of use.

Today's process developers require systems that can offer automation, reduce turnover time, and meet industry requirements such as the process analytical technology (PAT) initiative. Users in the biopharmaceutical manufacturing space, in turn, are constantly challenged to meet new regulatory standards, while increasing product yield and reducing hands-on time. A basic rocker is still sufficient for many applications, but an increasing number of users are seeking more advanced systems that provide accurate pH and dissolved oxygen (DO) control, perfusion capabilities, CFR Part 11 compliance, and can be connected to external surveillance solutions such as the DeltaV control system. The ReadyToProcess WAVE 25 bioreactor system was developed to meet all these requirements.

This application note shows the cell culture performance of the ReadyToProcess WAVE 25 system in fed-batch and perfusion applications. Solutions for how hands-on time can be reduced in bioprocessing are discussed.

Materials and methods

Cell cultures

In this study, ActiCHO™ Media System was used for culturing of the MAb-producing CHO DG44 cell line (licensed from Cellca GmbH, Laupheim, Germany). Bioreactor system setup is summarized in Table 1. Prior to inoculation, the Cellbag bioreactor chamber was inflated and filled with ActiCHO P CD production medium. The medium was heated to 37°C and pH 7 for 2 h, before cells were added by gravity flow to the culture chamber. Bioreactor system operation parameters are summarized in Table 2.

Table 1. Bioreactor system setup

	Fed batch	Perfusion
Bioreactor system	ReadyToProcess WAVE 25 and WAVE Bioreactor 20/50	ReadyToProcess WAVE 25
Cellbag bioreactor	20 L with optical pH and DO sensors	2 L with internal perfusion filter with optical pH and DO sensors
Medium volume at inoculation	6.2 L	1 L
Target cell concentration at inoculation	0.3×10^6 viable cells/mL	0.5×10^6 viable cells/mL

Table 2. Parameters used for operation

	Fed batch	Perfusion
Agitation rate (rpm)	22–27	25–40
Angle (°)	6–7	6–12
Rocking speed profile (smoothness)	15%	30%
Temperature (°C)	37	37
pH set point	N/A	7.2
pH control strategy	Controlled by constant CO_2 at 7.5%	Controlled by automatic CO_2 addition in response to pH measurement
DO set point (%)	60	30
DO control strategy	Controlled by O_2 and rocking speed	Controlled by O_2 and rocking speed
Gas flow rate (L/min)	0.2	0.2–0.5
Glucose target	> 2 g/L (on-demand feed from glucose stock solution)	> 2 g/L
Culture medium	ActiCHO P	ActiCHO P
Working Volume	10 L	1 L
Cellbag bioreactor	20 L	2 L with internal perfusion filter

Fed-batch cultures

From day 3, cells were fed once daily with 270 mL ActiCHO Feed-A CD and 30 mL of ActiCHO Feed-B CD. Final glucose concentration in the culture medium was maintained at ≥ 2 g/L by on-demand supplementation of glucose stock solution (400 g/L). For ReadyToProcess WAVE 25, feed addition was automated using the ***Method editor*** function of the UNICORN software, whereas for the WAVE Bioreactor 20/50 system, feed addition was handled manually. The overall culture time was 14 days, after which the culture volume was 10 L. The cultures were performed in duplicate and time for preparation, set-up, and daily interactions with the systems was recorded.

Perfusion culture

An exponential increase of perfusion rate, once every second hour, from 300 mL/d to 15.8 L/d was achieved using UNICORN ***Method editor***. Feed and harvest pumps were calibrated when initially filling the Cellbag bioreactor at a flow rate of 5 L/d using the auto calibration function. Perfusion was initiated 24 h post-inoculum and was maintained for seven days.

