

Señora

Directora de la Dirección de Nuevas Creaciones

Superintendencia de Industria y Comercio

Ministerio de Comercio, Industria y Turismo

E.

S.

D.

Re.:Expediente No. **NC2022/0012263**

Solicitud de Patente PCT entrada en Fase Nacional, para: **“POTENCIADOR DE FOTOSÍNTESIS A BASE DE NANOPARTÍCULAS HÍBRIDAS DE CARBONO, SU PROCESO DE OBTENCIÓN Y SU USO COMO NANOBIUESTIMULANTES Y NANO FERTILIZANTES EN CULTIVOS AGRÍCOLAS”**

Solicitantes: **EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA, FUNDAÇÃO UNIVERSIDADE DE BRASILIA y KRILLTECH SOLUÇÕES SUSTENTÁVEIS EM ENGENHARIA LTDA**

RESPUESTA A EXAMEN DE FONDO

Yo, **MARGARITA CASTELLANOS ABONDANO**, mayor de edad, identificada y domiciliada como aparece al pie de mi firma, obrando en mi calidad de apoderada de la sociedad Solicitante del asunto citado en la referencia, en atención a su oficio **No. 6680** notificado electrónicamente el 15 de abril de 2026, atentamente me permito dar respuesta al mismo de la siguiente manera:

1. Modificación a las Reivindicaciones

De acuerdo con lo establecido en el Artículo 34 de la Decisión 486 y en vista de las observaciones realizadas por el Examinador, se presenta en esta oportunidad un nuevo pliego reivindicatorio conformado por tres (3) reivindicaciones. Las modificaciones introducidas son las siguientes:

- En todas las reivindicaciones se adecuó el preámbulo a: “PROCESO DE OBTENCIÓN DEL POTENCIADOR DE FOTOSÍNTESIS” para ajustarlo a la

terminología empleada en la descripción (Pág. 17, Párr. [069], Pág. 18, Párr. [072] y Pág. 21, Párr. [086]).

- La reivindicación independiente 1 fue modificada conforme a las sugerencias del Examinador, con el fin de especificar los reactantes empleados, a saber: glucosa o ácido cítrico como fuente de carbono, e hidróxido de amonio o urea como fuente de nitrógeno; asimismo, se indica el uso de agua como solvente. Adicionalmente, se incorporan las condiciones de temperatura y presión correspondientes a las etapas de bombeo, conducción y recirculación. Esta materia encuentra soporte en las anteriores reivindicaciones 4 a 6 y en la descripción (pág. 17, párr. [069]; pág. 18, párr. [073]; y pág. 22, Ejemplo 1). Igualmente, y con el propósito de ajustarse a lo sugerido por esta oficina, se reformuló la redacción del proceso para estructurarlo en una secuencia de etapas.
- La reivindicación 2 ha sido modificada para definir el reactor tubular de flujo pistón (PFR), caracterizado por presentar baja rugosidad superficial, lo cual favorece el establecimiento de un régimen de flujo dinámico. Esta información se encuentra incorporada la descripción (pág. 19, párr. [076]).
- La reivindicación 3 fue ajustada para hacerla más concisa e incorporar la mención de los dopantes, correspondientes a macronutrientes seleccionados entre potasio, nitrógeno y fósforo. Esta modificación encuentra sustento en la descripción (pág. 14, párr. [052]; pág. 21, párr. [084]).
- Las anteriores reivindicaciones 4 a 6 no se incluyen en la presente versión, dado que la materia contenida en ellas ha sido incorporada en la reivindicación independiente.

Ninguna de las modificaciones realizadas amplía el alcance de la protección más allá de lo previsto en la solicitud tal y como se presentó originalmente. El Solicitante no renuncia a la materia que no haya sido incluida en esta oportunidad, y se reserva el derecho de buscar su protección con posterioridad.

2. Sobre la Claridad, Concisión y Soporte de las Reivindicaciones

El Examinador señaló que el pliego reivindicatorio no se encontraba suficientemente claro, en la medida en que los elementos esenciales de la invención, además de no estar adecuadamente definidos, estaban distribuidos en diferentes reivindicaciones.

Observó, además, que diversos términos empleados abarcaban un número excesivo de alternativas, lo que perjudicaba la delimitación clara del alcance de la materia reivindicada. En atención a tales objeciones, el pliego reivindicatorio fue íntegramente reformulado, con el objetivo de conferir mayor claridad a las reivindicaciones y definir de manera más específica los elementos esenciales de la invención, siempre en estricta consonancia con la memoria descriptiva, de modo que no se amplíe el alcance originalmente divulgado.

En todas las reivindicaciones, la expresión “PROCESO PARA LA PRODUCCIÓN DE ESTIMULADORES DE LA FOTOSÍNTESIS” fue sustituida por “PROCESO DE OBTENCIÓN DEL POTENCIADOR DE FOTOSÍNTESIS”, a fin de adecuar la terminología al título de la invención.

a. **Reivindicación 1**

El Examinador objetó la reivindicación independiente 1 por supuesta falta de claridad, concisión y sustento, indicando que *“los elementos esenciales de la invención, que deberían formar parte de la reivindicación independiente 1, se encuentran distribuidos en diferentes reivindicaciones”*. Asimismo, señaló que *“las definiciones de ‘puntos de carbono híbridos’, ‘macronutrientes’, ‘ácido-base parcial’, ‘soluciones ácidas y básicas’, ‘dopante’, ‘macronutriente’, ‘micronutriente’, ‘estimulantes’ y ‘matrices ricas en carbono’ comprenden un número muy amplio de alternativas, lo que impide establecer claramente el alcance de la protección”*.

El Solicitante disiente respetuosamente de dicha apreciación, por cuanto considera que el proceso, tal como fue originalmente redactado, resulta comprensible y reproducible para una persona experta en la materia.

No obstante, con el propósito de atender las observaciones formuladas por esta oficina, la reivindicación 1 fue reestructurada para incluir los elementos esenciales de la invención que se encontraban distribuidos en las antiguas reivindicaciones dependientes 4 a 6, además, la reivindicación 1 fue organizada en etapas sucesivas,

con el objeto de describir de forma más clara el funcionamiento de la metodología de flujo continuo que constituye el objeto de la presente solicitud.

En la primera etapa, se definió la composición de la solución ácida, que actúa como fuente de carbono, y de la solución básica, que actúa como fuente de nitrógeno, así como el solvente empleado. En particular, la solución ácida quedó delimitada como comprendiendo ácido cítrico o glucosa, y la solución básica como comprendiendo urea o hidróxido de amonio, conforme al párrafo [073] de la memoria descriptiva. El uso de agua como solvente fue establecido con base en el Ejemplo 1.

En la segunda etapa, se incorporó expresamente la proporción estequiométrica entre los reactivos, correspondiente a 2 moles de carbono por cada 1 mol de amoníaco, en consonancia con la estequiometría presentada en el Ejemplo 1, en el que se emplean glucosa e hidróxido de amonio como materias primas. Dicha relación se mantiene igualmente cuando se emplean ácido cítrico o urea, los cuales, si bien no se ejemplifican, se encuentran descritos en la memoria descriptiva. Asimismo, se especificó en esta etapa la conducción de la mezcla reaccional hacia un segundo recipiente mezclador, al cual se adiciona el flujo de recirculación proveniente de la cuarta etapa y, opcionalmente, los elementos dopantes, de acuerdo con el Ejemplo 2, que ilustra la implementación de dicho recipiente.

En la tercera etapa, se describe el bombeo de la mezcla de reactivos y recirculación hacia un reactor tubular de flujo pistón (PFR), en el cual la reacción tiene lugar a una presión de 5 bar y una temperatura de 150 °C, valor preferente indicado en el párrafo [075] de la memoria descriptiva.

En la cuarta etapa, se establece la conducción del efluente hacia un reactor de tanque agitado continuo (CSTR), donde se produce la separación, por diferencia de densidades, entre el producto final y la fracción no reaccionada, siendo esta última redirigida al segundo recipiente mezclador definido en la segunda etapa.

Finalmente, la quinta etapa describe la recirculación de la mezcla hasta la estabilización del proceso, la cual se alcanza cuando el sistema llega a la temperatura de 150 °C establecida, lo que ocurre en un intervalo de entre 0,5 y 2 horas.

De este modo, la reivindicación 1 refleja de manera clara, ordenada y encadenada el proceso descrito en la memoria descriptiva, en particular en los párrafos [075],

[077] y [078], incorporando los elementos esenciales que esta oficina consideró insuficientemente delimitados.

En relación con la supuesta ausencia de mención expresa a la recirculación, se precisa que dicha inconsistencia obedece a un error de traducción. En efecto, en los párrafos [077] y [078] de la memoria descriptiva, donde figura el término “reflujo”, debe entenderse “recirculación”, en concordancia con la descripción técnica del proceso reivindicado.

En virtud de lo anterior, la reivindicación 1 es clara, autosuficiente y se encuentra debidamente sustentada en la memoria descriptiva tal como fue presentada, por lo que el Solicitante solicita respetuosamente el levantamiento de la objeción formulada.

b. Reivindicaciones 2 y 3

El Examinador ha objetado las reivindicaciones 2 y 3 por una supuesta falta de concisión y sustento. En atención a lo requerido, dichas reivindicaciones fueron modificadas.

En la nueva reivindicación 2, se describe con mayor detalle las características del reactor tubular de flujo pistón (PFR) empleado en el proceso, en concordancia con lo señalado en el párrafo [076] de la memoria descriptiva.

Por su parte, la nueva reivindicación 3 fue reformulada para definir los dopantes. En este sentido, dado que la adición de dopantes constituye una etapa opcional dentro del proceso de síntesis del potenciador de la fotosíntesis objeto de la presente solicitud, se consideró que esta característica no corresponde a un elemento esencial del proceso. En consecuencia, no se incorporó en la reivindicación independiente 1, sino en la reivindicación dependiente 3.

En la memoria descriptiva, los dopantes se definen inicialmente en el párrafo [075] *overflow* como macronutrientes, micronutrientes y estimulantes. Posteriormente, los macronutrientes y micronutrientes se enumeran en el párrafo [084], y su incorporación a los *carbon dots* se describe en el Ejemplo 2. Considerando que los dopantes efectivamente ejemplificados, tal como lo señaló el Examinador, corresponden a potasio, nitrógeno y fósforo, dichos elementos fueron incorporados como parte de la materia reivindicada en la reivindicación 3.

Como resultado de esta reestructuración, el pliego reivindicatorio presenta con mayor claridad el proceso reivindicado, sin omitir elementos relevantes para la adecuada reproducción del objeto de la solicitud de patente.

De esta manera, se considera que el capítulo reivindicatorio cumple con los requisitos de claridad y concisión previstos en el artículo 30 de la Decisión 486.

Adicionalmente con respecto a las objeciones por artículo 28 en donde se refiere que no se describe la mejor manera de llevar a la práctica la invención, es oportuno aclarar que, mediante las limitaciones sustanciales incorporadas a las nuevas reivindicaciones; particularmente, en lo relativo a la definición específica de los reactantes como: glucosa o ácido cítrico como fuente de carbono e, hidróxido de amonio o urea como fuente de nitrógeno y, aclarando que, la no mención expresa del término recirculación, corresponde a un error de traducción como se hace evidente, en los párrafos [077] y [078] de la memoria descriptiva, en donde se evidencia el término “reflujo”, que debe leerse como “recirculación” para que el proceso se defina de manera apropiada con relación al campo técnico y para que haya coherencia el lenguaje empleado como se reivindica actualmente. Se debería dar por superada cualquier objeción al respecto ya que, mediante la información incorporada cualquier persona capacitada en la materia técnica podría ejecutar la invención como se encuentra actualmente reivindicada.

Por lo tanto, se considera que el pliego reivindicatorio cumple con los requisitos establecidos en el Artículo 30 de la Decisión 486.

3. Sobre la Descripción

Mediante las modificaciones introducidas a las reivindicaciones, es posible establecer que el alcance ahora reivindicado encuentra el debido soporte en la descripción, cumpliendo así con lo requerido por el Artículo 28 de la Decisión 486.

4. Sobre el Nivel Inventivo

El Examinador objetó las antiguas reivindicaciones 1-6 (actuales reivindicaciones 1-3) por una supuesta falta de nivel inventivo frente a los documentos D2 (CN108160013) y D3 (*Lignos, I. (2020). Continuous Multistage Synthesis and Functionalization of Sub-100 nm Silica Nanoparticles in 3D-Printed Continuous Stirred-Tank Reactor Cascades. American Chemical Society, 12(5), 6699–6706*). El Solicitante disiente respetuosamente de dicha apreciación.

En primer lugar, es importante resaltar que el pliego reivindicatorio ha sido sustancialmente modificado, de modo que las actuales reivindicaciones están dirigidas a un **proceso para la obtención de un potenciador de la fotosíntesis, en el cual se han incorporado explícitamente todas las características y condiciones esenciales del proceso**. En particular, la reivindicación independiente ha sido reformulada para incluir: (i) los reactantes específicos, es decir, glucosa o ácido cítrico como fuente de carbono, y urea o hidróxido de amonio como fuente de nitrógeno; (ii) el uso de agua como solvente; (iii) las condiciones de temperatura y presión asociadas a las etapas de bombeo, reacción y recirculación; y (iv) la estructuración del proceso en una secuencia de etapas técnicas claramente definidas.

En virtud de esta reformulación, ninguno de los documentos D2 y D3, considerados individualmente o en combinación, anticipa ni hace evidente la solución actualmente reivindicada. Por consiguiente, la objeción formulada no resulta aplicable al pliego reivindicatorio vigente.

En el oficio 6680, el Examinador considera que D2 constituye el estado de la técnica más cercano y que las presuntas diferencias podrían derivarse de D3. Sin embargo, **este análisis ignora diferencias esenciales tanto en la naturaleza química como en la arquitectura del proceso**.

El documento D2, correspondiente a la patente CN108160013, de junio de 2018, tal como señala el Examinador, describe la obtención de materiales poliméricos con liberación controlada de *carbon quantum dots* mediante microencapsulación. A diferencia de la presente solicitud, **D2 no tiene como objetivo la reivindicación de una nueva metodología de síntesis de *carbon-dots*, sino la microencapsulación de *carbon-dots*, con miras a obtener un sistema de liberación controlada** cuya metodología de síntesis se describe (Pág. 2, Reiv. 1). En este contexto, la síntesis de los *carbon-dots* es meramente accesorio y se realiza mediante un proceso hidrotermal convencional en lote, utilizando ácido cítrico y etilendiamina en medio acuoso. Se trata de una técnica ampliamente conocida en la literatura, sin que D2 aporte una contribución metodológica novedosa ni reivindique dicha etapa como elemento central.

Además, el Examinador afirma que la única diferencia entre el método de síntesis descrito en D2 y el método definido en la reivindicación 1 de la presente solicitud radica en la etapa de descarga del flujo de material proveniente del reactor tubular PFR hacia un reactor CSTR, sin evidencia de que tal modificación implique un efecto técnico distinto.

El Solicitante disiente respetuosamente de tal afirmación ya que, al comparar ambos métodos de síntesis, queda evidente que **son procesos sustancialmente distintos**. En primer lugar, el proceso conforme a la solicitud actual emplea como fuente de carbono no solo ácido cítrico, sino que también puede realizarse a partir de glucosa, aspecto que D2 no menciona. Asimismo, D2 señala que la solución básica es etilendiamina, mientras que la solicitud utiliza hidróxido de amonio y urea, que tampoco han sido contemplados como reactantes en el método de D2.