Analyses

Sampling of the perfusion culture was performed during rocking. Viable cell density was determined using the standard trypan blue exclusion method. IgG product titers were determined using the Biacore™ T200 system. Glucose, lactose, and glutamine levels were measured using the Bioprofile Flex™ analyzer (Nova Biomedical Corp., Waltham, MA, USA). The ABL5 blood gas analyzer (Radiometer Medical ApS, Brønshøj, Denmark) was used for measurement of culture pH, CO_2, and O_2 levels. All measurements were performed according to the manufacturers' instructions.

Results and discussion

Reduced hands-on time in a fed-batch process

To compare the overall performance of ReadyToProcess WAVE 25 with its precursor WAVE Bioreactor 20/50, fed-batch cultures of the MAb-producing CHO DG44 cell line were run in parallel in both systems. The results show an equivalent cell culture performance (Fig 2). The viable cell density reached a maximum of 15.7×10^6 viable cells/mL using ReadyToProcess WAVE 25 and 15.9×10^6 viable cells/mL using the WAVE Bioreactor 20/50 system. Cell viability was maintained above 90% for 11 days of culture, after which a gradual decline was observed. The obtained MAb yields were up to 3.9 g/L with ReadyToProcess WAVE 25 and up to 3.8 g/L using the WAVE Bioreactor 20/50 system. Nutrient and metabolite concentrations were comparable between the cultures (Fig 3), and the culture pH and DO were shown to be stable over the culture period for both bioreactor systems (Fig 4).

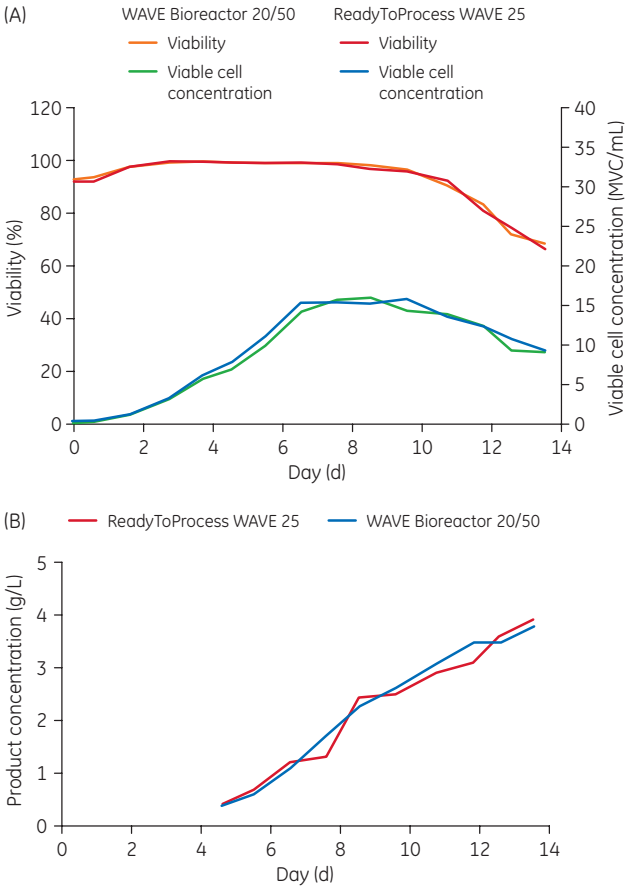

Fig 2. (A) Viable cell density, cell viability, and (B) product titers were comparable between ReadyToProcess WAVE 25 and WAVE Bioreactor 20/50 bioreactor systems.

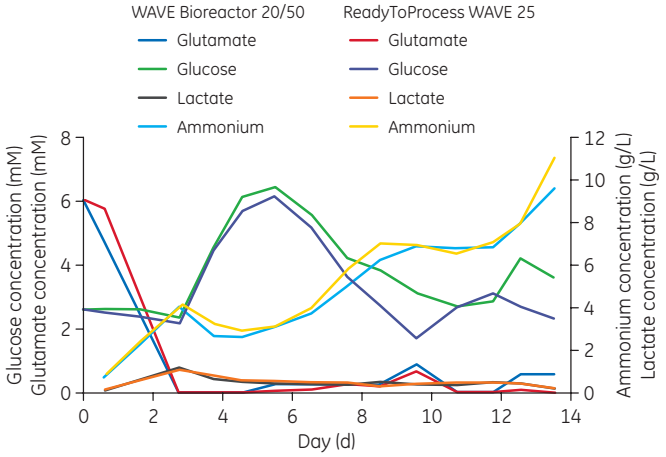

Fig 3. Culture nutrient and metabolite concentrations were similar for ReadyToProcess WAVE 25 and WAVE Bioreactor 20/50 bioreactor systems.