Además, la reacción descrita en D2 para la formación de los *carbon-dots* ocurre mediante una metodología de lote, en donde los reactivos son adicionados conjuntamente al solvente en un único reactor y sometidos a calentamiento hasta la formación del producto. **El documento no presenta evidencia alguna de la utilización de la configuración en flujo continuo que implica un reactor tubular de flujo pistón (PFR) seguido de un reactor de tanque agitado continuo (CSTR)**; tampoco describe etapas integradas de adición de dopantes en el mismo proceso, recirculación de la fracción no convertida o separación entre el producto terminado y el producto incompleto.

Así, no se trata de una simple adaptación puntual de un método conocido para obtener una alternativa equivalente. **La metodología descrita en la presente solicitud de patente constituye, por el contrario, una evolución técnica más compleja, en la que una ruta clásica de lote se convierte en un proceso continuo en flujo, apto para proporcionar mayor eficiencia operacional, adecuación a la producción continua a gran escala y mejores condiciones para obtener un producto más uniforme.**

Respecto al efecto técnico, el Examinador sostiene que: *“No hay evidencia de que el incluir específicamente la etapa de descargar el flujo de material que sale del reactor tubular en un reactor de tanque agitado continuo, bajo el mismo patrón de calentamiento, proporcione un método con algún efecto técnico diferente al que ya proporcionaba el objeto de D2, puesto que puntos cuánticos de carbono con alta intensidad de fluorescencia ya habían sido previstos en dicho documento (resumen) y en la descripción tampoco se evidencia un efecto asociado con esta etapa.”*

En este caso, el análisis de nivel inventivo realizado en el oficio **incurre en un error metodológico, consistente en aplicar un razonamiento en retrospectiva (“hindsight”)**, lo cual resulta contrario a las directrices establecidas tanto en la Guía para el examen de patentes de la Superintendencia de Industria y Comercio, en su

numeral 2.13 (pp. 71–79)¹, como en el Manual Andino para el examen de patentes, numeral 11 (pp. 184–205)². De acuerdo con estas directrices, el examen de nivel inventivo debe realizarse evitando cualquier reconstrucción *ex post facto* de la invención, debiendo el Examinador situarse en la perspectiva de una persona normalmente versada en la materia en la fecha de presentación, sin conocimiento de la solución reivindicada. En particular, la Guía establece que el análisis no puede apoyarse en un conocimiento posterior ni en la propia invención para demostrar su obviedad, prohibiendo expresamente el uso de razonamientos retrospectivos o reconstrucciones artificiales basadas en el resultado mismo de la invención. En el mismo sentido, el Manual Andino precisa que **el Examinador no debe evaluar la inventiva con conocimiento del resultado ni partir de la solución técnica reivindicada**, sino exclusivamente del estado de la técnica existente antes de la fecha de presentación, evitando cualquier análisis *ex post facto*.

Sin embargo, en el análisis objeto de respuesta, **el Examinador parte de una caracterización de la invención centrada en el resultado final**, es decir, la obtención de puntos cuánticos de carbono con alta fluorescencia (como se refiere en el párrafo citado del oficio de manera textual) **y es a partir de dicho resultado que concluye que no existe un efecto técnico adicional frente a D2**. Este enfoque constituye precisamente un ejemplo de **análisis retrospectivo**, en la medida en que toma como punto de partida la solución aportada por la solicitud y la utiliza para reconstruir artificialmente el estado de la técnica, en lugar de identificar objetivamente las diferencias técnicas reales y evaluar su contribución desde la perspectiva del experto sin conocimiento previo de la invención. Conforme a las reglas del método problema-solución, tal aproximación es incorrecta, ya que **el problema técnico debe formularse sin incluir elementos de la solución** ni resultados obtenidos, debiendo derivarse exclusivamente de las diferencias técnicas entre la invención y el estado de la técnica más cercano.

Adicionalmente, el planteamiento del Examinador incurre en un segundo error al formular inadecuadamente el problema técnico objetivo. Al equiparar implícitamente la invención con la mera obtención de puntos cuánticos de carbono, **el análisis reduce el problema técnico a uno ya resuelto en el estado de la técnica**,

¹ Superintendencia de Industria y Comercio. (s.f.). *Guía para el examen de solicitudes de patente de invención y modelo de utilidad* (Dirección de Nuevas Creaciones). Superintendencia de Industria y Comercio.

² Comunidad Andina. (2022). *Manual andino para el examen de patentes* (Secretaría General de la Comunidad Andina).

particularmente en D2, lo que inevitablemente conduce a una conclusión de obviedad. Sin embargo, tanto la Guía como el Manual Andino establecen que **el problema técnico debe reflejar el efecto técnico derivado de las características diferenciadoras de la invención**, y no un resultado genérico previamente conocido. En este caso, la invención no se limita a obtener puntos de carbono, sino que propone un proceso específico que permite controlar simultáneamente la nucleación, el crecimiento y la funcionalización superficial de dichas nanopartículas, lo que conduce a propiedades estructurales, ópticas y funcionales particulares que no están contempladas en el estado de la técnica citado.

En relación con lo anterior, el análisis impugnado omite considerar adecuadamente el efecto técnico atribuible a las diferencias técnicas introducidas por el proceso reivindicado. De acuerdo con la doctrina andina y las directrices de la SIC, **el examen de nivel inventivo debe centrarse precisamente en determinar si las características diferenciadoras producen un efecto técnico verificable** y no evidente, **ya que es dicho efecto el que permite definir correctamente el problema técnico objetivo y evaluar la obviedad de la solución**. Asimismo, dichas directrices establecen que las características técnicas deben analizarse en conjunto y no de manera aislada, ya que la actividad inventiva puede residir en la interacción funcional entre ellas. No obstante, el Examinador evalúa de manera fragmentada una de las etapas del proceso (la transición del reactor tubular al CSTR), sin considerar la configuración integral del sistema y la interacción entre sus etapas, lo que desvirtúa el análisis técnico requerido.

Por el contrario, a partir de la descripción de la solicitud, particularmente de los ejemplos experimentales y las figuras asociadas, se evidencia que el proceso reivindicado permite obtener nanopartículas con propiedades técnicas específicas, tales como un tamaño controlado y distribución homogénea, la incorporación efectiva de dopantes en la estructura carbonosa, propiedades ópticas diferenciadas que incluyen absorción en la región verde del espectro y emisión en longitudes de onda específicas, así como estabilidad coloidal en condiciones de alta fuerza iónica. De manera particularmente relevante, **se demuestra que estas características se traducen en efectos técnicos cuantificables en aplicaciones agrícolas, incluyendo incrementos significativos en la eficiencia fotosintética y en el uso del agua**. Tales resultados constituyen efectos técnicos verificables y funcionales, directamente atribuibles al control del proceso de síntesis, y no meras ventajas abstractas o especulativas.

En ese sentido, la invención reivindicada tampoco puede considerarse una mera alternativa de diseño basada en la selección arbitraria de configuraciones de reactores, sino que define un proceso integrado que permite obtener nanopartículas tipo *carbon-dots* con propiedades fisicoquímicas y funcionales específicas, las cuales no son predecibles a partir del estado de la técnica citado.

En particular, **la configuración del proceso**, que comprende la dosificación controlada de precursores, su procesamiento bajo flujo continuo **en un reactor tubular de tipo flujo pistón (PFR) bajo condiciones de temperatura y presión controladas, seguido de un reactor de tanque agitado continuo (CSTR) con recirculación y separación, permite un control simultáneo de la nucleación, el crecimiento y la funcionalización superficial de las nanopartículas**. Este control estructural y superficial es determinante en la obtención de materiales con propiedades diferenciadas.

La descripción demuestra que las nanopartículas obtenidas mediante este proceso presentan tamaño controlado y morfología homogénea (≈ 5 nm), como se evidencia en el Ejemplo 2 y en las Figuras asociadas de microscopía electrónica. Asimismo, se evidencia la incorporación efectiva de dopantes como fósforo dentro de la matriz carbonosa, según análisis XPS, como se muestra en la Figura 7 incluida a continuación, lo que demuestra que el proceso no solo genera partículas, sino que controla su composición estructural. Más aún, el proceso permite generar nanopartículas con propiedades ópticas específicas, caracterizadas por una absorción en la región verde (~ 532 nm) y emisión alrededor de 645 nm. Esta propiedad es particularmente relevante, dado que corresponde a una región espectral pobremente aprovechada por la clorofila natural, lo que **permite un mejor aprovechamiento de la radiación solar incidente y un aumento directo en la eficiencia fotosintética de las plantas**.

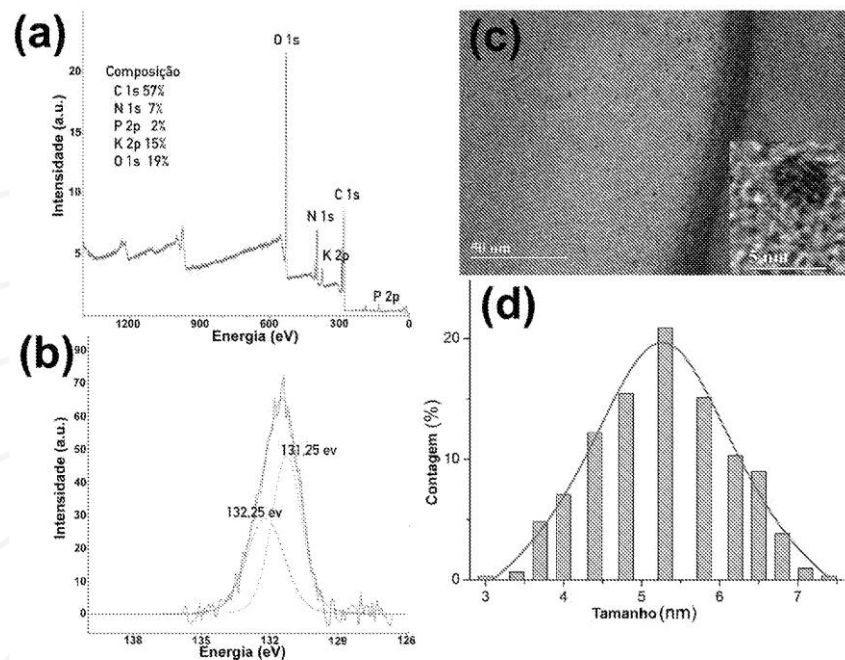


Figura 7

Estos efectos no son meramente teóricos, sino que han sido demostrados experimentalmente. Tal como se evidencia en las Figuras 13 y 14, que se anexan a continuación, en donde se demuestra que, el uso de los *carbon-dots* obtenidos mediante el proceso reivindicado conduce a **un incremento significativo en la tasa fotosintética y en la eficiencia del uso del agua en cultivos agrícolas, alcanzando mejoras del orden de hasta un 60% en fotosíntesis y aproximadamente un 50% en eficiencia hídrica.**

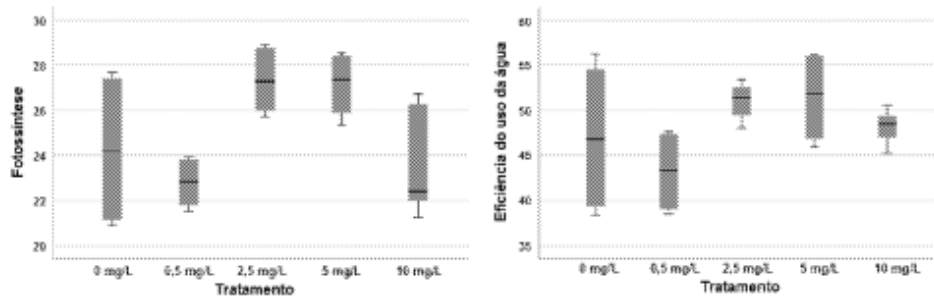


Figura 13

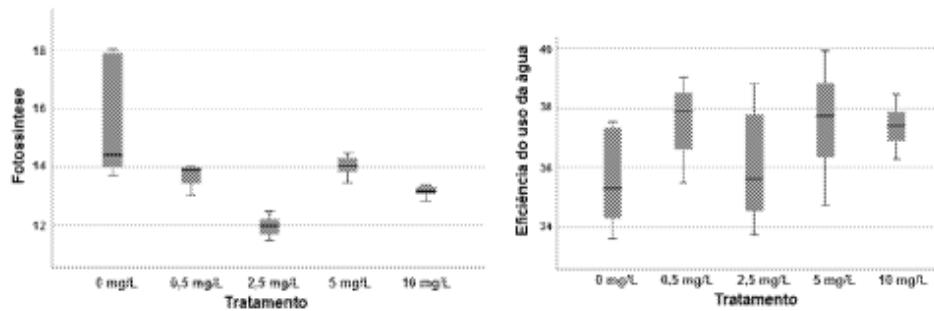


Figura 14

De manera consistente, en otros sistemas experimentales se observa también **un aumento significativo en la fotosíntesis neta y en la eficiencia del uso del agua** como queda claro en la Figura 18 que se incluye a continuación.

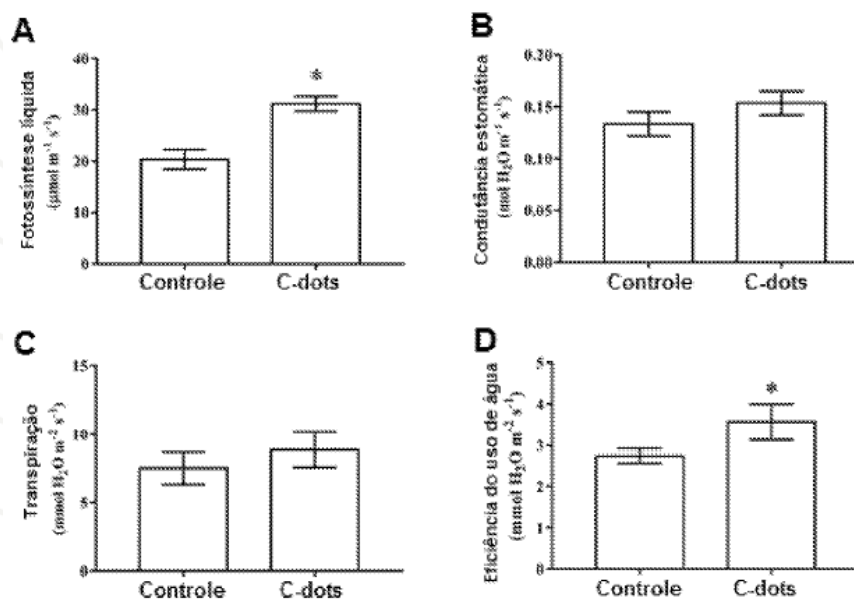


Figura 18

Adicionalmente, las nanopartículas obtenidas presentan **alta estabilidad coloidal incluso en condiciones de elevada fuerza iónica**, lo cual ha sido demostrado mediante mediciones de potencial zeta y diámetro hidrodinámico como se evidencia en la Figura 8, que se incluye enseguida, **evidenciando la capacidad del sistema para actuar como vehículo eficiente de nutrientes**. Este comportamiento está directamente relacionado con la presencia de grupos funcionales superficiales controlados durante el proceso de síntesis.

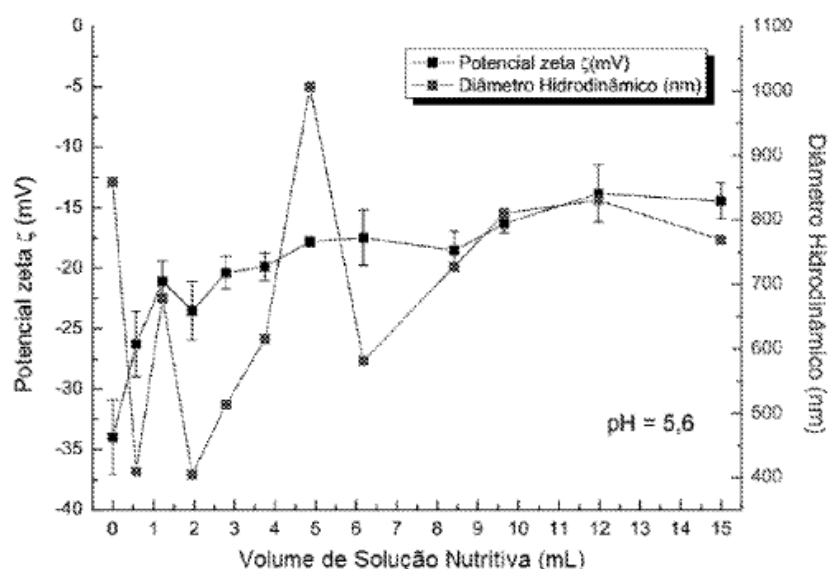


Figura 8

Por tanto, se observa una relación causal directa entre las condiciones del proceso reivindicado y las propiedades finales del material obtenido, las cuales a su vez determinan efectos técnicos concretos y cuantificables en aplicaciones agrícolas. Ni el documento D2 ni el documento D3 enseñan ni sugieren que la combinación específica de etapas de proceso reivindicadas permita obtener partículas con estas propiedades espectrales, estructurales y funcionales, y mucho menos que dichas propiedades conduzcan a mejoras significativas en variables fisiológicas de las plantas, tales como la eficiencia fotosintética o el uso del agua.

En consecuencia, la invención no puede ser considerada como una mera combinación de características conocidas, sino que constituye una solución técnica no evidente que permite obtener un material con propiedades emergentes derivadas del control específico del proceso, las cuales no serían previsibles para una persona versada en la materia a partir del estado de la técnica citado.

Ahora bien, continuando con el análisis del Examinador, en lo que respecta al documento D3, titulado “*Continuous Multistage Synthesis and Functionalization of Sub-100 nm Silica Nanoparticles in 3D-Printed Continuous Stirred-Tank Reactor Cascades*”, que describe un método de preparación de nanopartículas a partir de cascadas de reactores CSTR y que fue indicado por el Examinador como documento complementario apto para suplir la etapa de descarga del flujo de material proveniente del reactor tubular PFR hacia un reactor CSTR, que no estaría descrita en D2. Es a lugar disentir de tal afirmación, en la medida que, **la metodología descrita en D3 no emplea un reactor tubular de flujo pistón (PFR), sino cascadas sucesivas de reactores CSTR en serie**; entonces, lo que se describe en D3 son etapas de descarga de material de un reactor CSTR hacia otro reactor CSTR.

Para iniciar la discusión acerca de las diferencias entre D3 y el presente invento, establecemos aquellas que son más obvias. El documento D3 describe una metodología para la síntesis de nanopartículas de SiO₂, que son partículas inorgánicas de silicio, sintetizadas a partir de la hidrólisis de tetraortosilicato (TEOS), un compuesto organometálico, utilizando una solución básica de amoníaco. En cambio, la metodología descrita en la presente solicitud de patente está destinada a la síntesis de *carbon-dots*, nanopartículas de carbono sintetizadas a partir de la condensación, polimerización y carbonización de fuentes orgánicas de carbono.

Aunque la presencia de amoníaco en ambas reacciones pueda sugerir una similitud entre ellas, su función química en cada reacción es sustancialmente distinta. **En D3, el amoníaco actúa en la hidrólisis alcalina del TEOS**, una reacción química de ruptura de moléculas utilizando agua en presencia de iones hidroxilo. **En el presente invento, el amoníaco actúa en reacciones de condensación**, que son reacciones químicas que unen moléculas liberando agua, haciendo que la estructura de la fuente de amoníaco incorpore el producto.

En ese sentido es oportuno señalar que, las reacciones de condensación como parte de las metodologías de síntesis “*bottom up*” de *carbon-dots* ya están descritas en la literatura, se trae como ejemplo los artículos “*Hydrothermal Synthesis of Photoluminescent Nanocarbon from Hydroxylic Acids and Amines*” (*J. Solut. Chem.* 2016, 45, 1560-1570) en el cual se refiere la formación de puntos cuánticos de carbono dopados con nitrógeno (NCD) con luminiscencia azul mediante una reacción de policondensación entre un grupo carboxilo y un grupo amino, resultado de la deshidratación de aminoácidos mediante tratamiento hidrotermal que da lugar a la formación de polímeros de gran tamaño. como se ilustra en la imagen a continuación (3.3 Formation mechanism of NCDs from amino acids y Fig. 4)

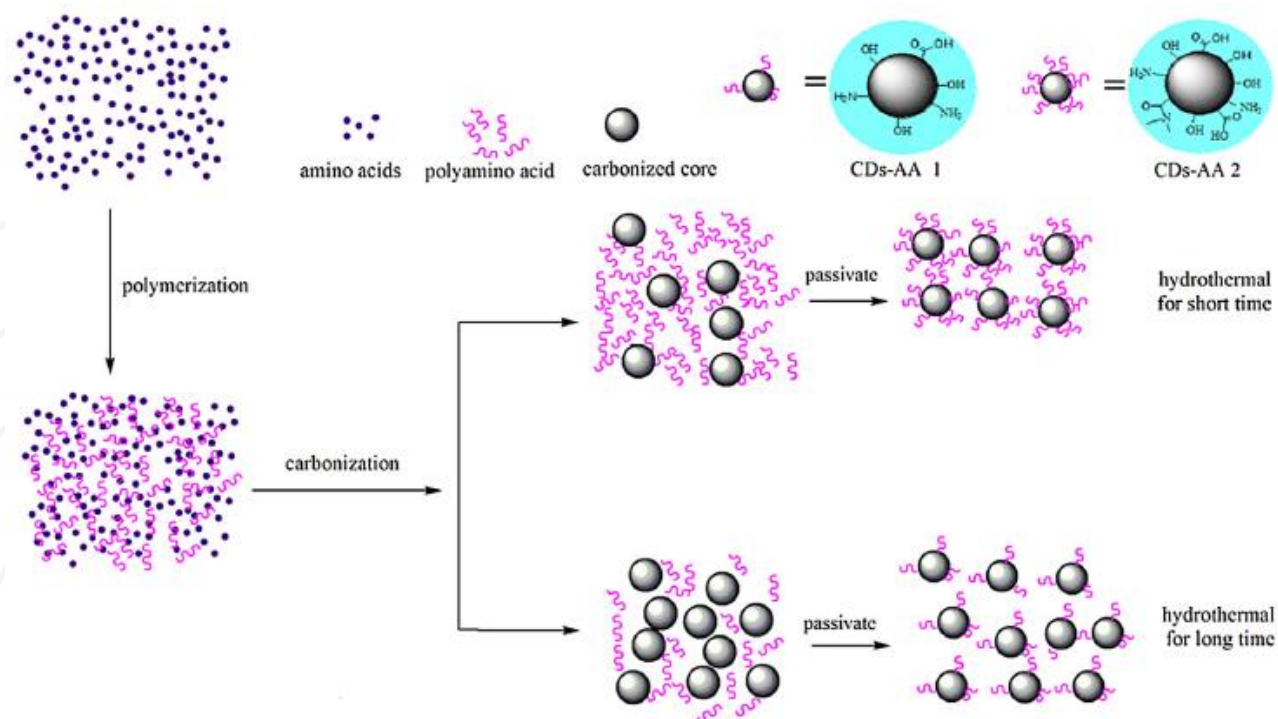


Fig. 4. Schematic formation of NCDs-Asp through dehydration, polymerization, carbonization and passivation.

Asimismo, el artículo *"Kinetics of nitrogen-doped carbon dot formation via hydrothermal synthesis"* (New J. Chem. 2016, 40, 5555-5561) en donde se describe la formación de *carbon dots* dopados con nitrógeno (N-CDs) a partir de ácido cítrico y urea mediante calentamiento en agua como solvente en un reactor cerrado a temperaturas de 130-180 °C tal y como se esquematiza en la Figura 8 que se incluye a continuación:

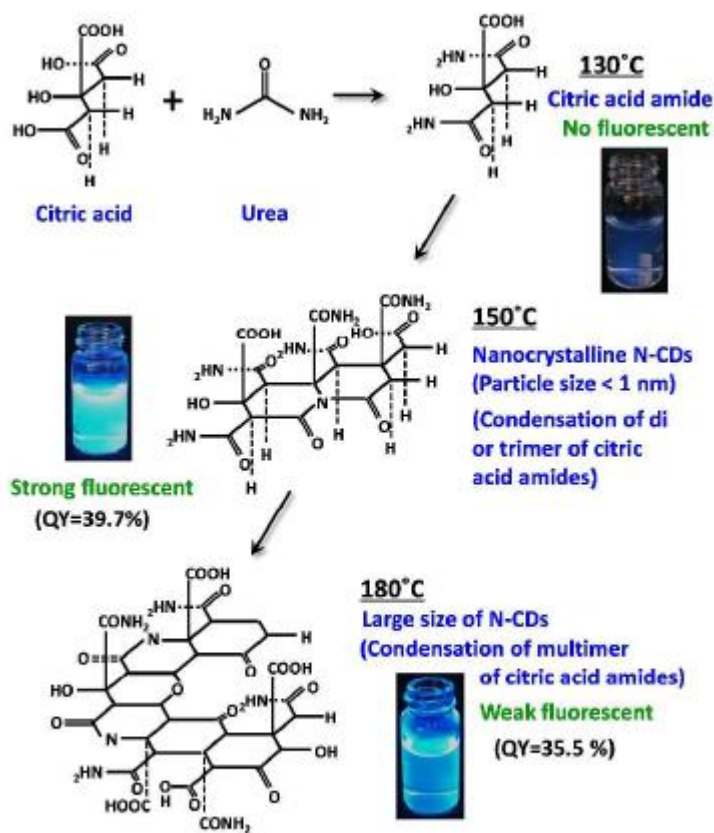


Fig. 8 Proposed mechanism of the formation of N-CDs via hydrothermal reaction of citric acid and urea.

A pesar de que D3 no utilice un reactor tubular PFR, si es capaz de desarrollar un proceso de síntesis en flujo, con todos los beneficios que acompañan a ese tipo de proceso; sin embargo, esto se debe a que **el proceso de D3 opera a escala miniaturizada, con cada reactor teniendo apenas 3.2 mL** y, aunque esto parezca una diferencia fácilmente superada mediante el escalado del proceso con reactores de volúmenes mayores, **el propio autor deja claro que el bajo volumen de los reactores es lo que los vuelve eficientes**. Esto porque uno de los puntos más importantes de la metodología de D3 es posibilita parametrizar reacciones, pero esto no es posible en la producción a gran escala.

En los pasajes: *“Importantly, 3-D printing allows for minimizing the chamber volume, and correspondingly the reagent consumption during parametric screening...”* (Introducción, pág. 6700) y *“Last, the synthesis is typically performed in laboratory flask-reactors, with inherent limitations regarding spatial/temporal variations of parameters inside the reaction mixture due to large operating volumes and the necessity to perform multistep chemistries”* (Pág. 6699, Introducción), el autor hace

explicito lo analizado anteriormente con relación a la necesidad de miniaturizar los reactores y, por ende, la reacción, para obtener los resultados deseados. Además, en los pasajes *“Specifically, the homogeneous concentration and temperature profiles, realized by strong agitation in each chamber, can result in nearly ideal mixing and accurate predictability of reaction conversions.”* (Introducción, pág. 6700), *“In addition, by using eight miniature CSTRs in series, the RTD profile becomes sharp, eliminating the tail of the outlet concentration profile because the CSTR module approaches the behavior of a plug flow reactor.”* y *“The nearly ideal CSTR cascade performance is essential for the design of NPs with narrow size distributions”* (Resultados y discusión; Pág. 6702), se aclara que, además de la disposición de reactores en serie, **la miniaturización es una parte integral de la aproximación del proceso al comportamiento de un reactor de flujo continuo PFR**. Este comportamiento depende de la discretización del sistema en múltiples etapas de pequeño volumen, donde, a menor volumen de las etapas, el proceso se aproxima más al comportamiento de un reactor PFR.

Por lo tanto, **el escalado a reactores de mayor volumen distanciaría cada vez más la reacción del comportamiento de un reactor PFR**. El equilibrio de temperatura y el ajuste fino del final de la reacción para controlar la etapa de crecimiento de la partícula, y, en consecuencia, el tamaño del producto, **se volverían menos eficientes a medida que se incrementa el volumen de los reactores**.

En conclusión, **D3 no describe la etapa específica** invocada por el Examinador, ya que se refiere a un material nanoparticulado de naturaleza química distinta y depende, en gran medida, de una configuración miniaturizada que no se puede trasladar fácilmente a una escala mayor. Por esta razón, dicho documento no hace obvia la metodología reivindicada en la presente solicitud de patente.

En este sentido, conforme al método de problema-solución, la correcta formulación del problema técnico debería orientarse a proporcionar un proceso capaz de controlar las etapas de nucleación, crecimiento y funcionalización de *carbon dots* de manera que se obtengan nanopartículas con propiedades estructurales y ópticas específicas que mejoren la eficiencia fotosintética.

Desde esta perspectiva, ni el documento D2 ni el documento D3 sugieren o motivan a una persona normalmente versada en la materia a adoptar la combinación específica de condiciones y etapas reivindicadas para alcanzar dicho efecto técnico. En particular, D2 se limita a describir la síntesis de puntos de carbono sin un control estructural y funcional comparable, mientras que D3 se refiere a configuraciones de

reactores sin relación con la obtención de nanopartículas con propiedades ópticas o biológicas específicas. **En consecuencia, la solución reivindicada no puede considerarse evidente a la luz del estado de la técnica.**

Finalmente, es importante destacar que, tras las modificaciones introducidas en las reivindicaciones, **el proceso actualmente definido incorpora de manera explícita todas las características técnicas esenciales que permiten establecer la relación causal entre las condiciones de operación del sistema y las propiedades del material obtenido.** Por ello, no es procedente calificar la invención como una mera alternativa de diseño carente de efecto técnico, ya que dicha afirmación ignora tanto la evidencia experimental contenida en la descripción como los criterios metodológicos establecidos en la normativa aplicable. Por el contrario, la invención se configura como una solución técnica que produce un efecto técnico verificable y no evidente, cumpliendo así con el requisito de nivel inventivo establecido en el artículo 18 de la Decisión 486.

Con base en los comentarios anteriores, las presentes reivindicaciones son inventivas respecto a las referencias citadas. El Solicitante solicita respetuosamente que se retire esta objeción.

Consecuentemente, la presente solicitud cumple con el requisito de nivel inventivo establecido en el artículo 18 de la Decisión 486.

Habiéndose superado todas las objeciones formuladas por el Examinador, se solicita respetuosamente que se proceda con la pronta concesión de la patente.

5. Conclusión

En vista de las modificaciones introducidas en esta oportunidad y los argumentos que se presentan, es posible establecer que las reivindicaciones cumplen con los requerimientos de la Decisión 486.

6. Anexos

- Nuevo pliego reivindicatorio conformado por tres (3) reivindicaciones.
- Copia del recibo por concepto de modificaciones a las reivindicaciones.
- Artículo: Yang, J., Chen, W., Liu, X., Zhang, Y., & Bai, Y. (2017). Hydrothermal synthesis and photoluminescent mechanistic investigation of highly fluorescent nitrogen doped carbon dots from amino acids.