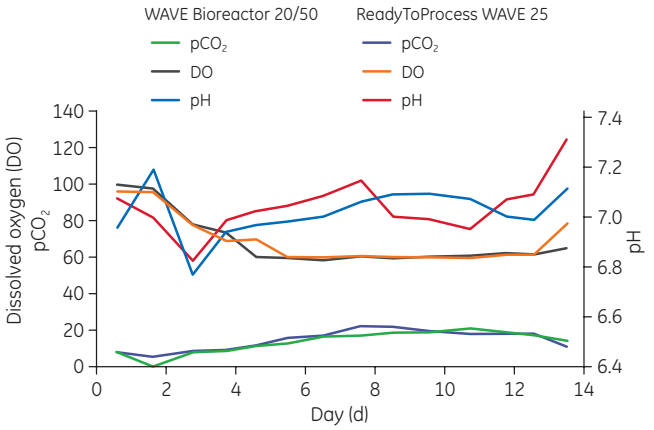

Fig 4. Culture pH and DO were shown to be stable over the culture period for both ReadyToProcess WAVE 25 and WAVE Bioreactor 20/50 bioreactor systems.

A significantly shorter overall hands-on time was achieved with ReadyToProcess WAVE 25 as compared with WAVE Bioreactor 20/50 (Table 3 and Fig 5). One part of the time saving can be attributed to the ergonomic design of the ReadyToProcess WAVE 25 system. Tray attachment to the rocker is easily performed and does not require any separate tools and the Cellbag bioreactor is rapidly and correctly installed with a snap-lock mechanism. A large part of the reduced time for manual handling can also be ascribed to the automated process settings enabled by the UNICORN control software. For example, PID* parameters for pH control are automatically set and updated by the software based on culture weight, gas flow set point, and Cellbag size. Feed additions were handled manually with the WAVE Bioreactor 20/50 system. For ReadyToProcess WAVE 25, the daily feed additions were automated using the ***Method Editor*** function. Automated feed additions contribute to the simplicity of ReadyToProcess WAVE 25 by further reducing the daily hands-on time.

* Proportional-integral-derivative

Table 3. Recorded time for system set up and operation

Activity*	Times repeated	ReadyToProcess WAVE 25 (min)	WAVE Bioreactor 20/50 (min)
Changing tray	1	0.35	6.53
Setting up bag, filter heater, pH and DO sensors	1	2.66	3.91
Entering of all process parameters	1	2.47	8.98
Inflating the Cellbag bioreactor	1	5.94	11.98
Medium fill and inoculation	1	27	27
Daily manual interactions, sampling and analysis	14	20	20
Feeding	11	2.4	9.8
Harvesting	1	5.47	6.72
Total time spent during culture		323 (5.4 h)	426 (7.1 h)

* Time for non value-adding activities, such as moving and picking up equipment, are excluded. With multiple reactors, many activities can be performed in parallel.