Materials Research Bulletin, 89, 26–32.

<https://doi.org/10.1016/j.materresbull.2017.01.013>

- Artículo: Ogi, T., Aishima, K., Permatasari, F. A., Iskandar, F., Tanabe, E., & Okuyama, K. (2016). Kinetics of nitrogen-doped carbon dot formation via hydrothermal synthesis. *New Journal of Chemistry*. <https://doi.org/10.1039/C6NJ00009F>

Señora Directora,



Margarita Castellanos Abondano

C.C. No. **39.779.654** de Bogotá

T.P.A. No. **70.819** del C.S.J.

Calle 117 No. 5A-35 - Bogotá, D.C.

Teléfono: 7560770

Bogotá, D.C.
MCA/pm/jms
P5813



Hydrothermal synthesis and photoluminescent mechanistic investigation of highly fluorescent nitrogen doped carbon dots from amino acids



Juan Yang¹, Weilong Chen¹, Xueping Liu, Yao Zhang, Yan Bai*

Department of Chemistry, Jinan University, Guangzhou 510632, People's Republic of China

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ABSTRACT

A green and facile method was developed to synthesize nitrogen doped carbon dots (NCDs) with blue-luminescence by hydrothermal treatment of different amino acids (aspartic acid, glutamic, proline, threonine, valine, phenylalanine, arginine and serine). The NCDs were characterized by various methods and the highest quantum yield reached 41.3%. The formation and surface passivation of NCDs were accomplished simultaneously via dehydration, polymerization, carbonization, and passivation process. The passivation degree of NCDs was controlled by the hydrothermal time. NCDs had good aqueous dispersibility, high stability, and preeminent photoluminescent properties. The emission of NCDs was highly dependent on excitation energy, as the excitation wavelength increased from 300 to 470 nm, the emission peak position shifted from blue to green region with the intensity decreased. These features resulted from the fact that the surface passivation could form the additional interband and we explained the relationship between luminescent properties and energy band in detail.

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1. Introduction

Fluorescent carbon dots (CDs) have received considerable attention because of their attractive photoelectric properties and potential applications in the fields of bioimaging [1,2], detecting [3–5], diagnosing [6], and catalysis [7] etc. Compared to organic dyes and semiconductor quantum dots, CDs offer the advantages of low toxicity, excellent biocompatibility, and good stability for biological application [8]. Thus, they have been proposed to be promising substitutes of fluorescent semiconductor quantum dots in the future.

CDs are generally consisted of three common elements: carbon, hydrogen and oxygen [9]. Some protein-rich biomass materials [3], and carbohydrates [10] usually used as the precursors for the synthesis of CDs. Moreover, hetero atoms (N and S) doped in CDs, especially N could effectively improve the fluorescence emission and introduce some new surface states [11,12]. Zhang et al. reported the CDs with different N contents synthesized by using CCl_4 and NaNH_2 as starting materials, showed high quantum yields (QY) [13].

Amino acids, biologically important organic compounds possessing both amino and carboxyl groups, could act as perfect molecular precursor for synthesizing N doped carbon dots (NCDs). Hsu et al. reported a simple hydrothermal approach for the preparation of CDs from glycine and the quantum yields reached up to 30.6% [14]. Meanwhile, Jiang et al. likewise used amino acids (histidine and alanine) as the source for producing NCDs by microwave irradiation in the presence of acid or alkali [15]. Furthermore, Yang' group reported a hydrothermal approach for the preparation of NCDs from L-tryptophan and L-serine in the weak alkaline water, whose highest QY reached 61.12% in a low pH [16] and their latest study about SNCDs showed that the chemical structure and photoluminescence (PL) were heavily influenced by the synthesis pH [17]. Although great efforts have been made to synthesize NCDs from N containing organic compounds, the systematic on using different kinds of amino acids to synthesis NCDs were still limited. Therefore, developing a simple, convenient and friendly route for synthesizing of NCDs from different amino acids is a worthy project.

Herein, we developed a one-step hydrothermal method for synthesizing highly fluorescent N doped carbon dots from amino acids. The different types of amino acids were used as carbon sources as well as passivating agents for synthesizing NCDs without any other additives, and a family of NCDs obtained from

* Corresponding author.

E-mail address: tbaiyan@jnu.edu.cn (Y. Bai).

¹ These authors contributed equally to this work.

the corresponding amino acids. Our results revealed that the surface of NCDs was passivated by amounts of carboxyl, hydroxyl and amino groups at their surface, and the NCDs exhibited good water soluble and stability as well as high QY. The mechanism behind the green and blue PL was also discussed.

2. Experiment section

2.1. Chemicals and materials

Aspartic acid ($C_4H_7NO_4$, Asp), glutamic ($C_5H_9NO_4$, Glu), proline ($C_5H_9NO_2$, Pro), threonine ($C_4H_9NO_3$, Thr), valine ($C_5H_{11}NO_2$, Val), phenylalanine ($C_9H_{11}NO_2$, Phe), arginine ($C_6H_{11}N_4O_2$, Arg) and serine ($C_3H_7NO_3$, Ser) were from Shanghai Bio Science & Technology Co. Ltd. (China). Sodium chloride, sodium hydroxide and hydrochloric acid were bought from Guangzhou Chemical Reagent Factory (China). All of the chemicals used in this work were analytical grade and used as received without further purification. Water was purified using a Milli-Q-system (Millipore, Bedford, MA, USA).

2.2. Characterization

Transmission electron microscope (TEM) images were obtained from JEM-2100F transmission electron microscope (JEOL Ltd., Japan) at an acceleration voltage of 200.00 kV. Atomic force microscope (AFM) images were taken in Bioscope CatylystNano-scope-V atomic force microscope (Bruker, USA). X-ray diffraction (XRD) patterns were obtained from a MSAL-XD2 X-ray diffraction (Beijing, China). The Fourier transform infrared spectra (FTIR) were measured by a Nicolet 6700 spectrometer (Bruker, USA). Fluorescence measurements were performed on a 970 CRT fluorescence spectrophotometer (Shanghai, China). UV–visible absorption spectra were recorded by a Cary 5000 UV–visible spectrophotometer (Varian, USA). Element analysis were measured by a EA2400 II CHNS/O Elemental Analyzer (PE, USA). The experimental characterizations were performed at room temperature.

2.3. Synthesis and functionalization of NCDs from amino acids

The fluorescent NCDs were synthesized by one-step hydrothermal method. In a typical synthesis, 0.1331 g Asp was dissolved into 20 mL water and stirred vigorously for 20 min. Then transferred the

clear solution into 50 mL Teflon-lined stainless-steel autoclave and heated at 180 °C for a fixed time. After cooling to room temperature, the centrifugation (11,000 rpm, 30 min) was followed and the supernatant containing fluorescent NCDs-Asp was collected. Finally, the products were collected by dialysis against deionized water through a dialysis membrane (500 MWCO) for 24 h. The powder of NCDs-Asp was obtained by lyophilisation. Other fluorescent NCDs, such as NCDs-Val, NCDs-Phe, NCDs-Arg, NCDs-Pro, NCDs-Thr, NCDs-Ser, NCDs-Glu, were similarly synthesized.

3. Result and discussion

3.1. Characterization of NCDs formed from amino acids

The formation of NCDs was confirmed by AFM and TEM measurements. AFM images of NCDs-Asp and NCDs-Glu are shown in Fig. 1. The topographic heights NCDs-Asp were mostly less than 1.5 nm, with an average height of about 1.0 nm (Fig. 1a and b), while the average heights of NCDs-Glu was 2.0 nm with a distribution in the range of 1.0–2.5 nm (Fig. 1c and d). The results suggested that NCDs-Asp were single-layered, similar to those observed in functionalized graphene CDs with only one layer, while NCDs-Glu were multi-layered graphene CDs (2–3 layers) [18–20]. TEM images of NCDs-Asp and NCDs-Glu are shown in Fig. 1(e and g), the NCDs were uniform and monodisperse nanoparticles with near spherical shape and an interlayer spacing of about 0.21 nm (NCDs-Asp, insert in Fig. 1e) and 0.23 nm (NCDs-Glu, insert in Fig. 1g) which was close to the (102) graphite peak, indicating the formation of the graphite structures [21]. Meanwhile, a statistical histogram of NCDs size distributions was in a narrow range, varying from 2.8–6.3 nm (NCDs-Asp, Fig. 1f) and 3.0–5.0 nm (NCDs-Glu, Fig. 1h).

Furthermore, the XRD and FTIR analysis of products in different preparation stages was carried out. As shown in Fig. 2, a series of significant changes in XRD patterns of products were observed at different hydrothermal time. For example, in Fig. 2a, some peaks in the XRD pattern of Asp disappeared gradually with the hydrothermal time increasing, leaving only one broad and weakened peak at around 25° for 18 h, which matched up with (002) facet of the graphite-like structure (PDF 08-0415) [15,22]. The result suggested that Asp gradually transformed into the NCDs-Asp which had the thin structure and was also consistent with the AFM result.

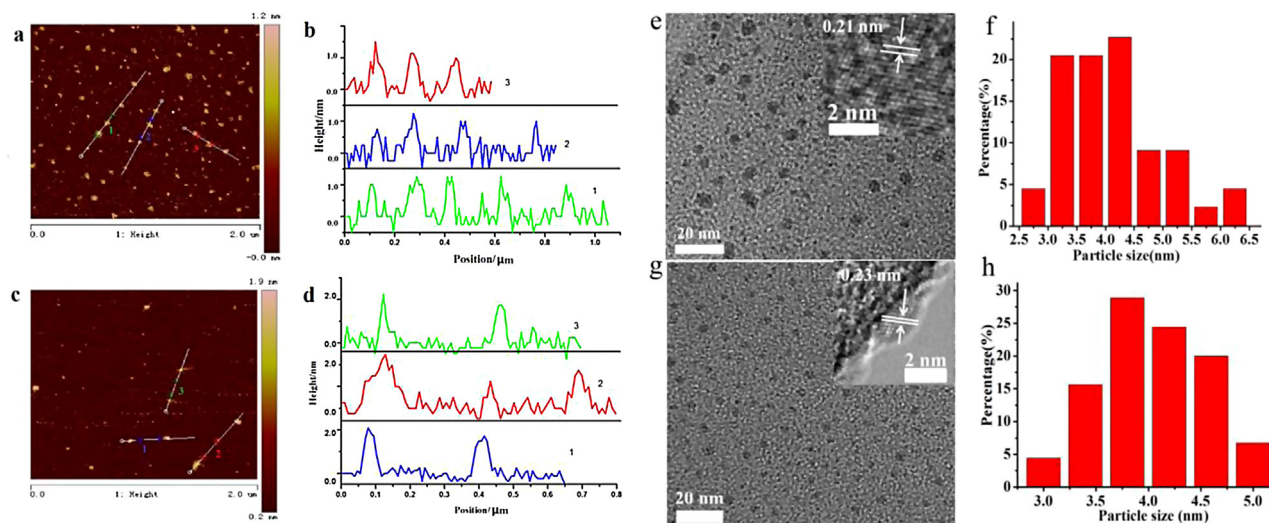


Fig. 1. AFM images of NCDs-Asp (a) and NCDs-Glu (c) on mica substrate; Height profiles of NCDs-Asp (b) and NCDs-Glu (d) corresponding to the AFM image. TEM images of NCDs-Asp (e) and NCDs-Glu (g) and size distribution histograms of NCDs-Asp (f) and NCDs-Glu (h).

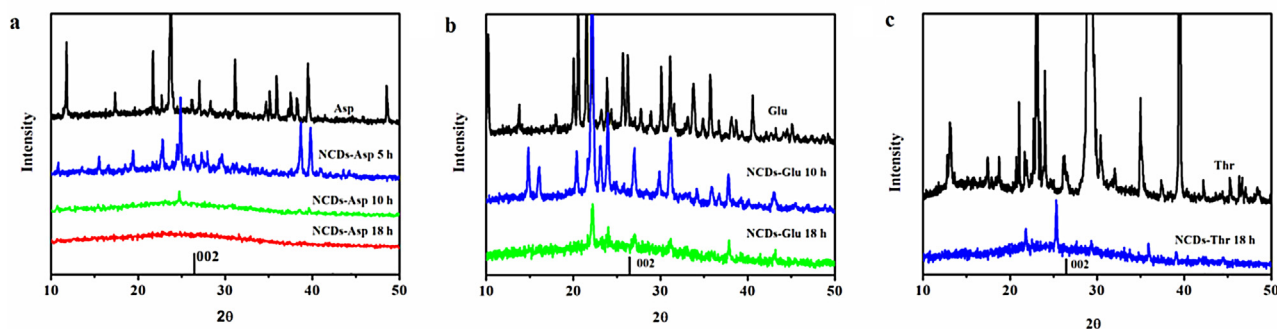


Fig. 2. XRD patterns of amino acids (Asp, Glu, Thr) and corresponding NCDs in different hydrothermal time. (a) Asp and NCDs-Asp synthesized by hydrothermal treatment 5, 10 and 18 h; (b) Glu and NCDs-Glu synthesized by hydrothermal treatment 10 and 18 h; (c) Thr and NCDs-Thr synthesized by hydrothermal treatment 18 h. Vertical lines show the positions of graphite (PDF 08-0415).

Compared with NCDs-Asp, peaks of amino acids were still observed in the XRD patterns of some NCDs for hydrothermal treatment 18 h, such as NCDs-Glu (Fig. 2b), NCDs-Thr (Fig. 2c). These results showed that the time played a pivotal role in the hydrothermal synthesis of NCDs at 180 °C.

Chemical and structural information about the products was also obtained by the FTIR. The spectra of amino acids and their hydrothermal products in different hydrothermal times are presented in Fig. 3 and there follows a detailed description of the hydrothermal process of Asp. As shown in Fig. 3a, when the Asp was hydrothermal treated within 5 h, the absorption bands of the products displayed several characteristics of Asp, such as the overlapping absorption band of —OH and N—H stretching vibration at 3000 cm^{-1} and C—H asymmetrical and symmetrical stretching vibration of —CH₂ at 2950 and 2850 cm^{-1} , indicating that the product contain large amounts of incompletely reacted Asp. As hydrothermal time prolonged, some absorption bands of Asp disappeared gradually and the FTIR spectra of products exhibited obvious characteristics of condensation reaction and carbonization. For example, the absorption bands around 1580 cm^{-1} and 1700 cm^{-1} were bending vibration of amide II (in-plane N—H bending) and amide I (C=O stretching vibration) and the absorption band around 1400 cm^{-1} assigned to amide III (C—N in amide bonds), which corresponding to the FTIR of GQDs [23]. In addition, a broad band around 3100–3600 cm^{-1} caused by —OH stretching vibration overlapped with N—H stretching vibration of the amide bond, which was wider than Asp and a blue shift due to the formation of the amide group. Finally, after 30 h, the main absorption bands were not changed except in the area of 400–1000 cm^{-1} , indicating that Asp has been carbonized completely in this condition [24]. These results approved that polycondensation reaction happened between carboxyl and amino groups at first, then the hydrothermal products enriched. Other

NCDs made from amino acids also showed the similar results, such as NCDs-Glu and NCDs-Thr (Fig. 3b and c).