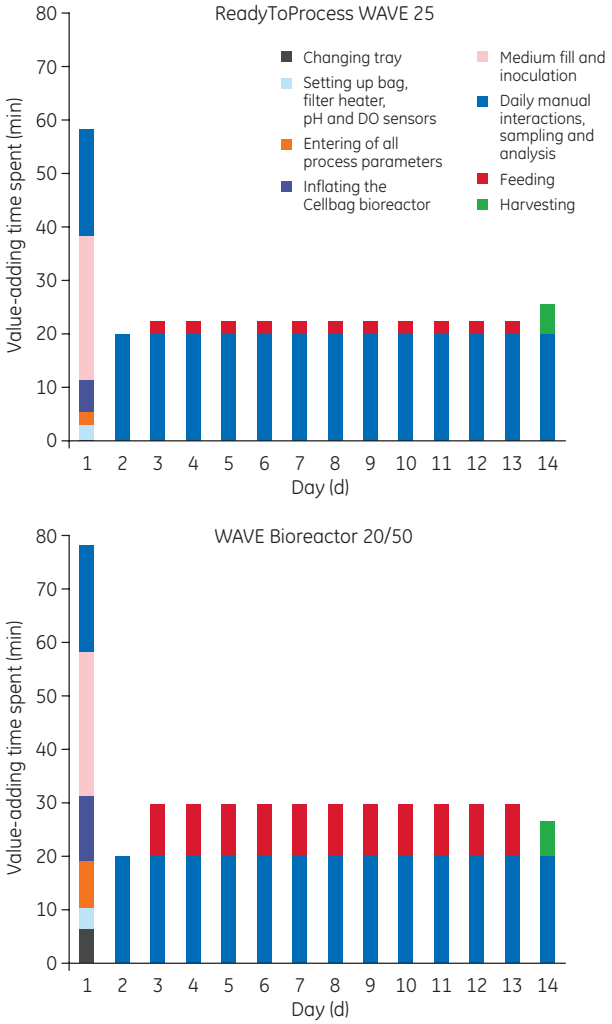

Fig 5. ReadyToProcess WAVE 25 enabled reduced hands-on time over the whole process compared with the WAVE Bioreactor 20/50 system.

Facilitated perfusion culturing, using intelligent control software

The perfusion culture was set up in ReadyToProcess WAVE 25 controlled by the UNICORN software. For an automated culture process, an exponentially increasing perfusion rate from 300 mL/d up to 15. 8 L/d was programmed using ***Method editor***. A total of 24 L medium was perfused over the culture period (Fig 6). In only seven days, a total of 115 × 10^9 viable cells were achieved at an average doubling rate of 23 h (specific growth rate; μ = 0.03 h^{-1}), while keeping the cell viability above 97% throughout the culture. With such cell numbers, a 1 L perfusion culture could in theory be split 1:500 and thus be used to directly inoculate a 500 L bioreactor at 0.2 × 10^6 cells/mL, omitting the need for intermediate seed cultures (Fig 7).

Continuous perfusion enabled steady-state levels of nutrients and metabolites over the culture period (Fig 8). Culture pH was controlled by regulating headspace CO_2 levels and DO was maintained stable above the set point 30% by increasing O_2 and rocking speed (Fig 9 and 10).

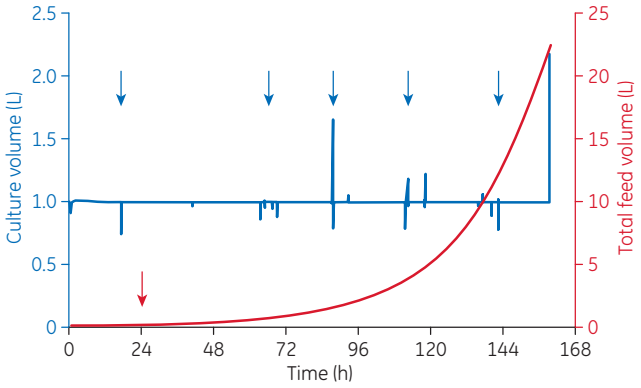

Fig 6. Perfusion culturing in ReadyToProcess WAVE 25 bioreactor system. Perfusion was started 24 h post-inoculation (red arrow). Over the seven-day culture, 25 L ActiCHO P medium was exchanged using an exponentially increasing perfusion rate of 90 pL/cell/d, which corresponds to 300 mL/d at perfusion start and 15.8 L/d at the end of the culture. Blue arrows indicate fluctuations due to operator interactions with the system.