3.2. Measurement for quantum yield and elemental analysis

Although CDs have become attractive materials recently, their application was limited by low QY (often less than 10%) [22,25]. NCDs commonly have more perfect QY than CDs without N doping [3,26]. The compositions and QYs of the NCDs synthesized by the hydrothermal treatment 18 h at 180 °C are shown in Table 1. The N content of NCDs was more than 10% and the ratio of N/C was higher, notably, NCDs-Asp displayed the QY of 41.3% which was close to the value reported by Li et al. [27]. Meanwhile, a series of optical image of NCDs solution under 365 nm UV lamp (Fig. S1, Supporting information) also showed the bright blue luminescence which was strong enough to be easily seen with the naked eyes. In addition, the formation and surface properties of NCDs were probably responsible for the QY, so the synthesis condition, especially hydrothermal time, played an important role in this work. The hydrothermal time for 18 h could ensure to synthesize NCDs with blue-luminescence from amino acids, but not the best synthesis condition for all kinds of NCDs. For example, compared with NCDs-

Table 1
Elemental Analysis and PL QYs of NCDs (hydrothermal time 18 h).

	C (%)	H (%)	N (%)	O (%) ^a	Ratio of N/C	QY (%) ^b
NCDs-Asp	34.14	4.82	10.40	50.64	30.46	41.3 ± 0.06
NCDs-Glu	45.02	5.37	10.58	39.03	23.50	13.3 ± 0.07
NCDs-Thr	38.91	6.88	13.67	40.54	35.13	14.9 ± 0.06
NCDs-Pro	43.83	7.98	10.52	37.67	24.00	23.2 ± 0.04

^a Calculated.

^b The values = Average ± Standard deviation; Experiment number n = 3.

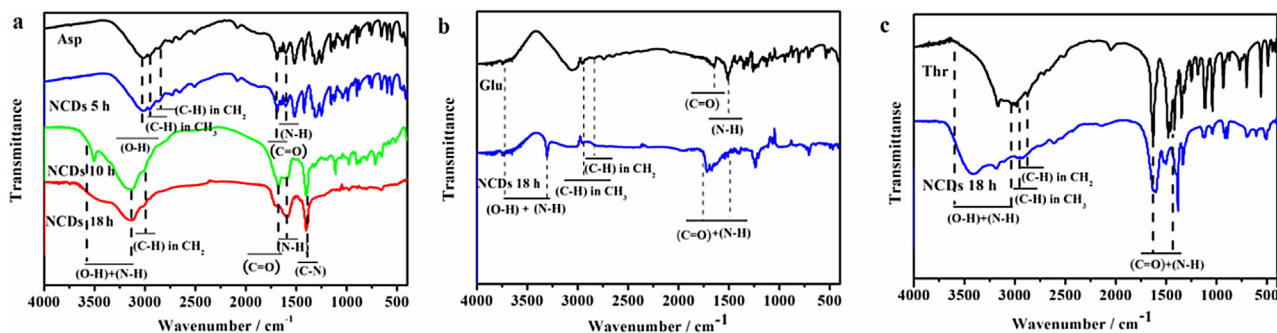


Fig. 3. FTIR spectra of amino acids and corresponding NCDs in different hydrothermal time. (a) Asp and NCDs-Asp; (b) Glu and NCDs-Glu; (c) Thr and NCDs-Thr.

Asp, luminescence intensities of NCDs-Glu and NCDs-Thr synthesized by hydrothermal treatment 18 h were not the strongest (Fig. S1), especially, NCDs-Thr presented a minor QYs though its N content and its ratio of N/C were higher than three other NCDs. The reason may be that most of the N elements were not doped into carbon framework in a hydrothermal treatment 18 h and made little contribution to improve the emission [11]. Meanwhile, the results of XRD and FTIR also showed that the degree of surface passivation for the three NCDs was different (see Figs. 2 and 3). Therefore, the QYs in Table 1 were not necessarily the highest. As described above, NCDs synthesized from different amino acids showed different QYs and the amino acid was considered to be an excellent material for NCDs.

3.3. Formation mechanism of NCDs from amino acids

The CDs commonly have a carbon core and an amorphous shell with oxygen-containing groups [11]. For the synthesizing of NCDs, amino acids were carbon and nitrogen source, as well as providing surface passivating groups such as $-\text{NH}_2$, $-\text{COOH}$ and $-\text{OH}$, thereby generating a carbon core bond with nitrogen [13] and an amorphous shell with amino groups and oxygen-containing groups. According to the proposed formation mechanism of CDs by Li's group [28], the NCDs synthesized from amino acids proceed via four stages, including dehydration, polymerization, carbonization and surface passivation as depicted in Fig. 4. Take NCDs-Asp for an example, the formation process of NCDs for the hydrothermal treatment at 180°C is described as follows. In the primary stage of hydrothermal treatment, large-sized polymers from the dehydration of amino acids were formed and polycondensation reaction between carboxyl and amino groups has been approved by FTIR spectra above. As shown in UV-vis spectra (Fig. S2, see Supporting information), in a short hydrothermal time like 3 h, an absorption band appeared in the 250–300 nm range and then it shifted to the long wavelength upon further increasing hydrothermal time, revealing the polymerization of Asp [14,29] and formation of NCDs-Asp. In addition, after 6 h treatment, the

polymers began to carbonize (Fig. S3a, see Supporting information). Subsequently, large-sized particles began to shrink by continuous carbonization process, leading to a short single burst of nucleation (Fig. S3b and c). With the prolongation of hydrothermal time, very uniform and monodisperse NCDs-Asp were obtained (Fig. S3d). Similar results have been reported by Ding's group [30]. In the process of hydrothermal treatment, N elements were doped into carbon framework to form the NCDs core with graphene-like carbon rings structures and the N-doping amount increased with prolonged hydrothermal reaction time [13]. In addition, the core of NCDs peripherally modified with amino groups and oxygen-containing groups to form the shell of surface passivation. As amino groups and oxygen-containing groups were unstable and tended to leave the surface after longer hydrothermal reaction time, the surface passivation was time-varying process. As shown in Fig. S1, the PL intensities of NCDs also changed with time and stronger fluorescence intensities were observed in the NCDs synthesized at 15–20 h, indicating that appropriate surface passivation could result in a significant increase in PL intense [27]. The NCDs had more surface traps at the beginning of the formation, then gradually tended to be perfected through modification of amino groups and oxygen-containing groups, so the PL of NCDs strengthen gradually. After a certain time, surface traps increased again as the modifying groups left the surface and the PL of NCDs weakened gradually, which indicated that NCDs were partially passivated or almost non-passivated when hydrothermally treated overtime. The dynamic change process in the formation and surface passivation of NCDs were illustrated in the XRD and FTIR. In addition, compared to the partly-passivated or almost non-passivated ones, the emission peaks of fully-passivated NCDs lightly shifted to the short wavelength direction. This phenomenon resulted from $-\text{OH}$ groups bounding on the surface of NCDs [13], because most of the $-\text{COOH}$ on the surface turned into $-\text{OH}$ in hydrothermal reaction. These results demonstrated that both the carbonization including N-doping and surface passivation were controlled by the treatment time at a constant hydrothermal temperature of 180°C .

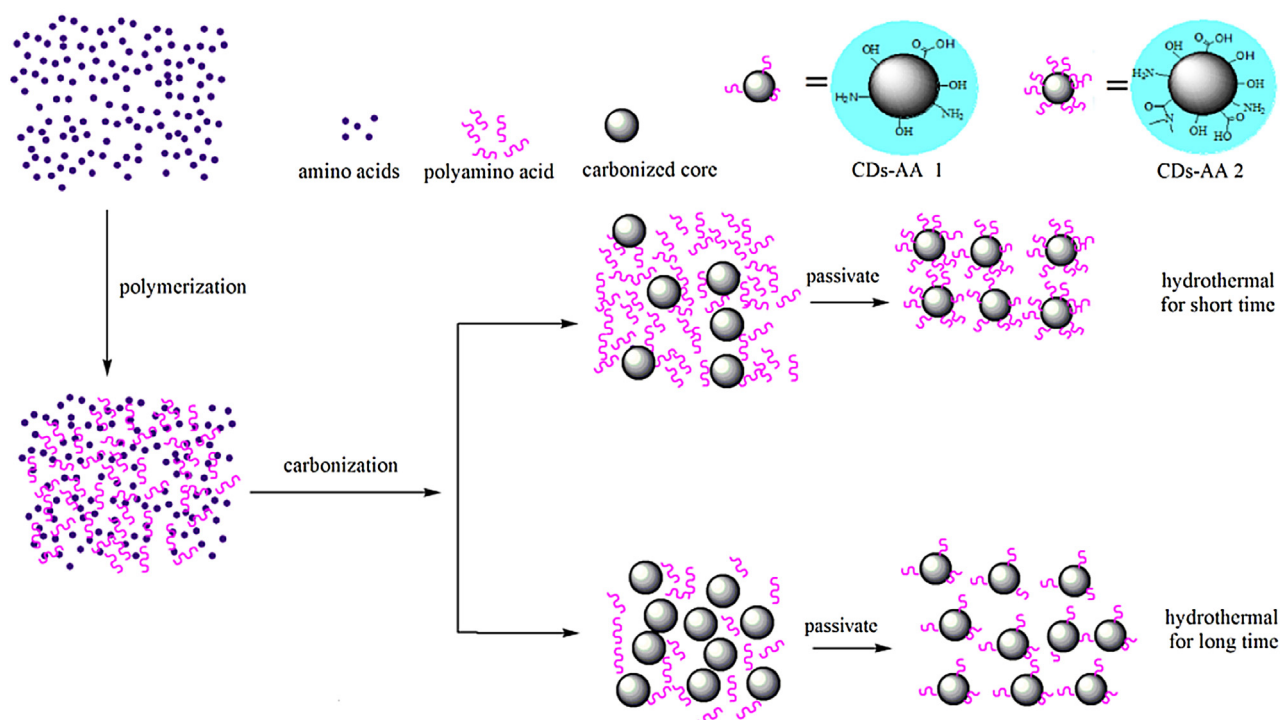


Fig. 4. Schematic formation of NCDs-Asp through dehydration, polymerization, carbonization and passivation.

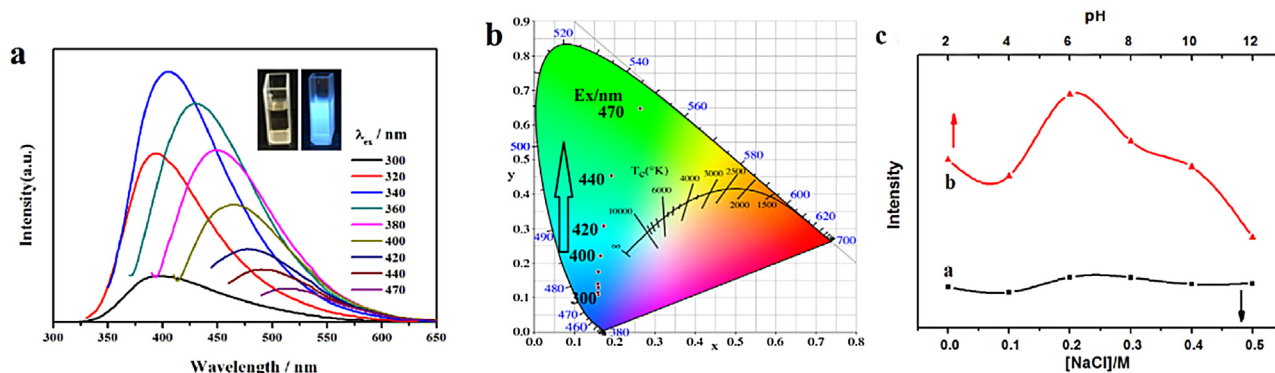


Fig. 5. PL spectra (a) and corresponding color coordinate (b) of the NCDs-Asp at different excitation wavelengths and effects of the NaCl concentration and pH on the PL intensity of NCDs-Asp (c). Inset in (a) is the photograph of the NCDs-Asp under daylight (left) and 365 nm UV light (right).

Therefore, according to the experimental results, a comprehensive consideration of the N-doping amount and surface passivation degree, we chose 18 h as the hydrothermal reaction time, unless otherwise specified.

3.4. Photoluminescent properties of NCDs formed from amino acids

Like most luminescent CDs, the NCDs displayed the excitation wavelength dependent fluorescence behavior. For example, when the excitation wavelength was changed from 300 to 470 nm, the corresponding PL emission peaks of NCDs-Asp (hydrothermal time 15 h) shifted from 397 to 513 nm (Fig. 5a). Meanwhile, the intensity of emission peaks became weak gradually with emission color range from blue to green in the corresponding Commission International d'Eclairage coordinates (CIE) of the samples (Fig. 5b) and the strongest emission peak at 405 nm (λ_{ex} = 340 nm) in accordance with Table S2 (see Supporting information). Moreover, the PL intensity of NCDs slightly change with NaCl concentrations (0.1–0.5 M) and changed with pH varying from 2 to 12 as shown in Fig. 5c. Other NCDs also had the similar PL properties (Fig. 6) and the strongest emission peaks of the NCDs occurred at 400–470 nm with a Stokes shift of 64–80 nm.

Herein, we investigated the PL mechanism of NCDs. First of all, we explored the outer electronic transition from a bonding orbital to antibonding orbital, such as π - π^* and n - π^* transition in NCDs by investigating the characteristics of UV–vis spectra. As shown in Fig. 6, the UV–vis spectra of NCDs exhibited wide electronic band

gap in the UV region (230–350 nm) with a tail extending into the visible range and with an absorption peak at around 270 nm, which was typical of that generally reported for other CDs [27,31]. More importantly, the absorption peak at around 270 nm was attributed to π - π^* transition of aromatic structure C–C bonds for carbon core [31], while the absorption band at 300–320 nm was attributed to n - π^* transition of C=O bonds or other connected group [32]. Since amino and hydroxy groups, as surface passivation groups, were electron donating and could enhance the conjugation degree of NCDs, the absorption peak of π - π^* transition was red-shift. The UV–vis spectra indicated that the larger energy band of NCDs was due to electronic states with well-defined conjugate aromatic structure and surface passivation.

Subsequently, in order to better understand the PL mechanism of NCDs formed from amino acids, we further explored the energy transformation in PL process. The more information about the excitation energy (E_x), emission energy (E_m) and the optical band gaps (E_g) of NCDs are shown in Table S1 (Supporting information). As shown above, from maximum excitation wavelength, the lowest direct absorption edge of NCDs was found to be 3.10–3.76 eV (average 3.47 eV). Further, from the broad absorption of NCDs we estimated the apparent optical band gap energy. The direct optical band gap energy of NCDs was found to be around 3.77–5.17 eV (average 4.50 eV). In fact, the optical band gap was corresponded to the energy difference between the highest occupied molecular orbital and lowest unoccupied molecular orbital (HOMO–LUMO gap, that is E_g). It is notable that the value of the optical band gap

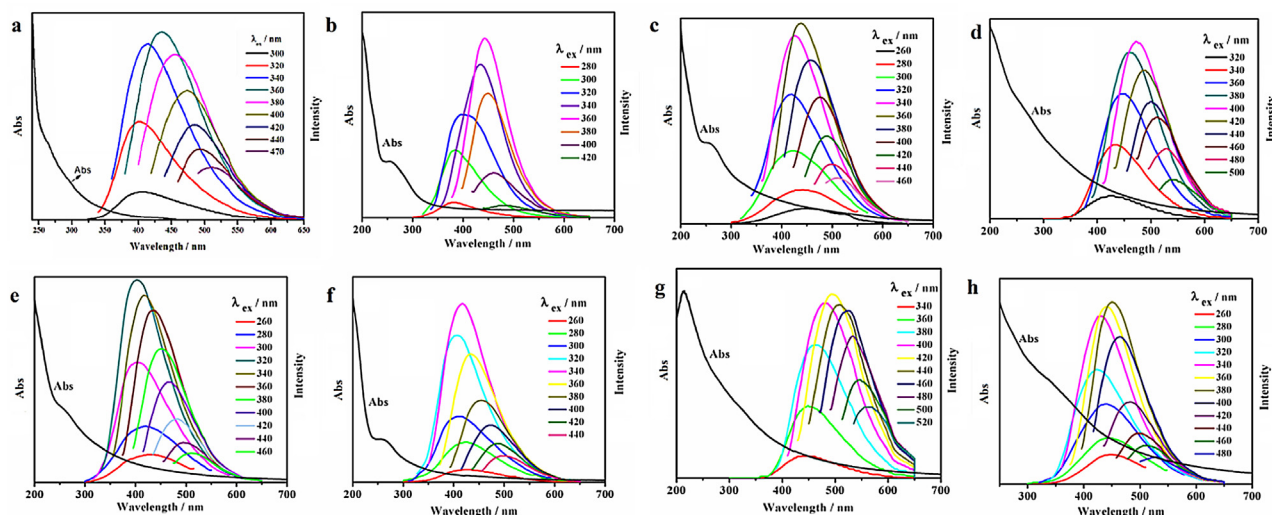
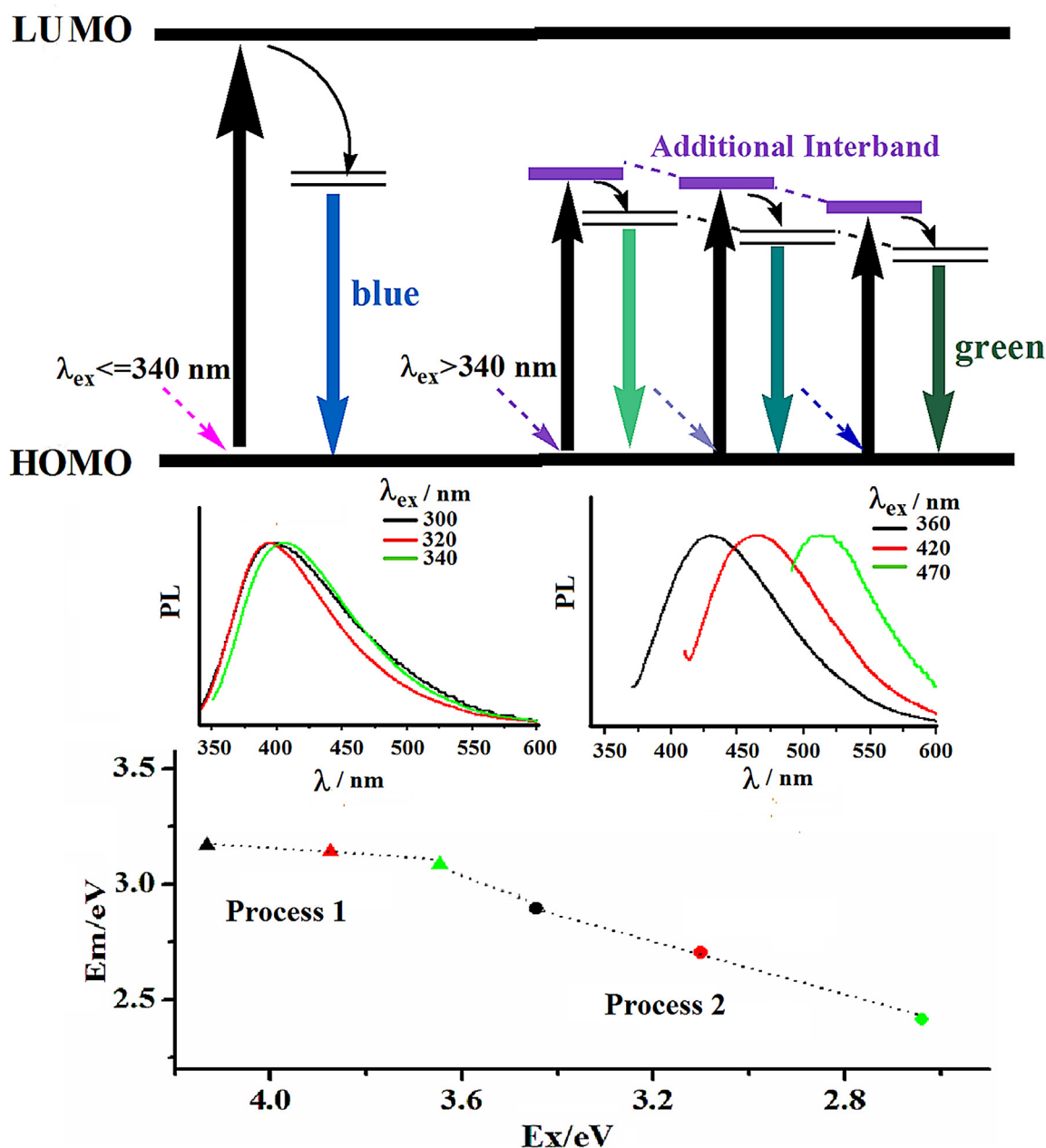


Fig. 6. UV–vis absorption and PL emission spectra of NCDs synthesized by different amino acids: Glu (a), Val (b), Phe (c), Arg (d), Pro (e), Thr (f), Cys (g), Ser (h).

(4.50 eV) of NCDs was greater than its excitation energy (3.47 eV, Table S1), indicating that there was an additional interband in between HOMO and LUMO. Kumar et al. suggested that an additional interband around 3.28 eV could be formed within the energy gap of amino-functionalized graphene quantum dot by p orbital hybridization in between C–N atoms at the edge sites [33]. Since the excitation energy of the strongest emission intensity of NCDs was closed to the value of 3.28 eV, we inferred that a new interstate energy level at around 3.10–3.76 eV (the excitation energy, Table S1) was formed in between the HOMO and LUMO states of NCDs like functionalized graphene quantum dots. Since NCDs had much larger energy band (4.50 eV) and an additional interband (3.28 eV) within the energy gap, the NCDs possessed the PL mainly in range of blue to green section [31,33]. In order to explain this result, take NCDs-Asp for an example, the possible PL

emission mechanism of NCDs was schematically shown in Scheme 1. In the case, the PL was contributed by two different transition processes, namely, transition process 1 and 2. For transition process 1, the PL was band to band transition, that is, the transitions from the HOMO to LUMO by higher excitation energy ($\lambda_{\text{ex}} < \lambda_{\text{ex}}(\text{max})$), E_{ex} was closed to HOMO–LUMO gap, which was the blue emission and not excitation-dependent PL behavior. For transition process 2, the PL was interstate or defect induced emission, in which the transition from the HOMO to the additional interband occurred under the lower excitation energy ($\lambda_{\text{ex}} \geq \lambda_{\text{ex}}(\text{max})$) and the PL was strong excitation-dependent. The surface passivation of NCDs could form the additional interband which reduced the excitation energy of the system compared with that in the transition from band to band, and which was also related to the excitation-dependent PL behavior.



Scheme 1. Schematic representation of the proposed energy band diagram model based on NCDs-Asp to explain the possible PL emission mechanism of NCDs. The emission peak intensity of NCDs-Asp was normalized in the PL spectra.

4. Conclusion

We have successfully developed a green and facile approach to synthesize N doped carbon dots by one step hydrothermal treatment of different amino acids. The formation and surface passivation of NCDs were accomplished simultaneously. The NCDs possessed single or few layers graphene-like carbon rings structures and surface passivation by amino and oxygen-containing groups and showed excellent aqueous dispersibility, and considerable stability. Furthermore, the PL emission of NCDs exhibited higher QYs (13.3–41.3%). Significantly, the research showed a strong correlation between the surface passivation and the PL feature of NCDs. The surface passivation could form the additional interband which played a pivotal role in reducing the excitation energy of NCDs, leading to the PL emission from the blue to green region in excitation wavelength dependence. A family of NCDs based on amino acids was attractive because of its simplicity and favorable optical properties.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.materresbull.2017.01.013>.

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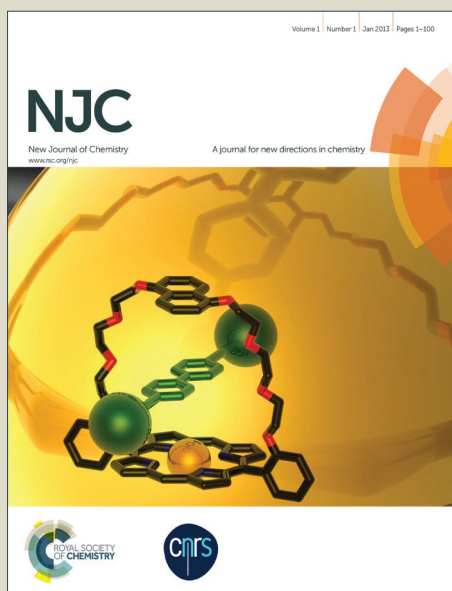
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Kinetics of nitrogen-doped carbon dot formation via hydrothermal synthesis

Takashi Ogi^{a,*}, Kana Aishima^a, Fitri Aulia Permatasari^a, Ferry Iskandar^b, Eishi Tanabe^c, and Kikuo Okuyama^a

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Carbon Dots (CDs) have attracted great attention because of their unique luminescence properties, chemical inertness, thermal stability, high water solubility, low toxicity, and ease of functionalization. Here, the kinetics of nitrogen-doped CD (N-CD) formation by hydrothermal synthesis were evaluated in an attempt to realize rapid and efficient production of N-CDs. A series of N-CDs was synthesized using various heating rates, reaction times, reaction temperatures, and precursor concentrations. Characterization of the series of N-CDs indicated that N-CD formation is a first-order reaction with a reaction rate constant of 0.634 min⁻¹. In addition, systematic investigation revealed that synthesis temperature is a more important factor to obtain highly fluorescent N-CDs than reaction time. Citric acid amide are formed by reaction between citric acid and urea at 130 °C and N-CD consisting of two or three citric acid amide molecules are formed through the dehydration, deammoniation and dehydrogenation of citric acid at 150 °C. By adjusting operating conditions, N-CDs with a highest quantum yield of 39.7% could be produced at a production rate of 50 g h⁻¹ with a reaction time of 16 min. The N-CDs were then embedded in polyvinyl alcohol (PVA) nanofibers. The luminescent intensity of the N-CD–PVA composite nanofibers was more than twice that of the N-CDs in solution.

1. Introduction

Carbon-related fluorescent materials including carbon nanotubes,¹ nanodiamond,² carbon dots (CDs),^{3–6} graphene quantum dots,^{7–11} and metal–carbon oxynitrides^{12–15} have attracted growing interest in recent years because of their unique properties such as low toxicity, intense luminescence, and chemical inertness. In particular, CDs are regarded as an emerging functional material because of their numerous novel chemical and physical properties, and their facile and inexpensive preparation. CDs can be prepared by several bottom-up methods¹⁶ including combustion methods,¹⁷ pyrolysis in concentrated acid,¹⁸ microwave heating,¹⁹ electrochemical synthesis,²⁰ hydrothermal synthesis,^{21–23} carbonization in a microreactor,²⁴ ultrasonic synthesis,⁶ microwave–hydrothermal synthesis,^{25,26} and plasma–hydrothermal synthesis.²⁷ Of these methods, hydrothermal reactions are attractive as a simple, low-cost, one-step route to fabricate CDs.

Heteroatom doping of CDs during their hydrothermal formation has recently received an increasing amount of attention as an effective method to enhance the luminescent

properties of CDs by increasing their quantum yield (QY) and tuning their emission wavelength. Dong *et al.*²⁸ synthesized nitrogen and sulfur co-doped CDs (N,S-CDs) with a high fluorescence QY of 73% from citric acid (CA) and L-cysteine by hydrothermal treatment at 200 °C for 180 min. Meanwhile, Wu *et al.*²⁹ used natural proteins (*Bombyx mori* silk) to produce nitrogen-doped CDs (N-CDs) with a QY of 13.9% in a one-step hydrothermal reaction at 190 °C for 180 min. Zhu and co-workers³⁰ realized a multicolor ink using N-CDs prepared by hydrothermal treatment of CA and ethylenediamine precursors at 150–300 °C for 300 min. Yang *et al.*³¹ also synthesized N-CDs with a QY of 13.5% from ammonium citrate at 160 °C for 360 min. Xu and colleagues³² fabricated sulfur-doped CDs (S-CDs) using sodium citrate and sodium thiosulfate. S-CDs prepared by hydrothermal reaction at 200 °C for 360 min exhibited a high QY of 67%. In other related work, Shen *et al.*³³ produced boron-doped CDs with a QY of 50% from boric acid and ethylenediamine heated at 260 °C for 720 min that can be used as light-emitting diode converter materials.

As indicated above, the QY and formation mechanism of heteroatom-doped CDs prepared by hydrothermal synthesis have been well studied. However, the kinetics of heteroatom-doped CDs formation in hydrothermal synthesis have never been discussed. Thus, the understanding of the required (minimum) reaction time and operating temperature for the formation of highly fluorescent heteroatom-doped CDs is still unsatisfactory. Both reaction time and operating temperature need to be considered to efficiently synthesize heteroatom-

^a Department of Chemical Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan

^b Department of Physics, Institute of Technology Bandung, Ganesha 10, Bandung 40132, West Java, Indonesia.

^c Hiroshima Prefectural Institute of Industrial Science and Technology, 3-10-32 Kagamiyama, Higashi-Hiroshima 739-0046, Japan

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doped CDs, particularly from the viewpoint of minimizing energy and operating costs. A short reaction time is advantageous because hydrothermal synthesis generally uses a large amount of energy. In other words, the reaction time determines the operating cost of this process. Nevertheless, the current literature lacks reports considering the scale up of this type of heteroatom-doped CDs fabrication process.

Recently, we focused on the transient nature of nitrogen-doped carbonaceous fluorescent material formation during hydrothermal reaction of CA and urea as starting materials.³⁴ We measured the photoluminescence (PL) properties of the products as a function of reaction time during the preparation of carbonaceous fluorescent materials using a custom-made autoclave apparatus. We found that stable nanocrystalline CDs with a high QY of 43.6% could be synthesized with a relatively short reaction time of 90 min. Nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) analyses revealed that the molecular structure of the nanocrystalline CDs prepared after 90 min of reaction was a condensate of two or three citric acid amides. However, further investigation including consideration of kinetics is still needed to unambiguously determine the minimum reaction time and operating temperature for CD formation. Therefore, as a continuation of our previous work, here we investigate the dependence of reaction time, heating rate, and precursor concentration, and operating temperature on the PL and chemical structure of N-CDs. In addition, the minimum required operating temperature for the formation of highly fluorescent N-CDs by hydrothermal synthesis is evaluated through a series of experiments. N-CD–polyvinyl alcohol (PVA) composite fluorescent nanofibers are also synthesized.

2. Experimental

2.1. Synthesis of N-CDs

To investigate the time dependence of N-CD formation including the evolution of chemical structure and luminescent properties, we developed the experimental apparatus for hydrothermal synthesis shown in Fig. S1. In this study, CA (Sigma-Aldrich, St. Louis, MO, USA) and urea (Sigma-Aldrich) were used as precursors for the synthesis of N-CDs. The precursor solutions were prepared by dissolving CA and urea in pure water at 25 °C for 10 min. The total concentration of CA and urea in the precursor solutions was varied from 0.0254 to 1.27 mol L⁻¹. The pH of the initial solutions was 3.0. The precursor solutions were transferred into a 50-mL stainless steel autoclave and heated at 130–180 °C while being stirred. To obtain the time course of N-CD formation, aliquots of the reaction solution were periodically withdrawn and their optical and physical properties were analyzed without further treatment like surface passivation.