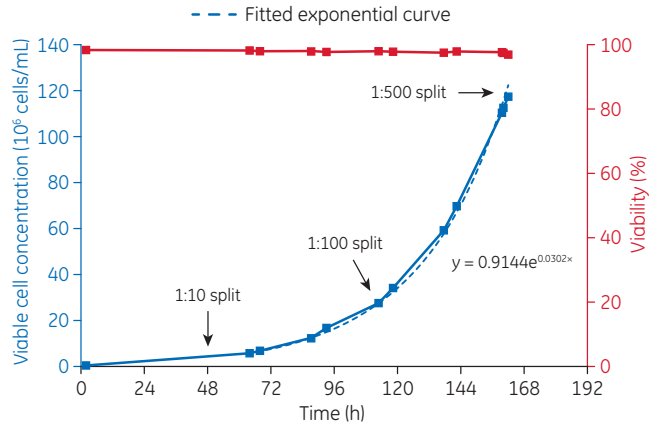

Fig 7. Cell counts of up to 115 × 10^6 viable cells/mL, corresponding to a split ratio of 1:500, were achieved using the ReadyToProcess WAVE 25 system.

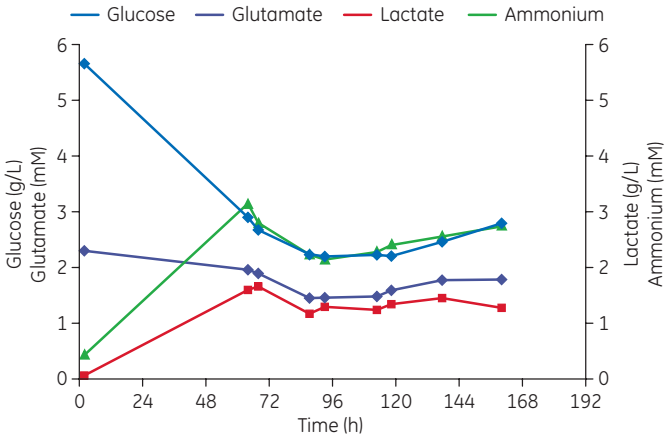

Fig 8. Continuous perfusion at increasing rate resulted in steady-state levels of the nutrients and metabolites.

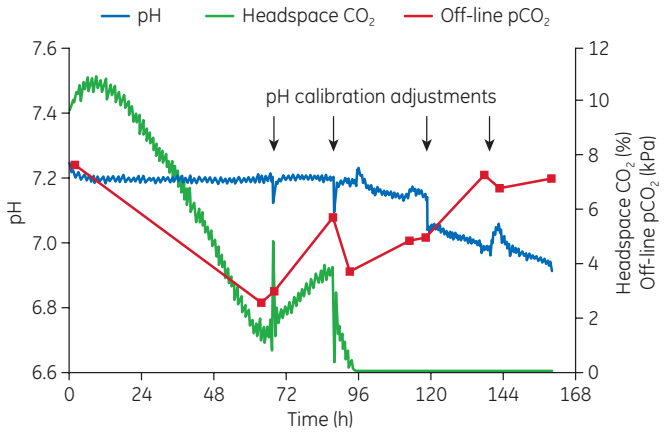

Fig 9. Culture pH was accurately maintained at 7.2 over 96 h of culture by regulating headspace CO_2 levels. A gradual drop to pH 6.9 was observed when the CO_2 concentration in the gas mix could not be reduced further and CO_2 levels in the medium started to increase in response to the high cell density. Blue arrows indicate pH calibration.