2.2. Synthesis of N-CD–PVA composite nanofibers

An electrospinning method was used to produce N-CD–PVA composite fibers. PVA (12.5 g, M_w = 500, Kanto Chemical Co., Inc., Japan) was dissolved in the synthesized N-CD solution (50 g, 20 wt%). The produced solution was electrospun using a

similar setup to that described in our previous work.³⁵ The voltage, syringe distance, and precursor flow rate were kept constant at 15 kV, 20 cm, and 1.0 $\mu\text{L min}^{-1}$, respectively.

2.3. Materials characterization

PL and PL excitation (PLE) spectra were measured using a spectrofluorophotometer (RF 5300PC, Shimadzu, Kyoto, Japan) equipped with a xenon lamp. The internal QY was measured using an absolute PL QY measurement system (C9920 02, Hamamatsu Photonics, Shizuoka, Japan) with a barium sulfate-coated integrating sphere and a xenon lamp as the light source. All PL analyses were performed at room temperature under excitation at 365 nm unless otherwise stated. The calculated errors of the experimental results were less than 10%. Elemental composition and functional group analyses were performed on an X-ray photoelectron spectrometer (PHI Quantera II, Ulvac-PHI, Inc.) and FTIR spectrometer (IRAffinity-1S, Shimadzu), respectively. ¹H NMR spectra were acquired on a Bruker Avance 600 spectrometer equipped with a triple-resonance cryoprobe at 25 °C. Sample morphology was observed by transmission electron microscopy (TEM; JEM-3000F, JEOL, Tokyo, Japan) at an accelerating voltage of 300 kV and field emission microscopy (FESEM; S-5000, Hitachi Corp., Tokyo, Japan) at 20 kV. Particle diameter was measured by dynamic light scattering (DLS; Zetasizer Nano, Malvern Instruments Ltd., Worcestershire, UK).

3. Results and discussion

3.1. Effect of heating rate on the optical properties of N-CDs

N-CDs were synthesized at heating rates of 2.0, 3.0, 4.0, 5.0, and 9.6 °C min⁻¹ using the same initial concentration (1.27 mol L⁻¹). To observe the change of optical properties of the N-CDs with reaction time, solution samples were collected every 10 min from 40 to 120 min after beginning heating (experimental start).

Fig. 1 shows digital photographs of the samples collected at different reaction times under visible or UV light at a constant heating rate of 4.0 °C min⁻¹. The obtained samples were colorless and transparent under visible light. Under UV irradiation, blue emission was obviously observed by the naked eye for the samples collected after over 33 min of reaction. The emission intensity increased with reaction time until it reached maximum PL intensity at around 33 min, and then began to decrease. According to the previous report³⁴, a long reaction time increased the size of the CDs, resulting in a decrease of emission intensity. This behaviour is supported by an investigation of the effect of initial precursor concentration on the luminescent properties of CDs (Details are provided in the supporting information SI-2). For all precursor concentrations, the emission intensity increased rapidly as the reaction time lengthened until the maximum PL intensity was reached. The reaction time needed to achieve maximum PL intensity is longer at lower precursor concentration. This is because the reaction rate of CA and urea is quite slow when their initial concentrations are low.

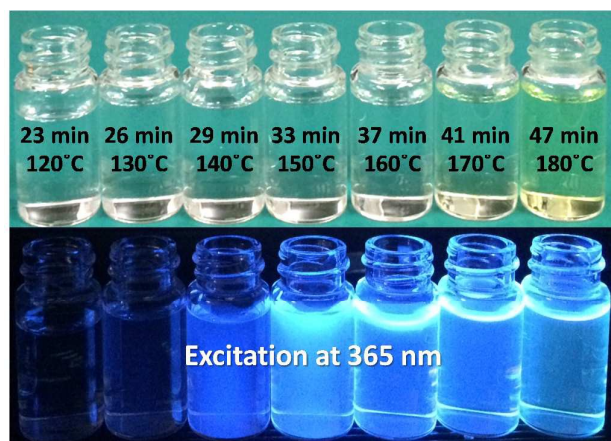


Fig. 1 Digital photographs under visible (top) and ultraviolet (bottom) light demonstrating the effect of reaction time on the optical properties of N-CDs. Initial precursor concentration: 1.27 mol L^{-1} , heating rate: $4.0^\circ\text{C min}^{-1}$. The heating rate was conducted from the gradient of Figure 2(a) which indicates relations of reaction time and solution temperature.

Fig. 2(a) shows the time courses of the PL intensity of N-CDs and solution temperature for each heating rate. When the heating rate was increased, the reaction time required to obtain the maximum PL intensity shortened (Fig. 2(b)). This is because the solution rapidly attained the required temperature for N-CD formation at higher heating rate. According to Fig. 2(a), the solution temperatures that gave the maximum emission intensity were in the range of $150\text{--}170^\circ\text{C}$. This result indicates that N-CD formation is affected more strongly by the solution temperature than the reaction time or precursor concentration. The QYs and absorption ratios of the N-CDs were also almost the same regardless of heating rate (Table 1). This indicates that the PL intensity of N-CDs does not depend on the heating rate. In other words, highly luminescent N-CDs can be obtained even using a high heating rate. This is a considerable advantage from the viewpoint of rapid N-CDs production. In this experiment, N-CDs displaying high PL intensity (QY=33.5%) were obtained after 16 min of reaction using a heating rate of $9.6^\circ\text{C min}^{-1}$. This reaction time (16 min) is much shorter than those used to prepare other heteroatom-doped CDs.^{27-29,31-33} We also confirmed that the N-CDs possess high photostability, as shown in Fig. 3; solutions retained their high QYs for at least 1 week under atmospheric conditions (25°C , 1 atm.) without further treatment.

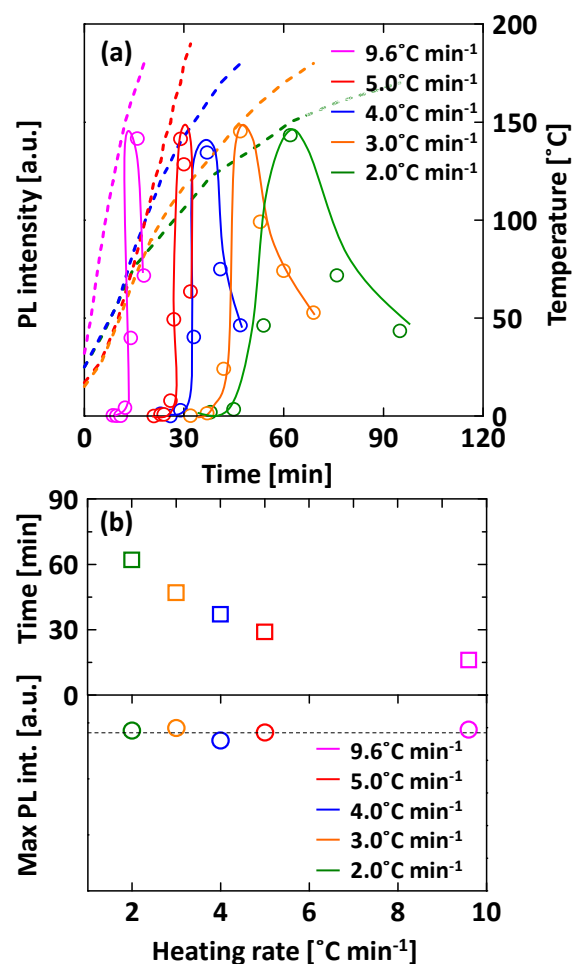


Fig. 2 Dependence of PL characteristics of N-CDs synthesized at different heating rates on reaction time. (a) PL intensity, (b) maximum PL intensity and reaction time to obtain maximum PL intensity. Initial concentration: 1.27 mol L^{-1} .

Table 1 Quantum yields (QYs) and absorption ratios (Abs.) of samples prepared using different heating rates

Heating rate [$^\circ\text{C min}^{-1}$]	Reaction time [min]	QY [%]	Abs. [%]
1.6	70	30.7	97.6
2.0	62	33.4	97.1
3.0	47	33.5	97.3
4.0	37	31.8	97.7
5.0	29	33.5	96.9
9.6	16	33.5	96.8

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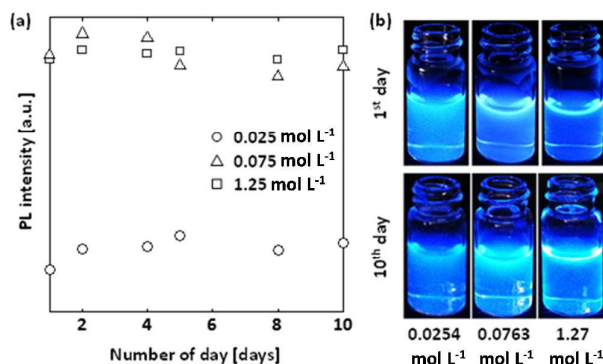


Fig. 3 Photostability of N-CDs prepared using different initial precursor concentrations. (a) PL intensity, and (b) digital photograph under 365 nm irradiation. 1 week under atmospheric conditions (25 °C, 1 atm.)

3.2. Kinetic analysis of highly fluorescent N-CD formation

Based on the above results, we calculated the reaction rate constant of N-CD formation. In this study, we used trial-and-error approach to properly determine the reaction rate constant because strongly influenced physical properties in the N-CDs formation reaction are unclear. In the calculation, it was assumed that N-CD formation was a first-order reaction and occurred in the temperature range of 130–160 °C. Moreover, because N-CD formation is affected by the solution temperature, the following equations were used.

$$t = 0.0047T^2 - 0.33T + 8.7 \quad (1)$$

$$r = -dC_{CA}/dt = -dC_{CA}/(0.0094T - 0.33)dT = kC_{CA} \quad (2)$$

$$\ln C_{CA} = -k(0.0047(T_2 - T_0^2) - 0.33(T - T_0)) + \ln C_{CA0} \quad (3)$$

Here, r is the reaction rate [mol min^{-1}], C_{CA} is the concentration of citric acid [mol], k is the reaction rate constant [min^{-1}], t is the reaction time [min] and T is the solution temperature [$^{\circ}\text{C}$]. Equation 1 is the relation of solution temperature and reaction time derived from Fig. 2(a). In addition, k was calculated as the gradient of a plot of $\ln C_{CA}$ versus $0.0047(T_2 - T_0^2) - 0.33(T - T_0)$ in Eq. (3). Meanwhile, C_{CA} was determined from the ratio of peak intensities in ^1H NMR spectra of the samples. In this measurement, we used the samples synthesized at 130, 140, 150 and 160 °C with a fixed precursor concentration of 1.27 mol L^{-1} and heating rate of $2.0 \text{ }^{\circ}\text{C min}^{-1}$. The ^1H NMR spectrum for each sample is depicted in Fig. 4(a). Table 2 lists C_{CA} calculated for each solution temperature, and Fig. 4(b) shows the plot of $\ln C_{CA}$ against X , where X is $(0.0047(T_2 - T_0^2) - 0.33(T - T_0))$ in Eq. (3). The plot was a straight line, so N-CD generation is a first-order reaction. Then, k was calculated to be 0.095 min^{-1} from the gradient of the graph (Fig. 4(b)). Therefore, in the solution temperature range from 130 to 160 °C, the reaction rate of N-CD formation is expressed as $r = 0.095 C_{CA}$. This means that if we produce 1 m^3 of N-CD suspension (12.8 wt%) per hour using a batch reactor with a heating rate of $2.0 \text{ }^{\circ}\text{C min}^{-1}$, the required reactor volume is 0.525 m^3 (=525 L). However, if we use a heating rate of $5.0 \text{ }^{\circ}\text{C min}^{-1}$, the required reactor volume can

be downsized to 0.079 m^3 (=79 L) (calculation details are provided in the supporting information SI-3). Such scale down of the reactor could be effective to minimize the energy consumption and cost of N-CD production.

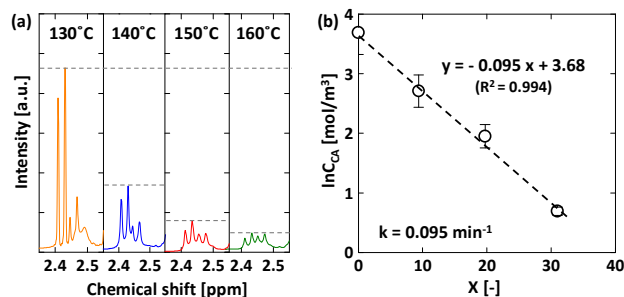


Fig. 4 Calculation of the reaction rate constant of N-CD production. (a) ^1H NMR measurements, and (b) plotted results. The calculated errors of the experimental results were less than 10%.

Table 2 Concentration of citric acid (C_{CA}) and the values of X ($0.0047(T_2 - T_0^2) - 0.33(T - T_0)$) at various solution temperatures.

Temp. [$^{\circ}\text{C}$]	C_{CA} [mol m^{-3}]	$\ln C_{CA}$ [mol m^{-3}]	X [-]
130	40	3.69	0.00
140	15	2.71	9.39
150	7.0	1.95	19.7
160	2.0	0.693	31.0

3.3. Effects of solution temperature on N-CD formation

Section 3.1 indicated that N-CD synthesis is affected more strongly by solution temperature than reaction time. To more clearly elucidate this finding, the effects of synthesis temperature on N-CD formation were investigated. In this series of experiments, the precursor concentration was kept constant at 0.127 mol L^{-1} . Fig. 5 shows the dependence of the PL properties of the N-CD solutions on reaction time at different temperatures (corresponding PL and PLE spectra are presented in Fig. S4). When the synthesis temperature was below 150 °C, PL intensity decreased after reaching its maximum value and the reaction time needed to achieve maximum PL intensity was relatively long (60 min). This result indicates that even at a low synthesis temperature, highly fluorescent N-CDs can be synthesized by extending the reaction time. Since the C-N bonding contributes additional electron to the π system on N-CD³⁶, the slight decrease of PL intensity when the synthesis temperature over 160 °C might be attributed to the slightly conversion C-N bonding into N-graphitic³⁷. It was supposed by the N1s spectra shift to the higher binding energy (Fig. 6e). However, taking experimental error bar into consideration, these decrease are quite small. Thus, the reaction time does not affect the luminescence properties of the solutions after nanocrystalline N-CD production. Accordingly, solution temperature has a larger effect on N-CD synthesis than reaction time.

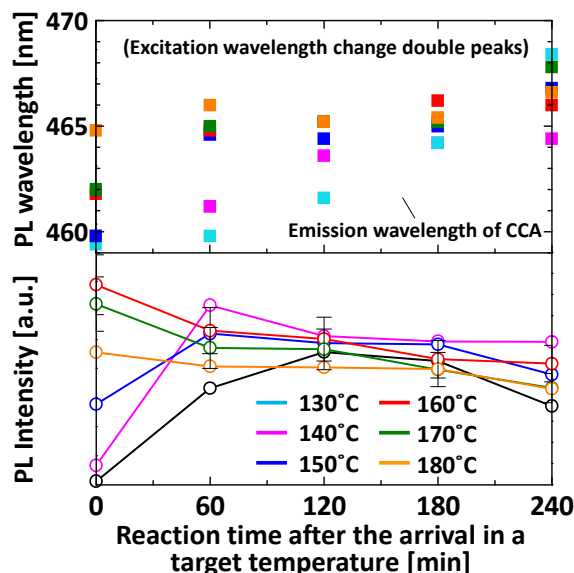


Fig. 5 Dependence of the PL properties of N-CDs prepared at different temperatures on reaction time. Initial concentration: 0.127 mol L^{-1} .