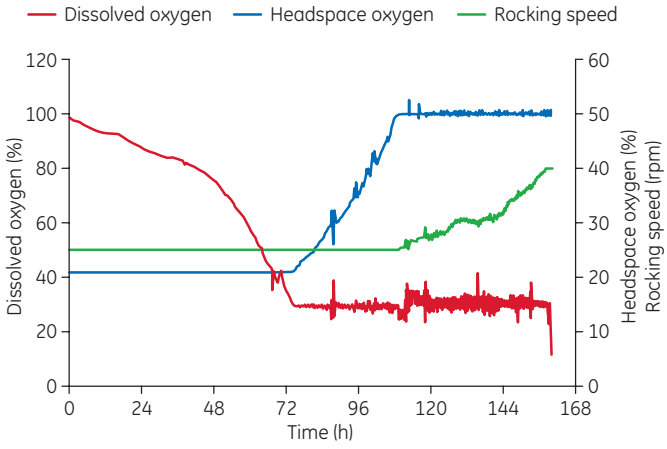

Fig 10. Dissolved oxygen was accurately controlled using a cascade strategy based on adjusting the percentage of O_2 in the in-gas, followed by rocking speed adjustment.

The high degree of automation of the ReadyToProcess WAVE 25 system reduces hands-on time during perfusion applications. The system also allows for automated calibration of the feed and harvest pumps. Automated calibration is performed when starting the perfusion and can be repeated with desired frequency during the run to avoid decreased flow due to tubing wear-out. The use of a Cellbag bioreactor containing an internal floating filter eliminates the need for an external cell retention device.

The high cell densities achieved in perfusion cultures can, for example, be used for shortening of seed trains for inoculation of larger fed-batch culture vessels. However, the more adapted nutrient supply in perfusion cultures, compared with fed-batch processes, requires a more sophisticated process regulation. The possibility of using UNICORN control software greatly facilitates perfusion culturing in the ReadyToProcess WAVE 25 bioreactor system.

During the run, the software automatically regulates process parameters against the settings. In addition, the UNICORN software allows for real-time monitoring of the process and run data can be reliably stored for subsequent visualization or export to, for example, Microsoft® Excel® for further analysis. The UNICORN software is suitable for use in a manner that complies with 21 CFR Part 11 and good automated manufacturing practice (GAMP) 5, enabling use of ReadyToProcess WAVE 25 in a regulated environment.

Conclusions

This application note shows that ReadyToProcess WAVE 25 exhibits similar excellent culture performance as its precursor WAVE Bioreactor 20/50, while reducing hands-on time by its higher degree of automation. With its user-friendly design, ReadyToProcess WAVE 25 allows for quick set-up and minimizes time-consuming manual handling. In addition, using ReadyToProcess WAVE 25 minimizes the risk of operator error in pH calibrations and addition of feeds. Using UNICORN control software, culture processes can rapidly be created and process parameters and instructions, such as feed rate, are easily entered using ***Method editor***. The UNICORN software also accurately controls culture parameters. This feature makes ReadyToProcess WAVE 25 well-suited for applications that require more sophisticated process regulation, such as perfusion culturing.

Ordering information

Product	Code number
ReadyToProcess WAVE 25, rocker	28-9880-00
ReadyToProcess™ CBCU Full	29-0440-81
ReadyToProcess Pump 25	29-0320-03
Tray 10	29-0444-71
Tray 20	29-0444-73
Lid 10	29-0444-75
Lid 20	29-0444-76
UNICORN 6.3.2 WrkStn-pure-BP	29-0469-18
Cellbag 2 L	29-0153-08
Cellbag 20 L	29-0152-14
ActiCHO Level 1 CD Pwd Kit	29-0925-41
Biacore T200 system	28-9750-01

Related literature	Code number
ReadyToProcess WAVE 25, data file	29-0566-95
Disposable Cellbag bioreactors for WAVE Bioreactor systems, data file	28-9511-36
UNICORN 6 control software, data file	28-9573-46

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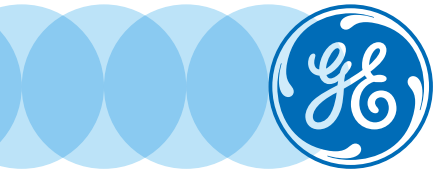

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29-1113-13 AA 09/2014