3.4. Dependence of the molecular structure of N-CDs on reaction time

The above results reveal that the luminescent properties of N-CDs are strongly influenced by the reaction temperature. Therefore, FTIR spectroscopy, ^1H NMR spectroscopy and X-ray photoelectron spectroscopy (XPS) analyses were conducted for periodically withdrawn aliquots of reaction solutions at reaction temperatures of 130, 150, and 180 $^{\circ}\text{C}$ to clarify the molecular structure of the luminescent species in the solutions. In this series of experiments, the precursor concentration and heating rate were constant at 1.27 mol L^{-1} and $1.6 \text{ }^{\circ}\text{C min}^{-1}$, respectively. Regarding PL and PLE properties, we confirmed the same phenomena as observed in Fig. 2(a); that is, maximum emission intensity was attained at 150 $^{\circ}\text{C}$ and it decreased as reaction time extended.

Fig. 6(a) shows FTIR spectra of the samples. The spectra of the samples prepared at 130 and 150 $^{\circ}\text{C}$ were almost the same, while new peaks appeared at 1345, 1266 and 868 cm^{-1} for the sample fabricated at 180 $^{\circ}\text{C}$. These new peaks indicate the formation of an ester or aromatic C-H bond. Therefore, the N-CD structure produced at 180 $^{\circ}\text{C}$ is different from that formed at lower temperature.

Fig 6(b) displays ^1H NMR spectra of the samples. The peaks of all samples are shifted to higher magnetic field from those of pure CA. This peak shift indicates an increase of hydrogen density. For the sample prepared at 130 $^{\circ}\text{C}$, four peaks similar to those of pure CA are observed, indicating the basic structure of CA is retained. As the solution temperature increases, peak intensities at 2.40, 2.43, 2.60, and 2.62 ppm, which correspond to the content of protons, decreased. This indicates the progress of CA condensation. For the sample prepared at a solution temperature of 180 $^{\circ}\text{C}$, a weak peak was

observed around 3.6 ppm. This peak might be attributed to an ether structure or split peak caused by allylic coupling. This result therefore indicates the deconstruction of the basic structure of CA through its condensation reaction at 180 $^{\circ}\text{C}$.

The XPS survey data for the samples (Fig. 6(c)) contain peaks at around 288, 399, and 532 eV that correspond to C1s, N1s, and O1s, respectively. The spectrum indicates that the samples are doped with nitrogen, which originated from urea. The high-resolution C1s spectrum contains four peaks at binding energies of 285.2, 286.7, 287.7 and 289.0 eV (Fig. 6(d)), which correspond to C-C, C=C, C-H; C-O, C-N; C=O, C=N; and O=C-O, O=C-N₂ bonds, respectively. For the sample prepared at 130 $^{\circ}\text{C}$, the peak intensity at 289.0 eV is higher than those of the other peaks, which suggests that the main species in the solution are urea and CA. For the sample prepared at 180 $^{\circ}\text{C}$, the peak intensities at 286.7 and 287.7 eV, which might be derived from graphene, are increased compared with those of the other peaks. Furthermore, a peak derived from graphitic C (283.8 eV) appears in the spectrum of the sample fabricated at 180 $^{\circ}\text{C}$. This result indicates the chemical species synthesized at 180 $^{\circ}\text{C}$ does not possess graphene structure, although aromatic rings are generated in the sample produced at 180 $^{\circ}\text{C}$. The high-resolution N1s spectra of the samples contain two peaks at 400.0 eV, which is attributed to C-N bonds, and 401.0 eV, which is assigned to graphitic N and N-H bonds (Fig. 6(e)). At higher solution temperature, the intensity of the peak at 401.0 eV derived from graphitic N increased; this result indicates N is doped into the CD structure.

Fig. 7 presents typical TEM images and DLS measurement results for samples prepared at solution temperatures of 150 and 180 $^{\circ}\text{C}$. The produced particles are well dispersed and partially crystallized. The particle size produced at 150 $^{\circ}\text{C}$ is about 2 nm (Fig. 7(a)) and between 10 and 40 nm when formed at 180 $^{\circ}\text{C}$ (Fig. 7(b)). However, it is difficult to declare the size of main chemical substance existed in the highest fluorescent sample is 2 nm due to the limitation of TEM resolution. In other words, the sample has a possibility of including chemical substances below 1 nm in size as indicated by NMR analysis. Regarding DLS result, we generally obtain larger size value compared to the actual size in case the sample is non-spherical or hydrated particle. From these facts, the size obtained from NMR is reasonable than that obtained from SEM and DLS analysis in this research.

Based on all of the above analyses, a possible formation mechanism of the luminescent species, *i.e.*, N-CDs, *via* hydrothermal reaction of CA and urea is illustrated in Fig. 8. Unlike in previous literature,³⁴ the mechanism suggested here is considered to depend on solution temperature. The proposed mechanism is as follows. When the solution temperature reached around 130 $^{\circ}\text{C}$, citric acid amide formed by reaction between CA and urea. Further heating caused the condensation of citric acid amide through the dehydration, deamination and dehydrogenation of CA. During this reaction, N-CDs consisting of two or three citric acid amide molecules formed at 150 $^{\circ}\text{C}$, as indicated by the NMR analysis. We observed the strongest PL intensity from the sample formed at this temperature. Further increasing solution

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temperature promoted condensation of citric acid amides to generate larger N-CDs.

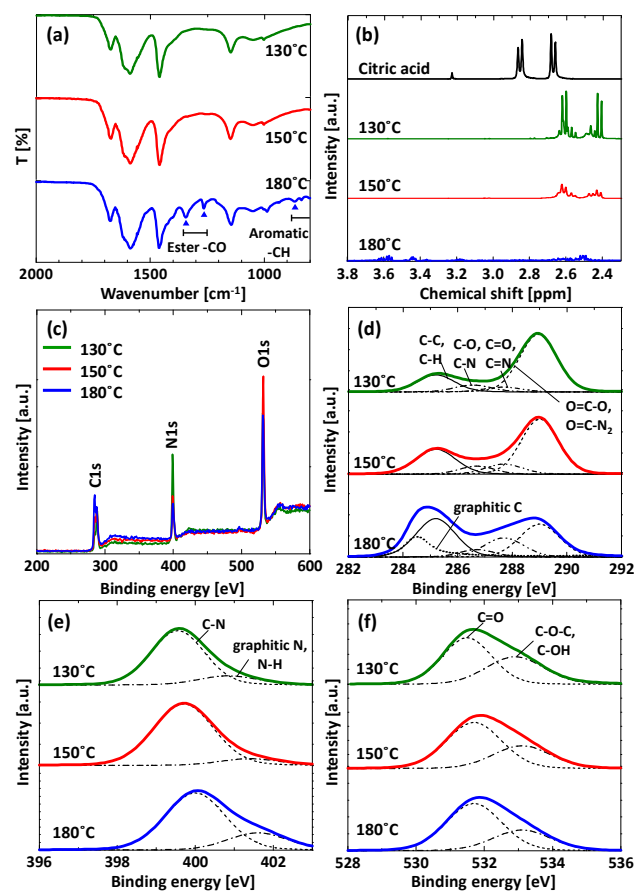


Fig. 6 Analyses of N-CD samples prepared at different temperatures. (a) FTIR spectra, (b) ^1H NMR spectra, and (c) XPS survey spectra along with corresponding (d) C1s, (e) N1s and (f) O1s spectra. Initial concentration: 1.27 mol L^{-1} , heating rate: $1.6^\circ\text{C min}^{-1}$.

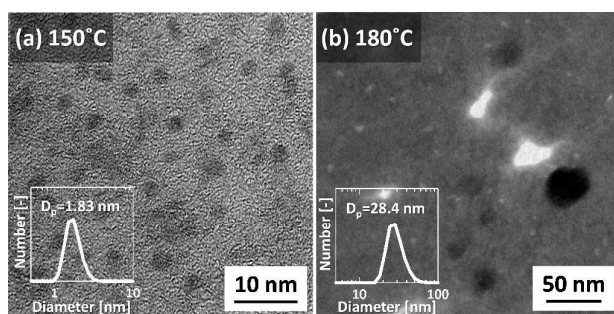


Fig. 7 TEM images of samples prepared at (a) 150 and (b) 180 $^\circ\text{C}$. Initial concentration: 1.27 mol L^{-1} , heating rate: $1.6^\circ\text{C min}^{-1}$.

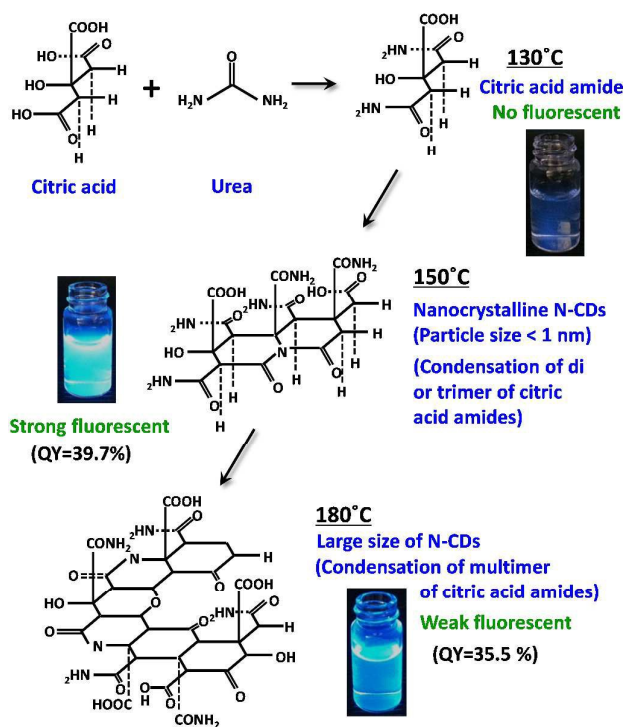


Fig. 8 Proposed mechanism of the formation of N-CDs *via* hydrothermal reaction of citric acid and urea.

3.5. Application of N-CDs in a fluorescent nanofibers

Fig. 9(a) and (b) depict photographs of a N-CD–PVA composite nanofiber mat synthesized *via* electrospinning of a dispersion of N-CDs in PVA. Under UV (365 nm) irradiation, the synthesized nanofiber mat exhibited dark blue emission (Fig. 9(b)). The scanning electron microscopy (SEM) image of the N-CD–PVA composite nanofibers in Fig. 9(c) indicates the fiber diameter is around 100 nm and uniform. Fig. 9(d) compares PL spectra of the N-CD–PVA nanofiber mat and N-CD colloidal solution. The nanofiber mat displays an emission peak at 449 nm and its intensity is more than twice that of the N-CDs in solution. This increase in emission intensity is attributed to the constraint of molecular movement of the functional groups that contribute to relaxation of the phosphor structure in solution, which results from hydrogen bonding among the amide, carbonyl, and hydroxyl groups in the N-CDs. The nanofiber mat containing N-CDs is mechanically stable, and can be easily molded into different geometries and sizes depending on the target application; *e.g.*, textured optical fibers and functional filters for sensing.

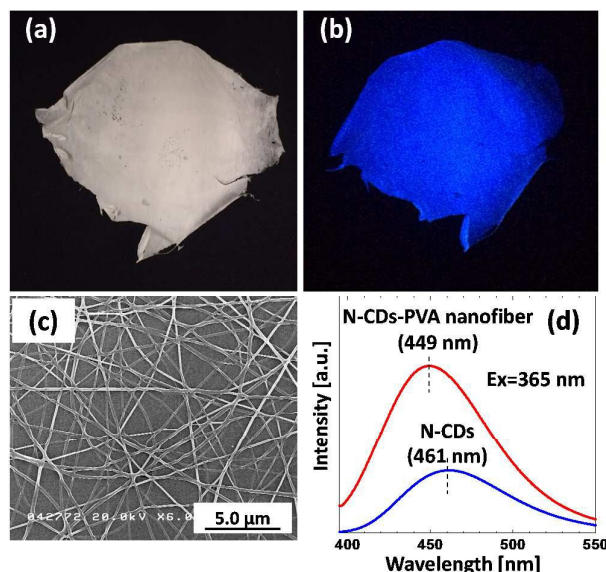


Fig. 9 N-CD-PVA composite nanofiber mat synthesized by electrospinning. Digital photographs of the mat under (a) visible and (b) UV (365 nm) light, (c) SEM image, and (d) fluorescence properties under excitation at 365 nm.

4. Conclusions

The chemical reaction rate in hydrothermal synthesis of highly luminescent nitrogen doped carbon dots was evaluated by investigating the effects of heating rate, synthesis temperature, and precursor concentration on the fluorescence properties and chemical structure of the nitrogen doped carbon dots produced. Nitrogen doped carbon dots with the highest quantum yield of 39.7% were prepared within 16 minutes by adjusting the heating rate, which is much faster than the reaction time for previously reported nitrogen doped carbon dots. Systematic assessment of the effects of various experimental parameters on the fluorescence properties of nitrogen doped carbon dots revealed that synthesis temperature is the most important factor determining the formation of stable, highly fluorescent nitrogen doped carbon dots. Based on this result, we suggested the mechanism of formation of nitrogen doped carbon dots via hydrothermal synthesis depend on solution temperature. Citric acid amide are formed by reaction between citric acid urea at 130 °C and further heating up to 150 °C caused the formation of nitrogen doped carbon dots consisting of two or three citric acid amide molecules through the dehydration, deamination and dehydrogenation of citric acid. Nitrogen doped carbon dots-polyvinyl alcohol composite nanofibers displayed high emission intensity compared with the nitrogen doped carbon dots suspensions. This indicates that the prepared nitrogen doped carbon dots-polymer composite nanofibers show potential for use in flexible optical and sensing applications.

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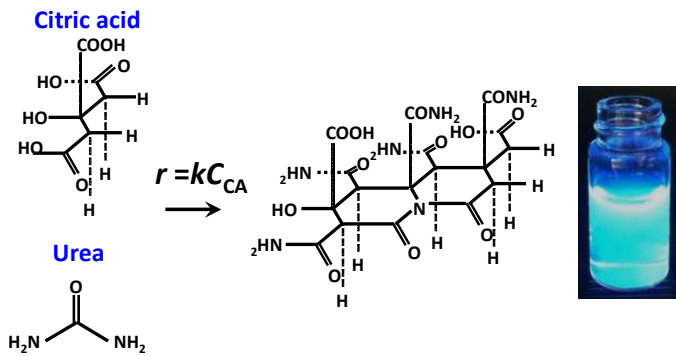
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Graphic Abstract:

How fast is the reaction of N-doped C-dot formation?





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NIT: 800.176.089-2

COMPROBANTE DE RECAUDO SISTEMA SIPI

Radicado: NC2022/0012263

Recibo: 26-269695

Fecha: 18/06/2026

CASTELLANOS & CO

NI: 8.001.746.411

Pago PSE No: 1273841

CUS: 401434166

Patente PCT

POTENCIADOR DE FOTOSÍNTESIS A BASE DE NANOPARTÍCULAS HÍBRIDAS DE CARBONO, SU PROCESO DE OBTENCIÓN Y SU USO COMO NANOBIIOESTIMULANTES Y NANO FERTILIZANTES EN CULTIVOS AGRÍCOLAS

Detalle del pago

Id.	Rentístico	Concepto y Servicio	Cant.	Val. Unit.	Subtotal
1	50005-01-03	Modificaciones / Correcciones al registro Patente PCT (DESCUENTO INTERNET)	1	\$ 236,000	\$ 236,000
TOTAL					\$ 236,000